1-1-2012

Structural Studies of the SMN-Gemin2 Complex

Kathryn Lorenz Sarachan
University of Pennsylvania, klorenz@mail.med.upenn.edu

Structural Studies of the SMN-Gemin2 Complex

Abstract
The proteins SMN and Gemin2 form the conserved core of the larger eponymous SMN complex, which also contains Gemins3-8 and unrip in an unknown stoichiometry. The complex facilitates the ordered assembly of seven Sm proteins onto a conserved site of an snRNA to form spliceosomal snRNP cores. A deficit in functional SMN as a consequence of deletions or loss-of-function mutations in the gene *SMN1* underlies the disease spinal muscular atrophy (SMA), a severe neurodegenerative disorder and leading genetic cause of infant mortality. Here, the NMR solution structure of the interacting domains of human SMN and Gemin2 is reported. The Gemin2-SMN heterodimer forms a novel all α-helical fold, comprising a single SMN helix embedded in an elongated, hydrophobic cavity of Gemin2. The interface between SMN and Gemin2 is mediated through highly conserved residues on the hydrophobic face of the amphipathic SMN helix and on four Gemin2 helices far separated in primary sequence. Mutations of interfacial residues predicted by the structure to be essential, but not of known disease mutations, disrupted the SMN-Gemin2 interaction. A number of biophysical studies also showed that unbound Gemin2 undergoes a significant conformational change upon SMN binding to become more compact, but belied reports of a Gemin2 self-interaction. A related set of investigations conducted on the full-length orthologous *S. pombe* Gemin2-SMN proteins showed that the complex occupies small, discrete oligomers that self-associate to form larger assemblies; its apparently large molecular weight by analytical size-exclusion chromatography results from an elongated, partially unstructured state. Together, these NMR and biophysical studies of SMN and Gemin2 provide the first high-resolution glimpse of the SMN-Gemin2 heterodimer, establish a framework for future structure-function studies investigating snRNP biogenesis and SMA pathogenesis and provide new valuable insight into the overall oligomerization state of the SMN complex.

Degree Type
Dissertation

Degree Name
Doctor of Philosophy (PhD)

Graduate Group
Biochemistry & Molecular Biophysics

First Advisor
Gregory D. Van Duyne

Keywords
Gemin2, SMN, spinal muscular atrophy

Subject Categories
Biochemistry | Biophysics

STRUCTURAL STUDIES OF THE SMN-GEMIN2 COMPLEX

Kathryn Lorenz Sarachan

A DISSERTATION

in

Biochemistry and Molecular Biophysics

Presented to the Faculties of the University of Pennsylvania

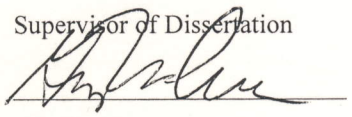
in

Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy

2012

Supervisor of Dissertation



Gregory D. Van Duyne
Professor of Biochemistry and Biophysics

Graduate Group Chairperson

Kathryn M. Ferguson
Associate Professor of Physiology

Dissertation Committee

Mark A. Lemmon, Professor of Biochemistry and Biophysics
S. Walter Englander, Professor of Biochemistry and Biophysics
Mitchell Lewis, Professor of Biochemistry and Biophysics
Ronen Marmorstein, Professor of Chemistry
A. Joshua Wand, Professor of Biochemistry and Biophysics
Linda K. Nicholson, Professor of Molecular Biology and Genetics, Cornell University

STRUCTURAL STUDIES OF THE SMN-GEMIN2 COMPLEX

Dedicated with love to my parents, who, when I called sobbing from college that first day to declare that leaving home had been “a BIG mistake,” insisted that I stick it out

And to my husband, who made me very glad I did.

Acknowledgments

It takes nothing less than a proverbial village to raise up a young scientist, and I would be remiss if I failed to acknowledge the guidance and support I was privileged to receive from numerous friends and mentors at every stage of this work.

I owe a resounding debt of gratitude to Gregory Van Duyne, my thesis advisor, for the enthusiasm, intellectual rigor and collegiality that distinguishes our lab in my mind from any other I've worked in. I appreciated your trust in allowing me to work with autonomy when the project called for it as much as I did your assistance – of which I was sometimes only too eager to avail myself! I know that the scientific reasoning I learned under your aegis will always be one of my most valued assets.

I extend thanks in equal measure to A. Joshua Wand, my *de facto* co-advisor, who graciously extended the hospitality of his expertise and his laboratory during collaboration on a lengthy and challenging endeavor. Grappling that project into submission will remain indelibly engraved on my memory as one of my proudest achievements. I am grateful for the intellectual challenge, the unwavering support and the happy occasion of having been adopted, as it were, into a second laboratory family.

I also acknowledge with thanks the other members of my thesis committee, Mark Lemmon, Mitch Lewis, and Ronen Marmorstein, who shared their time and their words of encouragement and thought-provoking perspectives. Walter Englander kindly offered his services as a reader of this dissertation; Linda Nicholson of Cornell University, who has been getting me out of academic scrapes since I was eighteen, generously agreed to serve as external reviewer.

Members of the Van Duyne laboratory, past and present, have always provided a sure source of companionship and support. Kushol Gupta never hesitated to share his wizardry with the tools of biophysics; he and Kay Perry excellently filled the roles both of mentors and friends. Renee Martin and Nisha Ninan shared with me the dubious "Team SMN" moniker and all the commiseration and enthusiasm and occasional all-nighters involved therein. My friendships with Karen Rutherford, Bob Sharp, Bryan Gibb, Peng Yuan, Scott Pesiridis, Kaushik Ghosh and Megan Neary have been a fun, stimulating, irreplaceable part of the fabric of my life at Penn, and I thank you.

With generosity, the Wand laboratory adopted a stray collaborating graduate student into their family; I acknowledge with particular gratitude Vonni Moorman's abiding friendship and support, the endless hours Kathy Valentine spent helping me design and

run NMR experiments and John Gledhill's very patient help with the rudiments of data processing. Without Cecilia Tommos' help, CNS would likely have caught my computer on fire.

The Howard Hughes Medical Institute and a Structural Biology Training Grant from the National Institutes of Health supported my graduate training financially.

Outside the hallowed halls of academe, I acknowledge with love the support of my husband Tom Sarachan, patient throughout this long sojourn in Philadelphia, and of my parents, Stephen and Mary Anne Lorenz, my sister Jennifer and my grandparents, James Lorenz and Margaret Elliott, whose unshakable confidence in the success of this work guaranteed it in the end. I am as blessed in my spouse's family as in my own, and also offer warm thanks to my mother and father-in-law, Lisa Miller and Ron Sarachan, and to my sisters-in-law, Meg and Annie.

For laughter and music and inimitable friendship, my gratitude extends to the Orphans Company and the Acoustic Mayhem, including but certainly not limited to Liz, Drew, Joy and Charlie, Denise and Nate, Kitty, Katie Jr., Lexi, Terri, Aly, Rose and Johanna; so many of my happiest memories of the last seven years are memories of the lot of us. And I offer my deepest affection to Brionna and Alysia, Luci and Noël, Lisbeth and Liam, Hill and Danaan, Fiona, Chloe and Deven and the many fine scholars and musicians I've had the pleasure of teaching at the Penn Alexander School. I could ask no greater pleasure or privilege than that of walking beside each of you a little ways.

AMDG

December 2011

ABSTRACT

STRUCTURAL STUDIES OF THE SMN-GEMIN2 COMPLEX

Kathryn Lorenz Sarachan

Gregory D. Van Duyne

The proteins SMN and Gemin2 form the conserved core of the larger eponymous SMN complex, which also contains Gemins3-8 and unrip in an unknown stoichiometry. The complex facilitates the ordered assembly of seven Sm proteins onto a conserved site of an snRNA to form spliceosomal snRNP cores. A deficit in functional SMN as a consequence of deletions or loss-of-function mutations in the gene *SMN1* underlies the disease spinal muscular atrophy (SMA), a severe neurodegenerative disorder and leading genetic cause of infant mortality. Here, the NMR solution structure of the interacting domains of human SMN and Gemin2 is reported. The Gemin2-SMN heterodimer forms a novel all α-helical fold, comprising a single SMN helix embedded in an elongated, hydrophobic cavity of Gemin2. The interface between SMN and Gemin2 is mediated through highly conserved residues on the hydrophobic face of the amphipathic SMN helix and on four Gemin2 helices far separated in primary sequence. Mutations of interfacial residues predicted by the structure to be essential, but not of known disease mutations, disrupted the SMN-Gemin2 interaction. A number of biophysical studies also showed that unbound Gemin2 undergoes a significant conformational change upon SMN binding to become more compact, but belied reports

of a Gemin2 self-interaction. A related set of investigations conducted on the full-length orthologous *S. pombe* Gemin2-SMN proteins showed that the complex occupies small, discrete oligomers that self-associate to form larger assemblies; its apparently large molecular weight by analytical size-exclusion chromatography results from an elongated, partially unstructured state. Together, these NMR and biophysical studies of SMN and Gemin2 provide the first high-resolution glimpse of the SMN-Gemin2 heterodimer, establish a framework for future structure-function studies investigating snRNP biogenesis and SMA pathogenesis and provide new valuable insight into the overall oligomerization state of the SMN complex.

Table of Contents

List of Tables

List of Illustrations

Chapter I: Introduction

1. Pre-mRNA splicing is mediated by the catalytic activity of snRNPs in the context of the spliceosome

Pre-mRNA splicing, the excision of non-coding intronic regions from nuclear mRNA precursors, is a multi-step process prerequisite to the expression of most eukaryotic genes (Burghes & Beattie, 2009). Prior to its discovery in 1977, the prevailing theory of "gene colinearity," first articulated by Crick in 1958 and combining insights about the molecular nature of DNA and Beadle and Tatum's 1941 "one gene-one enzyme" hypothesis, had described a gene is a sequence of nucleotides defining a sequence of amino acids (Beadle & Tatum, 1941; Crick, 1958). Although this notion accounts adequately for the nature of bacterial genes, Sharp and Roberts independently demonstrated by electron microscopy in 1977 that mRNA encoding a single adenoviral polypeptide hybridized to discontinuous segments of genomic DNA, suggesting compellingly that genetic material must "break apart and reform" (Berget *et al.*, 1977; Chow *et al.*, 1977). This paradigm, in which discontinuous genes consist of coding exons interleaved with noncoding introns and require splicing during pre-mRNA processing, was found to represent the norm in eukaryotes, although its prevalence and complexity varies. Only ten percent of *Saccharomyces cerevisiae* gene transcripts contain an intron, and never more than one; mammalian pre-mRNA transcripts, by contrast, contain an average of seven to eight introns and have been known to contain

more than fifty, with sizes ranging from eighty to 100,000 nucleotides (Burge *et al.*, 1999; Henry *et al.*, 1998).

Pre-mRNA splicing also serves as an important source of proteomic diversity. Shortly after Sharp and Roberts demonstrated the presence of discontinuous coding exons, researchers noted the existence of membrane-bound and secreted forms of an antibody that were encoded by the same gene (Early *et al.*, 1980); this differential exclusion and inclusion of exons, or "alternative splicing," was found to serve as a mechanism for the generation of variable forms of mRNA from a single pre-mRNA transcript in a regulated manner (Nilsen & Graveley, 2010). Initially considered exceptional, estimates for the number of genes employing alternative splicing to generate more than one protein isoforms have risen over time, with recent studies suggesting that 94-96% of pre-mRNA sequences containing more than one exon are processed to generate more than one mRNA (and protein) isoform (Pan *et al.*, 2008).

The sequence data delineating an intron destined for removal consists of short consensus sequences at the 5' and 3' splice sites, as well as a "branch site" located 18-40 nucleotides upstream of the 3' splice site (Figure 1.1A). These splice site and branch site sequences vary between common U2-type introns and the much rarer U12-type introns, found only in a subset of eukaryotes. In U2-type introns, constant GU and AG dinucleotides nearly always demarcate the 5' and 3' boundaries of the intron, with additional nucleotide positions at each splice site showing a variable degree of

conservation. The branchpoint sequence contains a loose consensus sequence of eight nucleotides flanking a highly conserved branchpoint adenosine and frequently followed in higher eukaryotes by a polypyrimidine tract (Burge *et al.*, 1999).

The basic chemistry describing the removal of non-coding introns from coding sequences consists of two sequential transesterification reactions (Figure 1.1B). In the first step, the 2' hydroxyl of the conserved branchpoint adenosine executes a nucleophilic attack on the 5' splice site, releasing the 5' exon and forming a cyclic "lariat intermediate" characterized by a 2'-5' phosphodiester bond between the 5' end of the intron and the branchpoint adenosine. Subsequently, the 3' hydroxyl of the free 5' exon attacks the 3' splice site to ligate the exons and free the intron, still in its cyclic "branched lariat" form (Burge *et al.*, 1999; Staley & Guthrie, 1998).

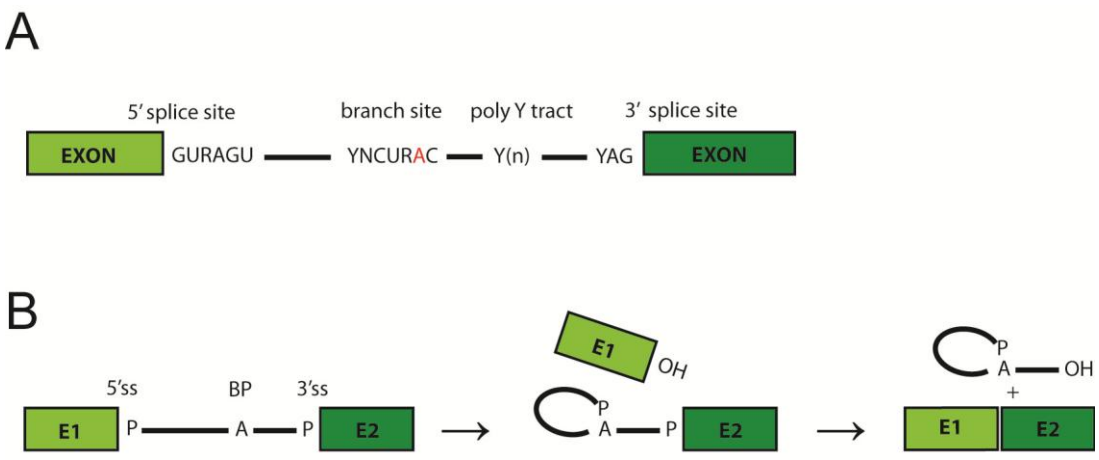


Figure 1.1 Pre-mRNA splicing by the U2-type spliceosome. A) Conserved sequences found at the 5′ and 3′ splice sites and branch site of U2-type pre-mRNA introns. Y depicts pyrimidines and R purines. The polypyrimidine tract is indicated. The branchpoint adenosine is colored red. B) Schematic representation of the two-step mechanism of pre-mRNA splicing. The branchpoint adenosine is indicated with an A.

Intronic excision from pre-mRNA is catalyzed by the spliceosome, a multi-megadalton ribonucleoprotein complex that acts to spatially orient the reactive groups of the pre-mRNA for the sequential steps in intron cleavage (Figure 1.3). A highly dynamic structure, the spliceosome varies constitutionally and conformationally over the course of the splicing reaction, involving perhaps as many as 300 total individual protein components (Ritchie *et al.,* 2009; Will & Lührmann, 2011). At its catalytic core lie five uridine-rich small nuclear ribonucleoprotein particles (U-snRNPs), each containing a unique, eponymous U-snRNA onto which seven Sm or Sm-like proteins assemble in a heptomeric ring, as well as several particle-specific accessory proteins (Raker *et al.,* 1996; Will & Lührmann, 2011). In an obvious parallel to the existence of distinct U2-type and U12-type introns, most eukaryotic cells contain two distinct spliceosomes; the U2-dependent ("major") and much less abundant U12-dependent ("minor") spliceosomes differ in their U-snRNP composition, the former including the U1, U2, and U5 snRNPs and the U4/U6 snRNP dimer and the latter sharing only U5 in addition to U11, U12 and U4atac/U6atac (Patel & Steitz, 2003; Will & Lührmann, 2005). In the case of the U1 snRNP, an analysis by mass spectrometry to determine the stoichiometry of all components indicated a single copy of each protein and RNA, and structures of the same particle based on single-particle electron microscopy and x-ray crystallography demonstrate that the U1 snRNA is threaded through a donut-shaped ring of Sm proteins (Figure 1.2) (Hochleitner *et al.,* 2004; Krummel *et al.*, 2010; Stark *et al.*, 2001).

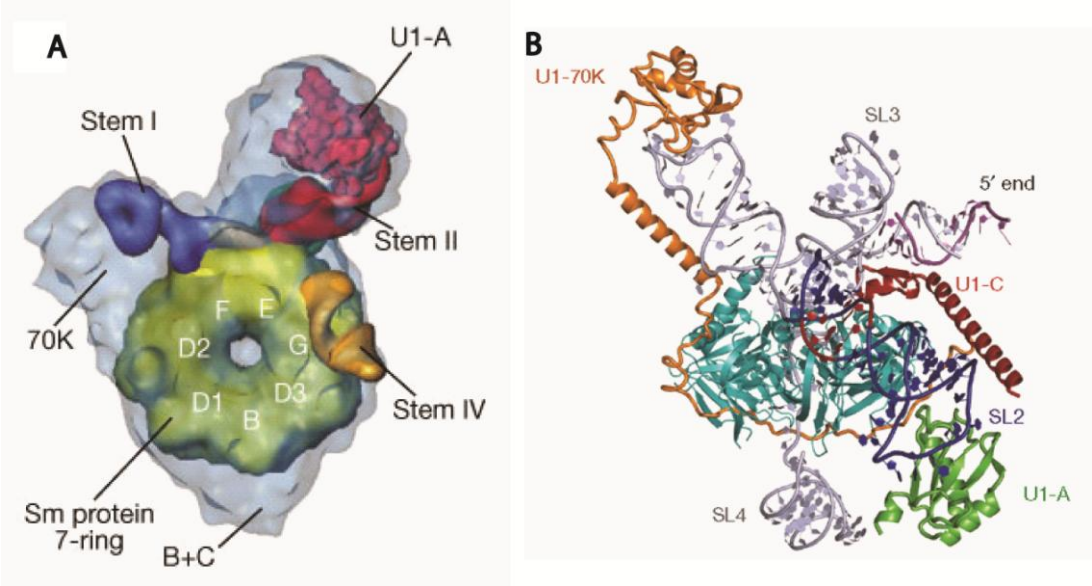


Figure 1.2. Models of the U1 snRNP. A) A cryoelectron microscopy model of the U1 snRNP. The Sm protein heptamer is colored yellow, U1-A red, and three stem loops of the U1 snRNA orange, red and blue. Positions of other snRNP components are indicated (Stark *et al.* 2001). B) 5.5Å x-ray crystallographic model (PDB: 3CW1) of the U1 snRNP. U1 small nuclear ribonucleic acid (snRNA) is colored in black, the seven Sm proteins in cyan, U1-70k in light brown, U1-A in green and U1-C in maroon. A 5′ splice-site mimic RNA is colored blue (Krummel *et al.* 2010).

Spliceosome assembly for catalysis of pre-mRNA splicing occurs by the ordered binding and release of snRNPs and numerous other splicing factors (Figure 1.3). In cross-intron splicing, describing the splicing of an intron whose length does not exceed 200-250 nucleotides, the spliceosome recognizes splice sites and assembles across the intron ("intron definition") in a series of discrete steps, all but the first of which are ATP dependent and require the action of DEAH-box RNA helicases (Fox-Walsh *et al.*, 2005; Ritchie *et al.*, 2009; Staley & Woolford, 2009). Following tight U1 snRNP association with the 5' splice site, non-snRNP protein factors including splicing factor 1 (SF1) and the heterodimer U2AF are recruited to the branch site and polypyrimidine tract and 3' splice site, respectively. The U2 snRNP then associates with the branch site, forming a

duplex between the U2 snRNA and the branch site and positioning the branchpoint adenosine, with an outward bulge, as the nucleophile for the first tranesterification reaction (the "prespliceosome"). The pre-assembled U4/U6,U5 tri-snRNP is recruited to the complex, which undergoes a series of major rearrangements: the disruption of U1 snRNP binding at the 5' splice site by the U6 snRNP; the disruption of U4/U6 snRNA base-pairing; and the formation of a U2/U6 snRNA structure which is believed, after catalytic activation by the DEAH-box RNA helicase Prp2, to form the active site of the spliceosome. After splicing, the spliceosome dissociates and, after remodeling, the released snRNPs are competent for additional splicing reactions (Ritchie *et al.*, 2009; Will & Lührmann, 2011).

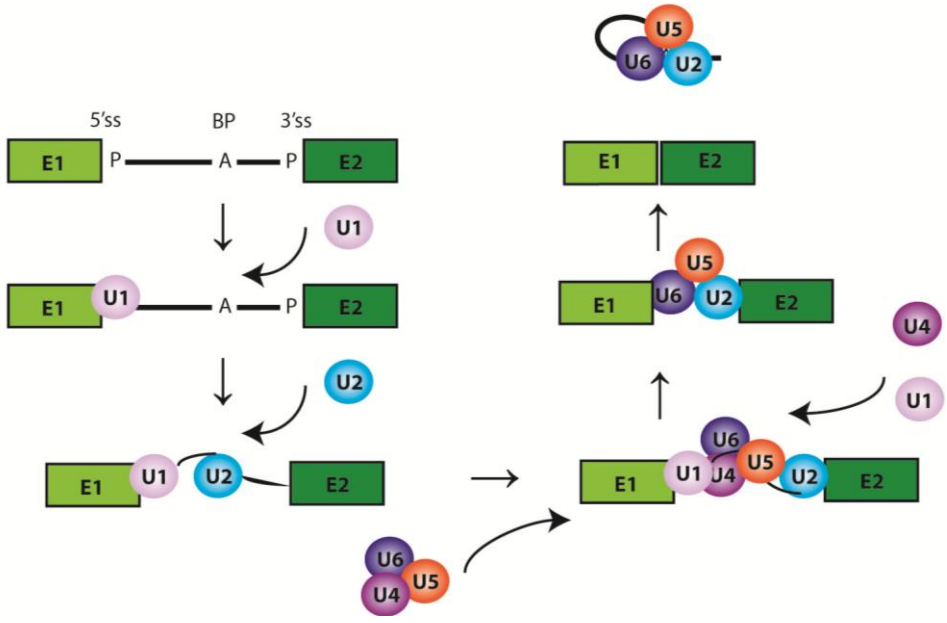


Figure 1.3 Canonical cross-intron assembly pathway of the U2-type spliceosome. The ordered interactions of the U-snRNPs (colored circles), but not of additional accessory proteins, are depicted interacting with exon (green boxes) and intron (black line) sequences.

Mammalian pre-mRNAs contain introns of lengths varying from a few hundred to several thousand nucleotides, while their exons maintain a roughly similar length of approximately 120 nucleotides (Ast, 2004; Deutsch & Long, 1999). Exons of increasing size are increasingly vulnerable to exon skipping unless their flanking introns are short; splice site definition is most efficient when either the exon or the intron is comparably short (Sterner *et al.*, 1996). In cases where intron lengths exceed 200-250 nucleotides, splice sites are recognized across the exon rather than across the intron, a process known as exon definition (Berget, 1995; Fox-Walsh *et al.*, 2005). The U1 snRNP binds to the 5' splice site downstream of the second exon, promoting the binding of U2AF with the polypyrimidine tract and 3' splice site of the upstream intron, thus promoting the recruitment of U2 snRNP to the branchpoint. The pairing of the defined 3' splice site with its upstream 5' counterpart is still poorly understood, but involves sequential disruption of the cross-exon complex and formation of a cross-intron prespliceosome (Reed, 2000; Will & Lührmann, 2011).

2. The biogenesis of spliceosomal snRNPs is mediated by the SMN complex

The assembly of the structural cores of the U1, U2, U4 and U5 snRNPs, as well as of the minor U11, U12 and U4atac snRNPs, involves the binding of a heteroheptameric ring of seven Sm proteins D1, D2, D3, B, E, F and G onto the conserved, uridine-rich "Sm site" of an snRNA (Burge *et al.*, 1999). This reaction proceeds spontaneously *in vitro*, in a hierarchical manner beginning with the formation of three Sm hetero-oligomers E/F/G, D1/D2 and D3/B, followed by the association of E/F/G and D1/D2

with the snRNA to form an intermediate structure to which the D3/B dimer binds to form the mature snRNP core (Raker *et al.*, 1996).

In vivo, however, U-snRNP biogenesis is an established function of the SMN complex (Figure 1.4) (Fischer *et al.*, 1997; Meister *et al.*, 2001a). This multi-component macromolecular machine counts as members at least nine proteins: the eponymous SMN; Gemins 2-8; and unr-interacting protein (unrip) (Baccon *et al.*, 2002; Carissimi *et al.*, 2005; Carissimi *et al.*, 2006a; Carissimi *et al.*, 2006b; Charroux *et al.*, 1999; Charroux *et al.*, 2000; Gubitz *et al.*, 2002; Liu *et al.*, 1997; Pellizzoni *et al.*, 2002a). The assembly reaction is thought to be more stringently regulated *in vivo* to prevent the promiscuous and potentially deleterious assembly of Sm cores on random, inappropriate RNA molecules (Palfi *et al.*, 2009; Pellizzoni *et al.*, 2002b). In keeping with its role as cytoplasmic mediator of snRNP assembly, the SMN complex is diffusely distributed in the cytoplasm; it can also be found in the nucleus, in discrete, specifically enriched nuclear bodies described as gems (Liu & Dreyfuss, 1996; Liu *et al.*, 1997).

The components of the SMN complex form a large stable species that sediments on sucrose gradients as a heterodisperse 30S-70S particle, suggesting a number of copies of one of more components (Gabanella *et al.*, 2005; Paushkin *et al.*, 2002). The SMN protein, as well as Gemin2 and Gemin8, have reported self-association activity, but the precise stoichiometry of those or any other components of the complex remains unknown (Lorson *et al.*, 1998; Ogawa *et al.*, 2007; Otter *et al.*, 2007; Young *et al.*, 2000). Protein-protein interaction studies, however, based on co-immunoprecipitations,

GST-pulldowns and yeast two-hybrid studies, suggest that the central part of the complex comprises SMN, Gemin7 and Gemin8, with Gemin2 and Gemin3 interacting with this backbone through direct contact with SMN, Gemin6 and unrip through Gemin7 and Gemin4 through Gemin8. A strong Gemin3-Gemin4 interaction has also been demonstrated, as well as a Gemin5 binding through Gemin2 (Otter *et al.*, 2007). Gemin2 also interacts directly with Gemin7 (Ogawa *et al.*, 2007). Alone among complex components, Gemin5's interaction with the SMN complex is substantially abrogated in the presence of high salt, and both Gemin5 and unrip co-immunoprecipitate weakly with epitope-tagged SMN (Otter *et al.*, 2007). Together with the observation that the two proteins exhibit compartment-specific localization, associating with the SMN complex in the cytoplasm but not in nuclear gems, a peripheral nature is usually ascribed to their interaction with the SMN complex (Battle *et al.*, 2007; Carissimi *et al.*, 2005; Grimmler *et al.*, 2005a; Hao *et al.*, 2007). All components of the complex except for unrip, however, are required for the complete assembly reaction; knockdown of SMN and Gemins2-8 in HeLa cells is reported to disrupt efficient snRNP assembly (Carissimi *et al.*, 2006a; Feng *et al.*, 2005; Ogawa *et al.*, 2007).

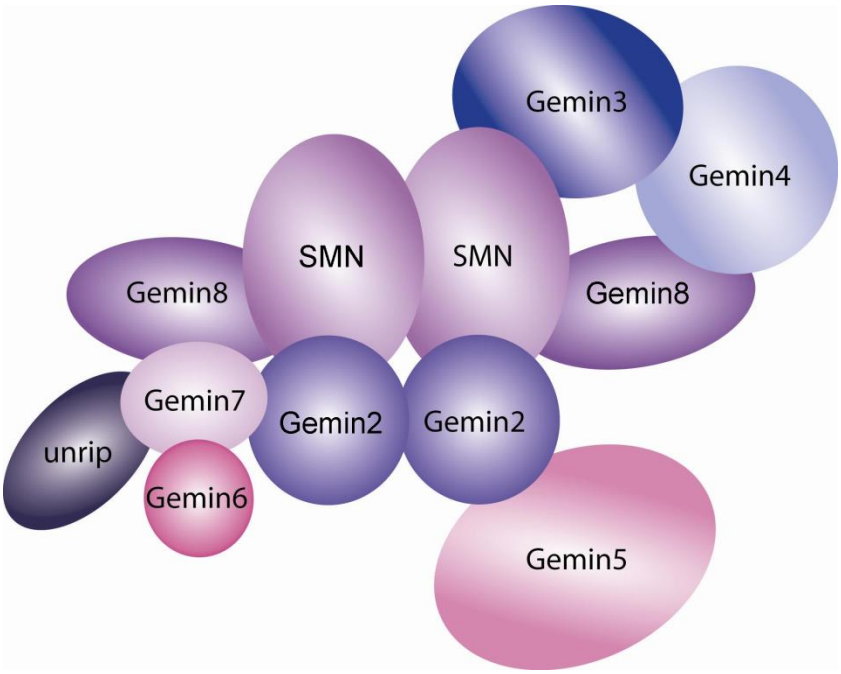


Figure 1.4 Schematic representation of the SMN complex. The central part of the complex comprises SMN, Gemin7 and Gemin8, with Gemin2 and Gemin3 interacting with this backbone through direct contact with SMN, Gemin6 and unrip through Gemin7 and Gemin4 through Gemin8. Gemin2 also binds Gemin5 and makes an interaction with Gemin7.

2.1 Both U-snRNAs and Sm proteins are escorted to the SMN complex by chaperone proteins

Before serving as substrates for SMN-chaperoned snRNP assembly, both nascent snRNAs and Sm proteins undergo upstream modifications before reaching their SMN complex destination under the aegis of specific protein escorts (Figure 1.5). After nuclear transcription by RNA polymerase II and co-transcriptional acquisition of a 7-methyloguanosine cap (m^7G) cap (Will & Lührmann, 2001), nascent "pre"-snRNAs interact with cap-binding complex (CBC) and phosphorylated adaptor for RNA export (PHAX); the latter acts as an adaptor for the addition of RanGTP and additional

interactions with the export receptor exportin I (XPO1). XPO1 interaction with the nuclear pore complex ensures export of the nucleic acid to the cytoplasm, where accessory factors dissociate for recycling back to the nucleus (Matera *et al.*, 2007). Gemin5, a member of the SMN complex, specifically recognizes the conserved Sm sites of snRNAs and binds them through its characteristic WD repeat motif with high affinity (Battle *et al.,* 2006; Lau *et al.*, 2009). Consistent with its role as a U-snRNA chaperone, immunoprecipitation of Gemin5 from cell extracts indicated that a fraction of the protein exists free of other SMN complex components; a recent study of the effects on SMN complex activity of protein synthesis inhibition demonstrated the accumulation of an snRNA-Gemin5 dimer, consistent with an independent intermediate in which Gemin5 serves as a chaperone of the RNA species (Battle *et al.,* 2007). It is the highly specific interaction between U-snRNAs and Gemin5, and the subsequent interaction of that subcomplex with the SMN complex, that enables the equally specific SMN complex recognition of its RNA targets (Yong *et al.*, 2010).

The Sm protein substrates of the SMN complex comprise SmB, D1, D2, D3, E, F and G; SmB' is alternate splicing isoform of SmB and differs only in the absence of eleven amino acid residues at the C-terminus (Meister *et al.*, 2001a). A highly conserved family with orthologues from mammals to prokaryotes, all of the Sm proteins, as well as the related LSm proteins, are characterized by a common fold, the "Sm domain," consisting of an N-terminal helix followed by a highly bent five-stranded antiparallel β-sheet. The Sm and LSm proteins display a tendency to self-oligomerize or interact with each other,

forming a head-to-tail continuous β-sheet through interactions made between edge β-strands of interacting proteins (Kambach *et al.*, 1999). Both *in vitro* and *in vivo,* Sm proteins form preferential heteromeric subcomplexes: D1/D2; D3/B; and E/F/G (Camasses *et al.,* 1998; Lehmeier *et al.*, 1994; Raker *et al.*, 1996). Based on biochemical data and the geometric and physical properties of the Sm D3/B and D1/D2 crystal structures, Kambach *et al.* proposed a model in which the seven Sm proteins assemble into a hetero-heptameric ring around a central cationic aperture large enough to accommodate a single-stranded RNA (Kambach *et al.*, 1999). This view was subsequently confirmed in images generated by cryo-electron microscopy and x-ray crystallography (Figure 1.2) (Krummel *et al.*, 2010; Stark *et al.*, 2001).

Three Sm proteins, D1, D3 and B, contain "RG motifs," arginine/glycine-rich regions C-terminal to their Sm domains that are symmetrically dimethylated prior to snRNP assembly; this modification is effected by the methylosome, a complex containing protein methyltransferase 5 (PRMT5), pICln and WD45/MEP50 (Brahms *et al.*, 2001; Friesen *et al.*, 2001a; Friesen *et al.*, 2001b). The conserved protein pICln competently binds all Sm protein monomers except SmE, as well as heterodimers D1/D2 and D3/B(B') in an otherwise unstable hetero-pentamer of D1/D2/E/F/G, and prevents their participation in snRNP assembly, presumably by averting their unregulated early self-association or interaction with RNA (Figure 1.5) (Chari *et al.*, 2008; Friesen *et al.*, 2001b; Meister *et al.*, 2001b; Pu *et al.*, 1999). It is suggested that pICln accompanies Sm D1, D3 and B to the methylosome, where its presence enhances PRMT5 methylation of

the Sm RG motifs, although it is unclear whether it recruits monomeric Sm proteins for this purpose or the dimers D1/D2 and D3/B (Pesiridis *et al.*, 2009; Pu *et al.*, 1999). Chari *et al.* showed that pICln also chaperones Sm proteins to the SMN complex when bound to the Sm dimer D3/B and the pentamer D1/D2/E/F/G; the latter is a long-lived species detectable by fractionation of HeLa extracts by gel filtration, while the former, although stable *in vitro* and necessary for snRNP assembly, is not detectable apart from the methylosome. Both biochemical studies and images based on electron microscopy indicate that pICln-Sm protein subcomplexes require, minimally, SMN and Gemin2 for efficient interaction and the dissociation of pICln after transfer of its Sm protein substrates, which is in turn necessary before the Sm protein-charged SMN complex becomes capable of proceeding in snRNP assembly (Chari *et al.*, 2008).

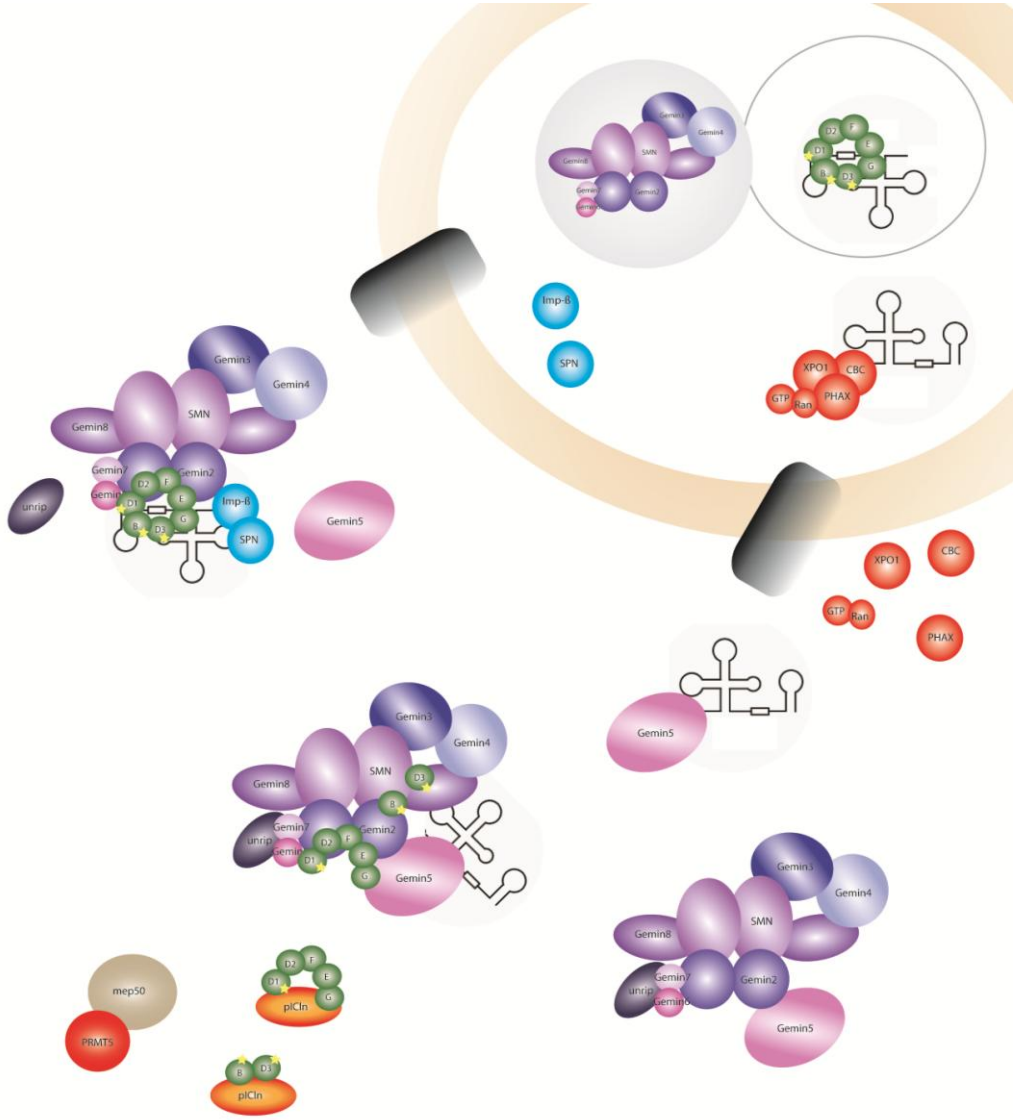


Figure 1.5. Overview of the survival motor neuron (SMN) functions in the assembly of mature spliceosome particles. Small nuclear RNAs (snRNAs) are transcribed by RNA polymerase II. After addition of a m^7G cap, binding is initiated by the nuclear export complex consisting of PHAX, XPO1/CRM1, RAN, and the cap-binding complex (CBC). Upon entering the cytoplasm, the nuclear export complex dissociates. Within the cytoplasm, pICln chaperones Sm proteins to the methylosome for PRMT5 methylation of the C-terminal tails of SmB, SmD1, and SmD3, then escorts the D1/D2/E/F/G and D3/B subcomplexes separately to the SMN complex. The SMN complex binds the chaperoned Sm protein pentamer, releasing pICln, then recruits the snRNA before completing the Sm protein ring with the D3/B dimer to achieve U-snRNP core assembly. The snRNA m^7G cap is hypermethylated, and the nuclear import complex consisting of snurportin and importin-β interacts with the m^3G cap and the SMN/snRNP complex and transports the entire complex into the nucleus. Upon entry, the SMN complex localizes to Gems and the snRNPs to Cajal bodies.

2.2 The SMN complex mediates snRNP assembly

The precise role played by each SMN complex component in the overall scheme of snRNP biogenesis remains unclear, although insights into the functions of certain members have begun to appear. Gemin5 is the established chaperone of U-snRNAs, responsible both for discriminately selecting its targets through high-affinity binding to their conserved Sm sites and for escorting those RNAs to the SMN complex and facilitating their binding there (Battle *et al.,* 2006; Lau *et al.*, 2009; Yong *et al.*, 2010). The crystal structure of the Gemin6-Gemin7 heterodimer demonstrates significant structural homology, for both proteins, to the conserved Sm fold of Sm proteins, suggesting a model in which Gemin6-Gemin7 substitutes for the SmD3/B heterodimer in the seven-membered Sm ring at some intermediate stage of snRNP assembly; a role for unrip in mediating this exchange has recently been imputed from pulldown assays showing disruption of a stable SMN-Gemin2-Gemin7 interaction in the presence of unrip (Ma *et al.,* 2005; Ogawa *et al.*, 2009). Sequence homology that indicates that Gemin3 is a DEAD-box helicase may ascribe to that protein a role in RNA unwinding activity (Charroux *et al.*, 1999).

The conserved catalytic core of the SMN complex, however, appears to consist of SMN and Gemin2. In *Schizosaccharomyces cerevisiae*, the perhaps simplest, most ancestral version of the SMN complex contains only those two proteins, and an SMN complex comprising only recombinant SMN and Gemin2 from *Drosophila melanogaster*, an

organism which does possess Gemin3 and Gemin5 orthologues, was fully competent to assemble snRNPs *in vitro* (Hannus *et al.,* 2000; Kroiss *et al.*, 2008).

SMN, a 294-residue, 32kDa protein (sometimes reported as 38kDa due to a tendency to run with an anomalously high apparent molecular weight in polyacrylimide gel electrophoresis experiments), is highly conserved evolutionarily among eukaryotes from humans to the fission yeast *S. pombe* (Liu *et al.,* 1997). SMN is essential for viability in all species tested to date: knockouts in yeast, worm, fly and mouse models result in lethality (Briese *et al.,* 2009; Chan *et al.*, 2003; Hannus *et al.*, 2000; Schrank *et al.,* 1997). Every SMN orthologue contains two regions of high sequence homology at its N- and C-termini, and metazoans share a third, central canonical Tudor domain (Liu *et al.,* 1997; Talbot *et al.*, 1998; Young *et al.*, 2000). The C-terminal region, including residues that map to exons 6 and 7, is characterized by an unusual YxxGYxxGYxxG motif and usually referred to as the "YG box" (Talbot *et al.,* 1998). A number of studies have pinpointed this region as necessary for SMN self-association that is, in turn, essential to its ability to assemble snRNPs (Liu *et al.,* 1997; Lorson *et al.*, 1998; Young *et al.*, 2000).

Two reports have also suggested an additional N-terminal region of SMN oligomerization (Ogawa *et al.,* 2007; Young *et al.*, 2000). The overall stoichiometry of SMN self-association, however, remains uncertain. Only the central Tudor domain is identifiable as a known structural motif based on sequence homology and has been

structurally characterized by both NMR spectroscopy and X-ray crystallography; like other Tudor domains, it forms a highly bent β-sheet consisting of five strands that form a barrel-like fold (Sprangers *et al.,* 2003; Talbot *et al.,* 1998). Several studies implicate it in Sm protein binding, highlighting in particular its preferential interactions with the symmetrically dimethylated arginine-rich C-terminal tails of Sm D1, D3 and B (Bühler *et al.,* 1999; Selenko *et al.,* 2001).

Gemin2 was identified early in the study of SMN as a core component of the SMN complex; its interaction with SMN remains intact even under stringent (high salt) conditions (Liu *et al.,* 1997). Coexpression of both proteins, but not expression of either protein alone, is required to reconstitute active SMN complex *in vitro,* and Gemin2 knockdown inhibits SMN complex-mediated snRNP assembly (Feng *et al.,* 2005; Kroiss *et al.,* 2008). The Gemin2-binding domain of SMN has been proposed to reside within the conserved N-terminal region of that protein (Liu *et al.,* 1997; Ogawa *et al.,* 2007; Young *et al.,* 2000).

Formerly referred to as SMN-interacting protein 1 (SIP1), Gemin2 itself is a 280-residue, 32 kDa protein even more highly conserved across evolutionary time than SMN; unlike that latter, an identifiable Gemin2 orthologue, Brr1, exists in the budding yeast *Saccharomyces cerevisiae* (Liu *et al.,* 1997; Noble & Guthrie, 1996a). Gemin2 is required for viability in mice, with homozygous knockout of the gene resulting in early embryonic lethality (Jablonka *et al.,* 2002). An analysis of Gemin2's primary sequence

reveals no immediately identifiable structural domains, although secondary structure predictions do propose an entirely α-helical fold. Isolating the SMN-binding domain of Gemin2 has proven challenging, although one report based on mammalian two-hybrid assays identified a Gemin2 fragment comprising the 180 C-terminal-most residues as the minimum necessary to bind SMN. Those researchers also reported a novel self-interaction for Gemin2, mapping to a slightly larger C-terminal fragment and purportedly acting to stabilize SMN's amino-terminal and carboxy-terminal self-association (Ogawa *et al.*, 2007).

Other protein interactions unrelated to Gemin2's role in snRNP assembly have also been imputed to the protein. In its SMN-bound form, Gemin2 is thought to play a key role in the repair of double-stranded DNA breaks by stimulating RAD51-mediated homologous pairing (Takaku *et al.*, 2011; Takizawa *et al.*, 2010). Gemin2 is also reported to interact with HIV-1 integrase, augmenting the assembly of reverse transcriptase onto viral RNA and facilitating HIV infection (Hamamoto *et al.*, 2006; Nishitsuji *et al.*, 2009).

2.3 Maturation of nascent assembled snRNP cores occurs in the nucleus

After SMN-mediated assembly, the snRNA component of the nascent snRNP cores undergoes two additional cytoplasmic processing steps (Figure 1.5). Hypermethylation under the auspices of trimeythlguanosine synthase-1 (TGS1) converts the m^7G cap to a 2,2,7-trimethylguanosine (TMG) cap, and the 3' end of the nucleic acid undergoes exonucleolytic removal of the 3′ trailer sequences (so-called "3' trimming"); both

processes proceed only in the context of snRNAs bound to the Sm protein heptamer. The TMG cap and the assembled Sm protein core appear to act together as a nuclear localization signal, signaling nuclear import of the snRNP core via importin-β (Imp-β), during which course of events snuportin-1 (Snp1) serves as the adaptor specifically recognizing the TMG, while the still-bound SMN complex apparently fills that role for the Sm core (Matera *et al.*, 2007). Upon nuclear re-entry, the SMN complex and snRNP core dissociate, with the former enriching cellular bodies known as gems for eventual recycling by an uncertain mechanism to the cytoplasm. The latter temporarily inhabit Cajal bodies, where they are believed to undergo additional stages of maturation, including addition accessory proteins specific to the U-snRNP species, U-snRNP coupling to create the U4/U6 di-snRNP and U4/U6,U5 tri-snRNP and site-specific pseudouridylation and 2'-O-ribose-methylation, before participation in pre-mRNA splicing (Nizami *et al.*, 2010).

The U6 and U6atac snRNPs follow a different biogenesis pathway that remains confined to the nucleus. After transcription by RNA III polymerase, the U6 and U6atac snRNAs receive a γ-monomethyl cap. The snRNA lacks a canonical Sm site, but a heptameric ring of LSm ("Sm-like") proteins 2-8 assembles on a uridine-rich 3' sequence (the "U tract") of the snRNA. The LSm proteins share the same structural Sm fold with the Sm proteins, and U6 and U6atac snRNP assembly has been proposed as a nuclear function for the SMN complex, but conclusive evidence of that functionality is lacking. Given that the LSm2-LSm8 heteromer, unlike its Sm protein counterpart, can

be isolated as a stable seven-membered species in the absence of U6 snRNA, assembly of the U6 snRNP may follow a very different format (Will & Lührmann, 2001). LSm proteins also participate in a wider repertoire of cellular activities than the spliceosome-specific Sm proteins; a complex comprising LSm1-LSm7 (rather than LSm2-Lsm8) has been documented to participate in mRNA degradation as part of an unrelated cytoplasmic complex, while another complex containing the Lsm2-Lsm7 proteins associates with snR5, a box H/ACA snoRNA that guides the site-specific pseudouridylation of rRNA (Bouveret *et al.*, 2000; Fernandez *et al.*, 2004; Spiller *et al.*, 2007; Tharun *et al.*, 2000).

3. Spinal muscular atrophy is caused by a deficit in functional SMN

Reduced levels of functional SMN lead to spinal muscular atrophy (SMA), an autosomal recessive neurodegenerative disorder characterized by degeneration of α-motor neurons in the anterior horn of the spinal cord, leading to progressive muscle weakness and wasting and, in severe cases, paralysis (Burghes & Beattie, 2009). The disease is typically subdivided into three types with onset during childhood and one type with adult onset; the most severe Type I SMN (Werdnig-Hoffmann disease) is typically diagnosed in the first months of life, and affected children are never able to sit or walk and have an average lifespan of eight months due to compromised breathing and airway protection. Types II and III SMA are less severe forms of the disease with later childhood onset; Type IV SMA is diagnosed in patients older than 30 (Zerres & Davies, 1999). SMA is the most common genetic cause of infant death, and affects 1 in 6000

live births with a carrier frequency of 1 in 35 for disease-causing mutations (D'Amico *et al.*, 2011).

The genetic underpinnings of SMA are well understood. The gene encoding the SMN protein was discovered in 1995 and exists in two copies mapped to chromosome 5q13 in the human genome, a telomeric copy (*SMN1*) and a centromeric copy (*SMN2*) present across a large inverted region of genomic duplication (Brzustowicz *et al.*, 1990; Brzustowicz *et al.*, 1992; Lefebvre *et al.*, 1995). The *SMN2* gene differs from its *SMN1* counterpart by a single base pair, a C→ T transition at position +6 in exon 7 (Kashima & Manley, 2003). It is uncertain whether this base pair change, otherwise translationally silent, creates an exonic splicing silencer of exon 7 or disrupts an existing exonic splice enhancer; under either description, however, it ultimately prevents of inclusion of exon 7 in the majority of transcripts (Cartegni & Krainer, 2002; Kashima & Manley, 2003). These transcripts, lacking exon 7, produce a truncated SMN protein (SMNΔ7) that lacks the final sixteen amino acids. It is incompetent for self-association and thought to be highly unstable and rapidly degraded (Lorson & Androphy, 2000). The inclusion of four residues from exon 8 at the C-terminus of SMNΔ7 transcripts, residues E-M-L-A, has been proposed to serve actively as a compelling degradation signal, or "degron"(Cho & Dreyfuss, 2010).

Most SMA cases are attributable to the homozygous loss of the *SMN1* gene or of exon 7 within it. The small percentage (estimated between 10-20%) of *SMN2* transcripts

containing exon 7 produces functional SMN protein, but at levels too low to fully compensate for the loss of *SMN1*. SMA is therefore the result of low levels of functional SMN protein, not its absence (Coovert *et al.,* 1997; Lefebvre *et al.,* 1997). Homozygous loss of both *SMN1* and *SMN2* has never been reported, presumably as a result of lethality. Indeed, all SMA patients carry at least one copy of *SMN2*, and homozygous deletion of *SMN1* in mice, which carry no *SMN2*, is embryonic lethal (Hsieh-Li *et al.,* 2000; Schrank *et al.,* 1997). Copy number of *SMN2* correlates with disease severity; the additive effect of combining the functional SMN produced by several *SMN2* genes confers a protective effect (Yamashita *et al.,* 2004).

Although large deletion or conversion mutations in *SMN1* characterize the genetic basis of SMA in most patients, some 5% of patients instead display small deletions, splice mutations and missense point mutations (Alías *et al.,* 2009). Certain missense mutations localizing to exons 6 and 7 of SMN, the reported oligomerization domain, severely disrupt the ability of SMN to self-associate *in vitro* (Lorson *et al.,* 1998; Pellizzoni *et al.,* 1999). When accompanied by only one or two copies of *SMN2*, these patient mutations result in severe SMA phenotypes (Bühler *et al.,* 1999; Lorson *et al.,* 1998). Another set of severe SMA missense mutations appears in the SMN Tudor domain, where they may disrupt Sm protein-SMN interactions (Bühler *et al.,* 1999; Cuscó *et al.,* 2004; Sun *et al.,* 2005). A small number of milder mutations appear elsewhere in the protein, including D30N (Type II) and D44V (Type III) in the N-terminal Gemin2-binding domain (Sun *et al.,* 2005).

The molecular pathogenesis of spinal muscular atrophy, in particular the description of the link between deficiency in a ubiquitously expressed housekeeping protein and a motor neuron-specific disease phenotype, has proven a controversial field of inquiry. Hypotheses explaining the functional role of SMN in the mechanism of SMA fall broadly into two categories. The first, sometimes called the "nucleocentric dogma," suggests that the disruption in snRNP biogenesis consequent to SMN deficiency causes deleterious effects in gene splicing, to which certain essential motor neuron proteins are particularly susceptible. In the second, it is proposed that SMN has a functional additional to its role in RNP assembly that is specific to motor neuron axons. Evidence exists to support both positions, and no clear consensus has yet emerged.

The first hypothesis predicts that an alteration in snRNP assembly will lead to a subsequent alteration in the amount or profile of snRNPs produced, then eliciting a change in splicing patterns (Eggert *et al.,* 2006; Gabanella *et al.*, 2007; Pellizzoni, 2007). Developed snRNP assembly assays have in fact demonstrated, in mice, a decrease in snRNP assembly activity concomitant with the severity of the SMA disease model (Gabanella *et al.*, 2007); in zebrafish, reintroduction of assembled snRNPs can rescue motor neuron defects caused by lower levels of SMN (Winkler *et al.*, 2005). Notably, in murine tissues from SMA-affected animals, reduced snRNP assembly also prompted uneven changes to snRNP profiles and a preferential reduction of snRNPs comprising the minor spliceosome, especially of U11 snRNP in the spinal cord,

although a target gene both spliced by the U12-spliceosome and possessing a functional implication in SMA has not been reported (Gabanella *et al.*, 2007; Zhang *et al.*, 2008). Although experiments using expression arrays and exon arrays have described hundreds of alterations in gene expression in SMA mice, these studies of tissues from older, terminally ill animals distinguish poorly between changes resulting from the disease state of the animal and those resulting from low SMN levels (Zhang *et al.*, 2008). While snRNP assembly does therefore appear to be altered in SMA, yielding reduced levels of certain snRNPs and perhaps altering splicing patterns across a number of genes, which target genes, if any, are specifically affected remains unknown.

An equally widespread counter-proposal holds that SMN exercises roles in axon outgrowth, guidance and/or the formation of neuromuscular junctions (NMJs) that are compromised in SMA (Briese *et al.*, 2005; Fan & Simard, 2002). Early immunohistochemical studies detected the presence of SMN in neural dendrites and axons (Béchade *et al.*, 1999; Jablonka *et al.*, 2002; Pagliardini *et al.*, 2000); GFP-SMN could also be visualized in granules that moved bidirectionally in neuronal processes, at speeds consistent with fast axonal transport (Hirokawa, 1998; Zhang *et al.*, 2003). SMN and Gemins, in the absence of Sm proteins, were later detected in both these mobile granules and larger, stationary counterparts, suggesting a role for SMN or the SMN complex outside of U-snRNP biogenesis (Zhang *et al.*, 2006). Another report demonstrated the presence of SMN in axons in the absence of Gemin2, strengthening

the claim for an SMN role in motor neurons separate from snRNP assembly (McWhorter *et al.*, 2008).

Additionally, SMN interacts and co-localizes in cultured cells with profilin IIa and hnRNP-R, both of which play important roles in neuronal axons and growth cones. Profilin IIa is involved in correct neurite outgrowth, while hnRNP-R binds β-actin mRNA, which in many neuron types must be transported to axon growth cones for local translation (Rossoll *et al.*, 2002; Rossoll *et al.*, 2003; Sharma *et al.*, 2005). Based on actin-specific anti-sense probes, both hnRNP-R, which contains both an SMN-interacting and an RNA-interacting domain, and SMN demonstrate involvement in β-actin mRNA translocation (Rossoll *et al.*, 2003). Impaired β-actin synthesis, perhaps the result of inhibited SMN function, is also suggested to underlie the reduced integration of voltage-gated Ca^{2+} channels observed in the growth cones of cultured model SMA cells (Jablonka *et al.*, 2007). An interaction with plastin 3, a stabilizer of β-actin, has also been proposed for SMN (Oprea *et al.*, 2008). A number of SMN mutations have been reported that fail to rescue motor axon outgrowth anomalies, but which retain SMN self-oligomerization and Sm binding capacity (Carrel *et al.*, 2006). (Perhaps more pertinently, however, snRNP assembly was unaddressed.) This body of data makes an axon-specific role for SMN underpins SMA a possibility, but conclusive evidence supporting that hypothesis, including a specific function for SMN and an explanation for the motor neuron specificity of the phenotype is still lacking.

A few reports also exist of a primary muscle effect. In *Drosophila* and mouse, SMN has been found at the actin-enriched I-band and actinin-enriched Z-disc of the sarcomere; in the latter organism, the entire SMN complex has been observed at the Z-disc (Rajendra *et al.*, 2007; Walker *et al.*, 2008). Expression of SMN in both neural and muscular tissues, but not muscles alone, dramatically improved the survival of severe SMA mice; in *smn*-knockout *Drosophila,* SMN expression in neural and mesodermal tissue evinced a similar decline in larval lethality (Chan *et al.*, 2003; Gavrilina *et al.*, 2008). Moreover, reduction of SMN in *Drosophila* mesodermal tissue had a much greater effect on adult viability than a similar reduction in neural tissue (Chang *et al.*, 2008). A possible muscle-specific role for SMN might include maintaining Z-disc integrity, signaling to the nucleus or localized transport of mRNPs at the sarcomeric Z-disc, and forms an intriguing additional field of study.

4. The *Schizosaccharomyces pombe* SMN and Gemin2 proteins form the simplest, most ancestral SMN complex

Schizosaccharomyces pombe is the most primitive organism with a recognizable SMN gene. After the discovery of human SMN as the disease gene for spinal muscular atrophy, bioinformatics searches in the late 1990s, Owen *et al.* identified a putative *S. pombe* orthologue demonstrating 25% sequence identity with its human counterpart (Lefebvre *et al.*, 1995; Owen *et al.*, 2000). Variously dubbed ySMN or yab8p and here described as spSMN, the 152 amino acid, 17.4 kDa protein is essential for *S. pombe* viability. It displays pronounced sequence conservation at its N- and C-termini,

recognizable as a Gemin2-binding domain and a YG box self-association domain (Owen *et al.*, 2000; Paushkin *et al.*, 2000). Its self-association can be demonstrated by GST-pulldowns and, in a remarkable example of structural conservation, extends to association with human SMN; it can also be disrupted by a deletion of the YG box, in analogy to similar deletions found in SMA disease mutants in its human counterpart (Owen *et al.*, 2000; Paushkin *et al.*, 2000). Unlike its counterparts in higher organisms, however, the *S. pombe* SMN lacks a signature Tudor domain.

S. pombe two-hybrid assays also identified a Gemin2 orthologue, the 235 amino acid, 27 kDa Yab8p-interacting protein (Yip1p; here spGemin2), which exhibits a sequence 20% identical and 45% similar to human Gemin2. Although lacking a region corresponding to human residues 152-179, it otherwise expresses homology throughout its primary sequence, with the most marked conservation at its N- and C-termini. spGemin2 displays robust affinity for spSMN in GST-pulldowns, but does not interact detectably with human SMN (Hannus *et al.*, 2000).

The *S. pombe* SMN complex functions, like its orthologues in higher organisms, as an assembly machine for spliceosomal snRNPs; using a strain of *S. pombe* containing an SMN temperature-degron allele and comparing with wild-type expression at the restrictive temperature, SMN disruption was demonstrably linked to a reduction in U-snRNA, Sm protein and U-snRNP levels(Campion *et al.*, 2010). The rudimentary

assembly of SMN and Gemin2 in *S. pombe* may represent the most evolutionarily basic SMN complex (Figure 1.6).

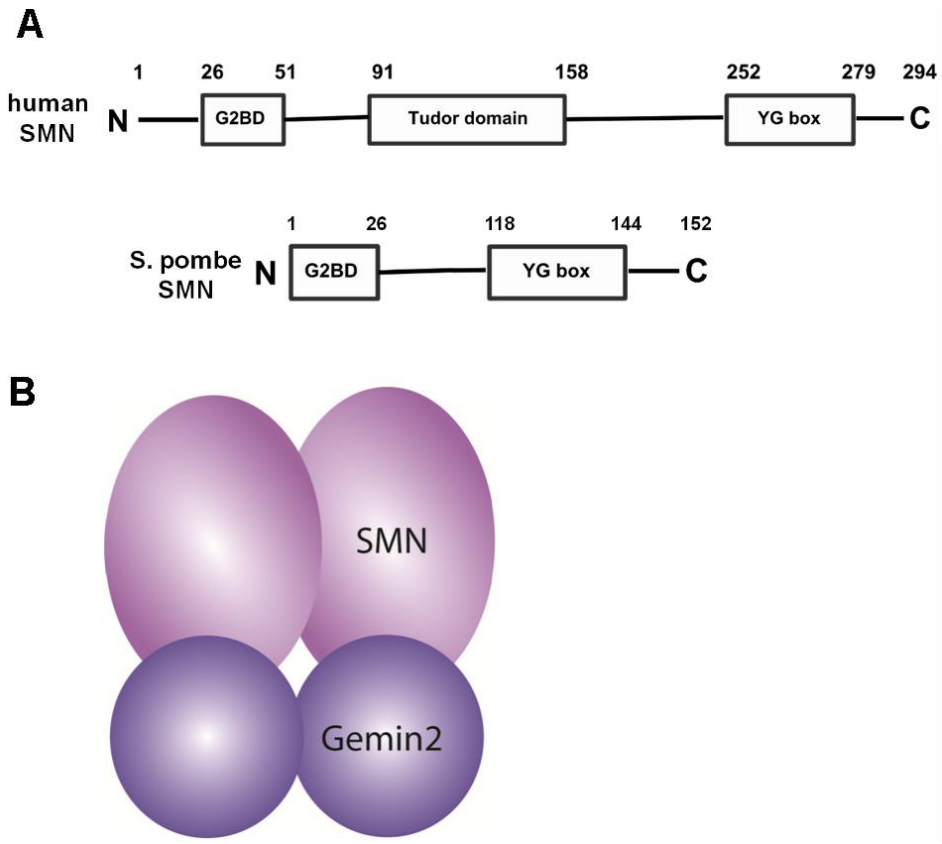


Figure 1.6. The *Schizosaccharomyces pombe* SMN complex A) The *S. pombe* SMN complex contains a Gemin2-binding domain and a YG box, but no Tudor domain. B) The *S. pombe* SMN complex contains only SMN and Gemin2.

5. Thesis objectives

The SMN complex contains at least nine protein members, among them Gemins2-8 and unrip and the eponymous SMN, the disease gene product in spinal muscular atrophy. An established function of the SMN complex lies in the assembly of seven Sm proteins onto an snRNA to form the structural core of a spliceosomal snRNP; the link between the role of SMN in that function or between another independent role of SMN and SMA remains uncertain.

A mounting body of evidence has begun to emerge detailing certain structural details and biochemical roles for members of the SMN complex, particularly relating to their roles in snRNP assembly. SMN demonstrates clear self-association, and failure of that oligomerization impedes SMN protein stability and catalysis of snRNP assembly and is linked to SMA pathogenesis. Gemin2 and Gemin8 are also thought to interact with themselves. One structural domain of SMN, its canonical Tudor domain, has been solved by both NMR and x-ray crystallography and is suggested to form a structural platform for the interaction of symmetrically dimethylated SmD1, D3 and B. Stable subunits of the SMN complex, comprising SMN-Gemin2-Gemin8, Gemin3-Gemin4-Gemin5, and Gemin6-Gemin7-unrip have been isolated *in vivo* and may represent assembly intermediates. Gemin5 has been pinpointed as a chaperone for newly transcribed nuclearly exported U-snRNAs, and Gemins 6 and 7 were demonstrated by protein x-ray crystallography to display an Sm-protein-like fold, bringing to mind a

hypothesis that they may act as an Sm dimer-surrogate in the construction of the Sm protein heteroheptamer.

This body of knowledge is still critically limited in several respects. Apart from the Tudor domain of SMN and the structure of the Gemin6-7 dimer, no atomic structures exist of any SMN complex components, severely handicapping efforts to understand the molecular basis of the complex's function in snRNP assembly and SMA pathogenesis. Functions have yet to be ascribed to several of the complex's proteins; despite the central, conserved role apparently played by SMN and Gemin2, the former remains largely undescribed and an absence of sequence homology or structural data for the latter has left its purpose wholly obscure. Without a structural knowledge of these two proteins, which form the catalytically competent core of the complex, a full understanding of the role of the SMN complex in biology and disease is unachievable.

With the goal of closing the gap in our knowledge of SMN and Gemin2, both in establishing a structural framework for those two proteins from which further studies probing their biological functions might be designed and in delineating the oligomeric state of SMN and Gemin2 and its relationship to disease, this dissertation undertakes three lines of experimental questioning. In the first, the NMR structure of the minimal binding domains of SMN and Gemin2 is presented, with accompanying mutational analysis of key SMN residues at the SMN-Gemin2 interface and a description of the role that two SMA patient mutations in that SMN construct play in that interaction. The

second describes a significant conformational change and stabilization of Gemin2 upon SMN binding and a low-resolution description of full-length, SMN-bound Gemin2. Finally, a third part characterizes the oligomerization state of the full-length SMN-Gemin2 hetero-oligomer from *Schizosaccharomyces pombe.*

The NMR structure of the minimal binding domains of SMN and Gemin2

In a previous, unpublished result, the Van Duyne laboratory at the University of Pennsylvania identified the minimal binding domains of Gemin2 and SMN using an in-house bacterial two-hybrid assay; these comprise a peptide of SMN containing residues 26-51 and a larger domain of Gemin2 including residues 95-280.

In the course of the investigations detailed in this dissertation, the SMN^{26-51}-$Gemin2^{95-280}$ interaction was confirmed using an *in vitro* fluorescence anisotropy binding assay and the minimal SMN-Gemin2 complex thus formed was probed biophysically by sedimentation velocity, sedimentation equilibrium and size-exclusion chromatography in-line with multi-angle light scattering. Its atomic structure was solved by triple-resonance multidimensional NMR spectroscopy based on backbone dihedral restraints, short-range NOE and long-range paramagnetic relaxation enhancement (PRE) distance restraints, small-angle scattering and residual dipolar coupling (RDC) orientational restraints. Gemin2 and SMN form a novel, all α-helical fold. The SMN-Gemin2 interface and two documented SMA patient mutations in that SMN construct were probed mutationally using fluorescent anisotropy binding assays, confirming the

essential nature of certain interfacial residues predicted by the NMR structure, while indicating no effect from either the D30N (Type II) or D44V (Type III) SMA on SMN-Gemin2.

The biophysical properties of Gemin2 in the presence and absence of SMN binding

To describe changes to Gemin2$^{95-280}$ upon SMN binding, the structure of unbound Gemin2$^{95-280}$ was compared to that of SMN$^{26-51}$-Gemin2$^{95-280}$. Sedimentation equilibrium and SEC-MALS indicated that both species exist as monomers in solution, but the radius of gyration, maximum dimension and stable shape reconstructions of unbound Gemin$^{95-280}$ in SAXS studies pointed to a much more extended shape. A ^{1}H-^{15}N HSQC of ^{15}N-labeled unbound Gemin2$^{95-280}$ showed line broadening and a collapse of peak dispersion, indicating incomplete folding. SMN may therefore play a role in stabilizing the structure of Gemin2.

The structure of the SMN$^{26-51}$-Gemin2$^{95-280}$ minimal complex and the SMN$^{26-51}$-Gemin2$^{12-280}$ (full-length) complex were also characterized biophysically for comparison. Contrary to reports of Gemin2 self-interaction, both of the Gemin2-SMN complexes, as well as unbound Gemin2$^{95-280}$ and Gemin2$^{12-280}$, are monomeric at all concentrations tested. The SMN$^{26-51}$-Gemin2$^{12-280}$ complex demonstrated a more elongated character than the globular minimal complex, attributable to its N-terminal region, which forms a rigid arm-like structure in SAXS shape reconstructions and suggests a number of functional hypotheses.

Determination of the stoichiometry of SMN and Gemin2 in the full-length S. pombe complex

An array of biophysical techniques was employed to determine the oligomeric state of the complex formed by the full-length *Schizosaccharomyces pombe* SMN and Gemin2 orthologues. Analytical size-exclusion chromatography suggested a high molecular weight species, but sedimentation equilibrium analytical ultracentrifugation studies provided, surprisingly, a superior description of the spSMN-Gemin2 complex as a dimer-tetramer equilibrium. SEC-MALS analysis over a range of protein concentrations also indicated that a dimer of the spSMN-Gemin2 heterodimer is actually the smallest discrete oligomer of the complex sampled at low concentrations. Small-angle x-ray scattering (SAXS) and sedimentation velocity experiments confirmed the extended nature of the particle, indicating a large radius of gyration and maximum dimension, and sequence analysis and the characteristic shape of a Kratky plot derived from SAXS data suggested that spSMN may possess a region of disorder. This model of SMN self-oligomerization yields the startling insight that, contrary its apparent behavior in self-exclusion chromatography and sucrose gradient fractionation experiments, the SMN complex forms small, discrete oligomers with unusual hydrodynamic properties.

Chapter II: The NMR structure of the minimal binding domains of SMN and Gemin2

Introduction

The SMN and Gemin2 proteins comprise the minimal catalytic machinery necessary to perform the critical function of spliceosomal U-snRNP assembly; the *S. pombe* SMN complex contains only those two members, and the more complex *D. melanogaster* SMN complex, reduced to SMN and Gemin2, is still competent for U-snRNP assembly *in vitro* (Campion *et al.*, 2010; Kroiss *et al.*, 2008). Gemin2, a 32 kDa, 269 residue protein, binds tightly and directly to SMN. Unlike SMN, however, with its known Tudor domain platform for Sm protein interaction and a tyrosine-glycine-rich oligomerization domain that plays a role in spinal muscular atrophy, the structure and function of Gemin2 remain nearly opaque.

A structural understanding of SMN and Gemin2 will provide insight into the role played by Gemin2, answers to mechanistic questions about the molecular details of SMN complex-mediated U-snRNP biogenesis and a structural framework off of which to build future structure-function studies. Here the structure of the complex formed by the minimal binding domains of Gemin2 and SMN is solved by heteronuclear multidimensional nuclear magnetic resonance spectroscopy. The hydrophobic interface between the two protein components is described, and the roles played by key conserved interfacial residues in binding are probed by mutagenesis. The two SMA patient

mutations within the SMN Gemin2-binding domain are shown, despite mixed reports in the literature, to have no effect on SMN-Gemin2 binding.

Results

Delineation of the minimal binding domains of human SMN and Gemin2

Despite a keen interest in SMN and Gemin2, owing to their evident importance in both cellular housekeeping and human disease, the considerable difficulty encountered expressing and purifying full-length human SMN and Gemin2 for biochemical studies limited the variety of experimental pathways open to pursuit, particularly structural studies for which stable monodisperse protein preparations are requisite. In an attempt to circumvent the difficulties inherent to full-length SMN and Gemin2, the minimal binding domains of the two proteins were sought instead; it was hoped that a minimal SMN-Gemin2 complex containing only the protein domains necessary and sufficient for binding might possess more tractable solution properties and permit structural studies to proceed.

An in-house bacterial two-hybrid assay based on the methods of Dmitrova *et al*. was employed for this purpose (Dmitrova *et al.*, 1998). Briefly, the lexA DNA-binding domain fusions were expressed from a *lac* promoter on compatible plasmids and the reporter construct was present on an F'-episome in strain CSH142, such that a positive interaction resulted in repression of *lacZ* transcription and white colonies on plates

containing X-gal or white-pink colonies on MacConkey agar (Figure 2.1; reviewed in more detail in Materials and Methods.).

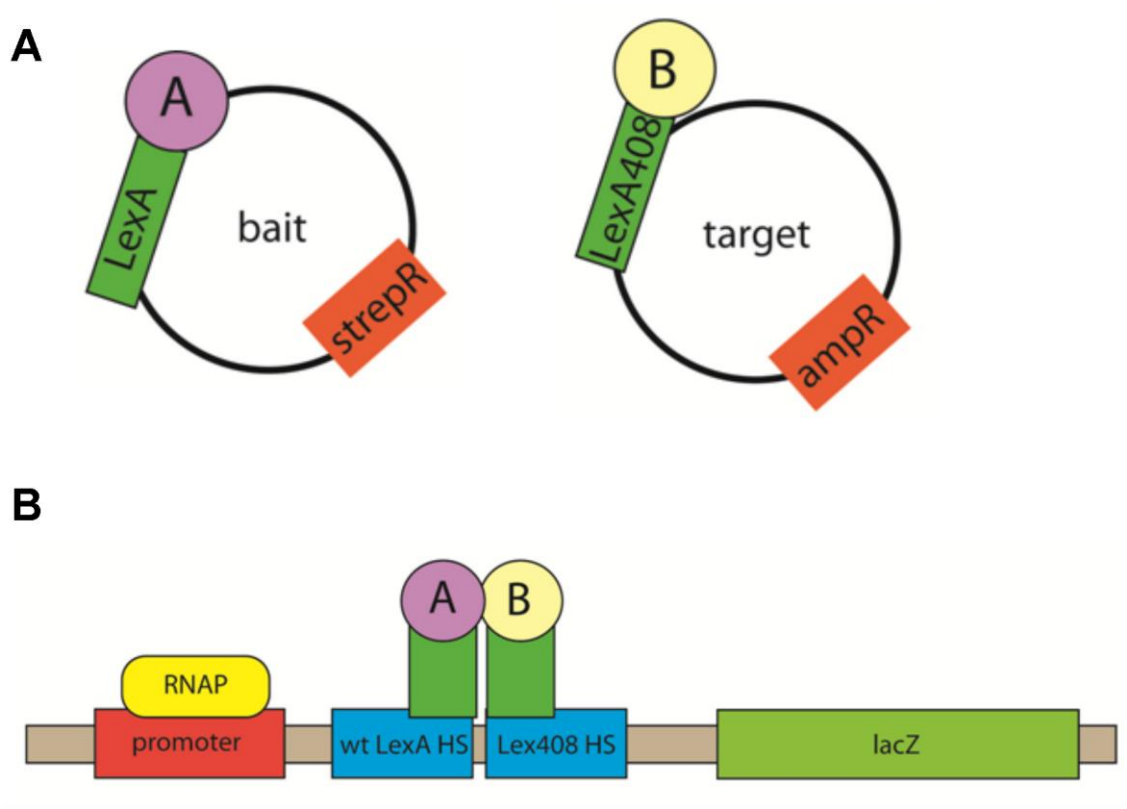


Figure 2.1 Schematic of bacterial two-hybrid assay system. A) Proteins of interest (A and B) are cloned in-frame with wildtype LexA (bait) and lexA408 (target) in a modified pETDuet in which the T7 promoter has been replaced by a lac promoter. B) After co-transformation into a reporter strain, a positive interaction between A and B results in repression of *lacZ* transcription and white colonies on plates containing X-gal.

Matthew Bernens, a technician in the Van Duyne laboratory at the University of Pennsylvania, performed the initial bacterial two-hybrid analyses and showed that an SMN fragment corresponding to exons 1-2b (residues 16-60) interacts robustly with Gemin2, as does a fragment corresponding to exons 1-3 (residues 16-156), consistent with reports that localized the Gemin2-binding domain to the N-terminus of SMN (Liu *et al.*, 1997; Ogawa *et al.*, 2007). No independent binding was observed, however, for

an SMN fragment containing exons 2b-3 (residues 52-156), contrary to one report that exon 2b mediates the SMN-Gemin2 interaction (Young *et al.*, 2000). Further truncations of the fragment containing residues 16-60 generated a minimal SMN fragment still capable of high-affinity binding to Gemin2 consisting of residues 31-51, approximately corresponding to exon 2a (Figure 2.2A,B).

The minimal SMN-binding domain of Gemin2 was identified at the same time as a region containing residues 95-280, corresponding roughly to the C-terminal two-thirds to the protein. No additional C-terminal truncations were supported. These results are in agreement with previous findings based on a mammalian two-hybrid assay that suggested a minimal Gemin2 binding domain of residues 101-280 (Figure 2.2A, B) (Ogawa *et al.*, 2007).

A

	G2 25-280	G2 47-280	G2 95-280	G2 165-280	G2 95-253
SMN 14-60	+	+	+	-	ND
SMN 14-156	+	+	+	-	-
SMN 31-156	ND	ND	+	ND	ND
SMN 31-51	+	ND	+	ND	ND
SMN 88-156	-	-	-	-	ND

	G2 100-280	G2 105-280	G2 110-280	G2 115-280	G2 120-280	G2 125-280
SMN 31-51	+	+	+	-	-	-

+: positive interaction; -: no interaction or weak interaction; ND: not determined

B

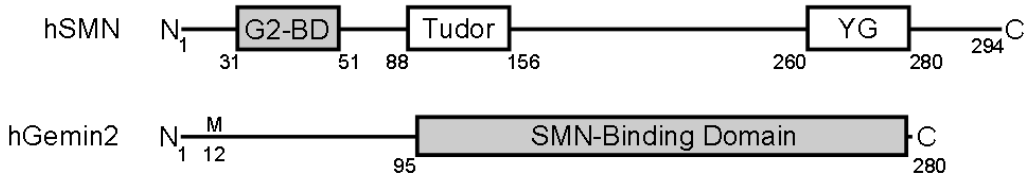


Figure 2.2 Domain boundaries of human SMN and Gemin2 A) Bacterial two-hybrid interactions between regions of hSMN and regions of hGemin2. B) Domain structures of SMN and Gemin2. The conserved Gemin2-binding domain, Tudor domain, and YG-box are shown for SMN. Minimal interaction domains are shaded. Gemin2 is assumed to initiate translation at M12, but numbering in this work follows that used elsewhere in the literature.

Under the auspices of this dissertation, the boundaries of the SMN Gemin2-binding peptide were expanded slightly to include residues 26-51. Serine residues 28 and 31 are reported to be phosphorylated in SMN *in vivo* and are suggested to modulate the efficiency of snRNP core assembly (Grimmler *et al.*, 2005b); since the affect of this post-translational modification on the interaction of SMN with Gemin2 was unknown, the residues were also included. Using the same bacterial two-hybrid system, the N-

terminal boundary of the Gemin2 SMN-binding domain was progressively truncated, and additional deletions of up to 15 residues from the N-terminus were tolerated. However, secondary structure predictions indicated these involved truncations of a long α-helix beginning at residue 100, and the Gemin2 boundaries of residues 95-280 were retained. Both SMN^{26-51} and $Gemin2^{95-280}$ could be expressed bacterially and purified, and both were soluble to the micromolar concentration range.

SMN^{26-51} binds $Gemin2^{95-280}$ and full-length Gemin2 with similar high affinity

Although SMN and Gemin2 have been reported qualitatively to interact with high-affinity, based on the resistance of their interaction to disruption in high salt (Liu *et al.*, 1997), a rigorous quantitative evaluation of their binding affinity has been lacking, due likely to the difficulty employing the full-length proteins in any experimental context. The minimal SMN^{26-51} and $Gemin2^{95-280}$ constructs, however, could be purified separately and demonstrated reasonable solution behavior, opening the door to a more thorough determination of the binding affinity with which the two proteins interact.

To quantify the affinity of the interaction between SMN^{26-51} and $Gemin2^{95-280}$, and to compare it to the interaction between SMN^{26-51} and full-length Gemin2, the fluorescence anisotropy of fluorescein-labeled SMN was measured against increasing concentrations of Gemin2.

For this and all experiments described, full-length Gemin2 refers to the Gemin2 construct comprising residues 12-280. The primary sequence for human Gemin2 contains two possible initiator methionines, Met1 and Met12. The first eleven amino acids, however, are not present in so closely related an organism as the mouse, nor does the upstream methionine Met1 have a strong Kozak consensus sequence, the mRNA sequence three codons prior to the initiator methionine that signals the translational start site to the ribosome. Gemin2$^{12\text{-}280}$ is therefore considered to be "full-length".

The anisotropy binding curves were fit with a two-state binding isotherm corrected for receptor depletion, due to the comparatively high concentration of SMN (15 nM) with respect to the dissociation constant (Kenakin, 1993). Both Gemin2$^{95\text{-}280}$ and Gemin2$^{12\text{-}280}$ bound the SMN peptide tightly, with similar affinities (3.3 ± 1.8 nM and 7.0 ± 2.5 nM, respectively; Figure 2.3), suggesting that the Gemin2$^{95\text{-}280}$ construct captures all the features of Gemin2 necessary to mediate its interaction with SMN.

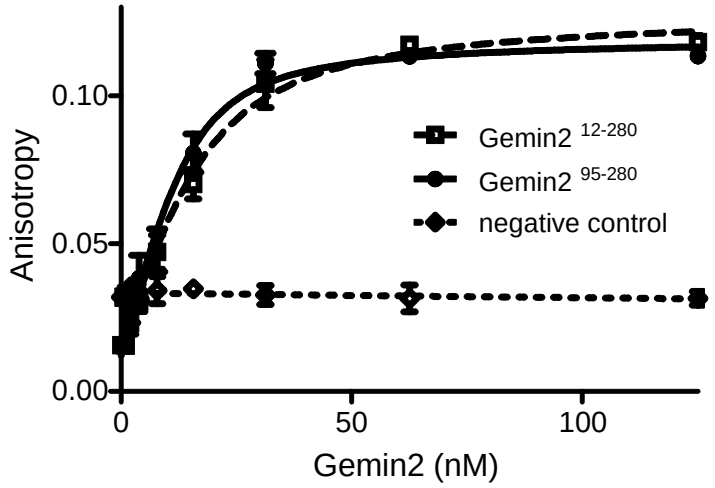


Figure 2.3 Binding of SMN$^{26-51}$ to Gemin2 or to Gemin2$^{95-280}$. Binding was based on fluorescence anisotropy of SMN N-terminally labeled with fluorescein isothiocyanate. The K_D values fit to the binding curves are 7.0±2.5 nM and 3.3±1.8 nM for Gemin2$^{12-280}$ and Gemin2$^{95-280}$, respectively. No binding was observed to a labeled, unrelated peptide (NC). Error bars represent one standard deviation from the mean, based on three replicate experiments.

Phosphorylation of SMN serines 28 and 31 has no effect on Gemin2 binding

A report by Grimmler *et al*. found that SMN serine residues 28 and 31 are phosphorylated *in vivo*, at levels measurably higher in the cytoplasm than in the nucleus. Their phosphorylation appeared to correlate with the ability of the SMN complex to efficiently assemble spliceosomal U-snRNPs (Grimmler *et al.*, 2005b). These residues fall within the boundaries of the minimal SMN Gemin2-binding domain used in this study, and it seemed within the realm of speculation that their phosphorylation state might influence the interaction between SMN and Gemin2.

The effect of the phosphorylation of SMN serine residues 28 and 31 on SMN-Gemin2 binding was tested using synthetic phosphorylated (pSer28,pSer31) and

dephosphorylated SMN peptides in the same fluorescence anisotropy binding assay described above. The anisotropy of fluorescein-labeled SMN peptide was measured against increasing concentrations of Gemin2$^{95-280}$, and the anisotropy binding curves were fit with a two-state binding isotherm corrected for receptor depletion. SMN$^{28-51}$ and SMN$^{28-51}$(pSer28,pSer31) bound Gemin2$^{95-280}$ tightly, with substantially similar affinities (9.0 ± 1.6 nM and 4.1 ± 0.7 nM, respectively; Figure 2.4 and Table 2.2). The phosphorylation state of SMN serines 28 and 31 appears to have very minor influence on Gemin2 binding.

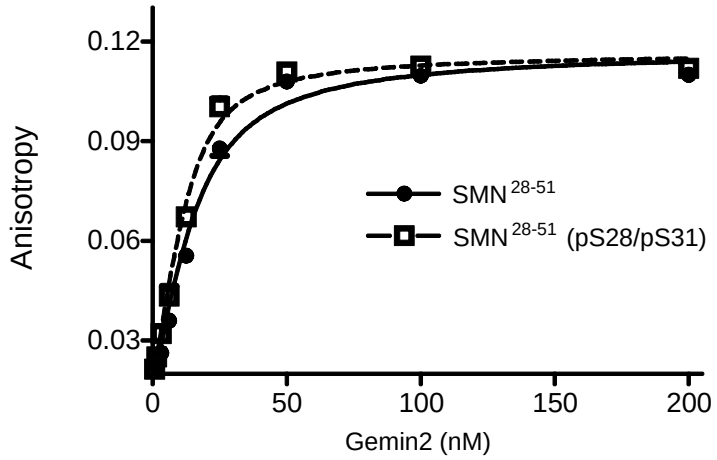


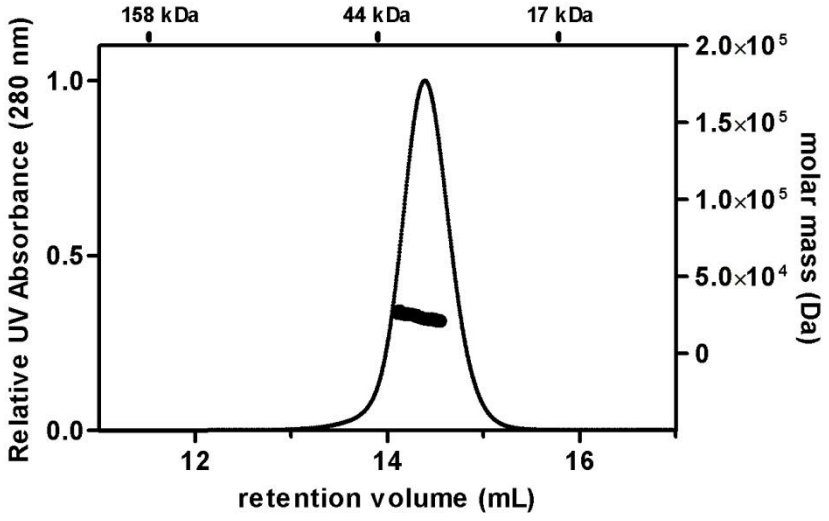
Figure 2.4 Binding of SMN$^{28-51}$ unphosphorylated and phosphorylated at Ser28 and Ser31 to Gemin2$^{95-280}$. The experiment employed the fluorescence anisotropy assay described in Figure 2.3. The K_D values fit to the binding curves are 9.0±1.6 nM and 4.1±0.7 nM for SMN$^{28-51}$ and SMN$^{28-51}$ (pSer28,pSer31), respectively. Error bars represent one standard deviation from the mean, based on three replicate experiments.

Purification and biophysical properties of the core SMN-Gemin2 complex

In an encouraging advance over the behavior of their full-length counterparts, both SMN$^{26-51}$ and Gemin2$^{95-280}$ as C-terminal Mxe intein-chitin binding domain-His6x

constructs were readily purified over sequential nickel NTA and chitin columns, followed by cleavage of the affinity tag, anion exchange chromatography, complex formation at room temperature in a molar excess of SMN^{26-51} and gel filtration.

A number of biophysical properties of the unexpectedly tractable core SMN-Gemin2 complex were described during preparation for a planned series of crystallization attempts. Both size-exclusion chromatography in-line with multi-angle light scattering (SEC-MALS) and sedimentation equilibrium analytical ultracentrifugation indicated that the complex formed a 1:1 heterodimer of SMN^{26-51} (3 kDa) and $Gemin2^{95-280}$ (21 kDa) in solution. SEC-MALS showed that the SMN^{26-51}-$Gemin2^{95-280}$ complex eluted as a single peak with an apparent molecular weight of 25 kDa with respect to globular molecular weight standards, while possessing a stable mass profile across the eluted peak with a weight-averaged molecular weight of 23.7 ± 0.1 kDa. Both values are highly consistent with the expected 24 kDa molar mass of a 1:1 SMN^{26-51}-$Gemin2^{95-280}$ complex (Figure 2.5).



.

Figure 2.5. Representative SEC-MALS analysis of the Gemin2$^{95-280}$-SMN$^{26-51}$ complex. The elution volumes of globular protein standards are shown above the panel.

Sedimentation equilibrium analytical ultracentrifugation data on SMN$^{26-51}$-Gemin2$^{95-280}$ were collected at three speeds and three concentrations at 20°C and best fit to a non-interacting single species model that produced an estimate of a molecular weight of 30 kDa (Figure 2.6). While slightly higher than the predicted molecular weight of a core SMN-Gemin2 heterodimer, it generally corroborated the SEC-MALS estimate of the molecular weight and certainly failed to suggest oligomerization; instead, the three-day room-temperature experiment, performed before trial and error had fully defined the requirements for SMN$^{26-51}$-Gemin2$^{95-280}$ stability, contained no reducing agent and likely experienced protein aggregation.

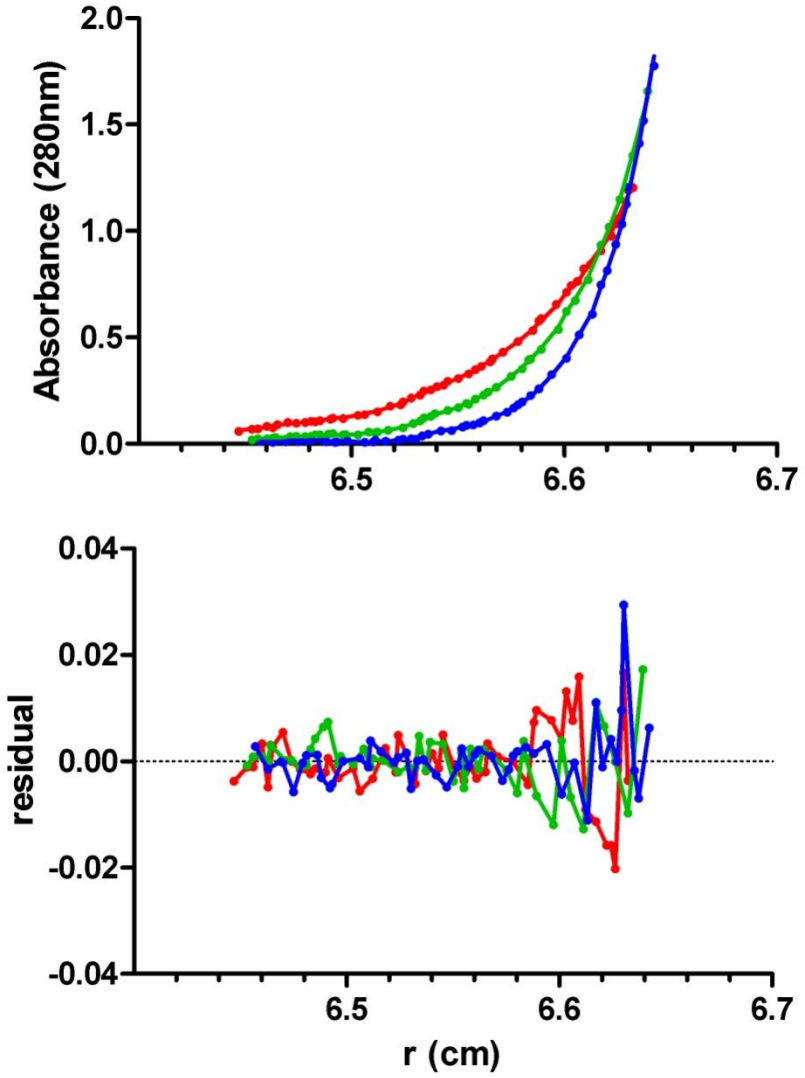


Figure 2.6 Representative sedimentation equilibrium analytical ultracentrifugation experiment on the core SMN-Gemin2 complex. Conducted at 4°C at 18 µM, 11 µM and 5 µM. Here the curves are fit to non-interacting single species model.

Sedimentation velocity analytical ultracentrifugation provided an evaluation of the sedimentation coefficient and frictional ratio f/f_o of the core SMN-Gemin2 complex. The frictional ratio is the ratio of the frictional coefficient *f* experienced by the molecule

when sedimenting to the theoretical frictional coefficient f_o for an ideal sphere of identical molecular weight and serves as a measure of particle elongation. It is related to the sedimentation coefficient through the Svedberg equation, where

$$f_o = 6\pi\eta r$$

for a perfect sphere of radius r in solution of viscosity η, and

$$f = \frac{M(1-\bar{v}\rho)}{N_a S}$$

where M is the molar mass of the particle, N_a Avogadro's number, S the sedimentation coefficient, v the partial specific volume and ρ the solvent density (Lebowitz *et al.*, 2002).

Sedimentation velocity data for $SMN^{26\text{-}51}$-$Gemin2^{95\text{-}280}$ at 25 μM was collected at 20°C and fit well to a continuous c(s) distribution yielding a single species with a sedimentation coefficient of 2.4S (Figure 2.7). From the sedimentation coefficient, the frictional ratio was calculated to be 1.27, very near the value of 1.2 cited for globular proteins and in accord with the observation that the complex evinced behavior in size-exclusion chromatography similar to that of globular molecular weight standards.

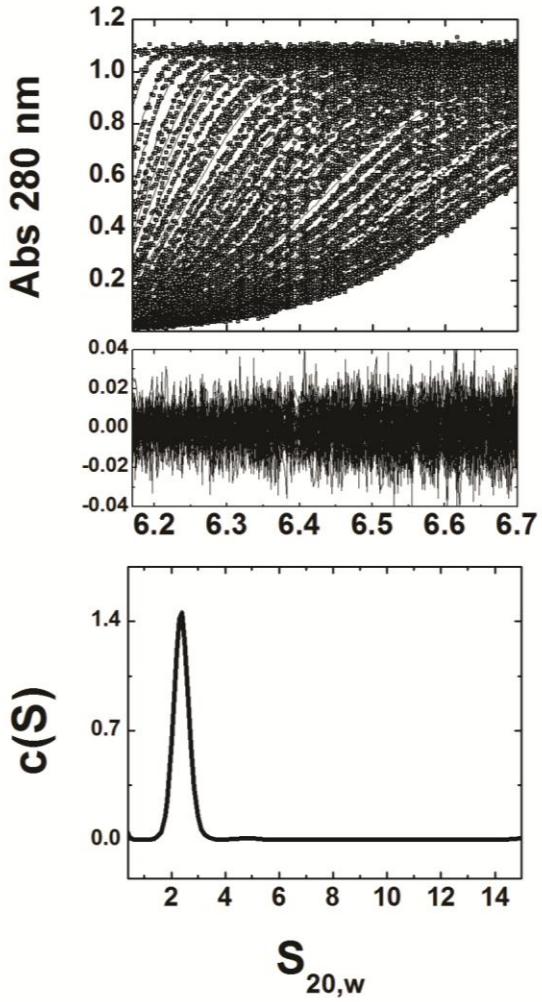


Figure 2.7 Sedimentation velocity analytical ultracentrifugation experiment on the SMN$^{26-51}$-Gemin2$^{95-280}$ complex at 20°C. Sedimentation velocity absorbance traces of SMN$^{26-51}$-Gemin2$^{95-280}$ at 280 nm occupy the top panel, with residuals of the experimental fits in the middle panel and a continuous sedimentation coefficient distribution (c(S)) of the protein in the bottom panel.

The SMN$^{26-51}$-Gemin2$^{95-280}$ complex formed a well-behaved, compact 1:1 heterodimer of the two proteins and appeared, and both SEC-MALS and sedimentation velocity experiments suggested monodisperse purified preparations. Protein crystallization experiments with the protein were undertaken, with an eye toward solving an elusive atomic structure, but neither the SMN$^{26-51}$-Gemin2$^{95-280}$ complex nor Gemin2$^{95-280}$ alone,

nor the complex formed by SMN^{26-51} with any of the additional Gemin2 N-terminal truncations tolerated by the bacterial two-hybrid interaction screen, nor orthologous protein constructs from *D. melanogaster* or *S. pombe* yielded crystal hits.

The solution structure of the core SMN-Gemin2 complex

After the core SMN^{26-51}-$Gemin2^{95-280}$ complex proved refractory to crystallization, an initial ^{1}H-^{15}N-HSQC of ^{15}N-labeled protein showed dispersed peaks with sharp linewidths (Figure 2.8), suggesting a possibility of success for structural studies by NMR spectroscopy, and heteronuclear multidimensional nuclear magnetic resonance spectroscopy using both ^{15}N- and $^{15}N/^{13}C$-enriched SMN^{26-51} and $Gemin2^{95-280}$ was employed to determine the solution structure of the core SMN-Gemin2 complex.

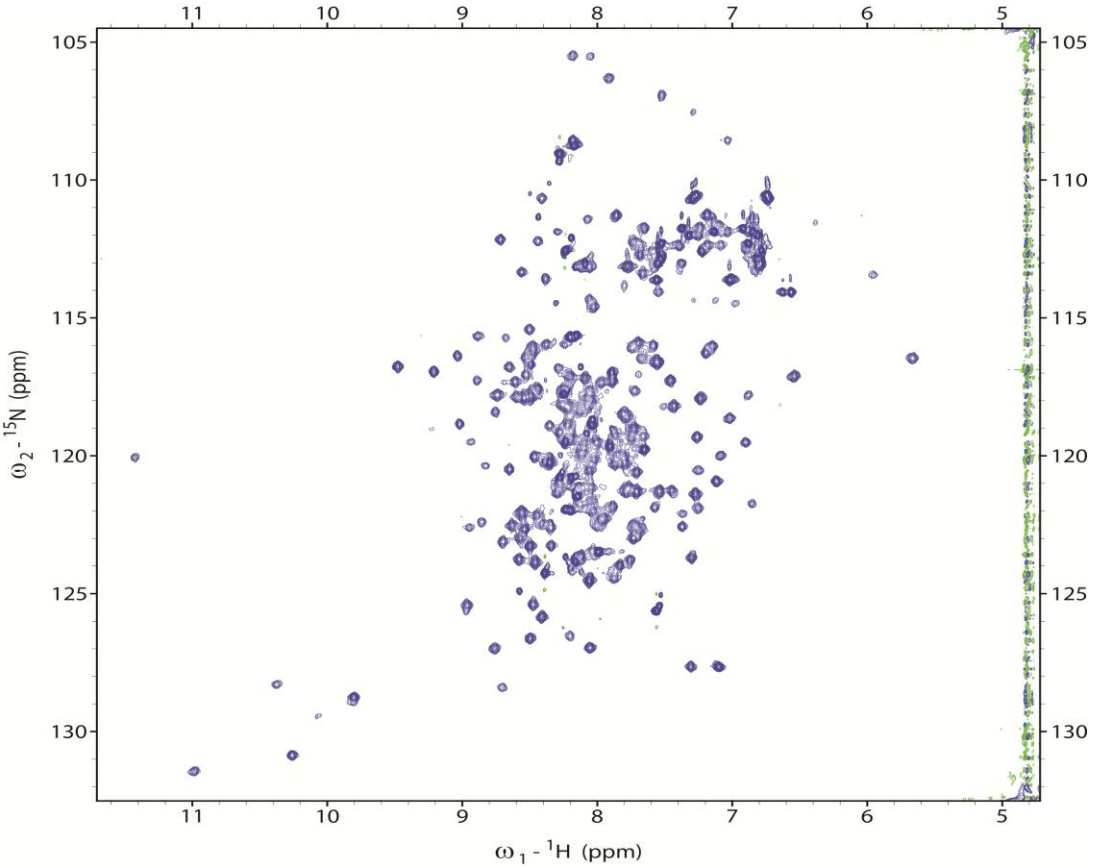


Figure 2.8 A two-dimensional ^{1}H-^{15}N HSQC of SMN$^{26-51}$-Gemin2$^{95-280}$

NMR structural studies proved non-trivial with respect to SMN$^{26-51}$-Gemin2$^{95-280}$; the complex's susceptibility to proteolysis hampered its stability for multi-day experiments at 25°C, as did a proclivity for oxidative precipitation only surmountable at very high concentrations of reducing agent, exchanged often for fresh buffer. At 24 kDa, the complex experienced many of the challenges to which solution NMR is heir with respect to large systems; weak signal due to line broadening made interpretation of some data challenging, and spectral overlap was sufficiently severe at times to require creative strategies for making backbone and sidechain peak assignments, and an unusual recourse was made to 4-dimensional through-space NOESY experiments to supplement the more typical 3D NOESY-HSQCs and to resolve uninterpretable areas of peak

degeneracy. The SMN^{26-51}-$Gemin2^{95-280}$ complex also experienced a broad dynamic range, and the wide range of peak intensities from different regions of the protein provided another complicating factor in data collection and analysis.

Comprehensive resonance assignments were obtained using triple resonance, total correlation spectroscopy and NOE-based strategies. Backbone assignments were obtained first from a standard suite of through-space experiments, HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HNCO and HN(CA)CO. Representative strip plots from the HNCA and HNCOCA, forming a "Cα walk" for Gemin2 residues K137-E142, are depicted in Figure 2.9 and make clear, if some strips, the difficulty imposed by spectral crowding.

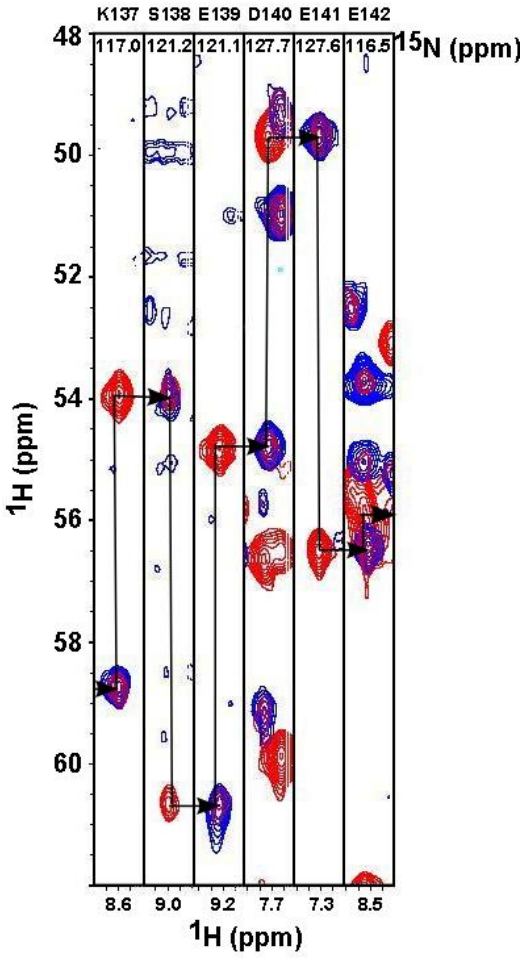


Figure 2.9 Triple resonance backbone assignment of Gemin2$^{95-280}$. Slices of the overlaid HNCA (red) and HNCOCA (blue) spectra of SMN$^{26-51}$-Gemin2$^{95-280}$ showing the connectivities for Gemin2 from K137-E142. The C_α connectivity is shown by a dotted black line.

Later experiments supplemented those listed above to facilitate assignments in areas of weak signal or spectral degeneracy. An ^{15}N HSQC of a sample in which only Gemin2 was labeled with ^{15}N-leucine enabled the assignment of most leucine backbone resonances, and a sample in which only SMN$^{26-51}$ was labeled resulted in complete

assignment of the SMN peptide backbone. Backbone assignments were complete to 97.3% of HN and N resonances and 94.4% of carbonyl carbon resonances.

Side chain assignments were obtained from H(CC)(CO)NH, (H)CC(CO)NH, HCCH-TOCSY and HCCH-COSY experiments. Assignment of all aliphatic sidechain proton and carbon resonances for SMN^{26-51} Ile33 is illustrated in Figure 10. While neither the HCCH-TOCSY nor the HCCH-COSY (Figure 10A,B) evince complexity due to spectral degeneracy, the substantial overlap of resonances characteristic of many regions of the SMN^{26-51}-$Gemin2^{95-280}$ spectra are in evidence in the HCCH-TOCSY and HCCH-COSY spectra, especially in the HCCH-TOCSY slices at the Hγ1 and Hγ2 H-C frequencies.

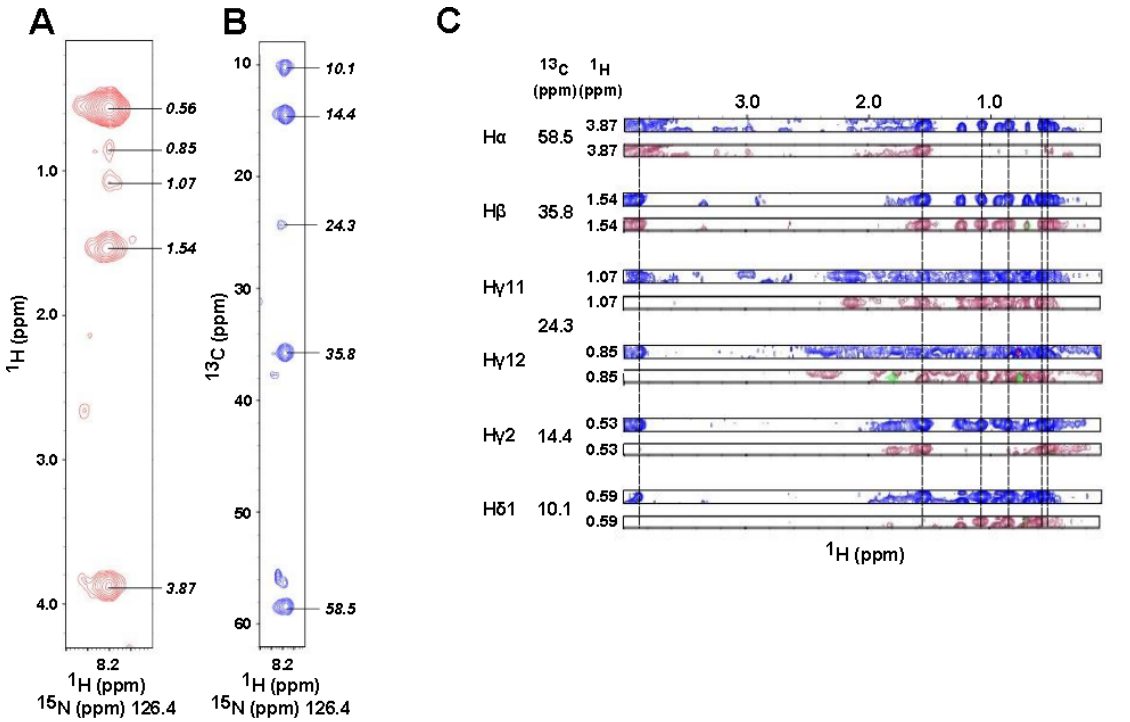


Figure 2.10 Triple resonance sidechain assignment of SMN$^{26-51}$ Ile33. A) H(CC)(CO)NH and B) (H)CC(CO)NH slices at the Trp34 backbone amide frequencies showing all Ile33 sidechain proton and carbon resonances, respectively. C) HCCH-TOCSY and HCCH-COSY slices showing all and immediately adjacent sidechain proton resonances, respectively, at each sidechain carbon frequency. The frequencies of each sidechain proton are depicted as dotted black lines.

Due to difficulty in making some sidechain assignments due to spectral degeneracy in some cases and to weak signal in others, some assignments were facilitated by three-dimensional ^{15}N and ^{13}C-edited NOESY-HSQC spectra. Stereo-specific side chain assignments of valine and leucine were determined from the ^{13}C-coupled carbon HSQC of trace-labeled protein sample, in which only the *pro-S* (Val Hγ2, Leu Hδ2) methyl resonances appear as singlets (Neri *et al.*, 1989); a representative expansion of overlaid HSQCs from fully labeled and trace-labeled samples appears as Figure 2.11. Aromatic side-chain assignments were made using three-dimensional aromatic-optimized ^{13}C NOESY-HSQC and HCCH-TOCSY experiments.

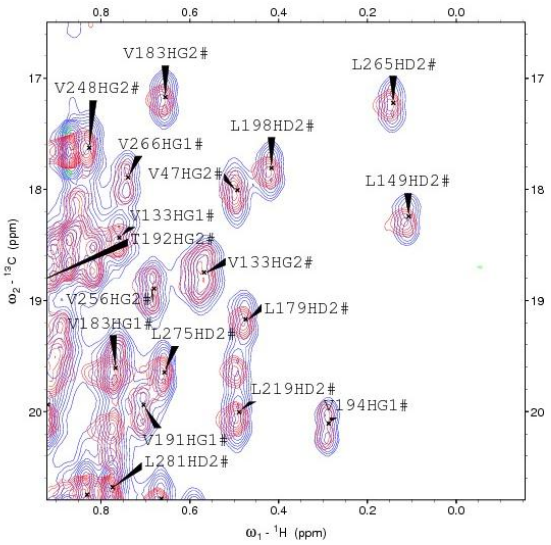


Figure 2.11 Stereospecific assignment of SMN$^{26-51}$-Gemin2$^{95-280}$ valine and leucine prochiral methyl groups. An overlay of the 'H-decoupled ^{1}H-^{13}C HSQC spectra of fully ^{13}C isotopically labeled (blue) and trace-labeled (red) SMN$^{26-51}$-Gemin2$^{95-280}$. The *pro-S* methyl groups (Hγ2 and Hδ2) are singlets in the spectrum from the trace-labeled sample.

Appendix A provides a table of all SMN$^{26-51}$-Gemin2$^{95-280}$ resonance assignments.

Structural restraints primarily consisted of the traditional combination of nuclear Overhauser effect (NOE) inter-proton distances derived from three and four-dimensional ^{15}N- and ^{13}C-edited NOE spectra and backbone dihedral restraints calculated based on chemical shifts, but the number of available NOE and dihedral restraints alone was too few compared to the size of the protein to obtain a structural ensemble of the desired precision. Several other sources of structural restraint, with an emphasis on those providing longer-distance restraints than the short-range 5 Å NOE, were introduced to improve ensemble precision. A constraint on overall molecular shape was imposed from small-angle X-ray scattering data. Residual dipolar couplings measured in a bacteriophage Pf1 liquid crystalline medium provided long-distance

orientational restraints. To create a visual example, both unaligned (both blue) and partially aligned (both red) SMN$^{26-51}$-Gemin2-$^{95-}$280, in-phase and anti-phase ^{1}H-^{15}N HSQC-IPAP spectra were added and subtracted, and the resulting spectra superimposed. The two resulting combined spectra were then overlaid on each other to illustrate changes in splitting obtained in Pf1 (Figure 2.12). The blue and red arrows indicate the aligned and unaligned splitting observed for SMN T37 and Gemin2 G203 and G212.

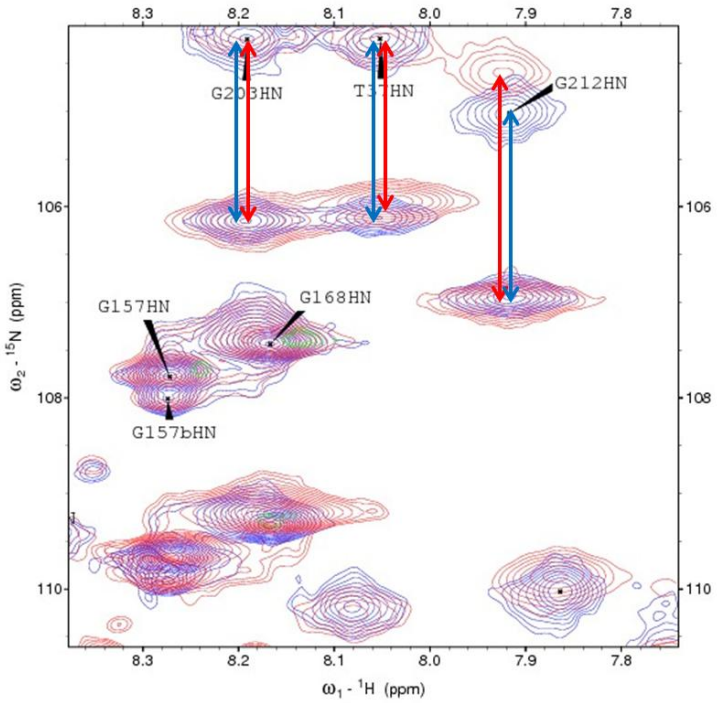


Figure 2.12 Residual dipolar couplings observed in partially aligned SMN$^{26-51}$-Gemin2$^{95-280}$. Spectra obtained by adding and subtracting in-phase and anti-phase ^{1}H-^{15}N HSQC-IPAP spectra were first overlaid for both unaligned (both red) and partially aligned (both blue) SMN$^{26-51}$-Gemin2-$^{95-280}$. The two resulting combined spectra were then overlaid to illustrate differences in splitting. The red and blue arrows indicate the aligned and unaligned splitting observed for SMN T37 and Gemin2 G203 and G212.

Tables of all structural restraints appear as Appendix B.

Long-range distance restraints were also obtained from paramagnetic relaxation enhancements (PREs) in which Cys241 of Gemin2(C154S/C221S/C264S) was coupled to the paramagnetic nitroxide spin label MTSSL (S-(2,2,5,5-tetramethyl-2,5-dihydro-^{1}H-pyrrol-3-yl)methyl methanesulfonothioate). Distance restraints from PREs are calculated based on attenuation of signal intensity in HSQC of resonances in proximity to the spin label, an effect reversed when the spin label is reduced; Figure 2.13 shows an example of the attenuation and recovery of the intensity of the R205 H-N resonance, based on its proximity to the MTSSL moiety, in of an overlay of ^{1}H-^{15}N HSQC spectra of oxidized and reduced Cys241-MTSSL-conjugated SMN$^{26\text{-}51}$-Gemin2$^{95\text{-}280}$ (C154S/C221S/C264S).

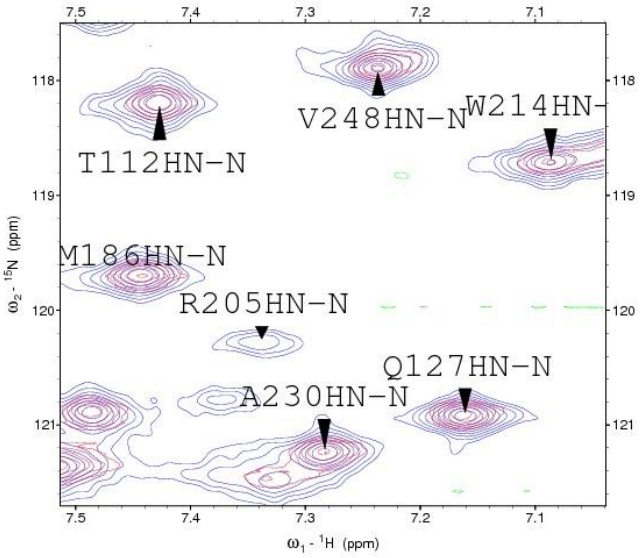


Figure 2.13 MTSSL-induced paramagnetic relaxation enhancement effects observed in the ^{1}H–^{15}N HSQC spectrum of SMN$^{26\text{-}51}$-Gemin2$^{95\text{-}280}$. Expansion of the ^{1}H–^{15}N HSQC spectra of oxidized (red) and reduced (blue) C241-MTSSL-conjugated SMN$^{26\text{-}51}$-Gemin2$^{95\text{-}280}$ (C154S/C221S/C264S). The cross-peak corresponding to R205 H-N decreases noticeably in intensity in the oxidized sample.

Design of the (C154S/C221S/C264S) mutant aimed to leave one conserved cysteine unmutated and accessible for spin labeling. Although C148 also remains unchanged in

this construct, NMR data from an $^{15}N/^{1}H$-edited HSQC of the paramagnetic labeled species displayed no attenuation of signal intensity of amide resonances of residues in near three-dimensional proximity to C148, suggesting that that buried residue remains unmodified.

The ensemble of structures was calculated using the XPLOR-NIH software program by simulated annealing molecular dynamics employing both torsion angle and Cartesian coordinate dynamics (Schwieters *et al.*, 2003; Schwieters *et al.*, 2006). Out of 400 trial structures calculated, the 32 with lowest energy comprise an ensemble that represents the structure of the SMN^{26-51}-$Gemin2^{95-280}$ heterodimer and possess a backbone RMSD of 0.59 Å. (Figure 2.14A) NMR structural and refinement statistics are summarized in Table 2.1.

Table 2.1. Summary of structural ensemble statistics

Experimental restraints	
NOE distance restraints	
All	1849
Intraresidue	616
Sequential ($\|i - j\| = 1$)	541
Medium-range ($1 < \|i - j\| < 5$)	453
Long-range ($\|i - j\| > 5$)	239
Inter-protein	50
PRE distance restraints	31
Hydrogen bond restraints	55
Dihedral angle restraints	
Φ	139
Ψ	134
^{15}N-^{1}H RDCs	84
RMSD from idealized covalent geometry	
Bonds (Å)	0.0040 ± 0.0001
Angles (°)	0.589 ± 0.006
Impropers (°)	0.441 ± 0.011
RMSD from solution NMR restraints	
NOE distance restraints (Å)	0.0210 ± 0.001
PRE distance restraints (Å)	0.0423 ± 0.009
Dihedral restraints (°)	0.787 ± 0.069
RDC restraints (Hz)	0.116 ± 0.014
SAXS data fit (χ)†	0.761 ± 0.292
RDC quality factor	0.311 ± 0.012
Precision of atomic coordinates	
Secondary structure ‡	
Backbone (N, Ca, C', O) (Å)	0.596 ± 0.096
Heavy atoms (Å)	1.056 ± 0.081
Ramachandran statistics	
Favored (%)	98.0
Allowed (%)	1.8
Disallowed (%)	0.2

† χ is the square root of the normalized χ^2 difference between observed and predicted scattering data

‡ The secondary elements used were as follows: Gemin2 residues 107-124,142-148, 180-185, 188-201, 210-220,229-246 and 259-269; SMN residues 38-48.

Gemin2$^{95\text{-}280}$ folds into a helical bundle containing seven α-helices, the locations of which correspond well to those predicted by secondary structure prediction algorithms. The Gemin2-binding domain of SMN also forms an α-helix that interacts with Gemin2 in an extended hydrophobic cleft formed by four helices of Gemin2 (Figure 2.14B: labeled 1, 3, 5 and 7) far separated in primary sequence.

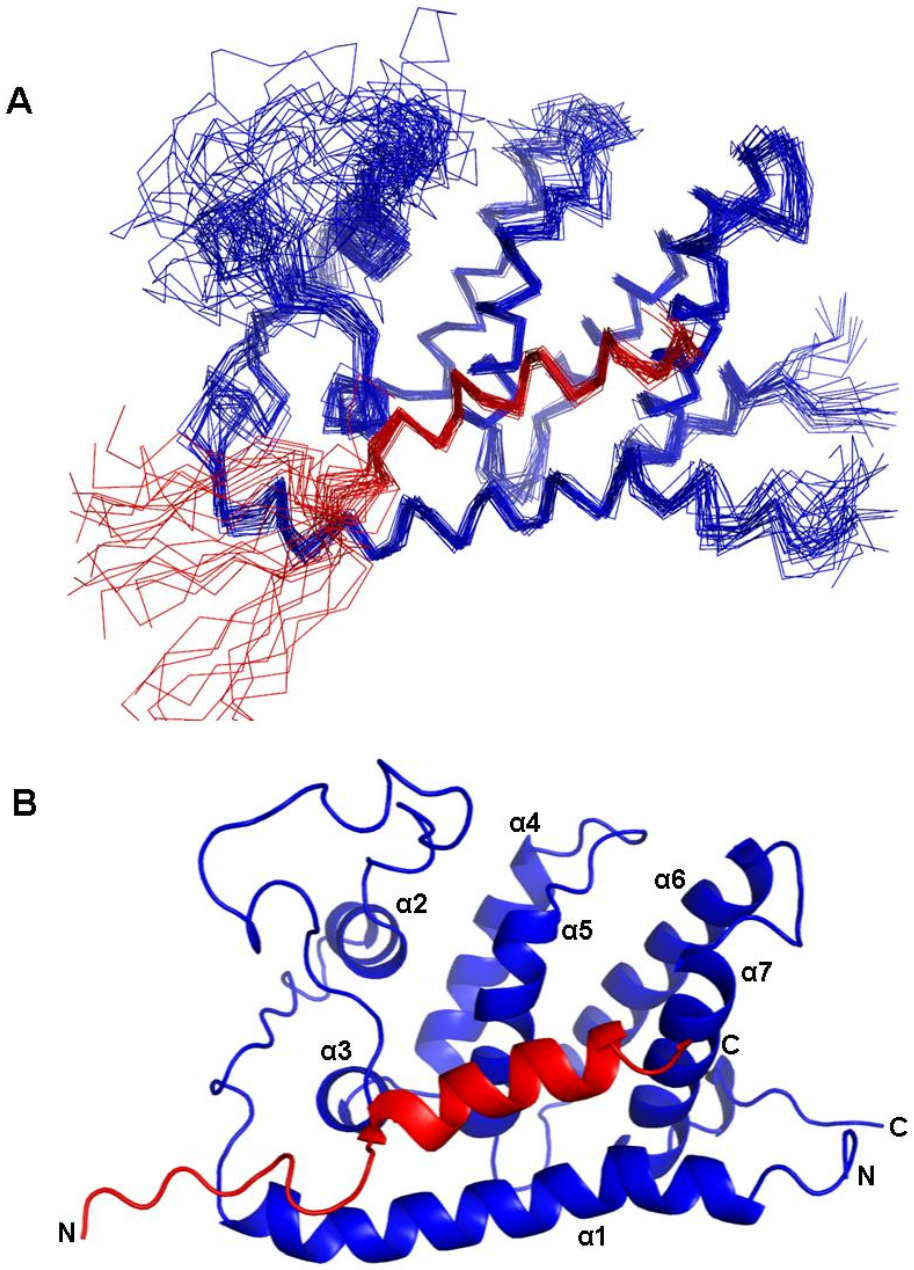


Figure 2.14 Structure of SMN bound to Gemin2. A) Overlay of Cα-traces for the 32 lowest energy structures. B) Cartoon drawing of the lowest energy structure. SMN^{26-51} is in red, $Gemin2^{95-280}$ is in blue. In B, the Gemin2 helices are numbered.

The structure is largely well-defined, with the notable exception of a large, poorly conserved loop in Gemin2 (residues 150-175) that is disordered and which demonstrates no long-range NOEs. This region of the protein is highly mobile in solution, as indicated by the relatively long ^{15}N T2 relaxation time constants calculated for it by Veronica Moorman, a graduate student in the Wand laboratory at the University of Pennsylvania (Figure 2.15). In addition, the first seven residues in the SMN peptide (residues 26-32) exhibit significant disorder, which is consistent with their lack of importance for Gemin2-binding in the bacterial two-hybrid assays described above.

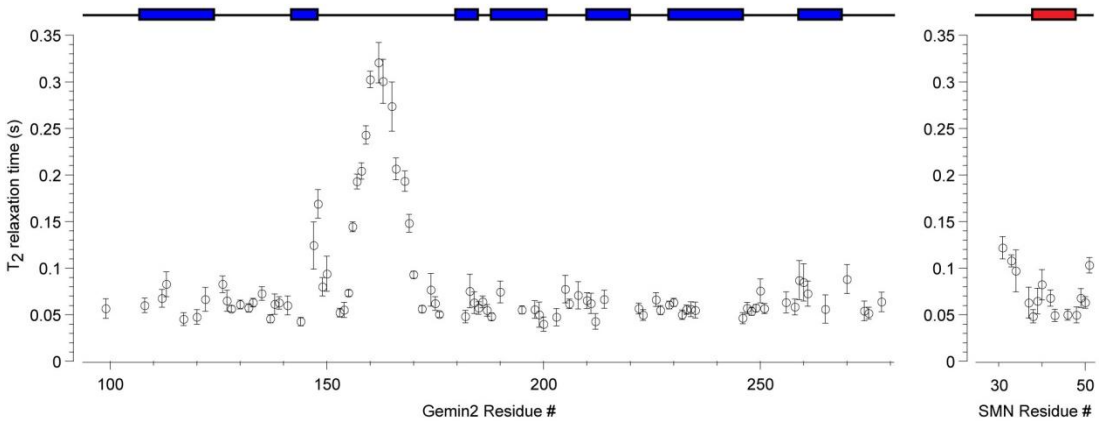


Figure 2.15 Backbone amide ^{15}N T2 relaxation times of Gemin2 and the bound SMN peptide. Obtained by Veronica Moorman in the Wand laboratory at the University of Pennsylvania at 750MHz (^{1}H). Helices are depicted above the graph as rectangles for reference. High T_2 times are indicative of increased backbone flexibility.

Neither the seven α-helices of Gemin2$^{95\text{-}280}$ alone nor the eight that make up the SMN$^{26\text{-}51}$-Gemin2$^{95\text{-}280}$ complex display any significant structural homology to known protein structures. Using the lowest-energy NMR structure as a query structure, searches of the Dali database yielded no persuasive matches (Holm & Sander, 1995; Sun *et al.*,

2005). Of those structures with scores indicating the highest degree of similarity, all had root-mean-squared deviations greater than 3 Å and typically treated as the region of structural homology the four-helix bundle formed by helices 4-7 of Gemin2, which is similar to that found in a number of helical sub-domains. Given this apparent lack of structural resemblance, the spatial arrangement of helices in Gemin2 and in the SMN-Gemin2 complex may be considered to comprise a unique protein fold.

The SMN-Gemin2 interface is mediated largely by hydrophobic interactions

Within the SMN$^{26\text{-}51}$ peptide, residues 37-50 form an α-helix with a strongly amphipathic character. Its hydrophobic face interacts with Gemin2 in a shallow, hydrophobic cavity formed by Gemin2 helices α1, α3, α5, and α7 (Figure 2.16).

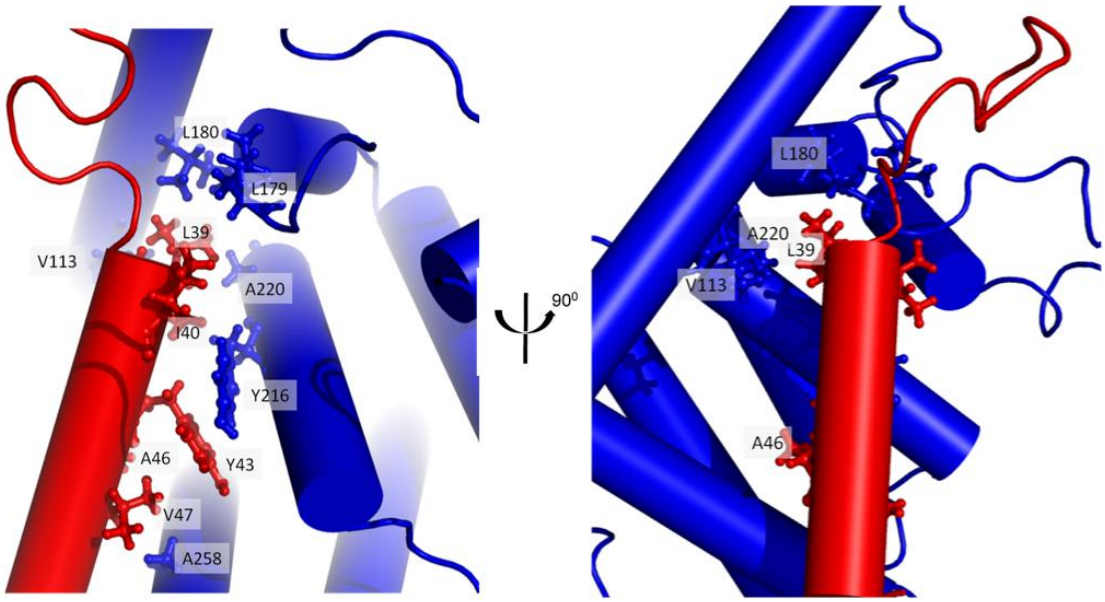


Figure 2.16 The conserved SMN-Gemin2 interface. A) Closeup of the interface. Gemin2 is depicted in blue and SMN in red. Key interfacial sidechains are labeled and represented as stick models. B) A 90° rotation of the view shown in panel A.

Fifty short-range proton-proton NOE distance restraints define the interface between SMN and Gemin2. Eleven of the 18 SMN residues from Ile33 through Phe50 are positioned close to the Gemin2 core domain, and SMN sequence alignments reveal that most of these residues involved in the interface with Gemin2 are conserved (Figure 2.17A) Leu39, Ala42, Tyr43, Ala46, and Val47 seem to play key roles mediating the hydrophobic interface; they are largely buried, with little solvent-accessible surface in members of the low energy structural ensemble. NOEs between Ile33, Asp36, Thr37, Ala38, Ile40 and Phe50 and Gemin2 are also observable. In the lowest-energy structure, the SMN-Gemin2 interface buries a total of 1800 $Å^2$ of solvent-accessible surface area, which is consistent with a high-affinity interaction. The Gemin2 residues involved in binding to SMN, many also highly conserved, are located throughout the sequence, explaining why a smaller SMN-binding domain has not been previously reported (Figure 2.17B).

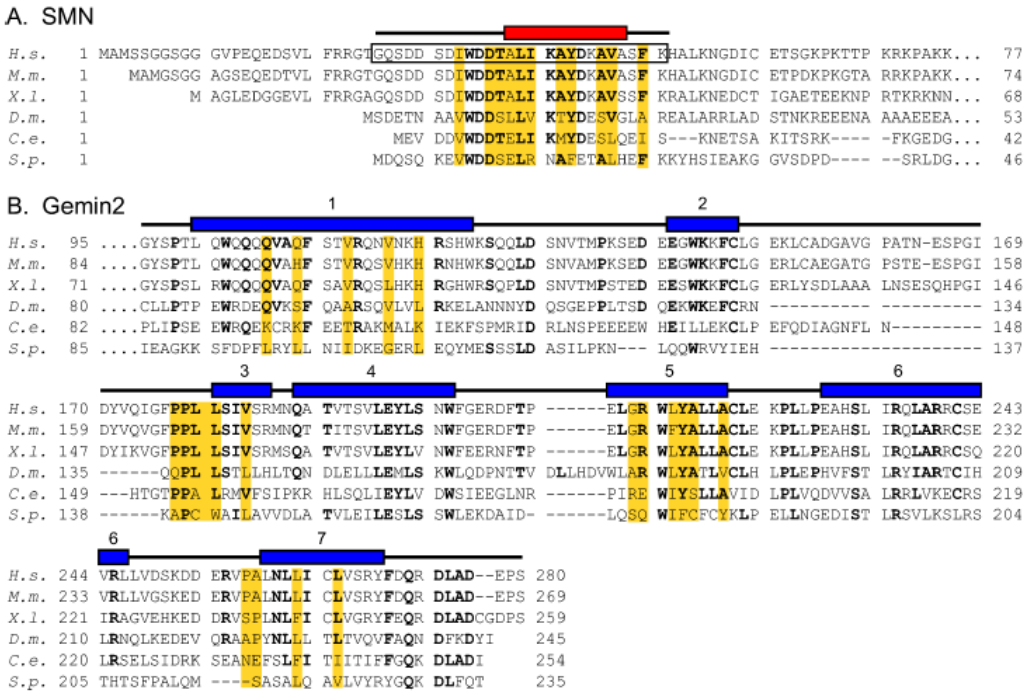


Figure 2.17 Sequence alignments of SMN and Gemin2 from a diverse set of organisms.
A) SMN alignment in the N-terminal region. B) Gemin2 alignment, with helices numbered as in Figure 2.14. Residues that are identical in four out of the six sequences are in bold font. Residues involved in the SMN-Gemin2 interface are shaded yellow. The sequences shown are human (*H.s.*), mouse (*M.m.*), frog (*X.l.*), fly (*D.m.)*, worm (*C.e.*), and fission yeast (*S.p.*)

At the N-terminus of the SMN helix, residues from the α3 helix of Gemin2 (Pro177, Pro178, Leu179, Leu180, Val183) and from helix α5 (Ala217, Ala220) contribute to a dense network of proton-proton NOEs with SMN Thr37, Ala38, Leu39 and Ile40. Helices α5 and α7 contribute interactions (Gly212, Leu215, Tyr216, Leu265) to SMN mid-helix residues (Tyr43, Ala46, Val47), and α7 (Pro257, Ala 258, Leu262, Leu265) caps the C-terminus. The α1 helix, running almost antiparallel to SMN, contributes interactions along its length (His120, Val117 and Val113 to Ala38 and Leu39; Val113, Gln109 and Gln106 to Ala42; Gln109 and Gln106 to Ala46) (Figure 2.16).

In addition, Gemin2's His120 interacts with Asp36 and Ile33 of SMN, beyond the N-terminal boundary of the SMN α-helix. Asp36, along with Trp34 and Asp35, is one of three invariably conserved residues that serve no immediately discernible role in mediating the SMN-Gemin2 interaction. Asp36, however, is directed towards the Gemin2 interface, where it could interact with His120 and/or Trp124. Another highly conserved SMN residue, Asp44, is oriented away from the hydrophobic interface but might be positioned to form a polar interaction with the sidechain of Arg213.

Mutation of key conserved interfacial residues confirms their importance in SMN-Gemin2 binding

Several highly conserved SMN residues appear to play key roles in mediating the hydrophobic SMN-Gemin2 interface, while other equally conserved residues serve no apparent role in protein-protein binding. To assess the effect of the most conserved SMN residues on Gemin2 binding, a series of SMN^{26-51} mutants were constructed and tested for binding to $Gemin2^{95-280}$ using the fluorescence anisotropy binding assay. Of those tested, binding affinity was reduced over 100-fold for Y43A, 20-fold for L39A, and 10-fold for A46N, all in accord with their apparent roles in Gemin2-binding. Other conserved residues, however, showed no effect on binding: W34A, D35A, D36A and K41A had dissociation constants similar to that of WT SMN^{26-51} (Figure 2.18; Table 2.2).

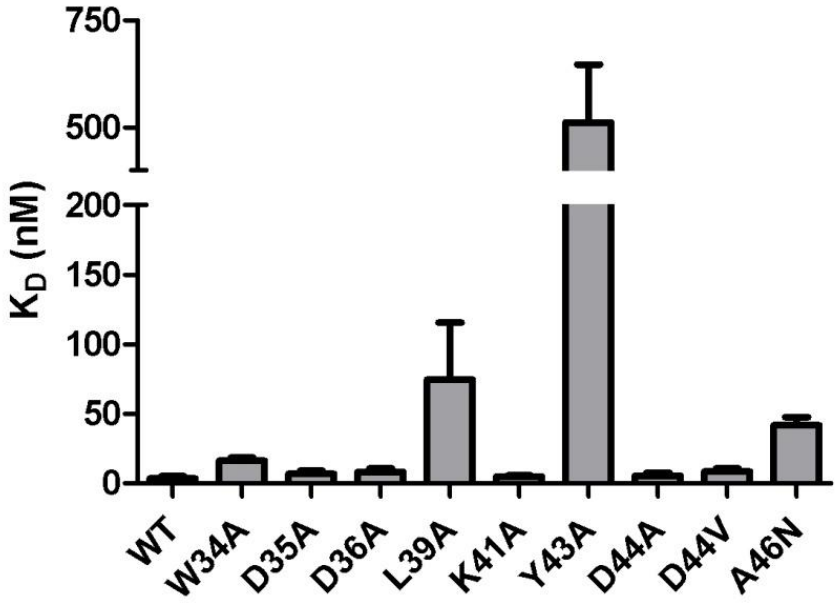


Figure 2.18 Binding of SMN$^{26-51}$ mutants to Gemin2$^{95-280}$. Using the fluorescence anisotropy assay described in Figure 2.3, each binding titration was repeated three times and the average dissociation constant is shown. Error bars represent one standard deviation from the mean.

Asp44, another highly conserved SMN residue, is the site of an SMA patient mutation in which valine is substituted at this position. An electrostatic interaction between Asp44 and Arg213 of Gemin2 seemed geometrically possible, but the likelihood of a significant energetic contribution from this interaction to binding was unclear. The mutants D44A and D44V were therefore probed for interaction with Gemin2. Both bound as well as wild-type SMN, suggesting that the functional defect in patients with the D44V mutation is unlikely to be an abrogation of SMN-Gemin2 binding.

Previous reports on the effect of the D44V SMN patient mutation on Gemin2 binding have been mixed; the original report of the mutation found no effect on binding, but later mammalian two-hybrid and GST pulldown studies of an SMN(exons1-5) construct

found D44V binding reduced compared to wildtype SMN (Ogawa *et al.*, 2007; Sun *et al.*, 2005; Zhang *et al.*, 2011).

Given the hydrophobic nature of the SMN-Gemin2 interface and the typical addition of detergents to reduce non-specific binding in buffers used in GST pull-down assays, it seemed possible that the discrepancy in results reported for the effect of D44V on Gemin2 binding might be the result of sensitivity to the presence of detergents, to temperature, or to both. To address this possibility, the same fluorescence anisotropy peptide binding experiments were repeated for SMN^{26-51} wildtype and D44V in the presence and absence of 0.05% Nonidet P40, both at 25°C and at 4°C.

Neither 4° conditions nor the presence of detergent at 25°C had any observable effect on wildtype SMN^{26-51} binding to Gemin2; a combination of the two effects, however, showed a mild reduction in binding affinity by about a factor of 25. Binding of SMN^{26-51} D44V to Gemin2 was also unaffected at at 4°C (K_D = 1.5±0.47 nM), but was reduced by a factor of ~500 at 25°C in the presence of detergent (K_D = 1.8±0.24 μM). When the two effects were combined, binding of D44V was nearly undetectable over the range of titrations, indicating a dissociation constant greater than 4 μM. The combined effects of low temperature and detergent observed for wild-type SMN^{26-51} are greatly amplified for the D44V mutant, and suggest that the abrogation of D44V binding reported in some studies may be a result seen only under the artefactual conditions of detergent and low

temperature and do not provide a realistic representation of the effect of the mutant on binding under physiological conditions. (Summarized in Table 2.2)

Table 2.2 SMN^{26-51}-$Gemin2^{95-280}$ dissociation constants

SMN (26-51)	Gemin2	Buffer[1]	Temperature (°C)	K_D (nM)[2]
Wild-type	12-280	PBS	25	7.0 ± 2.5
Wild-type	95-280	PBS	25	3.3 ± 1.8
Wild-type	95-280	PBS, 300 mM NaCl	25	5.2 ± 1.8
Wild-type	95-280	PBS, 600 mM NaCl	25	2.4 ± 1.5
Wild-type	95-280	PBS	4	2.3 ± 0.92
Wild-type	95-280	PBS + NP40	25	2.2 ± 1.7
Wild-type	95-280	PBS + NP40	4	180 ± 27
W34A	95-280	PBS	25	16 ± 2.2
D35A	95-280	PBS	25	6.8 ± 2.1
D36A	95-280	PBS	25	8.3 ± 2.5
L39A	95-280	PBS	25	74 ± 41
K41A	95-280	PBS	25	4.8 ± 1.0
Y43A	95-280	PBS	25	510 ± 130
D44A	95-280	PBS	25	5.3 ± 2.4
D44V	95-280	PBS	25	8.4 ± 2.4
D44V	95-280	PBS	4	1.5 ± 0.47
D44V	95-280	PBS + NP40	25	1800 ± 240
D44V	95-280	PBS + NP40	4	> 4000
A46N	95-280	PBS	25	42 ± 5.9
p28, p31	95-280	PBS	25	4.2 ± 1.3

[1]PBS = 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4, 2 mM DTT; NaCl concentration is modified where indicated. NP40 = Nonidet P40.
[2]The K_d values reported are based on 3-6 individual binding titrations. The K_D for SMN D44V in NP40-containing buffer at 4°C could not be fit, but a lower limit of 4 μM could be established.

The effect of ionic strength on the affinity of the SMN-Gemin2 interaction was also examined. Consistent with the hydrophobic nature of the interface, the binding affinity of SMN^{26-51} for $Gemin2^{95-280}$ in 300 mM or 600 mM NaCl (K_D = 5.2±1.8 nM and

2.4±1.5 nM, respectively) remains essentially unchanged from that observed in PBS buffer at 137 mM salt (K_D = 3.3±1.8 nM) (Table 2.2).

Discussion

Despite occupying the focal point of considerable research interest – due to the central role they play in spliceosomal U-snRNP biogenesis, but even more urgently to their vital urgent role in SMA pathogenesis – SMN and Gemin2 have eluded structural analysis at the atomic level for fifteen years after their discovery. Full-length SMN and Gemin2, expressed either bacterially or in Sf9 cells, proved largely intractable in solution: the former protein tends to aggregate; the latter expresses poorly, is minimally soluble and degrades readily (its tendency to bind all nitrocellulose membranes irreversibly also fails to recommend it). The coexpressed complex of the two full-length proteins elutes as a broad peak near the void volume of a Superdex 200 size exclusion chromatography column, suggesting that it forms a oligomer too large, and possibly too heterogeneous, for ready characterization.

The desire for an SMN-Gemin2 complex less refractory to all attempts at analysis stimulated the design of a minimal SMN-Gemin2 complex. The boundaries determined from bacterial two-hybrid experiments using SMN and Gemin2 truncations largely confirmed the locations of the binding domains reported in earlier studies. The minimal

binding domain of Gemin2, comprising residues 95-280, is closely consistent with results from a mammalian two-hybrid assay reported by Ogawa *et al* that pinpointed that domain as residues 101-280 (Ogawa *et al.*, 2007). This region includes nearly two-thirds of the polypeptide, suggesting a reason why a shorter functional Gemin2 construct has never been reported.

The 21-residue minimal Gemin2 binding domain of SMN, S31-K51, also corresponds broadly to previous reports: Liu *et al.* demonstrated that a peptide comprising SMN^{13-44} successfully competed with full-length SMN for Gemin2 binding, and Ogawa *et al.* positioned SMN's Gemin2 binding domain in exons 1-2b (residues 1-62) (Liu *et al.*, 1997; Otter *et al.*, 2007). By contrast, however, Young *et al.* (2000) used surface plasmon resonance experiments to identify exon 2b (residues 52-62) as the site of independent Gemin2 binding, a conclusion not supported by the results described in this dissertation (Young *et al.*, 2000).

The SMN^{26-51} and $Gemin2^{95-280}$ proteins both proved tractable to heterologous expression in *E. coli*; the protein heterodimer they formed could be purified to homogeneity and was amenable to biochemical and biophysical characterization. Although disappointingly refractory to the formation of diffraction-quality (or, indeed, any) protein crystals, a ^{1}H-^{15}N HSQC of the SMN^{26-51}- $Gemin2^{95-280}$ complex showed peak dispersion and sharp linewidths, the hallmarks of a folded protein potentially amenable to structure determination by NMR spectroscopy (Figure 2.8). That possibility

proved predictive in this case, and heteronuclear mulit-dimensional NMR was employed to solve the atomic-level structure of the SMN^{26-51}-$Gemin2^{95-280}$ heterodimer.

The NMR structure of SMN^{26-51}-$Gemin2^{95-280}$ reported in this work marks a substantial advance over the preceding fifteen years' research, representing the first view of the structure of Gemin2 and its binding interaction with the Gemin2-binding domain of SMN. Forming a helical bundle comprising seven α-helices, Gemin2 interacts with the single α-helix of SMN^{26-51} in an extended hydrophobic cavity formed by four helices, α1, α3, α5 and α7, far separated in primary sequence. The SMN helix displays a markedly amphipathic character, with a solvent-exposed polar face and a buried interfacial hydrophobic face (Figure 2.16).

Both $Gemin2^{95-280}$ in complex with SMN^{26-51} and taken alone appear to bear no significant structural homology to any other know protein structures. Using the lowest-energy member of the NMR ensemble as a representative structure, a search of the Dali structural database yielded no strong matches with or without the inclusion of the SMN peptide. The highest-scoring hits boasted fairly significant r.m.s. deviations (>3Å) and typically pinpointed the four-helix bundle comprising helices α4- α7, a motif encountered in a variety of helical protein folds, as the center of structural conservation. The spatial arrangement of helices in the structure of Gemin2 bound to SMN, then, appears to comprise a novel protein fold. Although the discovery of structural novelty in the study of heretofore uncharacterized Gemin2 failed to suggest immediately a

functional role for the protein as analogy to an obvious structural homologue might have done, the availability of a structural model for SMN-Gemin2 serves as a platform for design of the next generation of experiments, logical structure-function studies to probe the role of Gemin2 in U-snRNP assembly and in other reported protein-protein interactions.

Although the prediction of protein constructs that will crystallize resembles an art more than a science, certain characteristics of the SMN^{26-51}-$Gemin2^{95-280}$ heterodimer that became apparent from the NMR structure likely prejudiced early crystallization attempts. While most of the structure is fairly well-defined, it also contains two significant regions of disorder. A large, poorly conserved loop in Gemin2 (residues 150-175, a region entirely absent as recently evolutionarily as *Drosophila*) is entirely disordered, with no long-range NOEs observed. This region of the protein is highly mobile, as indicated from ^{15}N relaxation experiments performed and analyzed on SMN-Gemin2 by the Wand laboratory at the University of Pennsylvania, which yielded indicative long ^{15}N T_2 relaxation time constants for that region (Farrow *et al.,* 1994; Lipari & Szabo, 1982) (Figure 2.15)

.

In addition, the first seven residues in the SMN peptide (residues 26-32) are disordered, which is consistent with their lack of importance for Gemin2-binding as indicated by our interaction assays. An overall low order parameter O2 (0.6-0.8) was also found for

backbone amides in areas of helical structure, indicating a degree of backbone mobility that may have hampered crystallization (Figure 2.19).

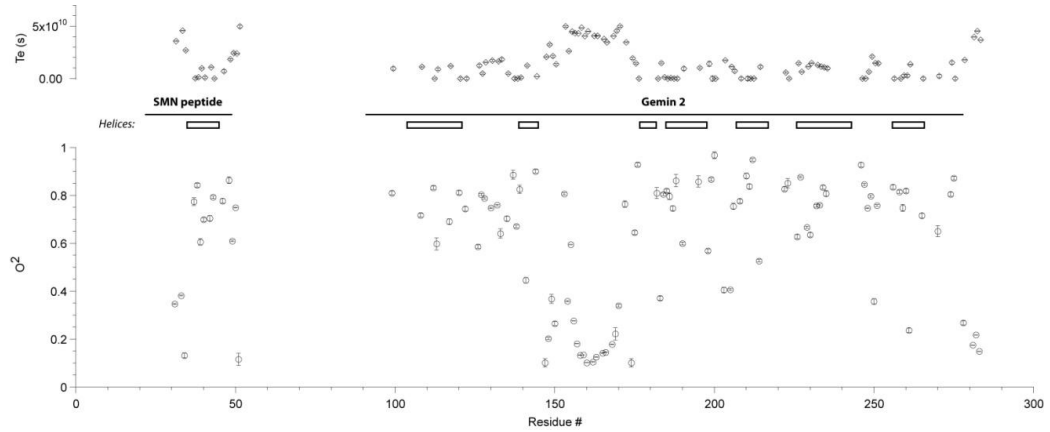


Figure 2.19 Backbone order parameters (O_2) of Gemin2 and the bound SMN peptide. Obtained by Veronica Moorman in the Wand laboratory at the University of Pennsylvania from T_1 and T_2 relaxation times at 750 MHz and 600 MHz (^{1}H). Helices are depicted above the graph as rectangles for reference. Order parameters near one imply rigidity; those near zero indicate a wide range of possible motion.

The importance of key conserved SMN residues at the hydrophobic interface with Gemin2 was confirmed in mutagenesis binding studies. Eleven SMN residues on the hydrophobic face of the amphipathic SMN helix mediate the interaction with Gemin2 in the cavity formed by the four α-helices α1, α3, α5 and α7; Leu39, Tyr43 and Ala46 exhibit strong evolutionary conservation and are essential for high affinity SMN-Gemin2 binding. The highly conserved Asp36 occupies a flanking position N-terminal to the beginning of the SMN helix and is possibly positioned to contribute a polar interaction with His120 or His124; its alanine mutation shows a mild effect on Gemin2 binding.

The position of helix α1 relative to SMN explains the observation made during delineation of domain boundaries that additional N-terminal deletions of Gemin2 beyond Gly95 to Phe110 still resulted in an SMN-Gemin2 interaction. Helix α1, comprising residues 100-124, runs roughly antiparallel to the SMN helix and contributes hydrophobic interactions with SMN along its length; it is apparent that the minor N-terminal truncations, including the loss of the contributions of Q106 and Q109 to the hydrophobic protein-peptide interface, still results in a detectable interaction between the two components, but that the cumulative loss of those residues plus the more centrally positioned and arguably more significant V113, V117 and H120 proves inimical to sustaining the SMN-Gemin2 interaction.

A number of conserved residues in the Gemin2-binding domain of SMN, however, serve no apparent role in Gemin2 binding. Conspicuous among them, Trp34 and Asp35 are invariantly conserved through evolutionary time, and Lys41 and Asp44 are invariant from humans to worms. No NOEs providing distance restraints were observed between the protons in these atoms and Gemin2, nor did alanine substitutions at any of their positions have an effect on the affinity of the SMN-Gemin2 interaction. These residues may form part of another binding surface or surfaces for other components of the SMN complex or its substrates. In considering other possible roles, the degree of evolutionary conservation evinced may provide certain clues. Trp34 and Asp35, for example, are conserved from mammals to *S. pombe*, an organism whose functional SMN complex consists only of SMN and Gemin2; those residues are therefore unlikely to play a role in

recruiting other Gemin components to the complex but may instead serve as part of an interaction platform for Sm protein substrates or for SMN or Gemin2 as part of the formation of higher-order oligomers.

Of the highly conserved SMN residues with no apparent role in binding, Asp44 has been singled out for examination as the site of a Type III (mild; adult onset) SMA missense mutation, in which valine is substituted as its position. Conclusions from a number of reports have been mixed with respect to the functional defect introduced by the D44V substitution. The original report of the mutation indicated no effect on Gemin2 binding based on *in vitro* GST pulldowns; the fluorescence anisotropy peptide binding studies employed in this work mirror that conclusion (Sun *et al.*, 2005). Ogawa *et al.*, however, reported that the interaction between Gemin2 and a truncated SMN (exons1-5) D44V construct was diminished with respect to wildtype binding in both GST pulldown experiments and mammalian two-hybrid assays (Sun *et al.*, 2005)(Sun et al., 2005){Sun2005}(Ogawa *et al.*, 2007). More recently, Zhang *et al.* also used *in vitro* pulldown assays of SMN produced by *in vitro* translation to demonstrate elimination of SMN D44V binding to Gemin2, a result considered attributable to disruption of a salt bridge formed at the SMN-Gemin2 interface between Asp44 and Gemin2 Arg213 (Zhang *et al.*, 2011).

The flat discrepancy between one set of conclusions recounting no effect of D44V on Gemin2 binding, including the peptide binding results reported here, and another

reporting an abrogation of that same interaction bore careful consideration. One pronounced difference between experiments relates to the conditions under which they were performed: in both studies reporting diminution of D44V binding to Gemin2 as the result of *in vitro* pulldowns, the binding experiments were performed at 4°C in the presence of non-denaturing detergent, standard experimental procedures used to minimize non-specific interactions and promote the stability of proteins. Given the categorically hydrophobic nature of the SMN-Gemin2 interface, it seemed possible that temperature and detergent might have an effect on the affinity of the interaction.

The same fluorescence anisotropy SMN peptide binding assays used to quantitate the affinity of wildtype and D44V SMN^{26-51} for $Gemin2^{95-280}$ in physiological buffer at room temperature were repeated at lower temperature, in the presence of detergents and under a combination of the two conditions, with startling results. Wildtype SMN^{26-51} binding to Gemin2 remains unaffected at 4°C and in 0.05% Nonidet P-40 at 25°C, but is reduced by a factor of ~25 when both conditions are present. The SMN D44V mutation also bound with wildtype affinity at 4°C, but was strikingly more sensitive to detergent: at 25°C with detergent present, Gemin2 binding was reduced by a factor of ~500, and at 4°C was so abrogated that no binding activity was observed over the range of Gemin2 concentrations titrated. It seems clear that the SMN D44V patient mutation only disturbs Gemin2 binding under the conditions of low temperature and high detergent concentration present in many standard *in vitro* binding assays. As neither condition seems likely to be operative *in vivo*, it seems a likely conclusion that the functional

deficiency introduced by the substitution of a valine at the Asp44 position in SMN is not a disruption of the SMN-Gemin2 binding interface.

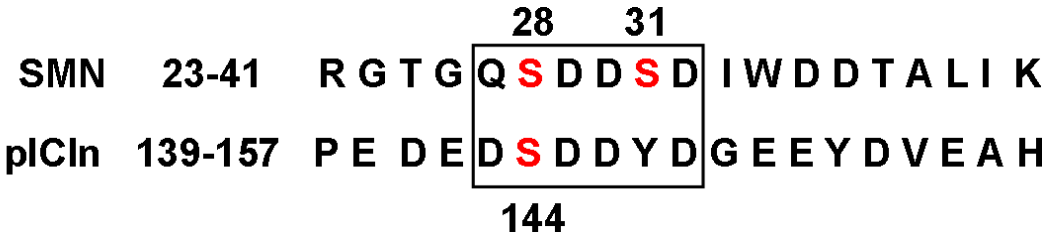
The possibility of a role for Asp44 in promoting higher-order SMN complex interactions cannot be ruled out, however. SMN is known to form higher order oligomers, an effect primarily attributed to its tyrosine-glycine rich exon6/exon7 "YG box" oligomerization domain (Lorson *et al.,* 1998; Pellizzoni *et al.,* 1999). Young *et al.* first proposed the possibility of an additional N-terminal exon 2 SMN self-interaction based on surface plasmon resonance binding experiments, and suggested an interaction of that region with SMN exon 4 (Young *et al.*, 2000). More trenchantly, Ogawa *et al.* (2007) also observed using mammalian two-hybrid assays the presence of an N-terminal SMN self-interaction that they reported required Gemin2 for stability and was significantly weakened in the SMN D44V mutant (Ogawa *et al.*, 2007). An *in vitro* dissociation assay showed a substantial impairment of SMN self-association in SMN D44V compared to wildtype; they also found SMN D44V mutants deficient in snRNP assembly in *in vitro* assembly assays. The authors conclude that an impairment in N-terminal SMN self-association underpins SMN in D44V patients.

SMN has also been shown to be a target for *in vivo* cleavage by calpain at a site that is located in exon4, in the C-terminal half of the protein. The functional significance of this observation is still unclear, but it is speculated to pertain to a specific role of SMN in the sarcomeric Z-discs of skeletal and cardiac muscle. The SMN D44V mutant

drastically decreases the efficiency of calpain cleavage, however, and this effect is dependent on SMN oligomerization (Walker *et al.*, 2008). Asp44, as well as other conserved residues in the Gemin2-binding region of SMN that play no role in binding, may be involved in higher order interactions within the oligomeric SMN complex. As mentioned above, the idea that the region encoded by exon 4 may interact with the Gemin2-binding domain has already been proposed by Young *et al*. The possibility that an N-terminal association of SMN plays a functional role and can be interrupted in the case of D44V seems an avenue of inquiry worthy of pursuit.

Another SMA patient missense mutation (Type II; intermediate) localizes to the region of SMN contained within residues 26-51 (Sun *et al.*, 2005), where an asparagine residue substitutes for Asp30. Unlike its D44V neighbor, however, the functional defect introduced by this patient mutation remains unclear. No binding assay to date has recorded any effect of the mutant on Gemin2 binding (Ogawa *et al.*, 2007; Sun *et al.*, 2005). Given that the NMR structure of the SMN-Gemin2 heterodimer indicates that the residue lies within an unstructured region of the SMN peptide, this result is unsurprising. Neither, however, did Ogawa *et al.* observe any effect of the mutation on SMN N-terminal self-interaction or SMN oligomerization stability in a dissociation assay. When introduced into an *in vitro* snRNP assembly assay, however, the D30N mutation exhibited an impairment of snRNP assembly at a level similar to that exhibited by D44V (Ogawa *et al.*, 2007).

The region of SMN contained in the peptide used for these structural studies also contains two known phosphorylation sites at Ser28 and Ser31 (Grimmler *et al.*, 2005b). Consistent with the observation from the NMR structure that both residues reside in the unstructured N-terminal portion of the peptide and play no obvious role in Gemin2 binding, fluorescence anisotropy binding assays comparing the avidity of the interaction of dephosphorylated and (pSer28,pSer31) SMN peptides for Gemin2 showed no difference. The phosphorylation motif containing the two serines was unknown before its discovery in this context and is shared only with a phosphorylation site in pICln, the molecular chaperone charged with delivering Sm proteins to the SMN complex for snRNP biogenesis. In this motif, relatively conserved in SMN, the first serine is proceeded by a polar residue and is succeeded by two aspartic acids and then, after a residue variously filled by another phosphoserine or a tyrosine, a third aspartic acid.



The D30N mutation occurs at the second aspartic acid position and may involve a disruption of the phosphorylation motif and a concomitant decrease in phosphorylated SMN and efficiency of snRNP assembly. Although the mechanism by which phosphorylation of Ser28 and Ser31 affects the catalytic activity of the SMN complex is unclear, a disturbance in its demonstrated role in snRNP assembly may explain the pathogenesis introduced by D30N.

Of the highly conserved residues in Gemin2, many play obvious roles in inter-helical packing and the formation of the SMN binding pocket. A number of others, however, are located on the protein's surface without close proximity to SMN and perhaps forming other functional interaction surfaces (Figure 2.20). These platforms for protein-protein interaction stand to play important roles in the structural organization of the SMN complex or in the molecular mechanics of snRNP assembly. Self-association has been imputed to the region of Gemin2 comprising the construct used in these studies (Ogawa *et al.*, 2007). It is also highly probable that Gemin2 interacts with one or several of the substrates of snRNP biogenesis. Chari *et al.* illustrated the composition of two snRNP assembly intermediates involving Gemin2 and SMN (Chari *et al.*, 2008). In the first, Gemin2 and SMN interact with a complex containing Sm proteins D1, D2, E, F, and G and the chaperone pICln. In the second, pICln dissociates, leaving a complex comprising SMN, Gemin2, and the D1/D2/E/F/G pentamer; the Sm D3/B dimer will later complete the heptameric Sm ring. The precise location of Gemin2 relative to the Sm proteins is unclear in the low-resolution electron microscopy images provided in the Chari *et al*. study; from that data, it seemed likely that conserved surface residues of Gemin2 might interact with one or several of them.

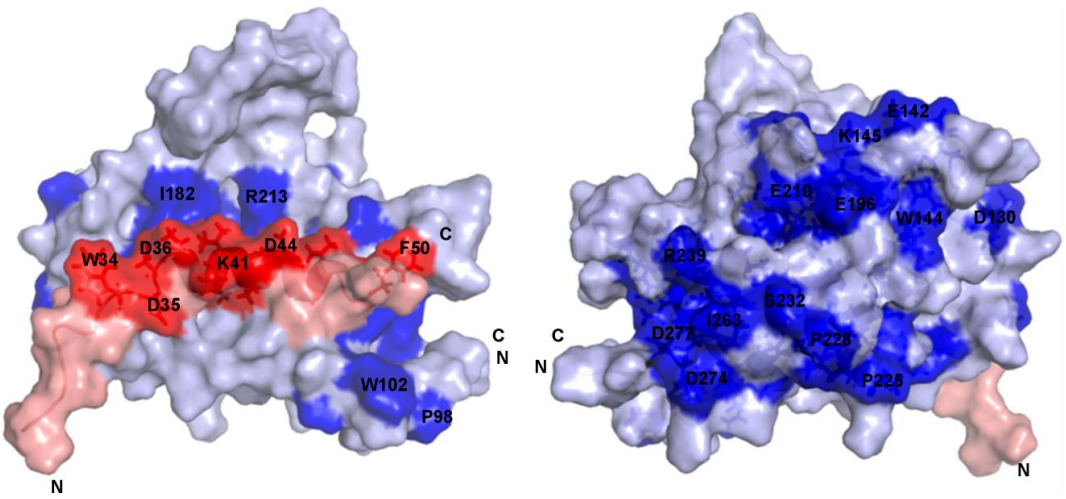


Figure 2.20 Conserved surfaces in the SMN-Gemin2 complex. A) View of the SMN-Gemin2 surface in the same orientation as that shown in Figure 2.9. B) View of the opposite side of the complex. Conserved residues (boldface type in Figure 2.12) are drawn in red for SMN and dark blue for Gemin2. Other residues are in pale red for SMN and pale blue for Gemin2. Selected conserved residues are labeled.

Indeed, during the preparation of this written dissertation, a 2.5Å crystal structure was published of the second snRNP assembly intermediate described above, consisting of Gemin2 bound to the Gemin2-binding domain of SMN and to the Sm D1/D2/E/F/G pentamer. As expected, a series of Gemin2-Sm protein interactions does appear in this structure; Gemin2 clearly coordinates direct interactions with several Sm proteins. Sm D1 and D2 appear to interact directly with the domain that corresponds to Gemin2$^{95\text{-}280}$ on the face opposite SMN binding, while E/F/G binds its extended N-terminal arm (Zhang *et al.*, 2011).

A superposition, based on backbone atoms in the eight α-helices of the NMR and crystal structures of Gemin2$^{95\text{-}280}$, illustrates regions of both significant similarity and noticeable dissimilarity (Figure 2.21A,B). Interestingly, the face of the protein that

appears to comprise the Sm D1/D2 binding surface shows the least difference between the two structures, with only slight orientational differences in the surface accessible regions of helices α4 and α7, and near perfect overlap of α5 and α6. This suggests that Gemin2 may undergo little structural change upon Sm protein binding, as the binding surface for the two Sm proteins appears to adopt the same configuration in the solution structure of the apo protein.

Other regions of the structures, however, evinced comparative differences that may result from crystal packing artifacts, the misinterpretion of crystallographic data or simple dissimilarity between the crystal and solution structures (Figure 2.21B). The latter is certainly a reasonable possibility given the comparatively low backbone amide order parameters evinced by the SMN-Gemin2 heterodimer in this study, indicating an unusual degree of backbone mobility. The position of the short α2 helix flanking the large unstructured G150-G175 loop is substantially translated between the two structures, and the crystal structure's α2 helix falls ~120 º out of register based on its violation of a large number of observed long-range NOEs. This helix forms lies at the surface of the protein and contains several conserved residues whose surface accessibility varies drastically between the two models, complicating attempts to assess their relative importance in helix-helix packing versus forming a solvent-accessible binding surface. The α3 and α7 helices also suffer a displacement in the crystal structure relative to their positions in the solution structure, which complicates interpretation of the Gemin2 SMN binding pocket. Indeed, Zhang *et al.* fail to mention the important

contribution either the α3 or the α7 helix makes to the SMN-Gemin2 interface (Zhang *et al.*, 2011). Some discrepancy is also visible between the two structures in the conformation of the α1 helix, which adopts a more bent, less ideal form in the NMR structure in response to RDC orientational restraints and to NOEs between its residues and those of SMN and helix α3. A systematic consideration of the differences between the crystal and NMR structures of SMN-Gemin2 will prove important in defining the structural model that best represents the two proteins and from which basis future experiments, especially structure-function studies probing the role of the complex in Sm protein binding and snRNP assembly, can most accurately be designed.

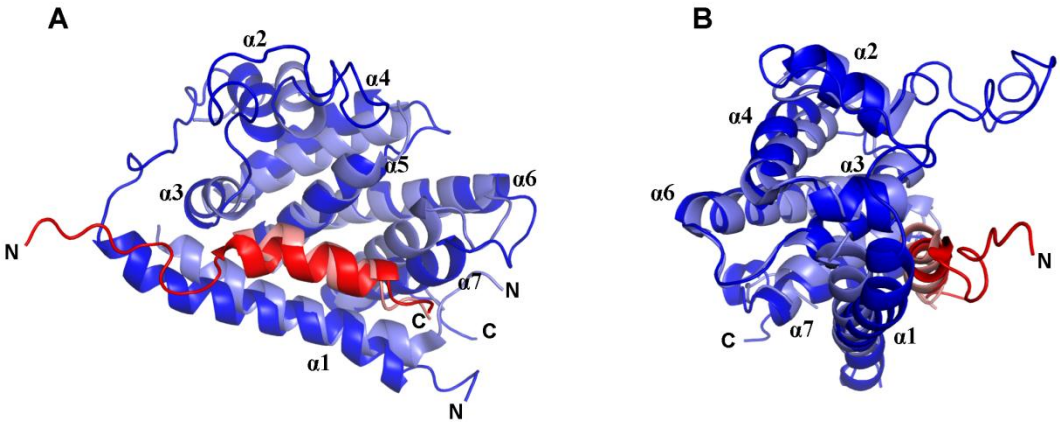


Figure 2.21 Comparison of the the NMR solution structure and x-ray crystal structure of SMN$^{26-51}$-Gemin2$^{95-280}$. A) Overlay of the NMR and x-ray crystal structures of SMN$^{26-51}$-Gemin2$^{95-280}$. SMN$^{26-51}$ is depicted in dark red for the NMR structure and in pink for the x-ray structure. The NMR structure of Gemin2$^{95-280}$ is in blue and the x-ray structure in light blue. B) A 90° rotation of the view in the first panel.

Gemin2 is also the established point of interaction at least two other Gemin components of the SMN complex. A direct interaction between Gemin2 and Gemin5, the snRNA chaperone, was established through co-immunoprecipitation and yeast two-hybrid assays and likely explains the early finding that Gemin2 mediates snRNA binding; the binding interface between Gemin2 and Gemin5 has yet to be defined (Lorson & Androphy, 1998; Otter *et al.*, 2007). Gemin2 has also been shown to bind Gemin7, perhaps in the context of the Gemin7-Gemin6 heterodimer that is thought to serve as a surrogate for the SmD3/B dimer in the nascent Sm D1/D2/E/F/G pentamer (Ma *et al.*, 2005). The Gemin2 Gemin7-binding domain appears to comprise residues 56-280, with a significant decrease in binding occurring after truncation to residues 101-280; this strongly implies that at least part of the binding surface crucial to Gemin2's interaction with Gemin7 lies along its N-terminal Sm protein-binding arm. The presence of SMN strongly enhances the stability of the Gemin2-Gemin7 interaction, suggesting that that interaction occurs in the context of the SMN-bound Gemin2 fold observed in the NMR and crystal structures (Ogawa *et al.*, 2009).

Apart from its well-documented role in snRNP assembly, Gemin2 has also been implicated in other biological processes, including HIV infection and homologous recombination (Hamamoto *et al.*, 2006; Takizawa *et al.*, 2010). In HIV-infected cells, following the reverse transcription of the viral genome by reverse transcriptase, HIV-1 integrase (HIV-1 IN) integrates the newly made cDNA copy into the host chromosome. HIV-1 IN is also reported to influence reverse transcription and thereby stimulate viral

cDNA synthesis, possibly by facilitating the loading of reverse transriptase onto viral RNA. This activity appears to be influenced by a direct interaction of Gemin2 with HIV-1 IN; interruption of the Gemin2-IN interaction, either by introducing mutations in HIV-1 IN, by competing for integrase binding with a peptide corresponding to Gemin2's IN binding domain or by siRNA depletion of Gemin2 abrogates reverse transcription activity. The possibility of SMN involvement in this process remains unclear, although other Gemins appear to be uninvolved (Hamamoto *et al.,* 2006; Nishitsuji *et al.*, 2009). The authors identify residues 137-238 of Gemin2 as its integrase binding domain, which, assuming a fold in this context similar to that assumed by the $SMN^{26-51}Gemin2^{95-280}$ complex, allows a partial visualization of the integrase binding surface (Figure 2.22). The integrase binding domain comprises Gemin2 helices α2, α3, α4, α5 and a portion of α6, as well as the large unstructured loop containing residues 150-175. If Gemin2 adopts the same fold in the context of HIV-1 IN binding that it does when binding SMN, this suggests that HIV-1 integrase, like the Sm D1-D2 dimer, interacts with Gemin2 on the face of the protein opposite its SMN binding site.

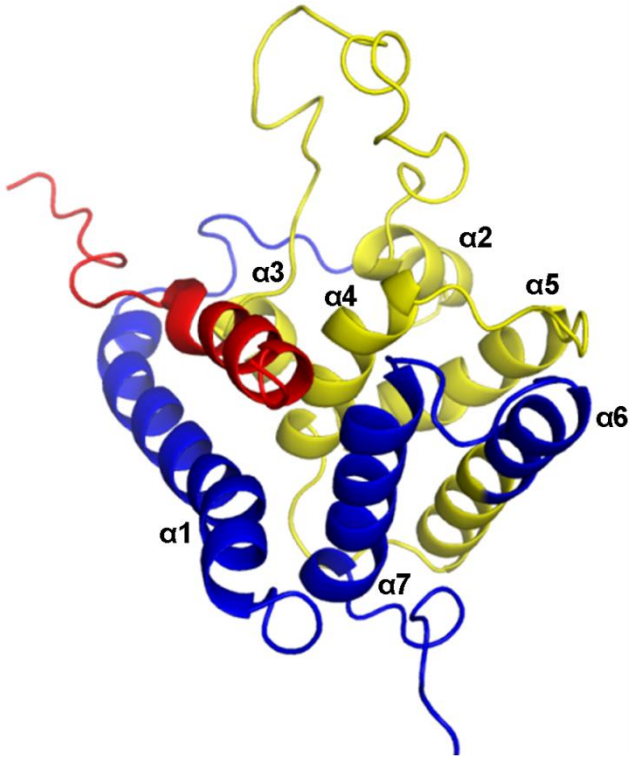


Figure 2.22 An illustration of the Gemin2 residues comprising the HIV-1 integrase binding domain. SMN is depicted in red, the INBD in yellow and Gemin2 residues not involved in HIV-1 integrase binding in blue. Gemin2 helices are labeled.

Another body of research has reported an interaction between Gemin2 and Rad51 (Takaku *et al.*, 2011; Takizawa *et al.*, 2010). Rad51, a eukaryotic recombinase, catalyzes the homologous pairing and strand exchanges reactions that make up part of the homologous recombination repair of double-stranded breaks in DNA. Gemin2 was demonstrated to enhance Rad51 activity in homologous pairing both *in vitro* and *in vivo.* The same results, as well as the appearance of DNA-binding activity and a significant increase in secondary duplex DNA capture by the RAD51-single-stranded DNA complex during homologous pairing, were reported for a fusion protein comprising full-length SMN joined at its C-terminus to Gemin2 at its N-terminus; the physiological

relevance of such a chimera has yet to be ascertained. The mode of Rad51 binding to Gemin2 in this case and the mechanics of its function in homologous recombination are uncertain, but it should now be straightforward to identify the regions of interest using the structural framework for Gemin2 reported in this work.

The SMN-Gemin2 structure reported here represents the achievement of a goal toward which effort has been applied for more than a decade. The protein fold of Gemin2 demonstrated obvious novelty, which failed to suggest function through structural homology but which established a vital framework for designing the next generation of structure-function experiments to probe the functional role of SMN-Gemin2 in snRNP assembly and SMA pathogenesis, as well as other biological roles for Gemin2.

Materials and Methods

DNA constructs

The DNA constructs employed in the experiments described in this chapter are listed in Table 2.3.

Table 2.3 Human SMN and Gemin2 expression constructs used in this study

expression construct	mutation	expression vector	ID#
SMN 26-51	WT	MxeDuet/-LEY**	1449
	W34A	MxeDuet/-LEY**	2256
	D35A	MxeDuet/-LEY**	2257
	D36A	MxeDuet/-LEY**	2258
	L39A	MxeDuet/-LEY**	2417
	K41A	MxeDuet/-LEY**	2259
	Y43A	MxeDuet/-LEY**	2418
	D44A	MxeDuet/-LEY**	2260
	D44V	MxeDuet/-LEY**	2261
	A46N	MxeDuet/-LEY**	2419
Gemin2 95-280	WT	MxeDuet*	987
	C154S/C221S/C264S	MxeDuet*	1525
Gemin2 12-280	WT	MxeDuet*	371

* MxeDuet denotes a modified pETDuet vector in which in-frame Mxe intein, chitin binding domain and hexahistidine tags have been added C-terminal to MCS II. Cloning into XhoI leaves a C-terminal L-E-Y

** MxeDuet/-LEY denotes an MxeDuet vector in which the C-terminal L-E-Y cloning tag has been removed by inverse PCR mutagenesis.

Human Gemin2$^{12\text{-}280}$ and Gemin2$^{95\text{-}280}$ were amplified by PCR and cloned into the NdeI and XhoI sites of pet21a (EMD Biosciences) and subsequently subcloned as an

NdeI/XhoI fragment into MCS II of a pETDuet (Novagen) expression vector modified to contain an in-frame C-terminal Mxe intein domain, chitin binding domain (New England Biosystems) and hexahistidine affinity tag. For this and all experiments described, full-length Gemin2 refers to the Gemin2 construct comprising residues 12-280.

Human SMN^{26-51} was originally amplified by PCR and cloned into MCS I of a petDUET expression vector; it was subsequently subcloned as an NdeI/XhoI fragment into MCS II of the same modified Mxe intein-chiting binding domain-His6x pETDuet vector described above. Cloning into the XhoI site of MCSII of this vector results in the inclusion of residues Leu-Glu-Tyr at the C-terminus of the protein just prior to the intein's thiol-reactive catalytic cysteine; all Gemin2 constructs described here retain the C-terminal LEY, while for the SMN^{26-51} peptide, inverse PCR mutagenesis was used to delete those residues. SMN K51 occupies the amino acid position immediately preceding the catalytic cysteine, rather than a well-tolerated tyrosine, but appears to have no effect on the efficiency of catalytic cleavage of the Mxe intein-CBD-His6x affinity tag from the SMN peptide.

Point mutations in SMN^{26-51} were generated by inverse PCR mutagenesis (New England BioLabs). To create the $Gemin2^{95-280}$ (C154S/C221S/C264S) mutant, the expression plasmid encoding wildtype $Gemin2^{95-280}$ in frame with C-terminal Mxe intein, chitin

binding domain and hexahistidine tags was used as a template for multiple simultaneous site-directed mutagenesis (Seyfang & Jin, 2004).

Bacterial Two-Hybrid Screens.

A modified version of the lexA-based bacterial two-hybrid assay based on the methods of Dmitrova *et al.* was used to test the interaction of Gemin2 and SMN truncations (Dmitrova *et al.*, 1998). The bait vector consisted of wildtype LexA (1-87) cloned upstream of the MCS in a modified pETDuet in which the T7 promoter has been replaced by a lac promoter and in which the standard MCS II has been deleted. The target vector contained the lexA408 (1-87) mutant in a similarly modified pCDFDuet vector. Human SMN and Gemin2 truncations (Table 2.2) were PCR amplified from existing expression constructs and cloned into BamHI/HindIII sites of the target and bait vectors. Both target and bait vectors were co-transformed into a reporter strain based on CSH142 in which the reporter construct was present on an F'-episome (Whipple, 1998). Positive transformants, as identified by growth on Luria-Bertani (LB) media containing ampicillin, kanamycin and streptomycin, were selected and transferred to either of two reporter media: LB/Amp/Kan/Strep containing X-gal and IPTG or MacConkey Base/Amp/Kan/Strep containing 1% lactose and IPTG and incubated overnight at 37°C. A positive interaction resulted in repression of *lacZ* transcription and, in the first case, white colonies on plates containing X-gal and in the second, white-pink colonies on MacConkey agar. A schematic of the bacterial two-hybrid system is depicted in Figure 2.18.

Expression and purification of human Gemin2 and SMN protein constructs

DNA expression constructs were co-transformed into *E. coli* BL21(DE3) cells; a culture grown from one positive transformant, identified by growth on Luria-Bertani media containing ampicillin and streptomycin, was used to create a 10% glycerol stock stored at -80°C. SMN^{26-51} and either $Gemin2^{12-280}$ or $Gemin2^{95-280}$ were expressed separately in Luria-Bertain (LB) cell cultures grown at 15°C for 20 hours after the addition of IPTG (isopropyl-β-D-thio-galactoside) to a final concentration of 0.65 mM.

For ^{15}N and $^{15}N/^{13}C$-enriched samples for NMR analysis, cultures grown in MOPS minimal medium with $^{15}NH_4Cl$ as the main nitrogen source and U-[$^{12}C/^{1}H$] or U-[$^{13}C/^{1}H$] glucose (Cambridge Isotope Laboratories) as the main carbon source were induced with IPTG and incubated at 15°C for 24 hours. For leucine-selective ^{15}N labeling, in which Gemin2 was uniformly enriched with ^{15}N-leucine, the Neidhardt *et al.* protocol for amino acid-supplemented MOPS was followed, substituting ^{15}N leucine (Cambridge Isotope Laboratories) for ^{14}N leucine (Neidhardt *et al.*, 1974). To create a fractionally ^{13}C labeled SMN-Gemin2 for stereo-specific methyl assignments, a mixture of 10% U-[$^{13}C/^{1}H$] glucose and 90% U-[$^{12}C/^{1}H$] glucose added to the MOPS minimal medium provided the main carbon source.

Cells were harvested by centrifugation at 4000 x g for 30 minutes at 4°C. Each liter of cells was resuspended in approximately 20ml of 50mM Na/K phosphate, pH 8.0,

300mM NaCl, 10mM imidazole, 0.5mM ethylenediaminetetraacetic acide (EDTA), 0.5mM 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF; Fisher Scientific), 10μM proteinase inhibitor E-64 (Sigma) and 2mM benzamidine (Fisher Scientific). Cells were lysed in two sequential passes through an Avestin Emulsiflex C-3 homogenizer at approximately 18,000 psi, and the crude lysate thereby obtained was cleared by centrifugation at 30590 x g at 4°C for 30 minutes.

Cleared lysate was filtered through grade 802 fluted filter paper (Whatman) and loaded onto 4ml (per 6 liters of cell culture) nickel nitriloacetic acid (Ni-NTA; Qiagen) in a 5.3 cm x 10cm Kontes column equilibrated with 50mM Na/K phosphate pH 8.0, 300mM NaCl, 10mM imidazole. The preparation was nutated at room temperature for 60 minutes; the resin and bound protein were then washed with ten column volumes of buffer containing 50mM Na/K phosphate, 300mM NaCl, 20mM imidazole and eluted in 50mM Na/K phosphate, 300mM NaCl, 250mM imidazole. The Ni-NTA eluant was loaded onto chitin resin equilibrated in a 2.5 cm x 10 cm Kontes column with 50mM Na/K phosphate, 300mM NaCl, 250mM imidazole and nutated for 90 minutes at room temperature. The resin and bound protein were washed with ten column volumes of 20mM Hepes pH 8.0, 300mM NaCl, 0.1mM EDTA.

Human Gemin2 and SMN^{26-51} proteins were cleaved from the C-terminal Mxe intein-chitin binding domain-hexahistidine tag by addition of 20mM Hepes pH 8.0, 300mM NaCl, 0.1mM EDTA, plus 50mM 2-mercaptoethanol (βME) acting as the cleavage-

inducing thiol reagent, to the protein-bound chitin resin followed by overnight (~16 hours) incubation at room temperature (18°C – 22°C). The preparation was then added to 4ml of fresh chitin resin equilibrated with 20mM Hepes pH 8.0, 300mM NaCl, 0.1mM EDTA, and the cleaved protein complex was collected in the flow-through.

Human Gemin2 and SMN^{26-51} proteins were then diluted three-fold in 20mM Tris pH 8.0, 10mM βME and were immediately loaded on a MonoQ HR 10/10 column (Pharmacia) equilibrated in 20mM Tris pH 8.0, 50mM NaCl, 2mM βME for purification by anion exchange chromatography. Proteins were eluted in a linear gradient of 50-450mM NaCl over 7 column volumes at 4ml/min. Gemin2 preparations eluted in a single peak well-separated from excess cleaved affinity tag; SMN^{26-51} and its cleaved affinity tag co-eluted.

When necessary, purified $Gemin2^{12-280}$ was stored after this purification step at -80°C after the addition of 40% glycerol. Final protein concentration was assessed using the absorbance at 280 nm (A_{280}), assuming an extinction coefficient of 42440 M^{-1} cm^{-1} based on amino acid compositional analysis by the ProtParam software tool on the ExPASy Proteomics Server (Swiss Institute of Bioinformatics) (Gasteiger *et al.*, 2003). $Gemin2^{12-280}$ was stored at concentrations ≤ 200 μM. This preparation typically yielded 3mg Gemin2 L^{-1} LB media.

SMN^{26-51} peak fractions collected after purification on MonoQ resin were then concentrated at 5000 x g at 4°C in 15ml Amicon Ultra 10,000 MWCO Centrifugal Filters (Millipore) to one milliliter for further purification by size exclusion chromatography at room temperature on a Superdex 75 HiLoad 16/60 column (Pharmacia) in 10mM Tris pH 8.0, 50mM NaCl, 2mM dithiothreitol (DTT) for biophysical studies, in 0.1M sodium bicarbonate, pH 9.0 for labeling with fluorescein-5-isothiocyanate (FITC) for binding studies or in 50mM Na/K phosphate, 50mM NaCl, 2mM DTT for NMR studies. Excess Mxe affinity tag was easily separated from SMN^{26-51}. $Gemin2^{95-280}$ intended for characterization as a single protein species rather than in complex with SMN^{26-51} was similarly purified by size exclusion chromatography after MonoQ elution. To form the human SMN^{26-51}-Gemin2 complexes, however, SMN^{26-51} purified by size exclusion chromatography was combined in molar excess with MonoQ-purified $Gemin2^{95-280}$ or $Gemin2^{12-280}$, and the resulting complex purified in a second step on Superdex-75.

When necessary, homogeneous purified SMN^{26-51}, $Gemin2^{95-280}$ or their complex was stored in its sizing buffer plus 10% glycerol at -80°C. Final protein concentration was assessed using the absorbance at 280 nm (A_{280}), assuming extinction coefficients of 36690 M^{-1} cm^{-1} (human $Gemin2^{95-280}$) and 6990 M^{-1} cm^{-1} (human SMN^{26-51}) based on amino acid compositional analysis by the ProtParam software tool on the ExPASy Proteomics Server (Swiss Institute of Bioinformatics) (Gasteiger *et al.*, 2003). SMN^{26-51} proved highly soluble and was stored at concentrations ≥ 1mM. $Gemin2^{95-280}$ was stored

at concentrations ≤ 200 μM. These preparations typically yielded 3mg Gemin2$^{95-280}$ and 1mg SMN$^{26-51}$ L^{-1} LB media, and 1.5mg Gemin2 and 0.5mg SMN$^{26-51}$ L^{-1} in MOPS minimal media.

Purification of synthetic SMN$^{28-51}$ and SMN$^{28-51}$(pSer28,pSer31) peptides

Synthetic SMN$^{28-51}$ and SMN$^{28-51}$ phosphorylated at positions Ser28 and Ser31 (SMN$^{28-51}$(pSer28,pSer31)) were obtained from the W. M. Keck Foundation Biotechnology Resource Laboratory (Yale University, New Haven, CT, USA). Both peptides were solubilized in 0.1% trifluoroacetic acid (TFA) and purified by reverse-phase HPLC on a Vydac 218TP1010 (10 x 250 mm) protein and peptide C18 column (Grace Division Discovery Sciences, Deerfield Park, IL) at 1ml/min over a linear gradient of 20%-42% acetonitrile (MeCN) for 30 minutes. All solvents contained 0.1% TFA. Mass analysis of peak fractions by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry confirmed the presence and identities of the desired peptides.

SMN$^{26-51}$-Gemin2$^{95-280}$ (C154S/C221S/C264S) Spin Labeling with MTSSL

A 3-fold molar excess of MTSSL ((1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-methyl)-methanethiosulfonate, Toronto Research Chemicals, North York, ON) was added from a concentrated stock in acetonitrile to ^{15}N-labeled SMN$^{26-51}$-Gemin2$^{95-280}$ (C154S/C221S/C264S) at 0.3mM in 50mM Na/K phosphate, pH 6.5, 50mM NaCl, 2mM DTT, 50 μM EDTA, 0.2mM sodium azide buffer and incubated overnight in the

dark at 4°C with constant stirring. The unreacted spin label was removed by extensive buffer exchange into 50mM Na/K phosphate, pH 6.5, 50mM NaCl, 50mM DTT, 50 µM EDTA, 0.2mM sodium azide and 10% D2O.

Fluorescence Anisotropy Binding Assays

Purified SMN^{26-51} peptides and an unrelated 32-mer control peptide in 0.1M sodium bicarbonate, pH 9.0 were concentrated in Amicon Ultra 0.5ml 3000 MWCO centrifugal filters at 14,000 rpm at 4°C to ≥ 5mg/ml. The relatively high pH favors labeling of the amino terminus. Amine-reactive fluorescein-5-isothiocyanate (FITC; Invitrogen) was dissolved to 10mg/ml in dimethylsulfoxide (DMSO) and a volume equal to 1/10 the volume of each concentrated SMN^{26-51} peptide solution was added to that solution in an amber Eppendorf tube for light protection. The labeling reaction was further protected from light with aluminum foil and nutated for one hour at room temperature. The reaction was quenched by addition of a volume of 1.5M hydroxylamine, pH 8.5 equal to 1/10 the volume of the original concentrated SMN^{26-51} peptide solution and nutated as before for one hour.

Unreacted FITC label was removed by buffer exchange into 1x PBS (10 mM Na_2HPO_4, 2 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4) at 2ml/min over a 5ml HiTrap desalting column. Protein and free FITC peak elution was monitored using UV absorbance at 280 nm (A_{280}) and 495 nm (A_{495}), and final FITC-labeled peptide concentration was assessed using A_{280} and A_{495} values following the formula

$$A_{protein} = A_{280} - A_{495}(CF)$$

where the correction factor CF is defined as (A_{280} free dye / A_{max} free dye) and is equivalent to 0.3 for fluorescein-5-isothiocyanate.

For binding studies, FITC-SMN peptides were diluted into PBS to which 2mM DTT was added, and Gemin2 was dialyzed into the same buffer in D-Tube Dialyzers, Midi 6-8kDa MWCO (Novagen). Binding reactions (100 µl) containing 20µl 75nM FITC-labeled SMN (15nM final) and 80µl of increasing concentrations of Gemin2 (for a final concentration range of 0–1 or 0–4 µM) were analyzed using a Beacon 2000 fluorescence polarization instrument (Panvera) to record millipolarization values, a ratio of light intensities providing a measure of the extent of molecular rotation during the period between excitation and emission. Millipolarization values were converted to polarization by a factor of 1000 and then converted to anisotropy using the following equation:

$$Anisotropy = \frac{2 * polarization}{(3 - polarization)}$$

Data were fit to a simple binding isotherm corrected for receptor depletion (Kenakin, 1993). Typically, the equation for a simple binding isotherm assumes that the total titrated concentration of receptor (here Gemin2) approximates the concentration of free receptor.

$$B = \frac{R_T B_T}{(K_D + R_T)}$$

where B = [total concentration complex], R_T = total concentration of receptor (Gemin2) and L_T = total concentration of ligand (SMN).

Because the concentration of FITC-peptide (15nM) so nearly approximates the dissociation constant of complex, the total receptor (Gemin2) concentration is depleted at concentrations near K_D and cannot be used as a good approximation of the free receptor concentration across the range of concentrations tested.

An explicit correction for receptor depletion was made according to the formula

$$A = A_f + (A_b - A_f) \text{ x } (L_T + K_D + R_T) - \frac{[(L_T - K_D - R_T)^2 - 4L_T R_T]^{0.5}}{2L_T}$$

where A = the experimental anisotropy, A_f = the anisotropy for the free ligand (here FITC-SMN), A_b = the anisotropy for the fully bound ligand, R_T = the total receptor (Gemin2) concentration and L_T = the total added concentration of the ligand and where B was converted to anisotropy according to

$$[ligand\ bound] = \frac{L_T(A - A_f)}{(A_b - A_f)} = B$$

To determine K_D, non-linear least squares regression was performed using GraphPad Prism version 5.04 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com) with L_T fixed at 15.0nM.

NMR Spectroscopy and Analysis

All NMR samples were prepared in Shigemi NMR tubes (Shigemi, Inc.) in buffer containing 50mM Na/K phosphate, pH 6.5, 50 mM NaCl, 50 mM DTT, 50 µM EDTA, 0.2mM sodium azide and either 90% H2O/10% D2O or 99.99% D2O. Spectra were collected at 25°C at either 750MHz or 500MHz (^{1}H) on Bruker AVANCE III NMR spectrometers equipped with triple resonance (H/N/C) cryo-probes. Proton chemical shifts were referenced to DSS in the buffer, while ^{13}C and ^{15}N chemical shifts were indirectly referenced. Data were processed using FELIX (Molecular Simulations, San Diego, CA, USA) and analyzed in SPARKY (Goddard & Kneller, 2008).

Backbone amide ^{1}H and ^{15}N, Cα, C=O, and side-chain Cβ resonances were obtained using the HNCA, HN(CO)CA , HNCACB, CBCA(CO)NH, HNCO and HN(CA)CO experiments; 97.3% of HN and N resonances were assigned and 94.4% of carbonyl carbon resonances. Side chain assignments were obtained from H(CCCO)NNH, (H)CC(CO)NNH and HCCH-TOCSY experiments, facilitated by three-dimensional ^{15}N and ^{13}C-edited NOESY-HSQC spectra, ^{15}N and ^{13}C HSQC spectra of a sample in which only SMN was labeled assisted in the assignment of SMN peptide resonances. These experiments are described in a review by Sattler *et al.*, and references therein (Sattler *et al.*, 1999). To aid in the assignment of aliphatic sidechains, an ^{15}N HSQC of a sample in which only Gemin2 was labeled with ^{15}N-leucine, and stereo-specific side chain assignments of valine and leucine were determined from the ^{13}C HSQC of trace-labeled protein sample (Neidhardt *et al.*, 1974; Neri *et al.*, 1989). Of aliphatic residues,

7/7 isoleucine, 19/24 leucine and 14/14 valine side-chains were completely assigned. Aromatic side-chain assignments were made using three-dimensional aromatic-optimized ^{13}C NOESY-HSQC and HCCH-TOCSY experiments; 4/6 tryptophan, 6/6 tyrosine and 4/6 phenylalanine side-chains were fully assigned. All resonance assignments may be found listed in Appendix A.

NOE distance restraints were derived, in H_2O, from 3D ^{13}C-edited NOESY-HSQC (Diercks *et al.*, 1999) and from 4D HNCH-NOESY-HSQC experiments and, in D_2O, from a 4D HCCH-NOESY-HMQC (Ross *et al.,* 1993; Vuister *et al.,* 1993). All mixing times were 110 ms.

NOE-derived inter-proton distances in each NOESY spectrum were sorted into classes, corresponding to approximate distance ranges based on the volumes of "calibration NOEs" representing known short-range distances in secondary structure elements. The volumes of peaks in each NOESY spectrum representing a certain short-range interaction ($HN_i \rightarrow HA_i$, $HN_i \rightarrow HA_{i-1}$ and $HA_i \rightarrow HB_{i+3}$ as appropriate) were represented as histograms, as in Figure 2.23.

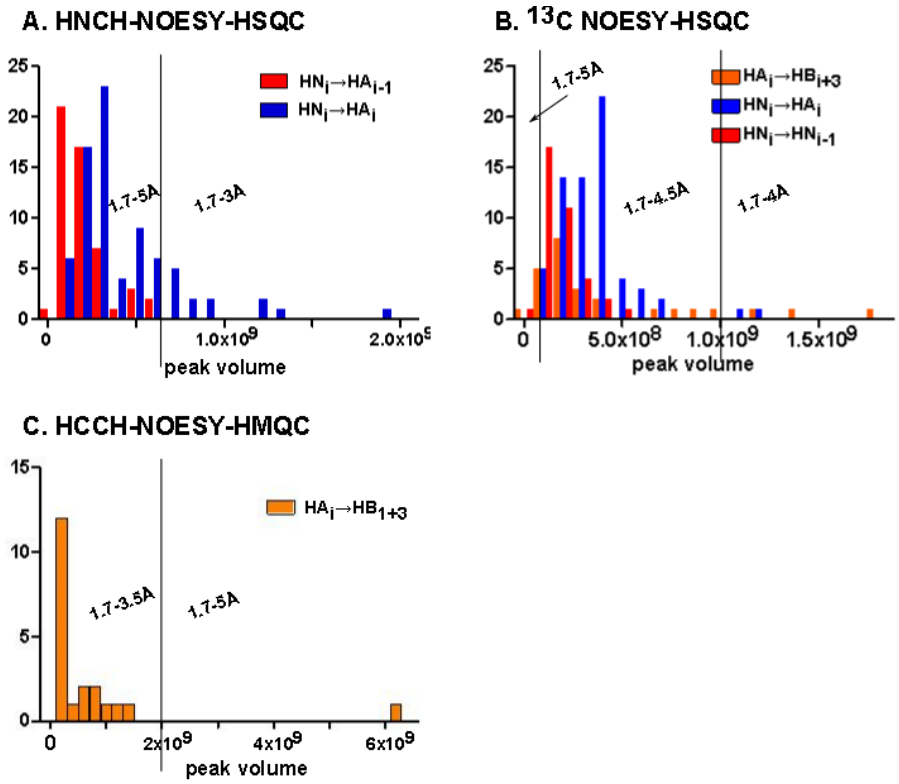


Figure 2.23. Peak volumes of NOEs used in distance calibration. Histograms of NOEs used to calibrate distance restraint limits for observed NOEs in the HNCH-NOESY-HSQC, ^{13}C NOESY-HSQC and HCCH-NOESY-HMQC experiments. Cutoff volumes are indicated with black arrows and the respective distance ranges are depicted in slanted text.

Based on the known $HN_i \rightarrow HA_i$ (2.7 Å), $HN_i \rightarrow HA_{i-1}$ (3.5Å) and $HA_i \rightarrow HB_{i+3}$ (3.6Å) distances in alpha helices (Wüthrich, 1986) and considering the volumes of various NOESY peaks corresponding to those interactions, bins were established at 1.8-3.0 Å (strong), 1.8-3.5Å (medium-strong), 1.8-4.0Å (medium), 1.8-4.5 Å (medium-weak) and 1.8-5.0Å (weak). All inter-proton restraints derived from the aromatic-optimized ^{13}C NOESY-HSQC were classified into the 1.8-5.0 Å distance range. A distance restraint involving one or two methyl groups was afforded an empirical correction of 0.5 Å per methyl group added to its upper distance boundary (Clore *et al.*, 1987).

Backbone torsion angle restraints were derived from chemical shifts using the TALOS+ program (Shen *et al.*, 2009). Hydrogen bonds were entered on the basis of secondary structure as indicated by calculated backbone torsion angles. For residual dipolar couplings, ^{15}N-labeled protein was aligned in bacteriophage Pf1 (ASLA Biotech) to a final concentration of 12 mg/ml. $^{1}D_{HN}$ couplings were obtained from a 2D ^{1}H-^{15}N HSQC IPAP (Ottiger *et al.*, 1998). SAXS data were collected at APS BioCAT beamline 18-ID and PRE distance restraints were derived from the attenuation of signal intensity of amide resonances in ^{1}H/^{15}N HSQCs collected under identical conditions in the presence of (paramagnetic) SMN$^{26-51}$-Gemin2$^{95-280}$ (C154S/C221S/C264S) labeled with MTSL at Cys241 and, after reduction with a 3-fold molar excess of ascorbic acid, its diamagnetic analogue (Battiste & Wagner, 2000).

NMR Structure Calculations

Generation of a ‘‘first stage’’ model comprised a standard Cartesian simulated annealing protocol performed using the Crystallography and NMR System (CNS) software, beginning from an extended structure and including active energy terms for bonds, angles, impropers, repulsive-only non-bonded interactions, NOEs from the ^{13}C NOESY-HSQC and backbone dihedral angles (Brünger *et al.*, 1998).

Structures were then calculated using the torsion angle/Cartesian space simulated annealing molecular dynamics refinement protocol within the python interface of the program XPLOR-NIH 2.26 (Schwieters *et al.*, 2003; Schwieters *et al.*, 2006). The first

ensemble used the lowest-energy "first stage" CNS model as a starting structure and was calculated using all NOEs, backbone dihedral angles and hydrogen bonds. These restraints may be found listed in Appendix B, Tables 1-3.

In every structure calculation, the trajectory was started at 3000K for 25ps and cooled down linearly in 10K temperature steps to 0K, first with the variable time step internal variable dynamics module (IVM) and then by Cartesian minimization. Each temperature step was propagated for 1000 simulation steps or 2ps, whichever was less. A quartic van der Waals repulsion term for the non-bonded contacts was used, in which the atomic radii were scaled from the van der Waals values multiplicatively, from 0.90 to 0.80. In the first round, the distance restraint force constants derived from NOEs and inferred hydrogen bonds were ramped multiplicatively from 2 to 30 kcal/Å^2 using a hard-square potential. Backbone dihedral angle (φ and ψ) restraints derived from chemical shifts using the software program TALOS+ operated under a force constant at 10 kcal mol^{-1} rad^{-2} at high temperature and were enforced during cooling by a strong force constant unramped at 200 kcal mol^{-1} rad^{-2}.

Subsequent rounds added a small-angle x-ray scattering (SAXS) term, residual dipolar couplings (RDCs) and distance restraints derived from paramagnetic relaxation enhancements (PREs). After each new class of restraint was added, eight structures were calculated, of which the lowest energy structure served as the starting structure for the

next round of refinement. The complete and sparsened SAXS data appear in Appendix B, Table 4.

The experimental SAXS data extended from 0.007 to 0.17Å^{-1} and were sparsened to 50 data points prior to input for the refinement calculations, in which a force constant of 40 kcal/ Å^{-2} was employed. Residual dipolar coupling (RDC) restraints describe the orientation of a given bond relative to an overall alignment tensor, described as D^{AB}

$$D^{AB}(\theta,\varphi) = D_a^{AB}\left\{\left(3\cos\ ^2\theta - 1\right) + \frac{3}{2}R(\sin\ ^2\theta\cos 2\varphi)\right\}$$

where the axial (D_a^{AB})and rhombic (D_r^{AB})components of the traceless second rank diagonal tensor are given as

$$D_a^{AB} = \frac{1}{3}\left[D_{zz}^{AB} - \frac{(D_{xx}^{AB} + D_{yy}^{AB})}{2}\right]$$

$$D_r^{AB} = \frac{1}{3}\left[D_{xx}^{AB} - D_{yy}^{AB}\right]$$

with

$$|D_{zz}^{AB}| > \left|D_{yy}^{AB}\right| \geq |D_{xx}^{AB}|$$

and rhombicity (R) defined as

$$R = \frac{D_r^{AB}}{D_a^{AB}} > 0$$

A histogram of measured RDCs from a sample provides an estimate of D_{yy} and D_{zz} (the extremes of the plotted data) and D_{xx} (the highest probability dipolar coupling value), as in Figure 2.24 (Clore *et al.*, 1998).

.

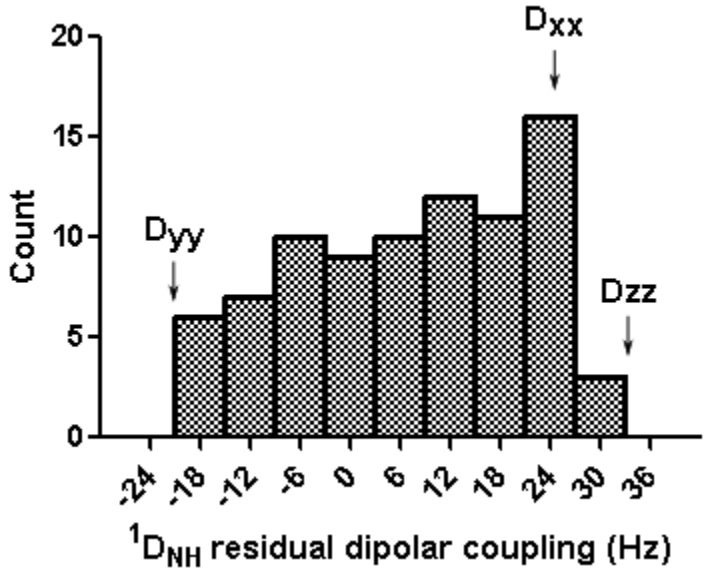


Figure 2.24 A histogram of $^{1}D_{NH}$ RDCs for SMN$^{26-51}$-Gemin2$^{95-280}$. The values of D_{xx}, D_{yy} and D_{zz} are indicated by black arrows.

Based on the values of D_{xx}, D_{yy} and D_{zz} (24 Hz, -21 Hz and 34 Hz, respectively) obtained from the histogram of RDCs, the calculated values for D_a and R were calculated as -10.6 and 1.23.

After addition of the SAXS term, an ensemble of eight structures was generated by XPLOR-NIH incorporating the RDC restraints. When the value of the alignment tensor was permitted to float, becoming optimized over the course of each calculation, the values obtained for D_a and R were calculated to be -10.56 and 0.35. While the estimate of the axial component from the histogram closely mirrored that calculated by XPLOR-NIH, the estimate of rhombicity varied rather more. RDC distance restraints were implemented using a hard-square potential with a force constant ramped multiplicatively from 0.03 to 3.0 kcal/Hz^2 for $^{1}D_{NH}$ couplings during the 3000 → 0 K cool down. The

components of the alignment tensor were first allowed to vary; the alignment tensor common to the lowest-energy structures in that round was fixed in the subsequent round. RDC restraints are listed in Appendix B, Table 5.

Paramagnetic relaxation enhancements based on observed attenuation of peak intensities in an 1H-15N HSQC were added last. The ratio of the intensities of each resonance is given by

$$\frac{I_{ox}}{I_{red}} = \frac{R2\exp(-R2^{sp}t)}{R2 + R2^{sp}}$$

where R2 is the experimentally determined transverse relaxation rate of the reduced sample and $R2^{sp}$ is the relaxation rate enhancement.

Solving for $R2^{sp}$ for each resonance allows the calculation of its distance from the spin label as

$$r = \left[\frac{K}{R2^{sp}}\left(4\tau_c + \frac{3\tau_c}{1+\omega_h^2\tau_c^2}\right)\right]^{1/6}$$

where τ_c is the correlation time for the electron-nuclear interaction, ω_h is the Larmor frequency and K is 1.23 x 10-32 cm^6 s^{-2} (Battiste & Wagner, 2000) .

Only two classes of PRE distance restraints were used for structure calculations; peaks with an intensity ratio >0.5 were restrained as the calculated distance ± 3.0 Å, while those with intensity ratios <0.5 were assigned a distance of 15 Å, with a 3.0 Å upper

bound and no lower bound. In XPLOR-NIH, the PRE distance restraint force was ramped multiplicatively from 2 to 50 kcal/Å^2 using a hard-square potential.

The evolution of all force constants over the sequential rounds of ensemble calculation as classes of restraints were added is summarized in Table 2.4.

Table 2.4. Force constants employed at each stage of structural refinement in XPLOR-NIH.

	stage of refinement				
	NOE/dihedrals	**SAXS**	**RDCs (variable)**	**RDCs(fixed)**	**PREs**
NOE	**2→30**	**2→50**	**2→50**	**2→50**	**2→50**
hbond	**2→30**	**2→50**	**2→50**	**2→50**	**2→50**
backbone dihedrals	**200**	**200**	**200**	**200**	**200**
SAXS		**40**	**40**	**40**	**40**
RDCs			**0.3→3**	**0.3→3**	**0.3→3**
PREs					**2→50**
Rama database potential	**0.002→1**	**0.002→1**	**0.002→1**	**0.002→1**	**0.002→1**
angles	**0.4→1**	**0.4→1**	**0.4→1**	**0.4→1**	**0.4→1**
impropers	**0.1→1**	**0.1→1**	**0.1→1**	**0.1→1**	**0.1→1**

In the final stage of model refinement, four hundred structures were generated, of which the 32 lowest energy structures form the ensemble. The final XPLOR-NIH python script employed can be found in Appendix C. Structure figures were generated using PyMOL (Delano, 2002).

There were no persistent NOE violations above 0.5 Å, dihedral angle violations above 5° or RDC violations above 0.5 Hz.

Small-angle x-ray scattering

Samples were dialyzed against 100% D_2O buffer (20mM Na/K PO_4, 50 mM NaCl, 2mM DTT) and were centrifuged at 10,000 x g for ten minutes at 4^oC immediately before data collection. X-ray radiation at 12 kEV was used to collect 10-15 individual one second exposures while the sample was oscillated in a quartz capillary to minimize radiation damage. Data were corrected for the intensity of the incident radiation and reduced to provide one-dimensional intensity profiles as a function of Q (which is equal to $4\pi\sin\theta/\lambda$, where 2θ is the scattering angle) using the data software Igor Pro. Accessible scattering was recorded in the range of $0.006 < Q < 0.35$ Å-1. Exposures were examined for radiation damage before averaging and subsequent subtraction of buffer scatter.

Averaged scattering curves from the SAXS and WAXS detectors were scaled and merged in PRIMUS; scattering from a matching buffer solution was subtracted from the data and corrected for the incident intensity of x-rays. Replicate exposures were examined carefully for evidence of radiation damage by Guinier analysis and Kratky plot analysis. Silver behenate powder was used to locate the beam center and to calibrate the sample-to-detector distance.

Evidence of the monodispersity of the preparation was gained by analysis of linearity in the Guinier region of the scattering data and agreement of the *I*(0) and *Rg* values

determined with inverse Fourier transform analysis by the programs GNOM and AUTOGNOM (Semenyuk & Svergun D, 1991). Molecular mass was derived from *I*(0) measurements, using the forward scatter from bovine serum albumin as a control, and was consistent with the molecular masses determined by centrifugation and SEC-MALS. When fitting manually, the maximum diameter of the particle (*D*max) was adjusted in 10-Å increments in GNOM to maximize the goodness-of-fit parameter. This analysis also yielded determinations of *Rg* and *I*(0).

Sedimentation velocity

Sedimentation velocity ultracentrifugation experiments were performed at 20 °C with an XL-A analytical ultracentrifuge (Beckman Coulter) and a TiAn60 rotor with two-channel charcoal-filled Epon centerpieces and quartz windows. Complete sedimentation velocity profiles of SMN^{26-51}-$Gemin2^{95-280}$ (25 μM, optical density of 0.6 at A_{280}) in 1x PBS pH 7.4 were collected every 60 seconds at 42,000 rpm for 200 scans, followed by data analysis using the continuous size-distribution (c(s)) analysis of the Lamm equation in SEDFIT (Schuck & Rossmanith, 2000). After optimizing meniscus position and fitting limits, the sedimentation coefficient (s) and best-fit frictional ratio (f/f_0) were determined by iterative least squares analysis.

Sedimentation equilibrium

Sedimentation equilibrium ultracentrifugation (SE) experiments were performed at 4°C with an XL-A analytical ultracentrifuge (Beckman Coulter) and a TiAn60 rotor with

six-channel charcoal-filled Epon centerpieces and quartz windows. Radial absorption scan data at 280 nm for three protein concentrations were measured (18.0 μM, 11 μM, 5 μM; 0.8OD, 0.5OD, 0.2OD) at 20 and 22 h for each of three different rotor speeds (22,000, 28,000 and 32,000 rpm). Comparison of radial absorption scans verified that equilibrium had been reached. Data were analyzed using the programs SEDFIT and SEDPHAT and fit variously to a single species model and to several models of self-association, with the best solutions exhibiting small goodness of fit and low and randomly distributed residual errors (Schuck & Rossmanith, 2000; Vistica *et al.*, 2004).

Size exclusion chromatography in-line with multi-angle light scattering

The absolute molecular weight of the SMN^{26-51}-$Gemin2^{95-280}$ oligomer was determined using multi-angle light scattering (MALS) in-line with a Superdex 75 10/300 column (GE Healthcare). The column was calibrated using the following proteins (Bio-Rad): thyroglobulin (670,000 Da), bovine gamma globulin (158,000 Da), chicken ovalbumin (44,000 Da), equine myoglobin (17,000 Da) and vitamin B-12 (1350 Da). Blue dextran (Sigma) was used to define the void volume of the column.

SMN^{26-51}-$Gemin2^{95-280}$, 20μl at 10 mg/ml was injected onto the column at 0.5 ml/min in buffer containing 1x PBS, pH 7.0 and 2mM dithiothreitol at room temperature. The scattered light intensity of the column eluant was recorded at 16 different angles using a DAWN-HELEOS MALS detector (Wyatt Technology Corp.) operating at 658 nm after calibration with 20μl of 20mg/ml lysozyme (Sigma). Protein concentration of the eluant

was determined using an in-line Optilab DSP interferometric refractometer (Wyatt Technology Corp.). The weight-averaged molecular weight of species within defined chromatographic peaks was calculated using the ASTRA software version 5.2 (Wyatt Technology Corp.), by construction of Debye plots ($KC/R\,\Theta$ *versus* $\sin^2[\Theta/2]$) at one-second data intervals. The weight-averaged molecular weight was then calculated at each point of the chromatographic trace from the Debye plot intercept, and an overall average molecular weight was calculated by averaging across the peak.

Chapter III: The biophysical properties of Gemin2 in the presence and absence of SMN binding

Introduction

Gemin2 plays a key role in SMN complex-mediated assembly of spliceosomal snRNPs. Gemin2's structure and function eluded description until recently, when NMR and x-ray crystal structures permitted development of a model in which a single α-helix of SMN binds the globular domain of residues 95-280 of Gemin2 on one face, while all five components of the Sm D1/D2/E/F/G pentamer bind the opposite face and along Gemin2's extended N-terminal arm.

Here, solution properties of Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ are compared to those of unbound Gemin2$^{95\text{-}280}$ to illustrate changes in the structure of the latter adopted upon SMN binding. Addressing the recent report of a functionally significant Gemin2-Gemin2 interaction (Ogawa *et al.*, 2007), the oligomerization states of Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ and Gemin2$^{95\text{-}280}$, as well as those of full-length Gemin2$^{12\text{-}280}$ both unbound and bound to SMN$^{26\text{-}51}$, are described. A comparison is also offered between low-resolution solution structures of the minimal Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ and full-length Gemin2$^{12\text{-}280}$-SMN$^{26\text{-}51}$ complexes.

Results

Gemin2$^{95-280}$, Gemin2$^{12-280}$ and their SMN$^{26-51}$-bound complexes are monomeric species in solution

In a recent report, Ogawa *et al.* proposed a Gemin2 self-interaction, which they attributed to residues 56-280 and suggested is necessary for the stabilization of SMN self-association at its N-terminal oligomerization domain (Ogawa *et al.*, 2007). In the current x-ray crystallographic model of full-length Gemin2 in complex with SMN$^{26-62}$, the reported Gemin2 self-association domain obliges a joint contribution from the globular SMN-binding domain of residues 95-280 and from the N-terminal Sm-protein binding arm to the creation or stabilization of the putative Gemin2 self-interaction surface (Zhang *et al.*, 2011). No evidence of Gemin2 dimerization, however, was apparent in the NMR studies of its minimal complex with SMN or in the crystal structure of Gemin2 bound both to a minimal SMN construct and its Sm protein substrates, leaving unclear the circumstances under which a Gemin2-Gemin2 interaction occurs.

In an effort to establish the oligomeric state and protein stoichiometry of several Gemin2 and Gemin2-SMN$^{26-51}$ species in solution, analytical size-exclusion chromatography in-line with multi-angle light scattering (SEC-MALS) was used to determine the absolute molecular weights of Gemin2$^{95-280}$, Gemin2$^{12-280}$ and those

species bound to SMN^{26-51}. For this and all experiments described, full-length Gemin2 refers to the Gemin2 construct comprising residues 12-280.

A simple interpretation of the analytical size exclusion chromatography elution profiles of each species in comparison with those of globular molecular weight standards and apparent molecular weight. As mentioned in Chapter II, $Gemin2^{95-280}$-SMN^{26-51}, injected at 10 mg/ml (410 μM), elutes as a single peak with an approximate molecular weight of 25 kDa, consistent with a 1:1 heterodimer of one $Gemin2^{95-280}$ molecule (21 kDa) and one SMN^{26-51} molecule (3 kDa). This is entirely consistent with observations about the complex made from its NMR solution structure and from its analysis by sedimentation equilibrium (also presented in Chapter II). Unbound $Gemin2^{95-280}$ at 1.7 mg/ml (79 μM)**,** however, elutes as a single peak with an apparent molecular weight of 60 kDa, consistent with a trimer of globular 21 kDa monomers (Figure 3.1).

Proteins with non-standard hydrodynamic properties due to an elongated shape or conformational flexibility can elute in size-exclusion chromatography experiments at the abbreviated retention times characteristic of larger globular particles. To discriminate between oligomerization and non-globular shape as the basis of elution from a size exclusion chromatography column at a large apparent molecular weight, a measure of the absolute molecular weights for the same Gemin2 species were determined considering multi-angle light scattering data coupled to size exclusion chromatography (SEC-MALS) on a Superdex 75 10/300 column. The weight-averaged molecular weight

of the Gemin2$^{95-280}$-SMN$^{26-51}$ complex was calculated to be 23.7 ± 0.1 kDa, again confirming its established nature as a single 1:1 heterodimer in solution. Despite the suggestion from its elution profile that it forms an oligomer in solution, Gemin2$^{95-280}$ showed a weight-averaged molecular weight of 22.9 ± 0.1 kDa, indicating that its solution state is also monomeric (Figure 3.1).

Full-length Gemin2$^{12-280}$ bound to SMN$^{26-51}$ contains all the residues of Gemin2 reported involved in Gemin2 self-interaction. Injected at 1.4 mg/ml (41 μM), it elutes as a single peak from a Superdex 75 10/300 size exclusion chromatography column, with an apparent molecular weight of 94 kDa, suggesting a trimer of 1:1 heterodimers consisting of one Gemin2$^{12-280}$ molecule (32 kDa) and one SMN$^{26-51}$ molecule (3 kDa). Its weight-averaged molecular weight based SEC-MALS analysis, however, was calculated to be 33.7 ± 0.4 kDa, again consistent with a 1:1 heterodimer of 35 kDa and displaying no evidence of oligomerization (Figure 3.1).

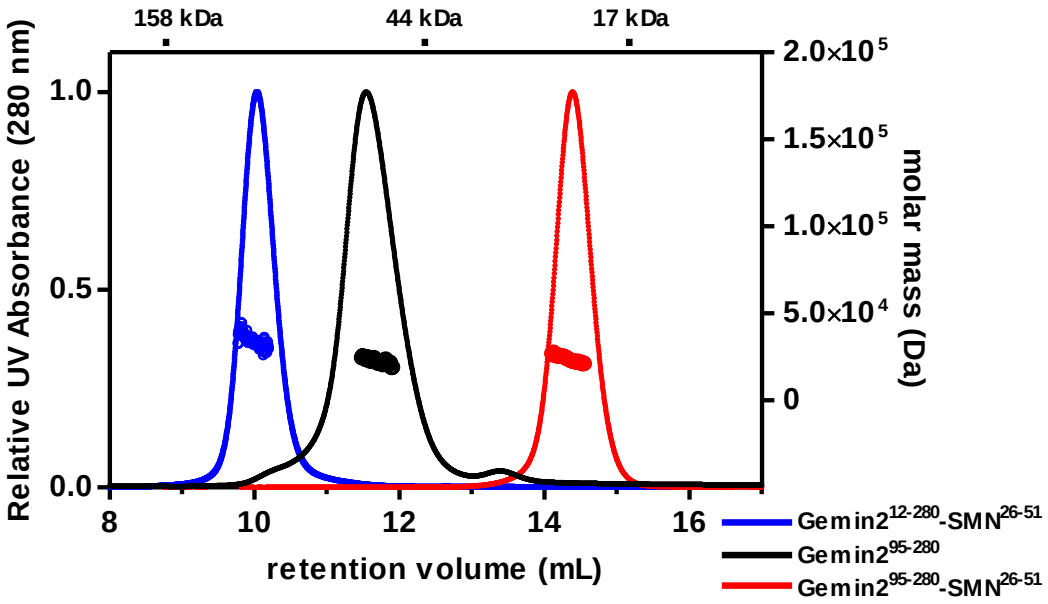


Figure 3.1 Representative SEC-MALS analyses of Gemin2 and Gemin2-SMN$^{26-51}$ complexes. The elution profiles depict Gemin2$^{12-280}$-SMN$^{26-51}$ (blue), Gemin2$^{95-280}$ (black) and Gemin2$^{95-280}$-SMN$^{26-51}$ (red) from a Superdex 75 10/300 column. The elution volumes of globular protein standards are shown above the panel. None of the three proteins show evidence of higher order oligomers.

In all previous attempts at its study, unbound full-length Gemin2$^{12-280}$ evinced resoundingly poor solution properties, including poor solubility, a tendency toward aggregation, susceptibility to proteolysis and a severe disposition toward promiscuous and irreversible binding to a number of chemical substrates, including all nitrocellulose membranes and dextran agarose sizing resin. Attempts to manipulate it experimentally remained very limited. Nisha Ninan, however, a graduate student in the Gregory Van Duyne laboratory at the University of Pennsylvania, recently succeeded in performing SEC-MALS analysis on the protein in buffer containing 5% glycerol. Gemin2$^{12-280}$ too elutes as a single peak with a weight-averaged molecular weight calculated to be 29.2 ± 0.3 kDa, in good agreement with its calculated molecular weight of 32 kDa. In evident

disagreement with the study by Ogawa *et al.*, Gemin2 dimerization was not apparent at the relatively high protein concentrations tested in these analyses for either Gemin2$^{95\text{-}280}$ or Gemin2$^{12\text{-}280}$, either unbound or bound to a minimal SMN peptide.

Gemin2$^{95\text{-}280}$ assumes an elongated structure in the absence of SMN binding

The disparity in the apparent and absolute molecular weights of Gemin2$^{95\text{-}280}$ species studied by SEC-MALS could be the result of unusual hydrodynamic properties, such as an elongated shape or conformational heterogeneity. To better understand the conformation adopted by Gemin2$^{95\text{-}280}$ in the presence and absence of SMN$^{26\text{-}51}$, sedimentation velocity analysis and small-angle x-ray scattering were employed to evaluate structural parameters such as the frictional ratio (f/f_o), radius of gyration (R_g) (a description of the mass distribution of the macromolecule around its center of gravity) and maximum dimension (D_{max}), all indicators of the extent of a particle's elongation.

Sedimentation velocity analytical ultracentrifugation data was collected for Gemin2$^{95\text{-}280}$ and Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ at 20°C and 8.2 µM and 18.3 µM, respectively, and fit to a continuous c(s) distribution yielding estimates for their sedimentation coefficients ($S_{20,w\text{-}}$) of 3.0 and 2.4. It becomes possible to calculate the frictional ratio f/f_o, the ratio of the frictional coefficient from the sedimentation coefficient, as discussed in Chapter II; the frictional ratio of a protein also correlates with its shape, with f/f_o values ~1.25 typically indicating a globular protein (Janson, 2011). Here, comparison of f/f_o values determined for Gemin2$^{95\text{-}280}$ and Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ is illustrative. Compared to the f/f_o of 1.25

calculated for the Gemin2$^{95-280}$-SMN$^{26-51}$ complex, Gemin2$^{95-280}$ has a value of 1.5, suggesting a relative elongation of that species (Figure 3.2).

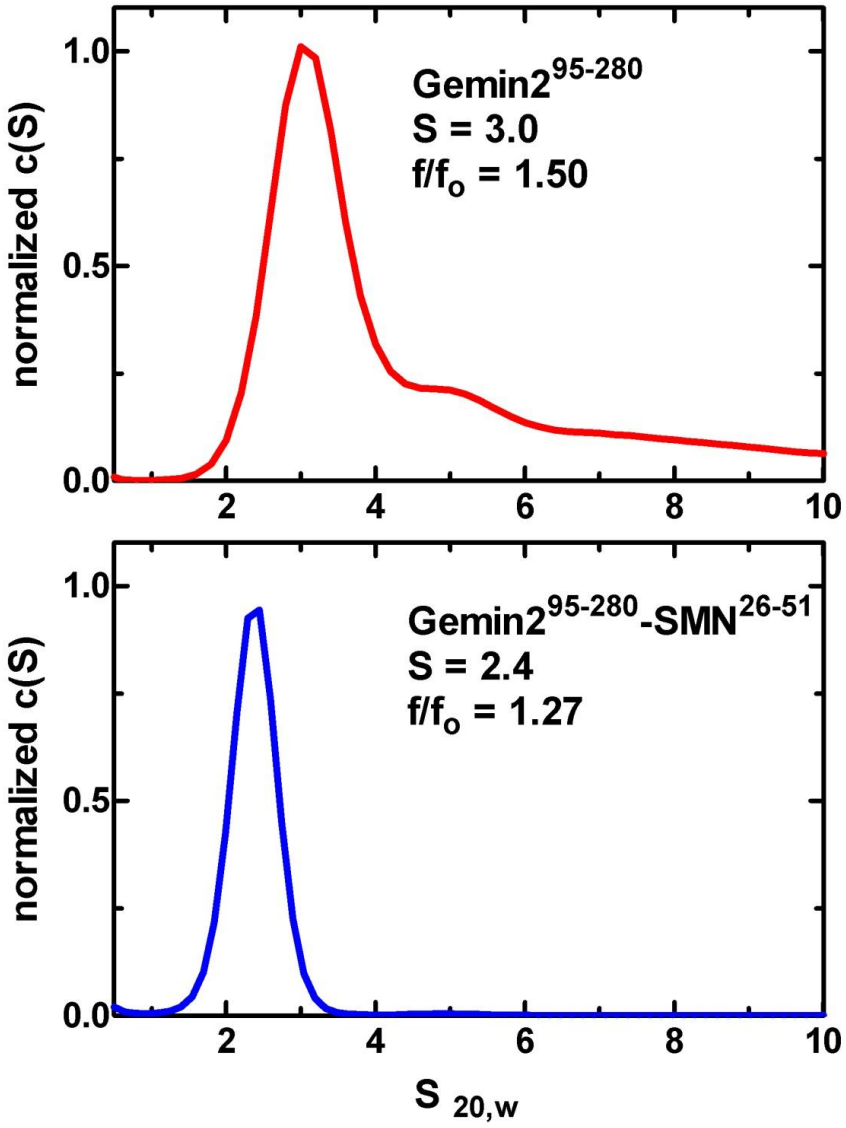


Figure 3.2 Sedimentation velocity analysis of Gemin2$^{95-280}$ and Gemin2$^{95-280}$-SMN$^{26-51}$
Representative c(S) analyses of Gemin2$^{95-280}$ (top; red) and Gemin2$^{95-280}$-SMN$^{26-51}$ (bottom; blue) from sedimentation velocity data.

A number of useful structural parameters related to the overall shape of a scattering particle can be derived from small-angle scattering experiments, including small-angle x-ray scattering. Radii of gyration for Gemin2$^{95-280}$ and Gemin2$^{95-280}$-SMN$^{26-51}$ were calculated by Guinier analysis of the SAXS scattering curve at very low scattering angles (Figure 3.3A), with values calculated as 25.6 ± 0.38 Å and 19.4 ± 0.13 Å, respectively, indicating that unbound Gemin2$^{95-280}$ occupies more extended conformation in solution than it does when bound to SMN$^{26-51}$. A summary of results for Gemin2$^{95-280}$-SMN$^{26-51}$ and unbound Gemin2$^{95-280}$ is presented in Table 3.1**.**

Table 3.1 Gemin2 structural parameters derived from small-angle x-ray scattering analyses

Sample	**Conc. (mg/mL)**	**Rg (A)**	**I(0)**	**Q**	**D_{max} (Å)**
Gemin2$^{95-280}$	0.5	25.6 ± 0.38	31.8 ± 0.41	0.017 - 0.35	75
Gemin2$^{95-280}$-SMN$^{26-51}$	3.6	19.4 ± 0.13	267.1 ± 1.40	0.025-0.35	54
Gemin2$^{12-280}$-SMN$^{26-51}$	8.5	29.1 ± 0.10	286.1 ± 0.56	0.011-0.32	134

P(r) curves, distance distribution functions that describe the probability of an interatomic vector of length r and that reach zero at the particle's maximum dimension

(D_{max}), were calculated from the collected intensity using an inverse Fourier transform (Figure 3.3B).

One measure of the elongation of a given protein is to compare its D_{max} to that of an ideal sphere of the same molar mass. The volume of protein of molecular mass M (Da), assuming an average partial specific volume for most proteins of v = 0.73 cm^3/g, can be calculated as:

$$V(\mathrm{nm}^3) = \frac{(0.73\,\frac{\mathrm{cm}^3}{\mathrm{g}})(10^{21}\frac{\mathrm{nm}^3}{\mathrm{cm}^3})}{6.023\mathrm{x}10^{23}\frac{\mathrm{Da}}{\mathrm{g}}}\ \mathrm{x}\ \mathrm{M(Da)}$$

Treating this volume as the most compact possible globular model, a sphere, one can calculate an approximate D_{max} as twice the sphere's radius. For Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ and unbound Gemin2$^{95\text{-}280}$, these values are 61 Å and 57 Å, respectively (Erickson, 2009).

Based on P(r) curves generated from SAXS intensity data, the experimental D_{max} values for the two species were determined to be 54 Å and 75 Å for Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ and Gemin2$^{95\text{-}280}$. The D_{max} value obtained for Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ is consistent with its known globular character; unbound Gemin2$^{95\text{-}280}$ has a much longer maximum dimension than its peptide-bound counterpart, further supporting the notion that the Gemin2-SMN heterodimer occupies a compact shape and confirming a much elongated character, comparatively, for the unbound Gemin2$^{95\text{-}280}$ (Figure 3.3B).

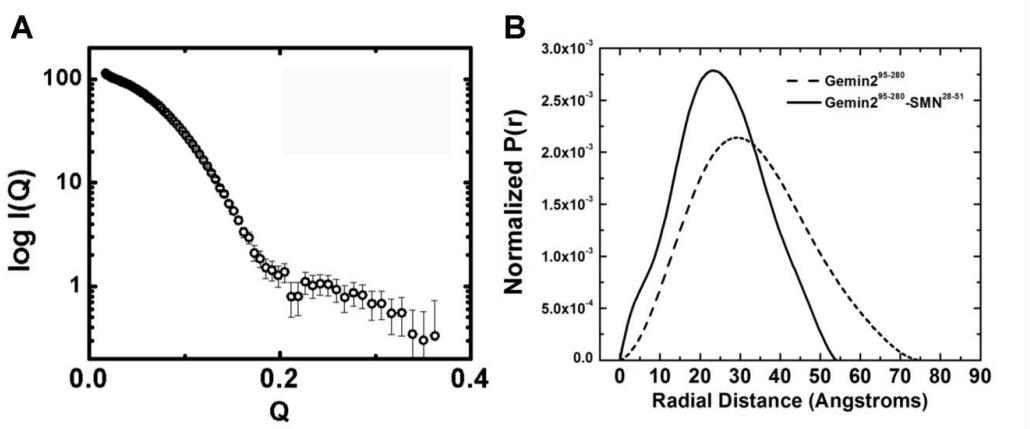


Figure 3.3 SAXS analysis of Gemin2$^{95\text{-}280}$ and Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$. A) Small-angle scattering data from Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$. Shown in black circles is the recorded intensity as a function of Q. B) P(r) shape functions for Gemin2$^{95\text{-}280}$ (dotted line) and Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ (solid line).

The Gemin2$^{95\text{-}280}$ structure adopted in the presence of SMN$^{26\text{-}51}$ is destabilized in its absence

The apparent elongation of Gemin2$^{95\text{-}280}$ in the absence of SMN might be visualized either as an ordered rearrangement of secondary structure elements into another, more extended stable protein fold or as the destabilization and partial unfolding of a portion of that protein. Certain characteristics of the ^{1}H-^{15}N HSQC spectrum of a protein or the Kratky analysis of its small-angle scattering data are consistently indicative of protein unfolding and were considered here. Two-dimensional ^{1}H-^{15}N HSQC spectra of Gemin2$^{95\text{-}280}$ and Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ were recorded at 25°C at 750 MHz (^{1}H) and overlain to illustrate the differences between them (Figure 3.4). The HSQC spectrum of Gemin2$^{95\text{-}280}$ shows substantial spectral changes relative to that of the Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ complex, including line broadening and a loss of peak dispersion. The number of discrete peaks (51) is far fewer than expected for the molecule (271 including sidechains); they largely correspond to known assignments in the unstructured loop

comprising residues 150-175. These spectral characteristics are highly suggestive of at least partial protein unfolding and conformational flexibility, suggesting that the structure of Gemin2$^{95\text{-}280}$ is poorly defined when SMN is not bound.

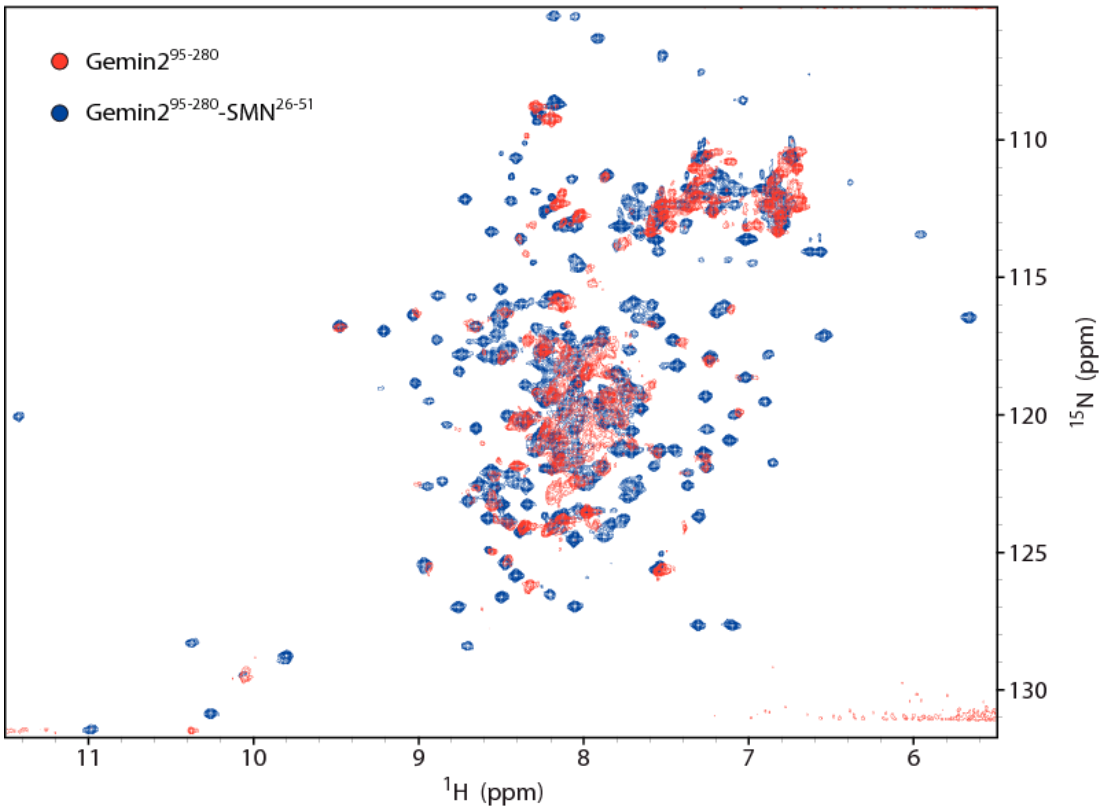


Figure 3.4 Overlay of $^{1}H/^{15}N$ HSQC spectra of Gemin2$^{95\text{-}280}$. Overlay of $^{1}H/^{15}N$ HSQC spectra of Gemin2$^{95\text{-}280}$ (red) and Gemin2$^{95\text{-}280}$ bound to SMN$^{26\text{-}51}$ (blue).

A Kratky analysis of small angle scattering data plots the intensity of the signal times the square of the scattering vector Q versus Q (where $Q = \frac{4\pi \sin\theta}{\lambda}$; 2θ is the scattering angle and λ is the wavelength) and serves as a diagnostic measure of protein unfolding. The Kratky curve of a folded protein displays a symmetric peak at low values of Q,

approaching zero with increasing Q. In the case of a disordered polypeptide, the Kratky curve instead increases linearly as Q increases (Doniach, 2001). Here, while the Kratky plot of the scattering data from the Gemin2$^{95-280}$-SMN$^{26-51}$ complex conforms to the curve typical of a folded protein, the curve of the Kratky plot from the scattering data measured for unbound Gemin2$^{95-280}$ shows clear characteristics of both the folded and unfolded theoretical curves and supports evidence from the ^{1}H-^{15}N HSQC spectrum that Gemin2$^{95-280}$ is partially unfolded (Figure 3.5).

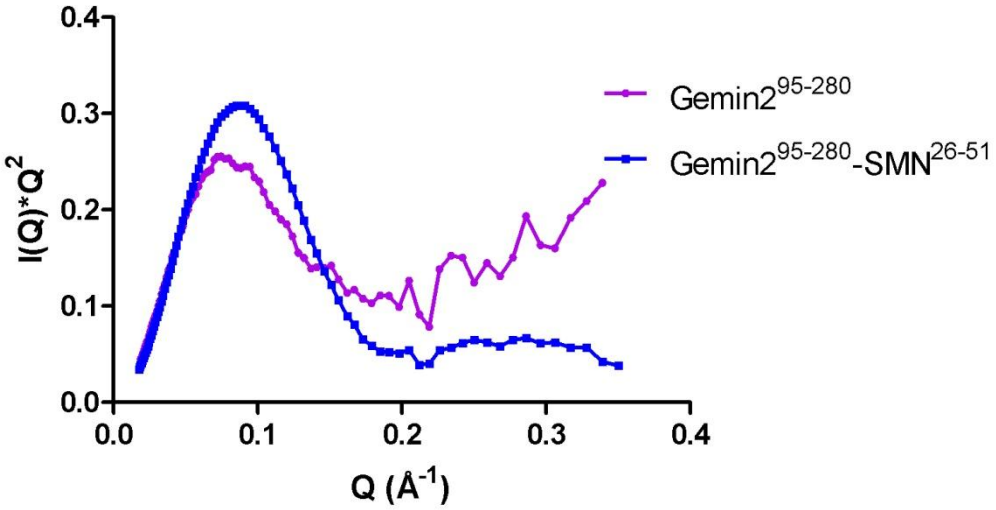


Figure 3.5 Kratky plot of unbound Gemin2$^{95-280}$ and the Gemin2$^{95-280}$-SMN$^{26-51}$ complex

Although the results of SEC-MALS analysis, the HSQC overlay and D_{max} values from SAXS and Kratky analysis clearly indicate that unbound Gemin2$^{95-280}$ adopts a partially disordered and comparatively elongated conformation relative to the Gemin2-SMN heterodimer, shape reconstructions based on SAXS scattering data were calculated to visualize the differences between the low-resolution solution structures of the two macromolecular species.

Shape reconstructions of Gemin2$^{95-280}$ and Gemin2$^{95-280}$-SMN$^{26-51}$ demonstrate a conformational change in Gemins$^{95-280}$ upon SMN binding

To extend the comparison of the conformations adopted by Gemin2$^{95-280}$-SMN$^{26-51}$ and Gemin2$^{95-280}$, low-resolution shape reconstructions from an ensemble of dummy residues were generated from their small-angle x-ray scattering data using GASBOR (Svergun *et al.*, 2001). For Gemin2$^{95-280}$-SMN$^{26-51}$, this approach reproducibly yielded envelopes with good correlations between experimental and calculated scattering data (sqrt(χ) ~1.3) (Figure 3.6A). The ensemble of envelopes generated from multiple iterations of the reconstruction process also agreed well with one another; normalized spatial discrepancy (NSD) values between the simulated shapes were calculated to establish the reliability of the solution and here ranged from 0.89 to 0.97 (where a value of unity corresponds to identity between two structures, and values below 1 indicate a high degree of overlap). The averaged molecular envelope of the Gemin2$^{95-280}$-SMN$^{26-51}$ heterodimer is a fairly compact prolate ellipsoid, entirely consistent with the NMR solution structure of the model (which, indeed, used this scattering data as a restraint) (Figure 3.6B).

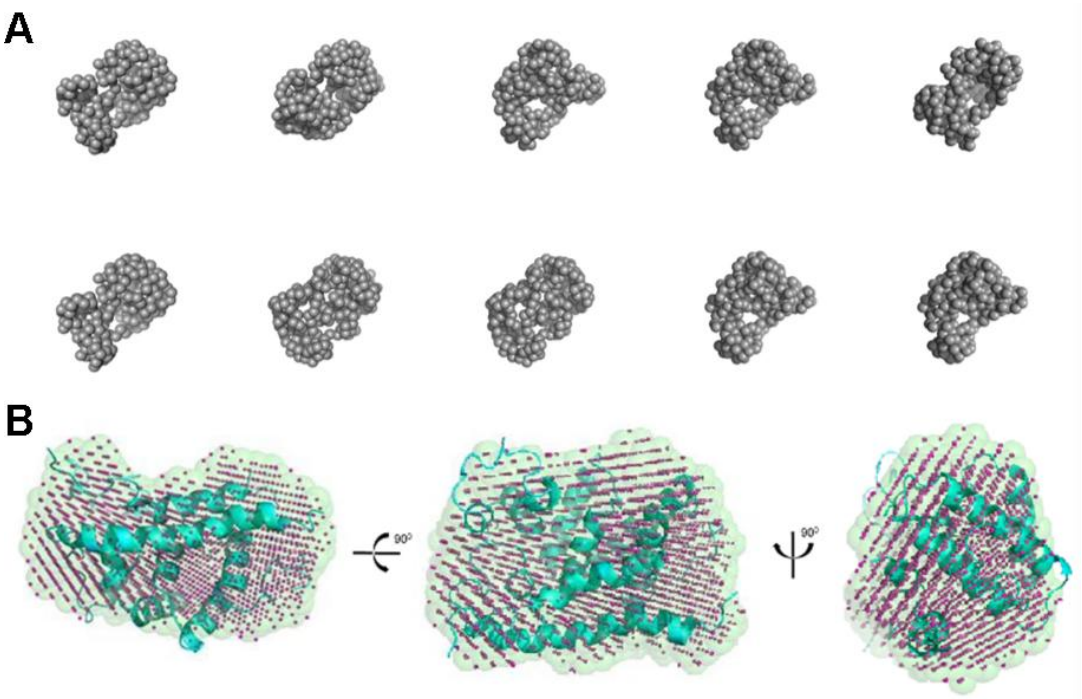


Figure 3.6 SAXS shape reconstructions of Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$. A) Ten independent *ab initio* envelopes generated from SAXS data using GASBOR. B) Orthogonal views of the NMR structure of Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ (cyan) docked into an *ab initio* averaged molecular envelope calculated by DAMAVER (purple spheres) and filtered by DAMFILT (light blue spheres).

A similar GASBOR analysis of the unbound Gemin2$^{95\text{-}280}$ scattering data yielded shape envelopes with (sqrt(χ) ~0.4) and NSD values ranging from 1.25 – 1.47, the latter value suggesting the envelopes comprising the ensemble varied more with respect to each other than did those calculated for Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ (Figure 3.7A). The filtered averaged molecular envelope adopted the appearance of an elongated prolate ellipsoid (Figure 3.7B). An important caveat in the interpretation of the averaged envelope of a biological macromolecule that exhibits some intrinsic disorder, however, is that the averaging and filtering step of shape reconstruction often removes relevant features of these systems sampled in only some shape reconstructions, establishing a misleading single shape.

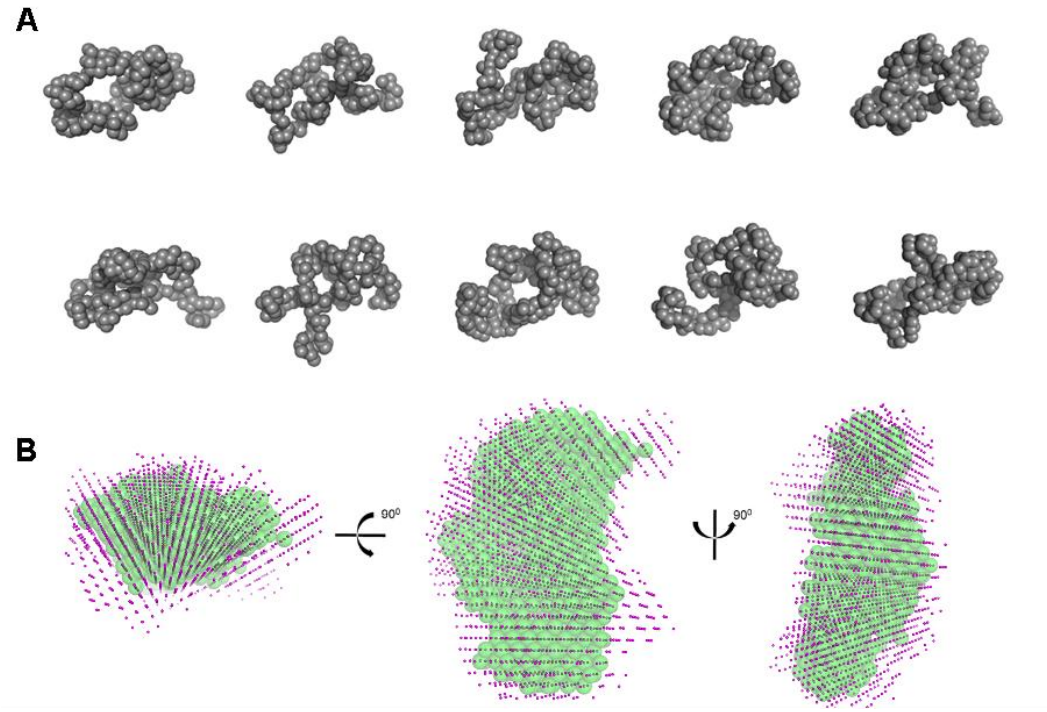


Figure 3.7 SAXS shape reconstructions of unbound Gemin2$^{95-280}$. A) Ten independent *ab initio* envelopes generated from SAXS using GASBOR data. B) Orthogonal views of the *ab initio* averaged molecular envelope calculated by DAMAVER (purple spheres) and filtered by DAMFILT (green spheres).

The solution conformation Gemin$^{12-280}$-SMN$^{26-51}$ is extended, and the N-terminal region of Gemin$^{12-280}$ forms a rigid arm

A recent crystal structure of Gemin2 bound to an SMN peptide and the Sm D1/D2/E/F/G pentamer represented a step forward in understanding the molecular function of Gemin2 in the context of the SMN complex; after escort to SMN and Gemin2 by the chaperone pICln, it appears that Gemin2 coordinates the pentamer before snRNA binding and addition of the Sm D3/B dimer to complete the Sm protein heptamer, forming the spliceosomal U-snRNP core. In particular, it is noteworthy that

Sm protein interactions with Gemin2 take place on its globular SMN-binding domain on the face opposite SMN binding, as well as along an extended region corresponding to the N-terminal 83 residues of the protein (Zhang *et al.*, 2011). Although that N-terminal binding arm appears to possess some helical character when bound to its Sm protein substrates, the conformation Gemin2 adopts in the absence of its substrates has yet to be explored.

Small-angle x-ray scattering analysis was applied to a description of the structural parameters of Gemin2$^{12\text{-}280}$-SMN$^{26\text{-}51}$ and to the *ab initio* calculation of a low-resolution molecular envelope. Although a comparable analysis of data from unbound Gemin2$^{12\text{-}280}$ might have served as an additional source of insight, the poor solubility and stability of that protein of precluded its inclusion in SAXS analysis. Guinier analysis of Gemin2$^{12\text{-}280}$-SMN$^{26\text{-}51}$ demonstrated an R_g of 29.1 ± 0.10 Å; from a P(r) distance distribution function, D_{max} was calculated to be 134 Å (Table 3.1). Given the theoretical D_{max} of 69 Å for a perfect 35 kDa sphere of specific density v = 0.73 cm^3/g, it is obvious that Gemin2$^{12\text{-}280}$, even bound to SMN, adopts an extended conformation. Given previous SEC-MALS analysis showing that Gemin2$^{12\text{-}280}$-SMN$^{26\text{-}51}$ forms a 1:1 heterodimer in solution but elutes with a smaller retention volume than predicted for a globular monomer, the confirmation of its elongated character is unsurprising.

GASBOR was employed to generate shape reconstructions from the Gemin2$^{12\text{-}280}$-SMN$^{26\text{-}51}$ scattering data (Svergun *et al.*, 2001). Independent shape reconstructions

showed good agreement, with sqrt(χ) values near 1.3 and NSD values ranging from 0.98-1.05, indicating a very stable structural solution (Figure 3.8A). The averaged molecular envelope of the Gemin2$^{12-280}$-SMN$^{26-51}$ heterodimer displayed a globular domain consistent with the region of the protein (residues 95-280) bound to SMN$^{26-51}$ that was solved by NMR spectroscopy. Surprisingly, however, the N-terminal region forms a rigid arm-like protrusion reproduced stably in each shape reconstruction and their averaged structure (Figure 3.8B). It appears that residues 12-94 of Gemin2 do indeed extend away from the globular SMN binding domain to interact with the Sm proteins; indeed, the NMR structure of the Gemin2 SMN-binding domain bound to SMN$^{26-51}$ docks readily by rigid body modeling into the globular domain of the Gemin2$^{12-280}$-SMN$^{26-51}$ molecular envelope, with its N-terminal-most helix positioned to extend into region of the envelope likely representing the N-terminal Sm protein-binding arm (Figure 3.8C). The shape reconstruction invites speculation that the N-terminal region of Gemin2 serves as a preformed binding surface, rather than adopting upon Sm protein binding what modest structure is evidenced in the crystal structure.

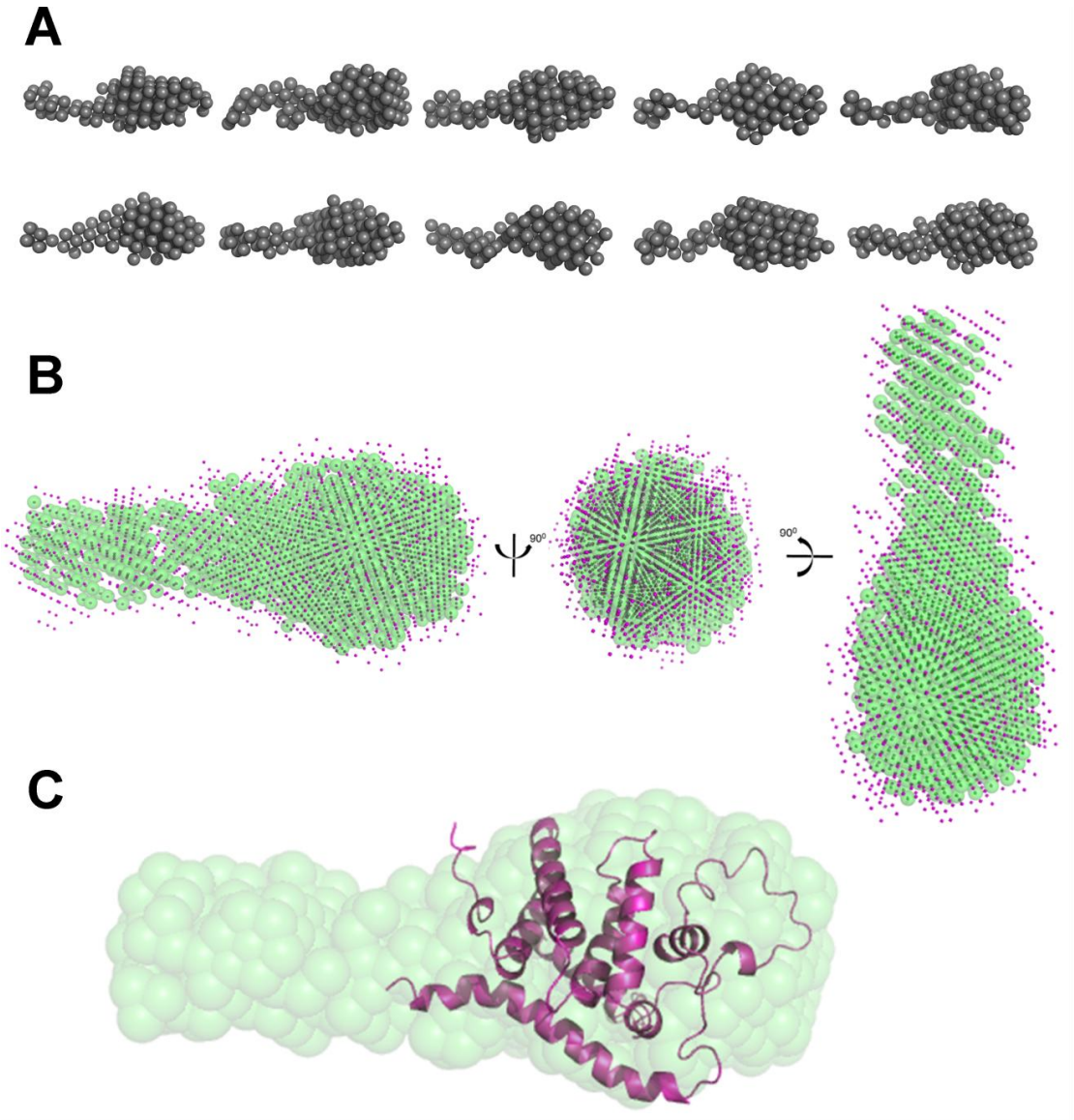


Figure 3.8 SAXS shape reconstructions of Gemin2$^{12\text{-}280}$-SMN$^{26\text{-}51}$. A) Ten independent *ab initio* envelopes generated from SAXS using GASBOR data. B) Orthogonal views of the *ab initio* averaged molecular envelope calculated by DAMAVER (purple spheres) and filtered by DAMFILT (green spheres). C) View of the NMR structure of Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ (purple) docked into an *ab initio* averaged molecular envelope calculated by DAMAVER and filtered by DAMFILT (green spheres).

Discussion

Gemin2 appears to play its crucial role in binding a subset of Sm proteins during snRNP biogenesis in the context of a tight binding interaction with the protein SMN. Based on the NMR solution structure of the minimal Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ heterodimer, it is apparent that the seven α–helices of Gemin2$^{95\text{-}280}$ adopt a fold unlikely to remain stable in the absence of the SMN helix. Here the stoichiometry and conformational states of Gemin2$^{95\text{-}280}$ and Gemin2$^{12\text{-}280}$ were examined as a source of insight into the likely requirement that Gemin2 must "fold on" to SMN to gain both stability and its functionally relevant structural fold, and to consider the implications of this constraint on Gemin2's involvement in processes and in organisms apart from SMN.

Ogawa *et al.* recently proposed a self-interaction for Gemin2 attributed to residues 56-280, which they suggest acts to stabilize the established SMN oligomer (Lorson *et al.*, 1998; Ogawa *et al.*, 2007). In the x-ray crystallographic model of Zhang *et al.*, the reported self-association domain implies the joint contribution of the globular SMN-binding domain of residues 95-280 and the N-terminal Sm-protein binding arm to the creation or stabilization of a Gemin2 self-interaction surface (Zhang *et al.*, 2011). A number of experiments in the Ogawa *et al.* study were cited as evidence in support of Gemin2 dimerization, including size-exclusion chromatography, a mammalian two-hybrid assay and *in* vitro pulldowns (Ogawa *et al.*, 2007). Results gained here over the course of this dissertation, however, especially those obtained from absolute molecular

weight calculations based on SEC-MALS experiments, cast doubt on some of the interpretations offered by Ogawa *et al*.

Among the evidence advanced in support of the dimerization of full-length 32 kDa Gemin2 included the 66 kDa apparent molecular weight with which it elutes from an analytical size-exclusion chromatography column (Ogawa *et al.*, 2007). Indeed, analytical size exclusion chromatography experiments conducted here demonstrated that Gemin2$^{95\text{-}280}$, Gemin2$^{12\text{-}280}$-SMN$^{26\text{-}51}$ and Gemin2$^{12\text{-}280}$ all elute with apparent molecular weights in excess of those expected for monomers. However, both molecular oligomerization and non-standard hydrodynamic properties readily explain the elution of a peak in a size-exclusion chromatography experiment with an unusually abbreviated retention time. Here, the addition of multi-angle light scattering to the size-exclusion chromatography analysis allows the calculation of absolute molecular weights for the protein species comprising each elution peak; this SEC-MALS analysis neatly confirms that both Gemin2$^{95\text{-}280}$ and Gemin2$^{12\text{-}280}$, alone or in complex with SMN$^{26\text{-}51}$, exist as exclusive monomers in solution at micromolar concentrations, suggesting a misinterpretation of the sizing profile of full-length Gemin2 in the Ogawa *et al.* study.

Positive results obtained using mammalian two-hybrid assays and interaction assays involving both *in vitro* synthesized Gemin2 from rabbit reticulocyte lysate and bacterially expressed GST and His-tagged protein are also cited in support of a Gemin2 self-interaction, a conclusion that runs contrary to the observation of Gemin2's

monomeric solution state made here. An indirect association through the established self-interaction of endogenous SMN readily explains an apparent Gemin2-Gemin2 interaction in the context of mammalian two-hybrid experiments or pulldowns involving protein synthesized from rabbit reticulosyte lysate (Lorson *et al.,* 1998; Young *et al.*, 2000).

A note of caution must also be struck when considering any experiment employing purified full-length Gemin2 in solution, such as the *in vitro* pulldowns using GST-Gemin2 and His-tagged Gemin2 furnished as evidence of a direct and specific Gemin2 self-association. Full-length Gemin2 was observed over the course of this dissertation work to exhibit exceptionally poor behavior in solution either as an N-terminal GST-fusion or in its wildtype state; it is poorly soluble, inclined to proteolysis and aggregation and, perhaps germane to this discussion, extremely prone to promiscuous and irreversible binding to a large number of targets (including dextran-agarose size-exclusion resin and all nitrocellulose-based membranes). Indeed, within the context of these studies, only at low protein concentrations in high ionic strength buffer containing glycerol could full-length Gemin2 be stabilized for SEC-MALS analysis. It seems necessary to consider that the interaction described as a relevant direct Gemin2-Gemin2 binding event may actually result, in the physiological ionic-strength buffer conditions described, from a non-specific interaction of full-length His-tagged Gemin2 with GST-Gemin2, the GST tag of its protein target or with the glutathione agarose itself.

In the NMR structure of Gemin2$^{95-280}$-SMN$^{26-51}$, careful consideration of the arrangement of the seven α-helices of Gemin2 with respect to the bound SMN helix draws the attention of the observer to the long α1 helix, which runs antiparallel to SMN and makes numerous hydrophobic contacts with it along its length, but very few contacts with any other regions of Gemin2 (Figure 3.9). The likelihood that that helix would maintain its position in the absence of SMN and any other intermolecular anchors seems remote, and a model suggests itself in which Gemin2 adopts a less restrained conformation when unbound, before "folding on" to SMN.

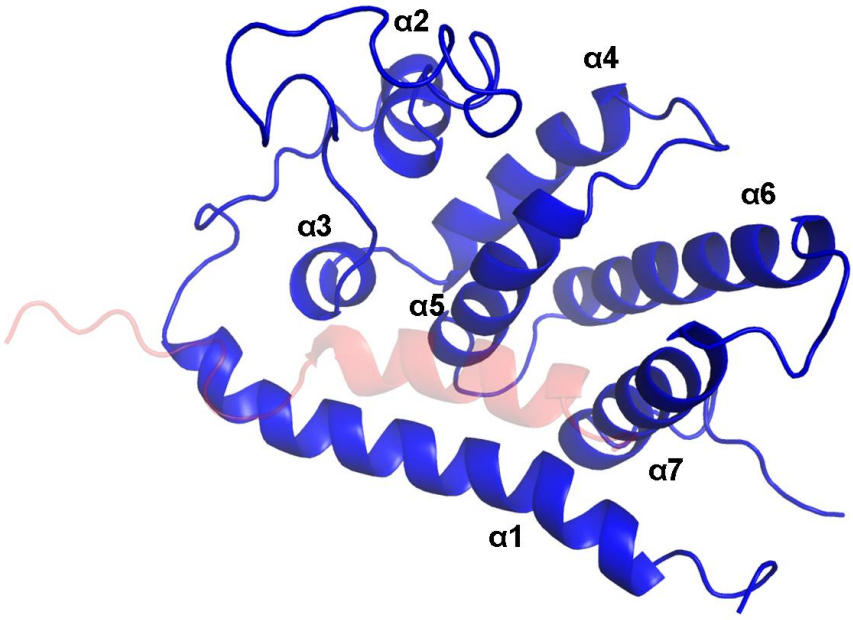


Figure 3.9 The α1 helix of Gemin2 primarily interacts with SMN. A view of the Gemin2$^{95-280}$-SMN$^{26-51}$ complex in which SMN (faded red) is de-emphasized to draw attention to the position of the α1 helix, forming very few interactions with other regions of Gemin2 despite an extensive interface with SMN.

The experiments detailed in this chapter describing both the Gemin2$^{95-280}$-SMN$^{26-51}$ complex and unbound Gemin2$^{95-280}$ support a model in which the latter is stabilized upon SMN binding. Both analytical ultracentrifugation and small-angle x-ray scattering studies allowed the calculation of basic structural parameters that indicate an elongation of Gemin2$^{95-280}$ relative to the fairly compact structure adopted by Gemin2$^{95-280}$-SMN$^{26-51}$, with the former evincing a significantly larger frictional ratio and radius of gyration than its SMN-bound counterpart. Calculation of the molecular envelopes of the two species based on small-angle x-ray scattering add to the body of evidence supporting a substantial conformational change in Gemin2 upon SMN binding, showing more extended shape reconstructions comprising a much more variable ensemble from the Gemin2$^{95-280}$ scattering data than those determined for Gemin2$^{95-280}$-SMN$^{26-51}$.

The apparent elongation of unbound Gemin2$^{95-280}$ could be interpreted as a destabilization and partial unfolding of the N-terminal 30-40 residues of the construct, as examination of the α1 helix comprising those residues in the context of SMN binding might suggest. Alternatively, an ordered rearrangement of Gemin2's helices into a different, more extended but similarly stable protein fold could elicit a similar modification of its structural parameters. Evidence from both the ^{1}H-^{15}N HSQC of unbound Gemin2$^{95-280}$ and the Kratky analysis of its scattering data argue for the acquisition of disorder in the absence of SMN. Compared to the broad peak dispersion and sharp linewidths characteristic of a folded, soluble protein and present in the ^{1}H-^{15}N HSQC of Gemin$^{95-280}$ bound to SMN$^{26-51}$, the same spectrum of Gemin2$^{95-280}$ alone

shows considerable line broadening and a loss of peak dispersion, typically hallmarks of protein unfolding (Figure 3.4). Although peak broadening might also have resulted from Gemin2 self-association, SEC-MALS analysis demonstrating that Gemin2$^{95\text{-}280}$ exists as a monomer in solution establishes that that hypothesis is not operative in this case; the alterations in the characteristics of the HSQC spectrum of Gemin2 in the absence of bound SMN are more likely the result of a gain in conformational flexibility relative to the folded heterodimer.

Kratky analysis of the two sets of scattering data corroborates the notion that Gemin2 acquires a partially unfolded character in the absence of SMN. The Kratky curve ($I(Q)*Q^2$ *v.* Q, where Q is the scattering vector) characteristically increases monotonically for random coil, while peaking and tapering to zero for a folded protein; while the Kratky plot of Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ complex scattering data conforms to the second set of criteria, confirming its folded, globular nature, Gemin2$^{95\text{-}280}$ alone demonstrates features of both theoretical curves, suggesting that it is partially but not entirely folded when unbound (Figure 3.5).

The biological relevance of partially unfolded Gemin2 in the absence of SMN is uncertain; indeed, it may not exist in appreciable quantities in the cell in the absence of its stabilizing binding partner. Destabilization of its fold in the absence of SMN could explain the repeated observation that a decrease in cellular SMN levels yields a concomitant decrease in Gemin2 (Jablonka *et al.*, 2002; Shpargel & Matera, 2005).

Other cellular functions have been attributed to Gemin2, however, and the mutual involvement of SMN in those cases is uncertain, inviting speculation about additional binding partners that could bind in place of SMN to stabilize the Gemin2 fold or the possibility of Gemin2 operating outside of the fold observed in its SMN-defined structure in some circumstances. In the reported interaction of Gemin2 with HIV-1 integrase (IN), for example, by which it facilitates the assembly of reverse transcriptase on viral RNA, the direct interaction between Gemin2 and IN is mapped to residues that would comprise the face of Gemin2 opposite SMN binding in the current NMR and crystallographic models. The involvement of SMN in the process is uncertain; SMN knockdown depletes cellular Gemin2 so efficiently that it became difficult to distinguish between a direct and an indirect effect of SMN on HIV infection (Hamamoto *et al.*, 2006; Nishitsuji *et al.*, 2009). Without SMN, it seems unlikely that the five α-helices (α2-α6) contained in the IN-binding domain of Gemin2 could adopt their SMN-bound conformation, predicting the presence of a non-SMN binding partner to stabilize Gemin2.

Gemin2 is also reported to bind Rad51 directly *in* vivo and to contribute to the ability of that latter to repair double-stranded DNA breaks through homologous recombination. Although the presence of an SMN-Gemin2 chimera (in which the C-terminus of the former was fused to the N-terminus of the latter) was reported to further enhance Rad51-mediated homologous pairing, the structural improbability of the engineered molecule makes a true evaluation of the role of SMN in the process difficult. In its

contribution to homologous recombination as in HIV-1 infection, however, Gemin2 is also likely to require an unidentified binding partner to stabilize its fold if SMN is indeed absent.

Returning to the discussion of the role of Gemin2 in snRNP biogenesis, another question of Gemin2 function in the absence of SMN invites speculation. Both SMN and Gemin2 are highly conserved proteins, consistent with the key functional role they play in cellular housekeeping, and sequence alignments reveal clear orthologues of both proteins from mammals to the fission yeast *S. pombe*. Gemin2, however, also possesses a yet more primitive orthologue named Brr1 in the family of budding yeasts related to and including *Saccharomyces cerevisiae*, even though these organisms lack a recognizable SMN (Liu *et al.*, 1997; Noble & Guthrie, 1996a). Brr1 is demonstrably involved in snRNP biogenesis, fortifying its claim as a functional relative of Gemin2 (Noble & Guthrie, 1996b). An alignment of Brr1 sequences from several species of budding yeast to human Gemin2 demonstrates the strongest conservation in regions corresponding to α-helices in human Gemin2 (Figure 3.10).

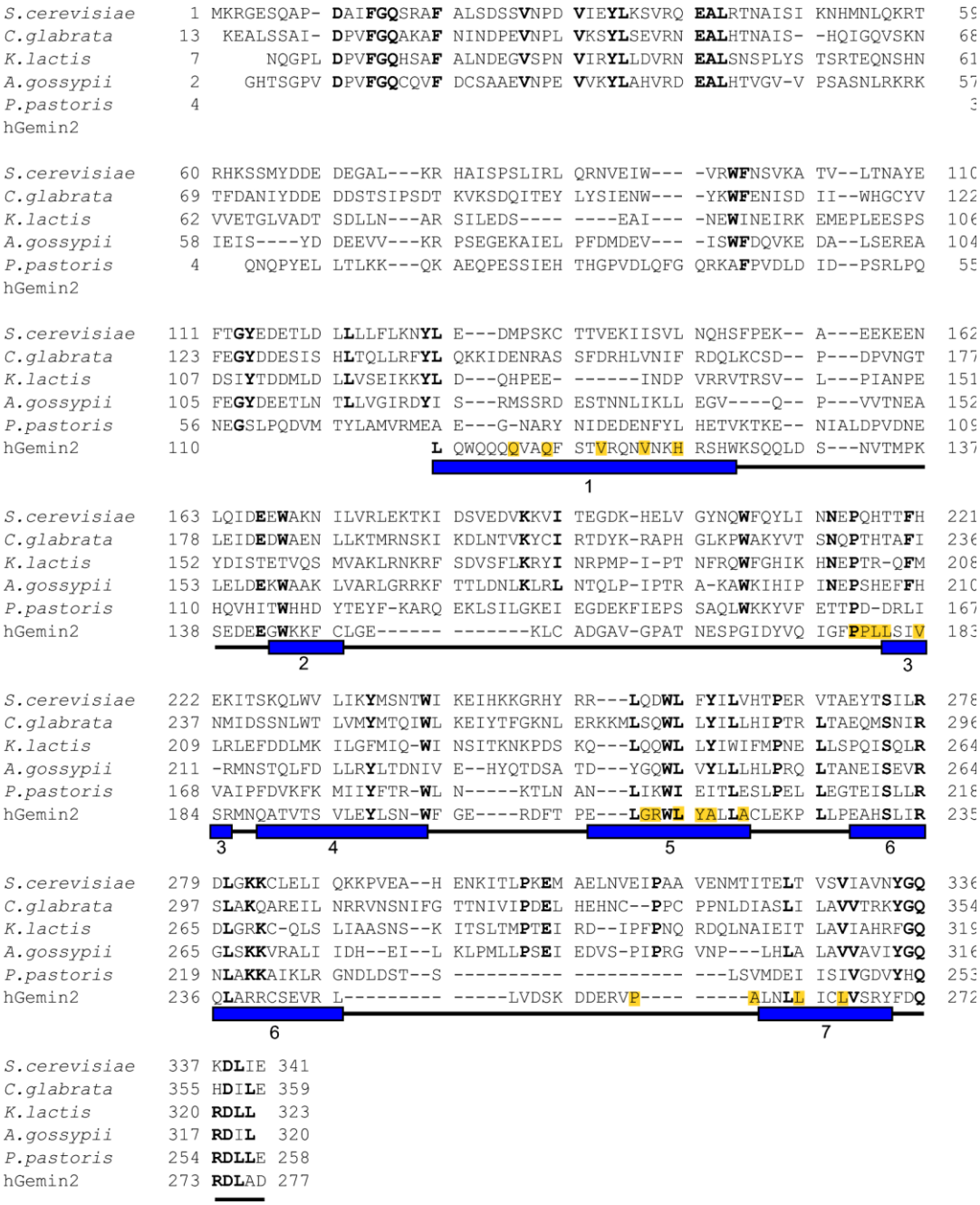


Figure 3.10 Alignment of Brr1 orthologues with human Gemin2. Gregory Van Duyne, Ph.D., performed the sequence alignment, obtained from four iterations of psi-Blast (Altschul *et al.*, 1997) searching with *S. cerevisiae* Brr1 as query sequence. Locations of helices are shown for Gemin2 and the regions in Gemin2 that are close to bound SMN are shaded yellow.

The same careful consideration of the arrangement of the seven α-helices of Gemin2 that suggested the possibility of instability in the absence of the SMN peptide also provides a lens through which to consider the unusual situation of Brr1. Although the residues that are closely involved in binding SMN are not strictly conserved in Brr1, they are similar in type, suggesting that they would also form a hydrophobic binding surface in Brr1 with a character similar to the SMN binding cleft that could similarly accommodate an amphipathic helix. A heretofore-unidentified SMN or SMN-like binding partner may fill that role. It is also possible that Brr1 may fill it itself. In this alternative model, the Brr1 α1 helix fills the binding pocket itself rather than flanking a binding helix from another protein; it is spatially positioned to do so, and shows the least sequence conservation in this region, perhaps consistent with an alternative role in *S. cerevisiae.*

Zhang *et al.* recently presented the x-ray crystallographic structure of full-length Gemin2 bound to an SMN peptide as well as to the Sm D1/D2/E/F/G pentamer. This structure established, for the first time, a functional role for Gemin2 in coordinating five Sm proteins as a pentameric intermediate until the binding of the snRNA component of spliceosomal U-snRNPs and addition of the Sm D3/B dimer to complete the canonical Sm protein heptameric ring that characterizes of those particles. Structurally, two separate domains of Gemin2 interact with the Sm pentamer; Sm D1 and D2 bind the globular C-terminal SMN-binding domain, on the face opposite the SMN peptide, while Sm E, F and G, though contiguous with D1 and D2, appear to interact with an extended

N-terminal domain of Gemin2 comprising approximately residues 12-100 that is ill-defined in the crystal structure but which appears to evince some α-helical character (Zhang *et al.*, 2011).

Gemin2 receives its Sm protein pentamer substrate from the chaperone pICln, which dissociates from the SMN-Gemin2 complex after delivery of its cargo (Chari *et al.*, 2008). Before this interaction, however, the conformation occupied by Gemin2 and by its N-terminal Sm protein-binding arm remains unexplored. The comparatively poor definition of that region in the crystal structure and its relative paucity of apparent secondary structure make a prediction of its structure in the absence of Sm proteins difficult.

In low-resolution shape reconstructions of Gemin2$^{12-280}$-SMN$^{26-51}$ based on small-angle x-ray scattering data, the N-terminal Sm protein-binding region forms a tubular armlike protrusion that extends away from the SMN-binding domain and which can be reproduced in each shape reconstruction and in their filtered average structure (Figure 3.8A,B). Docking the Gemin2$^{95-280}$-SMN$^{26-51}$ NMR structure by rigid body modeling into the globular domain of the Gemin2$^{12-280}$-SMN$^{26-51}$ molecular envelope positions the α1 helix to extend the region of the envelope likely representing the N-terminal Sm protein-binding arm (Figure 3.8C). Although the lack of electron density in regions of the Gemin2 Sm protein binding arm in the crystal structure suggested conformational flexibility, a state expected to persist in the absence of Sm protein binding, that region

appears to occupy a fixed extended conformation in the molecular envelopes generated from SAXS data. Gemin2 may create a pre-formed binding surface for its Sm substrates rather than adopting that structure upon binding, a structure that would theoretically be available for interactions with other SMN complex components as well.

The solution studies described here propose a clear role for SMN binding in stabilization of the tertiary fold of Gemin2, while suggesting that, contrary to previous reports, Gemin2 does not detectably self-interact either alone or bound to SMN. The low-resolution solution structure of full-length Gemin2 bound to SMN^{26-51} revealed a defined extended conformation of the Gemin2 Sm protein-binding arm, broadening structural understanding of the role of Gemin2 in snRNP assembly and contributing to the body of knowledge that will permit analysis of its roles in other biological systems.

Materials and Methods

DNA constructs

The construction of all molecular clones used in this chapter was detailed in the Materials and Methods section of Chapter II and is summarized in Table 2.2.

Expression and purification of human Gemin2 and SMN protein constructs

Gemin2$^{95-280}$, Gemin2$^{95-280}$-SMN$^{26-51}$ and Gemin2$^{12-280}$-SMN$^{26-51}$ were expressed and purified as described in the Materials and Methods section of Chapter II.

Size-exclusion chromatography in-line with multi-angle light scattering

The absolute molecular weights of Gemin2$^{95-280}$ and the SMN$^{26-51}$-Gemin2$^{95-280}$ and SMN$^{26-51}$-Gemin2$^{12-280}$ complexes were determined using multi-angle light scattering (MALS) in-line with a Superdex 75 10/300 column (GE Healthcare). The column was calibrated using the following proteins (Bio-Rad): thyroglobulin (670,000 Da), bovine gamma globulin (158,000 Da), chicken ovalbumin (44,000 Da), equine myoglobin (17,000 Da) and vitamin B-12 (1350 Da). Blue dextran (Sigma) was used to define the void volume of the column.

Purified proteins, 20µl at 1-10mg/ml, were injected onto the column at 0.5 ml/min in buffer containing PBS (10 mM Na_2HPO_4, 2 mM KH_2PO4, 137 mM NaCl, 2.7 mM KCl, pH 7.4) plus 2mM dithiothreitol (DTT) at room temperature. The scattered light

intensity of the column eluant was recorded at 16 different angles using a DAWN-HELEOS MALS detector (Wyatt Technology Corp.) operating at 658 nm after calibration with 20µl of 20mg/ml lysozyme (Sigma). Protein concentration of the eluant was determined using an in-line Optilab DSP interferometric refractometer (Wyatt Technology Corp.). The weight-averaged molecular weight of species within defined chromatographic peaks was calculated using the ASTRA software version 5.2 (Wyatt Technology Corp.), by construction of Debye plots ($KC/R\,\Theta$ *versus* $\sin^2[\Theta/2]$) at one-second data intervals. The weight-averaged molecular weight was then calculated at each point of the chromatographic trace from the Debye plot intercept, and an overall average molecular weight was calculated by averaging across the peak.

NMR Spectroscopy and Analysis

NMR samples of ^{15}N-labeled SMN$^{26\text{-}51}$-Gemin2$^{95\text{-}280}$ and Gemin2$^{95\text{-}280}$ were prepared in Shigemi NMR tubes in buffer containing 50mM Na/K phosphate, pH 6.5, 50 mM NaCl, 50 mM DTT, 50 µM EDTA, 0.2mM sodium azide and 95% H_2O/5% D_2O. Two-dimensional ^{1}H/^{15}N heteronuclear single quantum correlation (HSQC) spectra were collected at 25°C at 750MHz (^{1}H) on a Bruker AVANCE III NMR spectrometer equipped with a cryo-probe. Proton chemical shifts were referenced to DSS in the buffer, while ^{15}N chemical shifts were indirectly referenced. Data were processed using FELIX (Molecular Simulations, San Diego, CA, USA) and analyzed in SPARKY (Goddard & Kneller, 2008).

Sedimentation Velocity

Sedimentation velocity ultracentrifugation experiments were performed at 20 °C with an XL-A analytical ultracentrifuge (Beckman Coulter) and a TiAn60 rotor with two-channel charcoal-filled Epon centerpieces and quartz windows. Complete sedimentation velocity profiles of Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ and Gemin2$^{95\text{-}280}$ (18.3 μM and 8.2 μM, optical density of 0.8 and 0.3 at $A_{280,}$ respectively) in 1x PBS pH 7.4 were collected every 60 seconds at 42,000 rpm for 200 scans, followed by data analysis using the continuous size-distribution (c(s)) analysis of the Lamm equation in SEDFIT (Schuck & Rossmanith, 2000). After optimizing meniscus position and fitting limits, the sedimentation coefficient (s) and best-fit frictional ratio (f/f_0) was determined by iterative least squares analysis.

Small-angle x-ray scattering

Small-angle x-ray scattering data for Gemin2$^{95\text{-}280}$ and Gemin2$^{95\text{-}280}$-SMN$^{26\text{-}51}$ were collected at the Advanced Photon Source beam line 18-ID (BIOCAT) by Kushol Gupta, Ph.D., who also provided assistance with subsequent data processing and analysis. Samples were dialyzed against 100% D_2O buffer (20mM Na/K PO_4, 50 mM NaCl, 2mM DTT) and were centrifuged at 10,000 x g for ten minutes at 4^{o}C immediately before data collection. X-ray radiation at 12 kEV was used to collect 10-15 individual one second exposures while the sample was oscillated in a quartz capillary to minimize radiation damage. Data were corrected for the intensity of the incident radiation and reduced to provide one-dimensional intensity profiles as a function of Q (which is equal

to $4\pi\sin\theta/\lambda$, where 2θ is the scattering angle) using the data software Igor Pro. Accessible scattering was recorded in the range of $0.006 < Q < 0.35$ Å^{-1}.

Small-angle x-ray scattering data for the Gemin2$^{12\text{-}280}$-SMN$^{26\text{-}51}$ complex were collected at the Advanced Light Source Beamline 12.3.1 (SIBYLS), also by Kushol Gupta, Ph.D. Samples were centrifuged at 3,000 rpm for 10 min at 4°C prior to data collection. Data were collected using a 96-well plate handling sample robot, as previously described (Hura *et al.*, 2009). All samples were characterized with 0.5, 1, and 6 second exposures at 20°C, at a wavelength of 1 Å. Data were automatically reduced using custom software to provide one-dimensional intensity profiles as a function of Q. Accessible scattering was recorded in the range of $0.010 < Q < 0.35$ Å−1.

In all cases, the forward scattering from the samples studied was recorded on a CCD detector and circularly averaged to yield one-dimensional intensity profiles as a function of Q. Scattering from a matching buffer solution was subtracted from the data, and corrected for the incident intensity of x-rays. Replicate exposures were examined carefully for evidence of radiation damage by Guinier analysis and Kratky plot analysis. Silver behenate powder was used to locate the beam center and to calibrate the sample-to-detector distance.

Evidence of the monodispersity of the preparation was gained by analysis of linearity in the Guinier region of the scattering data and agreement of the I(0) and R_g values

determined with inverse Fourier transform analysis by the program GNOM (Semenyuk & Svergun D, 1991). When fitting manually, the maximum diameter of the particle (D_{max}) was adjusted in 10-Å increments in GNOM to maximize the goodness-of-fit parameter. This analysis also yielded determinations of R_g and I(0).

Low-resolution shapes were determined from solution scattering data using the program GASBOR. The number of dummy residues used in shape reconstruction was prescribed prior to calculation as 189 for Gemin2$^{95-280}$, 214 for Gemin2$^{95-280}$-SMN$^{26-51}$ and 294 for Gemin2$^{12-280}$-SMN$^{26-51}$ based on knowledge of the amino acid composition of the proteins and the 1:1 stoichiometry of the Gemin2-SMN complexes. Ten independent calculations were performed for each data set using default parameters. No symmetry constraints were applied. The ten independent reconstructions were then averaged and filtered to a final consensus model using the DAMAVER suite of programs (Volkov & Svergun, 2003). Bead models were visualized in PyMOL (Delano, 2002).

Chapter IV: Oligomerization properties of the *Schizosaccharomyces pombe* SMN-Gemin2 complex

Introduction

The SMN protein is known to self-associate, a capacity that correlates convincingly with the ability of the SMN complex to catalyze the snRNP biogenesis (Lorson *et al.*, 1998; Pellizzoni *et al.*, 1999). Despite its obvious importance, however, neither the oligomerization state of the SMN protein nor the molecular role played by the SMN oligomer in snRNP assembly has been established. Certain experimental indicators point to an SMN multimer containing several subunits: the human SMN complex, purified from HeLa cells, sediments on sucrose gradients as a ribosome-sized 30S-70S species (Gabanella *et al.*, 2005; Paushkin *et al.*, 2002), and recombinant full-length SMN-Gemin2 complex elutes in a broad peak in the void volume in size exclusion chromatography experiments. Speculation has favored the hypothesis that the complex must be sizeable, is likely heterodisperse, and contains a large and possibly non-discrete number of each component (Gubitz *et al.*, 2004).

The search for a model system in which to study SMN oligomerization suggests *Schizosaccharomyces pombe*, the most primitive organism to possess an identifiable SMN orthologue. Unlike many higher organisms, which evince orthologues to several Gemins in addition to SMN, the rudimentary *S. pombe* SMN complex contains only SMN and Gemin2 (Campion *et al.*, 2010). Proving unexpectedly tractable in solution,

the *S. pombe* SMN-Gemin2 complex provides an excellent model system for the examination of the stoichiometry and oligomerization properties of SMN in the SMN complex.

Here, a battery of biophysical techniques is employed in the analysis of the purified spSMN-Gemin2 complex and allows the determination of its concentration-dependent oligomeric state and a description of its hydrodynamic properties.

Results

The spSMN-Gemin2 heterodimer provides a model system for studying the oligomerization of full-length SMN

Despite the evidently critical role that SMN self-association plays in SMN complex-mediated snRNP assembly and SMA pathogenesis, the exact oligomerization state of the self-interacting SMN protein has never been rigorously defined. Results from early size exclusion chromatography and sucrose gradient centrifugation experiments on both the native human SMN complex and the purified human SMN-Gemin2 heterodimer showed a very large apparent molecular mass relative to globular molecular weight standards and were interpreted to indicate a complex of substantial size comprising many copies of SMN and Gemin2 (Liu *et al.*, 1997; Pellizzoni *et al.*, 1999).

The complex formed by full-length *S. pombe* SMN and Gemin2, which forms the most rudimentary functional SMN complex and proved conveniently tractable to bacterial expression and purification, served as a model minimal SMN complex for analysis of its oligomerization state. The spSMN-Gemin2 complex was first examined by analytical size exclusion chromatography, yielding a single elution peak with an apparent molecular weight of ~600 kDa with respect to globular molecular weight standards. That predicted molecular mass, like many size predictions for the human SMN complex and SMN-Gemin2 heterodimer, corresponds to a very large oligomer, here approximately 13 copies of the 44.3 kDa spSMN-Gemin2 heterodimer (Figure 4.1). The Stokes radius (R_s; the radius of a hard sphere that diffuses at the same rate as the particle in question) for the complex was calculated, based on the recorded Stokes radii of molecular weight standards, to 78.5 Å, a sizeable value consistent with the notion of a large particle.

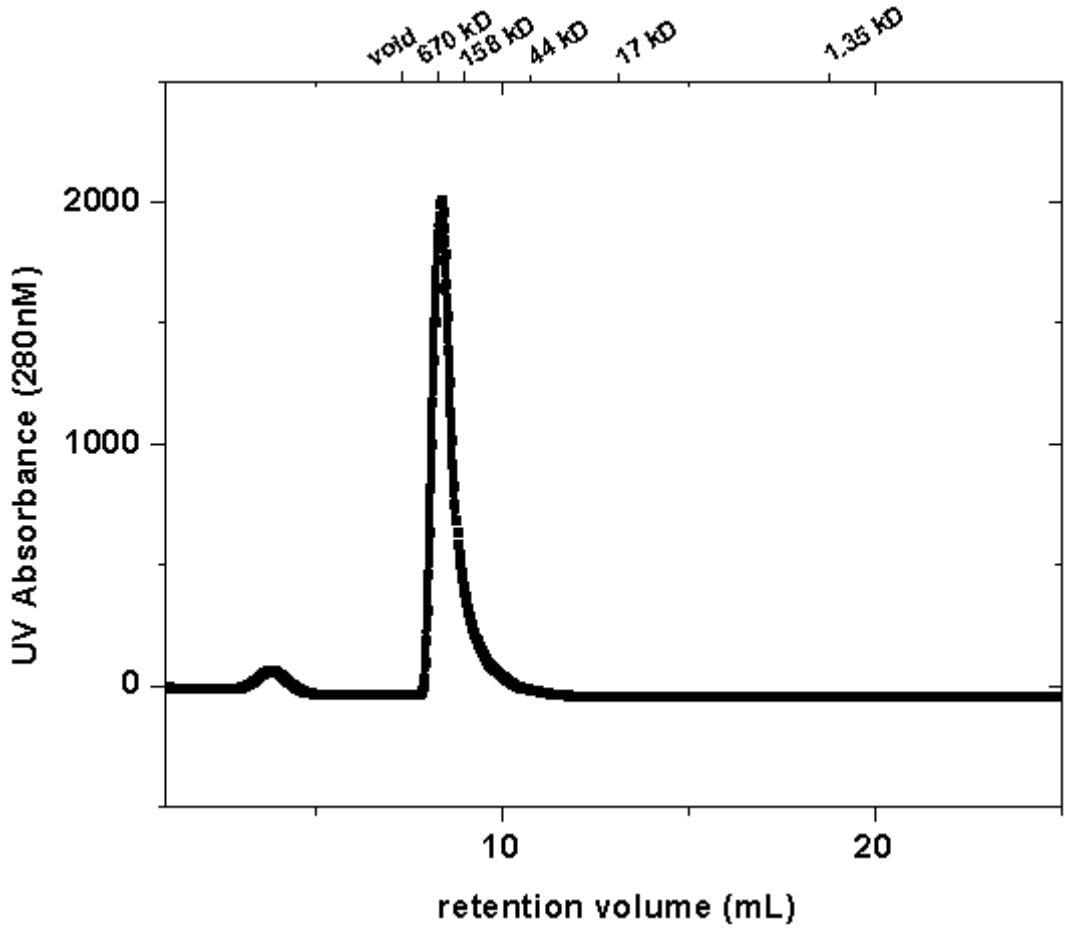


Figure 4.1 Analytical size exclusion chromatography profile of spSMN-Gemin2 on a Superdex 75 10/300 column. The elution volumes of globular protein standards are shown above the panel.

The spSMN-Gemin2 heterodimer occupies small, discrete oligomers that self-associate to form larger assemblies

To establish the absolute molecular weight, and thereby deduce the molecular inventory, of spSMN-Gemin2, the complex was examined by size-exclusion chromatography in-line with multi-angle light scattering (SEC-MALS) over a range of protein concentrations (Figure 4.2). At 10 mg/ml (226 µM), spSMN-Gemin2 eluted as a single peak with an apparent molecular weight of 310 kDa. Its mass profile across the peak

that varied from 210 kDa to 122 kDa, indicating a surprisingly smaller particle than recourse to molecular weight standards had suggested, as well as suggesting some heterodisperity in the masses of the molecules comprising the peak. Decreasing protein concentration to 5 mg/ml (113 μM) yielded a peak shift to an apparent molecular weight of 270 kDa and a mass profile spanning 187 to 87 kDa; another decrease to 2 mg/ml (46 μM) followed the same trend, with a peak shift to an apparent molecular weight of 190 kDa. At two mg/ml, however, the mass profile held a steady value of approximately 80 kDa, indicating the more uniform presence of a species possessing that molecular weight, intriguingly consistent with an 88 kDa spSMN-Gemin2 dimer. A further concentration decrease to 1 mg/ml (23 μM), the lowest concentration at which it was possible to extract meaningful light scattering data, demonstrated no change in the characteristics of the eluted peak, with the same retention time and mass profile indicative of a dimer of spSMN-Gemin2 heterodimers. The most fundamental oligomer of the spSMN-Gemin2 complex, then, far from the predicted enormous assembly, appears to be a dimer capable of forming higher-order oligomers in a concentration-dependent fashion. In an interesting instance of corroboration, dimerizations of an MBP fusion to the YG oligomerization domain of both human and *S. pombe* SMN were crystallized by Renee Martin, a graduate student in the Van Duyne laboratory at the University of Pennsylvania.(Martin, 2011)

Most trenchantly, no aspect of the SEC-MALS analysis of spSMN-Gemin2 substantiates the existence of any species possessing the large apparent molecular

weight with which the complex eluted in the original analytical size-exclusion chromatography experiment. Rather than comprising a large globular protein containing numerous copies of its component proteins, spSMN-Gemin2 appears to form a much smaller discrete oligomer or oligomers with large hydrodynamic radii, possibly due to an extended shape or to some intrinsic disorder.

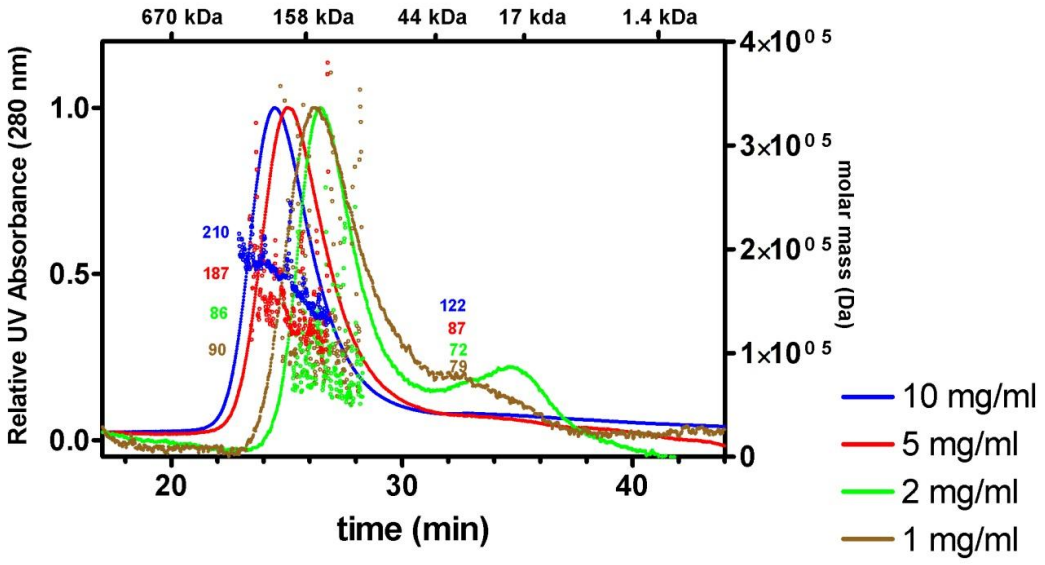


Figure 4.2 Representative SEC-MALS analyses of spSMN-Gemin2 injected at 10 mg/ml (blue), 5 mg/ml (red), 2 mg/ml (green) and 1 mg/ml (brown) on a Superdex 200 10/300 column.
The highest and lowest molecular weights sampled in each mass profile are labeled in the same color as the chromatogram for that protein concentration. The elution volumes of globular protein standards are shown above the panel.

A number of self-association models might explain a decreasing trend in the weight-averaged molecular weight of a species over a decreasing range of protein concentrations. Sedimentation equilibrium analytical ultracentrifugation was employed to identify models of self-interaction of the spSMN-Gemin2 heterodimer consistent with observed data for spSMN-Gemin2 and to eliminate incompatible models (Figure 4.3).

The variety of fitting models tested, with goodness-of-fit calculated here by reduced chi-squared values near one and small root-mean-square deviations, is summarized in Table 4.1. With data collected at three speeds and three concentrations (14.0μM, 8.8μM, 3.5μM), spSMN-Gemin2 was best fit by modeling the data as a dimer-tetramer equilibrium, an interchange of two and four spSMN-Gemin2 heterodimers with a dissociation constant (K_D) around 1.8μM. The addition of higher order species to the dimer-tetramer model (dimer-tetramer-hexamer or dimer-tetramer-octamer equilibria) perturbed the fit very little, but yielded calculated K_D values in the molar range for the secondary association, so high that no measurable higher order oligomers can be considered to be present. The addition of a monomer to the model (monomer-dimer-tetramer equilibrium) yielded a substantially worse fit to the data, as did the application of monomer-trimer, monomer-trimer-hexamer and monomer-tetramer models; all models assuming monomers were considered inappropriate descriptions of the spSMN-Gemin2 data.

Table 4.1 Fit statistics for *S. pombe* SMN-Gemin2 sedimentation equilibrium analytical ultracentrifugation data to a variety of models. Goodness-of-fit is indicated by the reduced chi squared value (χ_r^2).

Model	KD_1	KD_2	Fit (χ_r^2)	RMSD
2 – 4	1.8 μM	-	1.3	0.004
2 – 4 – 8	2.6 μM	152 mM	1.4	0.004
2 – 4 – 6	2.6 μM	> 1M	1.4	0.004
1 – 3	5.2 μM	-	6.4	0.008
1 – 3 – 6	1.1 μM	> 1 M	21	0.014
1 – 2 – 4	1μ-14	> 1 M	21	0.014
1 – 4	1.8 μM	-	10	0.009

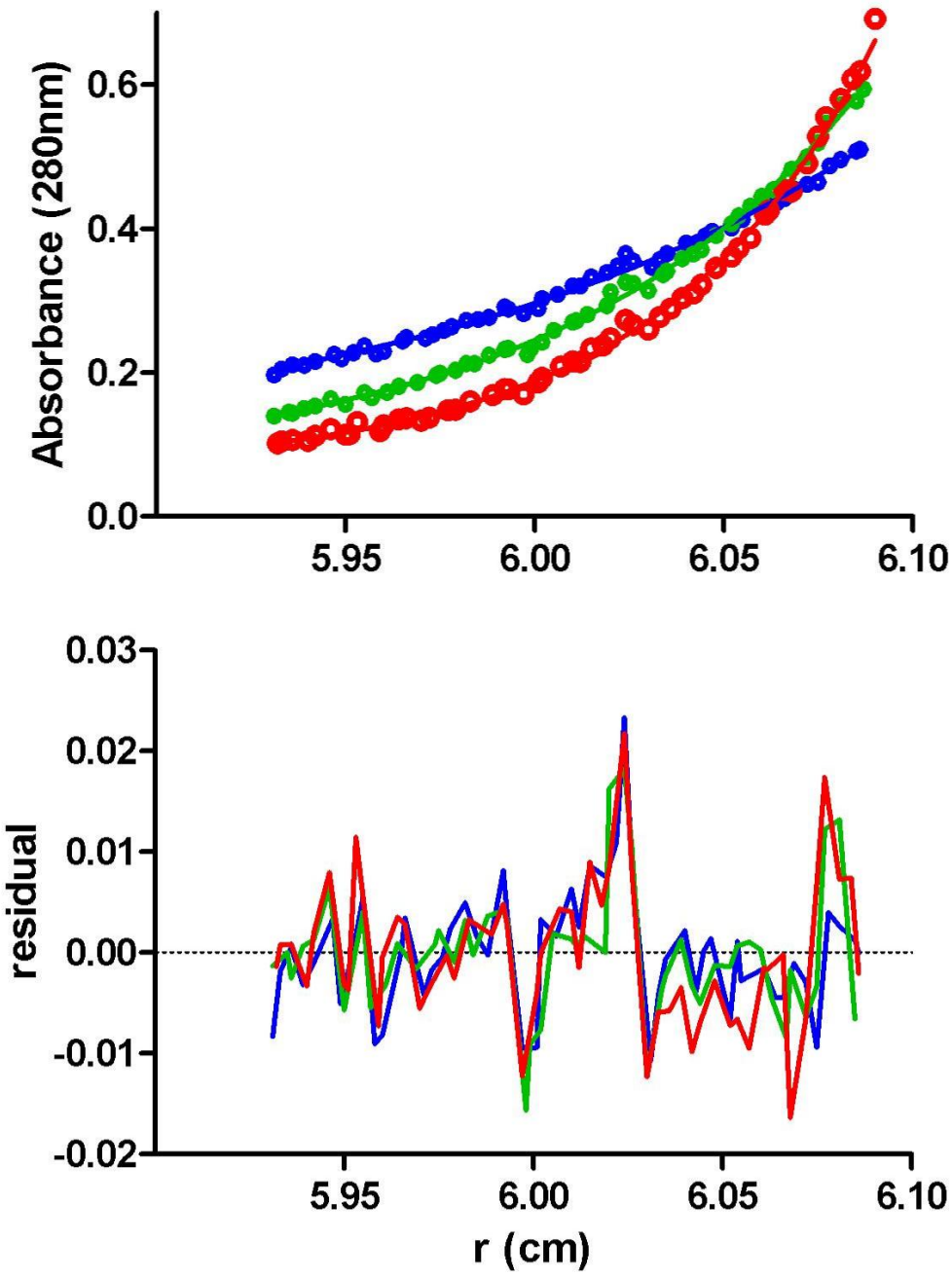


Figure 4.3 Representative sedimentation equilibrium analytical ultracentrifugation experiment on the *S. pombe* SMN-Gemin2 complex Experiments conducted at 4°C at 14 µM, 8.8 µM and 3.5 µM. Here the curves are fit to a dimer-tetramer $(spSMN\text{-}Gemin2)_2 \leftrightarrow (spSMN\text{-}Gemin2)_4$ equilibrium with residuals of the experimental fits depicted in the bottom panel.

The spSMN-Gemin2 complex possesses a partially disordered character

Given that the largest oligomer sampled by the spSMN-Gemin2 heterodimer at the concentrations used for analytical size exclusion chromatography is a 176 kDa tetramer, one explanation for spSMN-Gemin2's atypical elution profile is that the protein complex possesses either a structured but elongated shape or some regions of intrinsic disorder, granting it a large hydrodynamic radius.

Shape parameters derived from sedimentation velocity analytical ultracentrifugation and small-angle x-ray scattering confirm that spSMN-Gemin2 does not occupy a compact shape. Sedimentation velocity data for spSMN at 10 μM was collected at 20°C and fit well to a continuous c(s) distribution yielding a single species with a sedimentation coefficient of 6.5S. From the sedimentation coefficient, it becomes possible to calculate the frictional ratio f/f_o of a particle, as discussed mathematically in Chapter II.

The frictional ratio reflects the degree of elongation of a particle and carries a value of ~1.2 for a globular protein, increasing with the degree of elongation displayed by the particle. The weight-averaged frictional ratio for the 6.5S complex was calculated to be 1.67, indicating that spSMN-Gemin2 indeed possesses an extended conformation (Figure 4.4).

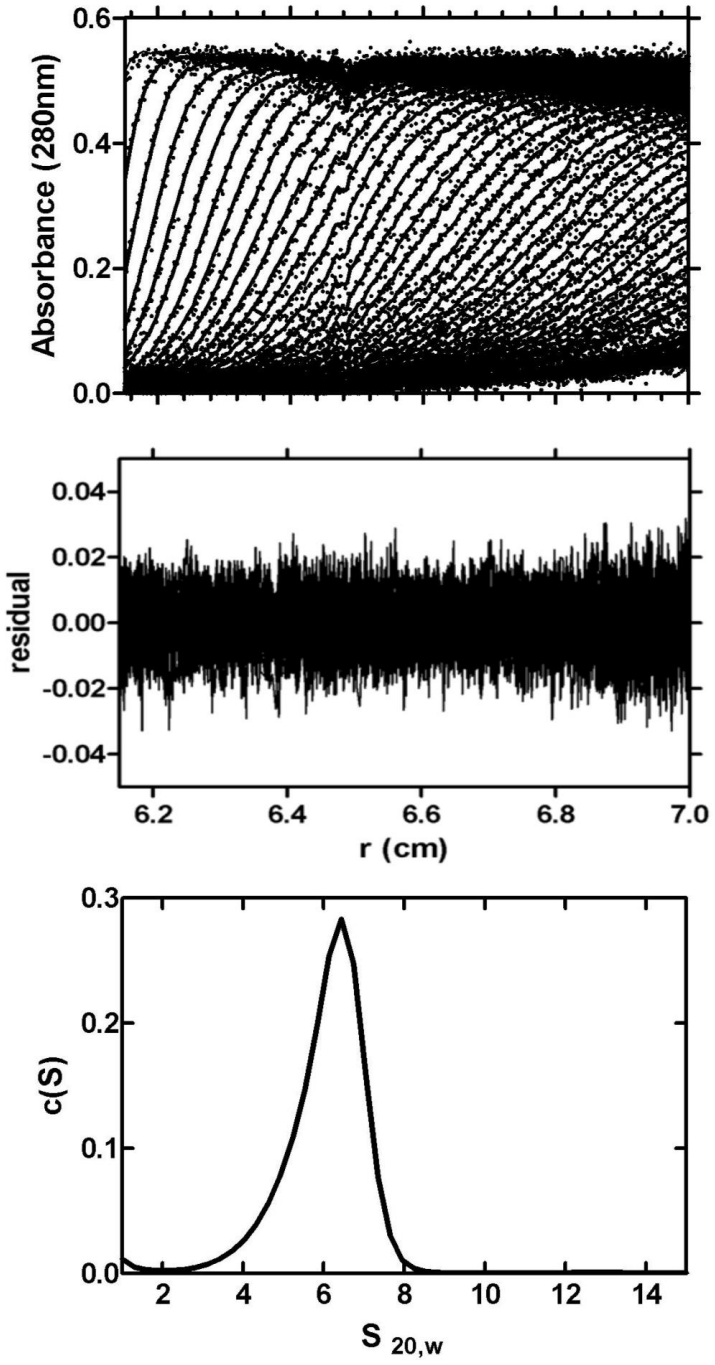


Figure 4.4 Sedimentation velocity analytical ultracentrifugation experiment on the *S. pombe* SMN-Gemin2 complex at 20°C. Sedimentation velocity absorbance traces of spSMN-Gemin2 at 280 nm occupy the top panel, with residuals of the experimental fits in the middle panel and a continuous sedimentation coefficient distribution (c(S)) of the protein in the bottom panel.

Small-angle x-ray scattering analysis was also employed to calculate parameters relevant to a consideration of the relative elongation of the spSMN-Gemin2 complex at 10 mg/ml (226 µM), a concentration at which the protein is predicted to form predominantly tetramers of the heterodimer. Based on Guinier analysis of the scattering curve at very low scattering angles, the spSMN-Gemin2 complex demonstrated a radius of gyration of 85.9 Å; a P(r) distance distribution function generated from the collected intensity using an inverse Fourier transform yielded a measure of the particle's maximum dimension of 260 Å (Figure 4.5). It must be recalled that for the case of a system in equilibrium, these values will represent averaged values over the ensemble. Even as ensemble averages, however, the calculated values are revealing. For a perfect sphere with a partial specific density *v* of 0.73 g/cm^3, the calculated D_{max} ranges from 93.4 Å for an 88.6 kDa dimer to 118.0 Å for a 177.2 kDa tetramer, significantly smaller values than the D_{max} calculated for the spSMN-Gemin2 protein complex.

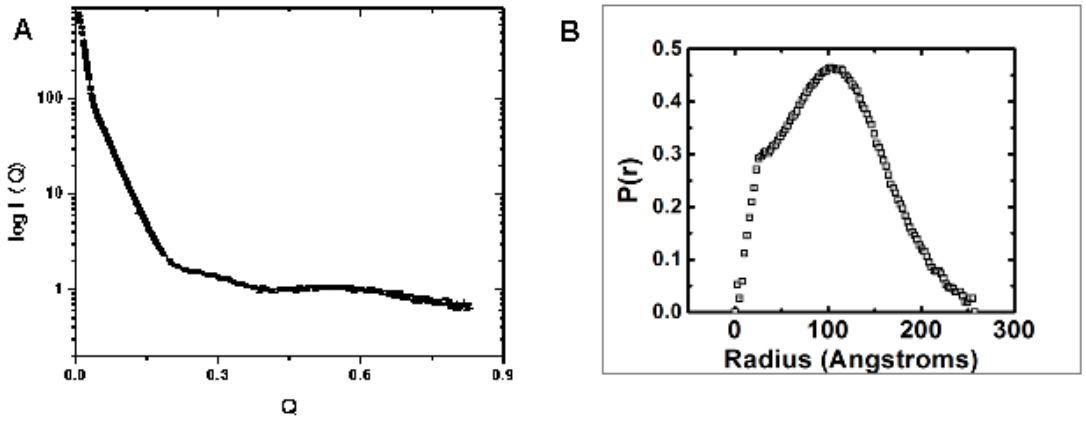


Figure 4.5 SAXS analysis of *S. pombe* SMN-Gemin2.
A) Small-angle scattering data from spSMN-Gemin2. Shown in black squares is the recorded intensity as a function of Q. B) P(r) shape functions for spSMN-Gemin2.

Both sedimentation velocity and SAXS shape parameters demonstrate clearly that spSMN-Gemin2 is an elongated species without indicating whether it possesses a disordered character. A simple examination of the primary sequence of spSMN, however, suggests that properties of that protein favor the latter interpretation. While a disorder prediction for spGemin2 by the DISOPRED2 server shows few unstructured regions, the same analysis performed on spSMN indicates an enormous region of predicted disorder (where the prediction of disorder is greater than 0.5) spanning the length of the protein from its conserved N-terminal region of Gemin2-binding to its C-terminal YG box oligomerization domain (Figure 4.6A, B) (Ward *et al.*, 2004).

A two-dimensional charge-hydropathy plot provides another means of identifying ordered and disordered proteins. Based on the observation that intrinsically disordered proteins often possess a combination of many charged residues and few hydrophobic ones, a charge-hydropathy plot graphs the mean net Kyte-Doolittle hydropathy (calculated here by the Davidson College GCAT server) of the protein against its mean net charge. Proteins with more disordered character and those that are more ordered are cluster in different segments of the plot and are largely segregated by a linear boundary. SMN falls (mean net hydropathy = 0.402; mean net charged calculated at neutral pH = 0.09) within the disordered region of the plot (Figure 4.6C) (Kyte & Doolittle, 1982; Uversky *et al.*, 2000).

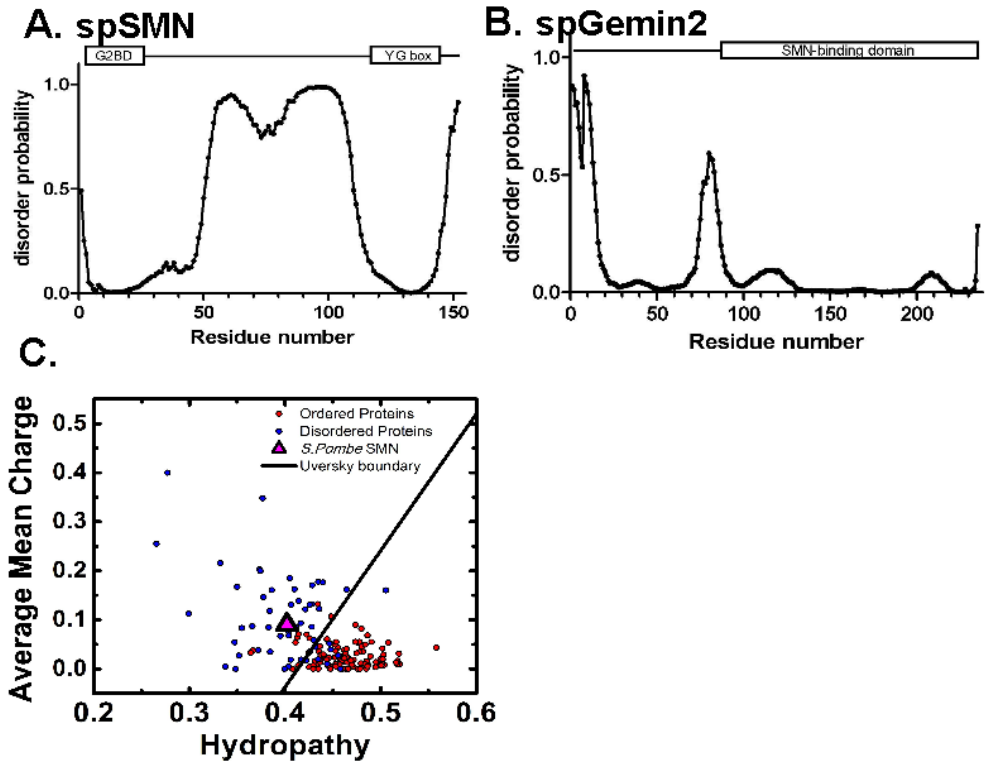


Figure 4.6 Disorder predictions for spSMN and spGemin2.
A) DISOPRED prediction of disorder for spSMN. A residue with a disorder probability > 0.5 is considered disordered. Known structural domains are depicted above the curve and correspond to the residues. B) DISOPRED disorder prediction for spGemin2. C) Charge-hydropathy plot for spSMN.

The disorder predicted for spSMN might be alleviated upon Gemin2 binding or self-association, a Kratky analysis was employed as an experimental diagnostic measure of protein disorder. Based on small angle scattering data, a Kratky plot depicts the intensity of the signal times the square of the scattering vector Q versus Q (where $Q = \frac{4\pi \sin\theta}{\lambda}$; 2θ is the scattering angle and λ is the wavelength) and displays, for globular proteins, a narrow symmetric peak at low Q and approaching zero as Q increases, but a curve that increases linearly with Q in the case of a fully disordered polypeptide (Doniach, 2001).

Broadening of the low Q peak is often observed for multi-domain proteins exhibiting some flexibility. The Kratky analysis of the spSMN-Gemin2 data at 10 mg/ml demonstrates characteristics of both theoretical curves, a peak at low Q that then climbs in value as Q increases (Figure 4.7). Some ordered structure is anticipated for the spSMN-Gemin2 complex, given that work presented earlier in this thesis supports a structured conformation for Gemin2 when bound to SMN; the characteristic increasing Kratky curve, however, indicates that the spSMN-Gemin2 complex is at least partially disordered, while the broad peak may prove an indicator that even the structured regions of the complex display some flexibility.

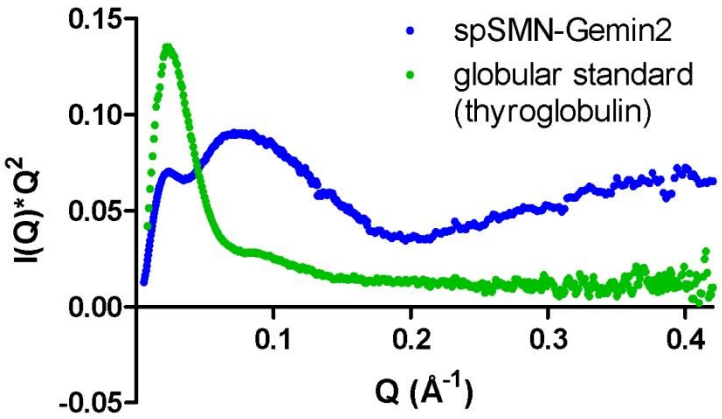


Figure 4.7 Kratky transformation of SAXS data from *S. pombe* SMN-Gemin2
S. pombe SMN-Gemin2 is depicted in blue and a globular thyroglobulin standard green.

Discussion

The self-association of SMN to form a higher-order oligomer is required for its activity in snRNP assembly. It is typically attributed to its tyrosine-glycine-rich exon6/exon7 "YG box" domain, although a region of N-terminal interaction has also been reported (Liu *et al.,* 1997; Lorson *et al.,* 1998; Ogawa *et al.*, 2007; Pellizzoni *et al.,* 1999; Young *et al.*, 2000). Certain missense mutations in the SMN YG box, identified as SMA disease mutations, demonstrably abolish SMN self-interaction *in vitro* and highlight the importance of correct oligomer formation for SMN function (Lorson *et al.,* 1998; Pellizzoni *et al.,* 1999). The precise stoichiometry assumed by SMN in the context of the SMN complex, however, remains unknown (Lorson *et al.,* 1998; Ogawa *et al.*, 2007; Otter *et al.*, 2007; Young *et al.*, 2000).

In early reports, fractionation of HeLa extracts over a size exclusion chromatography column suggested an overall molecular mass for the SMN complex greater than 300 kDa, while sucrose gradient centrifugation of the same complex from either HeLa or murine neural extracts indicated the presence of SMN in particles ranging from 30S-70S (Gabanella *et al.,* 2005; Liu *et al.,* 1997; Paushkin *et al.*, 2002). One study further observed coexpressed SMN and Gemin2, in the absence of other Gemins, forming a 40S particle (Paushkin *et al.*, 2002). The SMN complex has, as a result, been commonly (and perhaps fatalistically) concluded to possess an intractably large number of copies of both proteins, and few attempts have been made to better define its molecular

inventory. Still, given the critical importance of SMN oligomerization in the catalysis of snRNP assembly and in SMN pathogenesis, a more exact description of SMN stoichiometry offers a compelling goal.

Schizosaccharomyces pombe, the most primitive organism in which an orthologue to the human SMN protein can be found, provides an excellent model system for an inquiry into the oligomerization state of the SMN complex. It presents the most rudimentary SMN complex identifiable by sequence, consisting only of SMN and Gemin2, and has been shown functional for snRNP assembly (Campion *et al.*, 2010). Also favoring its choice as an experimental system, the bacterially expressed heterodimer, unlike that of its orthologues in higher organisms, is both stable and soluble in solution. It mirrors the behavior of its human counterpart in size exclusion chromatography experiments, eluting in or near the column void volume and again giving a first impression of a species with a very large molecular weight and a number of both protein components (Figure 4.1 shows a representative size exclusion profile for *S. pombe* SMN-Gemin2).

Multi-angle light scattering and analytical ultracentrifugation sedimentation equilibrium techniques were applied to the determination of the absolute molecular weight of the *S. pombe* SMN complex, from which its molecular inventory might be deduced. Unexpectedly, despite its short retention time and apparently large molecular weight by size exclusion chromatography, the model best fitting the available data for spSMN-

Gemin2 suggests that it occupies a dimer-tetramer equilibrium of the spSMN-Gemin2 heterodimer.

The behavior of spSMN-Gemin2 during a SEC-MALS experiment over a decreasing range of protein concentrations provide the first intimation both that the protein's molecular mass was much more limited than size exclusion chromatography alone had predicted, and that it appeared to reversibily associate in a concentration-dependent manner. spSMN-Gemin2 at concentrations ranging from 10 mg/ml to 1 mg/ml showed a concomitant increase in size exclusion retention time and a decrease in the molar masses sampled across each peak until a concentration of 2 mg/ml (46 μM), after which the peaks evinced the same elution profile and yielded mass calculations near 80 kDa, consistent with a dimer of spSMN-Gemin2 heterodimers as the basic operative unit of SMN oligomerization. The behavior of the protein over a 226 μM – 23 μM range of concentrations suggests a dissociation constant in the low micromolar range.

As a admonitory aside to the interested practitioner of SEC-MALS, the reported weight-averaged molecular weight across a peak may be misleading if the mass profile is not flat; spSMN-Gemin2 injected at 5 mg/ml is easily misinterpreted to comprise a trimer of heterodimers based on such a faulty analysis, and sooner consideration of the behavior of a concentration series of the protein might have spared some confusion.

A comparison of several models by which to fit spSMN-Gemin2 sedimentation equilibrium data allowed a numerical comparison of the goodness-of-fit of various models of self-interaction and helped eliminate or support models best describing the observed behavior of spSMN-Gemin2. Table 4.1 provides a summary of this analysis. The data were best fit under the assumption of a dimer-tetramer equilibrium. No addition of higher order oligomers (hexamers or octamers) improved the fit; the dissociation constants for the added hexamers and octamers fell in the high millimolar and molar range, respectively, suggesting them irrelevant. A clear deterioration in goodness-of-fit was observed upon the inclusion of monomers or trimers. A dimer-tetramer equilibrium correlates well with data observed in SEC-MALS experiments, where the mass profiles of the high concentration protein samples are easily interpreted as ranging from tetrameric to dimeric species. It is easy to imagine an extension of this dimer-tetramer paradigm to the SMN complex in higher organisms, and that possibility provides an exciting direction in which to take this research. In a promising bit of corroboration, Renee Martin, a previous graduate student in the Van Duyne laboratory at the University of Pennsylvania, solved the crystal structures of human and *S. pombe* YG box constructs fused to maltose binding protein for solubility that show clear dimerization (Martin, 2011).

Given the unquestionable result that spSMN-Gemin2 forms small, discrete oligomers, with data best explained by a dimer-tetramer equilibrium, its deceptive behavior in size exclusion chromatography experiments must originate instead in unusual hydrodynamic

properties. Several measures give credence to the notion of an extended shape, including SAXS measurements of a radius of gyration and maximum dimension greater than expected for a compact particle and a large frictional ratio by sedimentation velocity. A Kratky transformation of the SAXS scattering data, typically employed as a diagnostic for protein unfolding, shows evidence of a flexible, multi-domain molecule with partially disordered character. Particle elongation and flexibility, rather than a substantial molecular mass, account for the abbreviated retention time observed for spSMN-Gemin2 off of size exclusion chromatography columns; the long-held mistaken assumption of a substantial molecular mass for the SMN complex might serve as a cautionary tale for experimenters tempted to attribute size discrimination to molecular weight in experiments where it is correctly based on hydrodynamic volume.

Both experimental evidence from the SAXS Kratky plot and a consideration of the disorder predictions spSMN lend support to the notion that the protein exhibits some intrinsic disorder, even when bound to Gemin2 and in its preferred oligomerization state. The disorder prediction for the protein is striking, proposing that only its very N- and C-terminal binding domains have a strong predisposition to assume a structured fold. A number of functional implications are typically imputed to regions of disorder, including service as interfaces for intermolecular interactions due to their large available surface area. This could have fascinating implications for the involvement of SMN with its snRNP assembly substrates during biogenesis, as well as in its reported roles in other cellular housekeeping processes. These speculations may prove equally germane to the

discussion of human SMN; apart the addition of a Tudor domain lacking in the *S. pombe* orthologue, a disorder prediction for that protein bears a striking resemblance to that of spSMN and suggests that the regions of intrinsic disorder might serve an important function that has been retained evolutionarily. (Figure 4.8)

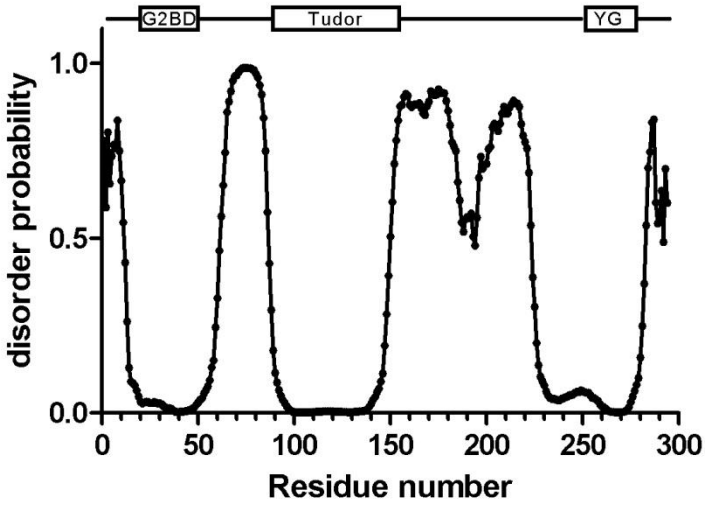


Figure 4.8 Disorder prediction for human SMN. DISOPRED prediction of disorder for spSMN. A residue with a disorder probability > 0.5 is considered disordered. Known structural domains are depicted above the curve and correspond to the residues.

The existing corpus of knowledge relating to SMN oligomerization bears consideration in light of the unexpected structural model of SMN association presented in this chapter. SMN possesses two identified regions of self-interaction, at its exon6/7 encoded tyrosine-glycine-rich "YG box" and an N-terminal region mapped to exons 1-2b (Ogawa *et al.*, 2007; Talbot *et al.*, 1998). The former, identified first and more extensively studied, is a stronger interaction requiring urea to disrupt; the latter is reported to be weaker, sufficiently prone to exchange to require no intervention to

separate units for surface plasmon resonance studies (Young *et al.*, 2000). Young *et al.* emphasize that any molecule with two self-association sites is theoretically capable of forming interactions of a higher order than dimers, and propose a model in which YG-mediated SMN dimers form tetramers or other oligomers through their second N-terminal self-interaction domain (Young *et al.*, 2000). Their prescient prediction of a role for the YG box in mediating the formation of core SMN dimers might be interpreted as consistent with the observation that deletion or disruption of YG box self-interaction abrogates the ability of SMN to assemble snRNPs and underscores severe disease phenotypes in many SMA patients, although the sole study to demonstrate this result biochemically showed only that YG box missense mutations disrupt SMN oligomerization, without conclusively demonstrating that SMN monomers result (Lorson *et al.*, 1998; Pellizzoni *et al.*, 1999).

The function of the N-terminal SMN oligomerization domain in formation of dimers of higher-order species is less clear. In human SMN, the SMA patient mutation of conserved Asp 44 to valine occurs within the SMN Gemin2 binding domain without affecting that interaction; instead its pathogenic influence is proposed to stem from a destabilization of the N-terminal SMN self-interaction (Ogawa *et al.*, 2007; Sun *et al.*, 2005). Conserved in all organisms including *S. pombe*, where a glutamic acid occupies that same amino acid position, Asp44 clearly plays an important role in some aspect of SMN complex function, although its D44V mutation results only in the mild, late-onset Type III form of SMA. It is tempting to speculate from the mild effect of the D44V

mutation on SMA pathogenesis that its predicted effect on N-terminal SMN self-interaction is a disruptive effect on the interaction of core SMN dimers forming a tetramer, although simple loss of an additional factor stabilizing the dimer is also possible. It should be straightforward to test that hypothesis in the context of the same SEC-MALS and sedimentation equilibrium experiments described, mutating the corresponding *S. pombe* glutamic acid, as well as other strictly conserved residues of unknown function in the same region.

Unexpectedly, in full contradiction to most stated opinions in the literature and to conclusions drawn from analytical size exclusion chromatography and sucrose gradient centrifugation, the oligomer formed by the self-association of the *S. pombe* SMN protein is shown here to consist not of an intractably large number of SMN units, but of discrete dimers of the spSMN-Gemin2 heterodimer that self-associate to form larger assemblies. This unanticipated, welcome insight into the higher order structure of SMN in its most rudimentary guise offers a model of SMN oligomerization applicable to higher organisms, and furnishes an additional perspective from which the structural role of oligomerization in snRNP biogenesis and the effects of SMA disease mutations in SMN may be probed.

Materials and Methods

DNA constructs

Full-length *Saccharomyces pombe* Gemin2 (Yip1p) (M1-T235) was amplified by PCR as an NdeI/XhoI fragment and cloned into the MCS II of Twin1B (New England Biosystems); it was later subcloned as an NdeI/XhoI fragment into the MCS II of a petDuet (Novagen) expression vector modified to contain an in-frame C-terminal Mxe intein domain (New England Biosystems), chitin binding domain and hexahistidine tag. Full-length *S. pombe* SMN (Yab8p) (M1-D152) was PCR amplified as an NcoI/BamHI fragment and cloned into the MCS of pET24dHT, and later subcloned into pGBKT7b. From this construct, it was then PCR amplified as an NcoI/NotI fragment and cloned into MCS I of pCDFDuet (Novagen).

Protein expression and purification

The two constructs were co-transformed into *E. coli* BL21(DE3) cells; a culture grown from one positive transformant, identified by growth on media containing ampicillin and streptomycin, was used to create a 10% glycerol stock stored at -80°C. spSMN and spGemin2 were co-expressed in Luria-Bertani (LB) cell cultures grown at 15°C for 20 hours after the addition of IPTG (isopropyl-β-D-thio-galactoside) to a final concentration of 0.5M.

Cells were harvested by centrifugation at 4000 x g for 30 minutes at 4°C. Each liter of cells was resuspended in approximately 20ml of 50mM Na/K phosphate, pH 8.0, 300mM NaCl, 10mM imidazole, 0.5mM ethylenediaminetetraacetic acide (EDTA), 0.5mM 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF; Fisher Scientific), 10μM proteinase inhibitor E-64 (Sigma) and 2mM benzamidine (Fisher Scientific). Cells were lysed in two sequential passes through an Avestin Emulsiflex C-3 homogenizer at approximately 18,000 psi, and the crude lysate thereby obtained was cleared by centrifugation at 16,000 rpm at 4°C for 30 minutes.

Cleared lysate was filtered through grade 802 fluted filter paper (Whatman) and loaded onto 4ml (per 6 liters of cell culture) nickel nitriloacetic acid (Ni-NTA; Qiagen) resin in a 5.3 cm x 10cm Kontes column equilibrated with 50mM Na/K phosphate pH 8.0, 300mM NaCl, 10mM imidazole. The preparation was nutated at room temperature for 60 minutes; the resin and bound protein were then washed with ten column volumes of buffer containing 50mM Na/K phosphate, 300mM NaCl, 20mM imidazole and eluted in 50mM Na/K phosphate, 300mM NaCl, 250mM imidazole. The Ni-NTA eluant was then loaded onto 8ml chitin resin equilibrated in a 2.5 cm x 10 cm Kontes column with 50mM Na/K phosphate, 300mM NaCl, 250mM imidazole and nutated for 90 minutes at room temperature. The resin and bound protein were washed with ten column volumes of 20mM Hepes pH 8.0, 300mM NaCl, 0.1mM EDTA.

spGemin2 bound to spSMN was cleaved from the C-terminal Mxe intein-chitin binding domain-hexahistidine tag by addition of 20mM Hepes pH 8.0, 300mM NaCl, 0.1mM EDTA, plus 50mM 2-mercaptoethanol acting as the cleavage-inducing thiol reagent, to the protein-bound chitin resin followed by overnight (~16 hours) incubation at room temperature. The cleaved preparation was then added to 4ml of fresh chitin resin equilibrated with 20mM Hepes pH 8.0, 300mM NaCl, 0.1mM EDTA, and the cleaved spSMN-Gemin2 complex was eluted in the same buffer.

Purified spGemin2-spSMN was concentrated at 5000 x g at 4°C in 15ml Amicon Ultra 10,000 MWCO Centrifugal Filters (Millipore) and further purified by size exclusion chromatography at room temperature on a Superdex 75 HiLoad 16/60 column (Pharmacia) in 10mM Tris pH 8.0, 50mM NaCl, 2mM dithiothreitol (DTT). When necessary, homogeneous purified protein was stored in that buffer plus 10% glycerol at -80°C. Final protein concentration was assessed using the absorbance at 280 nm (A_{280}), assuming an extinction coefficient of 56965 M^{-1} cm^{-1} based on amino acid compositional analysis by the ProtParam software tool on the ExPASy Proteomics Server (Swiss Institute of Bioinformatics). spSMN-Gemin2 proved moderately soluble and was typically concentrated to between 200μM and 500μM. A preparation in LB media yielded approximately 2 mg L^{-1}.

Analytical size exclusion chromatography

Purified *S. pombe* SMN-Gemin2 was concentrated as above to 1ml (~4 mg/ml; ~90μM) and injected onto an analytical Superdex 75 10/300 column (GE Healthcare) equilibrated in 10mM Tris pH 8.0, 50mM NaCl, 2mM DTT and run at room temperature at 0.4ml/min. The protein elution profile was monitored by absorbance at 280nm. Column calibration employed gel filtration standards (BioRad) containing thyroglobulin (670,000 Da), bovine gamma globulin (158,000 Da), chicken ovalbumin (44,000 Da), equine myoglobin (17,000 Da) and vitamin B-12 (1350 Da).

Sedimentation velocity

Sedimentation velocity ultracentrifugation experiments were performed at 20 °C with an XL-A analytical ultracentrifuge (Beckman Coulter) and a TiAn60 rotor with two-channel charcoal-filled Epon centerpieces and quartz windows. Complete sedimentation velocity profiles of *S. pombe* SMN-Gemin2 (10.5 μM, optical density of 0.6 at A_{280}) in 1x PBS pH 7.4 were collected every 60 seconds at 30,000 rpm for 500 scans, followed by data analysis using the continuous size-distribution (c(s)) analysis of the Lamm equation in SEDFIT (Schuck & Rossmanith, 2000). After optimizing meniscus position and fitting limits, the sedimentation coefficient (s) and best-fit frictional ratio (f/f_0) were determined by iterative least squares analysis.

Sedimentation equilibrium

Sedimentation equilibrium ultracentrifugation (SE) experiments were performed at 4°C with an XL-A analytical ultracentrifuge (Beckman Coulter) and a TiAn60 rotor with six-channel charcoal-filled Epon centerpieces and quartz windows. Radial absorption scan data at 280 nm for three protein concentrations were measured (14.0μM, 8.8μM, 3.5μM; 0.8OD, 0.5OD, 0.2OD) at 20 and 22 h for each of four different rotor speeds (12,000, 15,000, 18,000 and 21,000 rpm). Comparison of radial absorption scans verified that equilibrium had been reached. Data were analyzed using the programs SEDFIT and SEDPHAT and fit variously to a single species model and to several models of self-association, with the best solutions exhibiting small goodness of fit and low and randomly distributed residual errors (Schuck & Rossmanith, 2000; Vistica *et al.*, 2004).

Size exclusion chromatography in-line with multi-angle light scattering

The absolute molecular weight of the *S. pombe* SMN-Gemin2 oligomer was determined using multi-angle light scattering (MALS) in-line with a Superdex 200 10/300 column (GE Healthcare). The column was calibrated using the following proteins (Bio-Rad): thyroglobulin (670,000 Da), bovine gamma globulin (158,000 Da), chicken ovalbumin (44,000 Da), equine myoglobin (17,000 Da) and vitamin B-12 (1350 Da). Blue dextran (Sigma) was used to define the void volume of the column.

spSMN-Gemin2, 20µl at 1-10 mg/ml (23 µM – 226 µM), was injected onto the column at 0.5 ml/min in buffer containing 1x PBS, pH 7.0 and 2mM dithiothreitol at room temperature. The scattered light intensity of the column eluant was recorded at 16 different angles using a DAWN-HELEOS MALS detector (Wyatt Technology Corp.) operating at 658 nm after calibration with 20µl of 20mg/ml lysozyme (Sigma). Protein concentration of the eluant was determined using an in-line Optilab DSP interferometric refractometer (Wyatt Technology Corp.). The weight-averaged molecular weight of species within defined chromatographic peaks was calculated using the ASTRA software version 5.2 (Wyatt Technology Corp.), by construction of Debye plots (KC/R_Θ *versus* $\sin^2[\Theta/2]$) at one-second data intervals. The weight-averaged molecular weight was then calculated at each point of the chromatographic trace from the Debye plot intercept, and an overall average molecular weight was calculated by averaging across the peak.

Small-angle x-ray scattering

Small-angle x-ray scattering (SAXS) data were measured at beam line X9 at the National Synchrotron Light Source (Upton, NY). Sample scattering profiles were collected on two detectors with overlapping geometry: a Mar 165 CCD SAXS detector (Mar USA, Inc., Evanston, IL) 3.4 m from the sample, and a custom built Photonic Science CCD WAXS detector. Two-dimensional images were integrated using software developed at the beam line into one-dimensional intensity profiles as a function of momentum transfer Q (where $Q=4\pi\sin(\theta)/\lambda$, with 2θ the scattering angle and λ the

wavelength). Measurements were taken at 10°C with an X-ray wavelength of 0.855 Å; scattering profiles covered a Q range from 0.006 to 0.22 $Å^{-1}$. The sample holder was a 1-mm quartz capillary (Hampton Research, Aliso Viejo, CA) that was sealed across the evacuated beam path. Both ends of the capillary were open to allow the sample to flow continuously through to minimize radiation damage to the sample. The sample was centrifuged at 10,000 x *g* for 10 min at 4°C before measurements; each measurement required 30 µl of sample for three 10 µl, 60 second exposures. After each measurement, the capillary was washed repeatedly with buffer solution and purged with compressed nitrogen.

Averaged scattering curves from the SAXS and WAXS detectors were scaled and merged in PRIMUS; scattering from a matching buffer solution was subtracted from the data and corrected for the incident intensity of x-rays. Replicate exposures were examined carefully for evidence of radiation damage by Guinier analysis and Kratky plot analysis. Silver behenate powder was used to locate the beam center and to calibrate the sample-to-detector distance (Konarev *et al.*, 2003).

Evidence of the monodispersity of the preparation was gained by analysis of linearity in the Guinier region of the scattering data and agreement of the I(0) and R_g values determined with inverse Fourier transform analysis by the programs GNOM and AUTOGNOM. When fitting manually, the maximum diameter of the particle (D_{max})

was adjusted in 10-Å increments in GNOM to maximize the goodness-of-fit parameter. This analysis also yielded determinations of R_g (Semenyuk & Svergun D, 1991).

Chapter V: Conclusions and Future Perspectives

Despite a vital interest in achieving a molecular understanding of the structure and function of the SMN complex, an interest founded upon both the biochemist's interest in RNP biogenesis and the clinician's goal of treating SMA, descriptions of its stoichiometry and atomic structures of its components eluded research efforts for many years. This dissertation fills a crucial void in the available knowledge of the SMN complex, offering a detailed depiction of the binding domains of core components SMN and Gemin2, a description of their solution properties and the elucidation of the unanticipated oligomerization state of SMN.

The atomic structure of the complex formed by the minimal binding domains of human SMN and Gemin2 was solved by triple-resonance multidimensional NMR spectroscopy; it was shown to form an all α-helical fold bearing no significant structural homology to known proteins and characterized by a highly conserved hydrophobic SMN-Gemin2 interface. From this structural springboard, it becomes possible to plan and interpret the next generation of studies probing the role of Gemin2 in several cellular processes, many of them related to human health.

Gemin2 interactions are reported for several components of the SMN complex and its snRNP assembly substrates. The minimal SMN-Gemin2 complex demonstrates a number of conserved surface residues bearing no obvious role in SMN binding or the

stabilization of the Gemin2 fold; their judicious mutation provides an opportunity to probe the sites of binding for interacting proteins and to interpret them structurally, either in the context of the minimal SMN-Gemin2 complex or that of full-length Gemin2 binding minimal SMN. One recent structural study implicates full-length Gemin2 in Sm protein binding, positing that Sm D1 and D2 interact, in the context of the NMR structure described, with the face of Gemin2 opposite SMN binding (Zhang *et al.*, 2011). The Gemin2 residues mediating those interactions remain untested biochemically and bear consideration by mutational analysis, both to validate the structural model proposed and to provide some structural insight into Gemin2's function coordinating intermediates along the snRNP assembly pathway.

Gemin2 binding to Gemin7 has also been established, and may represent an interaction with the Gemin6-7 heterodimer though to mimic the Sm D3/B dimer and substitute for it, together with the Sm D1/D1/E/F/G pentamer, in a temporary assembly intermediate of the Sm heptamer. The known interaction of Sm B with Sm D1 in the final assembled snRNP suggests a region of the face of Gemin2, positioned to interact with the free face of Sm D1, where a Gemin7 interaction might occur (Krummel *et al.*, 2010). Pinpointing the binding site of Gemin7 relative to other Sm protein substrates would offer a more detailed glimpse of its role in Sm protein assembly. Gemin5, the snRNA chaperone, also makes its SMN complex contact through Gemin2; determining its binding site through mutational analysis might permit the development of a model for the transfer of snRNA to the SMN complex and into coordination with the Sm proteins. In the case of a

sufficiently stable Gemin2-Gemin7 or Gemin2-Gemin5 interaction, small-angle scattering could also be usefully employed; the difference between shape reconstructions with and without the Gemin6-7 heterodimer or Gemin5 would substantiate its suggested point of interact with the complex. If tractable, a complex containing Gemin5 and snRNA could also be probed by small-angle neutron scattering, which carries the advantage of scattering protein and DNA components differently and allowing for their relative localization within the scattering particle.

As an aside, certain aspects of the minimal SMN-Gemin2 heterodimer suggest future avenues for the interested protein x-ray crystallographer, which might in turn prove a useful tool for rapid consideration of the structural effects of mutations. While protein crystallization for x-ray experiments always relies to some extent on a researcher's luck, certain protein structural features can be predicted to introduce a bias against crystal formation. Large disordered regions of a protein are well established to inhibit crystal packing and the formation of crystal contacts (Canaves *et al.*, 2004), and may have spelled the demise of this project as a crystallography candidate. The minimal SMN-Gemin2 construct contains both a 25-residue unstructured loop between α-helices α2 and α3 in Gemin2 and, in SMN, 10 disordered residues N-terminal to the α-helix binding Gemin2; the strategic trimming of the latter and replacement of the former with a shorter connector, such as the four-residue sequence that bridges the same interhelical gap in *Drosophila*, provide clear possibilities for initial attempts to design a crystallizable minimal SMN-Gemin2.

Despite reports to the contrary, neither full-length Gemin2 nor its minimal SMN binding domain, either bound to SMN or unbound, demonstrated the ability to dimerize (Ogawa *et al.*, 2007), but unbound Gemin2 was shown to undergo a substantial conformational change upon SMN binding, becoming more compact. In good agreement with a recent crystal structure, low resolution shape reconstructions of full-length Gemin2 bound to the minimal binding domain of SMN demonstrate that Gemin2's N-terminal region forms a rigid arm extended away from its globular SMN-binding domain and positioned to bind its Sm protein substrates (Zhang *et al.*, 2011).

The functional relevance of unbound Gemin2 is uncertain. The work presented here demonstrates a patent disordering of Gemin2 when unbound to SMN; it is also well-established, and perhaps a logical conclusion, that cellular knockdown of SMN yields a concomitant decrease in Gemin2 (Jablonka *et al.*, 2002; Shpargel & Matera, 2005). In Gemin2's roles outside of SMN complex-mediated snRNP assembly, however, the presence of SMN remains uncertain or dubiously confirmed. Gemin2 makes a documented interaction with Rad51, a protein that assists in the repair of double-stranded DNA breaks through homologous recombination. The presence of SMN was assessed using an SMN-Gemin2 chimera in which the C-terminus of the latter was fused to the N-terminus of the former; the sheer structural improbability of that molecule calls into question the conclusion offered that SMN is, in fact, necessary. A probe for that direct interaction using more logical Gemin2 and Gemin2-SMN constructs would

provide a more satisfying answer and lay the groundwork for further studies of Gemin2's enhancement of Rad51-mediated DNA repair, a process disrupted in some forms of human cancer.

Importantly, another non-RNP assembly role for Gemin2 involves a direct interaction with HIV-1 integrase (IN) during viral infection, in which it enhances the assembly of reverse transcriptase onto viral RNA and contributes to viral infectivity (Hamamoto *et al.,* 2006; Nishitsuji *et al.,* 2009). Despite its importance, few requirements for the interaction have been established. The presence of SMN during Gemin2-IN binding has yet to be confirmed or excluded, and it will be an important factor in establishing whether Gemin2 interacts with IN in its unbound conformation, bound to SMN or bound to another partner protein. Equally, the IN requirements for Gemin2 binding, given the IN inclination both to oligomerize and to interact tightly with host proteins (LEDGF), remain to be determined (Cherepanov *et al.*, 2003). A binding region of Gemin2 for IN, falling within its SMN-binding domain, has been identified; provided that Gemin2 assumes its SMN-bound fold in the context of IN interaction, it defines a binding surface on Gemin2 that might be probed mutationally. A clear understanding of the part played by Gemin2 in HIV pathogenesis bolsters the mechanistic understanding of integrase and reverse transcriptase function, and may open profitable avenues for the design of future therapeutics.

The intriguing molecular shape reconstructions of full-length Gemin2 bound to the minimal SMN binding domain suggest further SAXS experiments. Those two proteins, bound to the Sm pentamer, were observed in both a recent crystal structure and in electron microscopy shape reconstructions; small-angle x-ray scattering of that particle, containing the Sm pentamer, would give a solution envelope that could be compared both to the reported x-ray and EM structures and to the structure of SMN-bound Gemin2 alone and suggest, in the first case, any differences that might be relevant in solution, and in the second, changes to Gemin2 upon Sm binding. Another exciting target might comprise the SMN-bound Gemin2-Sm pentamer complex just described using an SMN construct extended to include the Tudor domain. That domain, absent only in *S. pombe*, has been implicated in interacting with the R-G rich tails of Sm D3, D1 and B when symmetrically dimethylated (Bühler *et al.*, 1999; Selenko *et al.*, 2001). It would be illustrative to visualize its position relative to the Sm pentamer, particularly Sm D1 and G to which B and D3 respectively bind, as well as to the binding site of Gemin7 if determined (Krummel *et al.*, 2010); it might become possible to visualize the mechanism by which Sm D3 and B are recruited to and incorporated into the pentameric Sm ring to close it as a heptamer.

The ability of SMN to oligomerize is a requirement for the ability of the SMN complex to catalyze snRNP assembly; disruption of oligomerization by SMN transcripts lacking exon 7 or carrying certain missense mutations is also linked convincingly to SMA pathogenesis. While plainly indispensible, however, the actual oligomerization state or

states occupied by SMN has never been revealed. The complex formed by the full-length *Schizosaccharomyces pombe* SMN and Gemin2 orthologues was shown to occupy a dimer-tetramer equilibrium, with a dimer the smallest discrete oligomer of the complex sampled at low concentrations. The protein appears partially disordered, however, contributing to its unusual hydrodynamic properties and large Stokes radius. The discovery that SMN is able to form small, discrete oligomers overturns a long-held assumption, based on sucrose gradient centrifugation and size exclusion chromatography behavior, that SMN forms a very large and possibly heterodisperse oligomer containing multiple copies of the protein.

Although multiple reports have identified two self-interaction sites for SMN, both of which contain known SMA disease mutations, their relative contribution to the formation of SMN dimers and higher order oligomers has yet to be probed. The *S. pombe* SMN-Gemin2 complex can serve as a tractable model system for examining the role that conserved residues and identified SMA disease mutations play in shifting or disrupting the dimer-tetramer equilibrium observed for wildtype protein. These observations could be usefully coupled to functional assessment of the SMN complex's continued capacity to assemble snRNPs; an *in vitro* gel shift snRNP assembly assay has been available for the study of the human SMN complex for over a decade, and the development of an analogous *in vitro* assembly assay using *S. pombe* Sm proteins and the recombinant SMN complex described here would enable complementary functional analysis of any oligomerization mutants tested.

Indeed, the N-terminal region of SMN provides several excellent targets for mutational analysis. A number of residues uninvolved in Gemin2 binding are highly conserved in *S. pombe*, included the invariant W9, D10, D11 triplet and, at the position of the D44V SMA patient mutation in humans, E19. The latter has featured in the literature due to the pathogenesis it introduces, and is specifically suggested to destabilize the N-terminal SMN interaction when mutated. A similar role can be hypothesized for the neighboring W9, D10 and D11. The significance of the N-terminal SMN interaction in stabilizing SMN dimers or tetramers, in contrast to that brokered by the YG box, is uncertain, but might be probed in the context of perturbation of SMN's oligomerization behavior and provide some structural insight into the function of SMN self-association in snRNP assembly and the deficiency in that ability that causes SMA.

Another matter of some importance addresses the applicability of the dimer-tetramer paradigm observed for *S. pombe* SMN-Gemin2 orthologues to higher organisms. Felicitously, early unpublished in-house data show that a construct comprising the human SMN YG box oligomerization domain fused to MBP for solubilization also inhabits a dimer-tetramer equilibrium; this construct was also crystallized as a dimer, suggesting at least some correspondence between the two orthologues. The construct's ability to access a tetramer without the putative N-terminal self-interaction domain casts some doubt on the notion that the YG box mediates dimerization and the N-terminal domain higher order oligomer formation, but may be fruitfully addressed in by the

development of a full-length or near full-length SMN construct exhibiting tractable solution behavior in complex with Gemin2 and naturally possessing both domains.

Finally, the global structure of and orientation of dimeric subunits within each SMN tetramer offers a fascinating question. One can easily imagine the interaction of four similarly-oriented subunits, interacting in parallel and able to exchange with each other when reforming dimers; one can as readily imagine the interaction of two indivisible dimers in an orientation that never allows the exchange of subunits. Two experiments might be instructive. A simple exchange experiment differently tags separate pools of SMN-Gemin2 complexes before combining them; the evolution of mixed oligomers after some equilibration indicates the ability of the subunits to exchange. Low-resolution SAXS envelopes might also assist in supporting or eliminating potential models, particularly in the context of the MBP-YG box human chimeras mentioned above at concentrations favoring tetramer; the comparatively large MBP moieties might be identifiable obviously on a shared side or on opposite sides of the oligomerization domain, giving some indication of dimer-dimer polarity.

The biophysical studies of human SMN and Gemin2 described here mark a noteworthy advance in the structural understanding of the atomic foundations of their interaction, their solution behavior and the discrete oligomerization states occupied by SMN. The structural frameworks they provide will motivate the next generation of structure-function studies on the role of both proteins in many aspects of cellular housekeeping,

and represent a valuable tool for the continued study of U-snRNP biogenesis and SMA pathogenesis.

Appendix A. Backbone and sidechain resonance assignments for SMN$^{26-51}$ and Gemin2$^{95-280}$.

Table A1. Resonance assignments for SMN$^{26-51}$. Standard deviation to the mean is indicated except in cases where indicated chemical shift is based on a single assignment.

Column 1: atom number
Column 2: residue name
Column 3: atom name
Column 4: chemical shift
Column 5: ambiguity code

The ambiguity code for assignments follows the nomenclature of the Biological Magnetic Resonance Bank as follows:

1: unique
2: ambiguity of geminal atoms or geminal methyl proton groups
3: aromatic atoms on opposite sides of a symmetric ring

1	GLN27	C	176.3182 ± 0.053	1	54	ILE33	CA	61.2072 ± 0.136	1
2	GLN27	CA	56.4212 ± 0.078	1	55	ILE33	CB	38.5862 ± 0.077	1
3	GLN27	CB	29.5252 ± 0.079	1	56	ILE33	CD1	12.8622 ± 0.059	1
4	GLN27	CG	33.7362 ± 0.106	1	57	ILE33	CG1	27.0312 ± 0.076	1
5	GLN27	HA	4.3762 ± 0.007	1	58	ILE33	CG2	17.1432 ± 0.054	1
6	GLN27	HB2	2.0682 ± 0.037	2	59	ILE33	HA	3.9562 ± 0.010	1
7	GLN27	HB3	2.0682 ± 0.037	2	60	ILE33	HB	1.6242 ± 0.008	1
8	GLN27	HE21	7.6222	2	61	ILE33	HD1	0.6782 ± 0.014	1
9	GLN27	HE22	6.8642	2	62	ILE33	HG12	1.1512 ± 0.005	2
10	GLN27	HG2	2.3852 ± 0.008	2	63	ILE33	HG13	0.9382 ± 0.008	2
11	GLN27	HG3	2.3852 ± 0.008	2	64	ILE33	HG2	0.6232 ± 0.008	1
12	GLN27	NE2	112.6672	1	65	ILE33	H	7.7332 ± 0.006	1
13	SER28	C	174.2782 ± 0.038	1	66	ILE33	N	119.8342 ± 0.056	1
14	SER28	CA	58.4752 ± 0.059	1	67	TRP34	CA	56.3412 ± 0.119	1
15	SER28	CB	63.6532 ± 0.187	1	68	TRP34	CB	29.1072 ± 0.098	1
16	SER28	HA	4.4652 ± 0.009	1	69	TRP34	CD1	126.9502 ± 0.074	1
17	SER28	HB2	3.8702 ± 0.020	2	70	TRP34	CE3	121.0332	1
18	SER28	HB3	3.8702 ± 0.020	2	71	TRP34	CH2	124.5102 ± 0.055	1
19	SER28	H	8.5672 ± 0.008	1	72	TRP34	CZ2	114.3972 ± 0.142	1
20	SER28	N	116.7872 ± 0.056	1	73	TRP34	CZ3	121.1832 ± 0.103	1
21	ASP29	C	176.0802 ± 0.023	1	74	TRP34	HA	4.6562 ± 0.015	1
22	ASP29	CA	54.4522 ± 0.075	1	75	TRP34	HB2	3.1802 ± 0.014	2
23	ASP29	CB	41.1052 ± 0.090	1	76	TRP34	HB3	2.9762 ± 0.017	2
24	ASP29	HA	4.6162 ± 0.010	1	77	TRP34	HD1	7.0362 ± 0.007	1
25	ASP29	HB2	2.6982 ± 0.015	2	78	TRP34	HE1	9.8822 ± 0.011	1
26	ASP29	HB3	2.6982 ± 0.015	2	79	TRP34	HE3	7.2512 ± 0.008	1
27	ASP29	H	8.3132 ± 0.008	1	80	TRP34	HH2	6.9712 ± 0.006	1
28	ASP29	N	122.0502 ± 0.050	1	81	TRP34	H	8.2762 ± 0.012	1
29	ASP30	C	176.5802 ± 0.029	1	82	TRP34	HZ2	7.1742 ± 0.009	1
30	ASP30	CA	545642 ± 0.033	1	83	TRP34	HZ3	6.6452 ± 0.004	1
31	ASP30	CB	40.9832 ± 0.088	1	84	TRP34	N	126.5562 ± 0.094	1
32	ASP30	HA	4.6082 ± 0.002	1	85	TRP34	NE1	128.8832 ± 0.015	1
33	ASP30	HB2	2.6942 ± 0.011	2	86	ASP35	CA	52.6222 ± 0.079	1
34	ASP30	HB3	2.6942 ± 0.011	2	87	ASP35	HA	4.3942	1
35	ASP30	H	8.2792 ± 0.007	1	88	ASP35	H	8.0062 ± 0.002	1
36	ASP30	N	120.9312 ± 0.057	1	89	ASP35	N	123.4902 ± 0.061	1
37	SER31	C	174.4182 ± 0.019	1	90	ASP36	C	178.8332	1
38	SER31	CA	59.1542 ± 0.066	1	91	ASP36	CA	56.0282 ± 0.006	1
39	SER31	CB	63.7802 ± 0.089	1	92	ASP36	CB	41.6252	1
40	SER31	HA	4.3162 ± 0.007	1	93	ASP36	HB2	2.7772	2
41	SER31	HB2	3.8392 ± 0.008	2	94	ASP36	HB3	2.7772	2
42	SER31	HB3	3.8392 ± 0.008	2	95	THR37	C	175.1652	1
43	SER31	H	8.2442 ± 0.008	1	96	THR37	CA	61.0172 ± 0.103	1
44	SER31	N	115.7282 ± 0.030	1	97	THR37	CB	68.7452 ± 0.117	1
45	ASP32	C	175.8962 ± 0.047	1	98	THR37	CG2	22.2062 ± 0.069	1
46	ASP32	CA	54.3932 ± 0.082	1	99	THR37	HA	4.2582 ± 0.009	1
47	ASP32	CB	41.0082 ± 0.091	1	100	THR37	HB	4.5552 ± 0.010	1
48	ASP32	HA	4.5752 ± 0.012	1	101	THR37	HG2	1.0952 ± 0.009	1
49	ASP32	HB2	2.5582 ± 0.014	2	102	THR37	H	8.1502 ± 0.013	1
50	ASP32	HB3	2.5582 ± 0.014	2	103	THR37	N	105.6422 ± 0.074	1
51	ASP32	H	8.2802 ± 0.005	1	104	ALA38	C	180.1702 ± 0.005	1
52	ASP32	N	122.0392 ± 0.118	1	105	ALA38	CA	56.4082 ± 0.090	1
53	ILE33	C	175.7422 ± 0.054	1	106	ALA38	CB	19.1602 ± 0.090	1

107	ALA38	HA	3.7022 ± 0.010	1
108	ALA38	HB	0.9572 ± 0.022	1
109	ALA38	H	7.4592 ± 0.008	1
110	ALA38	N	122.7182 ± 0.095	1
111	LEU39	C	178.8352	1
112	LEU39	CA	57.7352 ± 0.079	1
113	LEU39	CB	41.3182 ± 0.091	1
114	LEU39	CD1	25.9042 ± 0.115	1
115	LEU39	CD2	23.4942 ± 0.130	1
116	LEU39	CG	26.2562 ± 0.462	1
117	LEU39	HA	4.2102 ± 0.012	1
118	LEU39	HB2	1.8162 ± 0.003	2
119	LEU39	HB3	1.3442 ± 0.035	2
120	LEU39	HD1	0.7652 ± 0.014	1
121	LEU39	HD2	0.7492 ± 0.007	1
122	LEU39	HG	1.5232 ± 0.008	1
123	LEU39	H	8.7672 ± 0.009	1
124	LEU39	N	115.8382 ± 0.091	1
125	ILE40	C	177.2072	1
126	ILE40	CA	65.7822 ± 0.099	1
127	ILE40	CB	38.7682 ± 0.090	1
128	ILE40	CD1	15.8972 ± 0.077	1
129	ILE40	CG1	29.8282 ± 0.138	1
130	ILE40	CG2	18.2612 ± 0.061	1
131	ILE40	HA	3.4062 ± 0.009	1
132	ILE40	HB	1.9022 ± 0.008	1
133	ILE40	HD1	0.6682 ± 0.008	1
134	ILE40	HG12	1.2972 ± 0.010	2
135	ILE40	HG13	0.7632 ± 0.011	2
136	ILE40	HG2	0.8052 ± 0.007	1
137	ILE40	H	6.9472 ± 0.008	1
138	ILE40	N	121.8492 ± 0.080	1
139	LYS41	C	179.5032 ± 0.025	1
140	LYS41	CA	59.1542 ± 0.122	1
141	LYS41	CB	32.7692 ± 0.116	1
142	LYS41	CD	29.4142 ± 0.101	1
143	LYS41	CE	41.8922 ± 0.182	1
144	LYS41	CG	25.8412 ± 0.100	1
145	LYS41	HA	4.0302 ± 0.007	1
146	LYS41	HB2	1.6982 ± 0.025	2
147	LYS41	HB3	1.6982 ± 0.025	2
148	LYS41	HD2	1.5652 ± 0.015	2
149	LYS41	HD3	1.5652 ± 0.015	2
150	LYS41	HE2	2.9122 ± 0.007	2
151	LYS41	HE3	2.9122 ± 0.007	2
152	LYS41	HG2	1.4342 ± 0.025	2
153	LYS41	HG3	1.4342 ± 0.025	2
154	LYS41	H	7.8332 ± 0.008	1
155	LYS41	N	116.2512 ± 0.108	1
156	ALA42	C	180.1362 ± 0.052	1
157	ALA42	CA	55.3872 ± 0.110	1
158	ALA42	CB	18.5062 ± 0.067	1
159	ALA42	HA	4.0452 ± 0.008	1
160	ALA42	HB	1.7262 ± 0.006	1
161	ALA42	H	7.9652 ± 0.008	1
162	ALA42	N	121.9532 ± 0.063	1
163	TYR43	C	176.0512 ± 0.022	1
164	TYR43	CA	61.6942 ± 0.070	1
165	TYR43	CB	37.4292 ± 0.223	1
166	TYR43	CD1	129.3572	3
167	TYR43	CD2	129.3572	3
168	TYR43	CE1	118.2222 ± 0.193	3
169	TYR43	CE2	118.2222 ± 0.193	3
170	TYR43	HA	3.1322 ± 0.009	1
171	TYR43	HB2	2.8382 ± 0.018	2
172	TYR43	HB3	2.2602 ± 0.018	2
173	TYR43	HD1	6.9172 ± 0.009	3
174	TYR43	HD2	6.9172 ± 0.009	3
175	TYR43	HE1	6.5322 ± 0.012	3
176	TYR43	HE2	6.5322 ± 0.012	3
177	TYR43	HH	9.4152	1
178	TYR43	H	7.6602 ± 0.007	1
179	TYR43	N	121.9492 ± 0.057	1
180	ASP44	C	179.7612	1
181	ASP44	CA	57.3412 ± 0.162	1
182	ASP44	CB	39.6102 ± 0.134	1
183	ASP44	HA	4.1292 ± 0.009	1
184	ASP44	HB2	2.7062 ± 0.019	2
185	ASP44	HB3	2.5442 ± 0.013	2
186	ASP44	H	8.4362 ± 0.011	1
187	ASP44	N	119.0592 ± 0.089	1
188	LYS45	C	178.7742	1
189	LYS45	CA	59.0192 ± 0.089	1
190	LYS45	CB	32.7652 ± 0.074	1
191	LYS45	CD	29.4792 ± 0.109	1
192	LYS45	CE	42.0312 ± 0.118	1
193	LYS45	CG	25.0872 ± 0.034	1
194	LYS45	HA	4.0172 ± 0.010	1
195	LYS45	HB2	1.8762 ± 0.014	2
196	LYS45	HB3	1.8762 ± 0.014	2
197	LYS45	HD2	1.6462 ± 0.012	2
198	LYS45	HD3	1.6462 ± 0.012	2
199	LYS45	HE2	2.9382 ± 0.013	2
200	LYS45	HE3	2.9382 ± 0.013	2
201	LYS45	HG2	1.4232 ± 0.035	2
202	LYS45	HG3	1.4232 ± 0.035	2
203	LYS45	H	8.2082 ± 0.012	1
204	LYS45	N	119.9972 ± 0.064	1
205	ALA46	C	181.1622	1
206	ALA46	CA	55.4272 ± 0.111	1
207	ALA46	CB	19.4412 ± 0.116	1
208	ALA46	HA	4.1612 ± 0.011	1
209	ALA46	HB	1.4392 ± 0.011	1
210	ALA46	H	7.8482 ± 0.007	1
211	ALA46	N	123.9042 ± 0.136	1
212	VAL47	C	177.5272	1

213	VAL47	CA	65.3162 ± 0.111	1
214	VAL47	CB	31.6872 ± 0.071	1
215	VAL47	CG1	21.3262 ± 0.164	1
216	VAL47	CG2	20.8682 ± 0.140	1
217	VAL47	HA	3.7202 ± 0.008	1
218	VAL47	HB	2.0192 ± 0.006	1
219	VAL47	HG1	0.6442 ± 0.017	1
220	VAL47	HG2	0.5922 ± 0.015	1
221	VAL47	H	8.1392 ± 0.009	1
222	VAL47	N	114.4472 ± 0.114	1
223	ALA48	C	178.3502 ± 0.019	1
224	ALA48	CA	53.4062 ± 0.076	1
225	ALA48	CB	18.7752 ± 0.095	1
226	ALA48	HA	4.3132 ± 0.010	1
227	ALA48	HB	1.4802 ± 0.016	1
228	ALA48	H	7.5392 ± 0.007	1
229	ALA48	N	121.3842 ± 0.044	1
230	SER49	C	174.2942 ± 0.009	1
231	SER49	CA	59.5382 ± 0.087	1
232	SER49	CB	64.0162 ± 0.122	1
233	SER49	HA	4.4122 ± 0.007	1
234	SER49	HB2	4.1122 ± 0.009	2
235	SER49	HB3	4.0072 ± 0.008	2
236	SER49	H	7.7712 ± 0.006	1
237	SER49	N	112.8012 ± 0.075	1
238	PHE50	C	173.8712 ± 0.022	1
239	PHE50	CA	59.0542 ± 0.092	1
240	PHE50	CB	39.6632 ± 0.123	1
241	PHE50	CD1	131.6612 ± 0.251	3
242	PHE50	CD2	131.6612 ± 0.251	3
243	PHE50	CE1	131.2702 ± 0.345	3
244	PHE50	CE2	131.2702 ± 0.345	3
245	PHE50	CZ	132.3432 ± 0.133	1
246	PHE50	HA	4.0472 ± 0.013	1
247	PHE50	HB2	2.9072 ± 0.012	2
248	PHE50	HB3	3.0252 ± 0.020	2
249	PHE50	HD1	6.6312 ± 0.019	3
250	PHE50	HD2	6.6312 ± 0.019	3
251	PHE50	HE1	6.9842 ± 0.024	3
252	PHE50	HE2	6.9842 ± 0.024	3
253	PHE50	H	7.7872 ± 0.006	1
254	PHE50	HZ	6.6392 ± 0.021	1
255	PHE50	N	122.7832 ± 0.054	1
256	LYS51	C	180.4482	1
257	LYS51	CA	57.6472 ± 0.128	1
258	LYS51	CB	34.0762 ± 0.074	1
259	LYS51	CD	29.1962 ± 0.151	1
260	LYS51	CE	42.0912 ± 0.155	1
261	LYS51	CG	24.5472 ± 0.047	1
262	LYS51	HA	3.9482 ± 0.009	1
263	LYS51	HB2	1.7432 ± 0.013	2
264	LYS51	HB3	1.6392 ± 0.010	2
265	LYS51	HD2	1.6532 ± 0.010	2
266	LYS51	HD3	1.6532 ± 0.010	2
267	LYS51	HE2	2.9742 ± 0.015	2
268	LYS51	HE3	2.9742 ± 0.015	2
269	LYS51	HG2	1.3382 ± 0.014	2
270	LYS51	HG3	1.3382 ± 0.014	2
271	LYS51	H	7.1822 ± 0.007	1
272	LYS51	N	127.7332 ± 0.063	1

Table A2. Resonance assignments for Gemin2$^{95\text{-}280}$. Standard deviation to the mean is indicated except in cases where indicated chemical shift is based on a single assignment.

Column 1: atom number
Column 2: residue name
Column 3: atom name
Column 4: chemical shift
Column 5: ambiguity code

The ambiguity code for assignments follows the nomenclature of the Biological Magnetic Resonance Bank as follows:

1: unique
2: ambiguity of geminal atoms or geminal methyl proton groups
3: aromatic atoms on opposite sides of a symmetric ring

1	GLY95	CA	44.9772	1	54	LEU100	HB2	1.6982 ± 0.008	2
2	GLY95	HA2	3.9192	2	55	LEU100	HB3	1.6612 ± 0.005	2
3	GLY95	HA3	3.9192	2	56	LEU100	HD1	0.9442 ± 0.013	2
4	TYR96	C	175.1852	1	57	LEU100	HD2	0.9442 ± 0.013	2
5	TYR96	CA	57.5772 ± 0.100	1	58	LEU100	HG	1.6382 ± 0.006	1
6	TYR96	CB	38.9752 ± 0.092	1	59	LEU100	H	9.0262 ± 0.010	1
7	TYR96	CD1	133.0652 ± 0.008	3	60	LEU100	N	122.8122 ± 0.122	1
8	TYR96	CD2	133.0652 ± 0.008	3	61	GLN101	C	178.2192 ± 0.003	1
9	TYR96	CE1	118.4692 ± 0.030	3	62	GLN101	CA	59.8032 ± 0.062	1
10	TYR96	CE2	118.4692 ± 0.030	3	63	GLN101	CB	28.1372 ± 0.033	1
11	TYR96	HA	4.5002 ± 0.008	1	64	GLN101	CG	34.0682 ± 0.042	1
12	TYR96	HB2	2.9202 ± 0.008	2	65	GLN101	HA	4.1262 ± 0.031	1
13	TYR96	HB3	2.7422 ± 0.011	2	66	GLN101	HB2	2.1352 ± 0.021	2
14	TYR96	HD1	6.9452 ± 0.003	3	67	GLN101	HB3	2.1352 ± 0.021	2
15	TYR96	HD2	6.9452 ± 0.003	3	68	GLN101	HG2	2.4642 ± 0.022	2
16	TYR96	HE1	6.7192 ± 0.001	3	69	GLN101	HG3	2.4642 ± 0.022	2
17	TYR96	HE2	6.7192 ± 0.001	3	70	GLN101	H	8.5802 ± 0.010	1
18	SER97	C	172.7472	1	71	GLN101	N	117.9082 ± 0.059	1
19	SER97	CA	56.4282 ± 0.038	1	72	TRP102	C	178.6852 ± 0.033	1
20	SER97	CB	63.5542 ± 0.131	1	73	TRP102	CA	62.5792 ± 0.102	1
21	SER97	HA	4.4452 ± 0.011	1	74	TRP102	CB	29.0292 ± 0.167	1
22	SER97	HB2	3.7182 ± 0.011	2	75	TRP102	CD1	128.4322 ± 0.105	1
23	SER97	HB3	3.6292 ± 0.006	2	76	TRP102	HA	4.1552 ± 0.024	1
24	SER97	H	7.9762 ± 0.009	1	77	TRP102	HB2	3.5092 ± 0.017	2
25	SER97	N	117.1652 ± 0.075	1	78	TRP102	HB3	3.3782 ± 0.029	2
26	PRO98	C	177.2812	1	79	TRP102	HD1	7.3862 ± 0.008	1
27	PRO98	CA	62.2432 ± 0.108	1	80	TRP102	HE1	10.3452	1
28	PRO98	CB	32.6372 ± 0.119	1	81	TRP102	H	8.2122 ± 0.008	1
29	PRO98	CD	50.5782	1	82	TRP102	N	121.7842 ± 0.108	1
30	PRO98	CG	27.7632 ± 0.300	1	83	TRP102	NE1	130.9702	1
31	PRO98	HA	4.6082 ± 0.006	1	84	GLN103	C	178.0502	1
32	PRO98	HB2	2.1362 ± 0.005	2	85	GLN103	CA	60.0482 ± 0.100	1
33	PRO98	HB3	1.9312 ± 0.008	2	86	GLN103	CB	27.8082 ± 0.050	1
34	PRO98	HD2	3.6012	2	87	GLN103	CG	34.0942 ± 0.003	1
35	PRO98	HD3	3.6012	2	88	GLN103	HA	3.9802 ± 0.020	1
36	PRO98	HG2	2.0392 ± 0.029	2	89	GLN103	HB2	2.0882 ± 0.002	2
37	PRO98	HG3	2.0392 ± 0.029	2	90	GLN103	HB3	2.0882 ± 0.002	2
38	THR99	C	175.8502 ± 0.035	1	91	GLN103	HG2	2.4822 ± 0.015	2
39	THR99	CA	61.8532 ± 0.101	1	92	GLN103	HG3	2.4822 ± 0.015	2
40	THR99	CB	70.6512 ± 0.102	1	93	GLN103	H	8.3782 ± 0.009	1
41	THR99	CG2	22.0732 ± 0.077	1	94	GLN103	N	117.0242 ± 0.100	1
42	THR99	HA	4.2432 ± 0.006	1	95	GLN104	C	179.6752 ± 0.031	1
43	THR99	HB	4.7452 ± 0.011	1	96	GLN104	CA	59.0032 ± 0.068	1
44	THR99	HG2	1.4182 ± 0.006	1	97	GLN104	CB	28.0172 ± 0.074	1
45	THR99	H	8.6342 ± 0.011	1	98	GLN104	CG	33.9302 ± 0.098	1
46	THR99	N	113.4402 ± 0.100	1	99	GLN104	HA	3.9182 ± 0.018	1
47	LEU100	C	179.1522	1	100	GLN104	HB2	2.1172 ± 0.020	2
48	LEU100	CA	58.1272 ± 0.166	1	101	GLN104	HB3	2.1172 ± 0.020	2
49	LEU100	CB	41.2702 ± 0.104	1	102	GLN104	HE21	7.3432 ± 0.001	2
50	LEU100	CD1	23.9992 ± 0.129	2	103	GLN104	HE22	6.8222 ± 0.005	2
51	LEU100	CD2	23.9992 ± 0.129	2	104	GLN104	HG2	2.5272 ± 0.007	2
52	LEU100	CG	27.1102 ± 0.067	1	105	GLN104	HG3	2.3772 ± 0.006	2
53	LEU100	HA	4.0962 ± 0.007	1	106	GLN104	H	8.6912 ± 0.009	1

107	GLN104N	117.4402 ± 0.102	1
108	GLN104NE2	110.5112 ± 0.072	1
109	GLN105C	178.9732 ± 0.016	1
110	GLN105CA	58.7562 ± 0.108	1
111	GLN105CB	27.8812 ± 0.129	1
112	GLN105CG	33.8302 ± 0.074	1
113	GLN105HA	4.0482 ± 0.009	1
114	GLN105HB2	2.1012 ± 0.027	2
115	GLN105HB3	2.1012 ± 0.027	2
116	GLN105HG2	2.4692 ± 0.024	2
117	GLN105HG3	2.4692 ± 0.024	2
118	GLN105H	8.1522 ± 0.008	1
119	GLN105N	120.6432 ± 0.097	1
120	GLN106C	180.3832 ± 0.031	1
121	GLN106CA	57.2482 ± 0.126	1
122	GLN106CB	25.9742 ± 0.070	1
123	GLN106CG	30.8512 ± 0.183	1
124	GLN106HA	3.8992 ± 0.020	1
125	GLN106HB3	1.6342 ± 0.012	2
126	GLN106HB2	1.6342 ± 0.012	2
127	GLN106HE21	6.4742 ± 0.003	2
128	GLN106HE22	6.4742 ± 0.003	2
129	GLN106HG2	1.2742 ± 0.010	2
130	GLN106HG3	0.6082 ± 0.009	2
131	GLN106H	8.0602 ± 0.011	1
132	GLN106N	117.5282 ± 0.067	1
133	GLN106NE2	111.5252 ± 0.119	1
134	VAL107C	177.3352 ± 0.024	1
135	VAL107CA	67.4612 ± 0.077	1
136	VAL107CB	31.6682 ± 0.109	1
137	VAL107CG1	24.5732 ± 0.089	1
138	VAL107CG2	22.0312 ± 0.105	1
139	VAL107HA	3.5102 ± 0.015	1
140	VAL107HB	2.1942 ± 0.004	1
141	VAL107HG1	1.0252 ± 0.008	1
142	VAL107HG2	1.1132 ± 0.014	1
143	VAL107H	8.1212 ± 0.012	1
144	VAL107N	122.0912 ± 0.048	1
145	ALA108C	181.0952 ± 0.018	1
146	ALA108CA	55.3992 ± 0.059	1
147	ALA108CB	17.8942 ± 0.101	1
148	ALA108HA	4.2142 ± 0.010	1
149	ALA108HB	1.5182 ± 0.009	1
150	ALA108H	7.8212 ± 0.009	1
151	ALA108N	123.1582 ± 0.144	1
152	GLN109C	178.5272 ± 0.015	1
153	GLN109CA	58.5542 ± 0.097	1
154	GLN109CB	28.2922 ± 0.080	1
155	GLN109CG	33.9002 ± 0.099	1
156	GLN109HA	4.1302 ± 0.016	1
157	GLN109HB2	2.1232 ± 0.014	2
158	GLN109HB3	2.1232 ± 0.014	2
159	GLN109HG2	2.5102 ± 0.026	2
160	GLN109HG3	2.5102 ± 0.026	2
161	GLN109H	8.1832 ± 0.010	1
162	GLN109N	117.3562 ± 0.122	1
163	PHE110 C	177.0322	1
164	PHE110 CA	60.2962 ± 0.094	1
165	PHE110 CB	39.8762 ± 0.149	1
166	PHE110 CD1	133.1052	3
167	PHE110 CD2	133.1052	3
168	PHE110 HA	4.4142 ± 0.013	1
169	PHE110 HB2	3.1272 ± 0.016	2
170	PHE110 HB3	2.6602 ± 0.010	2
171	PHE110 HD1	6.8302	3
172	PHE110 HD2	6.8302	3
173	PHE110 H	8.2272 ± 0.010	1
174	PHE110 N	121.2282 ± 0.112	1
175	SER111 C	177.3822	1
176	SER111 CA	62.1412 ± 0.149	1
177	SER111 CB	62.8152 ± 0.102	1
178	SER111 HA	3.8312 ± 0.011	1
179	SER111 HB2	4.1832 ± 0.010	2
180	SER111 HB3	4.1832 ± 0.010	2
181	SER111 H	8.2222 ± 0.010	1
182	SER111 N	113.3222 ± 0.089	1
183	THR112C	176.0282	1
184	THR112CA	66.6682 ± 0.093	1
185	THR112CB	68.5902 ± 0.104	1
186	THR112CG2	22.2242 ± 0.118	1
187	THR112HA	3.9192 ± 0.009	1
188	THR112HB	4.3062 ± 0.012	1
189	THR112HG2	1.2372 ± 0.009	1
190	THR112H	7.5292 ± 0.009	1
191	THR112N	118.3302 ± 0.122	1
192	VAL113C	178.4492 ± 0.013	1
193	VAL113CA	67.1932 ± 0.116	1
194	VAL113CB	31.8562 ± 0.045	1
195	VAL113CG1	20.9912 ± 0.076	1
196	VAL113CG2	22.7932 ± 0.082	1
197	VAL113HA	3.6342 ± 0.011	1
198	VAL113HB	2.1912 ± 0.006	1
199	VAL113HG1	0.9292 ± 0.003	1
200	VAL113HG2	1.2582 ± 0.005	1
201	VAL113H	7.9672 ± 0.012	1
202	VAL113N	124.5372 ± 0.091	1
203	ARG114C	179.3502 ± 0.014	1
204	ARG114CA	60.0022 ± 0.150	1
205	ARG114CB	30.3912 ± 0.108	1
206	ARG114CD	41.4922	1
207	ARG114CG	27.1032 ± 0.166	1
208	ARG114HA	3.5332 ± 0.016	1
209	ARG114HB2	1.7802 ± 0.011	2
210	ARG114HB3	1.4682 ± 0.008	2
211	ARG114HD2	2.2952	2
212	ARG114HD3	2.2952	2

213	ARG114	HG2	1.6432 ± 0.004	2	266	LYS119	HE2	2.8402 ± 0.020	2
214	ARG114	HG3	0.4392 ± 0.012	2	267	LYS119	HE3	2.8402 ± 0.020	2
215	ARG114	H	8.2132 ± 0.016	1	268	LYS119	HG2	1.2662 ± 0.013	2
216	ARG114	N	118.4812 ± 0.126	1	269	LYS119	HG3	1.0332 ± 0.015	2
217	GLN115	C	178.7002 ± 0.035	1	270	LYS119	H	8.2832 ± 0.010	1
218	GLN115	CA	58.8282 ± 0.085	1	271	LYS119	N	119.4252 ± 0.109	1
219	GLN115	CB	28.2302 ± 0.116	1	272	HIS120	C	175.9552 ± 0.049	1
220	GLN115	CG	34.0102 ± 0.128	1	273	HIS120	CA	56.4612 ± 0.059	1
221	GLN115	HA	4.0892 ± 0.017	1	274	HIS120	CB	31.1242 ± 0.092	1
222	GLN115	HB2	2.1662 ± 0.020	2	275	HIS120	CD2	120.1972 ± 0.041	1
223	GLN115	HB3	2.1662 ± 0.020	2	276	HIS120	CE1	138.4402 ± 0.069	1
224	GLN115	HG2	2.4652 ± 0.031	2	277	HIS120	HA	5.1222 ± 0.012	1
225	GLN115	HG3	2.4652 ± 0.031	2	278	HIS120	HB2	3.6712 ± 0.013	2
226	GLN115	H	7.8222 ± 0.010	1	279	HIS120	HB3	3.4502 ± 0.019	2
227	GLN115	N	119.1792 ± 0.117	1	280	HIS120	HD2	6.9002 ± 0.008	1
228	ASN116	C	178.1002	1	281	HIS120	HE1	7.7572 ± 0.003	1
229	ASN116	CA	56.0522 ± 0.113	1	282	HIS120	H	7.6562 ± 0.021	1
230	ASN116	CB	37.5262 ± 0.111	1	283	HIS120	N	114.2002 ± 0.148	1
231	ASN116	HA	4.3342 ± 0.017	1	284	ARG121	C	177.9342 ± 0.017	1
232	ASN116	HB2	2.5832 ± 0.016	2	285	ARG121	CA	61.2632 ± 0.080	1
233	ASN116	HB3	2.5832 ± 0.016	2	286	ARG121	CB	30.3982 ± 0.055	1
234	ASN116	H	7.9382 ± 0.013	1	287	ARG121	CD	43.5912 ± 0.097	1
235	ASN116	N	120.0542 ± 0.058	1	288	ARG121	CG	28.9722 ± 0.115	1
236	VAL117	C	178.1032 ± 0.006	1	289	ARG121	HA	4.2622 ± 0.008	1
237	VAL117	CA	66.9072 ± 0.168	1	290	ARG121	HB2	2.3332 ± 0.010	2
238	VAL117	CB	31.5752 ± 0.070	1	291	ARG121	HB3	1.9762 ± 0.013	2
239	VAL117	CG1	21.2182 ± 0.126	1	292	ARG121	HD2	3.4332 ± 0.022	2
240	VAL117	CG2	24.0142 ± 0.086	1	293	ARG121	HD3	3.3722 ± 0.026	2
241	VAL117	HA	3.6992 ± 0.007	1	294	ARG121	HG2	1.9682	2
242	VAL117	HB	2.1982 ± 0.007	1	295	ARG121	HG3	1.7192 ± 0.012	2
243	VAL117	HG1	0.9302 ± 0.008	1	296	ARG121	H	7.8262 ± 0.020	1
244	VAL117	HG2	1.0442 ± 0.013	1	297	ARG121	N	122.6482 ± 0.073	1
245	VAL117	H	8.5252 ± 0.010	1	298	SER122	C	176.5282	1
246	VAL117	N	122.2512 ± 0.077	1	299	SER122	CA	61.9742 ± 0.087	1
247	ASN118	C	178.0862 ± 0.013	1	300	SER122	CB	62.3892 ± 0.073	1
248	ASN118	CA	56.5012 ± 0.157	1	301	SER122	HA	4.1012 ± 0.016	1
249	ASN118	CB	38.9542 ± 0.104	1	302	SER122	HB2	3.9692 ± 0.013	2
250	ASN118	HA	4.4342 ± 0.015	1	303	SER122	HB3	3.9692 ± 0.013	2
251	ASN118	HB2	2.8502 ± 0.008	2	304	SER122	H	8.7932 ± 0.014	1
252	ASN118	HB3	2.8502 ± 0.008	2	305	SER122	N	112.2832 ± 0.080	1
253	ASN118	H	7.7852 ± 0.008	1	306	HIS123	C	177.8902 ± 0.003	1
254	ASN118	N	116.0502 ± 0.077	1	307	HIS123	CA	59.2622 ± 0.092	1
255	LYS119	C	178.5862 ± 0.029	1	308	HIS123	CB	29.2352 ± 0.040	1
256	LYS119	CA	59.1382 ± 0.144	1	309	HIS123	CE1	137.5602 ± 0.063	1
257	LYS119	CB	32.8842 ± 0.100	1	310	HIS123	HA	4.0862 ± 0.029	1
258	LYS119	CD	29.3842 ± 0.087	1	311	HIS123	HB2	2.9462 ± 0.020	2
259	LYS119	CE	41.7572 ± 0.097	1	312	HIS123	HB3	2.9462 ± 0.020	2
260	LYS119	CG	24.7682 ± 0.092	1	313	HIS123	HD2	7.0272	1
261	LYS119	HA	4.0432 ± 0.008	1	314	HIS123	HE1	7.3892 ± 0.005	1
262	LYS119	HB2	1.7792 ± 0.013	2	315	HIS123	H	7.8772 ± 0.008	1
263	LYS119	HB3	1.5112 ± 0.013	2	316	HIS123	N	121.3222 ± 0.151	1
264	LYS119	HD2	1.5392 ± 0.016	2	317	TRP124	C	178.2752 ± 0.010	1
265	LYS119	HD3	1.5392 ± 0.016	2	318	TRP124	CA	60.3682 ± 0.081	1

319	TRP	124	CB	29.6882 ± 0.034	1	372	GLN	128	CG	33.7472 ± 0.106	1
320	TRP	124	CD1	128.3502 ± 0.069	1	373	GLN	128	HA	4.1682 ± 0.011	1
321	TRP	124	CH2	122.7542 ± 0.025	1	374	GLN	128	HB2	2.0222 ± 0.008	2
322	TRP	124	CZ2	120.8332 ± 0.094	1	375	GLN	128	HB3	2.0222 ± 0.008	2
323	TRP	124	HA	4.1882 ± 0.023	1	376	GLN	128	HG2	2.3972 ± 0.010	2
324	TRP	124	HB2	3.1032 ± 0.017	2	377	GLN	128	HG3	2.3972 ± 0.010	2
325	TRP	124	HB3	3.1032 ± 0.017	2	378	GLN	128	H	8.6152 ± 0.007	1
326	TRP	124	HD1	7.5542 ± 0.003	1	379	GLN	128	N	122.7902 ± 0.082	1
327	TRP	124	HE1	10.4712 ± 0.008	1	380	LEU	129	C	177.3272 ± 0.008	1
328	TRP	124	HH2	7.1892 ± 0.001	1	381	LEU	129	CA	54.3672 ± 0.052	1
329	TRP	124	H	7.8952 ± 0.009	1	382	LEU	129	CB	43.2722 ± 0.326	1
330	TRP	124	HZ2	7.3332 ± 0.003	1	383	LEU	129	CD1	24.1962 ± 0.171	2
331	TRP	124	N	120.2172 ± 0.106	1	384	LEU	129	CD2	24.1962 ± 0.171	2
332	TRP	124	NE1	128.3712 ± 0.024	1	385	LEU	129	CG	26.8262 ± 0.090	1
333	LYS	125	C	176.3172 ± 0.036	1	386	LEU	129	HA	4.3932 ± 0.010	1
334	LYS	125	CA	58.5492 ± 0.066	1	387	LEU	129	HB2	1.6082	2
335	LYS	125	CB	32.2442 ± 0.088	1	388	LEU	129	HB3	1.5552 ± 0.012	2
336	LYS	125	CD	29.4082 ± 0.201	1	389	LEU	129	HD1	0.8512 ± 0.010	2
337	LYS	125	CE	42.0512 ± 0.107	1	390	LEU	129	HD2	0.8512 ± 0.010	2
338	LYS	125	CG	24.9632 ± 0.162	1	391	LEU	129	HG	1.6732 ± 0.013	1
339	LYS	125	HA	4.0852 ± 0.022	1	392	LEU	129	H	8.4782 ± 0.011	1
340	LYS	125	HB2	1.8032 ± 0.016	2	393	LEU	129	N	125.9012 ± 0.052	1
341	LYS	125	HB3	1.8032 ± 0.016	2	394	ASP	130	C	177.4252 ± 0.025	1
342	LYS	125	HD2	1.6682 ± 0.018	2	395	ASP	130	CA	55.0882 ± 0.054	1
343	LYS	125	HD3	1.6682 ± 0.018	2	396	ASP	130	CB	41.9682 ± 0.109	1
344	LYS	125	HE2	2.9532 ± 0.024	2	397	ASP	130	HA	4.5272 ± 0.012	1
345	LYS	125	HE3	2.9532 ± 0.024	2	398	ASP	130	HB2	2.7462 ± 0.014	2
346	LYS	125	HG2	1.4162 ± 0.027	2	399	ASP	130	HB3	2.7462 ± 0.014	2
347	LYS	125	HG3	1.4162 ± 0.027	2	400	ASP	130	H	8.5752 ± 0.010	1
348	LYS	125	H	8.5192 ± 0.016	1	401	ASP	130	N	123.3372 ± 0.058	1
349	LYS	125	N	117.7372 ± 0.062	1	402	SER	131	C	174.6322	1
350	SER	126	C	174.6632 ± 0.023	1	403	SER	131	CA	60.4652 ± 0.110	1
351	SER	126	CA	58.8162 ± 0.025	1	404	SER	131	CB	63.1702 ± 0.100	1
352	SER	126	CB	64.0642 ± 0.051	1	405	SER	131	HA	4.2022 ± 0.012	1
353	SER	126	HA	4.4042 ± 0.004	1	406	SER	131	HB2	3.9392 ± 0.008	2
354	SER	126	HB2	3.8832 ± 0.005	2	407	SER	131	HB3	3.9392 ± 0.008	2
355	SER	126	HB3	3.8832 ± 0.005	2	408	SER	131	H	8.5572 ± 0.010	1
356	SER	126	H	7.2792 ± 0.015	1	409	SER	131	N	117.8912 ± 0.050	1
357	SER	126	N	111.4372 ± 0.076	1	410	ASN	132	C	174.7532 ± 0.024	1
358	GLN	127	C	175.6752 ± 0.029	1	411	ASN	132	CA	53.6732 ± 0.117	1
359	GLN	127	CA	54.5982 ± 0.059	1	412	ASN	132	CB	38.3342 ± 0.114	1
360	GLN	127	CB	29.0162 ± 0.130	1	413	ASN	132	HA	4.6722 ± 0.011	1
361	GLN	127	CG	33.1082 ± 0.066	1	414	ASN	132	HB2	2.9632 ± 0.015	2
362	GLN	127	HA	4.1432 ± 0.004	1	415	ASN	132	HB3	2.8512 ± 0.009	2
363	GLN	127	HB2	2.1462 ± 0.030	2	416	ASN	132	H	8.6202 ± 0.008	1
364	GLN	127	HB3	1.9492 ± 0.015	2	417	ASN	132	N	118.0462 ± 0.077	1
365	GLN	127	HG2	2.2122 ± 0.007	2	418	VAL	133	C	174.8932 ± 0.033	1
366	GLN	127	HG3	2.0702 ± 0.011	2	419	VAL	133	CA	62.5492 ± 0.079	1
367	GLN	127	H	7.2142 ± 0.013	1	420	VAL	133	CB	31.8792 ± 0.152	1
368	GLN	127	N	121.0652 ± 0.074	1	421	VAL	133	CG1	21.2162 ± 0.071	1
369	GLN	128	C	176.2522 ± 0.009	1	422	VAL	133	CG2	21.5092 ± 0.076	1
370	GLN	128	CA	56.2792 ± 0.129	1	423	VAL	133	HA	3.7872 ± 0.011	1
371	GLN	128	CB	29.1232 ± 0.073	1	424	VAL	133	HB	2.1352 ± 0.008	1

425	VAL133	HG1	0.8512 ± 0.019 1
426	VAL133	HG2	0.6652 ± 0.011 1
427	VAL133	H	7.7982 ± 0.011 1
428	VAL133	N	121.4042 ± 0.028 1
429	THR134	C	173.7982 1
430	THR134	CA	60.7952 ± 0.082 1
431	THR134	CB	69.5482 ± 0.109 1
432	THR134	CG2	21.4242 ± 0.119 1
433	THR134	HA	4.2312 ± 0.008 1
434	THR134	HB	3.8802 ± 0.007 1
435	THR134	HG2	0.9922 ± 0.012 1
436	THR134	H	8.2062 ± 0.010 1
437	THR134	N	123.7512 ± 0.085 1
438	MET135	C	172.9952 1
439	MET135	CA	53.7142 ± 0.100 1
440	MET135	CB	32.1752 ± 0.041 1
441	MET135	CE	17.1812 ± 0.045 1
442	MET135	CG	32.2362 ± 0.088 1
443	MET135	HA	3.3302 ± 0.007 1
444	MET135	HB2	2.1612 ± 0.088 2
445	MET135	HB3	2.0222 ± 0.015 2
446	MET135	HE	2.1482 ± 0.005 1
447	MET135	HG2	1.6202 ± 0.018 2
448	MET135	HG3	1.6202 ± 0.018 2
449	MET135	H	8.5712 ± 0.009 1
450	MET135	N	126.7302 ± 0.049 1
451	PRO136	C	175.4962 ± 0.018 1
452	PRO136	CA	61.5362 ± 0.082 1
453	PRO136	CB	30.4772 ± 0.116 1
454	PRO136	CD	48.8102 ± 0.108 1
455	PRO136	CG	26.5822 ± 0.154 1
456	PRO136	HA	4.0712 ± 0.009 1
457	PRO136	HB2	1.7952 ± 0.005 2
458	PRO136	HB3	0.4422 ± 0.013 2
459	PRO136	HD2	2.3482 ± 0.010 2
460	PRO136	HD3	0.3352 ± 0.089 2
461	PRO136	HG2	0.8782 ± 0.012 2
462	PRO136	HG3	-0.1008 ± 0.011 2
463	LYS137	C	179.9642 ± 0.001 1
464	LYS137	CA	56.6512 ± 0.043 1
465	LYS137	CB	32.2932 ± 0.078 1
466	LYS137	CD	28.8272 ± 0.063 1
467	LYS137	CE	41.9882 ± 0.119 1
468	LYS137	CG	25.4372 ± 0.050 1
469	LYS137	HA	4.2522 ± 0.006 1
470	LYS137	HB2	2.1582 ± 0.010 2
471	LYS137	HB3	1.7112 ± 0.010 2
472	LYS137	HD2	1.7282 ± 0.014 2
473	LYS137	HD3	1.7282 ± 0.014 2
474	LYS137	HE2	3.0262 ± 0.003 2
475	LYS137	HE3	3.0262 ± 0.003 2
476	LYS137	HG2	1.6342 ± 0.003 2
477	LYS137	HG3	1.5692 ± 0.002 2
478	LYS137	H	8.7352 ± 0.005 1
479	LYS137	N	120.6472 ± 0.086 1
480	SER138	C	175.1712 1
481	SER138	CA	63.3242 ± 0.119 1
482	SER138	CB	62.8602 ± 0.125 1
483	SER138	HA	4.1702 ± 0.021 1
484	SER138	HB2	3.8982 ± 0.022 2
485	SER138	HB3	3.8982 ± 0.022 2
486	SER138	H	9.0942 ± 0.009 1
487	SER138	N	118.9292 ± 0.076 1
488	GLU139	C	175.7382 ± 0.018 1
489	GLU139	CA	57.5432 ± 0.105 1
490	GLU139	CB	28.8012 ± 0.033 1
491	GLU139	CG	35.9312 ± 0.169 1
492	GLU139	HA	4.3722 ± 0.012 1
493	GLU139	HB2	2.1652 ± 0.004 2
494	GLU139	HB3	1.7452 ± 0.003 2
495	GLU139	HG2	2.3322 ± 0.005 2
496	GLU139	HG3	2.2552 ± 0.002 2
497	GLU139	H	9.3032 ± 0.006 1
498	GLU139	N	117.1372 ± 0.063 1
499	ASP140	C	173.7752 1
500	ASP140	CA	52.4452 ± 0.092 1
501	ASP140	CB	39.8642 ± 0.113 1
502	ASP140	HA	4.6262 ± 0.019 1
503	ASP140	HB2	3.2742 ± 0.010 2
504	ASP140	HB3	2.3712 ± 0.015 2
505	ASP140	H	7.8542 ± 0.008 1
506	ASP140	N	121.2752 ± 0.084 1
507	GLU141	C	177.6952 ± 0.035 1
508	GLU141	CA	59.2412 ± 0.104 1
509	GLU141	CB	29.7792 ± 0.177 1
510	GLU141	CG	35.4032 ± 0.061 1
511	GLU141	HA	2.3172 ± 0.013 1
512	GLU141	HB2	1.6562 ± 0.010 2
513	GLU141	HB3	1.4532 ± 0.023 2
514	GLU141	HG2	1.9382 ± 0.018 2
515	GLU141	HG3	1.7362 ± 0.016 2
516	GLU141	H	7.3992 ± 0.009 1
517	GLU141	N	127.7902 ± 0.047 1
518	GLU142	C	179.4542 ± 0.011 1
519	GLU142	CA	58.8912 ± 0.064 1
520	GLU142	CB	29.0182 ± 0.085 1
521	GLU142	CG	36.3882 ± 0.106 1
522	GLU142	HA	3.9212 ± 0.037 1
523	GLU142	HB2	1.9992 ± 0.012 2
524	GLU142	HB3	1.9992 ± 0.012 2
525	GLU142	HG2	2.3322 ± 0.022 2
526	GLU142	HG3	2.3322 ± 0.022 2
527	GLU142	H	8.5562 ± 0.011 1
528	GLU142	N	116.4052 ± 0.123 1
529	GLY143	C	177.6242 ± 0.008 1
530	GLY143	CA	47.2912 ± 0.099 1

531	GLY143	HA2	3.6392 ± 0.014 2
532	GLY143	HA3	3.6392 ± 0.014 2
533	GLY143	H	8.2742 ± 0.007 1
534	GLY143	N	108.7152 ± 0.104 1
535	TRP144	C	178.4112 ± 0.015 1
536	TRP144	CA	61.9702 ± 0.066 1
537	TRP144	CB	30.1102 ± 0.135 1
538	TRP144	CD1	128.2382 ± 0.087 1
539	TRP144	CH2	123.6472 ± 0.087 1
540	TRP144	CZ2	115.1562 ± 0.182 1
541	TRP144	HA	4.1782 ± 0.016 1
542	TRP144	HB2	3.3022 ± 0.014 2
543	TRP144	HB3	3.0282 ± 0.014 2
544	TRP144	HD1	7.2712 ± 0.009 1
545	TRP144	HE1	8.1462 ± 0.006 1
546	TRP144	HH2	6.7262 ± 0.005 1
547	TRP144	H	8.4292 ± 0.007 1
548	TRP144	HZ2	7.0962 ± 0.004 1
549	TRP144	HZ3	6.4482 ± 0.002 1
550	TRP144	N	123.2572 ± 0.140 1
551	TRP144	NE1	127.0622 ± 0.034 1
552	LYS145	C	177.6482 ± 0.003 1
553	LYS145	CA	61.3962 ± 0.107 1
554	LYS145	CB	32.3202 ± 0.119 1
555	LYS145	CG	27.1042 ± 0.146 1
556	LYS145	HA	3.4672 ± 0.008 1
557	LYS145	HB2	1.8022 ± 0.024 2
558	LYS145	HB3	1.8022 ± 0.024 2
559	LYS145	HG2	1.6582 ± 0.014 2
560	LYS145	HG3	1.6582 ± 0.014 2
561	LYS145	H	7.9552 ± 0.008 1
562	LYS145	N	119.4302 ± 0.104 1
563	LYS146	C	179.4912 ± 0.028 1
564	LYS146	CA	59.4672 ± 0.085 1
565	LYS146	CB	32.5542 ± 0.063 1
566	LYS146	CD	29.5262 ± 0.192 1
567	LYS146	CE	42.0682 ± 0.147 1
568	LYS146	CG	26.2222 ± 0.083 1
569	LYS146	HA	3.7532 ± 0.019 1
570	LYS146	HB2	1.7592 ± 0.005 2
571	LYS146	HB3	1.7592 ± 0.005 2
572	LYS146	HD2	1.6642 ± 0.017 2
573	LYS146	HD3	1.6642 ± 0.017 2
574	LYS146	HE2	2.9182 ± 0.010 2
575	LYS146	HE3	2.9182 ± 0.010 2
576	LYS146	HG2	1.5252 ± 0.007 2
577	LYS146	HG3	1.2982 ± 0.005 2
578	LYS146	H	7.6502 ± 0.008 1
579	LYS146	N	116.6852 ± 0.042 1
580	PHE147	C	175.4162 ± 0.037 1
581	PHE147	CA	61.7822 ± 0.101 1
582	PHE147	CB	40.3962 ± 0.097 1
583	PHE147	CD1	133.3442 ± 0.065 3
584	PHE147	CD2	133.3442 ± 0.065 3
585	PHE147	CE1	130.8072 ± 0.199 3
586	PHE147	CE2	130.8072 ± 0.199 3
587	PHE147	HA	4.1482 ± 0.008 1
588	PHE147	HB2	3.0312 ± 0.023 2
589	PHE147	HB3	2.8242 ± 0.013 2
590	PHE147	HD1	7.1412 ± 0.005 3
591	PHE147	HD2	7.1412 ± 0.005 3
592	PHE147	HE1	6.8392 ± 0.017 3
593	PHE147	HE2	6.8392 ± 0.017 3
594	PHE147	H	8.4332 ± 0.010 1
595	PHE147	N	122.7912 ± 0.128 1
596	CYS148	C	175.1462 ± 0.025 1
597	CYS148	CA	62.2652 ± 0.129 1
598	CYS148	CB	27.8802 ± 0.142 1
599	CYS148	HA	4.0212 ± 0.008 1
600	CYS148	HB2	2.0102 ± 0.007 2
601	CYS148	HB3	1.5732 ± 0.011 2
602	CYS148	H	8.1422 ± 0.011 1
603	CYS148	N	113.2762 ± 0.120 1
604	LEU149	C	177.5342 ± 0.007 1
605	LEU149	CA	54.2522 ± 0.148 1
606	LEU149	CB	41.9742 ± 0.136 1
607	LEU149	CD1	23.2352 ± 0.084 1
608	LEU149	CD2	20.9562 ± 0.103 1
609	LEU149	CG	26.5072 ± 0.170 1
610	LEU149	HA	4.2182 ± 0.011 1
611	LEU149	HB2	1.3232 ± 0.013 2
612	LEU149	HB3	1.0082 ± 0.016 2
613	LEU149	HD1	-0.6808 ± 0.011 1
614	LEU149	HD2	0.1902 ± 0.007 1
615	LEU149	HG	0.9152 ± 0.009 1
616	LEU149	H	7.6832 ± 0.012 1
617	LEU149	N	112.7562 ± 0.105 1
618	GLY150	C	173.3752 ± 0.052 1
619	GLY150	CA	44.6662 ± 0.091 1
620	GLY150	HA2	3.9432 ± 0.016 2
621	GLY150	HA3	3.4892 ± 0.008 2
622	GLY150	H	7.6142 ± 0.009 1
623	GLY150	N	107.0922 ± 0.120 1
624	GLU151	CG	33.7612 1
625	GLU151	HG2	2.3612 2
626	GLU151	HG3	2.3612 2
627	GLU151	H	8.3402 ± 0.006 1
628	GLU151	N	118.3642 ± 0.144 1
629	LYS152	C	178.2312 1
630	LYS152	CA	58.8852 ± 0.070 1
631	LYS152	CB	32.2132 ± 0.176 1
632	LYS152	CD	29.2412 1
633	LYS152	CE	41.8662 ± 0.074 1
634	LYS152	CG	25.0712 ± 0.129 1
635	LYS152	HA	4.1062 ± 0.031 1
636	LYS152	HB2	1.8262 2

637	LYS152	HB3	1.8262	2	690	ALA158	H	8.0802 ± 0.010	1
638	LYS152	HE2	2.9622 ± 0.021	2	691	ALA158	N	123.5882 ± 0.064	1
639	LYS152	HE3	2.9622 ± 0.021	2	692	VAL159	C	176.3312 ± 0.013	1
640	LYS152	HG2	1.4442 ± 0.014	2	693	VAL159	CA	62.0912 ± 0.117	1
641	LYS152	HG3	1.4442 ± 0.014	2	694	VAL159	CB	32.9352 ± 0.059	1
642	LEU153	C	178.4632	1	695	VAL159	CG1	20.9582 ± 0.173	2
643	LEU153	CA	56.1882 ± 0.075	1	696	VAL159	CG2	20.9582 ± 0.173	2
644	LEU153	CB	42.1572 ± 0.108	1	697	VAL159	HA	4.1722 ± 0.004	1
645	LEU153	CD1	24.4172 ± 0.256	2	698	VAL159	HB	2.1012 ± 0.002	1
646	LEU153	CD2	24.4172 ± 0.256	2	699	VAL159	HG1	0.9532 ± 0.005	2
647	LEU153	CG	27.8492 ± 0.087	1	700	VAL159	HG2	0.9532 ± 0.005	2
648	LEU153	HA	4.3852 ± 0.007	1	701	VAL159	H	8.1182 ± 0.006	1
649	LEU153	HB2	1.6332 ± 0.015	2	702	VAL159	N	118.9252 ± 0.050	1
650	LEU153	HB3	1.6332 ± 0.015	2	703	GLY160	C	171.6202	1
651	LEU153	HD1	0.9862 ± 0.014	2	704	GLY160	CA	44.4732 ± 0.116	1
652	LEU153	HD2	0.9862 ± 0.014	2	705	GLY160	HA2	4.1782 ± 0.008	2
653	LEU153	HG	1.7302 ± 0.008	1	706	GLY160	HA3	4.0082 ± 0.012	2
654	LEU153	H	8.0752 ± 0.011	1	707	GLY160	H	8.3112 ± 0.007	1
655	LEU153	N	119.6572 ± 0.089	1	708	GLY160	N	112.6552 ± 0.049	1
656	CYS154	C	174.5262	1	709	PRO161	C	176.8082 ± 0.020	1
657	CYS154	CA	60.4722 ± 0.114	1	710	PRO161	CA	63.1012 ± 0.094	1
658	CYS154	CB	27.9452 ± 0.137	1	711	PRO161	CB	32.2032 ± 0.089	1
659	CYS154	HA	4.5482 ± 0.008	1	712	PRO161	CD	49.8352 ± 0.101	1
660	CYS154	HB2	2.9472 ± 0.007	2	713	PRO161	CG	27.2062 ± 0.128	1
661	CYS154	HB3	2.7162 ± 0.013	2	714	PRO161	HA	4.4222 ± 0.007	1
662	CYS154	H	7.6852 ± 0.011	1	715	PRO161	HB2	2.2592 ± 0.008	2
663	CYS154	N	116.1582 ± 0.070	1	716	PRO161	HB3	1.9322 ± 0.007	2
664	ALA155	C	177.3912 ± 0.014	1	717	PRO161	HB3	1.9322 ± 0.007	2
665	ALA155	CA	52.7272 ± 0.062	1	718	PRO161	HD2	3.6142 ± 0.003	2
666	ALA155	CB	19.3542 ± 0.109	1	719	PRO161	HD3	3.6142 ± 0.003	2
667	ALA155	HA	4.3802 ± 0.019	1	720	PRO161	HG2	1.9942 ± 0.011	2
668	ALA155	HB	1.4602 ± 0.013	1	721	PRO161	HG3	1.9942 ± 0.011	2
669	ALA155	H	7.9282 ± 0.008	1	722	ALA162	C	178.0262 ± 0.011	1
670	ALA155	N	124.1472 ± 0.089	1	723	ALA162	CA	52.4502 ± 0.047	1
671	ASP156	C	176.7962 ± 0.014	1	724	ALA162	CB	19.2402 ± 0.105	1
672	ASP156	CA	54.6302 ± 0.073	1	725	ALA162	HA	4.3802 ± 0.007	1
673	ASP156	CB	41.1692 ± 0.082	1	726	ALA162	HB	1.4002 ± 0.006	1
674	ASP156	HA	4.6082 ± 0.010	1	727	ALA162	H	8.4642 ± 0.007	1
675	ASP156	HB2	2.7032 ± 0.011	2	728	ALA162	N	124.3482 ± 0.056	1
676	ASP156	HB3	2.7032 ± 0.011	2	729	THR163	C	174.3552 ± 0.018	1
677	ASP156	H	8.3162 ± 0.006	1	730	THR163	CA	61.6702 ± 0.081	1
678	ASP156	N	119.6032 ± 0.128	1	731	THR163	CB	69.9712 ± 0.138	1
679	GLY157	C	173.8652 ± 0.018	1	732	THR163	CG2	21.5482 ± 0.093	1
680	GLY157	CA	45.4142 ± 0.113	1	733	THR163	HA	4.3282 ± 0.007	1
681	GLY157	HA2	3.9582 ± 0.017	2	734	THR163	HB	4.2412 ± 0.006	1
682	GLY157	HA3	3.9582 ± 0.017	2	735	THR163	HG2	1.1832 ± 0.005	1
683	GLY157	H	8.3592 ± 0.005	1	736	THR163	H	8.1662 ± 0.009	1
684	GLY157	N	109.2052 ± 0.065	1	737	THR163	N	113.1282 ± 0.029	1
685	ALA158	C	177.6422 ± 0.017	1	738	ASN164	C	174.9882 ± 0.016	1
686	ALA158	CA	52.4642 ± 0.063	1	739	ASN164	CA	53.4272 ± 0.077	1
687	ALA158	CB	19.3642 ± 0.086	1	740	ASN164	CB	38.9272 ± 0.137	1
688	ALA158	HA	4.3712 ± 0.006	1	741	ASN164	HA	4.6972 ± 0.006	1
689	ALA158	HB	1.3972 ± 0.008	1	742	ASN164	HB2	2.8312 ± 0.010	2

743	ASN164	HB3	2.7572 ± 0.010	2	796	ILE169	N	119.6912 ± 0.049	1
744	ASN164	H	8.4332 ± 0.007	1	797	ASP170	C	176.5152 ± 0.024	1
745	ASN164	N	120.3192 ± 0.055	1	798	ASP170	CA	53.2502 ± 0.147	1
746	GLU165	C	176.1772 ± 0.018	1	799	ASP170	CB	40.9852 ± 0.106	1
747	GLU165	CA	56.5772 ± 0.031	1	800	ASP170	HA	4.5522 ± 0.007	1
748	GLU165	CB	30.4172 ± 0.095	1	801	ASP170	HB2	2.6852 ± 0.014	2
749	GLU165	CG	36.2452 ± 0.060	1	802	ASP170	HB3	2.4792 ± 0.020	2
750	GLU165	HA	4.2892 ± 0.006	1	803	ASP170	H	8.1412 ± 0.005	1
751	GLU165	HB2	2.0542 ± 0.009	2	804	ASP170	N	124.5832 ± 0.041	1
752	GLU165	HB3	1.9242 ± 0.077	2	805	TYR171	C	177.2142 ± 0.018	1
753	GLU165	HG2	2.2152 ± 0.011	2	806	TYR171	CA	58.5772 ± 0.081	1
754	GLU165	HG3	2.2152 ± 0.011	2	807	TYR171	CB	36.9112 ± 0.064	1
755	GLU165	H	8.3552 ± 0.007	1	808	TYR171	CD1	133.0372 ± 0.144	3
756	GLU165	N	120.8682 ± 0.041	1	809	TYR171	CD2	133.0372 ± 0.144	3
757	SER166	C	172.6942	1	810	TYR171	CE1	118.1782 ± 0.222	3
758	SER166	CA	56.1982 ± 0.125	1	811	TYR171	CE2	118.1782 ± 0.222	3
759	SER166	CB	63.4072 ± 0.127	1	812	TYR171	HA	4.1352 ± 0.014	1
760	SER166	HA	4.7402 ± 0.004	1	813	TYR171	HB2	2.0772 ± 0.020	2
761	SER166	HB2	3.8482 ± 0.004	2	814	TYR171	HB3	1.9102 ± 0.024	2
762	SER166	HB3	3.8062 ± 0.001	2	815	TYR171	HD1	6.9812 ± 0.013	3
763	SER166	H	8.3262 ± 0.008	1	816	TYR171	HD2	6.9812 ± 0.013	3
764	SER166	N	117.8332 ± 0.022	1	817	TYR171	HE1	6.7482 ± 0.016	3
765	PRO167	C	177.2652 ± 0.023	1	818	TYR171	HE2	6.7482 ± 0.016	3
766	PRO167	CA	63.4222 ± 0.121	1	819	TYR171	H	8.0562 ± 0.009	1
767	PRO167	CB	32.0822 ± 0.069	1	820	TYR171	N	122.4142 ± 0.052	1
768	PRO167	CD	50.6682 ± 0.052	1	821	VAL172	CA	64.8612 ± 0.092	1
769	PRO167	CG	27.2242 ± 0.069	1	822	VAL172	CB	31.7682 ± 0.053	1
770	PRO167	HA	4.4292 ± 0.010	1	823	VAL172	CG1	21.4982 ± 0.042	1
771	PRO167	HB2	2.2532 ± 0.008	2	824	VAL172	CG2	22.2262 ± 0.116	1
772	PRO167	HB3	1.9482 ± 0.012	2	825	VAL172	HA	3.8292 ± 0.007	1
773	PRO167	HD2	3.7622 ± 0.004	2	826	VAL172	HB	2.1322 ± 0.014	1
774	PRO167	HD3	3.7092 ± 0.005	2	827	VAL172	HG1	0.9022 ± 0.017	1
775	PRO167	HG2	1.9972 ± 0.004	2	828	VAL172	HG2	1.0192 ± 0.011	1
776	PRO167	HG3	1.9972 ± 0.004	2	829	VAL172	H	7.8862 ± 0.007	1
777	GLY168	C	173.5902 ± 0.027	1	830	VAL172	N	120.3352 ± 0.098	1
778	GLY168	CA	45.0592 ± 0.082	1	831	GLN173	CA	57.9392 ± 0.098	1
779	GLY168	HA2	3.8962 ± 0.009	2	832	GLN173	CB	29.6892 ± 0.073	1
780	GLY168	HA3	3.8962 ± 0.009	2	833	GLN173	CG	33.8032 ± 0.073	1
781	GLY168	H	8.2582 ± 0.007	1	834	GLN173	HA	4.2002 ± 0.013	1
782	GLY168	N	108.8372 ± 0.052	1	835	GLN173	HB2	2.1012 ± 0.010	2
783	ILE169	C	175.2752 ± 0.016	1	836	GLN173	HB3	2.1012 ± 0.010	2
784	ILE169	CA	60.8782 ± 0.103	1	837	GLN173	HG2	2.3272 ± 0.021	2
785	ILE169	CB	38.6252 ± 0.071	1	838	GLN173	HG3	2.3272 ± 0.021	2
786	ILE169	CD1	13.0192 ± 0.197	1	839	GLN173	H	7.6212 ± 0.010	1
787	ILE169	CG1	27.1052 ± 0.090	1	840	GLN173	N	118.3042 ± 0.105	1
788	ILE169	CG2	17.5252 ± 0.039	1	841	ILE174	C	175.6882	1
789	ILE169	HA	4.0012 ± 0.011	1	842	ILE174	CA	61.9282 ± 0.111	1
790	ILE169	HB	1.6542 ± 0.008	1	843	ILE174	CB	39.3162 ± 0.066	1
791	ILE169	HD1	0.7832 ± 0.008	1	844	ILE174	CD1	14.1042 ± 0.128	1
792	ILE169	HG12	1.3192 ± 0.010	2	845	ILE174	CG1	26.1752 ± 0.113	1
793	ILE169	HG13	1.0122 ± 0.012	2	846	ILE174	CG2	17.9792 ± 0.075	1
794	ILE169	HG2	0.5832 ± 0.005	1	847	ILE174	HA	4.3942 ± 0.010	1
795	ILE169	H	7.9752 ± 0.008	1	848	ILE174	HB	2.0502 ± 0.014	1

849	ILE174	HD1	0.7892 ± 0.003	1
850	ILE174	HG12	1.5432 ± 0.017	2
851	ILE174	HG13	1.0222 ± 0.009	2
852	ILE174	HG2	0.8812 ± 0.010	1
853	ILE174	H	7.8822 ± 0.005	1
854	ILE174	N	114.0152 ± 0.123	1
855	GLY175	C	172.6152 ± 0.017	1
856	GLY175	CA	44.2822 ± 0.105	1
857	GLY175	HA2	3.6942 ± 0.012	2
858	GLY175	HA3	3.2482 ± 0.012	2
859	GLY175	H	7.8182 ± 0.006	1
860	GLY175	N	112.3212 ± 0.061	1
861	PHE176	C	176.9782	1
862	PHE176	CB	38.8482 ± 0.057	1
863	PHE176	CD1	132.5932 ± 0.127	3
864	PHE176	CD2	132.5932 ± 0.127	3
865	PHE176	CE1	131.4192 ± 0.148	3
866	PHE176	CE2	131.4192 ± 0.148	3
867	PHE176	HA	4.1882 ± 0.025	1
868	PHE176	HB2	2.4202 ± 0.019	2
869	PHE176	HB3	2.0332 ± 0.008	2
870	PHE176	HD1	6.7282 ± 0.009	3
871	PHE176	HD2	6.7282 ± 0.009	3
872	PHE176	HE1	7.1122 ± 0.011	3
873	PHE176	HE2	7.1122 ± 0.011	3
874	PHE176	H	8.6472 ± 0.007	1
875	PHE176	N	122.1882 ± 0.091	1
876	PRO177	CD	49.4552 ± 0.117	1
877	PRO177	CG	26.1712 ± 0.065	1
878	PRO177	HD2	3.3662 ± 0.005	2
879	PRO177	HD3	3.1712 ± 0.035	2
880	PRO177	HG2	1.9182 ± 0.003	2
881	PRO177	HG3	1.7202 ± 0.002	2
882	PRO178	C	174.1332	1
883	PRO178	CA	61.5752 ± 0.046	1
884	PRO178	CB	25.8942	1
885	PRO178	CD	48.6692 ± 0.096	1
886	PRO178	CG	30.9472 ± 0.055	1
887	PRO178	HD2	2.7862 ± 0.014	2
888	PRO178	HD3	2.1292 ± 0.016	2
889	PRO178	HG2	2.1422	2
890	PRO178	HG3	1.5002 ± 0.001	2
891	LEU179	C	178.3672	1
892	LEU179	CA	54.0632 ± 0.102	1
893	LEU179	CB	40.9902 ± 0.026	1
894	LEU179	CD1	24.5332 ± 0.088	1
895	LEU179	CD2	21.8992 ± 0.109	1
896	LEU179	CG	26.7122 ± 0.082	1
897	LEU179	HA	3.9502 ± 0.006	1
898	LEU179	HB2	1.7652	2
899	LEU179	HB3	1.2742	2
900	LEU179	HD1	0.9242 ± 0.006	1
901	LEU179	HD2	0.5602 ± 0.005	1
902	LEU179	HG	1.5352 ± 0.009	1
903	LEU179	H	8.1232 ± 0.010	1
904	LEU179	N	123.6092 ± 0.113	1
905	LEU180	CA	58.8072 ± 0.164	1
906	LEU180	CB	40.2762 ± 0.103	1
907	LEU180	CD1	25.8702 ± 0.099	1
908	LEU180	CD2	23.7372 ± 0.108	1
909	LEU180	CG	27.3842 ± 0.076	1
910	LEU180	HA	3.8282 ± 0.015	1
911	LEU180	HB2	1.1742 ± 0.011	2
912	LEU180	HB3	0.3592 ± 0.015	2
913	LEU180	HD1	0.5382 ± 0.013	1
914	LEU180	HD2	0.6752 ± 0.014	1
915	LEU180	HG	1.4002 ± 0.008	1
916	LEU180	H	8.7952 ± 0.010	1
917	LEU180	N	128.5722 ± 0.119	1
918	SER181	C	174.6422	1
919	SER181	CA	60.9872 ± 0.032	1
920	SER181	CB	61.7412	1
921	SER181	HB2	3.9352	2
922	SER181	HB3	3.9352	2
923	ILE182	C	177.8772	1
924	ILE182	CA	64.3612 ± 0.120	1
925	ILE182	CB	39.6462 ± 0.086	1
926	ILE182	CD1	13.8512 ± 0.778	1
927	ILE182	CG1	28.8372 ± 0.141	1
928	ILE182	CG2	17.2192 ± 0.082	1
929	ILE182	HA	4.1492 ± 0.006	1
930	ILE182	HB	2.1162 ± 0.009	1
931	ILE182	HD1	1.1822 ± 0.019	1
932	ILE182	HG12	1.8942 ± 0.012	2
933	ILE182	HG13	1.0432 ± 0.012	2
934	ILE182	HG2	1.3432 ± 0.007	1
935	ILE182	H	6.9842 ± 0.067	1
936	ILE182	N	119.6052 ± 0.041	1
937	VAL183	C	179.8932 ± 0.028	1
938	VAL183	CA	65.0752 ± 0.097	1
939	VAL183	CB	30.9862 ± 0.100	1
940	VAL183	CG1	22.2732 ± 0.062	1
941	VAL183	CG2	19.9172 ± 0.063	1
942	VAL183	HA	4.2432 ± 0.013	1
943	VAL183	HB	2.5142 ± 0.011	1
944	VAL183	HG1	0.8412 ± 0.007	1
945	VAL183	HG2	0.7412 ± 0.007	1
946	VAL183	H	8.4972 ± 0.007	1
947	VAL183	N	110.7972 ± 0.059	1
948	SER184	C	173.9842 ± 0.007	1
949	SER184	CA	62.2532 ± 0.119	1
950	SER184	CB	63.3382 ± 0.043	1
951	SER184	HA	4.1742 ± 0.011	1
952	SER184	HB2	3.8602 ± 0.006	2
953	SER184	HB3	3.8602 ± 0.006	2
954	SER184	H	8.5882 ± 0.011	1

955	SER184	N	115.5632 ± 0.079	1	1008	THR190	C	174.7502	1
956	ARG185	C	176.0212 ± 0.015	1	1009	THR190	CA	67.3932 ± 0.156	1
957	ARG185	CA	56.2032 ± 0.082	1	1010	THR190	CB	68.1052 ± 0.115	1
958	ARG185	CB	31.5152 ± 0.030	1	1011	THR190	CG2	21.4182 ± 0.155	1
959	ARG185	CG	28.6742	1	1012	THR190	HA	3.7102 ± 0.013	1
960	ARG185	HA	4.2432	1	1013	THR190	HB	4.1692 ± 0.006	1
961	ARG185	HB2	2.0032 ± 0.017	2	1014	THR190	HG2	1.0212 ± 0.012	1
962	ARG185	HB3	2.0032 ± 0.017	2	1015	THR190	H	8.2862 ± 0.010	1
963	ARG185	HD2	2.8252 ± 0.018	2	1016	THR190	N	117.2792 ± 0.088	1
964	ARG185	HD3	2.8252 ± 0.018	2	1017	VAL191	C	176.8822	1
965	ARG185	HG2	1.7672	2	1018	VAL191	CA	67.5272 ± 0.134	1
966	ARG185	HG3	1.7672	2	1019	VAL191	CB	32.1232 ± 0.089	1
967	ARG185	H	7.2382 ± 0.011	1	1020	VAL191	CG1	22.7052 ± 0.075	1
968	ARG185	N	116.2022 ± 0.102	1	1021	VAL191	CG2	23.7762 ± 0.122	1
969	MET186	C	175.7342 ± 0.013	1	1022	VAL191	HA	3.1862 ± 0.007	1
970	MET186	CA	55.8302 ± 0.107	1	1023	VAL191	HB	2.0182 ± 0.009	1
971	MET186	CB	34.7382 ± 0.112	1	1024	VAL191	HG1	0.7922 ± 0.007	1
972	MET186	CE	18.3622 ± 0.051	1	1025	VAL191	HG2	0.8992 ± 0.008	1
973	MET186	CG	32.2152 ± 0.055	1	1026	VAL191	H	8.6412 ± 0.007	1
974	MET186	HA	4.4162 ± 0.007	1	1027	VAL191	N	122.2392 ± 0.058	1
975	MET186	HB2	2.1272 ± 0.011	2	1028	THR192	C	175.6662	1
976	MET186	HB3	1.8902 ± 0.007	2	1029	THR192	CA	67.5332 ± 0.130	1
977	MET186	HE	1.6632 ± 0.007	1	1030	THR192	CB	68.2482 ± 0.092	1
978	MET186	HG2	2.3052 ± 0.003	2	1031	THR192	CG2	21.5812 ± 0.106	1
979	MET186	HG3	2.3052 ± 0.003	2	1032	THR192	HA	3.4592 ± 0.012	1
980	MET186	H	7.3492 ± 0.009	1	1033	THR192	HB	4.1162 ± 0.014	1
981	MET186	N	119.4792 ± 0.134	1	1034	THR192	HG2	1.0212 ± 0.005	1
982	ASN187	C	175.0222	1	1035	THR192	H	7.7452 ± 0.009	1
983	ASN187	CA	51.6252 ± 0.080	1	1036	THR192	N	111.8942 ± 0.093	1
984	ASN187	CB	37.5332 ± 0.191	1	1037	SER193	C	173.2262	1
985	ASN187	HA	4.7502 ± 0.011	1	1038	SER193	CA	62.7462 ± 0.137	1
986	ASN187	HB2	3.4792 ± 0.017	2	1039	SER193	CB	68.1622 ± 0.166	1
987	ASN187	HB3	3.1072 ± 0.012	2	1040	SER193	HA	3.7132 ± 0.024	1
988	ASN187	H	8.7782 ± 0.005	1	1041	SER193	HB2	4.1652 ± 0.011	2
989	ASN187	N	123.1862 ± 0.073	1	1042	SER193	HB3	4.1652 ± 0.011	2
990	GLN188	C	178.7142 ± 0.031	1	1043	SER193	H	7.8642 ± 0.010	1
991	GLN188	CA	59.0632 ± 0.098	1	1044	SER193	N	118.6862 ± 0.089	1
992	GLN188	CB	28.3472 ± 0.113	1	1045	VAL194	C	177.8452 ± 0.006	1
993	GLN188	CG	34.7792 ± 0.005	1	1046	VAL194	CA	66.9582 ± 0.077	1
994	GLN188	HA	3.8932 ± 0.020	1	1047	VAL194	CB	30.1142 ± 0.062	1
995	GLN188	HB2	2.0772 ± 0.038	2	1048	VAL194	CG1	22.8572 ± 0.071	1
996	GLN188	HB3	2.0772 ± 0.038	2	1049	VAL194	CG2	20.8482 ± 0.085	1
997	GLN188	HG2	2.5322 ± 0.008	2	1050	VAL194	HA	2.9002 ± 0.005	1
998	GLN188	HG3	2.5322 ± 0.008	2	1051	VAL194	HB	1.3162 ± 0.010	1
999	GLN188	H	8.8202 ± 0.012	1	1052	VAL194	HG1	0.3752 ± 0.006	1
1000	GLN188	N	117.9292 ± 0.082	1	1053	VAL194	HG2	-0.7728 ± 0.004	1
1001	ALA189	C	180.1982 ± 0.003	1	1054	VAL194	H	8.1852 ± 0.008	1
1002	ALA189	CA	55.1112 ± 0.114	1	1055	VAL194	N	120.0002 ± 0.122	1
1003	ALA189	CB	17.6592 ± 0.103	1	1056	LEU195	C	178.9742	1
1004	ALA189	HA	4.0442 ± 0.010	1	1057	LEU195	CA	58.5512 ± 0.157	1
1005	ALA189	HB	1.4032 ± 0.009	1	1058	LEU195	CB	41.6362 ± 0.063	1
1006	ALA189	H	8.5432 ± 0.013	1	1059	LEU195	CD1	24.5192 ± 0.089	1
1007	ALA189	N	123.9622 ± 0.101	1	1060	LEU195	CD2	25.6402 ± 0.101	1

1061	LEU195	CG	27.0242	1	1114	SER199	HA	4.3452 ± 0.041	1
1062	LEU195	HA	3.8212 ± 0.015	1	1115	SER199	HB2	4.0572 ± 0.008	2
1063	LEU195	HB2	1.7282	2	1116	SER199	HB3	4.0572 ± 0.008	2
1064	LEU195	HB3	1.6892 ± 0.019	2	1117	SER199	H	8.6132 ± 0.011	1
1065	LEU195	HD1	0.7832 ± 0.013	1	1118	SER199	N	117.1482 ± 0.113	1
1066	LEU195	HD2	0.7412 ± 0.011	1	1119	ASN200	C	178.8042	1
1067	LEU195	HG	1.5272 ± 0.015	1	1120	ASN200	CA	55.6382 ± 0.129	1
1068	LEU195	H	7.9602 ± 0.008	1	1121	ASN200	CB	36.9522 ± 0.107	1
1069	LEU195	N	120.3292 ± 0.097	1	1122	ASN200	HA	4.5332 ± 0.008	1
1070	GLU196	C	178.1342 ± 0.029	1	1123	ASN200	HB2	3.3232 ± 0.010	2
1071	GLU196	CA	59.9922 ± 0.059	1	1124	ASN200	HB3	3.0182 ± 0.011	2
1072	GLU196	CB	28.1552 ± 0.012	1	1125	ASN200	H	8.9372 ± 0.012	1
1073	GLU196	CG	35.3792 ± 0.122	1	1126	ASN200	N	122.4932 ± 0.120	1
1074	GLU196	HA	4.3992	1	1127	TRP201	C	177.9742	1
1075	GLU196	HB2	2.2102 ± 0.008	2	1128	TRP201	CA	61.5752 ± 0.105	1
1076	GLU196	HB3	1.9322 ± 0.013	2	1129	TRP201	CB	29.5152 ± 0.098	1
1077	GLU196	HG2	2.2172 ± 0.014	2	1130	TRP201	HA	4.2822 ± 0.010	1
1078	GLU196	HG3	2.2172 ± 0.014	2	1131	TRP201	HB2	3.1922 ± 0.004	2
1079	GLU196	H	7.7842 ± 0.008	1	1132	TRP201	HB3	3.1922 ± 0.004	2
1080	GLU196	N	122.8062 ± 0.052	1	1133	TRP201	HE1	11.5112	1
1081	TYR197	C	179.8722 ± 0.026	1	1134	TRP201	H	8.0972 ± 0.009	1
1082	TYR197	CA	57.8212 ± 0.203	1	1135	TRP201	N	122.6142 ± 0.056	1
1083	TYR197	CB	36.7862 ± 0.119	1	1136	TRP201	NE1	120.1722	1
1084	TYR197	CD1	125.1852	3	1137	PHE202	C	176.9842	1
1085	TYR197	CD2	125.1852	3	1138	PHE202	CA	55.4532 ± 0.156	1
1086	TYR197	CE1	114.8832 ± 0.095	3	1139	PHE202	CB	34.3682 ± 0.110	1
1087	TYR197	CE2	114.8832 ± 0.095	3	1140	PHE202	HA	4.5232 ± 0.018	1
1088	TYR197	HA	4.6572 ± 0.015	1	1141	PHE202	HB2	2.4052 ± 0.008	2
1089	TYR197	HB2	3.2432 ± 0.019	2	1142	PHE202	HB3	2.0482 ± 0.002	2
1090	TYR197	HB3	3.0292 ± 0.030	2	1143	PHE202	H	8.7662 ± 0.014	1
1091	TYR197	HD1	7.2382	3	1144	PHE202	N	117.5522 ± 0.096	1
1092	TYR197	HD2	7.2382	3	1145	GLY203	C	173.5372 ± 0.049	1
1093	TYR197	HE1	7.4542 ± 0.015	3	1146	GLY203	CA	46.9272 ± 0.066	1
1094	TYR197	HE2	7.4542 ± 0.015	3	1147	GLY203	HA2	3.9912 ± 0.007	2
1095	TYR197	H	8.4362 ± 0.007	1	1148	GLY203	HA3	3.5082 ± 0.015	2
1096	TYR197	N	120.4102 ± 0.075	1	1149	GLY203	H	8.2742 ± 0.005	1
1097	LEU198	C	179.5372	1	1150	GLY203	N	105.5652 ± 0.046	1
1098	LEU198	CA	57.6332 ± 0.069	1	1151	GLU204	C	175.8192	1
1099	LEU198	CB	40.8662 ± 0.205	1	1152	GLU204	CA	55.3432 ± 0.090	1
1100	LEU198	CD1	26.0062 ± 0.130	1	1153	GLU204	CB	31.8022 ± 0.068	1
1101	LEU198	CD2	20.5532 ± 0.069	1	1154	GLU204	CG	35.9982 ± 0.071	1
1102	LEU198	CG	26.6152 ± 0.197	1	1155	GLU204	HA	4.4742 ± 0.015	1
1103	LEU198	HA	4.2262 ± 0.011	1	1156	GLU204	HB2	2.0662 ± 0.013	2
1104	LEU198	HB2	1.7892	2	1157	GLU204	HB3	1.8002 ± 0.010	2
1105	LEU198	HB3	1.3012 ± 0.004	2	1158	GLU204	HG2	2.1442 ± 0.002	2
1106	LEU198	HD1	0.7452 ± 0.007	1	1159	GLU204	HG3	2.1442 ± 0.002	2
1107	LEU198	HD2	0.4972 ± 0.037	1	1160	GLU204	H	7.0802 ± 0.014	1
1108	LEU198	HG	1.8882 ± 0.014	1	1161	GLU204	N	114.6252 ± 0.042	1
1109	LEU198	H	8.9722 ± 0.008	1	1162	ARG205	C	173.8252	1
1110	LEU198	N	117.4342 ± 0.100	1	1163	ARG205	CA	54.2142 ± 0.116	1
1111	SER199	C	177.6632	1	1164	ARG205	CB	32.0102 ± 0.159	1
1112	SER199	CA	62.9392 ± 0.117	1	1165	ARG205	CD	42.0352 ± 0.096	1
1113	SER199	CB	62.7822 ± 0.066	1	1166	ARG205	CG	26.6392 ± 0.139	1

1167	ARG205 HA	4.4742 ± 0.008	1
1168	ARG205 HB2	1.2722 ± 0.011	2
1169	ARG205 HB3	0.5812 ± 0.007	2
1170	ARG205 HD2	2.5432 ± 0.005	2
1171	ARG205 HD3	2.0112 ± 0.008	2
1172	ARG205 HG2	0.8592 ± 0.006	2
1173	ARG205 HG3	0.2992 ± 0.013	2
1174	ARG205 H	7.3512 ± 0.014	1
1175	ARG205 N	120.6452 ± 0.113	1
1176	ASP206 C	175.5512 ± 0.009	1
1177	ASP206 CA	52.8862 ± 0.085	1
1178	ASP206 CB	42.3472 ± 0.119	1
1179	ASP206 HA	4.8152 ± 0.010	1
1180	ASP206 HB2	2.7212 ± 0.008	2
1181	ASP206 HB3	2.6522 ± 0.004	2
1182	ASP206 H	7.6352 ± 0.005	1
1183	ASP206 N	116.7722 ± 0.093	1
1184	PHE207 C	174.7032	1
1185	PHE207 CA	60.1712 ± 0.094	1
1186	PHE207 CB	40.8922 ± 0.139	1
1187	PHE207 CD1	131.1982 ± 0.168	3
1188	PHE207 CD2	131.1982 ± 0.168	3
1189	PHE207 CE1	131.2742 ± 0.085	3
1190	PHE207 CE2	131.2742 ± 0.085	3
1191	PHE207 CZ	129.1562 ± 0.026	1
1192	PHE207 HA	4.0322 ± 0.010	1
1193	PHE207 HB2	3.2382 ± 0.009	2
1194	PHE207 HB3	2.7742 ± 0.027	2
1195	PHE207 HD1	6.8212 ± 0.013	3
1196	PHE207 HD2	6.8212 ± 0.013	3
1197	PHE207 HE1	7.5272 ± 0.018	3
1198	PHE207 HE2	7.5272 ± 0.018	3
1199	PHE207 H	9.0162 ± 0.017	1
1200	PHE207 HZ	7.1722 ± 0.007	1
1201	PHE207 N	119.5792 ± 0.106	1
1202	THR208 C	173.4132	1
1203	THR208 CA	57.9532 ± 0.062	1
1204	THR208 CB	70.3422 ± 0.152	1
1205	THR208 CG2	22.3842 ± 0.106	1
1206	THR208 HA	4.8912 ± 0.013	1
1207	THR208 HB	4.6042 ± 0.008	1
1208	THR208 HG2	1.3632 ± 0.008	1
1209	THR208 H	5.7602 ± 0.012	1
1210	THR208 N	116.5522 ± 0.105	1
1211	PRO209 C	177.2412	1
1212	PRO209 CA	65.2672 ± 0.103	1
1213	PRO209 CB	32.1542 ± 0.061	1
1214	PRO209 CD	50.8612	1
1215	PRO209 CG	28.1492 ± 0.124	1
1216	PRO209 HA	4.2512 ± 0.009	1
1217	PRO209 HB2	2.5452 ± 0.011	2
1218	PRO209 HB3	1.8172 ± 0.004	2
1219	PRO209 HD2	3.8852	2
1220	PRO209 HD3	3.8852	2
1221	PRO209 HG2	2.1212 ± 0.021	2
1222	PRO209 HG3	2.1212 ± 0.021	2
1223	GLU210 C	179.2932 ± 0.017	1
1224	GLU210 CA	61.1742 ± 0.072	1
1225	GLU210 CB	28.3432 ± 0.196	1
1226	GLU210 CG	37.5942 ± 0.091	1
1227	GLU210 HA	3.8522 ± 0.010	1
1228	GLU210 HB2	1.9712 ± 0.010	2
1229	GLU210 HB3	1.8512 ± 0.014	2
1230	GLU210 HG2	2.6532 ± 0.011	2
1231	GLU210 HG3	2.2132 ± 0.009	2
1232	GLU210 H	8.9702 ± 0.007	1
1233	GLU210 N	115.8082 ± 0.077	1
1234	LEU211 C	178.1092	1
1235	LEU211 CA	57.5882 ± 0.093	1
1236	LEU211 CB	41.8052 ± 0.085	1
1237	LEU211 CD1	23.9462 ± 0.115	1
1238	LEU211 CD2	26.9192 ± 0.095	1
1239	LEU211 CG	27.0332 ± 0.079	1
1240	LEU211 HA	3.8482 ± 0.002	1
1241	LEU211 HB2	1.2682 ± 0.017	2
1242	LEU211 HB3	1.2682 ± 0.017	2
1243	LEU211 HD1	0.9222 ± 0.009	1
1244	LEU211 HD2	0.5992 ± 0.025	1
1245	LEU211 HG	1.1632 ± 0.018	1
1246	LEU211 H	6.9782 ± 0.011	1
1247	LEU211 N	117.9462 ± 0.096	1
1248	GLY212 C	174.5642	1
1249	GLY212 CA	48.8682 ± 0.119	1
1250	GLY212 HA2	4.2832 ± 0.009	2
1251	GLY212 HA3	3.4992 ± 0.011	2
1252	GLY212 H	8.0002 ± 0.008	1
1253	GLY212 N	106.4032 ± 0.063	1
1254	ARG213 C	179.3272 ± 0.004	1
1255	ARG213 CA	59.7722 ± 0.119	1
1256	ARG213 CB	29.4602 ± 0.217	1
1257	ARG213 CD	44.5022 ± 0.145	1
1258	ARG213 CG	29.0462 ± 0.223	1
1259	ARG213 HA	2.8902 ± 0.013	1
1260	ARG213 HB2	1.3042 ± 0.012	2
1261	ARG213 HB3	1.3042 ± 0.012	2
1262	ARG213 HD2	2.9952 ± 0.010	2
1263	ARG213 HD3	2.7802 ± 0.012	2
1264	ARG213 HG2	1.1532 ± 0.008	2
1265	ARG213 HG3	0.8222 ± 0.017	2
1266	ARG213 H	8.1652 ± 0.007	1
1267	ARG213 N	118.3092 ± 0.122	1
1268	TRP214 C	178.2372	1
1269	TRP214 CA	62.8102 ± 0.095	1
1270	TRP214 CB	30.0512 ± 0.086	1
1271	TRP214 CD1	126.4712 ± 0.105	1
1272	TRP214 CE3	120.4782 ± 0.131	1

1273	TRP214	CZ2	116.1242 ± 0.022	1	1326	LEU218	CD2	26.9142 ± 0.062	1
1274	TRP214	CZ3	121.9302 ± 0.162	1	1327	LEU218	CG	25.7592 ± 0.080	1
1275	TRP214	HA	3.9582 ± 0.015	1	1328	LEU218	HA	4.0182 ± 0.011	1
1276	TRP214	HB2	3.0892 ± 0.020	2	1329	LEU218	HB2	2.0612 ± 0.014	2
1277	TRP214	HB3	3.0892 ± 0.020	2	1330	LEU218	HB3	2.0612 ± 0.014	2
1278	TRP214	HD1	7.1492 ± 0.012	1	1331	LEU218	HD1	0.7762 ± 0.010	1
1279	TRP214	HE1	11.0672 ± 0.017	1	1332	LEU218	HD2	0.8492 ± 0.008	1
1280	TRP214	HE3	7.4422 ± 0.010	1	1333	LEU218	HG	1.2622 ± 0.002	1
1281	TRP214	HH2	7.1422	1	1334	LEU218	H	8.6172 ± 0.014	1
1282	TRP214	H	7.1212 ± 0.016	1	1335	LEU218	N	116.5712 ± 0.076	1
1283	TRP214	HZ2	7.4552 ± 0.004	1	1336	LEU219	C	179.9572	1
1284	TRP214	HZ3	6.8222 ± 0.014	1	1337	LEU219	CA	58.2892 ± 0.059	1
1285	TRP214	N	118.7412 ± 0.047	1	1338	LEU219	CB	41.1982 ± 0.164	1
1286	TRP214	NE1	131.5652 ± 0.038	1	1339	LEU219	CD1	25.8022 ± 0.069	1
1287	LEU215	C	178.1462	1	1340	LEU219	CD2	22.7452 ± 0.085	1
1288	LEU215	CA	58.6892 ± 0.122	1	1341	LEU219	CG	26.9192 ± 0.111	1
1289	LEU215	CB	42.0802 ± 0.104	1	1342	LEU219	HA	4.0332 ± 0.011	1
1290	LEU215	CD1	26.5102 ± 0.133	1	1343	LEU219	HB2	2.2272 ± 0.008	2
1291	LEU215	CD2	23.0462 ± 0.142	1	1344	LEU219	HB3	2.2272 ± 0.008	2
1292	LEU215	HA	4.0112 ± 0.007	1	1345	LEU219	HD1	0.7442 ± 0.008	1
1293	LEU215	HB2	2.2062 ± 0.020	2	1346	LEU219	HD2	0.5762 ± 0.006	1
1294	LEU215	HB3	1.2232 ± 0.015	2	1347	LEU219	HG	2.0542 ± 0.006	1
1295	LEU215	HD1	0.7812 ± 0.012	1	1348	LEU219	H	8.3422 ± 0.007	1
1296	LEU215	HD2	0.8632 ± 0.014	1	1349	LEU219	N	117.7672 ± 0.114	1
1297	LEU215	H	8.8332 ± 0.006	1	1350	ALA220	C	177.2572 ± 0.028	1
1298	LEU215	N	117.9522 ± 0.090	1	1351	ALA220	CA	54.6292 ± 0.084	1
1299	TYR216	C	176.1292	1	1352	ALA220	CB	17.5262 ± 0.057	1
1300	TYR216	CA	62.6162 ± 0.101	1	1353	ALA220	HA	3.6342 ± 0.010	1
1301	TYR216	CB	40.4572 ± 0.128	1	1354	ALA220	HB	1.2822 ± 0.009	1
1302	TYR216	CD1	133.1602 ± 0.108	3	1355	ALA220	H	8.5482 ± 0.007	1
1303	TYR216	CD2	133.1602 ± 0.108	3	1356	ALA220	N	120.1572 ± 0.067	1
1304	TYR216	CE1	118.2862 ± 0.046	3	1357	CYS221	C	175.4432 ± 0.023	1
1305	TYR216	CE2	118.2862 ± 0.046	3	1358	CYS221	CA	60.7302 ± 0.113	1
1306	TYR216	HA	3.8022 ± 0.016	1	1359	CYS221	CB	29.1762 ± 0.126	1
1307	TYR216	HB2	3.1972 ± 0.013	2	1360	CYS221	HA	4.2152 ± 0.007	1
1308	TYR216	HB3	3.0462 ± 0.015	2	1361	CYS221	HB2	3.0102 ± 0.010	2
1309	TYR216	HD1	6.8362 ± 0.011	3	1362	CYS221	HB3	3.0102 ± 0.010	2
1310	TYR216	HD2	6.8362 ± 0.011	3	1363	CYS221	H	7.3452 ± 0.009	1
1311	TYR216	HE1	6.5152 ± 0.017	3	1364	CYS221	N	112.0952 ± 0.085	1
1312	TYR216	HE2	6.5152 ± 0.017	3	1365	LEU222	C	176.7002 ± 0.012	1
1313	TYR216	H	8.6812 ± 0.009	1	1366	LEU222	CA	55.5412 ± 0.125	1
1314	TYR216	N	117.9892 ± 0.083	1	1367	LEU222	CB	43.1272 ± 0.075	1
1315	ALA217	C	179.0652 ± 0.051	1	1368	LEU222	CD1	24.2692 ± 0.089	1
1316	ALA217	CA	55.2652 ± 0.096	1	1369	LEU222	CD2	25.1292 ± 0.104	1
1317	ALA217	CB	20.3032 ± 0.082	1	1370	LEU222	CG	25.9812 ± 0.098	1
1318	ALA217	HA	3.9032 ± 0.011	1	1371	LEU222	HA	4.4432 ± 0.008	1
1319	ALA217	HB	1.3272 ± 0.009	1	1372	LEU222	HB2	1.7812 ± 0.016	2
1320	ALA217	H	8.1832 ± 0.009	1	1373	LEU222	HB3	1.6862 ± 0.006	2
1321	ALA217	N	119.7012 ± 0.113	1	1374	LEU222	HD1	0.6792 ± 0.011	1
1322	LEU218	C	179.7272	1	1375	LEU222	HD2	0.5332 ± 0.006	1
1323	LEU218	CA	58.4552 ± 0.162	1	1376	LEU222	HG	1.7032 ± 0.007	1
1324	LEU218	CB	41.0902 ± 0.166	1	1377	LEU222	H	7.3932 ± 0.011	1
1325	LEU218	CD1	25.9982 ± 0.150	1	1378	LEU222	N	123.8372 ± 0.058	1

1379	GLU	223	C	175.0482 ± 0.016	1	1432	LEU	227	CD1	25.1172 ± 0.069	1
1380	GLU	223	CA	56.5992 ± 0.037	1	1433	LEU	227	CD2	22.7062 ± 0.088	1
1381	GLU	223	CB	30.8682 ± 0.132	1	1434	LEU	227	CG	27.6322 ± 0.051	1
1382	GLU	223	CG	36.5602 ± 0.127	1	1435	LEU	227	HA	4.5632 ± 0.012	1
1383	GLU	223	HA	4.2872 ± 0.018	1	1436	LEU	227	HB2	1.8372 ± 0.011	2
1384	GLU	223	HB2	1.9482 ± 0.027	2	1437	LEU	227	HB3	1.6502 ± 0.012	2
1385	GLU	223	HB3	1.7662 ± 0.005	2	1438	LEU	227	HD1	0.9812 ± 0.014	1
1386	GLU	223	HG2	2.3392 ± 0.016	2	1439	LEU	227	HD2	1.0022 ± 0.009	1
1387	GLU	223	HG3	2.3392 ± 0.016	2	1440	LEU	227	HG	1.9372 ± 0.006	1
1388	GLU	223	H	8.8472 ± 0.008	1	1441	LEU	227	H	9.0302 ± 0.013	1
1389	GLU	223	N	127.1562 ± 0.049	1	1442	LEU	227	N	125.5332 ± 0.040	1
1390	LYS	224	C	175.1512	1	1443	PRO	228	C	179.4492 ± 0.004	1
1391	LYS	224	CA	54.2112 ± 0.087	1	1444	PRO	228	CA	66.3502 ± 0.141	1
1392	LYS	224	CB	33.6332 ± 0.046	1	1445	PRO	228	CB	31.9572 ± 0.163	1
1393	LYS	224	CG	25.4722 ± 0.361	1	1446	PRO	228	CD	50.1962 ± 0.161	1
1394	LYS	224	HA	4.4422 ± 0.009	1	1447	PRO	228	CG	27.7272	1
1395	LYS	224	HB2	1.8532	2	1448	PRO	228	HA	4.2952 ± 0.008	1
1396	LYS	224	HB3	1.6432	2	1449	PRO	228	HB2	2.4012 ± 0.005	2
1397	LYS	224	HE2	2.7852	2	1450	PRO	228	HB3	1.9342 ± 0.039	2
1398	LYS	224	HE3	2.7852	2	1451	PRO	228	HD2	3.9012 ± 0.018	2
1399	LYS	224	HG2	1.4622 ± 0.009	2	1452	PRO	228	HD3	3.8412 ± 0.019	2
1400	LYS	224	HG3	1.4622 ± 0.009	2	1453	PRO	228	HG2	1.9662 ± 0.011	2
1401	LYS	224	H	8.3492 ± 0.016	1	1454	PRO	228	HG3	1.9662 ± 0.011	2
1402	LYS	224	N	119.2842 ± 0.091	1	1455	GLU	229	C	178.7712 ± 0.015	1
1403	PRO	225	C	175.7132	1	1456	GLU	229	CA	59.8862 ± 0.099	1
1404	PRO	225	CA	61.8202 ± 0.065	1	1457	GLU	229	CB	28.5162 ± 0.107	1
1405	PRO	225	CB	34.3132 ± 0.043	1	1458	GLU	229	CG	36.5802 ± 0.073	1
1406	PRO	225	CD	50.6702	1	1459	GLU	229	HA	4.1212 ± 0.011	1
1407	PRO	225	CG	25.0282 ± 0.060	1	1460	GLU	229	HB2	2.0042 ± 0.002	2
1408	PRO	225	HA	4.5122 ± 0.007	1	1461	GLU	229	HB3	1.9492 ± 0.008	2
1409	PRO	225	HB2	2.4152 ± 0.007	2	1462	GLU	229	HG2	2.3692 ± 0.005	2
1410	PRO	225	HB3	2.0342 ± 0.012	2	1463	GLU	229	HG3	2.2872 ± 0.014	2
1411	PRO	225	HD2	3.5382	2	1464	GLU	229	H	9.5762 ± 0.009	1
1412	PRO	225	HD3	3.5382	2	1465	GLU	229	N	116.8972 ± 0.047	1
1413	PRO	225	HG2	1.9362 ± 0.009	2	1466	ALA	230	C	178.4642 ± 0.034	1
1414	PRO	225	HG3	1.9362 ± 0.009	2	1467	ALA	230	CA	54.7862 ± 0.114	1
1415	LEU	226	C	178.5582 ± 0.020	1	1468	ALA	230	CB	19.2942 ± 0.090	1
1416	LEU	226	CA	55.0792 ± 0.089	1	1469	ALA	230	HA	4.0632 ± 0.012	1
1417	LEU	226	CB	42.5832 ± 0.140	1	1470	ALA	230	HB	1.5482 ± 0.011	1
1418	LEU	226	CD1	26.3292 ± 0.052	1	1471	ALA	230	H	7.3632 ± 0.007	1
1419	LEU	226	CD2	23.9862 ± 0.066	1	1472	ALA	230	N	121.4902 ± 0.070	1
1420	LEU	226	CG	27.7812 ± 0.020	1	1473	HIS	231	C	176.5482 ± 0.013	1
1421	LEU	226	HA	4.3652 ± 0.013	1	1474	HIS	231	CA	60.0352 ± 0.186	1
1422	LEU	226	HB2	1.5712 ± 0.025	2	1475	HIS	231	CB	30.6362 ± 0.132	1
1423	LEU	226	HB3	1.5712 ± 0.025	2	1476	HIS	231	CD2	118.4872 ± 0.180	1
1424	LEU	226	HD1	0.9062 ± 0.006	1	1477	HIS	231	CE1	138.5532 ± 0.039	1
1425	LEU	226	HD2	0.8132 ± 0.005	1	1478	HIS	231	HA	4.0032 ± 0.016	1
1426	LEU	226	HG	1.8622 ± 0.008	1	1479	HIS	231	HB2	3.3322 ± 0.013	2
1427	LEU	226	H	8.7162 ± 0.006	1	1480	HIS	231	HB3	3.0712 ± 0.016	2
1428	LEU	226	N	122.6472 ± 0.075	1	1481	HIS	231	HD2	6.8932 ± 0.010	1
1429	LEU	227	C	176.4062	1	1482	HIS	231	HE1	7.8752 ± 0.002	1
1430	LEU	227	CA	53.8122 ± 0.095	1	1483	HIS	231	H	8.1602 ± 0.008	1
1431	LEU	227	CB	40.2022 ± 0.121	1	1484	HIS	231	N	118.0352 ± 0.071	1

1485	SER232	C	177.6042	1
1486	SER232	CA	61.5622 ± 0.142	1
1487	SER232	CB	62.6592 ± 0.096	1
1488	SER232	HA	4.0552 ± 0.008	1
1489	SER232	HB2	3.9532 ± 0.011	2
1490	SER232	HB3	3.9532 ± 0.011	2
1491	SER232	H	7.9522 ± 0.009	1
1492	SER232	N	111.4302 ± 0.069	1
1493	LEU233	C	179.9542 ± 0.006	1
1494	LEU233	CA	57.8822 ± 0.127	1
1495	LEU233	CB	42.6902 ± 0.109	1
1496	LEU233	CD1	25.8572 ± 0.078	1
1497	LEU233	CD2	24.3232 ± 0.081	1
1498	LEU233	CG	26.8522 ± 0.115	1
1499	LEU233	HA	4.1242 ± 0.018	1
1500	LEU233	HB2	1.9072 ± 0.015	2
1501	LEU233	HB3	1.3842 ± 0.010	2
1502	LEU233	HD1	0.8182 ± 0.009	1
1503	LEU233	HD2	0.8392 ± 0.015	1
1504	LEU233	HG	1.7352 ± 0.009	1
1505	LEU233	H	7.6332 ± 0.007	1
1506	LEU233	N	121.4872 ± 0.100	1
1507	ILE234	C	177.6282	1
1508	ILE234	CA	65.8372 ± 0.109	1
1509	ILE234	CB	36.7692 ± 0.162	1
1510	ILE234	CD1	15.7292 ± 0.090	1
1511	ILE234	CG1	25.9332 ± 0.344	1
1512	ILE234	CG2	17.6712 ± 0.086	1
1513	ILE234	HA	4.0222 ± 0.010	1
1514	ILE234	HB	1.8812 ± 0.009	1
1515	ILE234	HD1	0.6082 ± 0.011	1
1516	ILE234	HG12	1.7082 ± 0.010	2
1517	ILE234	HG13	1.7082 ± 0.010	2
1518	ILE234	HG2	0.6712 ± 0.006	1
1519	ILE234	H	8.4632 ± 0.013	1
1520	ILE234	N	113.7302 ± 0.082	1
1521	ARG235	C	178.2362	1
1522	ARG235	CA	60.3582 ± 0.111	1
1523	ARG235	HA	3.8122 ± 0.006	1
1524	ARG235	H	7.3392 ± 0.011	1
1525	ARG235	N	121.9942 ± 0.145	1
1526	GLN236	C	178.7592	1
1527	GLN236	CA	58.8022 ± 0.095	1
1528	GLN236	CB	28.1192 ± 0.108	1
1529	GLN236	CG	33.6992 ± 0.130	1
1530	GLN236	HA	4.0352 ± 0.014	1
1531	GLN236	HB2	2.2262 ± 0.010	2
1532	GLN236	HB3	2.2262 ± 0.010	2
1533	GLN236	HG2	2.4342 ± 0.014	2
1534	GLN236	HG3	2.4342 ± 0.014	2
1535	GLN236	H	7.8332 ± 0.008	1
1536	GLN236	N	119.3912 ± 0.069	1
1537	LEU237	C	179.0322	1
1538	LEU237	CA	58.0302 ± 0.096	1
1539	LEU237	CB	42.0652 ± 0.098	1
1540	LEU237	CD1	24.8012 ± 0.056	1
1541	LEU237	CD2	25.5162 ± 0.128	1
1542	LEU237	CG	27.5632 ± 0.071	1
1543	LEU237	HA	4.3472 ± 0.012	1
1544	LEU237	HB2	1.9622 ± 0.012	2
1545	LEU237	HB3	1.8412 ± 0.010	2
1546	LEU237	HD1	1.1642 ± 0.005	1
1547	LEU237	HD2	1.0302 ± 0.008	1
1548	LEU237	HG	1.7562 ± 0.019	1
1549	LEU237	H	8.3682 ± 0.010	1
1550	LEU237	N	121.1782 ± 0.093	1
1551	ALA238	C	179.5202 ± 0.023	1
1552	ALA238	CA	55.8942 ± 0.078	1
1553	ALA238	CB	18.1622 ± 0.108	1
1554	ALA238	HA	3.9512 ± 0.007	1
1555	ALA238	HB	1.5582 ± 0.009	1
1556	ALA238	H	8.1452 ± 0.007	1
1557	ALA238	N	121.7542 ± 0.081	1
1558	ARG239	C	179.0552 ± 0.004	1
1559	ARG239	CA	59.8832 ± 0.137	1
1560	ARG239	CB	29.5462 ± 0.081	1
1561	ARG239	CD	43.1742 ± 0.076	1
1562	ARG239	CG	27.9202 ± 0.176	1
1563	ARG239	HA	4.0102 ± 0.005	1
1564	ARG239	HB2	1.9202 ± 0.007	2
1565	ARG239	HB3	1.9202 ± 0.007	2
1566	ARG239	HD2	3.1862 ± 0.006	2
1567	ARG239	HD3	3.1862 ± 0.006	2
1568	ARG239	HG2	1.8372 ± 0.002	2
1569	ARG239	HG3	1.5922 ± 0.007	2
1570	ARG239	H	8.1372 ± 0.007	1
1571	ARG239	N	118.1202 ± 0.086	1
1572	ARG240	C	178.4912 ± 0.025	1
1573	ARG240	CA	57.8082 ± 0.145	1
1574	ARG240	H	8.3792 ± 0.009	1
1575	ARG240	N	121.3722 ± 0.050	1
1576	CYS241	C	176.5512 ± 0.019	1
1577	CYS241	CA	64.8452 ± 0.117	1
1578	CYS241	CB	27.3642 ± 0.161	1
1579	CYS241	HA	4.1782 ± 0.011	1
1580	CYS241	HB2	3.4092 ± 0.014	2
1581	CYS241	HB3	2.5502 ± 0.010	2
1582	CYS241	H	8.5652 ± 0.008	1
1583	CYS241	N	116.1842 ± 0.049	1
1584	SER242	CA	62.0002 ± 0.159	1
1585	SER242	CB	62.7692 ± 0.199	1
1586	SER242	HA	3.9862 ± 0.025	1
1587	SER242	HB2	4.0172 ± 0.006	2
1588	SER242	HB3	4.0172 ± 0.006	2
1589	SER242	H	8.1152 ± 0.008	1
1590	SER242	N	114.6972 ± 0.089	1

1591	GLU243	C	179.4842	1	1644	LEU247	CD1	25.3282 ± 0.043	1
1592	GLU243	CA	59.3942 ± 0.112	1	1645	LEU247	CD2	22.4942 ± 0.098	1
1593	GLU243	CB	30.0092 ± 0.150	1	1646	LEU247	CG	26.4862 ± 0.088	1
1594	GLU243	CG	36.3862 ± 0.096	1	1647	LEU247	HA	4.3392 ± 0.007	1
1595	GLU243	HA	4.0232 ± 0.010	1	1648	LEU247	HB2	1.9612 ± 0.009	2
1596	GLU243	HB2	2.1422 ± 0.024	2	1649	LEU247	HB3	1.6822 ± 0.018	2
1597	GLU243	HB3	2.1422 ± 0.024	2	1650	LEU247	HD1	0.9382 ± 0.008	1
1598	GLU243	HG2	2.4042 ± 0.017	2	1651	LEU247	HD2	0.8622 ± 0.017	1
1599	GLU243	HG3	2.1952 ± 0.014	2	1652	LEU247	HG	1.8352 ± 0.016	1
1600	GLU243	H	8.0432 ± 0.009	1	1653	LEU247	H	7.5482 ± 0.008	1
1601	GLU243	N	122.3802 ± 0.054	1	1654	LEU247	N	117.4332 ± 0.065	1
1602	VAL244	C	178.3042	1	1655	VAL248	C	176.0932 ± 0.001	1
1603	VAL244	CA	65.9672 ± 0.126	1	1656	VAL248	CA	62.7532 ± 0.076	1
1604	VAL244	CB	31.6012 ± 0.110	1	1657	VAL248	CB	32.1422 ± 0.077	1
1605	VAL244	CG1	22.5052 ± 0.105	2	1658	VAL248	CG1	22.0572 ± 0.071	1
1606	VAL244	CG2	22.5052 ± 0.105	2	1659	VAL248	CG2	20.3912 ± 0.061	1
1607	VAL244	HA	3.7232 ± 0.009	1	1660	VAL248	HA	4.0072 ± 0.005	1
1608	VAL244	HB	2.1842 ± 0.016	1	1661	VAL248	HB	2.2242 ± 0.004	1
1609	VAL244	HG1	1.1112 ± 0.008	2	1662	VAL248	HG1	0.9682 ± 0.014	1
1610	VAL244	HG2	1.1112 ± 0.008	2	1663	VAL248	HG2	0.9112 ± 0.007	1
1611	VAL244	H	8.3132 ± 0.014	1	1664	VAL248	H	7.3262 ± 0.009	1
1612	VAL244	N	118.3362 ± 0.077	1	1665	VAL248	N	118.0092 ± 0.062	1
1613	ARG245	C	177.2552 ± 0.012	1	1666	ASP249	C	176.1712 ± 0.024	1
1614	ARG245	CA	59.5862 ± 0.104	1	1667	ASP249	CA	55.0312 ± 0.123	1
1615	ARG245	CB	30.7942 ± 0.173	1	1668	ASP249	CB	42.0892 ± 0.110	1
1616	ARG245	CD	38.7672 ± 0.346	1	1669	ASP249	HA	4.6392 ± 0.009	1
1617	ARG245	CG	26.5342	1	1670	ASP249	HB2	2.7302 ± 0.015	2
1618	ARG245	HA	3.7282 ± 0.011	1	1671	ASP249	HB3	2.7302 ± 0.015	2
1619	ARG245	HB2	2.1422 ± 0.011	2	1672	ASP249	H	8.6532 ± 0.008	1
1620	ARG245	HB3	1.7402 ± 0.010	2	1673	ASP249	N	123.8892 ± 0.050	1
1621	ARG245	HD2	3.0192 ± 0.116	2	1674	SER250	C	174.6732	1
1622	ARG245	HD3	1.7142	2	1675	SER250	CA	57.6262 ± 0.094	1
1623	ARG245	HG2	1.5472	2	1676	SER250	CB	64.5362 ± 0.138	1
1624	ARG245	HG3	1.5472	2	1677	SER250	HA	4.5922 ± 0.005	1
1625	ARG245	H	8.1152 ± 0.007	1	1678	SER250	HB2	3.9642 ± 0.005	2
1626	ARG245	N	120.2122 ± 0.041	1	1679	SER250	HB3	3.8042 ± 0.007	2
1627	LEU246	C	178.2792 ± 0.017	1	1680	SER250	H	7.7592 ± 0.010	1
1628	LEU246	CA	56.7642 ± 0.112	1	1681	SER250	N	113.5092 ± 0.100	1
1629	LEU246	CB	42.3302 ± 0.060	1	1682	LYS251	C	175.8372 ± 0.021	1
1630	LEU246	CD1	25.0852 ± 0.084	1	1683	LYS251	CA	57.4902 ± 0.092	1
1631	LEU246	CD2	23.5012 ± 0.136	1	1684	LYS251	CB	32.1002 ± 0.145	1
1632	LEU246	CG	26.7852 ± 0.107	1	1685	LYS251	CD	29.1102 ± 0.062	1
1633	LEU246	HA	4.1892 ± 0.007	1	1686	LYS251	CE	42.0132	1
1634	LEU246	HB2	1.8852 ± 0.008	2	1687	LYS251	CG	24.5872 ± 0.140	1
1635	LEU246	HB3	1.6402 ± 0.009	2	1688	LYS251	HA	4.0652 ± 0.011	1
1636	LEU246	HD1	0.9512 ± 0.009	1	1689	LYS251	HB2	1.8062 ± 0.011	2
1637	LEU246	HD2	0.9192 ± 0.006	1	1690	LYS251	HB3	1.8062 ± 0.011	2
1638	LEU246	HG	1.8802 ± 0.004	1	1691	LYS251	HD2	1.6192 ± 0.010	2
1639	LEU246	H	7.2822 ± 0.010	1	1692	LYS251	HD3	1.6192 ± 0.010	2
1640	LEU246	N	116.3832 ± 0.070	1	1693	LYS251	HE2	2.9702	2
1641	LEU247	C	177.9302 ± 0.011	1	1694	LYS251	HE3	2.9702	2
1642	LEU247	CA	55.0242 ± 0.039	1	1695	LYS251	HG2	1.3952 ± 0.009	2
1643	LEU247	CB	42.2542 ± 0.104	1	1696	LYS251	HG3	1.3952 ± 0.009	2

1697	LYS251	H	8.6422 ± 0.008	1	1750	ALA258	CB	21.0152 ± 0.066	1
1698	LYS251	N	123.0892 ± 0.048	1	1751	ALA258	HA	3.9502 ± 0.017	1
1699	ASP252	C	175.4682 ± 0.034	1	1752	ALA258	HB	1.5802 ± 0.008	1
1700	ASP252	CA	54.1102 ± 0.086	1	1753	ALA258	H	6.6312 ± 0.017	1
1701	ASP252	CB	41.0982 ± 0.109	1	1754	ALA258	N	117.2522 ± 0.064	1
1702	ASP252	HA	4.6242 ± 0.006	1	1755	LEU259	C	178.6762	1
1703	ASP252	HB2	2.8042 ± 0.008	2	1756	LEU259	CA	58.0272 ± 0.109	1
1704	ASP252	HB3	2.5302 ± 0.008	2	1757	LEU259	CB	41.2562 ± 0.074	1
1705	ASP252	H	7.9762 ± 0.007	1	1758	LEU259	CD1	25.7082 ± 0.138	1
1706	ASP252	N	117.4312 ± 0.066	1	1759	LEU259	CD2	22.3252 ± 0.065	1
1707	ASP253	CA	55.1202 ± 0.136	1	1760	LEU259	CG	26.1092 ± 0.267	1
1708	ASP253	CB	43.6012 ± 0.109	1	1761	LEU259	HA	3.7592 ± 0.019	1
1709	ASP253	HA	4.4522 ± 0.005	1	1762	LEU259	HB2	1.7062 ± 0.010	2
1710	ASP253	HB2	2.7812 ± 0.013	2	1763	LEU259	HB3	1.0682 ± 0.023	2
1711	ASP253	HB3	2.7812 ± 0.013	2	1764	LEU259	HD1	0.6332 ± 0.008	1
1712	ASP253	H	7.4582 ± 0.014	1	1765	LEU259	HD2	0.5812 ± 0.008	1
1713	ASP253	N	122.2472 ± 0.055	1	1766	LEU259	HG	1.4382 ± 0.002	1
1714	GLU254	CB	29.6952	1	1767	LEU259	H	7.8172 ± 0.012	1
1715	GLU254	CA	58.6282 ± 0.001	1	1768	LEU259	N	117.7412 ± 0.068	1
1716	GLU254	CG	36.4722 ± 0.009	1	1769	ASN260	C	178.8722 ± 0.002	1
1717	GLU254	HG2	2.4172 ± 0.007	2	1770	ASN260	CA	55.7502 ± 0.076	1
1718	GLU254	HG3	2.4172 ± 0.007	2	1771	ASN260	CB	37.6152 ± 0.274	1
1719	GLU254	H	7.9012 ± 0.007	1	1772	ASN260	HA	4.1822 ± 0.011	1
1720	GLU254	N	126.0312 ± 0.004	1	1773	ASN260	HB2	2.8092 ± 0.019	2
1721	ARG255	C	177.3342	1	1774	ASN260	HB3	2.8092 ± 0.019	2
1722	ARG255	CA	58.5402 ± 0.019	1	1775	ASN260	H	8.4592 ± 0.009	1
1723	ARG255	HA	4.1872 ± 0.009	1	1776	ASN260	N	116.0852 ± 0.113	1
1724	ARG255	H	9.3132 ± 0.012	1	1777	LEU261	C	176.0742	1
1725	ARG255	N	119.1132 ± 0.085	1	1778	LEU261	CA	54.4222 ± 0.031	1
1726	VAL256	CA	69.4972 ± 0.098	1	1779	LEU261	CB	41.0842	1
1727	VAL256	CB	29.5012 ± 0.048	1	1780	LEU261	CD1	25.8212	1
1728	VAL256	CG1	25.3762 ± 0.080	1	1781	LEU261	CD2	22.2232 ± 0.046	1
1729	VAL256	CG2	21.6302 ± 0.052	1	1782	LEU261	HA	4.1632 ± 0.003	1
1730	VAL256	HA	3.3252 ± 0.013	1	1783	LEU261	HD1	0.7622	1
1731	VAL256	HB	2.3092 ± 0.008	1	1784	LEU261	HD2	0.9902 ± 0.007	1
1732	VAL256	HG1	1.0502 ± 0.010	1	1785	LEU261	H	8.4982 ± 0.017	1
1733	VAL256	HG2	0.7652 ± 0.004	1	1786	LEU261	N	122.5472 ± 0.149	1
1734	VAL256	H	7.7632 ± 0.007	1	1787	LEU262	C	178.9472	1
1735	VAL256	N	116.5952 ± 0.096	1	1788	LEU262	CA	58.9942 ± 0.087	1
1736	PRO257	C	177.7042 ± 0.024	1	1789	LEU262	CB	42.0382 ± 0.124	1
1737	PRO257	CA	66.1712 ± 0.135	1	1790	LEU262	CD1	26.9672 ± 0.060	1
1738	PRO257	CB	31.6692 ± 0.133	1	1791	LEU262	CD2	23.2352 ± 0.037	1
1739	PRO257	CD	49.2102 ± 0.215	1	1792	LEU262	CG	27.1322 ± 0.146	1
1740	PRO257	CG	28.4132 ± 0.176	1	1793	LEU262	HA	3.9132 ± 0.007	1
1741	PRO257	HA	3.5782 ± 0.014	1	1794	LEU262	HB2	2.3202 ± 0.018	2
1742	PRO257	HB2	2.2382 ± 0.019	2	1795	LEU262	HB3	1.2722 ± 0.014	2
1743	PRO257	HB3	1.8922 ± 0.014	2	1796	LEU262	HD1	0.8462 ± 0.003	1
1744	PRO257	HD2	3.5602 ± 0.023	2	1797	LEU262	HD2	0.8592 ± 0.005	1
1745	PRO257	HD3	3.5602 ± 0.023	2	1798	LEU262	HG	1.1452 ± 0.014	1
1746	PRO257	HG2	2.0682 ± 0.007	2	1799	LEU262	H	8.2952 ± 0.007	1
1747	PRO257	HG3	1.7362 ± 0.010	2	1800	LEU262	N	118.9242 ± 0.173	1
1748	ALA258	C	179.0462 ± 0.022	1	1801	ILE263	C	178.4962	1
1749	ALA258	CA	55.1222 ± 0.193	1	1802	ILE263	CA	66.9812 ± 0.112	1

1803	ILE263	CB	39.1302 ± 0.161	1
1804	ILE263	CD1	13.0342 ± 0.073	1
1805	ILE263	CG1	30.7262 ± 0.088	1
1806	ILE263	CG2	17.1952 ± 0.098	1
1807	ILE263	HA	3.4292 ± 0.010	1
1808	ILE263	HB	1.7512 ± 0.010	1
1809	ILE263	HD1	0.7452 ± 0.011	1
1810	ILE263	HG12	1.8982 ± 0.007	2
1811	ILE263	HG13	0.9672 ± 0.014	2
1812	ILE263	HG2	0.9492 ± 0.009	1
1813	ILE263	H	8.1372 ± 0.008	1
1814	ILE263	N	117.7762 ± 0.077	1
1815	CYS264	C	176.1242 ± 0.024	1
1816	CYS264	CA	61.5712 ± 0.079	1
1817	CYS264	CB	25.9552 ± 0.158	1
1818	CYS264	HA	4.3952 ± 0.013	1
1819	CYS264	HB2	3.4282 ± 0.013	2
1820	CYS264	HB3	3.1932 ± 0.008	2
1821	CYS264	H	8.1422 ± 0.010	1
1822	CYS264	N	121.2782 ± 0.184	1
1823	LEU265	C	180.2742 ± 0.041	1
1824	LEU265	CA	58.4272 ± 0.078	1
1825	LEU265	CB	40.7852 ± 0.112	1
1826	LEU265	CD1	24.2232 ± 0.087	1
1827	LEU265	CD2	19.9602 ± 0.093	1
1828	LEU265	CG	25.1422 ± 0.096	1
1829	LEU265	HA	3.9632 ± 0.018	1
1830	LEU265	HB2	1.7952 ± 0.015	2
1831	LEU265	HB3	0.8432 ± 0.023	2
1832	LEU265	HD1	0.0272 ± 0.009	1
1833	LEU265	HD2	0.2292 ± 0.008	1
1834	LEU265	HG	1.9662 ± 0.009	1
1835	LEU265	H	8.8362 ± 0.016	1
1836	LEU265	N	118.5062 ± 0.122	1
1837	VAL266	CA	68.2052 ± 0.101	1
1838	VAL266	CB	32.2562 ± 0.101	1
1839	VAL266	CG1	20.6462 ± 0.083	1
1840	VAL266	CG2	22.5882 ± 0.072	1
1841	VAL266	HA	3.8132 ± 0.011	1
1842	VAL266	HB	2.1332 ± 0.015	1
1843	VAL266	HG1	0.8272 ± 0.008	1
1844	VAL266	HG2	1.1492 ± 0.013	1
1845	VAL266	H	8.3072 ± 0.011	1
1846	VAL266	N	118.3512 ± 0.251	1
1847	SER267	C	175.5892	1
1848	SER267	CA	60.6182 ± 0.099	1
1849	SER267	CB	63.7782 ± 0.158	1
1850	SER267	HA	4.0512 ± 0.012	1
1851	SER267	HB2	3.6272 ± 0.023	2
1852	SER267	HB3	3.6272 ± 0.023	2
1853	SER267	H	8.5262 ± 0.011	1
1854	SER267	N	112.3602 ± 0.097	1
1855	ARG268	C	177.6812 ± 0.021	1
1856	ARG268	CA	56.4552 ± 0.083	1
1857	ARG268	CB	31.0502 ± 0.153	1
1858	ARG268	CD	43.0822 ± 0.060	1
1859	ARG268	CG	27.8812 ± 0.158	1
1860	ARG268	HA	4.5802 ± 0.017	1
1861	ARG268	HB2	1.9812	2
1862	ARG268	HB3	1.9812	2
1863	ARG268	HD2	3.1252 ± 0.049	2
1864	ARG268	HD3	3.1252 ± 0.049	2
1865	ARG268	HG2	1.8672 ± 0.006	2
1866	ARG268	HG3	1.8672 ± 0.006	2
1867	ARG268	H	8.7332 ± 0.010	1
1868	ARG268	N	116.8672 ± 0.141	1
1869	TYR269	C	175.9922 ± 0.029	1
1870	TYR269	CA	61.9272 ± 0.093	1
1871	TYR269	CB	39.0072 ± 0.105	1
1872	TYR269	CD1	133.1232 ± 0.131	3
1873	TYR269	CD2	133.1232 ± 0.131	3
1874	TYR269	CE1	118.0372 ± 0.120	3
1875	TYR269	CE2	118.0372 ± 0.120	3
1876	TYR269	HA	4.2972 ± 0.018	1
1877	TYR269	HB2	2.7632 ± 0.008	2
1878	TYR269	HB3	2.7632 ± 0.008	2
1879	TYR269	HD1	6.1582 ± 0.009	3
1880	TYR269	HD2	6.1582 ± 0.009	3
1881	TYR269	HE1	6.4032 ± 0.011	3
1882	TYR269	HE2	6.4032 ± 0.011	3
1883	TYR269	H	7.8232 ± 0.012	1
1884	TYR269	N	119.1132 ± 0.128	1
1885	PHE270	C	175.6422	1
1886	PHE270	CA	59.2032 ± 0.100	1
1887	PHE270	CB	38.3722 ± 0.203	1
1888	PHE270	CD1	132.4402 ± 0.049	3
1889	PHE270	CD2	132.4402 ± 0.049	3
1890	PHE270	HA	4.4732 ± 0.008	1
1891	PHE270	HB2	3.6082 ± 0.010	2
1892	PHE270	HB3	3.4322 ± 0.021	2
1893	PHE270	HD1	7.4142 ± 0.010	3
1894	PHE270	HD2	7.4142 ± 0.010	3
1895	PHE270	H	7.8512 ± 0.008	1
1896	PHE270	N	113.2532 ± 0.065	1
1897	ASP271	C	175.4212 ± 0.110	1
1898	ASP271	CA	56.3292 ± 0.051	1
1899	ASP271	CB	39.4892 ± 0.070	1
1900	ASP271	HA	4.3462 ± 0.006	1
1901	ASP271	HB2	3.0382 ± 0.005	2
1902	ASP271	HB3	2.6392 ± 0.007	2
1903	ASP271	H	6.9852 ± 0.010	1
1904	ASP271	N	112.6152 ± 0.114	1
1905	GLN272	C	176.5802 ± 0.020	1
1906	GLN272	CA	52.0562 ± 0.012	1
1907	GLN272	CB	26.2242	1
1908	GLN272	HA	4.7562 ± 0.001	1

1909	GLN272	HB2	1.8622	2	1962	ASP277	H	8.9062 ± 0.010	1
1910	GLN272	HB3	1.8622	2	1963	ASP277	N	120.4812 ± 0.042	1
1911	GLN272	H	7.8972 ± 0.011	1	1964	GLU278	C	174.4682	1
1912	GLN272	N	118.6042 ± 0.107	1	1965	GLU278	CA	53.4122 ± 0.046	1
1913	ARG273	C	177.2942 ± 0.022	1	1966	GLU278	CB	30.2652 ± 0.201	1
1914	ARG273	CA	59.1502 ± 0.085	1	1967	GLU278	CG	35.4312 ± 0.156	1
1915	ARG273	CB	30.2492 ± 0.078	1	1968	GLU278	HA	4.7242 ± 0.004	1
1916	ARG273	CD	42.9992 ± 0.089	1	1969	GLU278	HB2	2.0252 ± 0.013	2
1917	ARG273	CG	27.4102 ± 0.077	1	1970	GLU278	HB3	1.8782 ± 0.019	2
1918	ARG273	HA	3.8772 ± 0.011	1	1971	GLU278	HG2	2.2542	2
1919	ARG273	HB2	1.8672 ± 0.010	2	1972	GLU278	HG3	2.2032 ± 0.004	2
1920	ARG273	HB3	1.8672 ± 0.010	2	1973	GLU278	H	8.5482 ± 0.010	1
1921	ARG273	HD2	3.2152 ± 0.023	2	1974	GLU278	N	125.4112 ± 0.108	1
1922	ARG273	HD3	3.2152 ± 0.023	2	1975	PRO279	C	176.7742 ± 0.023	1
1923	ARG273	HG2	1.6802 ± 0.008	2	1976	PRO279	CA	63.2222 ± 0.080	1
1924	ARG273	HG3	1.6802 ± 0.008	2	1977	PRO279	CB	32.2372 ± 0.126	1
1925	ARG273	H	7.8002 ± 0.004	1	1978	PRO279	CD	50.6162 ± 0.044	1
1926	ARG273	N	120.7692 ± 0.056	1	1979	PRO279	CG	27.3532 ± 0.170	1
1927	ASP274	C	176.2872 ± 0.021	1	1980	PRO279	HA	4.4012 ± 0.022	1
1928	ASP274	CA	55.0132 ± 0.043	1	1981	PRO279	HB2	2.2692 ± 0.010	2
1929	ASP274	CB	38.7432 ± 0.080	1	1982	PRO279	HB3	1.9582	2
1930	ASP274	HA	4.3942 ± 0.002	1	1983	PRO279	HD2	3.7632 ± 0.006	2
1931	ASP274	HB2	2.7182 ± 0.011	2	1984	PRO279	HD3	3.7052 ± 0.010	2
1932	ASP274	HB3	2.7182 ± 0.011	2	1985	PRO279	HG2	1.9932 ± 0.025	2
1933	ASP274	H	9.1192 ± 0.010	1	1986	PRO279	HG3	1.2972	2
1934	ASP274	N	116.5312 ± 0.085	1	1987	SER280	C	174.3672 ± 0.018	1
1935	LEU275	C	175.7752	1	1988	SER280	CA	58.3112 ± 0.104	1
1936	LEU275	CA	53.9862 ± 0.162	1	1989	SER280	CB	63.7312 ± 0.113	1
1937	LEU275	CB	41.1892 ± 0.099	1	1990	SER280	HA	4.4002 ± 0.003	1
1938	LEU275	CD1	22.2572 ± 0.036	1	1991	SER280	HB2	3.8492 ± 0.011	2
1939	LEU275	CD2	22.3942 ± 0.073	1	1992	SER280	HB3	3.8492 ± 0.011	2
1940	LEU275	CG	26.8082 ± 0.203	1	1993	SER280	H	8.2812 ± 0.008	1
1941	LEU275	HA	4.4222 ± 0.007	1	1994	SER280	N	115.7672 ± 0.047	1
1942	LEU275	HB2	1.6302 ± 0.021	2	1995	LEU281	C	176.9132 ± 0.010	1
1943	LEU275	HB3	1.6302 ± 0.021	2	1996	LEU281	CA	55.0222 ± 0.086	1
1944	LEU275	HD1	0.7662 ± 0.014	1	1997	LEU281	CB	42.4992 ± 0.067	1
1945	LEU275	HD2	0.7372 ± 0.009	1	1998	LEU281	CD1	24.7422 ± 0.155	1
1946	LEU275	HG	1.5472 ± 0.013	1	1999	LEU281	CD2	23.4912 ± 0.108	1
1947	LEU275	H	7.7482 ± 0.007	1	2000	LEU281	CG	27.0192 ± 0.072	1
1948	LEU275	N	119.4492 ± 0.040	1	2001	LEU281	HA	4.3762 ± 0.005	1
1949	ALA276	C	178.1512 ± 0.012	1	2002	LEU281	HB2	1.5662 ± 0.022	2
1950	ALA276	CA	52.1932 ± 0.119	1	2003	LEU281	HB3	1.5662 ± 0.022	2
1951	ALA276	CB	19.7892 ± 0.070	1	2004	LEU281	HD1	0.9162 ± 0.006	1
1952	ALA276	HA	4.1932 ± 0.006	1	2005	LEU281	HD2	0.8592 ± 0.005	1
1953	ALA276	HB	1.2942 ± 0.006	1	2006	LEU281	HG	1.6072 ± 0.012	1
1954	ALA276	H	7.1832 ± 0.014	1	2007	LEU281	H	8.2412 ± 0.007	1
1955	ALA276	N	120.1382 ± 0.097	1	2008	LEU281	N	123.9482 ± 0.027	1
1956	ASP277	C	175.8322 ± 0.027	1	2009	GLU282	C	174.9142 ± 0.021	1
1957	ASP277	CA	54.4972 ± 0.133	1	2010	GLU282	CA	56.3692 ± 0.079	1
1958	ASP277	CB	41.1282 ± 0.109	1	2011	GLU282	CB	30.6572 ± 0.057	1
1959	ASP277	HA	4.6752 ± 0.005	1	2012	GLU282	CG	36.2672 ± 0.091	1
1960	ASP277	HB2	2.5662 ± 0.026	2	2013	GLU282	HA	4.2472 ± 0.003	1
1961	ASP277	HB3	2.5662 ± 0.026	2	2014	GLU282	HB2	1.9752 ± 0.012	2

2015	GLU	282	HB3	1.8162 ± 0.009 2
2016	GLU	282	HG2	2.1472 ± 0.013 2
2017	GLU	282	HG3	2.1472 ± 0.013 2
2018	GLU	282	H	8.2302 ± 0.005 1
2019	GLU	282	N	121.5522 ± 0.038 1
2020	TYR	283	C	180.2402 1
2021	TYR	283	CA	58.9982 ± 0.040 1
2022	TYR	283	CB	39.4582 ± 0.102 1
2023	TYR	283	CD1	133.5212 ± 0.168 3
2024	TYR	283	CD2	133.5212 ± 0.168 3
2025	TYR	283	CE1	118.1702 ± 0.119 3
2026	TYR	283	CE2	118.1702 ± 0.119 3
2027	TYR	283	HA	4.3662 ± 0.008 1
2028	TYR	283	HB2	3.0292 ± 0.008 2
2029	TYR	283	HB3	2.8852 ± 0.007 2
2030	TYR	283	HD1	7.0672 ± 0.013 3
2031	TYR	283	HD2	7.0672 ± 0.013 3
2032	TYR	283	HE1	6.7792 ± 0.013 3
2033	TYR	283	HE2	6.7792 ± 0.013 3
2034	TYR	283	H	7.6392 ± 0.007 1
2035	TYR	283	N	125.7052 ± 0.027 1

Appendix B. Structural restraints

Table B1. Nuclear Overhauser effect (NOE) distance restraints used in the XPLOR-NIH calculation of the SMN$^{26-51}$-Gemin2$^{95-280}$ ensemble. After the two atom selections, the observed distance is indicated in Angstroms, followed by increments defining its upper and lower bound.

From the 3D ^{15}N NOESY-HSQC:

```
assign (segid B and residue 38 and name HN) (segid B and residue 39 and name HN) 2.35 0.65 0.65
assign (segid B and residue 42 and name HN) (segid B and residue 43 and name HN) 2.35 0.65 0.65
assign (segid B and residue 43 and name HN) (segid B and residue 44 and name HN) 2.35 0.65 0.65
assign (segid B and residue 46 and name HN) (segid B and residue 47 and name HN) 2.35 0.65 0.65
assign (segid A and residue 102 and name HN) (segid A and residue 103 and name HN) 2.35 0.65 0.65
assign (segid A and residue 113 and name HN) (segid A and residue 114 and name HN) 2.35 0.65 0.65
assign (segid A and residue 114 and name HN) (segid A and residue 115 and name HN) 2.35 0.65 0.65
assign (segid A and residue 121 and name HN) (segid A and residue 122 and name HN) 2.35 0.65 0.65
assign (segid A and residue 190 and name HN) (segid A and residue 191 and name HN) 2.35 0.65 0.65
assign (segid A and residue 199 and name HN) (segid A and residue 200 and name HN) 2.35 0.65 0.65
assign (segid A and residue 214 and name HN) (segid A and residue 215 and name HN) 2.35 0.65 0.65
assign (segid A and residue 143 and name HN) (segid A and residue 144 and name HN) 2.35 0.65 0.65
assign (segid A and residue 231 and name HN) (segid A and residue 232 and name HN) 2.35 0.65 0.65
assign (segid A and residue 243 and name HN) (segid A and residue 244 and name HN) 2.35 0.65 0.65

assign (segid B and residue 37 and name HN) (segid B and residue 38 and name HN) 2.35 0.65 0.65
assign (segid B and residue 39 and name HN) (segid B and residue 40 and name HN) 2.35 0.65 0.65
assign (segid B and residue 40 and name HN) (segid B and residue 41 and name HN) 2.35 0.65 0.65
assign (segid B and residue 44 and name HN) (segid B and residue 45 and name HN) 2.35 0.65 0.65
assign (segid B and residue 45 and name HN) (segid B and residue 46 and name HN) 2.35 0.65 0.65
assign (segid B and residue 47 and name HN) (segid B and residue 48 and name HN) 2.35 0.65 0.65
assign (segid B and residue 48 and name HN) (segid B and residue 49 and name HN) 2.35 0.65 0.65
assign (segid A and residue 100 and name HN) (segid A and residue 101 and name HN) 2.35 0.65 0.65
assign (segid A and residue 101 and name HN) (segid A and residue 102 and name HN) 2.35 0.65 0.65
assign (segid A and residue 103 and name HN) (segid A and residue 104 and name HN) 2.35 0.65 0.65
assign (segid A and residue 104 and name HN) (segid A and residue 105 and name HN) 2.35 0.65 0.65
assign (segid A and residue 107 and name HN) (segid A and residue 108 and name HN) 2.35 0.65 0.65
assign (segid A and residue 108 and name HN) (segid A and residue 109 and name HN) 2.35 0.65 0.65
assign (segid A and residue 111 and name HN) (segid A and residue 112 and name HN) 2.35 0.65 0.65
assign (segid A and residue 112 and name HN) (segid A and residue 113 and name HN) 2.35 0.65 0.65
assign (segid A and residue 116 and name HN) (segid A and residue 117 and name HN) 2.35 0.65 0.65
```

```
assign (segid A and residue 117 and name HN) (segid A and residue 118 and name HN) 2.35 0.65 0.65
assign (segid A and residue 118 and name HN) (segid A and residue 119 and name HN) 2.35 0.65 0.65
assign (segid A and residue 119 and name HN) (segid A and residue 120 and name HN) 2.35 0.65 0.65
assign (segid A and residue 120 and name HN) (segid A and residue 121 and name HN) 2.35 0.65 0.65
assign (segid A and residue 122 and name HN) (segid A and residue 123 and name HN) 2.35 0.65 0.65
assign (segid A and residue 124 and name HN) (segid A and residue 125 and name HN) 2.35 0.65 0.65
assign (segid A and residue 141 and name HN) (segid A and residue 142 and name HN) 2.35 0.65 0.65
assign (segid A and residue 142 and name HN) (segid A and residue 143 and name HN) 2.35 0.65 0.65
assign (segid A and residue 144 and name HN) (segid A and residue 145 and name HN) 2.35 0.65 0.65
assign (segid A and residue 145 and name HN) (segid A and residue 146 and name HN) 2.35 0.65 0.65
assign (segid A and residue 146 and name HN) (segid A and residue 147 and name HN) 2.35 0.65 0.65
assign (segid A and residue 147 and name HN) (segid A and residue 148 and name HN) 2.35 0.65 0.65
assign (segid A and residue 182 and name HN) (segid A and residue 183 and name HN) 2.35 0.65 0.65
assign (segid A and residue 188 and name HN) (segid A and residue 189 and name HN) 2.35 0.65 0.65
assign (segid A and residue 189 and name HN) (segid A and residue 190 and name HN) 2.35 0.65 0.65
assign (segid A and residue 191 and name HN) (segid A and residue 192 and name HN) 2.35 0.65 0.65
assign (segid A and residue 194 and name HN) (segid A and residue 195 and name HN) 2.35 0.65 0.65
assign (segid A and residue 196 and name HN) (segid A and residue 197 and name HN) 2.35 0.65 0.65
assign (segid A and residue 197 and name HN) (segid A and residue 198 and name HN) 2.35 0.65 0.65
assign (segid A and residue 198 and name HN) (segid A and residue 199 and name HN) 2.35 0.65 0.65
assign (segid A and residue 200 and name HN) (segid A and residue 201 and name HN) 2.35 0.65 0.65
assign (segid A and residue 210 and name HN) (segid A and residue 211 and name HN) 2.35 0.65 0.65
assign (segid A and residue 211 and name HN) (segid A and residue 212 and name HN) 2.35 0.65 0.65
assign (segid A and residue 212 and name HN) (segid A and residue 213 and name HN) 2.35 0.65 0.65
assign (segid A and residue 213 and name HN) (segid A and residue 214 and name HN) 2.35 0.65 0.65
assign (segid A and residue 217 and name HN) (segid A and residue 218 and name HN) 2.35 0.65 0.65
assign (segid A and residue 220 and name HN) (segid A and residue 220 and name HA) 2.35 0.65 0.65
assign (segid A and residue 220 and name HN) (segid A and residue 221 and name HN) 2.35 0.65 0.65
assign (segid A and residue 229 and name HN) (segid A and residue 230 and name HN) 2.35 0.65 0.65
assign (segid A and residue 230 and name HN) (segid A and residue 231 and name HN) 2.35 0.65 0.65
assign (segid A and residue 232 and name HN) (segid A and residue 233 and name HN) 2.35 0.65 0.65
assign (segid A and residue 233 and name HN) (segid A and residue 234 and name HN) 2.35 0.65 0.65
assign (segid A and residue 234 and name HN) (segid A and residue 235 and name HN) 2.35 0.65 0.65
assign (segid A and residue 235 and name HN) (segid A and residue 236 and name HN) 2.35 0.65 0.65
assign (segid A and residue 236 and name HN) (segid A and residue 237 and name HN) 2.35 0.65 0.65
assign (segid A and residue 240 and name HN) (segid A and residue 241 and name HN) 2.35 0.65 0.65
assign (segid A and residue 241 and name HN) (segid A and residue 242 and name HN) 2.35 0.65 0.65
assign (segid A and residue 245 and name HN) (segid A and residue 246 and name HN) 2.35 0.65 0.65
assign (segid A and residue 246 and name HN) (segid A and residue 247 and name HN) 2.35 0.65 0.65
assign (segid A and residue 247 and name HN) (segid A and residue 248 and name HN) 2.35 0.65 0.65
assign (segid A and residue 255 and name HN) (segid A and residue 256 and name HN) 2.35 0.65 0.65
assign (segid A and residue 258 and name HN) (segid A and residue 259 and name HN) 2.35 0.65 0.65
assign (segid A and residue 259 and name HN) (segid A and residue 260 and name HN) 2.35 0.65 0.65
assign (segid A and residue 262 and name HN) (segid A and residue 263 and name HN) 2.35 0.65 0.65
```

```
assign (segid A and residue 264 and name HN) (segid A and residue 265 and name HN) 2.35 0.65 0.65
assign (segid A and residue 265 and name HN) (segid A and residue 266 and name HN) 2.35 0.65 0.65
assign (segid A and residue 266 and name HN) (segid A and residue 267 and name HN) 2.35 0.65 0.65
assign (segid A and residue 267 and name HN) (segid A and residue 268 and name HN) 2.35 0.65 0.65
assign (segid A and residue 268 and name HN) (segid A and residue 269 and name HN) 2.35 0.65 0.65

assign (segid B and residue 37 and name HA) (segid B and residue 38 and name HN) 2.85 1.15 1.15
assign (segid B and residue 39 and name HA) (segid B and residue 40 and name HN) 2.85 1.15 1.15
assign (segid B and residue 45 and name HA) (segid B and residue 46 and name HN) 2.85 1.15 1.15
assign (segid B and residue 47 and name HA) (segid B and residue 48 and name HN) 2.85 1.15 1.15
assign (segid A and residue 99 and name HA) (segid A and residue 100 and name HN) 2.85 1.15 1.15
assign (segid A and residue 99 and name HA) (segid A and residue 100 and name HN) 2.85 1.15 1.15
assign (segid A and residue 105 and name HA) (segid A and residue 106 and name HN) 2.85 1.15 1.15
assign (segid A and residue 107 and name HA) (segid A and residue 108 and name HN) 2.85 1.15 1.15
assign (segid A and residue 112 and name HA) (segid A and residue 113 and name HN) 2.85 1.15 1.15
assign (segid A and residue 114 and name HA) (segid A and residue 115 and name HN) 2.85 1.15 1.15
assign (segid A and residue 116 and name HA) (segid A and residue 117 and name HN) 2.85 1.15 1.15
assign (segid A and residue 117 and name HA) (segid A and residue 118 and name HN) 2.85 1.15 1.15
assign (segid A and residue 119 and name HA) (segid A and residue 120 and name HN) 2.85 1.15 1.15
assign (segid A and residue 120 and name HA) (segid A and residue 121 and name HN) 2.85 1.15 1.15
assign (segid A and residue 121 and name HA) (segid A and residue 122 and name HN) 2.85 1.15 1.15
assign (segid A and residue 141 and name HA) (segid A and residue 142 and name HN) 2.85 1.15 1.15
assign (segid A and residue 143 and name HA#) (segid A and residue 144 and name HN) 2.85 1.15 1.15
assign (segid A and residue 187 and name HA) (segid A and residue 188 and name HN) 2.85 1.15 1.15
assign (segid A and residue 190 and name HA) (segid A and residue 191 and name HN) 2.85 1.15 1.15
assign (segid A and residue 192 and name HA) (segid A and residue 193 and name HN) 2.85 1.15 1.15
assign (segid A and residue 197 and name HA) (segid A and residue 198 and name HN) 2.85 1.15 1.15
assign (segid A and residue 210 and name HA) (segid A and residue 211 and name HN) 2.85 1.15 1.15
assign (segid A and residue 219 and name HA) (segid A and residue 220 and name HN) 2.85 1.15 1.15
assign (segid A and residue 228 and name HA) (segid A and residue 229 and name HN) 2.85 1.15 1.15
assign (segid A and residue 236 and name HA) (segid A and residue 237 and name HN) 2.85 1.15 1.15
assign (segid A and residue 245 and name HA) (segid A and residue 246 and name HN) 2.85 1.15 1.15
assign (segid A and residue 246 and name HA) (segid A and residue 247 and name HN) 2.85 1.15 1.15
assign (segid A and residue 257 and name HA) (segid A and residue 258 and name HN) 2.85 1.15 1.15
assign (segid A and residue 264 and name HA) (segid A and residue 265 and name HN) 2.85 1.15 1.15
assign (segid A and residue 266 and name HA) (segid A and residue 267 and name HN) 2.85 1.15 1.15
assign (segid A and residue 269 and name HA) (segid A and residue 270 and name HN) 2.85 1.15 1.15
```

From the 3D ^{13}C NOESY-HSQC

```
assign (segid B and residue 43 and name HA) (segid B and residue 44 and name HN) 2.85 1.15 1.15
assign (segid B and residue 48 and name HA) (segid B and residue 49 and name HN) 2.85 1.15 1.15
```

```
assign (segid A and residue 106 and name HA) (segid A and residue 107 and name HN) 2.85 1.15 1.15
assign (segid A and residue 111 and name HA) (segid A and residue 112 and name HN) 2.85 1.15 1.15
assign (segid A and residue 113 and name HA) (segid A and residue 114 and name HN) 2.85 1.15 1.15
assign (segid A and residue 118 and name HA) (segid A and residue 119 and name HN) 2.85 1.15 1.15
assign (segid A and residue 182 and name HA) (segid A and residue 183 and name HN) 2.85 1.15 1.15
assign (segid A and residue 191 and name HA) (segid A and residue 192 and name HN) 2.85 1.15 1.15
assign (segid A and residue 194 and name HA) (segid A and residue 195 and name HN) 2.85 1.15 1.15
assign (segid A and residue 199 and name HA) (segid A and residue 200 and name HN) 2.85 1.15 1.15
assign (segid A and residue 200 and name HA) (segid A and residue 201 and name HN) 2.85 1.15 1.15
assign (segid A and residue 212 and name HA2) (segid A and residue 213 and name HN) 2.85 1.15 1.15
assign (segid A and residue 213 and name HA) (segid A and residue 214 and name HN) 2.85 1.15 1.15
assign (segid A and residue 220 and name HA) (segid A and residue 221 and name HN) 2.85 1.15 1.15
assign (segid A and residue 229 and name HA) (segid A and residue 230 and name HN) 2.85 1.15 1.15
assign (segid A and residue 232 and name HA) (segid A and residue 233 and name HN) 2.85 1.15 1.15
assign (segid A and residue 237 and name HA) (segid A and residue 238 and name HN) 2.85 1.15 1.15
assign (segid A and residue 247 and name HA) (segid A and residue 248 and name HN) 2.85 1.15 1.15
assign (segid A and residue 258 and name HA) (segid A and residue 259 and name HN) 2.85 1.15 1.15
assign (segid B and residue 38 and name HA) (segid B and residue 41 and name HB#) 3.10 1.40 1.40
assign (segid B and residue 39 and name HA) (segid B and residue 42 and name HB#) 3.35 1.65 1.65
assign (segid B and residue 39 and name HA) (segid B and residue 42 and name HB#) 3.35 1.65 1.65
assign (segid B and residue 40 and name HA) (segid B and residue 43 and name HB#) 3.10 1.40 1.40
assign (segid B and residue 41 and name HA) (segid B and residue 44 and name HB#) 3.10 1.40 1.40
assign (segid B and residue 41 and name HA) (segid B and residue 44 and name HB#) 3.10 1.40 1.40
assign (segid B and residue 42 and name HA) (segid B and residue 45 and name HB#) 3.10 1.40 1.40
assign (segid B and residue 43 and name HA) (segid B and residue 46 and name HB#) 3.10 1.40 1.40
assign (segid B and residue 43 and name HA) (segid B and residue 46 and name HB#) 3.10 1.40 1.40
assign (segid B and residue 43 and name HA) (segid B and residue 46 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 107 and name HA) (segid A and residue 110 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 113 and name HA) (segid A and residue 116 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 114 and name HA) (segid A and residue 117 and name HB) 3.10 1.40 1.40
assign (segid A and residue 115 and name HA) (segid A and residue 118 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 120 and name HA) (segid A and residue 123 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 190 and name HA) (segid A and residue 193 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 191 and name HA) (segid A and residue 194 and name HB) 3.10 1.40 1.40
assign (segid A and residue 192 and name HA) (segid A and residue 195 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 193 and name HA) (segid A and residue 196 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 194 and name HA) (segid A and residue 197 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 197 and name HA) (segid A and residue 200 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 210 and name HA) (segid A and residue 213 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 214 and name HA) (segid A and residue 217 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 214 and name HA) (segid A and residue 217 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 228 and name HA) (segid A and residue 231 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 238 and name HA) (segid A and residue 241 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 244 and name HA) (segid A and residue 247 and name HB#) 3.10 1.40 1.40
```

```
assign (segid A and residue 263 and name HA) (segid A and residue 266 and name HB) 3.10 1.40 1.40
```

From the 4D HCCH NOESY-HMQC

```
assign (segid A and residue 118 and name HA) (segid A and residue 121 and name HB#) 2.60 0.90 0.90
assign (segid A and residue 118 and name HA) (segid A and residue 121 and name HB#) 2.60 0.90 0.90
assign (segid B and residue 44 and name HA) (segid B and residue 47 and name HB) 2.60 0.90 0.90
assign (segid A and residue 243 and name HA) (segid A and residue 246 and name HB#) 2.60 0.90 0.90
assign (segid A and residue 243 and name HA) (segid A and residue 246 and name HB#) 2.60 0.90 0.90
assign (segid A and residue 262 and name HA) (segid A and residue 265 and name HB#) 2.60 0.90 0.90
assign (segid A and residue 116 and name HA) (segid A and residue 119 and name HB#) 2.60 0.90 0.90
assign (segid A and residue 116 and name HA) (segid A and residue 119 and name HB#) 2.60 0.90 0.90
assign (segid A and residue 116 and name HA) (segid A and residue 119 and name HB#) 2.60 0.90 0.90
assign (segid A and residue 116 and name HA) (segid A and residue 119 and name HB#) 2.60 0.90 0.90
assign (segid A and residue 212 and name HA#) (segid A and residue 215 and name HB#) 2.60 0.90 0.90
```

From the 3D ^{13}C-NOESY HSQC

```
assign (segid B and residue 28 and name HA) (segid B and residue 29 and name HN) 3.10 1.40 1.40
assign (segid B and residue 28 and name HB#) (segid B and residue 28 and name HN) 3.10 1.40 1.40
assign (segid B and residue 28 and name HB#) (segid B and residue 28 and name HA)  3.10 1.40 1.40
assign (segid B and residue 29 and name HA) (segid B and residue 29 and name HB#) 2.85 1.15 1.15
assign (segid B and residue 29 and name HB#) (segid B and residue 29 and name HA) 2.85 1.15 1.15
assign (segid B and residue 29 and name HB#) (segid B and residue 29 and name HN) 3.10 1.40 1.40
assign (segid B and residue 30 and name HB#) (segid B and residue 30 and name HA) 3.10 1.40 1.40
assign (segid B and residue 30 and name HB#) (segid B and residue 30 and name HN) 3.35 1.65 1.65
assign (segid B and residue 29 and name HN) (segid B and residue 30 and name HA) 2.85 1.15 1.15
assign (segid B and residue 30 and name HA) (segid B and residue 30 and name HN) 3.35 1.65 1.65
assign (segid B and residue 30 and name HA) (segid B and residue 30 and name HB#) 2.85 1.15 1.15
assign (segid B and residue 31 and name HA) (segid B and residue 31 and name HB#) 2.85 1.15 1.15
assign (segid B and residue 31 and name HA) (segid B and residue 31 and name HN) 2.85 1.15 1.15
assign (segid B and residue 31 and name HA) (segid B and residue 32 and name HA) 3.35 1.65 1.65
assign (segid B and residue 31 and name HB#) (segid B and residue 31 and name HN) 3.10 1.40 1.40
assign (segid B and residue 32 and name HA) (segid B and residue 32 and name HB#) 2.85 1.15 1.15
assign (segid B and residue 32 and name HA) (segid B and residue 33 and name HN) 3.10 1.40 1.40
assign (segid B and residue 32 and name HB#) (segid B and residue 32 and name HA) 3.10 1.40 1.40
assign (segid B and residue 32 and name HB#) (segid B and residue 32 and name HN) 3.10 1.40 1.40
assign (segid B and residue 32 and name HB#) (segid B and residue 33 and name HA) 3.35 1.65 1.65
assign (segid B and residue 32 and name HB#) (segid B and residue 33 and name HN) 3.10 1.40 1.40
assign (segid B and residue 33 and name HA) (segid B and residue 33 and name HB) 3.10 1.40 1.40
assign (segid B and residue 33 and name HA) (segid B and residue 33 and name HD1#) 3.35 1.65 1.65
assign (segid B and residue 33 and name HA) (segid B and residue 33 and name HG1#) 3.10 1.40 1.40
```

```
assign (segid B and residue 33 and name HA) (segid B and residue 33 and name HG2#) 3.35 1.65 1.65
assign (segid B and residue 33 and name HA) (segid B and residue 33 and name HN) 3.35 1.65 1.65
assign (segid B and residue 33 and name HA) (segid B and residue 34 and name HN) 3.10 1.40 1.40
assign (segid B and residue 33 and name HB) (segid B and residue 33 and name HN) 2.85 1.15 1.15
assign (segid B and residue 33 and name HB) (segid B and residue 34 and name HN) 3.35 1.65 1.65
assign (segid B and residue 32 and name HA) (segid B and residue 33 and name HD1#) 3.35 1.65 1.65
assign (segid B and residue 32 and name HB#) (segid B and residue 33 and name HD1#) 3.35 1.65 1.65
assign (segid B and residue 33 and name HD1#) (segid B and residue 33 and name HA) 3.35 1.65 1.65
assign (segid B and residue 33 and name HD1#) (segid B and residue 33 and name HB) 3.35 1.65 1.65
assign (segid B and residue 33 and name HD1#) (segid B and residue 33 and name HN) 2.85 1.15 1.15
assign (segid B and residue 33 and name HD1#) (segid B and residue 34 and name HN) 3.35 1.65 1.65
assign (segid B and residue 33 and name HG1#) (segid B and residue 33 and name HB)  2.85 1.15 1.15
assign (segid B and residue 33 and name HG1#) (segid B and residue 33 and name HD1#) 3.10 1.40 1.40
assign (segid B and residue 33 and name HG1#) (segid B and residue 33 and name HN)  3.10 1.40 1.40
assign (segid B and residue 33 and name HG1#) (segid B and residue 34 and name HN)  3.10 1.40 1.40
assign (segid B and residue 33 and name HG1#) (segid B and residue 34 and name HN)  3.10 1.40 1.40
assign (segid B and residue 32 and name HB#) (segid B and residue 33 and name HG2#)  3.35 1.65 1.65
assign (segid B and residue 33 and name HG2#) (segid B and residue 33 and name HA)  3.10 1.40 1.40
assign (segid B and residue 33 and name HG2#) (segid B and residue 33 and name HB)  2.85 1.15 1.15
assign (segid B and residue 33 and name HG2#) (segid B and residue 33 and name HG1#) 3.10 1.40 1.40
assign (segid B and residue 33 and name HG2#) (segid B and residue 33 and name HG1#) 3.10 1.40 1.40
assign (segid B and residue 33 and name HG2#) (segid B and residue 33 and name HN)  3.10 1.40 1.40
assign (segid B and residue 33 and name HG2#) (segid B and residue 34 and name HN)  3.10 1.40 1.40
assign (segid B and residue 37 and name HA) (segid B and residue 37 and name HB)  3.10 1.40 1.40
assign (segid B and residue 37 and name HA) (segid B and residue 37 and name HG2#)  3.35 1.65 1.65
assign (segid B and residue 37 and name HB) (segid B and residue 37 and name HA)  3.10 1.40 1.40
assign (segid B and residue 37 and name HB) (segid B and residue 37 and name HG2#)  3.35 1.65 1.65
assign (segid B and residue 37 and name HB) (segid B and residue 40 and name HB)  3.10 1.40 1.40
assign (segid B and residue 37 and name HG2#) (segid B and residue 40 and name HG2#)  3.10 1.40 1.40
assign (segid B and residue 37 and name HG2#) (segid B and residue 40 and name HG2#)  3.10 1.40 1.40
assign (segid B and residue 38 and name HA) (segid B and residue 38 and name HB#) 3.10 1.40 1.40
assign (segid B and residue 38 and name HA) (segid B and residue 41 and name HN) 3.10 1.40 1.40
assign (segid B and residue 38 and name HB#) (segid B and residue 38 and name HA)  3.10 1.40 1.40
assign (segid B and residue 38 and name HB#) (segid B and residue 38 and name HN)  3.10 1.40 1.40
assign (segid B and residue 38 and name HB#) (segid A and residue 180 and name HD1#) 3.10 1.40 1.40
assign (segid B and residue 38 and name HB#) (segid A and residue 180 and name HD1#) 3.10 1.40 1.40
assign (segid B and residue 38 and name HB#) (segid A and residue 180 and name HD2#) 3.10 1.40 1.40
assign (segid B and residue 39 and name HA) (segid B and residue 39 and name HD2#)  3.35 1.65 1.65
assign (segid B and residue 39 and name HD2#) (segid B and residue 39 and name HA)  3.35 1.65 1.65
assign (segid B and residue 39 and name HD2#) (segid B and residue 39 and name HN)  3.35 1.65 1.65
assign (segid B and residue 39 and name HD2#) (segid B and residue 40 and name HN)  3.35 1.65 1.65
assign (segid B and residue 40 and name HA) (segid B and residue 40 and name HD1#)  3.35 1.65 1.65
assign (segid B and residue 40 and name HA) (segid B and residue 40 and name HG1#)  3.10 1.40 1.40
assign (segid B and residue 40 and name HA) (segid B and residue 40 and name HG1#)  3.10 1.40 1.40
```

```
assign (segid B and residue 40 and name HA) (segid B and residue 40 and name HG2#)  3.35 1.65 1.65
assign (segid B and residue 40 and name HB) (segid B and residue 40 and name HD1#)  3.35 1.65 1.65
assign (segid B and residue 40 and name HB) (segid B and residue 40 and name HG1#)  3.10 1.40 1.40
assign (segid B and residue 40 and name HB) (segid B and residue 40 and name HG2#)  3.35 1.65 1.65
assign (segid B and residue 40 and name HB) (segid B and residue 40 and name HN)  3.10 1.40 1.40
assign (segid B and residue 40 and name HB) (segid B and residue 41 and name HG#)  3.35 1.65 1.65
assign (segid B and residue 40 and name HB) (segid B and residue 41 and name HN)  3.10 1.40 1.40
assign (segid B and residue 37 and name HA) (segid B and residue 40 and name HD1#) 3.35 1.65 1.65
assign (segid B and residue 37 and name HA) (segid B and residue 40 and name HD1#) 3.35 1.65 1.65
assign (segid B and residue 37 and name HB) (segid B and residue 40 and name HD1#)  3.35 1.65 1.65
assign (segid B and residue 37 and name HB) (segid B and residue 40 and name HD1#)  3.35 1.65 1.65
assign (segid B and residue 39 and name HN) (segid B and residue 40 and name HD1#)  3.35 1.65 1.65
assign (segid B and residue 40 and name HD1#) (segid B and residue 40 and name HA)  3.35 1.65 1.65
assign (segid B and residue 40 and name HD1#) (segid B and residue 40 and name HB) 3.35 1.65 1.65
assign (segid B and residue 40 and name HD1#) (segid B and residue 40 and name HG1#) 3.35 1.65 1.65
assign (segid B and residue 40 and name HD1#) (segid B and residue 40 and name HG1#) 3.35 1.65 1.65
assign (segid B and residue 40 and name HD1#) (segid B and residue 40 and name HN)  3.35 1.65 1.65
assign (segid B and residue 40 and name HD1#) (segid B and residue 41 and name HG#)  3.35 1.65 1.65
assign (segid B and residue 40 and name HD1#) (segid B and residue 41 and name HN)  3.60 1.90 1.90
assign (segid B and residue 37 and name HG2#) (segid B and residue 40 and name HD1#)  3.10 1.40 1.40
assign (segid B and residue 37 and name HG2#) (segid B and residue 40 and name HD1#)  3.10 1.40 1.40
assign (segid B and residue 40 and name HG1#) (segid B and residue 40 and name HA)  3.10 1.40 1.40
assign (segid B and residue 40 and name HG1#) (segid B and residue 40 and name HB)  3.10 1.40 1.40
assign (segid B and residue 40 and name HG1#) (segid B and residue 40 and name HB)  3.10 1.40 1.40
assign (segid B and residue 40 and name HG1#) (segid B and residue 40 and name HD1#) 3.35 1.65 1.65
assign (segid B and residue 40 and name HG1#) (segid B and residue 40 and name HD1#) 3.35 1.65 1.65
assign (segid B and residue 40 and name HG1#) (segid B and residue 40 and name HG2#)  3.35 1.65 1.65
assign (segid B and residue 40 and name HG1#) (segid B and residue 40 and name HG2#)  3.35 1.65 1.65
assign (segid B and residue 40 and name HG1#) (segid B and residue 40 and name HN)  3.10 1.40 1.40
assign (segid B and residue 37 and name HB) (segid B and residue 40 and name HG2#)  3.35 1.65 1.65
assign (segid B and residue 37 and name HB) (segid B and residue 40 and name HG2#)  3.35 1.65 1.65
assign (segid B and residue 40 and name HG2#) (segid B and residue 40 and name HA)  3.35 1.65 1.65
assign (segid B and residue 40 and name HG2#) (segid B and residue 40 and name HB)  3.35 1.65 1.65
assign (segid B and residue 40 and name HG2#) (segid B and residue 40 and name HD1#) 3.60 1.90 1.90
assign (segid B and residue 40 and name HG2#) (segid B and residue 40 and name HG1#)  3.35 1.65 1.65
assign (segid B and residue 40 and name HG2#) (segid B and residue 40 and name HG1#)  3.35 1.65 1.65
assign (segid B and residue 40 and name HG2#) (segid B and residue 40 and name HN)  3.35 1.65 1.65
assign (segid B and residue 40 and name HG2#) (segid B and residue 41 and name HG#)  3.35 1.65 1.65
assign (segid B and residue 40 and name HG2#) (segid B and residue 41 and name HN)  3.35 1.65 1.65
assign (segid B and residue 41 and name HA) (segid B and residue 41 and name HB#)  3.10 1.40 1.40
assign (segid B and residue 41 and name HA) (segid B and residue 41 and name HD#)  3.10 1.40 1.40
assign (segid B and residue 41 and name HA) (segid B and residue 44 and name HN)  3.35 1.65 1.65
assign (segid B and residue 41 and name HA) (segid B and residue 41 and name HE#)  3.10 1.40 1.40
assign (segid B and residue 41 and name HB#) (segid B and residue 41 and name HA)  3.10 1.40 1.40
```

```
assign (segid B and residue 41 and name HB#) (segid B and residue 41 and name HN)  3.10 1.40 1.40
assign (segid B and residue 42 and name HA) (segid B and residue 42 and name HB#)  3.10 1.40 1.40
assign (segid B and residue 41 and name HN) (segid B and residue 42 and name HB#)  3.10 1.40 1.40
assign (segid B and residue 42 and name HB#) (segid B and residue 42 and name HA)  3.10 1.40 1.40
assign (segid B and residue 42 and name HB#) (segid B and residue 42 and name HN)  3.35 1.65 1.65
assign (segid B and residue 42 and name HB#) (segid B and residue 43 and name HA)  3.35 1.65 1.65
assign (segid B and residue 42 and name HB#) (segid B and residue 43 and name HB#)  3.35 1.65 1.65
assign (segid B and residue 42 and name HB#) (segid B and residue 43 and name HN)  3.35 1.65 1.65
assign (segid B and residue 42 and name HB#) (segid B and residue 45 and name HN)  3.35 1.65 1.65
assign (segid B and residue 43 and name HA) (segid B and residue 43 and name HB#)  3.10 1.40 1.40
assign (segid B and residue 43 and name HA) (segid B and residue 46 and name HN)  3.10 1.40 1.40
assign (segid B and residue 43 and name HB#) (segid B and residue 44 and name HN)  3.10 1.40 1.40
assign (segid B and residue 43 and name HB#) (segid B and residue 43 and name HA)  3.10 1.40 1.40
assign (segid B and residue 43 and name HB#) (segid B and residue 43 and name HN)  3.10 1.40 1.40
assign (segid B and residue 44 and name HA) (segid B and residue 44 and name HB#)  3.10 1.40 1.40
assign (segid B and residue 44 and name HB#) (segid B and residue 44 and name HA)  3.10 1.40 1.40
assign (segid B and residue 44 and name HB#) (segid B and residue 44 and name HN)  3.10 1.40 1.40
assign (segid B and residue 44 and name HB#) (segid B and residue 45 and name HN)  3.10 1.40 1.40
assign (segid B and residue 44 and name HB#) (segid B and residue 45 and name HN)  3.10 1.40 1.40
assign (segid B and residue 45 and name HA) (segid B and residue 45 and name HB#)  3.10 1.40 1.40
assign (segid B and residue 45 and name HA) (segid B and residue 45 and name HD#)  3.10 1.40 1.40
assign (segid B and residue 45 and name HA) (segid B and residue 45 and name HG#)  3.10 1.40 1.40
assign (segid B and residue 45 and name HA) (segid B and residue 45 and name HE#)  3.10 1.40 1.40
assign (segid B and residue 44 and name HN) (segid B and residue 45 and name HB#)  3.35 1.65 1.65
assign (segid B and residue 45 and name HB#) (segid B and residue 45 and name HA)  3.10 1.40 1.40
assign (segid B and residue 45 and name HB#) (segid B and residue 45 and name HN)  3.10 1.40 1.40
assign (segid B and residue 45 and name HB#) (segid B and residue 46 and name HN)  3.10 1.40 1.40
assign (segid B and residue 45 and name HD#) (segid B and residue 45 and name HN)  3.10 1.40 1.40
assign (segid B and residue 45 and name HD#) (segid B and residue 45 and name HG#)  3.10 1.40 1.40
assign (segid B and residue 45 and name HD#) (segid B and residue 46 and name HN)  3.10 1.40 1.40
assign (segid B and residue 45 and name HD#) (segid B and residue 45 and name HE#)  2.85 1.15 1.15
assign (segid B and residue 45 and name HE#) (segid B and residue 45 and name HA)  3.10 1.40 1.40
assign (segid B and residue 45 and name HE#) (segid B and residue 46 and name HB#)  3.10 1.40 1.40
assign (segid B and residue 45 and name HE#) (segid B and residue 45 and name HD#)  3.10 1.40 1.40
assign (segid B and residue 45 and name HG#) (segid B and residue 45 and name HA)  3.10 1.40 1.40
assign (segid B and residue 45 and name HG#) (segid B and residue 45 and name HD#)  3.10 1.40 1.40
assign (segid B and residue 45 and name HG#) (segid B and residue 45 and name HE#)  3.10 1.40 1.40
assign (segid B and residue 46 and name HA) (segid B and residue 46 and name HB#)  3.35 1.65 1.65
assign (segid B and residue 46 and name HB#) (segid B and residue 46 and name HA)  3.60 1.90 1.90
assign (segid B and residue 46 and name HB#) (segid B and residue 46 and name HN)  3.60 1.90 1.90
assign (segid B and residue 46 and name HB#) (segid B and residue 47 and name HA)  3.60 1.90 1.90
assign (segid B and residue 46 and name HB#) (segid B and residue 47 and name HN)  3.60 1.90 1.90
assign (segid B and residue 47 and name HA) (segid B and residue 47 and name HB)  3.10 1.40 1.40
assign (segid B and residue 47 and name HA) (segid B and residue 47 and name HG2#) 3.35 1.65 1.65
```

```
assign (segid B and residue 47 and name HB) (segid B and residue 47 and name HG2#)  3.35 1.65 1.65
assign (segid B and residue 47 and name HB) (segid B and residue 47 and name HN)  3.10 1.40 1.40
assign (segid B and residue 47 and name HB) (segid B and residue 48 and name HN)  3.10 1.40 1.40
assign (segid B and residue 44 and name HA) (segid B and residue 47 and name HG2#) 3.35 1.65 1.65
assign (segid B and residue 44 and name HA) (segid B and residue 47 and name HG2#) 3.35 1.65 1.65
assign (segid B and residue 44 and name HN) (segid B and residue 47 and name HG2#)  3.60 1.90 1.90
assign (segid B and residue 46 and name HB#) (segid B and residue 47 and name HG2#)  3.35 1.65 1.65
assign (segid B and residue 46 and name HN) (segid B and residue 47 and name HG2#)  3.60 1.90 1.90
assign (segid B and residue 47 and name HG2#) (segid B and residue 47 and name HA)  3.35 1.65 1.65
assign (segid B and residue 47 and name HG2#) (segid B and residue 47 and name HB)  3.35 1.65 1.65
assign (segid B and residue 47 and name HG2#) (segid B and residue 47 and name HN)  3.35 1.65 1.65
assign (segid B and residue 47 and name HG2#) (segid B and residue 48 and name HN)  3.35 1.65 1.65
assign (segid B and residue 47 and name HG2#) (segid A and residue 258 and name HB#)  3.35 1.65 1.65
assign (segid B and residue 47 and name HG2#) (segid A and residue 258 and name HB#)  3.35 1.65 1.65
assign (segid B and residue 45 and name HA) (segid B and residue 48 and name HB#)  3.35 1.65 1.65
assign (segid B and residue 48 and name HB#) (segid B and residue 48 and name HA)  3.10 1.40 1.40
assign (segid B and residue 47 and name HG2#) (segid B and residue 48 and name HB#)  3.85 2.15 2.15
assign (segid B and residue 45 and name HE#) (segid B and residue 48 and name HB#)  3.60 1.90 1.90
assign (segid B and residue 45 and name HB#) (segid B and residue 48 and name HB#)  3.60 1.90 1.90
assign (segid B and residue 49 and name HA) (segid B and residue 49 and name HB#)  3.10 1.40 1.40
assign (segid B and residue 49 and name HA) (segid B and residue 49 and name HB#)  3.10 1.40 1.40
assign (segid B and residue 49 and name HA) (segid B and residue 49 and name HN)  3.10 1.40 1.40
assign (segid B and residue 48 and name HB#) (segid B and residue 49 and name HA)  3.60 1.90 1.90
assign (segid B and residue 49 and name HB#) (segid B and residue 49 and name HN) 3.10 1.40 1.40
assign (segid B and residue 50 and name HA) (segid B and residue 50 and name HB#) 3.10 1.40 1.40
assign (segid B and residue 50 and name HA) (segid B and residue 51 and name HN)  2.85 1.15 1.15
assign (segid B and residue 50 and name HB#) (segid A and residue 258 and name HN) 3.10 1.40 1.40
assign (segid B and residue 50 and name HB#) (segid A and residue 257 and name HB#) 3.35 1.65 1.65
assign (segid B and residue 50 and name HB#) (segid A and residue 257 and name HG#) 3.35 1.65 1.65
assign (segid B and residue 50 and name HB#) (segid A and residue 257 and name HD#) 3.35 1.65 1.65
assign (segid B and residue 51 and name HA) (segid B and residue 51 and name HB#)  3.10 1.40 1.40
assign (segid B and residue 51 and name HA) (segid B and residue 51 and name HD#)  3.10 1.40 1.40
assign (segid B and residue 51 and name HA) (segid B and residue 51 and name HG#)  3.10 1.40 1.40
assign (segid B and residue 51 and name HA) (segid B and residue 51 and name HN)  3.10 1.40 1.40
assign (segid B and residue 51 and name HB#) (segid B and residue 51 and name HD#)  2.85 1.15 1.15
assign (segid B and residue 51 and name HB#) (segid B and residue 51 and name HD#)  2.85 1.15 1.15
assign (segid B and residue 51 and name HB#) (segid B and residue 51 and name HE#)  3.35 1.65 1.65
assign (segid B and residue 51 and name HB#) (segid B and residue 51 and name HG#)  3.10 1.40 1.40
assign (segid B and residue 51 and name HB#) (segid B and residue 51 and name HN)  3.10 1.40 1.40
assign (segid B and residue 51 and name HD#) (segid B and residue 51 and name HE#)  3.10 1.40 1.40
assign (segid B and residue 51 and name HD#) (segid B and residue 51 and name HG#)  2.85 1.15 1.15
assign (segid B and residue 51 and name HE#) (segid B and residue 51 and name HD#)  3.10 1.40 1.40
assign (segid B and residue 51 and name HE#) (segid B and residue 51 and name HG#)  3.10 1.40 1.40
assign (segid B and residue 51 and name HE#) (segid B and residue 51 and name HN)  3.10 1.40 1.40
```

```
assign (segid B and residue 51 and name HE#) (segid B and residue 51 and name HA)  3.10 1.40 1.40
assign (segid B and residue 48 and name HA) (segid B and residue 51 and name HG#)  3.10 1.40 1.40
assign (segid B and residue 51 and name HG#) (segid B and residue 51 and name HA)  3.10 1.40 1.40
assign (segid B and residue 51 and name HG#) (segid B and residue 51 and name HE#)  3.10 1.40 1.40
assign (segid B and residue 51 and name HG#) (segid B and residue 51 and name HN)  3.10 1.40 1.40
assign (segid A and residue 96 and name HA) (segid A and residue 96 and name HB#) 2.85 1.15 1.15
assign (segid A and residue 96 and name HA) (segid A and residue 96 and name HB#) 2.85 1.15 1.15
assign (segid A and residue 96 and name HA) (segid A and residue 97 and name HN)  3.10 1.40 1.40
assign (segid A and residue 96 and name HA) (segid A and residue 99 and name HB)  3.10 1.40 1.40
assign (segid A and residue 97 and name HA) (segid A and residue 97 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 97 and name HA) (segid A and residue 97 and name HN)  3.35 1.65 1.65
assign (segid A and residue 97 and name HB#) (segid A and residue 97 and name HA)  3.10 1.40 1.40
assign (segid A and residue 97 and name HB#) (segid A and residue 97 and name HN)  3.10 1.40 1.40
assign (segid A and residue 97 and name HB#) (segid A and residue 99 and name HG2#) 3.60 1.90 1.90
assign (segid A and residue 97 and name HB#) (segid A and residue 98 and name HA)  3.35 1.65 1.65
assign (segid A and residue 98 and name HA) (segid A and residue 98 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 98 and name HA) (segid A and residue 99 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 98 and name HA) (segid A and residue 99 and name HN)  3.10 1.40 1.40
assign (segid A and residue 98 and name HB#) (segid A and residue 98 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 99 and name HA) (segid A and residue 99 and name HB)  3.10 1.40 1.40
assign (segid A and residue 99 and name HA) (segid A and residue 99 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 99 and name HG2#) (segid A and residue 99 and name HA)  3.35 1.65 1.65
assign (segid A and residue 99 and name HG2#) (segid A and residue 99 and name HB)  3.35 1.65 1.65
assign (segid A and residue 99 and name HG2#) (segid A and residue 99 and name HN)  3.35 1.65 1.65
assign (segid A and residue 99 and name HG2#) (segid A and residue 100 and name HN)  3.35 1.65 1.65
assign (segid A and residue 100 and name HA) (segid A and residue 100 and name HD#)  3.35 1.65 1.65
assign (segid A and residue 100 and name HD#) (segid A and residue 100 and name HA)  3.35 1.65 1.65
assign (segid A and residue 100 and name HD#) (segid A and residue 100 and name HN)  3.35 1.65 1.65
assign (segid A and residue 102 and name HA) (segid A and residue 102 and name HN)  3.10 1.40 1.40
assign (segid A and residue 104 and name HA) (segid A and residue 104 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 104 and name HB#) (segid A and residue 104 and name HA)  3.10 1.40 1.40
assign (segid A and residue 104 and name HB#) (segid A and residue 105 and name HN)  3.10 1.40 1.40
assign (segid A and residue 105 and name HA) (segid A and residue 105 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 105 and name HA) (segid A and residue 105 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 106 and name HA) (segid A and residue 106 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 106 and name HA) (segid A and residue 106 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 107 and name HA) (segid A and residue 107 and name HB)  3.10 1.40 1.40
assign (segid A and residue 107 and name HA) (segid A and residue 107 and name HG1#) 3.35 1.65 1.65
assign (segid A and residue 107 and name HA) (segid A and residue 110 and name HN)  3.10 1.40 1.40
assign (segid A and residue 107 and name HB) (segid A and residue 107 and name HA)  3.10 1.40 1.40
assign (segid A and residue 107 and name HB) (segid A and residue 107 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 107 and name HB) (segid A and residue 107 and name HN)  3.10 1.40 1.40
assign (segid A and residue 107 and name HB) (segid A and residue 108 and name HN)  2.85 1.15 1.15
assign (segid A and residue 104 and name HG#) (segid A and residue 107 and name HG1#) 3.60 1.90 1.90
```

```
assign (segid A and residue 107 and name HG1#) (segid A and residue 107 and name HA)  3.35 1.65 1.65
assign (segid A and residue 107 and name HG1#) (segid A and residue 107 and name HB)  3.35 1.65 1.65
assign (segid A and residue 107 and name HG1#) (segid A and residue 107 and name HN)  3.35 1.65 1.65
assign (segid A and residue 107 and name HG1#) (segid A and residue 108 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 107 and name HG1#) (segid A and residue 108 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 107 and name HG1#) (segid A and residue 108 and name HN)  3.35 1.65 1.65
assign (segid A and residue 107 and name HG1#) (segid A and residue 265 and name HD2#) 3.60 1.90 1.90
assign (segid A and residue 107 and name HG1#) (segid A and residue 265 and name HD2#) 3.60 1.90 1.90
assign (segid A and residue 107 and name HG2#) (segid A and residue 107 and name HA)  3.35 1.65 1.65
assign (segid A and residue 107 and name HG2#) (segid A and residue 108 and name HN)  3.35 1.65 1.65
assign (segid A and residue 107 and name HG2#) (segid A and residue 265 and name HD2#)  3.60 1.90 1.90
assign (segid A and residue 107 and name HG2#) (segid A and residue 107 and name HB)  3.35 1.65 1.65
assign (segid A and residue 108 and name HA) (segid A and residue 108 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 108 and name HB#) (segid A and residue 108 and name HA) 3.10 1.40 1.40
assign (segid A and residue 108 and name HB#) (segid A and residue 108 and name HN) 3.10 1.40 1.40
assign (segid A and residue 108 and name HB#) (segid A and residue 109 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 108 and name HB#) (segid A and residue 109 and name HG#)  3.35 1.65 1.65
assign (segid A and residue 108 and name HB#) (segid A and residue 109 and name HN) 3.10 1.40 1.40
assign (segid A and residue 109 and name HA) (segid A and residue 109 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 109 and name HA) (segid A and residue 109 and name HG#)  2.85 1.15 1.15
assign (segid A and residue 109 and name HA) (segid A and residue 109 and name HN)  3.10 1.40 1.40
assign (segid A and residue 109 and name HB#) (segid A and residue 109 and name HA)  2.85 1.15 1.15
assign (segid A and residue 109 and name HB#) (segid A and residue 109 and name HG#)  2.85 1.15 1.15
assign (segid A and residue 109 and name HG#) (segid A and residue 109 and name HA)  2.85 1.15 1.15
assign (segid A and residue 109 and name HG#) (segid A and residue 109 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 110 and name HA) (segid A and residue 110 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 110 and name HA) (segid A and residue 113 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 110 and name HA) (segid A and residue 113 and name HN)  3.10 1.40 1.40
assign (segid A and residue 110 and name HB#) (segid A and residue 110 and name HA)  3.10 1.40 1.40
assign (segid A and residue 110 and name HB#) (segid A and residue 110 and name HN)  3.10 1.40 1.40
assign (segid A and residue 110 and name HB#) (segid A and residue 265 and name HD2#) 3.35 1.65 1.65
assign (segid A and residue 111 and name HA) (segid A and residue 111 and name HB#) 2.85 1.15 1.15
assign (segid A and residue 111 and name HB#) (segid A and residue 112 and name HN)  3.10 1.40 1.40
assign (segid A and residue 112 and name HA) (segid A and residue 112 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 111 and name HN) (segid A and residue 112 and name HB)  3.10 1.40 1.40
assign (segid A and residue 112 and name HB) (segid A and residue 112 and name HA)  3.10 1.40 1.40
assign (segid A and residue 112 and name HB) (segid A and residue 112 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 112 and name HB) (segid A and residue 112 and name HN)  3.10 1.40 1.40
assign (segid A and residue 112 and name HB) (segid A and residue 113 and name HN)  3.10 1.40 1.40
assign (segid A and residue 112 and name HG2#) (segid A and residue 112 and name HA) 3.35 1.65 1.65
assign (segid A and residue 112 and name HG2#) (segid A and residue 112 and name HN)  3.35 1.65 1.65
assign (segid A and residue 113 and name HA) (segid A and residue 113 and name HB)  3.10 1.40 1.40
assign (segid A and residue 113 and name HA) (segid A and residue 113 and name HG2#) 3.35 1.65 1.65
assign (segid A and residue 113 and name HA) (segid A and residue 113 and name HG1#)  3.60 1.90 1.90
```

```
assign (segid A and residue 113 and name HB) (segid A and residue 113 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 113 and name HB) (segid A and residue 114 and name HG#)  3.35 1.65 1.65
assign (segid A and residue 113 and name HB) (segid A and residue 113 and name HA)  3.10 1.40 1.40
assign (segid B and residue 42 and name HB#) (segid A and residue 113 and name HG1#)  3.60 1.90 1.90
assign (segid B and residue 42 and name HB#) (segid A and residue 113 and name HG1#)  3.60 1.90 1.90
assign (segid A and residue 110 and name HA) (segid A and residue 113 and name HG1#)  3.35 1.65 1.65
assign (segid A and residue 113 and name HG1#) (segid A and residue 113 and name HA)  3.35 1.65 1.65
assign (segid A and residue 113 and name HG1#) (segid A and residue 113 and name HB)  3.35 1.65 1.65
assign (segid A and residue 113 and name HG1#) (segid A and residue 113 and name HG2#) 3.35 1.65 1.65
assign (segid A and residue 113 and name HG1#) (segid A and residue 113 and name HN)  3.35 1.65 1.65
assign (segid B and residue 42 and name HB#) (segid A and residue 113 and name HG2#)  3.60 1.90 1.90
assign (segid B and residue 42 and name HB#) (segid A and residue 113 and name HG2#)  3.60 1.90 1.90
assign (segid A and residue 109 and name HG#) (segid A and residue 113 and name HG2#) 3.35 1.65 1.65
assign (segid A and residue 110 and name HN) (segid A and residue 113 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 113 and name HG2#) (segid A and residue 113 and name HA)  3.35 1.65 1.65
assign (segid A and residue 113 and name HG2#) (segid A and residue 113 and name HB)  3.35 1.65 1.65
assign (segid A and residue 113 and name HG2#) (segid A and residue 113 and name HN)  3.35 1.65 1.65
assign (segid A and residue 113 and name HG2#) (segid A and residue 114 and name HG#)  3.60 1.90 1.90
assign (segid A and residue 114 and name HA) (segid A and residue 114 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 114 and name HA) (segid A and residue 114 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 114 and name HA) (segid A and residue 117 and name HG1#)  3.35 1.65 1.65
assign (segid A and residue 114 and name HA) (segid A and residue 117 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 114 and name HA) (segid A and residue 117 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 114 and name HB#) (segid A and residue 114 and name HN)  3.10 1.40 1.40
assign (segid A and residue 114 and name HG#) (segid A and residue 114 and name HA)  3.35 1.65 1.65
assign (segid A and residue 114 and name HG#) (segid A and residue 114 and name HN)  3.10 1.40 1.40
assign (segid A and residue 115 and name HG#) (segid A and residue 115 and name HA)  3.35 1.65 1.65
assign (segid A and residue 116 and name HA) (segid A and residue 116 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 116 and name HB#) (segid A and residue 116 and name HA)  3.10 1.40 1.40
assign (segid A and residue 117 and name HA) (segid A and residue 117 and name HB)  3.10 1.40 1.40
assign (segid A and residue 117 and name HA) (segid A and residue 117 and name HG1#) 3.35 1.65 1.65
assign (segid A and residue 117 and name HA) (segid A and residue 117 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 117 and name HB) (segid A and residue 117 and name HA)  3.10 1.40 1.40
assign (segid A and residue 117 and name HG1#) (segid A and residue 117 and name HN)  3.35 1.65 1.65
assign (segid A and residue 117 and name HG1#) (segid A and residue 117 and name HA)  3.35 1.65 1.65
assign (segid A and residue 117 and name HG1#) (segid A and residue 117 and name HB)  3.35 1.65 1.65
assign (segid A and residue 117 and name HG2#) (segid A and residue 117 and name HA)  3.35 1.65 1.65
assign (segid A and residue 117 and name HG2#) (segid A and residue 117 and name HN)  3.35 1.65 1.65
assign (segid A and residue 118 and name HA) (segid A and residue 118 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 117 and name HB) (segid A and residue 118 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 117 and name HG1#) (segid A and residue 118 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 118 and name HB#) (segid A and residue 118 and name HA)  2.85 1.15 1.15
assign (segid A and residue 118 and name HB#) (segid A and residue 118 and name HN)  2.85 1.15 1.15
assign (segid A and residue 118 and name HB#) (segid A and residue 119 and name HN)  3.10 1.40 1.40
```

```
assign (segid A and residue 119 and name HA) (segid A and residue 119 and name HB#) 2.85 1.15 1.15
assign (segid A and residue 119 and name HA) (segid A and residue 119 and name HE#)  3.10 1.40 1.40
assign (segid A and residue 119 and name HA) (segid A and residue 119 and name HG#)  2.85 1.15 1.15
assign (segid A and residue 119 and name HB#) (segid A and residue 119 and name HG#)  2.85 1.15 1.15
assign (segid A and residue 119 and name HB#) (segid A and residue 119 and name HG#)  2.85 1.15 1.15
assign (segid A and residue 119 and name HB#) (segid A and residue 119 and name HG#)  2.85 1.15 1.15
assign (segid A and residue 119 and name HB#) (segid A and residue 119 and name HG#)  2.85 1.15 1.15
assign (segid A and residue 119 and name HB#) (segid A and residue 119 and name HG#)  2.85 1.15 1.15
assign (segid A and residue 119 and name HB#) (segid A and residue 119 and name HA)  3.10 1.40 1.40
assign (segid A and residue 119 and name HB#) (segid A and residue 119 and name HN)  3.10 1.40 1.40
assign (segid A and residue 119 and name HB#) (segid A and residue 120 and name HN)  3.10 1.40 1.40
assign (segid A and residue 119 and name HB#) (segid A and residue 120 and name HN)  3.10 1.40 1.40
assign (segid A and residue 119 and name HD#) (segid A and residue 119 and name HA)  3.10 1.40 1.40
assign (segid A and residue 119 and name HD#) (segid A and residue 119 and name HE#)  2.85 1.15 1.15
assign (segid A and residue 119 and name HG#) (segid A and residue 119 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 116 and name HA) (segid A and residue 119 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 118 and name HB#) (segid A and residue 119 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 118 and name HN) (segid A and residue 119 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 119 and name HG#) (segid A and residue 119 and name HA)  3.10 1.40 1.40
assign (segid A and residue 119 and name HG#) (segid A and residue 119 and name HN)  3.10 1.40 1.40
assign (segid A and residue 120 and name HA) (segid A and residue 120 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 120 and name HB#) (segid A and residue 120 and name HA)  3.10 1.40 1.40
assign (segid A and residue 120 and name HB#) (segid A and residue 120 and name HN)  3.10 1.40 1.40
assign (segid A and residue 121 and name HA) (segid A and residue 121 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 121 and name HA) (segid A and residue 121 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 122 and name HB#) (segid A and residue 122 and name HN)  3.10 1.40 1.40
assign (segid A and residue 123 and name HA) (segid A and residue 123 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 124 and name HA) (segid A and residue 124 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 124 and name HB#) (segid A and residue 124 and name HA)  3.10 1.40 1.40
assign (segid A and residue 125 and name HB#) (segid A and residue 125 and name HE#)  3.10 1.40 1.40
assign (segid A and residue 125 and name HB#) (segid A and residue 125 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 125 and name HB#) (segid A and residue 126 and name HN)  3.10 1.40 1.40
assign (segid A and residue 125 and name HD#) (segid A and residue 125 and name HA)  2.85 1.15 1.15
assign (segid A and residue 125 and name HD#) (segid A and residue 125 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 125 and name HD#) (segid A and residue 125 and name HE#)  3.10 1.40 1.40
assign (segid A and residue 125 and name HE#) (segid A and residue 125 and name HG#)  2.85 1.15 1.15
assign (segid A and residue 125 and name HE#) (segid A and residue 125 and name HD#)  2.85 1.15 1.15
assign (segid A and residue 125 and name HG#) (segid A and residue 125 and name HA)  3.10 1.40 1.40
assign (segid A and residue 125 and name HG#) (segid A and residue 125 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 125 and name HG#) (segid A and residue 125 and name HD#)  2.85 1.15 1.15
assign (segid A and residue 125 and name HG#) (segid A and residue 125 and name HE#)  3.10 1.40 1.40
assign (segid A and residue 126 and name HB#) (segid A and residue 126 and name HN)  3.10 1.40 1.40
assign (segid A and residue 126 and name HB#) (segid A and residue 126 and name HA) 3.10 1.40 1.40
```

```
assign (segid A and residue 127 and name HB#) (segid A and residue 127 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 127 and name HG#) (segid A and residue 127 and name HA)  3.10 1.40 1.40
assign (segid A and residue 127 and name HG#) (segid A and residue 127 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 128 and name HA) (segid A and residue 128 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 128 and name HA) (segid A and residue 128 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 128 and name HA) (segid A and residue 128 and name HN)  3.10 1.40 1.40
assign (segid A and residue 128 and name HB#) (segid A and residue 128 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 128 and name HB#) (segid A and residue 128 and name HN)  2.85 1.15 1.15
assign (segid A and residue 128 and name HG#) (segid A and residue 128 and name HA)  3.10 1.40 1.40
assign (segid A and residue 128 and name HG#) (segid A and residue 128 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 128 and name HG#) (segid A and residue 129 and name HN)  3.10 1.40 1.40
assign (segid A and residue 129 and name HA) (segid A and residue 130 and name HN)  3.10 1.40 1.40
assign (segid A and residue 129 and name HA) (segid A and residue 129 and name HN)  3.10 1.40 1.40
assign (segid A and residue 129 and name HB#) (segid A and residue 129 and name HN)  3.10 1.40 1.40
assign (segid A and residue 129 and name HD#) (segid A and residue 129 and name HN)  3.35 1.65 1.65
assign (segid A and residue 129 and name HD#) (segid A and residue 130 and name HN)  3.35 1.65 1.65
assign (segid A and residue 129 and name HG) (segid A and residue 129 and name HA)  2.85 1.15 1.15
assign (segid A and residue 129 and name HG) (segid A and residue 129 and name HD#)  3.35 1.65 1.65
assign (segid A and residue 129 and name HG) (segid A and residue 129 and name HN)  3.10 1.40 1.40
assign (segid A and residue 130 and name HA) (segid A and residue 130 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 130 and name HA) (segid A and residue 131 and name HN)  2.85 1.15 1.15
assign (segid A and residue 130 and name HA) (segid A and residue 130 and name HN)  2.85 1.15 1.15
assign (segid A and residue 131 and name HA) (segid A and residue 131 and name HN)  3.35 1.65 1.65
assign (segid A and residue 131 and name HA) (segid A and residue 132 and name HN)  3.35 1.65 1.65
assign (segid A and residue 131 and name HB#) (segid A and residue 131 and name HN)  3.10 1.40 1.40
assign (segid A and residue 132 and name HA) (segid A and residue 132 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 132 and name HA) (segid A and residue 132 and name HN)  3.10 1.40 1.40
assign (segid A and residue 132 and name HA) (segid A and residue 133 and name HN)  3.10 1.40 1.40
assign (segid A and residue 132 and name HB#) (segid A and residue 132 and name HN)  3.10 1.40 1.40
assign (segid A and residue 132 and name HB#) (segid A and residue 133 and name HN)  3.10 1.40 1.40
assign (segid A and residue 133 and name HA) (segid A and residue 133 and name HG1#)  3.35 1.65 1.65
assign (segid A and residue 133 and name HA) (segid A and residue 133 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 133 and name HA) (segid A and residue 134 and name HN)  2.85 1.15 1.15
assign (segid A and residue 133 and name HB) (segid A and residue 133 and name HA)  3.10 1.40 1.40
assign (segid A and residue 133 and name HB) (segid A and residue 133 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 133 and name HG2#) (segid A and residue 133 and name HA)  3.10 1.40 1.40
assign (segid A and residue 133 and name HG2#) (segid A and residue 133 and name HB)  3.10 1.40 1.40
assign (segid A and residue 133 and name HG2#) (segid A and residue 133 and name HG1#) 3.10 1.40 1.40
assign (segid A and residue 133 and name HG2#) (segid A and residue 133 and name HN)  3.10 1.40 1.40
assign (segid A and residue 134 and name HA) (segid A and residue 134 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 134 and name HA) (segid A and residue 134 and name HN)  3.10 1.40 1.40
assign (segid A and residue 134 and name HA) (segid A and residue 135 and name HN)  2.85 1.15 1.15
assign (segid A and residue 134 and name HB) (segid A and residue 134 and name HN)  3.10 1.40 1.40
assign (segid A and residue 134 and name HB) (segid A and residue 135 and name HN)  3.10 1.40 1.40
```

```
assign (segid A and residue 134 and name HG2#) (segid A and residue 134 and name HA) 3.35 1.65 1.65
assign (segid A and residue 134 and name HG2#) (segid A and residue 134 and name HB)  3.35 1.65 1.65
assign (segid A and residue 134 and name HG2#) (segid A and residue 135 and name HN)  3.35 1.65 1.65
assign (segid A and residue 135 and name HA) (segid A and residue 135 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 135 and name HA) (segid A and residue 135 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 135 and name HA) (segid A and residue 136 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 135 and name HB#) (segid A and residue 135 and name HA)  3.10 1.40 1.40
assign (segid A and residue 134 and name HG2#) (segid A and residue 135 and name HE#)  3.35 1.65 1.65
assign (segid A and residue 135 and name HE#) (segid A and residue 135 and name HA)  3.35 1.65 1.65
assign (segid A and residue 135 and name HE#) (segid A and residue 135 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 135 and name HE#) (segid A and residue 135 and name HN)  3.35 1.65 1.65
assign (segid A and residue 135 and name HE#) (segid A and residue 136 and name HD#)  3.60 1.90 1.90
assign (segid A and residue 135 and name HE#) (segid A and residue 182 and name HD1#)  3.10 1.40 1.40
assign (segid A and residue 135 and name HE#) (segid A and residue 182 and name HD1#)  3.10 1.40 1.40
assign (segid A and residue 135 and name HE#) (segid A and residue 182 and name HD1#)  3.10 1.40 1.40
assign (segid A and residue 135 and name HE#) (segid A and residue 182 and name HG2#)  3.10 1.40 1.40
assign (segid A and residue 135 and name HE#) (segid A and residue 182 and name HG2#)  3.10 1.40 1.40
assign (segid A and residue 135 and name HE#) (segid A and residue 182 and name HG2#)  3.10 1.40 1.40
assign (segid A and residue 136 and name HA) (segid A and residue 136 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 136 and name HA) (segid A and residue 136 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 136 and name HB#) (segid A and residue 136 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 136 and name HB#) (segid A and residue 136 and name HA)  3.10 1.40 1.40
assign (segid A and residue 136 and name HD#) (segid A and residue 136 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 135 and name HG#) (segid A and residue 136 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 136 and name HG#) (segid A and residue 136 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 136 and name HG#) (segid A and residue 136 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 137 and name HA) (segid A and residue 138 and name HN)  3.10 1.40 1.40
assign (segid A and residue 137 and name HA) (segid A and residue 137 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 137 and name HA) (segid A and residue 137 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 137 and name HB#) (segid A and residue 137 and name HD#)  2.85 1.15 1.15
assign (segid A and residue 137 and name HB#) (segid A and residue 137 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 137 and name HB#) (segid A and residue 138 and name HN)  3.10 1.40 1.40
assign (segid A and residue 137 and name HB#) (segid A and residue 138 and name HN)  3.10 1.40 1.40
assign (segid A and residue 137 and name HG#) (segid A and residue 137 and name HA)  3.10 1.40 1.40
assign (segid A and residue 137 and name HG#) (segid A and residue 137 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 137 and name HG#) (segid A and residue 137 and name HE#)  3.10 1.40 1.40
assign (segid A and residue 137 and name HG#) (segid A and residue 137 and name HN)  3.10 1.40 1.40
assign (segid A and residue 138 and name HA) (segid A and residue 138 and name HN)  3.10 1.40 1.40
assign (segid A and residue 138 and name HA) (segid A and residue 139 and name HN)  3.10 1.40 1.40
assign (segid A and residue 138 and name HB#) (segid A and residue 138 and name HN)  3.10 1.40 1.40
assign (segid A and residue 139 and name HA) (segid A and residue 139 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 139 and name HA) (segid A and residue 139 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 139 and name HA) (segid A and residue 139 and name HN)  3.10 1.40 1.40
assign (segid A and residue 139 and name HB#) (segid A and residue 139 and name HN)  3.10 1.40 1.40
```

```
assign (segid A and residue 139 and name HG#) (segid A and residue 139 and name HA)  3.10 1.40 1.40
assign (segid A and residue 139 and name HG#) (segid A and residue 139 and name HN)  3.10 1.40 1.40
assign (segid A and residue 140 and name HA) (segid A and residue 140 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 140 and name HA) (segid A and residue 140 and name HN)  3.10 1.40 1.40
assign (segid A and residue 140 and name HA) (segid A and residue 141 and name HA)  3.10 1.40 1.40
assign (segid A and residue 140 and name HA) (segid A and residue 141 and name HN)  3.10 1.40 1.40
assign (segid A and residue 140 and name HB#) (segid A and residue 140 and name HN)  3.10 1.40 1.40
assign (segid A and residue 141 and name HA) (segid A and residue 141 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 141 and name HA) (segid A and residue 141 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 141 and name HA) (segid A and residue 141 and name HN)  3.10 1.40 1.40
assign (segid A and residue 141 and name HA) (segid A and residue 144 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 141 and name HA) (segid A and residue 144 and name HN)  3.10 1.40 1.40
assign (segid A and residue 141 and name HB#) (segid A and residue 141 and name HN)  3.10 1.40 1.40
assign (segid A and residue 141 and name HB#) (segid A and residue 142 and name HN)  3.10 1.40 1.40
assign (segid A and residue 141 and name HG#) (segid A and residue 141 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 141 and name HG#) (segid A and residue 141 and name HA)  3.10 1.40 1.40
assign (segid A and residue 142 and name HG#) (segid A and residue 142 and name HN)  3.10 1.40 1.40
assign (segid A and residue 144 and name HB#) (segid A and residue 144 and name HN)  3.10 1.40 1.40
assign (segid A and residue 145 and name HA) (segid A and residue 146 and name HN)  3.35 1.65 1.65
assign (segid A and residue 145 and name HA) (segid A and residue 145 and name HN) 3.35 1.65 1.65
assign (segid A and residue 147 and name HA) (segid A and residue 147 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 147 and name HA) (segid A and residue 147 and name HN)  3.10 1.40 1.40
assign (segid A and residue 147 and name HB#) (segid A and residue 147 and name HN)  3.10 1.40 1.40
assign (segid A and residue 147 and name HB#) (segid A and residue 148 and name HN)  3.10 1.40 1.40
assign (segid A and residue 147 and name HB#) (segid A and residue 148 and name HN)  3.10 1.40 1.40
assign (segid A and residue 148 and name HA) (segid A and residue 148 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 148 and name HA) (segid A and residue 148 and name HN)  3.10 1.40 1.40
assign (segid A and residue 148 and name HA) (segid A and residue 149 and name HD1#) 3.10 1.40 1.40
assign (segid A and residue 148 and name HB#) (segid A and residue 148 and name HA)  2.85 1.15 1.15
assign (segid A and residue 148 and name HB#) (segid A and residue 148 and name HN)  3.10 1.40 1.40
assign (segid A and residue 149 and name HA) (segid A and residue 149 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 149 and name HA) (segid A and residue 149 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 149 and name HA) (segid A and residue 149 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 149 and name HA) (segid A and residue 149 and name HN)  3.10 1.40 1.40
assign (segid A and residue 149 and name HB#) (segid A and residue 149 and name HD1#) 3.60 1.90 1.90
assign (segid A and residue 149 and name HB#) (segid A and residue 149 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 149 and name HB#) (segid A and residue 149 and name HA)  3.10 1.40 1.40
assign (segid A and residue 148 and name HB#) (segid A and residue 149 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 149 and name HD1#) (segid A and residue 149 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 149 and name HD1#) (segid A and residue 149 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 149 and name HD1#) (segid A and residue 149 and name HG)  3.35 1.65 1.65
assign (segid A and residue 149 and name HD1#) (segid A and residue 198 and name HD2#) 3.60 1.90 1.90
assign (segid A and residue 148 and name HB#) (segid A and residue 149 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 149 and name HD2#) (segid A and residue 149 and name HA)  3.35 1.65 1.65
```

```
assign (segid A and residue 149 and name HD2#) (segid A and residue 149 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 149 and name HD2#) (segid A and residue 149 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 149 and name HD2#) (segid A and residue 149 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 149 and name HD2#) (segid A and residue 149 and name HG)  3.35 1.65 1.65
assign (segid A and residue 149 and name HD2#) (segid A and residue 149 and name HN)  3.35 1.65 1.65
assign (segid A and residue 149 and name HD2#) (segid A and residue 198 and name HD1#) 3.60 1.90 1.90
assign (segid A and residue 149 and name HD2#) (segid A and residue 198 and name HD2#)  3.60 1.90 1.90
assign (segid A and residue 149 and name HD2#) (segid A and residue 198 and name HD2#)  3.60 1.90 1.90
assign (segid A and residue 149 and name HG) (segid A and residue 149 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 149 and name HG) (segid A and residue 149 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 150 and name HA1) (segid A and residue 150 and name HA2)  2.85 1.15 1.15
assign (segid A and residue 150 and name HA1) (segid A and residue 150 and name HN)  3.10 1.40 1.40
assign (segid A and residue 150 and name HA2) (segid A and residue 150 and name HA1)  2.85 1.15 1.15
assign (segid A and residue 150 and name HA2) (segid A and residue 150 and name HN)  3.10 1.40 1.40
assign (segid A and residue 150 and name HA2) (segid A and residue 151 and name HN)  3.35 1.65 1.65
assign (segid A and residue 152 and name HE#) (segid A and residue 152 and name HG#) 3.10 1.40 1.40
assign (segid A and residue 153 and name HA) (segid A and residue 153 and name HD#) 3.35 1.65 1.65
assign (segid A and residue 153 and name HA) (segid A and residue 153 and name HN)  3.10 1.40 1.40
assign (segid A and residue 153 and name HA) (segid A and residue 154 and name HN)  3.10 1.40 1.40
assign (segid A and residue 153 and name HB#) (segid A and residue 153 and name HN)  3.10 1.40 1.40
assign (segid A and residue 153 and name HB#) (segid A and residue 153 and name HA)  3.10 1.40 1.40
assign (segid A and residue 153 and name HB#) (segid A and residue 154 and name HN)  3.10 1.40 1.40
assign (segid A and residue 153 and name HB#) (segid A and residue 153 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 149 and name HD2#) (segid A and residue 153 and name HD#) 3.60 1.90 1.90
assign (segid A and residue 153 and name HD#) (segid A and residue 153 and name HA)  3.35 1.65 1.65
assign (segid A and residue 153 and name HD#) (segid A and residue 153 and name HN)  3.35 1.65 1.65
assign (segid A and residue 153 and name HD#) (segid A and residue 153 and name HG)  3.10 1.40 1.40
assign (segid A and residue 153 and name HG) (segid A and residue 153 and name HA)  3.10 1.40 1.40
assign (segid A and residue 153 and name HG) (segid A and residue 153 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 153 and name HG) (segid A and residue 153 and name HN)  2.85 1.15 1.15
assign (segid A and residue 154 and name HA) (segid A and residue 154 and name HB#) 2.85 1.15 1.15
assign (segid A and residue 154 and name HA) (segid A and residue 154 and name HB#) 2.85 1.15 1.15
assign (segid A and residue 154 and name HA) (segid A and residue 154 and name HB#) 2.85 1.15 1.15
assign (segid A and residue 154 and name HA) (segid A and residue 154 and name HN)  3.10 1.40 1.40
assign (segid A and residue 154 and name HA) (segid A and residue 155 and name HN)  3.10 1.40 1.40
assign (segid A and residue 154 and name HB#) (segid A and residue 154 and name HA)  2.85 1.15 1.15
assign (segid A and residue 154 and name HB#) (segid A and residue 154 and name HA)  2.85 1.15 1.15
assign (segid A and residue 154 and name HB#) (segid A and residue 154 and name HA)  2.85 1.15 1.15
assign (segid A and residue 154 and name HB#) (segid A and residue 154 and name HN)  3.10 1.40 1.40
assign (segid A and residue 154 and name HB#) (segid A and residue 155 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 154 and name HB#) (segid A and residue 155 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 155 and name HA) (segid A and residue 155 and name HN)  3.10 1.40 1.40
assign (segid A and residue 155 and name HA) (segid A and residue 156 and name HN) 3.10 1.40 1.40
assign (segid A and residue 155 and name HA) (segid A and residue 155 and name HB#)  3.35 1.65 1.65
```

```
assign (segid A and residue 154 and name HA) (segid A and residue 155 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 155 and name HB#) (segid A and residue 155 and name HA) 3.10 1.40 1.40
assign (segid A and residue 155 and name HB#) (segid A and residue 155 and name HN)  3.35 1.65 1.65
assign (segid A and residue 155 and name HB#) (segid A and residue 156 and name HN)  3.35 1.65 1.65
assign (segid A and residue 156 and name HA) (segid A and residue 156 and name HN) 3.35 1.65 1.65
assign (segid A and residue 156 and name HB#) (segid A and residue 156 and name HA) 3.10 1.40 1.40
assign (segid A and residue 156 and name HB#) (segid A and residue 156 and name HN) 3.35 1.65 1.65
assign (segid A and residue 157 and name HA#) (segid A and residue 157 and name HN)  2.85 1.15 1.15
assign (segid A and residue 157 and name HA#) (segid A and residue 158 and name HN)  3.10 1.40 1.40
assign (segid A and residue 158 and name HA) (segid A and residue 158 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 158 and name HA) (segid A and residue 158 and name HN) 2.85 1.15 1.15
assign (segid A and residue 158 and name HA) (segid A and residue 159 and name HN)  3.10 1.40 1.40
assign (segid A and residue 158 and name HA) (segid A and residue 159 and name HG#)  3.35 1.65 1.65
assign (segid A and residue 158 and name HB#) (segid A and residue 158 and name HA)  3.10 1.40 1.40
assign (segid A and residue 158 and name HB#) (segid A and residue 158 and name HN)  3.35 1.65 1.65
assign (segid A and residue 158 and name HB#) (segid A and residue 159 and name HN)  3.35 1.65 1.65
assign (segid A and residue 158 and name HB#) (segid A and residue 160 and name HN)  3.35 1.65 1.65
assign (segid A and residue 159 and name HA) (segid A and residue 159 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 159 and name HA) (segid A and residue 159 and name HN)  3.10 1.40 1.40
assign (segid A and residue 159 and name HA) (segid A and residue 160 and name HN)  2.85 1.15 1.15
assign (segid A and residue 159 and name HB) (segid A and residue 159 and name HN)  3.10 1.40 1.40
assign (segid A and residue 159 and name HG#) (segid A and residue 159 and name HA) 2.85 1.15 1.15
assign (segid A and residue 159 and name HG#) (segid A and residue 159 and name HB)  3.10 1.40 1.40
assign (segid A and residue 160 and name HA1) (segid A and residue 160 and name HN)  3.10 1.40 1.40
assign (segid A and residue 160 and name HA2) (segid A and residue 160 and name HN)  2.85 1.15 1.15
assign (segid A and residue 161 and name HA) (segid A and residue 161 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 161 and name HA) (segid A and residue 162 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 161 and name HA) (segid A and residue 161 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 161 and name HA) (segid A and residue 161 and name HG#)  3.35 1.65 1.65
assign (segid A and residue 161 and name HA) (segid A and residue 162 and name HN)  3.10 1.40 1.40
assign (segid A and residue 161 and name HB#) (segid A and residue 161 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 161 and name HD#) (segid A and residue 161 and name HA)  3.10 1.40 1.40
assign (segid A and residue 161 and name HD#) (segid A and residue 161 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 161 and name HD#) (segid A and residue 161 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 161 and name HG#) (segid A and residue 161 and name HA)  3.10 1.40 1.40
assign (segid A and residue 161 and name HG#) (segid A and residue 161 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 162 and name HA) (segid A and residue 162 and name HN)  3.10 1.40 1.40
assign (segid A and residue 162 and name HA) (segid A and residue 163 and name HN)  3.35 1.65 1.65
assign (segid A and residue 162 and name HA) (segid A and residue 162 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 162 and name HB#) (segid A and residue 162 and name HN) 3.10 1.40 1.40
assign (segid A and residue 162 and name HB#) (segid A and residue 162 and name HA)  3.10 1.40 1.40
assign (segid A and residue 163 and name HA) (segid A and residue 163 and name HG2#)  3.10 1.40 1.40
assign (segid A and residue 163 and name HA) (segid A and residue 163 and name HN)  3.10 1.40 1.40
assign (segid A and residue 163 and name HA) (segid A and residue 164 and name HN)  3.10 1.40 1.40
```

```
assign (segid A and residue 163 and name HB) (segid A and residue 163 and name HG2#) 3.35 1.65 1.65
assign (segid A and residue 163 and name HB) (segid A and residue 163 and name HN)  3.35 1.65 1.65
assign (segid A and residue 163 and name HB) (segid A and residue 164 and name HN)  3.10 1.40 1.40
assign (segid A and residue 163 and name HG2#) (segid A and residue 163 and name HA) 3.35 1.65 1.65
assign (segid A and residue 163 and name HG2#) (segid A and residue 163 and name HB)  3.35 1.65 1.65
assign (segid A and residue 163 and name HG2#) (segid A and residue 163 and name HN)  3.35 1.65 1.65
assign (segid A and residue 164 and name HB#) (segid A and residue 164 and name HN)  3.10 1.40 1.40
assign (segid A and residue 164 and name HB#) (segid A and residue 164 and name HA)  3.10 1.40 1.40
assign (segid A and residue 165 and name HA) (segid A and residue 165 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 165 and name HA) (segid A and residue 165 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 165 and name HA) (segid A and residue 165 and name HN)  2.85 1.15 1.15
assign (segid A and residue 165 and name HB#) (segid A and residue 165 and name HA)  3.10 1.40 1.40
assign (segid A and residue 165 and name HB#) (segid A and residue 165 and name HG#)  2.85 1.15 1.15
assign (segid A and residue 165 and name HB#) (segid A and residue 165 and name HN)  2.85 1.15 1.15
assign (segid A and residue 166 and name HB#) (segid A and residue 166 and name HA)  3.10 1.40 1.40
assign (segid A and residue 166 and name HB#) (segid A and residue 166 and name HN) 3.10 1.40 1.40
assign (segid A and residue 167 and name HA) (segid A and residue 167 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 167 and name HA) (segid A and residue 167 and name HG#)  3.35 1.65 1.65
assign (segid A and residue 167 and name HA) (segid A and residue 167 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 167 and name HA) (segid A and residue 168 and name HN)  3.10 1.40 1.40
assign (segid A and residue 167 and name HA) (segid A and residue 168 and name HA#)  3.10 1.40 1.40
assign (segid A and residue 167 and name HB#) (segid A and residue 167 and name HA)  2.85 1.15 1.15
assign (segid A and residue 167 and name HB#) (segid A and residue 167 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 167 and name HB#) (segid A and residue 167 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 167 and name HB#) (segid A and residue 168 and name HN)  3.10 1.40 1.40
assign (segid A and residue 167 and name HD#) (segid A and residue 167 and name HA)  3.10 1.40 1.40
assign (segid A and residue 167 and name HD#) (segid A and residue 167 and name HA)  3.10 1.40 1.40
assign (segid A and residue 167 and name HD#) (segid A and residue 167 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 167 and name HD#) (segid A and residue 167 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 167 and name HD#) (segid A and residue 167 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 167 and name HD#) (segid A and residue 167 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 167 and name HD#) (segid A and residue 167 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 166 and name HA) (segid A and residue 167 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 167 and name HG#) (segid A and residue 167 and name HA)  3.10 1.40 1.40
assign (segid A and residue 168 and name HA#) (segid A and residue 168 and name HN)  2.85 1.15 1.15
assign (segid A and residue 168 and name HA#) (segid A and residue 169 and name HD1#) 3.60 1.90 1.90
assign (segid A and residue 168 and name HA#) (segid A and residue 169 and name HG1#)  3.10 1.40 1.40
assign (segid A and residue 168 and name HA#) (segid A and residue 169 and name HN)  2.85 1.15 1.15
assign (segid A and residue 169 and name HA) (segid A and residue 169 and name HB)  3.10 1.40 1.40
assign (segid A and residue 169 and name HA) (segid A and residue 169 and name HD1#) 3.35 1.65 1.65
assign (segid A and residue 169 and name HA) (segid A and residue 169 and name HG1#)  3.10 1.40 1.40
assign (segid A and residue 169 and name HA) (segid A and residue 169 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 169 and name HA) (segid A and residue 169 and name HN)  3.10 1.40 1.40
assign (segid A and residue 169 and name HA) (segid A and residue 170 and name HN)  2.85 1.15 1.15
```

```
assign (segid A and residue 169 and name HB) (segid A and residue 169 and name HA)  3.10 1.40 1.40
assign (segid A and residue 169 and name HB) (segid A and residue 169 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 169 and name HB) (segid A and residue 169 and name HG1#)  3.10 1.40 1.40
assign (segid A and residue 169 and name HB) (segid A and residue 169 and name HG2#)  2.85 1.15 1.15
assign (segid A and residue 169 and name HB) (segid A and residue 169 and name HN)  2.85 1.15 1.15
assign (segid A and residue 169 and name HB) (segid A and residue 170 and name HN)  3.10 1.40 1.40
assign (segid A and residue 169 and name HD1#) (segid A and residue 169 and name HA) 3.35 1.65 1.65
assign (segid A and residue 169 and name HD1#) (segid A and residue 169 and name HB)  3.35 1.65 1.65
assign (segid A and residue 169 and name HD1#) (segid A and residue 169 and name HG1#) 3.10 1.40 1.40
assign (segid A and residue 169 and name HD1#) (segid A and residue 169 and name HN)  3.60 1.90 1.90
assign (segid A and residue 169 and name HG1#) (segid A and residue 169 and name HA)  3.10 1.40 1.40
assign (segid A and residue 169 and name HG1#) (segid A and residue 169 and name HA)  3.10 1.40 1.40
assign (segid A and residue 169 and name HG1#) (segid A and residue 169 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 169 and name HG1#) (segid A and residue 169 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 169 and name HG1#) (segid A and residue 169 and name HN)  3.10 1.40 1.40
assign (segid A and residue 169 and name HG2#) (segid A and residue 169 and name HA)  3.10 1.40 1.40
assign (segid A and residue 169 and name HG2#) (segid A and residue 169 and name HB)  3.10 1.40 1.40
assign (segid A and residue 169 and name HG2#) (segid A and residue 169 and name HD1#) 3.35 1.65 1.65
assign (segid A and residue 169 and name HG2#) (segid A and residue 169 and name HG1#)  3.10 1.40 1.40
assign (segid A and residue 169 and name HG2#) (segid A and residue 169 and name HG1#)  3.10 1.40 1.40
assign (segid A and residue 169 and name HG2#) (segid A and residue 169 and name HN)  3.10 1.40 1.40
assign (segid A and residue 169 and name HG2#) (segid A and residue 170 and name HN)  3.10 1.40 1.40
assign (segid A and residue 170 and name HA) (segid A and residue 170 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 170 and name HB#) (segid A and residue 170 and name HA)  3.10 1.40 1.40
assign (segid A and residue 170 and name HB#) (segid A and residue 170 and name HN)  3.10 1.40 1.40
assign (segid A and residue 171 and name HA) (segid A and residue 171 and name HN)  3.10 1.40 1.40
assign (segid A and residue 172 and name HA) (segid A and residue 172 and name HB)  3.10 1.40 1.40
assign (segid A and residue 172 and name HA) (segid A and residue 172 and name HG1#) 3.35 1.65 1.65
assign (segid A and residue 172 and name HA) (segid A and residue 172 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 172 and name HA) (segid A and residue 172 and name HN)  3.10 1.40 1.40
assign (segid A and residue 172 and name HA) (segid A and residue 173 and name HG#)  3.35 1.65 1.65
assign (segid A and residue 172 and name HA) (segid A and residue 173 and name HN)  3.10 1.40 1.40
assign (segid A and residue 172 and name HB) (segid A and residue 172 and name HA)  2.85 1.15 1.15
assign (segid A and residue 172 and name HB) (segid A and residue 172 and name HG1#)  3.35 1.65 1.65
assign (segid A and residue 172 and name HB) (segid A and residue 172 and name HG2#)  3.10 1.40 1.40
assign (segid A and residue 172 and name HB) (segid A and residue 172 and name HN)  2.85 1.15 1.15
assign (segid A and residue 172 and name HB) (segid A and residue 173 and name HN)  3.10 1.40 1.40
assign (segid A and residue 172 and name HG2#) (segid A and residue 172 and name HB) 3.10 1.40 1.40
assign (segid A and residue 173 and name HA) (segid A and residue 173 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 173 and name HA) (segid A and residue 173 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 172 and name HG1#) (segid A and residue 173 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 173 and name HB#) (segid A and residue 173 and name HN)  3.10 1.40 1.40
assign (segid A and residue 173 and name HB#) (segid A and residue 174 and name HN)  3.10 1.40 1.40
assign (segid A and residue 172 and name HG1#) (segid A and residue 173 and name HG#) 3.60 1.90 1.90
```

```
assign (segid A and residue 172 and name HG2#) (segid A and residue 173 and name HG#)  3.35 1.65 1.65
assign (segid A and residue 173 and name HG#) (segid A and residue 173 and name HA)  3.35 1.65 1.65
assign (segid A and residue 173 and name HG#) (segid A and residue 173 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 174 and name HA) (segid A and residue 174 and name HB)  2.85 1.15 1.15
assign (segid A and residue 174 and name HA) (segid A and residue 174 and name HG1#) 3.10 1.40 1.40
assign (segid A and residue 174 and name HA) (segid A and residue 174 and name HG1#) 3.10 1.40 1.40
assign (segid A and residue 174 and name HB) (segid A and residue 174 and name HA)  3.10 1.40 1.40
assign (segid A and residue 174 and name HB) (segid A and residue 174 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 174 and name HB) (segid A and residue 174 and name HG1#)  3.10 1.40 1.40
assign (segid A and residue 174 and name HB) (segid A and residue 174 and name HG1#)  3.10 1.40 1.40
assign (segid A and residue 174 and name HB) (segid A and residue 174 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 174 and name HD1#) (segid A and residue 174 and name HA)  3.35 1.65 1.65
assign (segid A and residue 174 and name HD1#) (segid A and residue 174 and name HB)  3.35 1.65 1.65
assign (segid A and residue 174 and name HD1#) (segid A and residue 174 and name HG1#) 3.35 1.65 1.65
assign (segid A and residue 174 and name HD1#) (segid A and residue 174 and name HG1#) 3.35 1.65 1.65
assign (segid A and residue 174 and name HG1#) (segid A and residue 174 and name HA)  3.10 1.40 1.40
assign (segid A and residue 174 and name HG1#) (segid A and residue 174 and name HB)  3.10 1.40 1.40
assign (segid A and residue 174 and name HG2#) (segid A and residue 174 and name HA)  3.35 1.65 1.65
assign (segid A and residue 174 and name HG2#) (segid A and residue 174 and name HB)  3.10 1.40 1.40
assign (segid A and residue 174 and name HG2#) (segid A and residue 174 and name HG1#) 3.35 1.65 1.65
assign (segid A and residue 174 and name HG2#) (segid A and residue 174 and name HG1#) 3.35 1.65 1.65
assign (segid A and residue 175 and name HA1) (segid A and residue 175 and name HA2)  2.85 1.15 1.15
assign (segid A and residue 175 and name HA1) (segid A and residue 175 and name HN)  3.10 1.40 1.40
assign (segid A and residue 175 and name HA1) (segid A and residue 176 and name HN)  3.10 1.40 1.40
assign (segid A and residue 175 and name HA2) (segid A and residue 175 and name HA1)  2.85 1.15 1.15
assign (segid A and residue 175 and name HA2) (segid A and residue 175 and name HN)  3.10 1.40 1.40
assign (segid A and residue 175 and name HA2) (segid A and residue 176 and name HN)  3.10 1.40 1.40
assign (segid A and residue 176 and name HB#) (segid A and residue 176 and name HA)  3.10 1.40 1.40
assign (segid A and residue 176 and name HB#) (segid A and residue 176 and name HN)  3.10 1.40 1.40
assign (segid A and residue 179 and name HA) (segid A and residue 179 and name HN)  3.10 1.40 1.40
assign (segid A and residue 179 and name HA) (segid A and residue 180 and name HN)  3.10 1.40 1.40
assign (segid A and residue 179 and name HB#) (segid A and residue 179 and name HD1#) 3.10 1.40 1.40
assign (segid A and residue 179 and name HB#) (segid A and residue 179 and name HD2#) 3.35 1.65 1.65
assign (segid A and residue 179 and name HB#) (segid A and residue 179 and name HA)  3.10 1.40 1.40
assign (segid A and residue 179 and name HD1#) (segid A and residue 179 and name HD2#) 3.60 1.90 1.90
assign (segid A and residue 179 and name HD2#) (segid A and residue 179 and name HD1#) 3.60 1.90 1.90
assign (segid A and residue 179 and name HD2#) (segid A and residue 179 and name HG)  3.10 1.40 1.40
assign (segid A and residue 179 and name HD2#) (segid A and residue 180 and name HN)  3.35 1.65 1.65
assign (segid A and residue 179 and name HG) (segid A and residue 179 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 179 and name HG) (segid A and residue 179 and name HN)  3.10 1.40 1.40
assign (segid A and residue 179 and name HG) (segid A and residue 180 and name HN)  3.10 1.40 1.40
assign (segid A and residue 179 and name HD1#) (segid A and residue 179 and name HG)  3.10 1.40 1.40
assign (segid A and residue 180 and name HA) (segid A and residue 180 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 180 and name HB#) (segid A and residue 180 and name HD2#)  3.35 1.65 1.65
```

```
assign (segid A and residue 180 and name HB#) (segid A and residue 181 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 180 and name HD1#) (segid A and residue 180 and name HA) 3.60 1.90 1.90
assign (segid A and residue 180 and name HD1#) (segid A and residue 180 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 180 and name HD1#) (segid A and residue 180 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 180 and name HD1#) (segid A and residue 180 and name HG)  3.35 1.65 1.65
assign (segid A and residue 180 and name HD1#) (segid A and residue 180 and name HN)  3.35 1.65 1.65
assign (segid A and residue 180 and name HD2#) (segid A and residue 180 and name HA)  3.35 1.65 1.65
assign (segid A and residue 180 and name HD2#) (segid A and residue 180 and name HG)  3.35 1.65 1.65
assign (segid A and residue 180 and name HD2#) (segid A and residue 183 and name HB)  3.60 1.90 1.90
assign (segid A and residue 180 and name HD2#) (segid A and residue 180 and name HN)  3.60 1.90 1.90
assign (segid A and residue 180 and name HG) (segid A and residue 180 and name HA)  3.10 1.40 1.40
assign (segid A and residue 180 and name HG) (segid A and residue 180 and name HD1#) 3.35 1.65 1.65
assign (segid A and residue 180 and name HG) (segid A and residue 180 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 180 and name HG) (segid A and residue 180 and name HN)  3.10 1.40 1.40
assign (segid A and residue 182 and name HA) (segid A and residue 182 and name HB)  3.10 1.40 1.40
assign (segid A and residue 182 and name HA) (segid A and residue 182 and name HG1#) 3.35 1.65 1.65
assign (segid A and residue 182 and name HA) (segid A and residue 182 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 182 and name HB) (segid A and residue 182 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 182 and name HB) (segid A and residue 182 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 182 and name HB) (segid A and residue 182 and name HN)  3.10 1.40 1.40
assign (segid A and residue 182 and name HB) (segid A and residue 183 and name HN)  3.10 1.40 1.40
assign (segid A and residue 179 and name HG) (segid A and residue 182 and name HD1#) 3.35 1.65 1.65
assign (segid A and residue 181 and name HB#) (segid A and residue 182 and name HD1#) 3.60 1.90 1.90
assign (segid A and residue 182 and name HD1#) (segid A and residue 182 and name HA)  3.35 1.65 1.65
assign (segid A and residue 182 and name HD1#) (segid A and residue 182 and name HB)  3.35 1.65 1.65
assign (segid A and residue 182 and name HD1#) (segid A and residue 182 and name HG1#)  3.35 1.65 1.65
assign (segid A and residue 182 and name HD1#) (segid A and residue 182 and name HG1#)  3.35 1.65 1.65
assign (segid A and residue 182 and name HD1#) (segid A and residue 182 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 182 and name HD1#) (segid A and residue 182 and name HN)  3.10 1.40 1.40
assign (segid A and residue 182 and name HD1#) (segid A and residue 183 and name HN)  3.35 1.65 1.65
assign (segid A and residue 182 and name HG1#) (segid A and residue 182 and name HD1#) 3.35 1.65 1.65
assign (segid A and residue 182 and name HG1#) (segid A and residue 182 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 182 and name HG1#) (segid A and residue 182 and name HN)  3.10 1.40 1.40
assign (segid A and residue 182 and name HG2#) (segid A and residue 182 and name HA)  3.35 1.65 1.65
assign (segid A and residue 182 and name HG2#) (segid A and residue 182 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 182 and name HG2#) (segid A and residue 182 and name HG1#)  3.35 1.65 1.65
assign (segid A and residue 182 and name HG2#) (segid A and residue 182 and name HG1#)  3.35 1.65 1.65
assign (segid A and residue 182 and name HG2#) (segid A and residue 182 and name HN)  3.35 1.65 1.65
assign (segid A and residue 182 and name HG2#) (segid A and residue 183 and name HN)  3.10 1.40 1.40
assign (segid A and residue 182 and name HB) (segid A and residue 183 and name HA)  3.10 1.40 1.40
assign (segid A and residue 183 and name HA) (segid A and residue 183 and name HB)  2.85 1.15 1.15
assign (segid A and residue 183 and name HA) (segid A and residue 183 and name HG1#) 3.10 1.40 1.40
assign (segid A and residue 183 and name HA) (segid A and residue 183 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 183 and name HA) (segid A and residue 184 and name HN)  3.35 1.65 1.65
```

```
assign (segid A and residue 183 and name HB) (segid A and residue 183 and name HA)  3.10 1.40 1.40
assign (segid A and residue 183 and name HB) (segid A and residue 183 and name HG1#)  3.35 1.65 1.65
assign (segid A and residue 183 and name HB) (segid A and residue 183 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 183 and name HG1#) (segid A and residue 183 and name HB)  3.10 1.40 1.40
assign (segid A and residue 180 and name HA) (segid A and residue 183 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 182 and name HB) (segid A and residue 183 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 182 and name HG1#) (segid A and residue 183 and name HG2#) 3.60 1.90 1.90
assign (segid A and residue 182 and name HG2#) (segid A and residue 183 and name HG2#)  3.60 1.90 1.90
assign (segid A and residue 182 and name HG2#) (segid A and residue 183 and name HG2#)  3.60 1.90 1.90
assign (segid A and residue 182 and name HN) (segid A and residue 183 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 183 and name HG2#) (segid A and residue 183 and name HA)  3.35 1.65 1.65
assign (segid A and residue 183 and name HG2#) (segid A and residue 183 and name HB)  3.35 1.65 1.65
assign (segid A and residue 183 and name HG2#) (segid A and residue 183 and name HN)  3.35 1.65 1.65
assign (segid A and residue 183 and name HG2#) (segid A and residue 184 and name HN)  3.35 1.65 1.65
assign (segid A and residue 183 and name HG2#) (segid A and residue 186 and name HE#)  3.60 1.90 1.90
assign (segid A and residue 183 and name HG2#) (segid A and residue 186 and name HE#)  3.60 1.90 1.90
assign (segid A and residue 184 and name HA) (segid A and residue 184 and name HN) 2.85 1.15 1.15
assign (segid A and residue 184 and name HA) (segid A and residue 185 and name HN)  3.10 1.40 1.40
assign (segid A and residue 185 and name HA) (segid A and residue 185 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 185 and name HB#) (segid A and residue 185 and name HN)  3.10 1.40 1.40
assign (segid A and residue 185 and name HB#) (segid A and residue 186 and name HN)  3.10 1.40 1.40
assign (segid A and residue 186 and name HE#) (segid A and residue 186 and name HA)  3.35 1.65 1.65
assign (segid A and residue 186 and name HE#) (segid A and residue 186 and name HN)  3.35 1.65 1.65
assign (segid A and residue 187 and name HB#) (segid A and residue 187 and name HA) 3.10 1.40 1.40
assign (segid A and residue 188 and name HA) (segid A and residue 188 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 188 and name HG#) (segid A and residue 188 and name HA)  3.10 1.40 1.40
assign (segid A and residue 189 and name HA) (segid A and residue 189 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 189 and name HB#) (segid A and residue 189 and name HN) 3.10 1.40 1.40
assign (segid A and residue 189 and name HB#) (segid A and residue 190 and name HN)  3.35 1.65 1.65
assign (segid A and residue 190 and name HB) (segid A and residue 190 and name HN)  3.10 1.40 1.40
assign (segid A and residue 190 and name HB) (segid A and residue 190 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 190 and name HG2#) (segid A and residue 190 and name HA) 3.35 1.65 1.65
assign (segid A and residue 190 and name HG2#) (segid A and residue 194 and name HG2#) 3.60 1.90 1.90
assign (segid A and residue 186 and name HE#) (segid A and residue 191 and name HA) 3.35 1.65 1.65
assign (segid A and residue 186 and name HE#) (segid A and residue 191 and name HA) 3.35 1.65 1.65
assign (segid A and residue 191 and name HA) (segid A and residue 191 and name HG1#) 3.35 1.65 1.65
assign (segid A and residue 191 and name HA) (segid A and residue 191 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 191 and name HB) (segid A and residue 191 and name HG1#)  3.35 1.65 1.65
assign (segid A and residue 191 and name HB) (segid A and residue 191 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 191 and name HG1#) (segid A and residue 191 and name HA)  3.35 1.65 1.65
assign (segid A and residue 191 and name HG1#) (segid A and residue 191 and name HB)  3.35 1.65 1.65
assign (segid A and residue 191 and name HG1#) (segid A and residue 191 and name HN)  3.35 1.65 1.65
assign (segid A and residue 191 and name HG1#) (segid A and residue 192 and name HN)  3.35 1.65 1.65
assign (segid A and residue 191 and name HG1#) (segid A and residue 194 and name HG1#) 3.85 2.15 2.15
```

```
assign (segid A and residue 149 and name HD1#) (segid A and residue 191 and name HG2#)  3.60 1.90 1.90
assign (segid A and residue 149 and name HD2#) (segid A and residue 191 and name HG2#)  3.60 1.90 1.90
assign (segid A and residue 191 and name HG2#) (segid A and residue 191 and name HA)  3.35 1.65 1.65
assign (segid A and residue 191 and name HG2#) (segid A and residue 191 and name HN)  3.35 1.65 1.65
assign (segid A and residue 191 and name HG2#) (segid A and residue 192 and name HN)  3.35 1.65 1.65
assign (segid A and residue 192 and name HA) (segid A and residue 192 and name HB)  3.10 1.40 1.40
assign (segid A and residue 192 and name HA) (segid A and residue 192 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 192 and name HA) (segid A and residue 195 and name HD#)  3.35 1.65 1.65
assign (segid A and residue 192 and name HB) (segid A and residue 192 and name HA)  3.10 1.40 1.40
assign (segid A and residue 192 and name HB) (segid A and residue 192 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 192 and name HG2#) (segid A and residue 192 and name HA)  3.35 1.65 1.65
assign (segid A and residue 192 and name HG2#) (segid A and residue 192 and name HB)  3.10 1.40 1.40
assign (segid A and residue 192 and name HG2#) (segid A and residue 192 and name HN)  3.35 1.65 1.65
assign (segid A and residue 193 and name HA) (segid A and residue 194 and name HN)  3.35 1.65 1.65
assign (segid A and residue 194 and name HA) (segid A and residue 194 and name HB)  3.10 1.40 1.40
assign (segid A and residue 194 and name HA) (segid A and residue 194 and name HG1#) 3.35 1.65 1.65
assign (segid A and residue 194 and name HA) (segid A and residue 194 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 194 and name HA) (segid A and residue 197 and name HN)  3.35 1.65 1.65
assign (segid A and residue 194 and name HB) (segid A and residue 194 and name HG1#)  3.35 1.65 1.65
assign (segid A and residue 194 and name HB) (segid A and residue 194 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 194 and name HB) (segid A and residue 195 and name HN)  3.10 1.40 1.40
assign (segid A and residue 194 and name HB) (segid A and residue 194 and name HN)  3.10 1.40 1.40
assign (segid A and residue 191 and name HB) (segid A and residue 194 and name HG1#)  3.35 1.65 1.65
assign (segid A and residue 194 and name HG1#) (segid A and residue 194 and name HA)  3.35 1.65 1.65
assign (segid A and residue 194 and name HG1#) (segid A and residue 194 and name HB)  3.35 1.65 1.65
assign (segid A and residue 194 and name HG1#) (segid A and residue 194 and name HG2#) 3.60 1.90 1.90
assign (segid A and residue 194 and name HG1#) (segid A and residue 194 and name HN)  3.35 1.65 1.65
assign (segid A and residue 194 and name HG1#) (segid A and residue 195 and name HD#)  3.60 1.90 1.90
assign (segid A and residue 194 and name HG1#) (segid A and residue 195 and name HN)  3.35 1.65 1.65
assign (segid A and residue 186 and name HE#) (segid A and residue 194 and name HG2#)  3.60 1.90 1.90
assign (segid A and residue 191 and name HA) (segid A and residue 194 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 191 and name HG1#) (segid A and residue 194 and name HG2#) 3.85 2.15 2.15
assign (segid A and residue 194 and name HG2#) (segid A and residue 194 and name HA)  3.35 1.65 1.65
assign (segid A and residue 194 and name HG2#) (segid A and residue 194 and name HB)  3.35 1.65 1.65
assign (segid A and residue 194 and name HG2#) (segid A and residue 194 and name HG1#) 3.60 1.90 1.90
assign (segid A and residue 194 and name HG2#) (segid A and residue 194 and name HN)  3.35 1.65 1.65
assign (segid A and residue 194 and name HG2#) (segid A and residue 195 and name HN)  3.35 1.65 1.65
assign (segid A and residue 195 and name HB#) (segid A and residue 195 and name HN)  3.10 1.40 1.40
assign (segid A and residue 195 and name HB#) (segid A and residue 196 and name HN)  3.10 1.40 1.40
assign (segid A and residue 198 and name HA) (segid A and residue 198 and name HD2#)  3.60 1.90 1.90
assign (segid A and residue 198 and name HA) (segid A and residue 198 and name HN)  3.10 1.40 1.40
assign (segid A and residue 198 and name HA) (segid A and residue 199 and name HN)  3.10 1.40 1.40
assign (segid A and residue 198 and name HB#) (segid A and residue 198 and name HD2#) 3.60 1.90 1.90
assign (segid A and residue 194 and name HG1#) (segid A and residue 198 and name HD1#) 3.60 1.90 1.90
```

```
assign (segid A and residue 198 and name HD1#) (segid A and residue 198 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 198 and name HD1#) (segid A and residue 198 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 198 and name HD1#) (segid A and residue 198 and name HN)  3.35 1.65 1.65
assign (segid A and residue 198 and name HD1#) (segid A and residue 199 and name HN)  3.35 1.65 1.65
assign (segid A and residue 149 and name HB#) (segid A and residue 198 and name HD2#)  3.10 1.40 1.40
assign (segid A and residue 149 and name HG) (segid A and residue 198 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 198 and name HD2#) (segid A and residue 198 and name HA)  3.35 1.65 1.65
assign (segid A and residue 198 and name HD2#) (segid A and residue 198 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 198 and name HD2#) (segid A and residue 198 and name HD1#)  3.60 1.90 1.90
assign (segid A and residue 198 and name HD2#) (segid A and residue 198 and name HN)  3.35 1.65 1.65
assign (segid A and residue 198 and name HG) (segid A and residue 198 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 198 and name HG) (segid A and residue 198 and name HN)  3.10 1.40 1.40
assign (segid A and residue 199 and name HA) (segid A and residue 199 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 200 and name HA) (segid A and residue 200 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 200 and name HB#) (segid A and residue 200 and name HA)  3.10 1.40 1.40
assign (segid A and residue 200 and name HB#) (segid A and residue 200 and name HA)  3.10 1.40 1.40
assign (segid A and residue 200 and name HB#) (segid A and residue 200 and name HN)  3.10 1.40 1.40
assign (segid A and residue 200 and name HB#) (segid A and residue 201 and name HN)  3.10 1.40 1.40
assign (segid A and residue 202 and name HA) (segid A and residue 202 and name HN) 3.10 1.40 1.40
assign (segid A and residue 202 and name HA) (segid A and residue 203 and name HA2) 3.35 1.65 1.65
assign (segid A and residue 202 and name HB#) (segid A and residue 202 and name HA)  2.85 1.15 1.15
assign (segid A and residue 202 and name HB#) (segid A and residue 203 and name HA2)  3.10 1.40 1.40
assign (segid A and residue 203 and name HA1) (segid A and residue 203 and name HA2)  2.85 1.15 1.15
assign (segid A and residue 203 and name HA1) (segid A and residue 203 and name HN)  3.10 1.40 1.40
assign (segid A and residue 203 and name HA1) (segid A and residue 204 and name HN)  3.35 1.65 1.65
assign (segid A and residue 203 and name HA2) (segid A and residue 203 and name HA1)  2.85 1.15 1.15
assign (segid A and residue 203 and name HA2) (segid A and residue 203 and name HN)  3.10 1.40 1.40
assign (segid A and residue 204 and name HA) (segid A and residue 204 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 204 and name HA) (segid A and residue 204 and name HN)  3.10 1.40 1.40
assign (segid A and residue 204 and name HA) (segid A and residue 205 and name HN)  3.10 1.40 1.40
assign (segid A and residue 204 and name HG#) (segid A and residue 204 and name HA)  2.85 1.15 1.15
assign (segid A and residue 204 and name HG#) (segid A and residue 204 and name HN)  3.10 1.40 1.40
assign (segid A and residue 204 and name HG#) (segid A and residue 205 and name HN)  3.10 1.40 1.40
assign (segid A and residue 205 and name HA) (segid A and residue 205 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 205 and name HA) (segid A and residue 205 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 205 and name HA) (segid A and residue 205 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 205 and name HA) (segid A and residue 205 and name HN)  3.10 1.40 1.40
assign (segid A and residue 205 and name HA) (segid A and residue 206 and name HN)  3.10 1.40 1.40
assign (segid A and residue 205 and name HB#) (segid A and residue 205 and name HA)  3.10 1.40 1.40
assign (segid A and residue 205 and name HB#) (segid A and residue 205 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 205 and name HB#) (segid A and residue 205 and name HN)  3.35 1.65 1.65
assign (segid A and residue 204 and name HN) (segid A and residue 205 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 205 and name HD#) (segid A and residue 205 and name HA)  3.10 1.40 1.40
assign (segid A and residue 205 and name HD#) (segid A and residue 205 and name HB#)  3.10 1.40 1.40
```

```
assign (segid A and residue 205 and name HD#) (segid A and residue 205 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 205 and name HG#) (segid A and residue 205 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 206 and name HB#) (segid A and residue 206 and name HA)  2.85 1.15 1.15
assign (segid A and residue 206 and name HB#) (segid A and residue 206 and name HN)  3.10 1.40 1.40
assign (segid A and residue 206 and name HB#) (segid A and residue 207 and name HN)  3.10 1.40 1.40
assign (segid A and residue 207 and name HA) (segid A and residue 207 and name HN)  3.10 1.40 1.40
assign (segid A and residue 207 and name HA) (segid A and residue 208 and name HN)  3.10 1.40 1.40
assign (segid A and residue 207 and name HB#) (segid A and residue 207 and name HA)  3.35 1.65 1.65
assign (segid A and residue 207 and name HB#) (segid A and residue 207 and name HN)  3.10 1.40 1.40
assign (segid A and residue 208 and name HA) (segid A and residue 208 and name HG2#) 3.35 1.65 1.65
assign (segid A and residue 207 and name HN) (segid A and residue 208 and name HB)  3.10 1.40 1.40
assign (segid A and residue 208 and name HB) (segid A and residue 208 and name HG2#) 3.35 1.65 1.65
assign (segid A and residue 149 and name HD2#) (segid A and residue 208 and name HG2#) 3.85 2.15 2.15
assign (segid A and residue 207 and name HN) (segid A and residue 208 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 208 and name HG2#) (segid A and residue 208 and name HA)  3.35 1.65 1.65
assign (segid A and residue 208 and name HG2#) (segid A and residue 208 and name HB)  3.35 1.65 1.65
assign (segid A and residue 208 and name HG2#) (segid A and residue 208 and name HN)  3.35 1.65 1.65
assign (segid A and residue 153 and name HD#) (segid A and residue 208 and name HG2#) 3.85 2.15 2.15
assign (segid A and residue 210 and name HA) (segid A and residue 210 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 210 and name HA) (segid A and residue 210 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 210 and name HB#) (segid A and residue 211 and name HN)  3.35 1.65 1.65
assign (segid A and residue 210 and name HB#) (segid A and residue 211 and name HN)  3.35 1.65 1.65
assign (segid A and residue 210 and name HG#) (segid A and residue 210 and name HA)  3.10 1.40 1.40
assign (segid A and residue 210 and name HG#) (segid A and residue 211 and name HN)  3.35 1.65 1.65
assign (segid A and residue 208 and name HN) (segid A and residue 211 and name HD2#) 3.60 1.90 1.90
assign (segid A and residue 208 and name HN) (segid A and residue 211 and name HD1#) 3.60 1.90 1.90
assign (segid A and residue 208 and name HG2#) (segid A and residue 211 and name HD2#) 3.85 2.15 2.15
assign (segid A and residue 212 and name HA1) (segid A and residue 212 and name HA2)  3.10 1.40 1.40
assign (segid A and residue 212 and name HA2) (segid A and residue 212 and name HA1)  3.10 1.40 1.40
assign (segid A and residue 213 and name HA) (segid A and residue 213 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 213 and name HA) (segid A and residue 213 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 213 and name HB#) (segid A and residue 213 and name HA)  3.10 1.40 1.40
assign (segid A and residue 213 and name HB#) (segid A and residue 213 and name HG#) 3.10 1.40 1.40
assign (segid A and residue 213 and name HB#) (segid A and residue 213 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 213 and name HB#) (segid A and residue 213 and name HN)  3.10 1.40 1.40
assign (segid A and residue 213 and name HB#) (segid A and residue 214 and name HN)  3.10 1.40 1.40
assign (segid A and residue 213 and name HD#) (segid A and residue 213 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 213 and name HD#) (segid A and residue 213 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 213 and name HG#) (segid A and residue 213 and name HA)  3.10 1.40 1.40
assign (segid A and residue 213 and name HG#) (segid A and residue 213 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 213 and name HG#) (segid A and residue 213 and name HN)  3.10 1.40 1.40
assign (segid A and residue 214 and name HB#) (segid A and residue 214 and name HN)  3.10 1.40 1.40
assign (segid A and residue 215 and name HA) (segid A and residue 215 and name HN)  3.35 1.65 1.65
assign (segid A and residue 215 and name HD1#) (segid A and residue 215 and name HG)  3.60 1.90 1.90
```

```
assign (segid A and residue 215 and name HD1#) (segid A and residue 215 and name HN)  3.60 1.90 1.90
assign (segid A and residue 215 and name HD2#) (segid A and residue 215 and name HG)  3.60 1.90 1.90
assign (segid A and residue 215 and name HD2#) (segid A and residue 215 and name HA)  3.60 1.90 1.90
assign (segid A and residue 215 and name HD2#) (segid A and residue 215 and name HN)  3.60 1.90 1.90
assign (segid A and residue 216 and name HA) (segid A and residue 216 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 216 and name HB#) (segid A and residue 216 and name HA)  3.10 1.40 1.40
assign (segid A and residue 216 and name HB#) (segid A and residue 216 and name HN)  3.35 1.65 1.65
assign (segid A and residue 217 and name HA) (segid A and residue 217 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 217 and name HB#) (segid A and residue 217 and name HA) 3.10 1.40 1.40
assign (segid A and residue 217 and name HB#) (segid A and residue 217 and name HN)  3.35 1.65 1.65
assign (segid A and residue 217 and name HB#) (segid A and residue 218 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 217 and name HB#) (segid A and residue 218 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 217 and name HB#) (segid A and residue 218 and name HN)  3.35 1.65 1.65
assign (segid A and residue 218 and name HA) (segid A and residue 218 and name HD2#)  3.10 1.40 1.40
assign (segid A and residue 218 and name HB#) (segid A and residue 218 and name HN)  3.10 1.40 1.40
assign (segid A and residue 218 and name HB#) (segid A and residue 219 and name HN)  3.10 1.40 1.40
assign (segid A and residue 219 and name HA) (segid A and residue 219 and name HD2#) 3.35 1.65 1.65
assign (segid A and residue 219 and name HB#) (segid A and residue 219 and name HA)  3.35 1.65 1.65
assign (segid A and residue 219 and name HB#) (segid A and residue 219 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 219 and name HB#) (segid A and residue 219 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 219 and name HB#) (segid A and residue 219 and name HG)  3.35 1.65 1.65
assign (segid A and residue 219 and name HB#) (segid A and residue 219 and name HN)  3.10 1.40 1.40
assign (segid A and residue 219 and name HB#) (segid A and residue 220 and name HN)  3.10 1.40 1.40
assign (segid A and residue 219 and name HD1#) (segid A and residue 219 and name HA) 3.35 1.65 1.65
assign (segid A and residue 219 and name HD1#) (segid A and residue 219 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 219 and name HD1#) (segid A and residue 219 and name HG)  3.10 1.40 1.40
assign (segid A and residue 219 and name HD1#) (segid A and residue 219 and name HN)  3.35 1.65 1.65
assign (segid A and residue 219 and name HD2#) (segid A and residue 219 and name HA)  3.10 1.40 1.40
assign (segid A and residue 219 and name HD2#) (segid A and residue 219 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 219 and name HD2#) (segid A and residue 219 and name HG)  3.35 1.65 1.65
assign (segid A and residue 219 and name HD2#) (segid A and residue 219 and name HN)  3.35 1.65 1.65
assign (segid A and residue 219 and name HG) (segid A and residue 219 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 219 and name HG) (segid A and residue 219 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 219 and name HG) (segid A and residue 219 and name HN)  3.10 1.40 1.40
assign (segid A and residue 220 and name HA) (segid A and residue 220 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 217 and name HN) (segid A and residue 220 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 220 and name HB#) (segid A and residue 220 and name HA)  3.35 1.65 1.65
assign (segid A and residue 220 and name HB#) (segid A and residue 220 and name HN)  3.35 1.65 1.65
assign (segid A and residue 220 and name HB#) (segid A and residue 221 and name HA)  3.35 1.65 1.65
assign (segid A and residue 220 and name HB#) (segid A and residue 221 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 221 and name HA) (segid A and residue 221 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 221 and name HB#) (segid A and residue 221 and name HA)  3.10 1.40 1.40
assign (segid A and residue 221 and name HB#) (segid A and residue 221 and name HN)  3.10 1.40 1.40
assign (segid A and residue 222 and name HA) (segid A and residue 222 and name HD1#)  3.35 1.65 1.65
```

```
assign (segid A and residue 222 and name HA) (segid A and residue 222 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 222 and name HA) (segid A and residue 223 and name HN)  2.85 1.15 1.15
assign (segid A and residue 222 and name HA) (segid A and residue 222 and name HN)  3.10 1.40 1.40
assign (segid A and residue 222 and name HB#) (segid A and residue 222 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 222 and name HB#) (segid A and residue 222 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 222 and name HB#) (segid A and residue 222 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 222 and name HB#) (segid A and residue 222 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 222 and name HB#) (segid A and residue 222 and name HN)  3.10 1.40 1.40
assign (segid A and residue 219 and name HA) (segid A and residue 222 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 222 and name HD1#) (segid A and residue 222 and name HA)  3.35 1.65 1.65
assign (segid A and residue 222 and name HD1#) (segid A and residue 222 and name HD2#)  3.10 1.40 1.40
assign (segid A and residue 222 and name HD1#) (segid A and residue 222 and name HG)  3.10 1.40 1.40
assign (segid A and residue 222 and name HD1#) (segid A and residue 222 and name HN)  3.35 1.65 1.65
assign (segid A and residue 222 and name HD1#) (segid A and residue 223 and name HN)  3.60 1.90 1.90
assign (segid A and residue 222 and name HD1#) (segid A and residue 230 and name HB#)  3.60 1.90 1.90
assign (segid A and residue 222 and name HD1#) (segid A and residue 230 and name HB#)  3.60 1.90 1.90
assign (segid A and residue 219 and name HA) (segid A and residue 222 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 222 and name HD2#) (segid A and residue 222 and name HA)  3.35 1.65 1.65
assign (segid A and residue 222 and name HD2#) (segid A and residue 222 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 222 and name HD2#) (segid A and residue 222 and name HG)  3.10 1.40 1.40
assign (segid A and residue 222 and name HD2#) (segid A and residue 222 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 222 and name HD2#) (segid A and residue 222 and name HN)  3.35 1.65 1.65
assign (segid A and residue 222 and name HD2#) (segid A and residue 223 and name HN)  3.35 1.65 1.65
assign (segid A and residue 222 and name HD2#) (segid A and residue 230 and name HB#)  3.60 1.90 1.90
assign (segid A and residue 222 and name HD2#) (segid A and residue 230 and name HB#)  3.60 1.90 1.90
assign (segid A and residue 222 and name HG) (segid A and residue 222 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 222 and name HG) (segid A and residue 222 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 222 and name HG) (segid A and residue 222 and name HN)  3.10 1.40 1.40
assign (segid A and residue 223 and name HA) (segid A and residue 223 and name HN)  3.10 1.40 1.40
assign (segid A and residue 223 and name HA) (segid A and residue 224 and name HN)  3.35 1.65 1.65
assign (segid A and residue 223 and name HB#) (segid A and residue 223 and name HG#)  2.85 1.15 1.15
assign (segid A and residue 223 and name HB#) (segid A and residue 223 and name HN)  3.10 1.40 1.40
assign (segid A and residue 223 and name HG#) (segid A and residue 223 and name HA)  2.85 1.15 1.15
assign (segid A and residue 223 and name HG#) (segid A and residue 223 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 224 and name HA) (segid A and residue 224 and name HN)  3.10 1.40 1.40
assign (segid A and residue 226 and name HA) (segid A and residue 227 and name HN)  2.85 1.15 1.15
assign (segid A and residue 226 and name HB#) (segid A and residue 227 and name HN)  3.10 1.40 1.40
assign (segid A and residue 226 and name HB#) (segid A and residue 226 and name HN)  3.10 1.40 1.40
assign (segid A and residue 226 and name HD1#) (segid A and residue 226 and name HG)  3.60 1.90 1.90
assign (segid A and residue 226 and name HD1#) (segid A and residue 226 and name HN)  3.60 1.90 1.90
assign (segid A and residue 226 and name HD1#) (segid A and residue 227 and name HN)  3.60 1.90 1.90
assign (segid A and residue 226 and name HD1#) (segid A and residue 226 and name HA)  3.60 1.90 1.90
assign (segid A and residue 226 and name HD2#) (segid A and residue 226 and name HA)  3.60 1.90 1.90
assign (segid A and residue 226 and name HD2#) (segid A and residue 226 and name HN)  3.60 1.90 1.90
```

```
assign (segid A and residue 226 and name HD2#) (segid A and residue 227 and name HN)  3.60 1.90 1.90
assign (segid A and residue 227 and name HA) (segid A and residue 227 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 227 and name HA) (segid A and residue 227 and name HD1#)  3.60 1.90 1.90
assign (segid A and residue 227 and name HA) (segid A and residue 227 and name HN)  3.35 1.65 1.65
assign (segid A and residue 227 and name HA) (segid A and residue 228 and name HD#)  3.35 1.65 1.65
assign (segid A and residue 227 and name HB#) (segid A and residue 227 and name HA)  3.35 1.65 1.65
assign (segid A and residue 227 and name HB#) (segid A and residue 227 and name HD1#)  3.60 1.90 1.90
assign (segid A and residue 227 and name HD1#) (segid A and residue 227 and name HN)  3.60 1.90 1.90
assign (segid A and residue 227 and name HD1#) (segid A and residue 227 and name HA)  3.60 1.90 1.90
assign (segid A and residue 227 and name HD1#) (segid A and residue 227 and name HG)  3.60 1.90 1.90
assign (segid A and residue 227 and name HD2#) (segid A and residue 227 and name HA)  3.60 1.90 1.90
assign (segid A and residue 227 and name HD2#) (segid A and residue 227 and name HN)  3.60 1.90 1.90
assign (segid A and residue 227 and name HG) (segid A and residue 227 and name HA)  3.35 1.65 1.65
assign (segid A and residue 227 and name HG) (segid A and residue 227 and name HD1#)  3.60 1.90 1.90
assign (segid A and residue 227 and name HG) (segid A and residue 227 and name HN)  3.35 1.65 1.65
assign (segid A and residue 228 and name HA) (segid A and residue 228 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 228 and name HA) (segid A and residue 228 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 228 and name HA) (segid A and residue 228 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 228 and name HA) (segid A and residue 228 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 228 and name HA) (segid A and residue 228 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 228 and name HA) (segid A and residue 231 and name HN)  3.10 1.40 1.40
assign (segid A and residue 228 and name HB#) (segid A and residue 228 and name HA)  2.85 1.15 1.15
assign (segid A and residue 228 and name HB#) (segid A and residue 228 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 228 and name HB#) (segid A and residue 228 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 228 and name HB#) (segid A and residue 229 and name HN)  3.10 1.40 1.40
assign (segid A and residue 229 and name HA) (segid A and residue 229 and name HB#) 2.85 1.15 1.15
assign (segid A and residue 229 and name HB#) (segid A and residue 229 and name HN)  3.10 1.40 1.40
assign (segid A and residue 229 and name HB#) (segid A and residue 230 and name HN)  3.10 1.40 1.40
assign (segid A and residue 229 and name HB#) (segid A and residue 229 and name HA)  2.85 1.15 1.15
assign (segid A and residue 229 and name HG#) (segid A and residue 229 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 229 and name HG#) (segid A and residue 229 and name HN)  3.10 1.40 1.40
assign (segid A and residue 229 and name HG#) (segid A and residue 230 and name HN)  3.10 1.40 1.40
assign (segid A and residue 229 and name HG#) (segid A and residue 229 and name HA)  2.85 1.15 1.15
assign (segid A and residue 230 and name HA) (segid A and residue 230 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 229 and name HN) (segid A and residue 230 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 230 and name HB#) (segid A and residue 230 and name HA)  3.35 1.65 1.65
assign (segid A and residue 230 and name HB#) (segid A and residue 230 and name HN)  3.35 1.65 1.65
assign (segid A and residue 230 and name HB#) (segid A and residue 231 and name HB#)  3.60 1.90 1.90
assign (segid A and residue 231 and name HA) (segid A and residue 231 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 231 and name HB#) (segid A and residue 231 and name HA)  3.10 1.40 1.40
assign (segid A and residue 231 and name HB#) (segid A and residue 231 and name HA)  3.10 1.40 1.40
assign (segid A and residue 231 and name HB#) (segid A and residue 231 and name HN)  3.10 1.40 1.40
assign (segid A and residue 231 and name HB#) (segid A and residue 232 and name HN)  3.10 1.40 1.40
assign (segid A and residue 232 and name HB#) (segid A and residue 232 and name HN)  2.85 1.15 1.15
```

```
assign (segid A and residue 232 and name HB#) (segid A and residue 233 and name HN)  3.10 1.40 1.40
assign (segid A and residue 233 and name HA) (segid A and residue 233 and name HD1#)  3.10 1.40 1.40
assign (segid A and residue 233 and name HB#) (segid A and residue 233 and name HA)  3.10 1.40 1.40
assign (segid A and residue 233 and name HB#) (segid A and residue 233 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 233 and name HB#) (segid A and residue 233 and name HN)  3.10 1.40 1.40
assign (segid A and residue 233 and name HB#) (segid A and residue 234 and name HN)  3.10 1.40 1.40
assign (segid A and residue 233 and name HD1#) (segid A and residue 233 and name HA)  3.35 1.65 1.65
assign (segid A and residue 233 and name HD1#) (segid A and residue 233 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 233 and name HD1#) (segid A and residue 233 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 233 and name HD1#) (segid A and residue 233 and name HG)  3.35 1.65 1.65
assign (segid A and residue 233 and name HG) (segid A and residue 233 and name HA)  3.10 1.40 1.40
assign (segid A and residue 233 and name HG) (segid A and residue 233 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 233 and name HG) (segid A and residue 233 and name HD1#)  3.10 1.40 1.40
assign (segid A and residue 233 and name HG) (segid A and residue 233 and name HN)  3.10 1.40 1.40
assign (segid A and residue 233 and name HG) (segid A and residue 234 and name HN)  3.10 1.40 1.40
assign (segid A and residue 234 and name HA) (segid A and residue 234 and name HD1#) 3.35 1.65 1.65
assign (segid A and residue 234 and name HA) (segid A and residue 234 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 234 and name HA) (segid A and residue 237 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 234 and name HB) (segid A and residue 234 and name HA)  3.10 1.40 1.40
assign (segid A and residue 234 and name HB) (segid A and residue 234 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 234 and name HB) (segid A and residue 234 and name HG1#)  3.10 1.40 1.40
assign (segid A and residue 234 and name HB) (segid A and residue 234 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 234 and name HD1#) (segid A and residue 270 and name HA)  3.60 1.90 1.90
assign (segid A and residue 234 and name HD1#) (segid A and residue 270 and name HB#)  3.60 1.90 1.90
assign (segid A and residue 233 and name HN) (segid A and residue 234 and name HG2#)  3.60 1.90 1.90
assign (segid A and residue 234 and name HG2#) (segid A and residue 234 and name HA)  3.60 1.90 1.90
assign (segid A and residue 234 and name HG2#) (segid A and residue 234 and name HB)  3.35 1.65 1.65
assign (segid A and residue 234 and name HG2#) (segid A and residue 234 and name HG1#) 3.35 1.65 1.65
assign (segid A and residue 234 and name HG2#) (segid A and residue 234 and name HN)  3.35 1.65 1.65
assign (segid A and residue 234 and name HG2#) (segid A and residue 235 and name HN)  3.35 1.65 1.65
assign (segid A and residue 236 and name HA) (segid A and residue 236 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 236 and name HA) (segid A and residue 236 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 236 and name HB#) (segid A and residue 236 and name HG#)  2.85 1.15 1.15
assign (segid A and residue 236 and name HB#) (segid A and residue 236 and name HN)  2.85 1.15 1.15
assign (segid A and residue 236 and name HB#) (segid A and residue 237 and name HN)  2.85 1.15 1.15
assign (segid A and residue 236 and name HG#) (segid A and residue 236 and name HN)  3.10 1.40 1.40
assign (segid A and residue 236 and name HG#) (segid A and residue 237 and name HN)  3.10 1.40 1.40
assign (segid A and residue 237 and name HA) (segid A and residue 237 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 237 and name HA) (segid A and residue 237 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 237 and name HA) (segid A and residue 237 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 237 and name HB#) (segid A and residue 237 and name HA)  3.10 1.40 1.40
assign (segid A and residue 237 and name HB#) (segid A and residue 237 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 237 and name HB#) (segid A and residue 237 and name HN)  3.10 1.40 1.40
assign (segid A and residue 237 and name HB#) (segid A and residue 238 and name HN)  3.10 1.40 1.40
```

```
assign (segid A and residue 237 and name HB#) (segid A and residue 238 and name HN)  3.10 1.40 1.40
assign (segid A and residue 237 and name HD1#) (segid A and residue 237 and name HA)  3.35 1.65 1.65
assign (segid A and residue 237 and name HD1#) (segid A and residue 237 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 237 and name HD1#) (segid A and residue 237 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 237 and name HD1#) (segid A and residue 237 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 237 and name HD1#) (segid A and residue 237 and name HG)  3.10 1.40 1.40
assign (segid A and residue 237 and name HD1#) (segid A and residue 237 and name HN)  3.35 1.65 1.65
assign (segid A and residue 237 and name HD1#) (segid A and residue 238 and name HN)  3.35 1.65 1.65
assign (segid A and residue 237 and name HD2#) (segid A and residue 237 and name HA)  3.35 1.65 1.65
assign (segid A and residue 237 and name HD2#) (segid A and residue 237 and name HG)  3.10 1.40 1.40
assign (segid A and residue 237 and name HD2#) (segid A and residue 237 and name HN)  3.35 1.65 1.65
assign (segid A and residue 237 and name HG) (segid A and residue 237 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 237 and name HG) (segid A and residue 237 and name HN)  3.10 1.40 1.40
assign (segid A and residue 238 and name HB#) (segid A and residue 238 and name HA)  3.35 1.65 1.65
assign (segid A and residue 238 and name HB#) (segid A and residue 266 and name HG1#)  3.60 1.90 1.90
assign (segid A and residue 238 and name HB#) (segid A and residue 266 and name HG1#)  3.60 1.90 1.90
assign (segid A and residue 238 and name HB#) (segid A and residue 266 and name HG2#)  3.60 1.90 1.90
assign (segid A and residue 241 and name HA) (segid A and residue 241 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 241 and name HA) (segid A and residue 244 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 241 and name HA) (segid A and residue 245 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 241 and name HB#) (segid A and residue 241 and name HN)  3.35 1.65 1.65
assign (segid A and residue 241 and name HB#) (segid A and residue 245 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 241 and name HB#) (segid A and residue 242 and name HN)  3.35 1.65 1.65
assign (segid A and residue 243 and name HB#) (segid A and residue 243 and name HA)  3.10 1.40 1.40
assign (segid A and residue 243 and name HB#) (segid A and residue 243 and name HG#)  2.85 1.15 1.15
assign (segid A and residue 243 and name HB#) (segid A and residue 243 and name HN)  3.10 1.40 1.40
assign (segid A and residue 243 and name HB#) (segid A and residue 244 and name HN)  3.10 1.40 1.40
assign (segid A and residue 243 and name HG#) (segid A and residue 243 and name HA)  2.85 1.15 1.15
assign (segid A and residue 243 and name HG#) (segid A and residue 243 and name HN)  3.10 1.40 1.40
assign (segid A and residue 243 and name HG#) (segid A and residue 244 and name HN)  3.10 1.40 1.40
assign (segid A and residue 243 and name HG#) (segid A and residue 247 and name HD1#) 3.60 1.90 1.90
assign (segid A and residue 244 and name HA) (segid A and residue 244 and name HB) 3.10 1.40 1.40
assign (segid A and residue 244 and name HA) (segid A and residue 244 and name HG#)  3.35 1.65 1.65
assign (segid A and residue 244 and name HB) (segid A and residue 244 and name HA)  3.10 1.40 1.40
assign (segid A and residue 244 and name HB) (segid A and residue 244 and name HN)  3.10 1.40 1.40
assign (segid A and residue 244 and name HB) (segid A and residue 244 and name HG2#)  3.10 1.40 1.40
assign (segid A and residue 244 and name HB) (segid A and residue 245 and name HN)  3.10 1.40 1.40
assign (segid A and residue 207 and name HA) (segid A and residue 244 and name HG#)  3.35 1.65 1.65
assign (segid A and residue 207 and name HB#) (segid A and residue 244 and name HG#)  3.35 1.65 1.65
assign (segid A and residue 244 and name HG#) (segid A and residue 244 and name HA)  3.35 1.65 1.65
assign (segid A and residue 244 and name HG#) (segid A and residue 245 and name HA)  3.35 1.65 1.65
assign (segid A and residue 244 and name HG#) (segid A and residue 244 and name HB)  3.35 1.65 1.65
assign (segid A and residue 244 and name HG#) (segid A and residue 244 and name HN)  3.35 1.65 1.65
assign (segid A and residue 244 and name HG#) (segid A and residue 245 and name HN)  3.10 1.40 1.40
```

```
assign (segid A and residue 244 and name HG#) (segid A and residue 247 and name HN)  3.35 1.65 1.65
assign (segid A and residue 245 and name HA) (segid A and residue 245 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 245 and name HA) (segid A and residue 245 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 245 and name HB#) (segid A and residue 245 and name HN)  3.10 1.40 1.40
assign (segid A and residue 245 and name HB#) (segid A and residue 246 and name HN)  3.10 1.40 1.40
assign (segid A and residue 246 and name HA) (segid A and residue 246 and name HB#) 2.85 1.15 1.15
assign (segid A and residue 246 and name HA) (segid A and residue 246 and name HD2#) 3.10 1.40 1.40
assign (segid A and residue 246 and name HA) (segid A and residue 246 and name HG)  3.10 1.40 1.40
assign (segid A and residue 246 and name HB#) (segid A and residue 246 and name HA)  3.10 1.40 1.40
assign (segid A and residue 246 and name HB#) (segid A and residue 246 and name HG)  3.10 1.40 1.40
assign (segid A and residue 246 and name HB#) (segid A and residue 246 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 246 and name HB#) (segid A and residue 246 and name HN)  2.85 1.15 1.15
assign (segid A and residue 246 and name HB#) (segid A and residue 247 and name HD1#) 3.10 1.40 1.40
assign (segid A and residue 246 and name HB#) (segid A and residue 246 and name HD1#)  3.10 1.40 1.40
assign (segid A and residue 246 and name HD1#) (segid A and residue 246 and name HA)  3.35 1.65 1.65
assign (segid A and residue 246 and name HD1#) (segid A and residue 246 and name HG)  3.10 1.40 1.40
assign (segid A and residue 246 and name HD1#) (segid A and residue 246 and name HN)  3.35 1.65 1.65
assign (segid A and residue 246 and name HD2#) (segid A and residue 246 and name HA)  3.10 1.40 1.40
assign (segid A and residue 246 and name HD2#) (segid A and residue 246 and name HG)  3.35 1.65 1.65
assign (segid A and residue 246 and name HD2#) (segid A and residue 246 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 246 and name HD2#) (segid A and residue 246 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 246 and name HD2#) (segid A and residue 246 and name HN)  3.35 1.65 1.65
assign (segid A and residue 246 and name HD2#) (segid A and residue 247 and name HN)  3.35 1.65 1.65
assign (segid A and residue 246 and name HG) (segid A and residue 246 and name HA)  3.10 1.40 1.40
assign (segid A and residue 246 and name HG) (segid A and residue 246 and name HN)  3.10 1.40 1.40
assign (segid A and residue 247 and name HB#) (segid A and residue 247 and name HA) 3.10 1.40 1.40
assign (segid A and residue 247 and name HB#) (segid A and residue 247 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 247 and name HB#) (segid A and residue 247 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 247 and name HB#) (segid A and residue 247 and name HN)  3.10 1.40 1.40
assign (segid A and residue 247 and name HB#) (segid A and residue 248 and name HN)  3.10 1.40 1.40
assign (segid A and residue 247 and name HD1#) (segid A and residue 247 and name HN)  3.35 1.65 1.65
assign (segid A and residue 247 and name HD1#) (segid A and residue 247 and name HA)  3.35 1.65 1.65
assign (segid A and residue 247 and name HD2#) (segid A and residue 247 and name HA)  3.10 1.40 1.40
assign (segid A and residue 247 and name HD2#) (segid A and residue 247 and name HG)  3.10 1.40 1.40
assign (segid A and residue 247 and name HD2#) (segid A and residue 247 and name HN)  3.35 1.65 1.65
assign (segid A and residue 247 and name HD2#) (segid A and residue 248 and name HN)  3.35 1.65 1.65
assign (segid A and residue 247 and name HG) (segid A and residue 247 and name HA)  3.35 1.65 1.65
assign (segid A and residue 247 and name HG) (segid A and residue 247 and name HN)  3.35 1.65 1.65
assign (segid A and residue 248 and name HA) (segid A and residue 248 and name HB)  3.10 1.40 1.40
assign (segid A and residue 248 and name HA) (segid A and residue 248 and name HG1#) 3.35 1.65 1.65
assign (segid A and residue 248 and name HA) (segid A and residue 248 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 248 and name HA) (segid A and residue 248 and name HN)  3.10 1.40 1.40
assign (segid A and residue 248 and name HA) (segid A and residue 249 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 248 and name HA) (segid A and residue 249 and name HN)  3.10 1.40 1.40
```

```
assign (segid A and residue 248 and name HB) (segid A and residue 248 and name HA)  3.10 1.40 1.40
assign (segid A and residue 248 and name HB) (segid A and residue 248 and name HG1#)  3.10 1.40 1.40
assign (segid A and residue 248 and name HB) (segid A and residue 248 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 248 and name HB) (segid A and residue 248 and name HN)  3.10 1.40 1.40
assign (segid A and residue 248 and name HB) (segid A and residue 249 and name HN)  3.10 1.40 1.40
assign (segid A and residue 248 and name HG1#) (segid A and residue 248 and name HB) 3.10 1.40 1.40
assign (segid A and residue 248 and name HG1#) (segid A and residue 248 and name HN)  3.35 1.65 1.65
assign (segid A and residue 248 and name HG1#) (segid A and residue 249 and name HN)  3.35 1.65 1.65
assign (segid A and residue 248 and name HG2#) (segid A and residue 248 and name HA)  3.35 1.65 1.65
assign (segid A and residue 248 and name HG2#) (segid A and residue 248 and name HN)  3.35 1.65 1.65
assign (segid A and residue 248 and name HG2#) (segid A and residue 249 and name HN)  3.35 1.65 1.65
assign (segid A and residue 249 and name HA) (segid A and residue 249 and name HN)  3.10 1.40 1.40
assign (segid A and residue 249 and name HA) (segid A and residue 250 and name HN)  3.10 1.40 1.40
assign (segid A and residue 249 and name HB#) (segid A and residue 250 and name HN)  3.10 1.40 1.40
assign (segid A and residue 249 and name HB#) (segid A and residue 250 and name HA)  3.35 1.65 1.65
assign (segid A and residue 250 and name HA) (segid A and residue 250 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 250 and name HA) (segid A and residue 250 and name HN)  3.10 1.40 1.40
assign (segid A and residue 250 and name HA) (segid A and residue 251 and name HN)  2.85 1.15 1.15
assign (segid A and residue 250 and name HB#) (segid A and residue 250 and name HA)  2.85 1.15 1.15
assign (segid A and residue 250 and name HB#) (segid A and residue 250 and name HN)  3.10 1.40 1.40
assign (segid A and residue 250 and name HB#) (segid A and residue 251 and name HN)  2.85 1.15 1.15
assign (segid A and residue 250 and name HB#) (segid A and residue 251 and name HN)  2.85 1.15 1.15
assign (segid A and residue 250 and name HB#) (segid A and residue 251 and name HN)  2.85 1.15 1.15
assign (segid A and residue 250 and name HB#) (segid A and residue 253 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 251 and name HA) (segid A and residue 251 and name HG#) 3.10 1.40 1.40
assign (segid A and residue 251 and name HB#) (segid A and residue 251 and name HE#)  3.10 1.40 1.40
assign (segid A and residue 251 and name HB#) (segid A and residue 251 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 251 and name HB#) (segid A and residue 251 and name HN)  3.10 1.40 1.40
assign (segid A and residue 251 and name HB#) (segid A and residue 252 and name HN)  3.10 1.40 1.40
assign (segid A and residue 251 and name HD#) (segid A and residue 251 and name HE#) 3.10 1.40 1.40
assign (segid A and residue 251 and name HD#) (segid A and residue 251 and name HG#) 3.10 1.40 1.40
assign (segid A and residue 251 and name HE#) (segid A and residue 251 and name HA) 3.10 1.40 1.40
assign (segid A and residue 251 and name HE#) (segid A and residue 251 and name HD#) 3.10 1.40 1.40
assign (segid A and residue 251 and name HG#) (segid A and residue 251 and name HN)  3.10 1.40 1.40
assign (segid A and residue 252 and name HA) (segid A and residue 252 and name HN)  3.10 1.40 1.40
assign (segid A and residue 252 and name HB#) (segid A and residue 252 and name HA) 3.10 1.40 1.40
assign (segid A and residue 253 and name HA) (segid A and residue 253 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 253 and name HA) (segid A and residue 253 and name HN)  3.10 1.40 1.40
assign (segid A and residue 253 and name HA) (segid A and residue 255 and name HN)  3.10 1.40 1.40
assign (segid A and residue 253 and name HB#) (segid A and residue 253 and name HA)  3.10 1.40 1.40
assign (segid A and residue 253 and name HB#) (segid A and residue 253 and name HN)  3.10 1.40 1.40
assign (segid A and residue 256 and name HA) (segid A and residue 256 and name HB)  3.10 1.40 1.40
assign (segid A and residue 256 and name HA) (segid A and residue 256 and name HG1#) 3.35 1.65 1.65
assign (segid A and residue 256 and name HA) (segid A and residue 256 and name HG2#)  3.35 1.65 1.65
```

```
assign (segid A and residue 256 and name HB) (segid A and residue 256 and name HG1#)  3.35 1.65 1.65
assign (segid A and residue 256 and name HB) (segid A and residue 256 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 256 and name HB) (segid A and residue 256 and name HN)  3.10 1.40 1.40
assign (segid A and residue 255 and name HN) (segid A and residue 256 and name HG1#)  3.35 1.65 1.65
assign (segid A and residue 256 and name HG1#) (segid A and residue 256 and name HA)  3.35 1.65 1.65
assign (segid A and residue 256 and name HG1#) (segid A and residue 256 and name HB)  3.35 1.65 1.65
assign (segid A and residue 256 and name HG1#) (segid A and residue 256 and name HN)  3.35 1.65 1.65
assign (segid A and residue 256 and name HG2#) (segid A and residue 256 and name HA) 3.35 1.65 1.65
assign (segid A and residue 256 and name HG2#) (segid A and residue 256 and name HB)  3.35 1.65 1.65
assign (segid A and residue 256 and name HG2#) (segid A and residue 256 and name HG1#) 3.60 1.90 1.90
assign (segid A and residue 256 and name HG2#) (segid A and residue 256 and name HN)  3.35 1.65 1.65
assign (segid A and residue 257 and name HA) (segid A and residue 257 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 257 and name HA) (segid A and residue 257 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 257 and name HA) (segid A and residue 257 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 257 and name HA) (segid A and residue 257 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 257 and name HB#) (segid A and residue 258 and name HN)  3.10 1.40 1.40
assign (segid A and residue 257 and name HB#) (segid A and residue 258 and name HN)  3.10 1.40 1.40
assign (segid A and residue 257 and name HG#) (segid A and residue 258 and name HN)  3.10 1.40 1.40
assign (segid A and residue 257 and name HG#) (segid A and residue 258 and name HN)  3.10 1.40 1.40
assign (segid A and residue 258 and name HA) (segid A and residue 258 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 258 and name HB#) (segid A and residue 258 and name HA)  3.35 1.65 1.65
assign (segid A and residue 258 and name HB#) (segid A and residue 258 and name HN)  3.35 1.65 1.65
assign (segid A and residue 258 and name HB#) (segid A and residue 259 and name HN)  3.35 1.65 1.65
assign (segid A and residue 259 and name HA) (segid A and residue 259 and name HN)  3.35 1.65 1.65
assign (segid A and residue 259 and name HA) (segid A and residue 260 and name HN)  3.35 1.65 1.65
assign (segid A and residue 259 and name HD2#) (segid A and residue 259 and name HA)  3.60 1.90 1.90
assign (segid A and residue 259 and name HD2#) (segid A and residue 259 and name HN)  3.60 1.90 1.90
assign (segid A and residue 259 and name HD2#) (segid A and residue 260 and name HA)  3.60 1.90 1.90
assign (segid A and residue 262 and name HA) (segid A and residue 262 and name HD2#)  3.60 1.90 1.90
assign (segid A and residue 262 and name HD1#) (segid A and residue 262 and name HN) 3.60 1.90 1.90
assign (segid A and residue 262 and name HD2#) (segid A and residue 265 and name HD1#) 3.85 2.15 2.15
assign (segid A and residue 262 and name HD2#) (segid A and residue 265 and name HD2#) 3.85 2.15 2.15
assign (segid A and residue 263 and name HA) (segid A and residue 263 and name HG1#)  3.10 1.40 1.40
assign (segid A and residue 263 and name HA) (segid A and residue 266 and name HN)  3.10 1.40 1.40
assign (segid A and residue 263 and name HB) (segid A and residue 263 and name HA)  3.10 1.40 1.40
assign (segid A and residue 263 and name HB) (segid A and residue 263 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 263 and name HB) (segid A and residue 263 and name HG1#)  3.35 1.65 1.65
assign (segid A and residue 263 and name HB) (segid A and residue 263 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 263 and name HD1#) (segid A and residue 263 and name HB)  3.35 1.65 1.65
assign (segid A and residue 263 and name HD1#) (segid A and residue 263 and name HG1#) 3.35 1.65 1.65
assign (segid A and residue 263 and name HD1#) (segid A and residue 263 and name HG2#)  3.60 1.90 1.90
assign (segid A and residue 263 and name HD1#) (segid A and residue 263 and name HN)  3.35 1.65 1.65
assign (segid A and residue 263 and name HD1#) (segid A and residue 266 and name HB)  3.35 1.65 1.65
assign (segid A and residue 263 and name HG1#) (segid A and residue 263 and name HD1#) 3.35 1.65 1.65
```

```
assign (segid A and residue 263 and name HG1#) (segid A and residue 263 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 263 and name HG1#) (segid A and residue 263 and name HN)  3.10 1.40 1.40
assign (segid A and residue 263 and name HG2#) (segid A and residue 263 and name HA)  3.35 1.65 1.65
assign (segid A and residue 263 and name HG2#) (segid A and residue 263 and name HB)  3.35 1.65 1.65
assign (segid A and residue 263 and name HG2#) (segid A and residue 263 and name HD1#) 3.60 1.90 1.90
assign (segid A and residue 263 and name HG2#) (segid A and residue 264 and name HN)  3.35 1.65 1.65
assign (segid A and residue 263 and name HG2#) (segid A and residue 263 and name HN)  3.35 1.65 1.65
assign (segid A and residue 264 and name HA) (segid A and residue 264 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 261 and name HA) (segid A and residue 264 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 264 and name HB#) (segid A and residue 264 and name HA)  3.10 1.40 1.40
assign (segid A and residue 264 and name HB#) (segid A and residue 264 and name HA)  3.10 1.40 1.40
assign (segid A and residue 264 and name HB#) (segid A and residue 264 and name HN)  3.10 1.40 1.40
assign (segid A and residue 264 and name HB#) (segid A and residue 265 and name HN)  3.10 1.40 1.40
assign (segid A and residue 264 and name HB#) (segid A and residue 265 and name HN)  3.10 1.40 1.40
assign (segid A and residue 265 and name HA) (segid A and residue 265 and name HD1#) 3.35 1.65 1.65
assign (segid A and residue 265 and name HA) (segid A and residue 265 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 265 and name HB#) (segid A and residue 265 and name HA) 3.10 1.40 1.40
assign (segid A and residue 265 and name HB#) (segid A and residue 265 and name HD1#) 3.60 1.90 1.90
assign (segid A and residue 265 and name HB#) (segid A and residue 265 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 265 and name HB#) (segid A and residue 265 and name HN)  3.10 1.40 1.40
assign (segid A and residue 265 and name HB#) (segid A and residue 266 and name HN)  3.10 1.40 1.40
assign (segid A and residue 265 and name HB#) (segid A and residue 265 and name HG)  3.10 1.40 1.40
assign (segid A and residue 265 and name HD1#) (segid A and residue 265 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 265 and name HD1#) (segid A and residue 265 and name HD2#)  3.60 1.90 1.90
assign (segid A and residue 265 and name HD1#) (segid A and residue 265 and name HG)  3.35 1.65 1.65
assign (segid A and residue 265 and name HD1#) (segid A and residue 265 and name HN)  3.35 1.65 1.65
assign (segid A and residue 265 and name HD1#) (segid A and residue 266 and name HG2#) 3.60 1.90 1.90
assign (segid A and residue 265 and name HD1#) (segid A and residue 266 and name HG2#) 3.60 1.90 1.90
assign (segid A and residue 265 and name HD1#) (segid A and residue 269 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 265 and name HD2#) (segid A and residue 265 and name HA)  3.35 1.65 1.65
assign (segid A and residue 265 and name HD2#) (segid A and residue 265 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 265 and name HD2#) (segid A and residue 265 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 265 and name HD2#) (segid A and residue 265 and name HD1#)  3.35 1.65 1.65
assign (segid A and residue 265 and name HD2#) (segid A and residue 265 and name HG)  3.35 1.65 1.65
assign (segid A and residue 265 and name HD2#) (segid A and residue 265 and name HN)  3.35 1.65 1.65
assign (segid A and residue 265 and name HG) (segid A and residue 265 and name HA)  3.35 1.65 1.65
assign (segid A and residue 265 and name HG) (segid A and residue 265 and name HD1#)  3.60 1.90 1.90
assign (segid A and residue 265 and name HG) (segid A and residue 265 and name HD2#)  3.60 1.90 1.90
assign (segid A and residue 266 and name HA) (segid A and residue 266 and name HB)  3.10 1.40 1.40
assign (segid A and residue 266 and name HA) (segid A and residue 266 and name HG1#) 3.35 1.65 1.65
assign (segid A and residue 266 and name HA) (segid A and residue 266 and name HG2#)  3.35 1.65 1.65
assign (segid A and residue 266 and name HA) (segid A and residue 270 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 266 and name HB) (segid A and residue 266 and name HN)  3.10 1.40 1.40
assign (segid A and residue 263 and name HA) (segid A and residue 266 and name HG1#)  3.35 1.65 1.65
```

```
assign (segid A and residue 266 and name HG1#) (segid A and residue 266 and name HG2#)  3.60 1.90 1.90
assign (segid A and residue 266 and name HG1#) (segid A and residue 266 and name HN)  3.35 1.65 1.65
assign (segid A and residue 266 and name HG1#) (segid A and residue 267 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 266 and name HG2#) (segid A and residue 266 and name HA)  3.35 1.65 1.65
assign (segid A and residue 266 and name HG2#) (segid A and residue 266 and name HG1#)  3.60 1.90 1.90
assign (segid A and residue 266 and name HG2#) (segid A and residue 267 and name HN)  3.35 1.65 1.65
assign (segid A and residue 267 and name HA) (segid A and residue 267 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 269 and name HA) (segid A and residue 269 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 269 and name HB#) (segid A and residue 269 and name HN)  3.10 1.40 1.40
assign (segid A and residue 270 and name HA) (segid A and residue 270 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 270 and name HA) (segid A and residue 270 and name HN)  3.10 1.40 1.40
assign (segid A and residue 270 and name HA) (segid A and residue 271 and name HN)  3.10 1.40 1.40
assign (segid A and residue 270 and name HB#) (segid A and residue 270 and name HN)  3.10 1.40 1.40
assign (segid A and residue 271 and name HA) (segid A and residue 271 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 271 and name HA) (segid A and residue 271 and name HN)  2.85 1.15 1.15
assign (segid A and residue 271 and name HB#) (segid A and residue 271 and name HN)  3.10 1.40 1.40
assign (segid A and residue 273 and name HA) (segid A and residue 273 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 273 and name HA) (segid A and residue 273 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 273 and name HA) (segid A and residue 273 and name HN)  2.85 1.15 1.15
assign (segid A and residue 273 and name HA) (segid A and residue 274 and name HN)  3.10 1.40 1.40
assign (segid A and residue 273 and name HB#) (segid A and residue 273 and name HA)  3.10 1.40 1.40
assign (segid A and residue 273 and name HB#) (segid A and residue 273 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 273 and name HB#) (segid A and residue 273 and name HG#)  2.85 1.15 1.15
assign (segid A and residue 273 and name HB#) (segid A and residue 273 and name HN)  2.85 1.15 1.15
assign (segid A and residue 273 and name HB#) (segid A and residue 274 and name HN)  3.10 1.40 1.40
assign (segid A and residue 273 and name HD#) (segid A and residue 273 and name HA)  3.10 1.40 1.40
assign (segid A and residue 273 and name HD#) (segid A and residue 273 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 273 and name HG#) (segid A and residue 273 and name HA)  3.10 1.40 1.40
assign (segid A and residue 273 and name HG#) (segid A and residue 273 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 273 and name HG#) (segid A and residue 273 and name HD#)  2.85 1.15 1.15
assign (segid A and residue 273 and name HG#) (segid A and residue 273 and name HN)  3.10 1.40 1.40
assign (segid A and residue 273 and name HG#) (segid A and residue 274 and name HN)  3.10 1.40 1.40
assign (segid A and residue 274 and name HA) (segid A and residue 274 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 274 and name HB#) (segid A and residue 274 and name HA)  3.10 1.40 1.40
assign (segid A and residue 274 and name HB#) (segid A and residue 274 and name HN)  3.35 1.65 1.65
assign (segid A and residue 274 and name HB#) (segid A and residue 275 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 274 and name HB#) (segid A and residue 275 and name HD2#)  3.60 1.90 1.90
assign (segid A and residue 275 and name HA) (segid A and residue 275 and name HN)  3.10 1.40 1.40
assign (segid A and residue 275 and name HA) (segid A and residue 276 and name HN)  3.10 1.40 1.40
assign (segid A and residue 275 and name HB#) (segid A and residue 275 and name HN)  3.10 1.40 1.40
assign (segid A and residue 276 and name HA) (segid A and residue 276 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 276 and name HA) (segid A and residue 276 and name HN)  3.10 1.40 1.40
assign (segid A and residue 276 and name HA) (segid A and residue 277 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 276 and name HA) (segid A and residue 277 and name HN)  3.35 1.65 1.65
```

```
assign (segid A and residue 276 and name HA) (segid A and residue 278 and name HN)  3.35 1.65 1.65
assign (segid A and residue 275 and name HN) (segid A and residue 276 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 276 and name HB#) (segid A and residue 276 and name HA)  3.10 1.40 1.40
assign (segid A and residue 276 and name HB#) (segid A and residue 276 and name HN)  3.10 1.40 1.40
assign (segid A and residue 276 and name HB#) (segid A and residue 277 and name HN)  3.10 1.40 1.40
assign (segid A and residue 277 and name HA) (segid A and residue 278 and name HN)  3.35 1.65 1.65
assign (segid A and residue 277 and name HB#) (segid A and residue 277 and name HN)  3.10 1.40 1.40
assign (segid A and residue 277 and name HB#) (segid A and residue 277 and name HA) 3.10 1.40 1.40
assign (segid A and residue 279 and name HA) (segid A and residue 279 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 279 and name HA) (segid A and residue 279 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 279 and name HA) (segid A and residue 279 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 279 and name HA) (segid A and residue 280 and name HN)  2.85 1.15 1.15
assign (segid A and residue 279 and name HB#) (segid A and residue 279 and name HA)  3.10 1.40 1.40
assign (segid A and residue 279 and name HB#) (segid A and residue 279 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 279 and name HB#) (segid A and residue 280 and name HN)  3.10 1.40 1.40
assign (segid A and residue 279 and name HD#) (segid A and residue 280 and name HN)  3.10 1.40 1.40
assign (segid A and residue 279 and name HD#) (segid A and residue 279 and name HA)  3.10 1.40 1.40
assign (segid A and residue 279 and name HD#) (segid A and residue 279 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 279 and name HD#) (segid A and residue 279 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 279 and name HG#) (segid A and residue 279 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 279 and name HG#) (segid A and residue 279 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 279 and name HG#) (segid A and residue 280 and name HN)  3.35 1.65 1.65
assign (segid A and residue 279 and name HG#) (segid A and residue 279 and name HA)  3.10 1.40 1.40
assign (segid A and residue 280 and name HA) (segid A and residue 280 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 280 and name HA) (segid A and residue 281 and name HD2#)  3.35 1.65 1.65
assign (segid A and residue 280 and name HA) (segid A and residue 281 and name HG)  3.10 1.40 1.40
assign (segid A and residue 280 and name HA) (segid A and residue 281 and name HN)  3.35 1.65 1.65
assign (segid A and residue 280 and name HA) (segid A and residue 280 and name HN)  3.10 1.40 1.40
assign (segid A and residue 280 and name HB#) (segid A and residue 280 and name HA)  2.85 1.15 1.15
assign (segid A and residue 280 and name HB#) (segid A and residue 280 and name HN)  3.10 1.40 1.40
assign (segid A and residue 280 and name HB#) (segid A and residue 281 and name HN)  3.35 1.65 1.65
assign (segid A and residue 280 and name HB#) (segid A and residue 281 and name HG) 3.10 1.40 1.40
assign (segid A and residue 281 and name HA) (segid A and residue 281 and name HD1#) 3.35 1.65 1.65
assign (segid A and residue 281 and name HA) (segid A and residue 282 and name HN)  2.85 1.15 1.15
assign (segid A and residue 281 and name HA) (segid A and residue 281 and name HN)  3.10 1.40 1.40
assign (segid A and residue 281 and name HD1#) (segid A and residue 281 and name HA) 3.35 1.65 1.65
assign (segid A and residue 281 and name HD1#) (segid A and residue 281 and name HB#)  3.35 1.65 1.65
assign (segid A and residue 281 and name HD1#) (segid A and residue 281 and name HG)  3.10 1.40 1.40
assign (segid A and residue 281 and name HD1#) (segid A and residue 282 and name HN)  3.60 1.90 1.90
assign (segid A and residue 281 and name HD1#) (segid A and residue 281 and name HN)  3.35 1.65 1.65
assign (segid A and residue 281 and name HD2#) (segid A and residue 281 and name HB#)  3.10 1.40 1.40
assign (segid A and residue 281 and name HG) (segid A and residue 281 and name HA)  3.10 1.40 1.40
assign (segid A and residue 281 and name HG) (segid A and residue 281 and name HN)  3.10 1.40 1.40
assign (segid A and residue 281 and name HG) (segid A and residue 281 and name HD1#)  3.10 1.40 1.40
```

```
assign (segid A and residue 281 and name HG) (segid A and residue 281 and name HD2#)  3.10 1.40 1.40
assign (segid A and residue 281 and name HG) (segid A and residue 282 and name HN)  3.35 1.65 1.65
assign (segid A and residue 282 and name HA) (segid A and residue 282 and name HB#) 3.10 1.40 1.40
assign (segid A and residue 282 and name HA) (segid A and residue 282 and name HG#)  3.10 1.40 1.40
assign (segid A and residue 282 and name HA) (segid A and residue 282 and name HN)  3.35 1.65 1.65
assign (segid A and residue 282 and name HA) (segid A and residue 283 and name HN)  2.85 1.15 1.15
assign (segid A and residue 282 and name HB#) (segid A and residue 282 and name HN)  3.10 1.40 1.40
assign (segid A and residue 282 and name HB#) (segid A and residue 283 and name HN)  3.10 1.40 1.40
assign (segid A and residue 282 and name HG#) (segid A and residue 282 and name HA)  3.10 1.40 1.40
assign (segid A and residue 282 and name HG#) (segid A and residue 282 and name HB#)  2.85 1.15 1.15
assign (segid A and residue 282 and name HG#) (segid A and residue 282 and name HN)  3.10 1.40 1.40
assign (segid A and residue 282 and name HG#) (segid A and residue 283 and name HN)  3.10 1.40 1.40
assign (segid A and residue 283 and name HA) (segid A and residue 283 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 283 and name HA) (segid A and residue 283 and name HN)  3.10 1.40 1.40
assign (segid A and residue 283 and name HB#) (segid A and residue 283 and name HA)  2.85 1.15 1.15
assign (segid A and residue 283 and name HB#) (segid A and residue 283 and name HD#)  3.10 1.40 1.40
assign (segid A and residue 283 and name HB#) (segid A and residue 283 and name HN)  3.10 1.40 1.40
```

From the 4D HCCH-NOESY-HMQC

```
assign (segid B and residue 27 and name HA) (segid B and residue 27 and name HB#)          3.350  1.650  1.650
assign (segid B and residue 27 and name HA) (segid B and residue 27 and name HG#)          3.350  1.650  1.650
assign (segid B and residue 27 and name HA) (segid B and residue 28 and name HB#)          3.350  1.650  1.650
assign (segid B and residue 27 and name HG#) (segid B and residue 27 and name HA)          3.350  1.650  1.650
assign (segid B and residue 27 and name HG#) (segid B and residue 27 and name HB#)         2.600  0.900  0.900
assign (segid B and residue 27 and name HG#) (segid B and residue 28 and name HB#)         3.350  1.650  1.650
assign (segid B and residue 28 and name HA) (segid B and residue 28 and name HB#)          2.600  0.900  0.900
assign (segid B and residue 28 and name HA) (segid B and residue 29 and name HA)           3.350  1.650  1.650
assign (segid B and residue 28 and name HA) (segid B and residue 29 and name HB#)          3.350  1.650  1.650
assign (segid B and residue 33 and name HB) (segid B and residue 33 and name HG1#)         2.600  0.900  0.900
assign (segid B and residue 33 and name HB) (segid B and residue 33 and name HG1#)         2.600  0.900  0.900
assign (segid B and residue 33 and name HB) (segid B and residue 33 and name HG2#)         2.900  1.200  1.200
assign (segid B and residue 31 and name HB#) (segid B and residue 33 and name HD1#)        3.350  1.650  1.650
assign (segid B and residue 33 and name HD1#) (segid B and residue 33 and name HG1#)       2.900  1.200  1.200
assign (segid B and residue 33 and name HD1#) (segid B and residue 33 and name HG1#)       2.900  1.200  1.200
assign (segid B and residue 33 and name HD1#) (segid B and residue 33 and name HG2#)       3.850  2.150  2.150
assign (segid B and residue 30 and name HB#) (segid B and residue 33 and name HG2#)        3.350  1.650  1.650
assign (segid B and residue 33 and name HG2#) (segid B and residue 33 and name HD1#)       3.850  2.150  2.150
assign (segid B and residue 34 and name HB#) (segid B and residue 34 and name HA)          3.350  1.650  1.650
assign (segid B and residue 37 and name HA) (segid B and residue 40 and name HG2#)         3.600  1.900  1.900
assign (segid B and residue 37 and name HA) (segid A and residue 179 and name HD2#)        3.600  1.900  1.900
assign (segid B and residue 37 and name HB) (segid A and residue 179 and name HD2#)        3.600  1.900  1.900
```

```
assign (segid B and residue 37 and name HG2#) (segid B and residue 37 and name HA)         3.600  1.900  1.900
assign (segid B and residue 37 and name HG2#) (segid B and residue 37 and name HB)         3.600  1.900  1.900
assign (segid B and residue 37 and name HG2#) (segid B and residue 40 and name HB)         3.600  1.900  1.900
assign (segid B and residue 37 and name HG2#) (segid B and residue 40 and name HG1#)       3.600  1.900  1.900
assign (segid B and residue 37 and name HG2#) (segid A and residue 179 and name HD2#)      3.850  2.150  2.150
assign (segid B and residue 38 and name HA) (segid B and residue 41 and name HD#)         3.350  1.650  1.650
assign (segid B and residue 38 and name HA) (segid B and residue 41 and name HE#)         3.350  1.650  1.650
assign (segid B and residue 38 and name HB#) (segid B and residue 39 and name HD2#)       3.850  2.150  2.150
assign (segid B and residue 39 and name HD2#) (segid B and residue 39 and name HB#)       3.600  1.900  1.900
assign (segid B and residue 39 and name HD2#) (segid B and residue 39 and name HD1#)      3.850  2.150  2.150
assign (segid B and residue 39 and name HD2#) (segid B and residue 39 and name HG)        3.600  1.900  1.900
assign (segid B and residue 39 and name HD2#) (segid B and residue 42 and name HB#)       3.850  2.150  2.150
assign (segid B and residue 39 and name HD2#) (segid A and residue 113 and name HA)       3.600  1.900  1.900
assign (segid B and residue 39 and name HD2#) (segid A and residue 113 and name HG1#)     3.850  2.150  2.150
assign (segid B and residue 39 and name HD2#) (segid A and residue 113 and name HG2#)     3.850  2.150  2.150
assign (segid B and residue 39 and name HD2#) (segid A and residue 117 and name HG2#)     3.850  2.150  2.150
assign (segid B and residue 39 and name HD2#) (segid A and residue 183 and name HG2#)     3.850  2.150  2.150
assign (segid B and residue 39 and name HD2#) (segid A and residue 217 and name HB#)      3.850  2.150  2.150
assign (segid B and residue 39 and name HD2#) (segid A and residue 220 and name HB#)      3.850  2.150  2.150
assign (segid B and residue 39 and name HD1#) (segid B and residue 40 and name HA)        3.600  1.900  1.900
assign (segid B and residue 40 and name HA) (segid B and residue 40 and name HB)          3.350  1.650  1.650
assign (segid B and residue 40 and name HD1#) (segid B and residue 40 and name HG2#)      3.850  2.150  2.150
assign (segid B and residue 40 and name HD1#) (segid A and residue 177 and name HG#)      3.600  1.900  1.900
assign (segid B and residue 40 and name HD1#) (segid A and residue 178 and name HD#)      3.600  1.900  1.900
assign (segid B and residue 40 and name HD1#) (segid A and residue 178 and name HG#)      3.600  1.900  1.900
assign (segid B and residue 40 and name HD1#) (segid A and residue 179 and name HD1#)     3.850  2.150  2.150
assign (segid B and residue 40 and name HD1#) (segid A and residue 179 and name HD2#)     3.850  2.150  2.150
assign (segid B and residue 40 and name HD1#) (segid A and residue 213 and name HD#)      3.600  1.900  1.900
assign (segid B and residue 39 and name HB#) (segid B and residue 40 and name HG1#)       3.350  1.650  1.650
assign (segid B and residue 40 and name HG2#) (segid B and residue 41 and name HA)        3.600  1.900  1.900
assign (segid B and residue 40 and name HG2#) (segid A and residue 179 and name HD2#)     3.850  2.150  2.150
assign (segid B and residue 40 and name HG2#) (segid A and residue 213 and name HA)       3.600  1.900  1.900
assign (segid B and residue 40 and name HG2#) (segid A and residue 213 and name HD#)      3.600  1.900  1.900
assign (segid B and residue 40 and name HG2#) (segid A and residue 213 and name HG#)      3.600  1.900  1.900
assign (segid B and residue 39 and name HD1#) (segid B and residue 42 and name HB#)       3.850  2.150  2.150
assign (segid B and residue 41 and name HE#) (segid B and residue 42 and name HB#)        3.350  1.650  1.650
assign (segid B and residue 42 and name HB#) (segid A and residue 109 and name HG#)       3.350  1.650  1.650
assign (segid B and residue 42 and name HB#) (segid A and residue 113 and name HA)        3.600  1.900  1.900
assign (segid B and residue 44 and name HB#) (segid B and residue 47 and name HG2#)       3.600  1.900  1.900
assign (segid B and residue 46 and name HB#) (segid B and residue 47 and name HG1#)       3.850  2.150  2.150
assign (segid B and residue 46 and name HB#) (segid A and residue 215 and name HD2#)      3.850  2.150  2.150
assign (segid B and residue 44 and name HA) (segid B and residue 47 and name HG1#)        3.600  1.900  1.900
assign (segid B and residue 47 and name HG1#) (segid B and residue 47 and name HA)        3.600  1.900  1.900
assign (segid B and residue 47 and name HG1#) (segid A and residue 258 and name HA)       3.600  1.900  1.900
```

```
assign (segid B and residue 47 and name HG1#) (segid A and residue 258 and name HB#)       3.850  2.150  2.150
assign (segid B and residue 47 and name HG2#) (segid A and residue 215 and name HD2#)      3.850  2.150  2.150
assign (segid B and residue 47 and name HG2#) (segid A and residue 258 and name HA)        3.600  1.900  1.900
assign (segid B and residue 48 and name HA) (segid B and residue 48 and name HB#)          2.900  1.200  1.200
assign (segid B and residue 47 and name HA) (segid B and residue 48 and name HB#)          3.600  1.900  1.900
assign (segid B and residue 50 and name HB#) (segid B and residue 50 and name HA)          2.600  0.900  0.900
assign (segid B and residue 50 and name HB#) (segid B and residue 50 and name HD#)         3.350  1.650  1.650
assign (segid B and residue 50 and name HB#) (segid B and residue 50 and name HE#)         3.350  1.650  1.650
assign (segid B and residue 51 and name HA) (segid B and residue 51 and name HB#)          2.600  0.900  0.900
assign (segid B and residue 51 and name HA) (segid B and residue 51 and name HE#)          3.350  1.650  1.650
assign (segid B and residue 51 and name HB#) (segid B and residue 51 and name HA)          2.600  0.900  0.900
assign (segid B and residue 51 and name HB#) (segid B and residue 51 and name HA)          2.600  0.900  0.900
assign (segid B and residue 51 and name HG#) (segid B and residue 51 and name HB#)         3.350  1.650  1.650
assign (segid B and residue 51 and name HG#) (segid B and residue 51 and name HD#)         2.600  0.900  0.900
assign (segid A and residue 96 and name HA) (segid A and residue 96 and name HB#)          2.600  0.900  0.900
assign (segid A and residue 99 and name HG2#) (segid A and residue 103 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 99 and name HG2#) (segid A and residue 103 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 101 and name HA) (segid A and residue 104 and name HB#)        2.600  0.900  0.900
assign (segid A and residue 102 and name HA) (segid A and residue 105 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 104 and name HA) (segid A and residue 107 and name HB)         3.350  1.650  1.650
assign (segid A and residue 105 and name HA) (segid A and residue 108 and name HB#)        3.600  1.900  1.900
assign (segid A and residue 106 and name HA) (segid A and residue 109 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 107 and name HA) (segid A and residue 107 and name HG2#)       3.600  1.900  1.900
assign (segid A and residue 107 and name HA) (segid A and residue 265 and name HD2#)       3.600  1.900  1.900
assign (segid A and residue 104 and name HA) (segid A and residue 107 and name HG1#)       3.600  1.900  1.900
assign (segid A and residue 107 and name HG1#) (segid A and residue 107 and name HG2#)     3.850  2.150  2.150
assign (segid A and residue 108 and name HA) (segid A and residue 111 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 107 and name HG2#) (segid A and residue 108 and name HB#)      3.850  2.150  2.150
assign (segid A and residue 108 and name HB#) (segid A and residue 111 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 109 and name HA) (segid A and residue 112 and name HB)         3.350  1.650  1.650
assign (segid A and residue 112 and name HA) (segid A and residue 112 and name HB)         3.350  1.650  1.650
assign (segid A and residue 112 and name HA) (segid A and residue 115 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 112 and name HA) (segid A and residue 115 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 109 and name HB#) (segid A and residue 112 and name HB)        3.350  1.650  1.650
assign (segid A and residue 112 and name HB) (segid A and residue 115 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 108 and name HB#) (segid A and residue 112 and name HG2#)      3.850  2.150  2.150
assign (segid A and residue 109 and name HA) (segid A and residue 112 and name HG2#)       3.600  1.900  1.900
assign (segid A and residue 109 and name HB#) (segid A and residue 112 and name HG2#)      3.350  1.650  1.650
assign (segid A and residue 112 and name HG2#) (segid A and residue 112 and name HB)       2.900  1.200  1.200
assign (segid A and residue 117 and name HG2#) (segid A and residue 117 and name HG1#)     3.850  2.150  2.150
assign (segid A and residue 117 and name HG2#) (segid A and residue 180 and name HD1#)     3.850  2.150  2.150
assign (segid A and residue 117 and name HG2#) (segid A and residue 183 and name HG2#)     3.850  2.150  2.150
assign (segid A and residue 117 and name HG2#) (segid A and residue 220 and name HB#)      3.850  2.150  2.150
assign (segid A and residue 119 and name HB#) (segid A and residue 119 and name HD#)       3.350  1.650  1.650
```

```
assign (segid A and residue 119 and name HB#) (segid A and residue 119 and name HE#)       3.350  1.650  1.650
assign (segid A and residue 119 and name HG#) (segid A and residue 119 and name HB#)       2.600  0.900  0.900
assign (segid A and residue 119 and name HG#) (segid A and residue 119 and name HD#)       2.600  0.900  0.900
assign (segid A and residue 119 and name HG#) (segid A and residue 119 and name HE#)       3.350  1.650  1.650
assign (segid A and residue 121 and name HA) (segid A and residue 124 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 117 and name HG1#) (segid A and residue 121 and name HD#)     3.600  1.900  1.900
assign (segid A and residue 118 and name HA) (segid A and residue 121 and name HD#)       3.350  1.650  1.650
assign (segid A and residue 121 and name HD#) (segid A and residue 121 and name HA)       3.350  1.650  1.650
assign (segid A and residue 121 and name HD#) (segid A and residue 121 and name HB#)      3.350  1.650  1.650
assign (segid A and residue 121 and name HD#) (segid A and residue 121 and name HG#)      3.350  1.650  1.650
assign (segid A and residue 127 and name HA) (segid A and residue 127 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 127 and name HA) (segid A and residue 127 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 127 and name HG#) (segid A and residue 131 and name HA)       3.350  1.650  1.650
assign (segid A and residue 131 and name HA) (segid A and residue 131 and name HB#)       2.600  0.900  0.900
assign (segid A and residue 132 and name HB#) (segid A and residue 132 and name HA)       2.600  0.900  0.900
assign (segid A and residue 132 and name HB#) (segid A and residue 132 and name HA)       2.600  0.900  0.900
assign (segid A and residue 130 and name HB#) (segid A and residue 133 and name HG1#)     3.350  1.650  1.650
assign (segid A and residue 133 and name HG1#) (segid A and residue 133 and name HA)      3.600  1.900  1.900
assign (segid A and residue 133 and name HG1#) (segid A and residue 133 and name HG2#)    3.850  2.150  2.150
assign (segid A and residue 129 and name HB#) (segid A and residue 133 and name HG2#)     3.350  1.650  1.650
assign (segid A and residue 129 and name HG) (segid A and residue 133 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 133 and name HG2#) (segid A and residue 135 and name HE#)     3.850  2.150  2.150
assign (segid A and residue 133 and name HG2#) (segid A and residue 182 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 133 and name HG2#) (segid A and residue 182 and name HG2#)    3.850  2.150  2.150
assign (segid A and residue 134 and name HB) (segid A and residue 134 and name HA)        2.600  0.900  0.900
assign (segid A and residue 134 and name HB) (segid A and residue 134 and name HG2#)      2.900  1.200  1.200
assign (segid A and residue 134 and name HA) (segid A and residue 135 and name HE#)       3.600  1.900  1.900
assign (segid A and residue 135 and name HE#) (segid A and residue 135 and name HB#)      3.600  1.900  1.900
assign (segid A and residue 135 and name HE#) (segid A and residue 182 and name HA)       3.600  1.900  1.900
assign (segid A and residue 135 and name HE#) (segid A and residue 183 and name HA)       3.600  1.900  1.900
assign (segid A and residue 135 and name HE#) (segid A and residue 183 and name HG1#)     3.850  2.150  2.150
assign (segid A and residue 135 and name HE#) (segid A and residue 183 and name HG2#)     3.850  2.150  2.150
assign (segid A and residue 135 and name HE#) (segid A and residue 185 and name HD#)      3.350  1.650  1.650
assign (segid A and residue 135 and name HE#) (segid A and residue 186 and name HA)       3.600  1.900  1.900
assign (segid A and residue 135 and name HE#) (segid A and residue 186 and name HE#)      3.850  2.150  2.150
assign (segid A and residue 145 and name HA) (segid A and residue 145 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 145 and name HA) (segid A and residue 198 and name HD2#)      3.600  1.900  1.900
assign (segid A and residue 146 and name HG#) (segid A and residue 146 and name HA)       3.350  1.650  1.650
assign (segid A and residue 146 and name HG#) (segid A and residue 146 and name HB#)      3.350  1.650  1.650
assign (segid A and residue 146 and name HG#) (segid A and residue 146 and name HD#)      3.350  1.650  1.650
assign (segid A and residue 146 and name HG#) (segid A and residue 146 and name HE#)      3.350  1.650  1.650
assign (segid A and residue 153 and name HA) (segid A and residue 154 and name HA)        3.350  1.650  1.650
assign (segid A and residue 153 and name HB#) (segid A and residue 154 and name HA)       3.350  1.650  1.650
assign (segid A and residue 154 and name HB#) (segid A and residue 155 and name HA)       3.350  1.650  1.650
```

```
assign (segid A and residue 155 and name HB#) (segid A and residue 156 and name HA)        3.600  1.900  1.900
assign (segid A and residue 155 and name HB#) (segid A and residue 156 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 156 and name HA) (segid A and residue 157 and name HA#)        3.350  1.650  1.650
assign (segid A and residue 158 and name HA) (segid A and residue 159 and name HA)        3.350  1.650  1.650
assign (segid A and residue 158 and name HB#) (segid A and residue 159 and name HA)       3.600  1.900  1.900
assign (segid A and residue 159 and name HA) (segid A and residue 159 and name HB)        2.600  0.900  0.900
assign (segid A and residue 159 and name HB) (segid A and residue 159 and name HA)        2.600  0.900  0.900
assign (segid A and residue 160 and name HA1) (segid A and residue 160 and name HA2)      2.600  0.900  0.900
assign (segid A and residue 160 and name HA1) (segid A and residue 161 and name HD#)      2.600  0.900  0.900
assign (segid A and residue 160 and name HA2) (segid A and residue 160 and name HA1)      2.600  0.900  0.900
assign (segid A and residue 160 and name HA2) (segid A and residue 161 and name HD#)      2.600  0.900  0.900
assign (segid A and residue 161 and name HB#) (segid A and residue 161 and name HA)       2.600  0.900  0.900
assign (segid A and residue 161 and name HB#) (segid A and residue 161 and name HG#)      2.600  0.900  0.900
assign (segid A and residue 160 and name HA1) (segid A and residue 161 and name HD#)      2.600  0.900  0.900
assign (segid A and residue 160 and name HA2) (segid A and residue 161 and name HD#)      2.600  0.900  0.900
assign (segid A and residue 161 and name HD#) (segid A and residue 161 and name HG#)      2.600  0.900  0.900
assign (segid A and residue 160 and name HA1) (segid A and residue 161 and name HG#)      3.350  1.650  1.650
assign (segid A and residue 160 and name HA2) (segid A and residue 161 and name HG#)      3.350  1.650  1.650
assign (segid A and residue 161 and name HG#) (segid A and residue 161 and name HD#)      2.600  0.900  0.900
assign (segid A and residue 163 and name HB) (segid A and residue 163 and name HA)        2.600  0.900  0.900
assign (segid A and residue 162 and name HB#) (segid A and residue 163 and name HG2#)     3.850  2.150  2.150
assign (segid A and residue 164 and name HA) (segid A and residue 164 and name HB#)       2.600  0.900  0.900
assign (segid A and residue 166 and name HA) (segid A and residue 167 and name HD#)       2.600  0.900  0.900
assign (segid A and residue 166 and name HB#) (segid A and residue 167 and name HD#)      3.350  1.650  1.650
assign (segid A and residue 167 and name HD#) (segid A and residue 167 and name HG#)      2.600  0.900  0.900
assign (segid A and residue 166 and name HA) (segid A and residue 167 and name HD#)       2.600  0.900  0.900
assign (segid A and residue 167 and name HG#) (segid A and residue 167 and name HB#)      2.600  0.900  0.900
assign (segid A and residue 167 and name HG#) (segid A and residue 167 and name HB#)      2.600  0.900  0.900
assign (segid A and residue 167 and name HG#) (segid A and residue 167 and name HD#)      2.600  0.900  0.900
assign (segid A and residue 169 and name HD1#) (segid A and residue 169 and name HG1#)    2.900  1.200  1.200
assign (segid A and residue 169 and name HD1#) (segid A and residue 169 and name HG1#)    2.900  1.200  1.200
assign (segid A and residue 169 and name HD1#) (segid A and residue 169 and name HG2#)    3.850  2.150  2.150
assign (segid A and residue 169 and name HG1#) (segid A and residue 169 and name HB)      3.350  1.650  1.650
assign (segid A and residue 173 and name HA) (segid A and residue 174 and name HA)        3.350  1.650  1.650
assign (segid A and residue 174 and name HA) (segid A and residue 174 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 174 and name HA) (segid A and residue 174 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 174 and name HD1#) (segid A and residue 174 and name HG2#)    3.850  2.150  2.150
assign (segid A and residue 173 and name HG#) (segid A and residue 174 and name HG2#)     3.350  1.650  1.650
assign (segid A and residue 174 and name HG2#) (segid A and residue 174 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 177 and name HD#) (segid A and residue 179 and name HD2#)     3.600  1.900  1.900
assign (segid A and residue 177 and name HG#) (segid A and residue 179 and name HD2#)     3.600  1.900  1.900
assign (segid A and residue 179 and name HD2#) (segid A and residue 179 and name HA)      3.600  1.900  1.900
assign (segid A and residue 179 and name HD2#) (segid A and residue 179 and name HB#)     3.600  1.900  1.900
assign (segid A and residue 179 and name HD2#) (segid A and residue 182 and name HD1#)    3.850  2.150  2.150
```

```
assign (segid A and residue 180 and name HD1#) (segid A and residue 183 and name HG2#)     3.850  2.150  2.150
assign (segid A and residue 117 and name HG1#) (segid A and residue 180 and name HD2#)     3.850  2.150  2.150
assign (segid A and residue 179 and name HG) (segid A and residue 180 and name HD2#)      3.600  1.900  1.900
assign (segid A and residue 180 and name HD2#) (segid A and residue 180 and name HB#)     3.600  1.900  1.900
assign (segid A and residue 180 and name HD2#) (segid A and residue 180 and name HB#)     3.600  1.900  1.900
assign (segid A and residue 180 and name HD2#) (segid A and residue 183 and name HG2#)     3.850  2.150  2.150
assign (segid A and residue 180 and name HD2#) (segid A and residue 217 and name HB#)     3.850  2.150  2.150
assign (segid A and residue 180 and name HD2#) (segid A and residue 220 and name HB#)     3.850  2.150  2.150
assign (segid A and residue 132 and name HB#) (segid A and residue 182 and name HD1#)     3.350  1.650  1.650
assign (segid A and residue 133 and name HA) (segid A and residue 182 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 133 and name HG1#) (segid A and residue 182 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 179 and name HD1#) (segid A and residue 182 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 182 and name HD1#) (segid A and residue 183 and name HG1#)    3.850  2.150  2.150
assign (segid A and residue 182 and name HD1#) (segid A and residue 183 and name HG2#)    3.850  2.150  2.150
assign (segid A and residue 182 and name HG2#) (segid A and residue 182 and name HB)      3.600  1.900  1.900
assign (segid A and residue 182 and name HG2#) (segid A and residue 183 and name HB)      3.600  1.900  1.900
assign (segid A and residue 182 and name HG2#) (segid A and residue 183 and name HG1#)    3.850  2.150  2.150
assign (segid A and residue 182 and name HG2#) (segid A and residue 186 and name HE#)     3.850  2.150  2.150
assign (segid A and residue 183 and name HA) (segid A and residue 186 and name HE#)       3.600  1.900  1.900
assign (segid A and residue 183 and name HG1#) (segid A and residue 183 and name HA)      3.600  1.900  1.900
assign (segid A and residue 183 and name HG1#) (segid A and residue 183 and name HG2#)    3.850  2.150  2.150
assign (segid A and residue 183 and name HG1#) (segid A and residue 186 and name HE#)     3.850  2.150  2.150
assign (segid A and residue 183 and name HG1#) (segid A and residue 217 and name HB#)     3.850  2.150  2.150
assign (segid A and residue 179 and name HG) (segid A and residue 183 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 180 and name HG) (segid A and residue 183 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 183 and name HG2#) (segid A and residue 183 and name HG1#)    3.850  2.150  2.150
assign (segid A and residue 183 and name HG2#) (segid A and residue 184 and name HA)      3.600  1.900  1.900
assign (segid A and residue 183 and name HG2#) (segid A and residue 217 and name HA)      3.600  1.900  1.900
assign (segid A and residue 183 and name HG2#) (segid A and residue 217 and name HB#)     3.850  2.150  2.150
assign (segid A and residue 183 and name HG2#) (segid A and residue 218 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 182 and name HD1#) (segid A and residue 186 and name HE#)     3.850  2.150  2.150
assign (segid A and residue 185 and name HD#) (segid A and residue 186 and name HE#)      3.350  1.650  1.650
assign (segid A and residue 186 and name HE#) (segid A and residue 186 and name HB#)      3.600  1.900  1.900
assign (segid A and residue 186 and name HE#) (segid A and residue 186 and name HG#)      3.350  1.650  1.650
assign (segid A and residue 186 and name HE#) (segid A and residue 190 and name HA)       3.600  1.900  1.900
assign (segid A and residue 186 and name HE#) (segid A and residue 190 and name HB)       3.600  1.900  1.900
assign (segid A and residue 186 and name HE#) (segid A and residue 190 and name HG2#)     3.850  2.150  2.150
assign (segid A and residue 186 and name HE#) (segid A and residue 191 and name HB)       3.600  1.900  1.900
assign (segid A and residue 186 and name HE#) (segid A and residue 191 and name HG2#)     3.850  2.150  2.150
assign (segid A and residue 186 and name HE#) (segid A and residue 194 and name HB)       3.600  1.900  1.900
assign (segid A and residue 186 and name HE#) (segid A and residue 194 and name HG1#)     3.850  2.150  2.150
assign (segid A and residue 188 and name HA) (segid A and residue 191 and name HB)       3.350  1.650  1.650
assign (segid A and residue 189 and name HA) (segid A and residue 192 and name HB)       3.350  1.650  1.650
assign (segid A and residue 189 and name HB#) (segid A and residue 189 and name HA)      2.900  1.200  1.200
```

```
assign (segid A and residue 189 and name HB#) (segid A and residue 190 and name HA)        3.600  1.900  1.900
assign (segid A and residue 189 and name HB#) (segid A and residue 192 and name HB)        3.600  1.900  1.900
assign (segid A and residue 189 and name HB#) (segid A and residue 192 and name HG2#)      3.850  2.150  2.150
assign (segid A and residue 189 and name HB#) (segid A and residue 222 and name HD1#)      3.850  2.150  2.150
assign (segid A and residue 189 and name HB#) (segid A and residue 227 and name HD1#)      3.850  2.150  2.150
assign (segid A and residue 189 and name HB#) (segid A and residue 227 and name HD2#)      3.850  2.150  2.150
assign (segid A and residue 190 and name HG2#) (segid A and residue 191 and name HA)       3.600  1.900  1.900
assign (segid A and residue 191 and name HA) (segid A and residue 191 and name HB)         3.350  1.650  1.650
assign (segid A and residue 186 and name HE#) (segid A and residue 191 and name HG1#)      3.850  2.150  2.150
assign (segid A and residue 191 and name HG1#) (segid A and residue 192 and name HA)       3.600  1.900  1.900
assign (segid A and residue 191 and name HG1#) (segid A and residue 221 and name HA)       3.600  1.900  1.900
assign (segid A and residue 191 and name HG1#) (segid A and residue 221 and name HB#)      3.350  1.650  1.650
assign (segid A and residue 191 and name HG1#) (segid A and residue 222 and name HB#)      3.600  1.900  1.900
assign (segid A and residue 191 and name HG1#) (segid A and residue 222 and name HD1#)     3.850  2.150  2.150
assign (segid A and residue 191 and name HG1#) (segid A and residue 222 and name HD2#)     3.850  2.150  2.150
assign (segid A and residue 191 and name HG1#) (segid A and residue 222 and name HG)       3.600  1.900  1.900
assign (segid A and residue 191 and name HG1#) (segid A and residue 230 and name HB#)      3.850  2.150  2.150
assign (segid A and residue 192 and name HB) (segid A and residue 227 and name HD1#)       3.600  1.900  1.900
assign (segid A and residue 189 and name HA) (segid A and residue 192 and name HG2#)       3.600  1.900  1.900
assign (segid A and residue 192 and name HG2#) (segid A and residue 222 and name HD2#)     3.850  2.150  2.150
assign (segid A and residue 192 and name HG2#) (segid A and residue 230 and name HB#)      3.850  2.150  2.150
assign (segid A and residue 190 and name HG2#) (segid A and residue 194 and name HG1#)     3.850  2.150  2.150
assign (segid A and residue 194 and name HG1#) (segid A and residue 198 and name HD2#)     3.850  2.150  2.150
assign (segid A and residue 194 and name HG1#) (segid A and residue 198 and name HG)       3.600  1.900  1.900
assign (segid A and residue 192 and name HA) (segid A and residue 195 and name HD1#)       3.600  1.900  1.900
assign (segid A and residue 194 and name HG1#) (segid A and residue 195 and name HD1#)     3.850  2.150  2.150
assign (segid A and residue 195 and name HD1#) (segid A and residue 195 and name HA)       3.600  1.900  1.900
assign (segid A and residue 195 and name HD1#) (segid A and residue 195 and name HB#)      3.600  1.900  1.900
assign (segid A and residue 195 and name HD1#) (segid A and residue 195 and name HD2#)     3.850  2.150  2.150
assign (segid A and residue 195 and name HD1#) (segid A and residue 195 and name HG)       3.600  1.900  1.900
assign (segid A and residue 195 and name HD1#) (segid A and residue 198 and name HD2#)     3.850  2.150  2.150
assign (segid A and residue 195 and name HD1#) (segid A and residue 199 and name HB#)      3.350  1.650  1.650
assign (segid A and residue 195 and name HD1#) (segid A and residue 219 and name HD2#)     3.850  2.150  2.150
assign (segid A and residue 195 and name HD1#) (segid A and residue 230 and name HA)       3.600  1.900  1.900
assign (segid A and residue 195 and name HD1#) (segid A and residue 234 and name HG2#)     3.850  2.150  2.150
assign (segid A and residue 198 and name HD2#) (segid A and residue 198 and name HG)       3.600  1.900  1.900
assign (segid A and residue 198 and name HD2#) (segid A and residue 199 and name HA)       3.600  1.900  1.900
assign (segid A and residue 198 and name HD2#) (segid A and residue 211 and name HD1#)     3.850  2.150  2.150
assign (segid A and residue 208 and name HB) (segid A and residue 208 and name HA)         3.350  1.650  1.650
assign (segid A and residue 209 and name HA) (segid A and residue 209 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 209 and name HB#) (segid A and residue 209 and name HA)        3.350  1.650  1.650
assign (segid A and residue 209 and name HB#) (segid A and residue 209 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 207 and name HA) (segid A and residue 211 and name HD2#)       3.600  1.900  1.900
assign (segid A and residue 211 and name HD2#) (segid A and residue 211 and name HA)       3.600  1.900  1.900
```

```
assign (segid A and residue 211 and name HD2#) (segid A and residue 211 and name HD1#)     3.850  2.150  2.150
assign (segid A and residue 211 and name HD2#) (segid A and residue 211 and name HG)       3.600  1.900  1.900
assign (segid A and residue 212 and name HA1) (segid A and residue 215 and name HB#)      2.600  0.900  0.900
assign (segid A and residue 213 and name HA) (segid A and residue 216 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 215 and name HA) (segid A and residue 218 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 217 and name HA) (segid A and residue 220 and name HB#)       3.600  1.900  1.900
assign (segid A and residue 215 and name HD2#) (segid A and residue 219 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 219 and name HD1#) (segid A and residue 219 and name HD2#)    3.850  2.150  2.150
assign (segid A and residue 219 and name HD1#) (segid A and residue 234 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 219 and name HD1#) (segid A and residue 234 and name HG2#)    3.850  2.150  2.150
assign (segid A and residue 219 and name HD1#) (segid A and residue 262 and name HD2#)    3.850  2.150  2.150
assign (segid A and residue 219 and name HD1#) (segid A and residue 266 and name HG2#)    3.850  2.150  2.150
assign (segid A and residue 219 and name HD2#) (segid A and residue 219 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 219 and name HD2#) (segid A and residue 220 and name HB#)     3.850  2.150  2.150
assign (segid A and residue 219 and name HD2#) (segid A and residue 222 and name HB#)     3.350  1.650  1.650
assign (segid A and residue 219 and name HD2#) (segid A and residue 222 and name HD2#)    3.850  2.150  2.150
assign (segid A and residue 219 and name HD2#) (segid A and residue 222 and name HG)      3.600  1.900  1.900
assign (segid A and residue 219 and name HD2#) (segid A and residue 234 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 219 and name HD2#) (segid A and residue 234 and name HG2#)    3.850  2.150  2.150
assign (segid A and residue 219 and name HD2#) (segid A and residue 237 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 219 and name HD2#) (segid A and residue 237 and name HD2#)    3.850  2.150  2.150
assign (segid A and residue 219 and name HD2#) (segid A and residue 262 and name HD2#)    3.850  2.150  2.150
assign (segid A and residue 219 and name HD2#) (segid A and residue 265 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 219 and name HD2#) (segid A and residue 266 and name HA)      3.600  1.900  1.900
assign (segid A and residue 219 and name HD2#) (segid A and residue 266 and name HG1#)    3.850  2.150  2.150
assign (segid B and residue 42 and name HB#) (segid A and residue 220 and name HB#)       3.850  2.150  2.150
assign (segid A and residue 113 and name HG1#) (segid A and residue 220 and name HB#)     3.850  2.150  2.150
assign (segid A and residue 113 and name HG2#) (segid A and residue 220 and name HB#)     3.850  2.150  2.150
assign (segid A and residue 219 and name HD1#) (segid A and residue 220 and name HB#)     3.850  2.150  2.150
assign (segid A and residue 222 and name HB#) (segid A and residue 222 and name HA)       3.350  1.650  1.650
assign (segid A and residue 222 and name HB#) (segid A and residue 222 and name HG)       3.350  1.650  1.650
assign (segid A and residue 219 and name HD2#) (segid A and residue 222 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 221 and name HA) (segid A and residue 222 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 222 and name HD1#) (segid A and residue 222 and name HB#)     3.350  1.650  1.650
assign (segid A and residue 222 and name HD1#) (segid A and residue 226 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 222 and name HD1#) (segid A and residue 234 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 222 and name HD1#) (segid A and residue 234 and name HG2#)    3.850  2.150  2.150
assign (segid A and residue 219 and name HD1#) (segid A and residue 222 and name HD2#)    3.850  2.150  2.150
assign (segid A and residue 222 and name HD2#) (segid A and residue 226 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 222 and name HD2#) (segid A and residue 234 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 222 and name HD2#) (segid A and residue 234 and name HG2#)    3.850  2.150  2.150
assign (segid A and residue 224 and name HA) (segid A and residue 225 and name HA)        3.350  1.650  1.650
assign (segid A and residue 224 and name HB#) (segid A and residue 225 and name HA)       3.350  1.650  1.650
assign (segid A and residue 225 and name HA) (segid A and residue 225 and name HB#)       3.350  1.650  1.650
```

```
assign (segid A and residue 225 and name HA) (segid A and residue 225 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 224 and name HA) (segid A and residue 226 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 226 and name HD1#) (segid A and residue 226 and name HB#)     3.350  1.650  1.650
assign (segid A and residue 226 and name HD1#) (segid A and residue 226 and name HD2#)    3.850  2.150  2.150
assign (segid A and residue 226 and name HD1#) (segid A and residue 230 and name HB#)     3.850  2.150  2.150
assign (segid A and residue 226 and name HD1#) (segid A and residue 234 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 226 and name HD1#) (segid A and residue 234 and name HG1#)    3.850  2.150  2.150
assign (segid A and residue 222 and name HD2#) (segid A and residue 226 and name HD2#)    3.850  2.150  2.150
assign (segid A and residue 226 and name HD2#) (segid A and residue 226 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 226 and name HD2#) (segid A and residue 226 and name HG)      3.600  1.900  1.900
assign (segid A and residue 226 and name HD2#) (segid A and residue 230 and name HB#)     3.850  2.150  2.150
assign (segid A and residue 226 and name HD2#) (segid A and residue 234 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 226 and name HD2#) (segid A and residue 234 and name HG2#)    3.850  2.150  2.150
assign (segid A and residue 227 and name HB#) (segid A and residue 227 and name HD2#)     3.600  1.900  1.900
assign (segid A and residue 227 and name HB#) (segid A and residue 227 and name HG)       3.350  1.650  1.650
assign (segid A and residue 227 and name HD2#) (segid A and residue 227 and name HB#)     3.600  1.900  1.900
assign (segid A and residue 227 and name HD2#) (segid A and residue 227 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 227 and name HD2#) (segid A and residue 227 and name HG)      3.600  1.900  1.900
assign (segid A and residue 227 and name HD2#) (segid A and residue 228 and name HA)      3.600  1.900  1.900
assign (segid A and residue 227 and name HD2#) (segid A and residue 228 and name HD#)     3.600  1.900  1.900
assign (segid A and residue 228 and name HD#) (segid A and residue 228 and name HB#)      3.350  1.650  1.650
assign (segid A and residue 229 and name HA) (segid A and residue 232 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 230 and name HA) (segid A and residue 233 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 192 and name HB) (segid A and residue 230 and name HB#)       3.600  1.900  1.900
assign (segid A and residue 226 and name HA) (segid A and residue 230 and name HB#)       3.600  1.900  1.900
assign (segid A and residue 226 and name HB#) (segid A and residue 230 and name HB#)      3.350  1.650  1.650
assign (segid A and residue 227 and name HD1#) (segid A and residue 230 and name HB#)     3.850  2.150  2.150
assign (segid A and residue 227 and name HD2#) (segid A and residue 230 and name HB#)     3.850  2.150  2.150
assign (segid A and residue 229 and name HG#) (segid A and residue 230 and name HB#)      3.350  1.650  1.650
assign (segid A and residue 230 and name HB#) (segid A and residue 233 and name HD1#)     3.850  2.150  2.150
assign (segid A and residue 230 and name HB#) (segid A and residue 234 and name HD1#)     3.850  2.150  2.150
assign (segid A and residue 230 and name HB#) (segid A and residue 234 and name HG2#)     3.850  2.150  2.150
assign (segid A and residue 231 and name HA) (segid A and residue 234 and name HB)        3.350  1.650  1.650
assign (segid A and residue 233 and name HB#) (segid A and residue 233 and name HD2#)     3.600  1.900  1.900
assign (segid A and residue 233 and name HB#) (segid A and residue 233 and name HG)       3.350  1.650  1.650
assign (segid A and residue 233 and name HB#) (segid A and residue 236 and name HB#)      3.350  1.650  1.650
assign (segid A and residue 233 and name HB#) (segid A and residue 237 and name HG)       3.350  1.650  1.650
assign (segid A and residue 192 and name HA) (segid A and residue 233 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 192 and name HG2#) (segid A and residue 233 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 229 and name HG#) (segid A and residue 233 and name HD1#)     3.350  1.650  1.650
assign (segid A and residue 230 and name HA) (segid A and residue 233 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 233 and name HD1#) (segid A and residue 233 and name HD2#)    3.850  2.150  2.150
assign (segid A and residue 233 and name HD1#) (segid A and residue 236 and name HG#)     3.350  1.650  1.650
assign (segid A and residue 230 and name HA) (segid A and residue 233 and name HD2#)      3.600  1.900  1.900
```

```
assign (segid A and residue 233 and name HD2#) (segid A and residue 233 and name HA)       3.600  1.900  1.900
assign (segid A and residue 233 and name HD2#) (segid A and residue 233 and name HB#)      3.600  1.900  1.900
assign (segid A and residue 233 and name HB#) (segid A and residue 234 and name HA)       3.350  1.650  1.650
assign (segid A and residue 234 and name HA) (segid A and residue 234 and name HB)        3.350  1.650  1.650
assign (segid A and residue 234 and name HA) (segid A and residue 237 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 234 and name HA) (segid A and residue 237 and name HG)        3.350  1.650  1.650
assign (segid A and residue 234 and name HA) (segid A and residue 266 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 234 and name HD1#) (segid A and residue 266 and name HG1#)    3.850  2.150  2.150
assign (segid A and residue 234 and name HD1#) (segid A and residue 266 and name HG2#)    3.850  2.150  2.150
assign (segid A and residue 233 and name HG) (segid A and residue 234 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 234 and name HG2#) (segid A and residue 234 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 234 and name HG2#) (segid A and residue 237 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 234 and name HG2#) (segid A and residue 237 and name HD2#)    3.850  2.150  2.150
assign (segid A and residue 234 and name HG2#) (segid A and residue 266 and name HG1#)    3.850  2.150  2.150
assign (segid A and residue 234 and name HG2#) (segid A and residue 266 and name HG2#)    3.850  2.150  2.150
assign (segid A and residue 235 and name HA) (segid A and residue 238 and name HB#)       3.600  1.900  1.900
assign (segid A and residue 236 and name HA) (segid A and residue 239 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 237 and name HB#) (segid A and residue 237 and name HG)       3.350  1.650  1.650
assign (segid A and residue 215 and name HD1#) (segid A and residue 237 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 215 and name HD2#) (segid A and residue 237 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 219 and name HD1#) (segid A and residue 237 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 234 and name HD1#) (segid A and residue 237 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 237 and name HD1#) (segid A and residue 263 and name HG2#)    3.850  2.150  2.150
assign (segid A and residue 215 and name HD2#) (segid A and residue 237 and name HD2#)    3.850  2.150  2.150
assign (segid A and residue 219 and name HD1#) (segid A and residue 237 and name HD2#)    3.850  2.150  2.150
assign (segid A and residue 234 and name HB) (segid A and residue 237 and name HD2#)      3.600  1.900  1.900
assign (segid A and residue 234 and name HD1#) (segid A and residue 237 and name HD2#)    3.850  2.150  2.150
assign (segid A and residue 237 and name HD2#) (segid A and residue 237 and name HB#)     3.600  1.900  1.900
assign (segid A and residue 237 and name HD2#) (segid A and residue 237 and name HD1#)    3.850  2.150  2.150
assign (segid A and residue 237 and name HD2#) (segid A and residue 238 and name HB#)     3.850  2.150  2.150
assign (segid A and residue 238 and name HA) (segid A and residue 238 and name HB#)       3.600  1.900  1.900
assign (segid A and residue 238 and name HA) (segid A and residue 263 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 238 and name HA) (segid A and residue 263 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 238 and name HA) (segid A and residue 266 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 234 and name HD1#) (segid A and residue 238 and name HB#)     3.850  2.150  2.150
assign (segid A and residue 237 and name HB#) (segid A and residue 238 and name HB#)      3.350  1.650  1.650
assign (segid A and residue 237 and name HD1#) (segid A and residue 238 and name HB#)     3.850  2.150  2.150
assign (segid A and residue 237 and name HG) (segid A and residue 238 and name HB#)       3.600  1.900  1.900
assign (segid A and residue 238 and name HB#) (segid A and residue 239 and name HA)       3.600  1.900  1.900
assign (segid A and residue 238 and name HB#) (segid A and residue 239 and name HD#)      3.350  1.650  1.650
assign (segid A and residue 238 and name HB#) (segid A and residue 263 and name HG2#)     3.850  2.150  2.150
assign (segid A and residue 238 and name HB#) (segid A and residue 266 and name HB)       3.600  1.900  1.900
assign (segid A and residue 238 and name HB#) (segid A and residue 275 and name HD1#)     3.850  2.150  2.150
assign (segid A and residue 244 and name HA) (segid A and residue 247 and name HD2#)      3.600  1.900  1.900
```

```
assign (segid A and residue 244 and name HA) (segid A and residue 247 and name HG)         3.350  1.650  1.650
assign (segid A and residue 243 and name HA) (segid A and residue 246 and name HD2#)       3.600  1.900  1.900
assign (segid A and residue 246 and name HD2#) (segid A and residue 246 and name HD1#)     3.850  2.150  2.150
assign (segid A and residue 245 and name HA) (segid A and residue 248 and name HG2#)       3.600  1.900  1.900
assign (segid A and residue 247 and name HG) (segid A and residue 248 and name HG2#)       3.600  1.900  1.900
assign (segid A and residue 248 and name HG2#) (segid A and residue 248 and name HB)       3.600  1.900  1.900
assign (segid A and residue 248 and name HG2#) (segid A and residue 248 and name HG1#)     3.850  2.150  2.150
assign (segid A and residue 248 and name HG2#) (segid A and residue 251 and name HA)       3.600  1.900  1.900
assign (segid A and residue 248 and name HG2#) (segid A and residue 253 and name HA)       3.600  1.900  1.900
assign (segid A and residue 248 and name HG2#) (segid A and residue 253 and name HB#)      3.350  1.650  1.650
assign (segid A and residue 248 and name HG2#) (segid A and residue 256 and name HG1#)     3.850  2.150  2.150
assign (segid A and residue 248 and name HG2#) (segid A and residue 259 and name HD1#)     3.850  2.150  2.150
assign (segid A and residue 250 and name HA) (segid A and residue 250 and name HB#)        2.600  0.900  0.900
assign (segid A and residue 248 and name HG1#) (segid A and residue 256 and name HA)       3.600  1.900  1.900
assign (segid A and residue 248 and name HG2#) (segid A and residue 256 and name HA)       3.600  1.900  1.900
assign (segid A and residue 256 and name HA) (segid A and residue 259 and name HD1#)       3.600  1.900  1.900
assign (segid A and residue 251 and name HA) (segid A and residue 256 and name HG2#)       3.600  1.900  1.900
assign (segid A and residue 255 and name HA) (segid A and residue 258 and name HB#)        3.600  1.900  1.900
assign (segid A and residue 258 and name HB#) (segid A and residue 262 and name HG)        3.600  1.900  1.900
assign (segid A and residue 259 and name HA) (segid A and residue 262 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 256 and name HG1#) (segid A and residue 259 and name HD1#)     3.850  2.150  2.150
assign (segid A and residue 256 and name HG2#) (segid A and residue 259 and name HD1#)     3.850  2.150  2.150
assign (segid A and residue 259 and name HD1#) (segid A and residue 259 and name HD2#)     3.850  2.150  2.150
assign (segid A and residue 259 and name HD1#) (segid A and residue 259 and name HG)       3.600  1.900  1.900
assign (segid A and residue 241 and name HB#) (segid A and residue 263 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 259 and name HD2#) (segid A and residue 263 and name HD1#)     3.850  2.150  2.150
assign (segid A and residue 260 and name HA) (segid A and residue 263 and name HD1#)       3.600  1.900  1.900
assign (segid A and residue 263 and name HD1#) (segid A and residue 263 and name HA)       3.600  1.900  1.900
assign (segid A and residue 263 and name HG2#) (segid A and residue 263 and name HG1#)     3.600  1.900  1.900
assign (segid A and residue 263 and name HG2#) (segid A and residue 266 and name HG2#)     3.850  2.150  2.150
assign (segid A and residue 263 and name HG2#) (segid A and residue 275 and name HD1#)     3.850  2.150  2.150
assign (segid A and residue 263 and name HG2#) (segid A and residue 276 and name HA)       3.600  1.900  1.900
assign (segid A and residue 263 and name HG2#) (segid A and residue 276 and name HB#)      3.850  2.150  2.150
assign (segid A and residue 264 and name HB#) (segid A and residue 268 and name HD#)       3.350  1.650  1.650
assign (segid B and residue 42 and name HB#) (segid A and residue 265 and name HD1#)       3.850  2.150  2.150
assign (segid B and residue 46 and name HB#) (segid A and residue 265 and name HD1#)       3.850  2.150  2.150
assign (segid A and residue 215 and name HD2#) (segid A and residue 265 and name HD1#)     3.850  2.150  2.150
assign (segid A and residue 265 and name HD1#) (segid A and residue 265 and name HA)       3.600  1.900  1.900
assign (segid A and residue 265 and name HD1#) (segid A and residue 266 and name HG1#)     3.850  2.150  2.150
assign (segid A and residue 215 and name HD2#) (segid A and residue 265 and name HD2#)     3.850  2.150  2.150
assign (segid A and residue 219 and name HD1#) (segid A and residue 265 and name HD2#)     3.850  2.150  2.150
assign (segid A and residue 266 and name HG1#) (segid A and residue 266 and name HA)       3.600  1.900  1.900
assign (segid A and residue 271 and name HB#) (segid A and residue 271 and name HA)        2.600  0.900  0.900
assign (segid A and residue 238 and name HB#) (segid A and residue 275 and name HD2#)      3.850  2.150  2.150
```

```
assign (segid A and residue 239 and name HA) (segid A and residue 275 and name HD2#)       3.600  1.900  1.900
assign (segid A and residue 239 and name HB#) (segid A and residue 275 and name HD2#)      3.350  1.650  1.650
assign (segid A and residue 239 and name HD#) (segid A and residue 275 and name HD2#)      3.350  1.650  1.650
assign (segid A and residue 239 and name HG#) (segid A and residue 275 and name HD2#)      3.600  1.900  1.900
assign (segid A and residue 263 and name HG2#) (segid A and residue 275 and name HD2#)     3.850  2.150  2.150
assign (segid A and residue 275 and name HD2#) (segid A and residue 275 and name HA)       3.600  1.900  1.900
assign (segid A and residue 275 and name HD2#) (segid A and residue 275 and name HB#)      3.350  1.650  1.650
assign (segid A and residue 275 and name HD2#) (segid A and residue 275 and name HG)       3.600  1.900  1.900
assign (segid A and residue 275 and name HD2#) (segid A and residue 276 and name HB#)      3.850  2.150  2.150
assign (segid A and residue 267 and name HA) (segid A and residue 276 and name HA)        3.350  1.650  1.650
assign (segid A and residue 267 and name HB#) (segid A and residue 276 and name HA)       3.350  1.650  1.650
assign (segid A and residue 267 and name HA) (segid A and residue 276 and name HB#)       3.600  1.900  1.900
assign (segid A and residue 267 and name HB#) (segid A and residue 276 and name HB#)      3.350  1.650  1.650
assign (segid A and residue 268 and name HA) (segid A and residue 276 and name HB#)       3.600  1.900  1.900
assign (segid A and residue 268 and name HG#) (segid A and residue 276 and name HB#)      3.350  1.650  1.650
assign (segid A and residue 273 and name HA) (segid A and residue 276 and name HB#)       3.600  1.900  1.900
assign (segid A and residue 273 and name HB#) (segid A and residue 276 and name HB#)      3.350  1.650  1.650
assign (segid A and residue 273 and name HD#) (segid A and residue 276 and name HB#)      3.350  1.650  1.650
assign (segid A and residue 273 and name HG#) (segid A and residue 276 and name HB#)      3.350  1.650  1.650
assign (segid A and residue 275 and name HD1#) (segid A and residue 276 and name HB#)     3.850  2.150  2.150
assign (segid A and residue 278 and name HA) (segid A and residue 278 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 278 and name HA) (segid A and residue 278 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 278 and name HA) (segid A and residue 279 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 278 and name HA) (segid A and residue 279 and name HD#)       2.600  0.900  0.900
assign (segid A and residue 278 and name HB#) (segid A and residue 279 and name HD#)      3.350  1.650  1.650
assign (segid A and residue 278 and name HG#) (segid A and residue 279 and name HD#)      3.350  1.650  1.650
assign (segid A and residue 278 and name HA) (segid A and residue 279 and name HD#)       3.350  1.650  1.650
assign (segid A and residue 281 and name HA) (segid A and residue 281 and name HB#)       2.600  0.900  0.900
assign (segid A and residue 281 and name HA) (segid A and residue 281 and name HD2#)      2.900  1.200  1.200
assign (segid A and residue 281 and name HA) (segid A and residue 281 and name HG)        2.600  0.900  0.900
assign (segid A and residue 281 and name HD2#) (segid A and residue 281 and name HA)      2.900  1.200  1.200
assign (segid A and residue 281 and name HD2#) (segid A and residue 281 and name HG)      2.900  1.200  1.200
assign (segid A and residue 279 and name HG#) (segid A and residue 282 and name HB#)      3.350  1.650  1.650
assign (segid A and residue 282 and name HB#) (segid A and residue 282 and name HA)       2.600  0.900  0.900
assign (segid A and residue 282 and name HB#) (segid A and residue 282 and name HG#)      2.600  0.900  0.900
assign (segid A and residue 282 and name HB#) (segid A and residue 282 and name HA)       2.600  0.900  0.900
assign (segid A and residue 283 and name HA) (segid A and residue 283 and name HB#)       2.600  0.900  0.900
assign (segid A and residue 283 and name HA) (segid A and residue 283 and name HB#)       2.600  0.900  0.900
assign (segid B and residue 46 and name HB#) (segid A and residue 262 and name HD2#)      3.85 2.15 2.15
assign (segid A and residue 261 and name HD1#) (segid A and residue 265 and name HD1#)    3.85 2.15 2.15
assign (segid A and residue 262 and name HD1#) (segid A and residue 265 and name HD1#)    3.85 2.15 2.15
assign (segid A and residue 238 and name HB#) (segid A and residue 262 and name HD1#)     3.85 2.15 2.15
assign (segid B and residue 46 and name HB#) (segid A and residue 262 and name HD1#)      3.85 2.15 2.15
assign (segid A and residue 258 and name HB#) (segid A and residue 262 and name HD1#)     3.85 2.15 2.15
```

```
assign (segid A and residue 262 and name HD1#) (segid A and residue 266 and name HG1#)     3.85 2.15 2.15
assign (segid A and residue 262 and name HD1#) (segid A and residue 262 and name HD2#)     3.85 2.15 2.15
assign (segid A and residue 262 and name HD1#) (segid A and residue 275 and name HD2#)     3.85 2.15 2.15
assign (segid B and residue 46 and name HB#) (segid A and residue 109 and name HG#)       3.60 1.90 1.90
assign (segid B and residue 46 and name HB#) (segid A and residue 106 and name HE22)      3.60 1.90 1.90
assign (segid A and residue 100 and name HD#) (segid A and residue 104 and name HE2#)     3.60 1.90 1.90
assign (segid A and residue 100 and name HB#) (segid A and residue 104 and name HE2#)     3.35 1.65 1.65
assign (segid A and residue 104 and name HE2#) (segid A and residue 108 and name HB#)     3.60 1.90 1.90
assign (segid A and residue 100 and name HG) (segid A and residue 104 and name HE2#)      3.35 1.65 1.65
assign (segid A and residue 135 and name HE#) (segid A and residue 190 and name HG2#)     3.85 2.15 2.15
assign (segid A and residue 186 and name HA) (segid A and residue 190 and name HG2#)      3.60 1.90 1.90
assign (segid A and residue 186 and name HB#) (segid A and residue 190 and name HG2#)     3.60 1.90 1.90
assign (segid A and residue 149 and name HD2#) (segid A and residue 211 and name HD1#)    3.85 2.15 2.15
assign (segid A and residue 149 and name HG) (segid A and residue 194 and name HG1#)      3.60 1.90 1.90
assign (segid B and residue 34 and name HA) (segid B and residue 35 and name HN)          3.35 1.65 1.65
assign (segid B and residue 35 and name HN) (segid B and residue 38 and name HB#)         3.60 1.90 1.90
```

From the 4D HNCH NOESY-HSQC

```
assign (segid B and residue 29 and name HA) (segid B and residue 30 and name HN)          2.350  0.650  0.650
assign (segid B and residue 30 and name HA) (segid B and residue 31 and name HN)          2.350  0.650  0.650
assign (segid B and residue 30 and name HB#) (segid B and residue 31 and name HN)         2.350  0.650  0.650
assign (segid B and residue 31 and name HN) (segid B and residue 31 and name HA)          2.350  0.650  0.650
assign (segid B and residue 31 and name HN) (segid B and residue 31 and name HB#)         2.350  0.650  0.650
assign (segid B and residue 31 and name HB#) (segid B and residue 32 and name HN)         2.350  0.650  0.650
assign (segid B and residue 32 and name HN) (segid B and residue 32 and name HB#)         2.350  0.650  0.650
assign (segid B and residue 32 and name HN) (segid B and residue 33 and name HA)          3.350  1.650  1.650
assign (segid B and residue 32 and name HN) (segid B and residue 33 and name HD1#)        3.600  1.900  1.900
assign (segid B and residue 32 and name HN) (segid B and residue 33 and name HG1#)        3.350  1.650  1.650
assign (segid B and residue 32 and name HN) (segid B and residue 33 and name HG2#)        3.600  1.900  1.900
assign (segid B and residue 31 and name HA) (segid B and residue 33 and name HN)          3.350  1.650  1.650
assign (segid B and residue 31 and name HB#) (segid B and residue 33 and name HN)         3.350  1.650  1.650
assign (segid B and residue 33 and name HN) (segid B and residue 33 and name HA)          2.350  0.650  0.650
assign (segid B and residue 33 and name HN) (segid B and residue 33 and name HD1#)        3.600  1.900  1.900
assign (segid B and residue 33 and name HN) (segid B and residue 33 and name HG2#)        2.600  0.900  0.900
assign (segid B and residue 32 and name HA) (segid B and residue 34 and name HN)          3.350  1.650  1.650
assign (segid B and residue 32 and name HB#) (segid B and residue 34 and name HN)         3.350  1.650  1.650
assign (segid B and residue 34 and name HN) (segid B and residue 34 and name HA)          3.350  1.650  1.650
assign (segid B and residue 34 and name HN) (segid B and residue 34 and name HB#)         3.350  1.650  1.650
assign (segid B and residue 36 and name HB#) (segid B and residue 37 and name HN)         3.350  1.650  1.650
assign (segid B and residue 37 and name HN) (segid B and residue 37 and name HA)          3.350  1.650  1.650
```

```
assign (segid B and residue 37 and name HN) (segid B and residue 37 and name HG2#)         3.600  1.900  1.900
assign (segid B and residue 37 and name HB) (segid B and residue 38 and name HN)          3.350  1.650  1.650
assign (segid B and residue 37 and name HG2#) (segid B and residue 38 and name HN)        3.600  1.900  1.900
assign (segid B and residue 38 and name HN) (segid B and residue 38 and name HA)          2.350  0.650  0.650
assign (segid B and residue 38 and name HN) (segid B and residue 38 and name HB#)         3.600  1.900  1.900
assign (segid B and residue 38 and name HN) (segid A and residue 180 and name HD1#)       3.600  1.900  1.900
assign (segid B and residue 38 and name HA) (segid B and residue 39 and name HN)          3.350  1.650  1.650
assign (segid B and residue 39 and name HN) (segid B and residue 39 and name HA)          3.350  1.650  1.650
assign (segid B and residue 39 and name HN) (segid B and residue 39 and name HB#)         3.350  1.650  1.650
assign (segid B and residue 39 and name HN) (segid B and residue 39 and name HD1#)       3.600  1.900  1.900
assign (segid B and residue 39 and name HN) (segid B and residue 39 and name HD2#)       3.600  1.900  1.900
assign (segid B and residue 39 and name HN) (segid B and residue 39 and name HG)          3.350  1.650  1.650
assign (segid B and residue 39 and name HN) (segid B and residue 42 and name HB#)         3.600  1.900  1.900
assign (segid B and residue 37 and name HG2#) (segid B and residue 40 and name HN)        3.600  1.900  1.900
assign (segid B and residue 40 and name HN) (segid B and residue 40 and name HA)          3.350  1.650  1.650
assign (segid B and residue 40 and name HN) (segid B and residue 40 and name HB)          3.350  1.650  1.650
assign (segid B and residue 40 and name HN) (segid B and residue 40 and name HD1#)       3.600  1.900  1.900
assign (segid B and residue 40 and name HN) (segid B and residue 40 and name HG1#)       3.350  1.650  1.650
assign (segid B and residue 40 and name HN) (segid B and residue 40 and name HG2#)       3.600  1.900  1.900
assign (segid B and residue 41 and name HN) (segid B and residue 41 and name HA)          2.350  0.650  0.650
assign (segid B and residue 41 and name HN) (segid B and residue 41 and name HB#)         3.350  1.650  1.650
assign (segid B and residue 41 and name HN) (segid B and residue 41 and name HD#)         3.350  1.650  1.650
assign (segid B and residue 41 and name HN) (segid B and residue 41 and name HE#)         3.350  1.650  1.650
assign (segid B and residue 41 and name HN) (segid B and residue 41 and name HG#)         3.350  1.650  1.650
assign (segid B and residue 38 and name HA) (segid B and residue 42 and name HN)          3.350  1.650  1.650
assign (segid B and residue 41 and name HA) (segid B and residue 42 and name HN)          3.350  1.650  1.650
assign (segid B and residue 41 and name HB#) (segid B and residue 42 and name HN)         3.350  1.650  1.650
assign (segid B and residue 41 and name HD#) (segid B and residue 42 and name HN)         3.350  1.650  1.650
assign (segid B and residue 41 and name HE#) (segid B and residue 42 and name HN)         3.350  1.650  1.650
assign (segid B and residue 41 and name HG#) (segid B and residue 42 and name HN)         3.350  1.650  1.650
assign (segid B and residue 42 and name HN) (segid B and residue 42 and name HA)          2.350  0.650  0.650
assign (segid B and residue 42 and name HN) (segid B and residue 42 and name HB#)         2.600  0.900  0.900
assign (segid B and residue 43 and name HN) (segid B and residue 43 and name HA)          2.350  0.650  0.650
assign (segid B and residue 43 and name HN) (segid B and residue 43 and name HB#)         3.350  1.650  1.650
assign (segid B and residue 44 and name HN) (segid B and residue 44 and name HA)          2.350  0.650  0.650
assign (segid B and residue 44 and name HN) (segid B and residue 44 and name HB#)         2.350  0.650  0.650
assign (segid B and residue 42 and name HA) (segid B and residue 45 and name HN)          3.350  1.650  1.650
assign (segid B and residue 45 and name HN) (segid B and residue 45 and name HA)          2.350  0.650  0.650
assign (segid B and residue 45 and name HN) (segid B and residue 45 and name HB#)         2.350  0.650  0.650
assign (segid B and residue 45 and name HN) (segid B and residue 45 and name HD#)         3.350  1.650  1.650
assign (segid B and residue 45 and name HN) (segid B and residue 45 and name HE#)         3.350  1.650  1.650
assign (segid B and residue 45 and name HN) (segid B and residue 45 and name HG#)         3.350  1.650  1.650
assign (segid B and residue 45 and name HE#) (segid B and residue 46 and name HN)         3.350  1.650  1.650
assign (segid B and residue 45 and name HG#) (segid B and residue 46 and name HN)         3.350  1.650  1.650
```

```
assign (segid B and residue 46 and name HN) (segid B and residue 46 and name HA)          2.350  0.650  0.650
assign (segid B and residue 46 and name HN) (segid B and residue 46 and name HB#)         2.600  0.900  0.900
assign (segid B and residue 44 and name HA) (segid B and residue 47 and name HN)          3.350  1.650  1.650
assign (segid B and residue 46 and name HA) (segid B and residue 47 and name HN)          3.350  1.650  1.650
assign (segid B and residue 47 and name HN) (segid B and residue 47 and name HA)          3.350  1.650  1.650
assign (segid B and residue 47 and name HN) (segid B and residue 47 and name HB)          3.350  1.650  1.650
assign (segid B and residue 47 and name HN) (segid B and residue 47 and name HG2#)        2.600  0.900  0.900
assign (segid B and residue 48 and name HN) (segid B and residue 48 and name HA)          2.350  0.650  0.650
assign (segid B and residue 48 and name HN) (segid B and residue 48 and name HB#)         2.600  0.900  0.900
assign (segid B and residue 45 and name HA) (segid B and residue 49 and name HN)          3.350  1.650  1.650
assign (segid B and residue 46 and name HA) (segid B and residue 49 and name HN)          3.350  1.650  1.650
assign (segid B and residue 47 and name HG2#) (segid B and residue 49 and name HN)        3.600  1.900  1.900
assign (segid B and residue 48 and name HB#) (segid B and residue 49 and name HN)         3.600  1.900  1.900
assign (segid B and residue 49 and name HN) (segid B and residue 49 and name HA)          2.350  0.650  0.650
assign (segid B and residue 49 and name HN) (segid B and residue 50 and name HB#)         3.350  1.650  1.650
assign (segid B and residue 48 and name HA) (segid B and residue 50 and name HN)          3.350  1.650  1.650
assign (segid B and residue 50 and name HN) (segid B and residue 50 and name HA)          2.350  0.650  0.650
assign (segid B and residue 50 and name HN) (segid B and residue 50 and name HB#)         2.350  0.650  0.650
assign (segid B and residue 50 and name HN) (segid B and residue 51 and name HG#)         3.350  1.650  1.650
assign (segid B and residue 48 and name HA) (segid B and residue 51 and name HN)          3.350  1.650  1.650
assign (segid B and residue 48 and name HB#) (segid B and residue 51 and name HN)         3.600  1.900  1.900
assign (segid B and residue 49 and name HA) (segid B and residue 51 and name HN)          3.350  1.650  1.650
assign (segid B and residue 50 and name HB#) (segid B and residue 51 and name HN)         2.350  0.650  0.650
assign (segid B and residue 51 and name HN) (segid B and residue 51 and name HA)          2.350  0.650  0.650
assign (segid B and residue 51 and name HN) (segid B and residue 51 and name HD#)         3.350  1.650  1.650
assign (segid B and residue 51 and name HN) (segid B and residue 51 and name HE#)         3.350  1.650  1.650
assign (segid B and residue 51 and name HN) (segid B and residue 51 and name HG#)         2.350  0.650  0.650
assign (segid A and residue 95 and name HA#) (segid A and residue 97 and name HN)         3.350  1.650  1.650
assign (segid A and residue 96 and name HB#) (segid A and residue 97 and name HN)         3.350  1.650  1.650
assign (segid A and residue 96 and name HE#) (segid A and residue 97 and name HN)         3.350  1.650  1.650
assign (segid A and residue 97 and name HN) (segid A and residue 97 and name HB#)         2.350  0.650  0.650
assign (segid A and residue 97 and name HN) (segid A and residue 98 and name HD#)         3.350  1.650  1.650
assign (segid A and residue 97 and name HN) (segid A and residue 98 and name HG#)         3.350  1.650  1.650
assign (segid A and residue 98 and name HB#) (segid A and residue 99 and name HN)         3.350  1.650  1.650
assign (segid A and residue 98 and name HG#) (segid A and residue 99 and name HN)         3.350  1.650  1.650
assign (segid A and residue 99 and name HN) (segid A and residue 99 and name HA)          3.350  1.650  1.650
assign (segid A and residue 99 and name HN) (segid A and residue 99 and name HB)          3.350  1.650  1.650
assign (segid A and residue 99 and name HN) (segid A and residue 99 and name HG2#)        3.600  1.900  1.900
assign (segid A and residue 98 and name HB#) (segid A and residue 100 and name HN)        3.350  1.650  1.650
assign (segid A and residue 98 and name HG#) (segid A and residue 100 and name HN)        3.350  1.650  1.650
assign (segid A and residue 99 and name HB) (segid A and residue 100 and name HN)         3.350  1.650  1.650
assign (segid A and residue 100 and name HN) (segid A and residue 100 and name HA)        3.350  1.650  1.650
assign (segid A and residue 100 and name HN) (segid A and residue 100 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 100 and name HN) (segid A and residue 100 and name HG)        3.350  1.650  1.650
```

```
assign (segid A and residue 100 and name HN) (segid A and residue 101 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 99 and name HG2#) (segid A and residue 101 and name HN)        3.600  1.900  1.900
assign (segid A and residue 100 and name HB#) (segid A and residue 101 and name HN)        3.350  1.650  1.650
assign (segid A and residue 100 and name HG) (segid A and residue 101 and name HN)         3.350  1.650  1.650
assign (segid A and residue 101 and name HN) (segid A and residue 101 and name HA)         2.350  0.650  0.650
assign (segid A and residue 101 and name HN) (segid A and residue 101 and name HG#)        2.350  0.650  0.650
assign (segid A and residue 101 and name HB#) (segid A and residue 102 and name HN)        2.350  0.650  0.650
assign (segid A and residue 101 and name HG#) (segid A and residue 102 and name HN)        3.350  1.650  1.650
assign (segid A and residue 102 and name HN) (segid A and residue 102 and name HA)         2.350  0.650  0.650
assign (segid A and residue 102 and name HN) (segid A and residue 102 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 102 and name HA) (segid A and residue 103 and name HN)         3.350  1.650  1.650
assign (segid A and residue 103 and name HN) (segid A and residue 103 and name HA)         3.350  1.650  1.650
assign (segid A and residue 103 and name HN) (segid A and residue 103 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 103 and name HN) (segid A and residue 103 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 100 and name HB#) (segid A and residue 104 and name HN)        3.350  1.650  1.650
assign (segid A and residue 103 and name HA) (segid A and residue 104 and name HN)         3.350  1.650  1.650
assign (segid A and residue 104 and name HN) (segid A and residue 104 and name HA)         2.350  0.650  0.650
assign (segid A and residue 104 and name HN) (segid A and residue 104 and name HB#)        2.350  0.650  0.650
assign (segid A and residue 104 and name HN) (segid A and residue 104 and name HG#)        2.350  0.650  0.650
assign (segid A and residue 102 and name HA) (segid A and residue 105 and name HN)         3.350  1.650  1.650
assign (segid A and residue 105 and name HN) (segid A and residue 105 and name HA)         2.350  0.650  0.650
assign (segid A and residue 105 and name HN) (segid A and residue 105 and name HB#)        2.350  0.650  0.650
assign (segid A and residue 105 and name HN) (segid A and residue 105 and name HG#)        2.350  0.650  0.650
assign (segid A and residue 105 and name HB#) (segid A and residue 106 and name HN)        3.350  1.650  1.650
assign (segid A and residue 105 and name HG#) (segid A and residue 106 and name HN)        3.350  1.650  1.650
assign (segid A and residue 106 and name HN) (segid A and residue 106 and name HA)         2.350  0.650  0.650
assign (segid A and residue 106 and name HN) (segid A and residue 106 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 106 and name HN) (segid A and residue 106 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 106 and name HN) (segid A and residue 107 and name HG1#)       3.600  1.900  1.900
assign (segid A and residue 106 and name HG#) (segid A and residue 107 and name HN)        3.350  1.650  1.650
assign (segid A and residue 107 and name HN) (segid A and residue 107 and name HA)         3.350  1.650  1.650
assign (segid A and residue 107 and name HN) (segid A and residue 107 and name HB)         3.350  1.650  1.650
assign (segid A and residue 107 and name HN) (segid A and residue 107 and name HG1#)       2.600  0.900  0.900
assign (segid A and residue 107 and name HN) (segid A and residue 107 and name HG2#)       3.600  1.900  1.900
assign (segid A and residue 108 and name HN) (segid A and residue 108 and name HB#)        2.600  0.900  0.900
assign (segid A and residue 109 and name HN) (segid A and residue 109 and name HA)         2.350  0.650  0.650
assign (segid A and residue 109 and name HN) (segid A and residue 109 and name HB#)        2.350  0.650  0.650
assign (segid A and residue 109 and name HN) (segid A and residue 112 and name HB)         3.350  1.650  1.650
assign (segid A and residue 109 and name HA) (segid A and residue 110 and name HN)         3.350  1.650  1.650
assign (segid A and residue 110 and name HN) (segid A and residue 110 and name HA)         3.350  1.650  1.650
assign (segid A and residue 110 and name HN) (segid A and residue 110 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 110 and name HN) (segid A and residue 112 and name HG2#)       3.600  1.900  1.900
assign (segid A and residue 110 and name HN) (segid A and residue 265 and name HD2#)       3.600  1.900  1.900
assign (segid A and residue 107 and name HA) (segid A and residue 111 and name HN)         3.350  1.650  1.650
```

```
assign (segid A and residue 107 and name HG1#) (segid A and residue 111 and name HN)       3.600  1.900  1.900
assign (segid A and residue 107 and name HG2#) (segid A and residue 111 and name HN)       3.600  1.900  1.900
assign (segid A and residue 108 and name HA) (segid A and residue 111 and name HN)        3.350  1.650  1.650
assign (segid A and residue 108 and name HB#) (segid A and residue 111 and name HN)       3.600  1.900  1.900
assign (segid A and residue 109 and name HB#) (segid A and residue 111 and name HN)       3.350  1.650  1.650
assign (segid A and residue 110 and name HA) (segid A and residue 111 and name HN)        3.350  1.650  1.650
assign (segid A and residue 110 and name HB#) (segid A and residue 111 and name HN)       3.350  1.650  1.650
assign (segid A and residue 111 and name HN) (segid A and residue 111 and name HA)        3.350  1.650  1.650
assign (segid A and residue 111 and name HN) (segid A and residue 111 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 108 and name HB#) (segid A and residue 112 and name HN)       3.600  1.900  1.900
assign (segid A and residue 109 and name HA) (segid A and residue 112 and name HN)        3.350  1.650  1.650
assign (segid A and residue 112 and name HN) (segid A and residue 112 and name HA)        2.350  0.650  0.650
assign (segid A and residue 112 and name HN) (segid A and residue 112 and name HB)        2.350  0.650  0.650
assign (segid A and residue 112 and name HN) (segid A and residue 112 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 112 and name HN) (segid A and residue 113 and name HB)        3.350  1.650  1.650
assign (segid A and residue 112 and name HN) (segid A and residue 115 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 109 and name HB#) (segid A and residue 113 and name HN)       3.350  1.650  1.650
assign (segid A and residue 109 and name HG#) (segid A and residue 113 and name HN)       3.350  1.650  1.650
assign (segid A and residue 113 and name HN) (segid A and residue 113 and name HA)        3.350  1.650  1.650
assign (segid A and residue 113 and name HN) (segid A and residue 113 and name HB)        3.350  1.650  1.650
assign (segid A and residue 113 and name HN) (segid A and residue 113 and name HG1#)      3.600  1.900  1.900
assign (segid A and residue 113 and name HN) (segid A and residue 113 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 113 and name HB) (segid A and residue 114 and name HN)        3.350  1.650  1.650
assign (segid A and residue 113 and name HG1#) (segid A and residue 114 and name HN)      3.600  1.900  1.900
assign (segid A and residue 114 and name HN) (segid A and residue 114 and name HA)        3.350  1.650  1.650
assign (segid A and residue 114 and name HN) (segid A and residue 114 and name HD#)       3.350  1.650  1.650
assign (segid A and residue 114 and name HN) (segid A and residue 114 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 112 and name HA) (segid A and residue 115 and name HN)        3.350  1.650  1.650
assign (segid A and residue 113 and name HA) (segid A and residue 116 and name HN)        3.350  1.650  1.650
assign (segid A and residue 115 and name HB#) (segid A and residue 116 and name HN)       2.350  0.650  0.650
assign (segid A and residue 115 and name HG#) (segid A and residue 116 and name HN)       3.350  1.650  1.650
assign (segid A and residue 116 and name HN) (segid A and residue 116 and name HA)        2.350  0.650  0.650
assign (segid A and residue 116 and name HN) (segid A and residue 116 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 116 and name HN) (segid A and residue 118 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 114 and name HA) (segid A and residue 117 and name HN)        3.350  1.650  1.650
assign (segid A and residue 116 and name HB#) (segid A and residue 117 and name HN)       3.350  1.650  1.650
assign (segid A and residue 117 and name HN) (segid A and residue 117 and name HA)        3.350  1.650  1.650
assign (segid A and residue 117 and name HN) (segid A and residue 117 and name HB)        3.350  1.650  1.650
assign (segid A and residue 117 and name HN) (segid A and residue 117 and name HG1#)      3.600  1.900  1.900
assign (segid A and residue 117 and name HN) (segid A and residue 117 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 117 and name HN) (segid A and residue 118 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 114 and name HA) (segid A and residue 118 and name HN)        3.350  1.650  1.650
assign (segid A and residue 115 and name HA) (segid A and residue 118 and name HN)        3.350  1.650  1.650
assign (segid A and residue 117 and name HB) (segid A and residue 118 and name HN)        3.350  1.650  1.650
```

```
assign (segid A and residue 117 and name HG1#) (segid A and residue 118 and name HN)       3.600  1.900  1.900
assign (segid A and residue 117 and name HG2#) (segid A and residue 118 and name HN)       3.600  1.900  1.900
assign (segid A and residue 118 and name HN) (segid A and residue 118 and name HA)        2.350  0.650  0.650
assign (segid A and residue 118 and name HN) (segid A and residue 118 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 118 and name HN) (segid A and residue 119 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 116 and name HA) (segid A and residue 119 and name HN)        3.350  1.650  1.650
assign (segid A and residue 119 and name HN) (segid A and residue 119 and name HD#)       3.350  1.650  1.650
assign (segid A and residue 119 and name HN) (segid A and residue 119 and name HG#)       2.350  0.650  0.650
assign (segid A and residue 116 and name HA) (segid A and residue 120 and name HN)        3.350  1.650  1.650
assign (segid A and residue 117 and name HA) (segid A and residue 120 and name HN)        3.350  1.650  1.650
assign (segid A and residue 117 and name HG2#) (segid A and residue 120 and name HN)      3.600  1.900  1.900
assign (segid A and residue 119 and name HD#) (segid A and residue 120 and name HN)       3.350  1.650  1.650
assign (segid A and residue 119 and name HE#) (segid A and residue 120 and name HN)       3.350  1.650  1.650
assign (segid A and residue 120 and name HN) (segid A and residue 120 and name HA)        3.350  1.650  1.650
assign (segid A and residue 120 and name HN) (segid A and residue 120 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 120 and name HB#) (segid A and residue 121 and name HN)       3.350  1.650  1.650
assign (segid A and residue 121 and name HN) (segid A and residue 121 and name HA)        3.350  1.650  1.650
assign (segid A and residue 121 and name HN) (segid A and residue 121 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 121 and name HN) (segid A and residue 121 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 119 and name HA) (segid A and residue 122 and name HN)        3.350  1.650  1.650
assign (segid A and residue 121 and name HB#) (segid A and residue 122 and name HN)       3.350  1.650  1.650
assign (segid A and residue 121 and name HD#) (segid A and residue 122 and name HN)       3.350  1.650  1.650
assign (segid A and residue 121 and name HG#) (segid A and residue 122 and name HN) 3.350  1.650  1.650
assign (segid A and residue 122 and name HN) (segid A and residue 122 and name HA)        2.350  0.650  0.650
assign (segid A and residue 122 and name HN) (segid A and residue 122 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 122 and name HN) (segid A and residue 125 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 120 and name HA) (segid A and residue 123 and name HN)        3.350  1.650  1.650
assign (segid A and residue 122 and name HA) (segid A and residue 123 and name HN)        3.350  1.650  1.650
assign (segid A and residue 122 and name HB#) (segid A and residue 123 and name HN)       2.350  0.650  0.650
assign (segid A and residue 123 and name HN) (segid A and residue 123 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 124 and name HN) (segid A and residue 124 and name HA)        3.350  1.650  1.650
assign (segid A and residue 124 and name HB#) (segid A and residue 125 and name HN)       3.350  1.650  1.650
assign (segid A and residue 125 and name HN) (segid A and residue 125 and name HA)        2.350  0.650  0.650
assign (segid A and residue 125 and name HN) (segid A and residue 125 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 125 and name HN) (segid A and residue 125 and name HD#)       3.350  1.650  1.650
assign (segid A and residue 125 and name HN) (segid A and residue 125 and name HE#)       3.350  1.650  1.650
assign (segid A and residue 125 and name HN) (segid A and residue 125 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 125 and name HA) (segid A and residue 126 and name HN)        3.350  1.650  1.650
assign (segid A and residue 125 and name HD#) (segid A and residue 126 and name HN)       3.350  1.650  1.650
assign (segid A and residue 125 and name HE#) (segid A and residue 126 and name HN)       3.350  1.650  1.650
assign (segid A and residue 125 and name HG#) (segid A and residue 126 and name HN)       3.350  1.650  1.650
assign (segid A and residue 126 and name HN) (segid A and residue 126 and name HA)        2.350  0.650  0.650
assign (segid A and residue 126 and name HN) (segid A and residue 126 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 126 and name HN) (segid A and residue 127 and name HA)        3.350  1.650  1.650
```

```
assign (segid A and residue 126 and name HN) (segid A and residue 127 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 124 and name HA) (segid A and residue 127 and name HN)        3.350  1.650  1.650
assign (segid A and residue 126 and name HA) (segid A and residue 127 and name HN)        3.350  1.650  1.650
assign (segid A and residue 126 and name HB#) (segid A and residue 127 and name HN)       3.350  1.650  1.650
assign (segid A and residue 127 and name HN) (segid A and residue 127 and name HA)        2.350  0.650  0.650
assign (segid A and residue 127 and name HA) (segid A and residue 128 and name HN)        2.350  0.650  0.650
assign (segid A and residue 128 and name HN) (segid A and residue 128 and name HA)        2.350  0.650  0.650
assign (segid A and residue 128 and name HN) (segid A and residue 128 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 128 and name HN) (segid A and residue 128 and name HG#)       2.350  0.650  0.650
assign (segid A and residue 128 and name HB#) (segid A and residue 129 and name HN)       2.350  0.650  0.650
assign (segid A and residue 129 and name HN) (segid A and residue 129 and name HA)        2.350  0.650  0.650
assign (segid A and residue 129 and name HN) (segid A and residue 129 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 129 and name HN) (segid A and residue 129 and name HG)        3.600  1.900  1.900
assign (segid A and residue 129 and name HN) (segid A and residue 133 and name HG1#)      3.600  1.900  1.900
assign (segid A and residue 128 and name HA) (segid A and residue 130 and name HN)        3.350  1.650  1.650
assign (segid A and residue 129 and name HB#) (segid A and residue 130 and name HN)       3.350  1.650  1.650
assign (segid A and residue 129 and name HG) (segid A and residue 130 and name HN)        3.350  1.650  1.650
assign (segid A and residue 130 and name HN) (segid A and residue 130 and name HA)        2.350  0.650  0.650
assign (segid A and residue 130 and name HN) (segid A and residue 130 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 130 and name HN) (segid A and residue 131 and name HA)        3.350  1.650  1.650
assign (segid A and residue 130 and name HN) (segid A and residue 133 and name HG1#)      3.600  1.900  1.900
assign (segid A and residue 130 and name HB#) (segid A and residue 131 and name HN)       2.350  0.650  0.650
assign (segid A and residue 131 and name HN) (segid A and residue 131 and name HA)        2.350  0.650  0.650
assign (segid A and residue 131 and name HN) (segid A and residue 131 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 131 and name HN) (segid A and residue 133 and name HG1#)      3.600  1.900  1.900
assign (segid A and residue 130 and name HB#) (segid A and residue 132 and name HN)       3.350  1.650  1.650
assign (segid A and residue 131 and name HB#) (segid A and residue 132 and name HN)       2.350  0.650  0.650
assign (segid A and residue 132 and name HN) (segid A and residue 132 and name HA)        2.350  0.650  0.650
assign (segid A and residue 132 and name HN) (segid A and residue 132 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 132 and name HN) (segid A and residue 133 and name HB)        3.350  1.650  1.650
assign (segid A and residue 132 and name HN) (segid A and residue 133 and name HG1#)      3.600  1.900  1.900
assign (segid A and residue 129 and name HB#) (segid A and residue 133 and name HN)       3.350  1.650  1.650
assign (segid A and residue 130 and name HB#) (segid A and residue 133 and name HN)       3.350  1.650  1.650
assign (segid A and residue 131 and name HA) (segid A and residue 133 and name HN)        3.350  1.650  1.650
assign (segid A and residue 133 and name HN) (segid A and residue 133 and name HA)        2.350  0.650  0.650
assign (segid A and residue 133 and name HN) (segid A and residue 133 and name HB)        3.350  1.650  1.650
assign (segid A and residue 133 and name HN) (segid A and residue 133 and name HG1#)      2.600  0.900  0.900
assign (segid A and residue 133 and name HN) (segid A and residue 133 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 133 and name HG1#) (segid A and residue 134 and name HN)      3.600  1.900  1.900
assign (segid A and residue 133 and name HG2#) (segid A and residue 134 and name HN)      3.600  1.900  1.900
assign (segid A and residue 134 and name HN) (segid A and residue 134 and name HB)        2.350  0.650  0.650
assign (segid A and residue 133 and name HG2#) (segid A and residue 135 and name HN)      3.600  1.900  1.900
assign (segid A and residue 135 and name HN) (segid A and residue 135 and name HA)        3.350  1.650  1.650
assign (segid A and residue 135 and name HN) (segid A and residue 135 and name HB#)       3.350  1.650  1.650
```

```
assign (segid A and residue 135 and name HN) (segid A and residue 135 and name HE#)        3.600  1.900  1.900
assign (segid A and residue 135 and name HN) (segid A and residue 135 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 134 and name HG2#) (segid A and residue 137 and name HN)       3.600  1.900  1.900
assign (segid A and residue 136 and name HB#) (segid A and residue 137 and name HN)        3.350  1.650  1.650
assign (segid A and residue 136 and name HD#) (segid A and residue 137 and name HN)        3.350  1.650  1.650
assign (segid A and residue 137 and name HN) (segid A and residue 137 and name HA)         3.350  1.650  1.650
assign (segid A and residue 137 and name HN) (segid A and residue 137 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 137 and name HN) (segid A and residue 137 and name HD#)        3.350  1.650  1.650
assign (segid A and residue 137 and name HN) (segid A and residue 137 and name HE#)        3.350  1.650  1.650
assign (segid A and residue 137 and name HN) (segid A and residue 137 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 137 and name HN) (segid A and residue 140 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 137 and name HD#) (segid A and residue 138 and name HN)        3.350  1.650  1.650
assign (segid A and residue 137 and name HG#) (segid A and residue 138 and name HN)        3.350  1.650  1.650
assign (segid A and residue 138 and name HN) (segid A and residue 138 and name HA)         3.350  1.650  1.650
assign (segid A and residue 138 and name HN) (segid A and residue 138 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 138 and name HN) (segid A and residue 139 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 137 and name HB#) (segid A and residue 139 and name HN)        3.350  1.650  1.650
assign (segid A and residue 137 and name HE#) (segid A and residue 139 and name HN)        3.350  1.650  1.650
assign (segid A and residue 137 and name HG#) (segid A and residue 139 and name HN)        3.350  1.650  1.650
assign (segid A and residue 138 and name HB#) (segid A and residue 139 and name HN)        3.350  1.650  1.650
assign (segid A and residue 139 and name HN) (segid A and residue 139 and name HA)         3.350  1.650  1.650
assign (segid A and residue 139 and name HN) (segid A and residue 139 and name HB#)        2.350  0.650  0.650
assign (segid A and residue 139 and name HN) (segid A and residue 139 and name HG#)        2.350  0.650  0.650
assign (segid A and residue 139 and name HN) (segid A and residue 140 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 136 and name HB#) (segid A and residue 140 and name HN)        3.350  1.650  1.650
assign (segid A and residue 137 and name HA) (segid A and residue 140 and name HN)         3.350  1.650  1.650
assign (segid A and residue 137 and name HB#) (segid A and residue 140 and name HN)        3.350  1.650  1.650
assign (segid A and residue 137 and name HG#) (segid A and residue 140 and name HN)        3.350  1.650  1.650
assign (segid A and residue 139 and name HA) (segid A and residue 140 and name HN)         3.350  1.650  1.650
assign (segid A and residue 139 and name HG#) (segid A and residue 140 and name HN)        3.350  1.650  1.650
assign (segid A and residue 140 and name HN) (segid A and residue 140 and name HA)         3.350  1.650  1.650
assign (segid A and residue 140 and name HN) (segid A and residue 140 and name HB#)        2.350  0.650  0.650
assign (segid A and residue 140 and name HB#) (segid A and residue 141 and name HN)        3.350  1.650  1.650
assign (segid A and residue 141 and name HN) (segid A and residue 141 and name HA)         3.350  1.650  1.650
assign (segid A and residue 141 and name HN) (segid A and residue 141 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 141 and name HN) (segid A and residue 141 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 141 and name HN) (segid A and residue 142 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 140 and name HA) (segid A and residue 142 and name HN)         3.350  1.650  1.650
assign (segid A and residue 141 and name HG#) (segid A and residue 142 and name HN)        3.350  1.650  1.650
assign (segid A and residue 142 and name HN) (segid A and residue 142 and name HA)         3.350  1.650  1.650
assign (segid A and residue 142 and name HN) (segid A and residue 142 and name HB#)        2.350  0.650  0.650
assign (segid A and residue 142 and name HN) (segid A and residue 142 and name HG#)        2.350  0.650  0.650
assign (segid A and residue 142 and name HN) (segid A and residue 143 and name HA#)        3.350  1.650  1.650
assign (segid A and residue 142 and name HA) (segid A and residue 143 and name HN)         3.350  1.650  1.650
```

```
assign (segid A and residue 142 and name HB#) (segid A and residue 143 and name HN)        2.350  0.650  0.650
assign (segid A and residue 142 and name HG#) (segid A and residue 143 and name HN)        3.350  1.650  1.650
assign (segid A and residue 143 and name HN) (segid A and residue 143 and name HA#)        3.350  1.650  1.650
assign (segid A and residue 136 and name HB#) (segid A and residue 144 and name HN)        3.350  1.650  1.650
assign (segid A and residue 136 and name HG#) (segid A and residue 144 and name HN)        3.350  1.650  1.650
assign (segid A and residue 144 and name HN) (segid A and residue 144 and name HA)         2.350  0.650  0.650
assign (segid A and residue 144 and name HN) (segid A and residue 144 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 144 and name HN) (segid A and residue 146 and name HD#)        3.350  1.650  1.650
assign (segid A and residue 145 and name HN) (segid A and residue 145 and name HA)         3.350  1.650  1.650
assign (segid A and residue 145 and name HN) (segid A and residue 145 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 145 and name HN) (segid A and residue 145 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 143 and name HA#) (segid A and residue 146 and name HN)        3.350  1.650  1.650
assign (segid A and residue 146 and name HN) (segid A and residue 146 and name HA)         2.350  0.650  0.650
assign (segid A and residue 146 and name HN) (segid A and residue 146 and name HB#)        2.350  0.650  0.650
assign (segid A and residue 146 and name HN) (segid A and residue 146 and name HD#)        3.350  1.650  1.650
assign (segid A and residue 146 and name HN) (segid A and residue 146 and name HE#)        3.350  1.650  1.650
assign (segid A and residue 146 and name HN) (segid A and residue 146 and name HG#)        2.350  0.650  0.650
assign (segid A and residue 146 and name HA) (segid A and residue 147 and name HN)         3.350  1.650  1.650
assign (segid A and residue 146 and name HB#) (segid A and residue 147 and name HN)        3.350  1.650  1.650
assign (segid A and residue 146 and name HE#) (segid A and residue 147 and name HN)        3.350  1.650  1.650
assign (segid A and residue 146 and name HG#) (segid A and residue 147 and name HN)        3.350  1.650  1.650
assign (segid A and residue 147 and name HN) (segid A and residue 147 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 136 and name HG#) (segid A and residue 148 and name HN)        3.350  1.650  1.650
assign (segid A and residue 145 and name HA) (segid A and residue 148 and name HN)         3.350  1.650  1.650
assign (segid A and residue 146 and name HA) (segid A and residue 148 and name HN)         3.350  1.650  1.650
assign (segid A and residue 148 and name HN) (segid A and residue 148 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 145 and name HA) (segid A and residue 149 and name HN)         3.350  1.650  1.650
assign (segid A and residue 146 and name HA) (segid A and residue 149 and name HN)         3.350  1.650  1.650
assign (segid A and residue 148 and name HB#) (segid A and residue 149 and name HN)        3.350  1.650  1.650
assign (segid A and residue 149 and name HN) (segid A and residue 149 and name HA)         2.350  0.650  0.650
assign (segid A and residue 149 and name HN) (segid A and residue 149 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 149 and name HN) (segid A and residue 149 and name HD1#)       3.600  1.900  1.900
assign (segid A and residue 149 and name HN) (segid A and residue 149 and name HD2#)       3.600  1.900  1.900
assign (segid A and residue 149 and name HN) (segid A and residue 149 and name HG)         3.350  1.650  1.650
assign (segid A and residue 149 and name HN) (segid A and residue 150 and name HA1)        3.350  1.650  1.650
assign (segid A and residue 149 and name HN) (segid A and residue 150 and name HA2)        3.350  1.650  1.650
assign (segid A and residue 146 and name HA) (segid A and residue 150 and name HN)         3.350  1.650  1.650
assign (segid A and residue 146 and name HB#) (segid A and residue 150 and name HN)        3.350  1.650  1.650
assign (segid A and residue 149 and name HA) (segid A and residue 150 and name HN)         3.350  1.650  1.650
assign (segid A and residue 149 and name HB#) (segid A and residue 150 and name HN)        3.350  1.650  1.650
assign (segid A and residue 149 and name HD1#) (segid A and residue 150 and name HN)       3.600  1.900  1.900
assign (segid A and residue 149 and name HD2#) (segid A and residue 150 and name HN)       3.600  1.900  1.900
assign (segid A and residue 150 and name HN) (segid A and residue 150 and name HA1)        3.350  1.650  1.650
assign (segid A and residue 150 and name HN) (segid A and residue 150 and name HA2)        3.350  1.650  1.650
```

```
assign (segid A and residue 150 and name HA1) (segid A and residue 151 and name HN)        2.350  0.650  0.650
assign (segid A and residue 151 and name HN) (segid A and residue 151 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 152 and name HE#) (segid A and residue 153 and name HN)        3.350  1.650  1.650
assign (segid A and residue 152 and name HG#) (segid A and residue 153 and name HN)        3.350  1.650  1.650
assign (segid A and residue 153 and name HN) (segid A and residue 153 and name HA)         2.350  0.650  0.650
assign (segid A and residue 153 and name HN) (segid A and residue 153 and name HB#)        2.350  0.650  0.650
assign (segid A and residue 153 and name HN) (segid A and residue 153 and name HG)         2.350  0.650  0.650
assign (segid A and residue 153 and name HN) (segid A and residue 154 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 153 and name HG) (segid A and residue 154 and name HN)         3.350  1.650  1.650
assign (segid A and residue 154 and name HN) (segid A and residue 154 and name HA)         2.350  0.650  0.650
assign (segid A and residue 154 and name HN) (segid A and residue 154 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 154 and name HN) (segid A and residue 155 and name HB#)        3.600  1.900  1.900
assign (segid A and residue 152 and name HA) (segid A and residue 155 and name HN)         2.350  0.650  0.650
assign (segid A and residue 152 and name HG#) (segid A and residue 155 and name HN)        3.350  1.650  1.650
assign (segid A and residue 153 and name HA) (segid A and residue 155 and name HN)         3.350  1.650  1.650
assign (segid A and residue 154 and name HB#) (segid A and residue 155 and name HN)        2.350  0.650  0.650
assign (segid A and residue 155 and name HN) (segid A and residue 155 and name HA)         2.350  0.650  0.650
assign (segid A and residue 155 and name HN) (segid A and residue 155 and name HB#)        2.600  0.900  0.900
assign (segid A and residue 155 and name HN) (segid A and residue 156 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 154 and name HA) (segid A and residue 156 and name HN)         3.350  1.650  1.650
assign (segid A and residue 156 and name HN) (segid A and residue 156 and name HA)         2.350  0.650  0.650
assign (segid A and residue 156 and name HN) (segid A and residue 156 and name HB#)        2.350  0.650  0.650
assign (segid A and residue 156 and name HN) (segid A and residue 157 and name HA#)        3.350  1.650  1.650
assign (segid A and residue 153 and name HA) (segid A and residue 157 and name HN)         3.350  1.650  1.650
assign (segid A and residue 155 and name HB#) (segid A and residue 157 and name HN)        3.600  1.900  1.900
assign (segid A and residue 156 and name HB#) (segid A and residue 157 and name HN)        2.350  0.650  0.650
assign (segid A and residue 157 and name HN) (segid A and residue 157 and name HA#)        2.350  0.650  0.650
assign (segid A and residue 157 and name HN) (segid A and residue 158 and name HA)         3.350  1.650  1.650
assign (segid A and residue 156 and name HB#) (segid A and residue 158 and name HN)        3.350  1.650  1.650
assign (segid A and residue 158 and name HN) (segid A and residue 158 and name HA)         2.350  0.650  0.650
assign (segid A and residue 158 and name HN) (segid A and residue 158 and name HB#)        2.600  0.900  0.900
assign (segid A and residue 156 and name HB#) (segid A and residue 159 and name HN)        3.350  1.650  1.650
assign (segid A and residue 157 and name HA#) (segid A and residue 159 and name HN)        3.350  1.650  1.650
assign (segid A and residue 159 and name HN) (segid A and residue 159 and name HA)         2.350  0.650  0.650
assign (segid A and residue 159 and name HN) (segid A and residue 159 and name HB)         2.350  0.650  0.650
assign (segid A and residue 158 and name HA) (segid A and residue 160 and name HN)         3.350  1.650  1.650
assign (segid A and residue 160 and name HN) (segid A and residue 160 and name HA1)        2.350  0.650  0.650
assign (segid A and residue 160 and name HN) (segid A and residue 160 and name HA2)        2.350  0.650  0.650
assign (segid A and residue 160 and name HN) (segid A and residue 161 and name HD#)        3.350  1.650  1.650
assign (segid A and residue 160 and name HN) (segid A and residue 161 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 160 and name HN) (segid A and residue 162 and name HB#)        3.600  1.900  1.900
assign (segid A and residue 160 and name HN) (segid A and residue 163 and name HB)         3.350  1.650  1.650
assign (segid A and residue 160 and name HA1) (segid A and residue 162 and name HN)        3.350  1.650  1.650
assign (segid A and residue 160 and name HA2) (segid A and residue 162 and name HN)        3.350  1.650  1.650
```

```
assign (segid A and residue 161 and name HD#) (segid A and residue 162 and name HN)        3.350  1.650  1.650
assign (segid A and residue 161 and name HG#) (segid A and residue 162 and name HN)        3.350  1.650  1.650
assign (segid A and residue 162 and name HN) (segid A and residue 162 and name HA)        2.350  0.650  0.650
assign (segid A and residue 162 and name HN) (segid A and residue 162 and name HB#)       2.600  0.900  0.900
assign (segid A and residue 162 and name HB#) (segid A and residue 163 and name HN)       3.600  1.900  1.900
assign (segid A and residue 163 and name HN) (segid A and residue 163 and name HA)        2.350  0.650  0.650
assign (segid A and residue 163 and name HN) (segid A and residue 163 and name HB)        2.350  0.650  0.650
assign (segid A and residue 163 and name HN) (segid A and residue 163 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 163 and name HN) (segid A and residue 164 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 163 and name HN) (segid A and residue 165 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 162 and name HB#) (segid A and residue 164 and name HN)       3.600  1.900  1.900
assign (segid A and residue 163 and name HG2#) (segid A and residue 164 and name HN)      3.600  1.900  1.900
assign (segid A and residue 164 and name HN) (segid A and residue 164 and name HA)        2.350  0.650  0.650
assign (segid A and residue 164 and name HN) (segid A and residue 164 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 163 and name HA) (segid A and residue 165 and name HN)        3.350  1.650  1.650
assign (segid A and residue 163 and name HB) (segid A and residue 165 and name HN)        3.350  1.650  1.650
assign (segid A and residue 163 and name HG2#) (segid A and residue 165 and name HN)      3.600  1.900  1.900
assign (segid A and residue 165 and name HN) (segid A and residue 165 and name HA)        2.350  0.650  0.650
assign (segid A and residue 165 and name HN) (segid A and residue 165 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 165 and name HN) (segid A and residue 166 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 165 and name HN) (segid A and residue 167 and name HD#)       3.350  1.650  1.650
assign (segid A and residue 164 and name HB#) (segid A and residue 166 and name HN)       3.350  1.650  1.650
assign (segid A and residue 165 and name HA) (segid A and residue 166 and name HN)        2.350  0.650  0.650
assign (segid A and residue 166 and name HN) (segid A and residue 166 and name HA)        2.350  0.650  0.650
assign (segid A and residue 166 and name HN) (segid A and residue 166 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 166 and name HN) (segid A and residue 167 and name HD#)       3.350  1.650  1.650
assign (segid A and residue 166 and name HA) (segid A and residue 168 and name HN)        3.350  1.650  1.650
assign (segid A and residue 166 and name HB#) (segid A and residue 168 and name HN)       3.350  1.650  1.650
assign (segid A and residue 167 and name HD#) (segid A and residue 168 and name HN)       3.350  1.650  1.650
assign (segid A and residue 167 and name HG#) (segid A and residue 168 and name HN)       3.350  1.650  1.650
assign (segid A and residue 168 and name HN) (segid A and residue 168 and name HA#)       2.350  0.650  0.650
assign (segid A and residue 168 and name HN) (segid A and residue 169 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 168 and name HN) (segid A and residue 169 and name HG1#)      3.350  1.650  1.650
assign (segid A and residue 168 and name HN) (segid A and residue 169 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 166 and name HB#) (segid A and residue 169 and name HN)       3.350  1.650  1.650
assign (segid A and residue 169 and name HN) (segid A and residue 169 and name HA)        2.350  0.650  0.650
assign (segid A and residue 169 and name HN) (segid A and residue 169 and name HB)        2.350  0.650  0.650
assign (segid A and residue 169 and name HN) (segid A and residue 169 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 169 and name HD1#) (segid A and residue 170 and name HN)      3.600  1.900  1.900
assign (segid A and residue 169 and name HG1#) (segid A and residue 170 and name HN)      3.350  1.650  1.650
assign (segid A and residue 170 and name HN) (segid A and residue 170 and name HA)        2.350  0.650  0.650
assign (segid A and residue 170 and name HN) (segid A and residue 170 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 170 and name HN) (segid A and residue 173 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 170 and name HN) (segid A and residue 173 and name HG#)       3.350  1.650  1.650
```

```
assign (segid A and residue 169 and name HG2#) (segid A and residue 171 and name HN)       3.600  1.900  1.900
assign (segid A and residue 170 and name HB#) (segid A and residue 171 and name HN)       3.350  1.650  1.650
assign (segid A and residue 171 and name HN) (segid A and residue 171 and name HA)        2.350  0.650  0.650
assign (segid A and residue 171 and name HN) (segid A and residue 174 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 171 and name HN) (segid A and residue 174 and name HG1#)      3.350  1.650  1.650
assign (segid A and residue 169 and name HG2#) (segid A and residue 172 and name HN)      3.600  1.900  1.900
assign (segid A and residue 170 and name HA) (segid A and residue 172 and name HN)        3.350  1.650  1.650
assign (segid A and residue 170 and name HB#) (segid A and residue 172 and name HN)       3.350  1.650  1.650
assign (segid A and residue 171 and name HB#) (segid A and residue 172 and name HN)       3.350  1.650  1.650
assign (segid A and residue 172 and name HN) (segid A and residue 172 and name HA)        2.350  0.650  0.650
assign (segid A and residue 172 and name HN) (segid A and residue 172 and name HB)        2.350  0.650  0.650
assign (segid A and residue 172 and name HN) (segid A and residue 174 and name HB)        3.350  1.650  1.650
assign (segid A and residue 172 and name HN) (segid A and residue 174 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 172 and name HN) (segid A and residue 174 and name HG1#)      3.350  1.650  1.650
assign (segid A and residue 172 and name HG1#) (segid A and residue 173 and name HN)      3.600  1.900  1.900
assign (segid A and residue 172 and name HG2#) (segid A and residue 173 and name HN)      3.600  1.900  1.900
assign (segid A and residue 173 and name HN) (segid A and residue 173 and name HA)        3.350  1.650  1.650
assign (segid A and residue 173 and name HN) (segid A and residue 173 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 173 and name HN) (segid A and residue 173 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 173 and name HN) (segid A and residue 174 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 173 and name HN) (segid A and residue 174 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 172 and name HA) (segid A and residue 174 and name HN)        3.350  1.650  1.650
assign (segid A and residue 172 and name HG1#) (segid A and residue 174 and name HN)      3.600  1.900  1.900
assign (segid A and residue 172 and name HG2#) (segid A and residue 174 and name HN)      3.600  1.900  1.900
assign (segid A and residue 173 and name HA) (segid A and residue 174 and name HN)        2.350  0.650  0.650
assign (segid A and residue 173 and name HG#) (segid A and residue 174 and name HN)       3.350  1.650  1.650
assign (segid A and residue 174 and name HN) (segid A and residue 174 and name HA)        3.350  1.650  1.650
assign (segid A and residue 174 and name HN) (segid A and residue 174 and name HB)        3.350  1.650  1.650
assign (segid A and residue 174 and name HN) (segid A and residue 174 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 174 and name HN) (segid A and residue 174 and name HG1#)      3.350  1.650  1.650
assign (segid A and residue 174 and name HN) (segid A and residue 174 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 171 and name HA) (segid A and residue 175 and name HN)        3.350  1.650  1.650
assign (segid A and residue 171 and name HB#) (segid A and residue 175 and name HN)       3.350  1.650  1.650
assign (segid A and residue 173 and name HG#) (segid A and residue 175 and name HN)       3.350  1.650  1.650
assign (segid A and residue 174 and name HB) (segid A and residue 175 and name HN)        2.350  0.650  0.650
assign (segid A and residue 174 and name HD1#) (segid A and residue 175 and name HN)      3.600  1.900  1.900
assign (segid A and residue 174 and name HG1#) (segid A and residue 175 and name HN)      3.350  1.650  1.650
assign (segid A and residue 174 and name HG2#) (segid A and residue 175 and name HN)      3.600  1.900  1.900
assign (segid A and residue 175 and name HN) (segid A and residue 175 and name HA1)       3.350  1.650  1.650
assign (segid A and residue 175 and name HN) (segid A and residue 175 and name HA2)       2.350  0.650  0.650
assign (segid A and residue 174 and name HG2#) (segid A and residue 176 and name HN)      3.600  1.900  1.900
assign (segid A and residue 176 and name HN) (segid A and residue 176 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 176 and name HN) (segid A and residue 182 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 179 and name HN) (segid A and residue 179 and name HD2#)      3.600  1.900  1.900
```

```
assign (segid A and residue 179 and name HN) (segid A and residue 179 and name HG)         3.350  1.650  1.650
assign (segid A and residue 179 and name HN) (segid A and residue 182 and name HD1#)       3.600  1.900  1.900
assign (segid A and residue 180 and name HN) (segid A and residue 180 and name HA)         3.350  1.650  1.650
assign (segid A and residue 180 and name HN) (segid A and residue 180 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 180 and name HN) (segid A and residue 180 and name HD1#)       3.600  1.900  1.900
assign (segid A and residue 180 and name HN) (segid A and residue 180 and name HD2#)       3.600  1.900  1.900
assign (segid A and residue 180 and name HN) (segid A and residue 180 and name HG)         3.350  1.650  1.650
assign (segid A and residue 182 and name HN) (segid A and residue 182 and name HA)         3.350  1.650  1.650
assign (segid A and residue 182 and name HN) (segid A and residue 182 and name HB)         3.350  1.650  1.650
assign (segid A and residue 182 and name HN) (segid A and residue 182 and name HD1#)       3.600  1.900  1.900
assign (segid A and residue 182 and name HN) (segid A and residue 182 and name HG1#)       3.350  1.650  1.650
assign (segid A and residue 182 and name HN) (segid A and residue 182 and name HG2#)       3.600  1.900  1.900
assign (segid A and residue 182 and name HG1#) (segid A and residue 183 and name HN)       3.350  1.650  1.650
assign (segid A and residue 183 and name HN) (segid A and residue 183 and name HA)         3.350  1.650  1.650
assign (segid A and residue 183 and name HN) (segid A and residue 183 and name HB)         3.350  1.650  1.650
assign (segid A and residue 183 and name HN) (segid A and residue 183 and name HG1#)       3.600  1.900  1.900
assign (segid A and residue 183 and name HN) (segid A and residue 183 and name HG2#)       3.600  1.900  1.900
assign (segid A and residue 184 and name HN) (segid A and residue 184 and name HA)         3.350  1.650  1.650
assign (segid A and residue 183 and name HA) (segid A and residue 185 and name HN)         3.350  1.650  1.650
assign (segid A and residue 184 and name HB#) (segid A and residue 185 and name HN)        3.350  1.650  1.650
assign (segid A and residue 185 and name HN) (segid A and residue 185 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 185 and name HN) (segid A and residue 185 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 135 and name HE#) (segid A and residue 186 and name HN)        3.600  1.900  1.900
assign (segid A and residue 183 and name HA) (segid A and residue 186 and name HN)         3.350  1.650  1.650
assign (segid A and residue 185 and name HD#) (segid A and residue 186 and name HN)        3.350  1.650  1.650
assign (segid A and residue 186 and name HN) (segid A and residue 186 and name HA)         3.350  1.650  1.650
assign (segid A and residue 186 and name HN) (segid A and residue 186 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 186 and name HN) (segid A and residue 186 and name HE#)        3.600  1.900  1.900
assign (segid A and residue 185 and name HD#) (segid A and residue 187 and name HN)        3.350  1.650  1.650
assign (segid A and residue 186 and name HB#) (segid A and residue 187 and name HN)        3.350  1.650  1.650
assign (segid A and residue 186 and name HE#) (segid A and residue 187 and name HN)        3.600  1.900  1.900
assign (segid A and residue 186 and name HG#) (segid A and residue 187 and name HN)        3.350  1.650  1.650
assign (segid A and residue 187 and name HN) (segid A and residue 187 and name HA)         3.350  1.650  1.650
assign (segid A and residue 187 and name HN) (segid A and residue 187 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 187 and name HN) (segid A and residue 190 and name HB)         3.350  1.650  1.650
assign (segid A and residue 187 and name HN) (segid A and residue 190 and name HG2#)       3.600  1.900  1.900
assign (segid A and residue 187 and name HB#) (segid A and residue 188 and name HN)        3.350  1.650  1.650
assign (segid A and residue 188 and name HN) (segid A and residue 188 and name HA)         3.350  1.650  1.650
assign (segid A and residue 188 and name HN) (segid A and residue 188 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 188 and name HN) (segid A and residue 188 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 188 and name HN) (segid A and residue 189 and name HB#)        3.600  1.900  1.900
assign (segid A and residue 188 and name HA) (segid A and residue 189 and name HN)         3.350  1.650  1.650
assign (segid A and residue 188 and name HG#) (segid A and residue 189 and name HN)        3.350  1.650  1.650
assign (segid A and residue 189 and name HN) (segid A and residue 189 and name HA)         2.350  0.650  0.650
```

```
assign (segid A and residue 189 and name HN) (segid A and residue 189 and name HB#)        2.600  0.900  0.900
assign (segid A and residue 189 and name HN) (segid A and residue 192 and name HB)         3.350  1.650  1.650
assign (segid A and residue 189 and name HA) (segid A and residue 190 and name HN)         3.350  1.650  1.650
assign (segid A and residue 190 and name HN) (segid A and residue 190 and name HA)         3.350  1.650  1.650
assign (segid A and residue 190 and name HN) (segid A and residue 190 and name HB)         3.350  1.650  1.650
assign (segid A and residue 190 and name HB) (segid A and residue 191 and name HN)         3.350  1.650  1.650
assign (segid A and residue 191 and name HN) (segid A and residue 191 and name HA)         3.350  1.650  1.650
assign (segid A and residue 191 and name HN) (segid A and residue 191 and name HG1#)       3.600  1.900  1.900
assign (segid A and residue 191 and name HN) (segid A and residue 191 and name HG2#)       3.600  1.900  1.900
assign (segid A and residue 192 and name HN) (segid A and residue 192 and name HA)         3.350  1.650  1.650
assign (segid A and residue 192 and name HN) (segid A and residue 192 and name HB)         3.350  1.650  1.650
assign (segid A and residue 192 and name HN) (segid A and residue 192 and name HG2#)       3.600  1.900  1.900
assign (segid A and residue 189 and name HA) (segid A and residue 193 and name HN)         3.350  1.650  1.650
assign (segid A and residue 189 and name HB#) (segid A and residue 193 and name HN)        3.600  1.900  1.900
assign (segid A and residue 190 and name HA) (segid A and residue 193 and name HN)         3.350  1.650  1.650
assign (segid A and residue 192 and name HB) (segid A and residue 193 and name HN)         3.350  1.650  1.650
assign (segid A and residue 193 and name HN) (segid A and residue 193 and name HA)         3.350  1.650  1.650
assign (segid A and residue 193 and name HN) (segid A and residue 194 and name HG2#)       3.600  1.900  1.900
assign (segid A and residue 193 and name HB#) (segid A and residue 194 and name HN)        3.350  1.650  1.650
assign (segid A and residue 194 and name HN) (segid A and residue 194 and name HA)         3.350  1.650  1.650
assign (segid A and residue 194 and name HN) (segid A and residue 194 and name HB)         3.350  1.650  1.650
assign (segid A and residue 194 and name HN) (segid A and residue 194 and name HG1#)       3.600  1.900  1.900
assign (segid A and residue 194 and name HN) (segid A and residue 194 and name HG2#)       3.600  1.900  1.900
assign (segid A and residue 195 and name HN) (segid A and residue 195 and name HB#)        2.350  0.650  0.650
assign (segid A and residue 195 and name HN) (segid A and residue 195 and name HD1#)       3.600  1.900  1.900
assign (segid A and residue 195 and name HN) (segid A and residue 195 and name HD2#)       3.600  1.900  1.900
assign (segid A and residue 195 and name HD1#) (segid A and residue 196 and name HN)       3.600  1.900  1.900
assign (segid A and residue 196 and name HN) (segid A and residue 196 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 196 and name HN) (segid A and residue 196 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 194 and name HG1#) (segid A and residue 197 and name HN)       3.600  1.900  1.900
assign (segid A and residue 195 and name HD2#) (segid A and residue 197 and name HN)       3.600  1.900  1.900
assign (segid A and residue 196 and name HB#) (segid A and residue 197 and name HN)        3.350  1.650  1.650
assign (segid A and residue 197 and name HN) (segid A and residue 197 and name HA)         3.350  1.650  1.650
assign (segid A and residue 197 and name HN) (segid A and residue 197 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 194 and name HG1#) (segid A and residue 198 and name HN)       3.600  1.900  1.900
assign (segid A and residue 195 and name HA) (segid A and residue 198 and name HN)         3.350  1.650  1.650
assign (segid A and residue 197 and name HB#) (segid A and residue 198 and name HN)        3.350  1.650  1.650
assign (segid A and residue 198 and name HN) (segid A and residue 198 and name HA)         3.350  1.650  1.650
assign (segid A and residue 198 and name HN) (segid A and residue 198 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 198 and name HN) (segid A and residue 198 and name HD1#)       3.600  1.900  1.900
assign (segid A and residue 198 and name HN) (segid A and residue 198 and name HD2#)       3.600  1.900  1.900
assign (segid A and residue 198 and name HN) (segid A and residue 198 and name HG)         3.350  1.650  1.650
assign (segid A and residue 198 and name HN) (segid A and residue 199 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 198 and name HB#) (segid A and residue 199 and name HN)        3.350  1.650  1.650
```

```
assign (segid A and residue 199 and name HB#) (segid A and residue 200 and name HN)        3.350  1.650  1.650
assign (segid A and residue 200 and name HN) (segid A and residue 200 and name HA)        3.350  1.650  1.650
assign (segid A and residue 200 and name HN) (segid A and residue 200 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 201 and name HN) (segid A and residue 201 and name HA)        2.350  0.650  0.650
assign (segid A and residue 201 and name HN) (segid A and residue 201 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 202 and name HA) (segid A and residue 203 and name HN)        3.350  1.650  1.650
assign (segid A and residue 203 and name HN) (segid A and residue 203 and name HA1)       2.350  0.650  0.650
assign (segid A and residue 203 and name HN) (segid A and residue 203 and name HA2)       2.350  0.650  0.650
assign (segid A and residue 203 and name HA2) (segid A and residue 204 and name HN)       3.350  1.650  1.650
assign (segid A and residue 204 and name HN) (segid A and residue 204 and name HA)        2.350  0.650  0.650
assign (segid A and residue 204 and name HN) (segid A and residue 204 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 204 and name HN) (segid A and residue 204 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 204 and name HB#) (segid A and residue 205 and name HN)       3.350  1.650  1.650
assign (segid A and residue 205 and name HN) (segid A and residue 205 and name HA)        2.350  0.650  0.650
assign (segid A and residue 205 and name HN) (segid A and residue 205 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 205 and name HN) (segid A and residue 205 and name HD#)       3.350  1.650  1.650
assign (segid A and residue 205 and name HN) (segid A and residue 205 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 205 and name HB#) (segid A and residue 206 and name HN)       3.350  1.650  1.650
assign (segid A and residue 205 and name HD#) (segid A and residue 206 and name HN)       3.350  1.650  1.650
assign (segid A and residue 205 and name HG#) (segid A and residue 206 and name HN)       3.350  1.650  1.650
assign (segid A and residue 206 and name HN) (segid A and residue 206 and name HA)        3.350  1.650  1.650
assign (segid A and residue 206 and name HN) (segid A and residue 206 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 206 and name HA) (segid A and residue 207 and name HN)        2.350  0.650  0.650
assign (segid A and residue 207 and name HN) (segid A and residue 207 and name HA)        3.350  1.650  1.650
assign (segid A and residue 207 and name HN) (segid A and residue 207 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 208 and name HN) (segid A and residue 208 and name HA)        3.350  1.650  1.650
assign (segid A and residue 208 and name HN) (segid A and residue 208 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 209 and name HA) (segid A and residue 210 and name HN)        3.350  1.650  1.650
assign (segid A and residue 209 and name HB#) (segid A and residue 210 and name HN)       3.350  1.650  1.650
assign (segid A and residue 209 and name HD#) (segid A and residue 210 and name HN)       3.350  1.650  1.650
assign (segid A and residue 209 and name HG#) (segid A and residue 210 and name HN)       3.350  1.650  1.650
assign (segid A and residue 210 and name HN) (segid A and residue 210 and name HA)        3.350  1.650  1.650
assign (segid A and residue 210 and name HN) (segid A and residue 210 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 210 and name HN) (segid A and residue 210 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 208 and name HG2#) (segid A and residue 211 and name HN)      3.600  1.900  1.900
assign (segid A and residue 211 and name HN) (segid A and residue 211 and name HA)        3.350  1.650  1.650
assign (segid A and residue 211 and name HN) (segid A and residue 211 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 211 and name HN) (segid A and residue 211 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 211 and name HN) (segid A and residue 211 and name HD2#)      3.600  1.900  1.900
assign (segid A and residue 211 and name HN) (segid A and residue 211 and name HG)        3.350  1.650  1.650
assign (segid A and residue 209 and name HA) (segid A and residue 212 and name HN)        3.350  1.650  1.650
assign (segid A and residue 211 and name HA) (segid A and residue 212 and name HN)        3.350  1.650  1.650
assign (segid A and residue 211 and name HB#) (segid A and residue 212 and name HN)       3.350  1.650  1.650
assign (segid A and residue 211 and name HD1#) (segid A and residue 212 and name HN)      3.600  1.900  1.900
```

```
assign (segid A and residue 211 and name HD2#) (segid A and residue 212 and name HN)       3.600  1.900  1.900
assign (segid A and residue 211 and name HG) (segid A and residue 212 and name HN)        3.350  1.650  1.650
assign (segid A and residue 212 and name HN) (segid A and residue 212 and name HA1)       3.350  1.650  1.650
assign (segid A and residue 212 and name HN) (segid A and residue 212 and name HA2)       3.350  1.650  1.650
assign (segid A and residue 212 and name HA1) (segid A and residue 213 and name HN)       3.350  1.650  1.650
assign (segid A and residue 213 and name HN) (segid A and residue 213 and name HA)        3.350  1.650  1.650
assign (segid A and residue 213 and name HN) (segid A and residue 213 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 213 and name HN) (segid A and residue 213 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 211 and name HB#) (segid A and residue 214 and name HN)       3.350  1.650  1.650
assign (segid A and residue 214 and name HN) (segid A and residue 214 and name HA)        3.350  1.650  1.650
assign (segid A and residue 214 and name HN) (segid A and residue 214 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 212 and name HA2) (segid A and residue 215 and name HN)       3.350  1.650  1.650
assign (segid A and residue 214 and name HA) (segid A and residue 215 and name HN)        3.350  1.650  1.650
assign (segid A and residue 214 and name HB#) (segid A and residue 215 and name HN)       3.350  1.650  1.650
assign (segid A and residue 215 and name HN) (segid A and residue 215 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 215 and name HN) (segid A and residue 215 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 215 and name HN) (segid A and residue 215 and name HD2#)      3.600  1.900  1.900
assign (segid A and residue 213 and name HA) (segid A and residue 216 and name HN)        3.350  1.650  1.650
assign (segid A and residue 215 and name HB#) (segid A and residue 216 and name HN)       3.350  1.650  1.650
assign (segid A and residue 215 and name HD1#) (segid A and residue 216 and name HN)      3.600  1.900  1.900
assign (segid A and residue 216 and name HN) (segid A and residue 216 and name HA)        3.350  1.650  1.650
assign (segid A and residue 216 and name HN) (segid A and residue 216 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 216 and name HN) (segid A and residue 217 and name HB#)       3.600  1.900  1.900
assign (segid A and residue 216 and name HB#) (segid A and residue 217 and name HN)       3.350  1.650  1.650
assign (segid A and residue 217 and name HN) (segid A and residue 217 and name HB#)       3.600  1.900  1.900
assign (segid A and residue 217 and name HA) (segid A and residue 218 and name HN)        3.350  1.650  1.650
assign (segid A and residue 218 and name HN) (segid A and residue 218 and name HA)        2.350  0.650  0.650
assign (segid A and residue 218 and name HN) (segid A and residue 218 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 218 and name HN) (segid A and residue 218 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 218 and name HN) (segid A and residue 218 and name HD2#)      3.600  1.900  1.900
assign (segid A and residue 219 and name HN) (segid A and residue 219 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 219 and name HN) (segid A and residue 219 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 219 and name HN) (segid A and residue 219 and name HD2#)      3.600  1.900  1.900
assign (segid A and residue 219 and name HN) (segid A and residue 219 and name HG)        2.350  0.650  0.650
assign (segid A and residue 217 and name HA) (segid A and residue 220 and name HN)        3.350  1.650  1.650
assign (segid A and residue 217 and name HB#) (segid A and residue 220 and name HN)       3.600  1.900  1.900
assign (segid A and residue 219 and name HD1#) (segid A and residue 220 and name HN)      3.600  1.900  1.900
assign (segid A and residue 219 and name HD2#) (segid A and residue 220 and name HN)      3.600  1.900  1.900
assign (segid A and residue 219 and name HG) (segid A and residue 220 and name HN)        3.350  1.650  1.650
assign (segid A and residue 220 and name HN) (segid A and residue 220 and name HB#)       3.600  1.900  1.900
assign (segid A and residue 220 and name HN) (segid A and residue 221 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 218 and name HA) (segid A and residue 221 and name HN)        3.350  1.650  1.650
assign (segid A and residue 220 and name HB#) (segid A and residue 221 and name HN)       3.600  1.900  1.900
assign (segid A and residue 221 and name HN) (segid A and residue 221 and name HA)        2.350  0.650  0.650
```

```
assign (segid A and residue 221 and name HN) (segid A and residue 221 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 221 and name HN) (segid A and residue 222 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 219 and name HA) (segid A and residue 222 and name HN)        3.350  1.650  1.650
assign (segid A and residue 220 and name HB#) (segid A and residue 222 and name HN)       3.600  1.900  1.900
assign (segid A and residue 221 and name HA) (segid A and residue 222 and name HN)        3.350  1.650  1.650
assign (segid A and residue 221 and name HB#) (segid A and residue 222 and name HN)       3.350  1.650  1.650
assign (segid A and residue 222 and name HN) (segid A and residue 222 and name HA)        3.350  1.650  1.650
assign (segid A and residue 222 and name HN) (segid A and residue 222 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 222 and name HN) (segid A and residue 222 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 222 and name HN) (segid A and residue 222 and name HD2#)      3.600  1.900  1.900
assign (segid A and residue 222 and name HN) (segid A and residue 222 and name HG)        3.350  1.650  1.650
assign (segid A and residue 222 and name HN) (segid A and residue 223 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 222 and name HB#) (segid A and residue 223 and name HN)       3.350  1.650  1.650
assign (segid A and residue 222 and name HG) (segid A and residue 223 and name HN)        3.350  1.650  1.650
assign (segid A and residue 223 and name HN) (segid A and residue 223 and name HA)        3.350  1.650  1.650
assign (segid A and residue 223 and name HN) (segid A and residue 223 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 224 and name HN) (segid A and residue 224 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 225 and name HA) (segid A and residue 226 and name HN)        2.350  0.650  0.650
assign (segid A and residue 225 and name HB#) (segid A and residue 226 and name HN)       3.350  1.650  1.650
assign (segid A and residue 225 and name HD#) (segid A and residue 226 and name HN)       3.350  1.650  1.650
assign (segid A and residue 225 and name HG#) (segid A and residue 226 and name HN)       3.350  1.650  1.650
assign (segid A and residue 226 and name HN) (segid A and residue 226 and name HA)        2.350  0.650  0.650
assign (segid A and residue 226 and name HN) (segid A and residue 226 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 226 and name HN) (segid A and residue 226 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 226 and name HN) (segid A and residue 226 and name HD2#)      3.600  1.900  1.900
assign (segid A and residue 226 and name HN) (segid A and residue 230 and name HB#)       3.600  1.900  1.900
assign (segid A and residue 227 and name HN) (segid A and residue 227 and name HA)        3.350  1.650  1.650
assign (segid A and residue 227 and name HN) (segid A and residue 227 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 227 and name HN) (segid A and residue 227 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 227 and name HN) (segid A and residue 227 and name HD2#)      3.600  1.900  1.900
assign (segid A and residue 227 and name HN) (segid A and residue 227 and name HG)        2.350  0.650  0.650
assign (segid A and residue 227 and name HN) (segid A and residue 228 and name HD#)       3.350  1.650  1.650
assign (segid A and residue 227 and name HN) (segid A and residue 230 and name HB#)       3.600  1.900  1.900
assign (segid A and residue 227 and name HA) (segid A and residue 229 and name HN)        3.350  1.650  1.650
assign (segid A and residue 227 and name HB#) (segid A and residue 229 and name HN)       3.350  1.650  1.650
assign (segid A and residue 227 and name HD1#) (segid A and residue 229 and name HN)      3.600  1.900  1.900
assign (segid A and residue 227 and name HD2#) (segid A and residue 229 and name HN)      3.600  1.900  1.900
assign (segid A and residue 228 and name HD#) (segid A and residue 229 and name HN)       3.350  1.650  1.650
assign (segid A and residue 229 and name HN) (segid A and residue 229 and name HA)        2.350  0.650  0.650
assign (segid A and residue 229 and name HN) (segid A and residue 229 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 229 and name HN) (segid A and residue 229 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 227 and name HB#) (segid A and residue 230 and name HN)       3.350  1.650  1.650
assign (segid A and residue 228 and name HA) (segid A and residue 230 and name HN)        3.350  1.650  1.650
assign (segid A and residue 230 and name HN) (segid A and residue 230 and name HA)        2.350  0.650  0.650
```

```
assign (segid A and residue 230 and name HN) (segid A and residue 230 and name HB#)        2.600  0.900  0.900
assign (segid A and residue 230 and name HN) (segid A and residue 231 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 230 and name HN) (segid A and residue 232 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 230 and name HB#) (segid A and residue 231 and name HN)        3.600  1.900  1.900
assign (segid A and residue 231 and name HN) (segid A and residue 231 and name HB#)        2.350  0.650  0.650
assign (segid A and residue 231 and name HN) (segid A and residue 232 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 228 and name HA) (segid A and residue 232 and name HN)         3.350  1.650  1.650
assign (segid A and residue 229 and name HB#) (segid A and residue 232 and name HN)        3.350  1.650  1.650
assign (segid A and residue 230 and name HA) (segid A and residue 232 and name HN)         3.350  1.650  1.650
assign (segid A and residue 230 and name HB#) (segid A and residue 232 and name HN)        3.600  1.900  1.900
assign (segid A and residue 232 and name HN) (segid A and residue 232 and name HB#)        2.350  0.650  0.650
assign (segid A and residue 232 and name HN) (segid A and residue 233 and name HD1#)       3.600  1.900  1.900
assign (segid A and residue 232 and name HN) (segid A and residue 233 and name HD2#)       3.600  1.900  1.900
assign (segid A and residue 232 and name HN) (segid A and residue 233 and name HG)         3.350  1.650  1.650
assign (segid A and residue 233 and name HN) (segid A and residue 233 and name HA)         2.350  0.650  0.650
assign (segid A and residue 233 and name HN) (segid A and residue 233 and name HD1#)       3.600  1.900  1.900
assign (segid A and residue 233 and name HN) (segid A and residue 233 and name HD2#)       3.600  1.900  1.900
assign (segid A and residue 233 and name HN) (segid A and residue 233 and name HG)         2.350  0.650  0.650
assign (segid A and residue 233 and name HN) (segid A and residue 236 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 230 and name HA) (segid A and residue 234 and name HN)         3.350  1.650  1.650
assign (segid A and residue 230 and name HB#) (segid A and residue 234 and name HN)        3.600  1.900  1.900
assign (segid A and residue 231 and name HA) (segid A and residue 234 and name HN)         3.350  1.650  1.650
assign (segid A and residue 232 and name HB#) (segid A and residue 234 and name HN)        3.350  1.650  1.650
assign (segid A and residue 233 and name HA) (segid A and residue 234 and name HN)         3.350  1.650  1.650
assign (segid A and residue 233 and name HD1#) (segid A and residue 234 and name HN)       3.600  1.900  1.900
assign (segid A and residue 233 and name HD2#) (segid A and residue 234 and name HN)       3.600  1.900  1.900
assign (segid A and residue 234 and name HN) (segid A and residue 234 and name HA)         3.350  1.650  1.650
assign (segid A and residue 234 and name HN) (segid A and residue 234 and name HB)         3.350  1.650  1.650
assign (segid A and residue 234 and name HN) (segid A and residue 234 and name HD1#)       3.600  1.900  1.900
assign (segid A and residue 234 and name HN) (segid A and residue 234 and name HG1#)       3.600  1.900  1.900
assign (segid A and residue 234 and name HN) (segid A and residue 234 and name HG2#)       3.600  1.900  1.900
assign (segid A and residue 234 and name HA) (segid A and residue 235 and name HN)         3.350  1.650  1.650
assign (segid A and residue 234 and name HB) (segid A and residue 235 and name HN)         3.350  1.650  1.650
assign (segid A and residue 234 and name HD1#) (segid A and residue 235 and name HN)       3.600  1.900  1.900
assign (segid A and residue 235 and name HN) (segid A and residue 235 and name HA)         3.350  1.650  1.650
assign (segid A and residue 235 and name HN) (segid A and residue 236 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 236 and name HN) (segid A and residue 236 and name HA)         2.350  0.650  0.650
assign (segid A and residue 236 and name HN) (segid A and residue 236 and name HB#)        2.350  0.650  0.650
assign (segid A and residue 236 and name HN) (segid A and residue 236 and name HG#)        2.350  0.650  0.650
assign (segid A and residue 234 and name HA) (segid A and residue 237 and name HN)         3.350  1.650  1.650
assign (segid A and residue 234 and name HG2#) (segid A and residue 237 and name HN)       3.600  1.900  1.900
assign (segid A and residue 237 and name HN) (segid A and residue 237 and name HA)         2.350  0.650  0.650
assign (segid A and residue 237 and name HN) (segid A and residue 237 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 237 and name HN) (segid A and residue 237 and name HD1#)       3.600  1.900  1.900
```

```
assign (segid A and residue 237 and name HN) (segid A and residue 237 and name HD2#)       3.600  1.900  1.900
assign (segid A and residue 237 and name HN) (segid A and residue 237 and name HG)        2.350  0.650  0.650
assign (segid A and residue 234 and name HA) (segid A and residue 238 and name HN)        3.350  1.650  1.650
assign (segid A and residue 238 and name HN) (segid A and residue 238 and name HA)        3.350  1.650  1.650
assign (segid A and residue 238 and name HN) (segid A and residue 238 and name HB#)       3.600  1.900  1.900
assign (segid A and residue 238 and name HB#) (segid A and residue 239 and name HN)       3.600  1.900  1.900
assign (segid A and residue 239 and name HN) (segid A and residue 239 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 239 and name HN) (segid A and residue 239 and name HD#)       3.350  1.650  1.650
assign (segid A and residue 239 and name HN) (segid A and residue 239 and name HG#)       2.350  0.650  0.650
assign (segid A and residue 238 and name HB#) (segid A and residue 240 and name HN)       3.600  1.900  1.900
assign (segid A and residue 239 and name HD#) (segid A and residue 240 and name HN)       3.350  1.650  1.650
assign (segid A and residue 238 and name HB#) (segid A and residue 241 and name HN)       3.600  1.900  1.900
assign (segid A and residue 241 and name HN) (segid A and residue 241 and name HA)        3.350  1.650  1.650
assign (segid A and residue 241 and name HN) (segid A and residue 241 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 239 and name HA) (segid A and residue 242 and name HN)        3.350  1.650  1.650
assign (segid A and residue 241 and name HA) (segid A and residue 242 and name HN)        3.350  1.650  1.650
assign (segid A and residue 242 and name HN) (segid A and residue 242 and name HA)        2.350  0.650  0.650
assign (segid A and residue 242 and name HN) (segid A and residue 242 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 242 and name HN) (segid A and residue 243 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 242 and name HN) (segid A and residue 263 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 242 and name HB#) (segid A and residue 243 and name HN)       3.350  1.650  1.650
assign (segid A and residue 243 and name HN) (segid A and residue 243 and name HA)        2.350  0.650  0.650
assign (segid A and residue 243 and name HN) (segid A and residue 243 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 243 and name HN) (segid A and residue 243 and name HG#)       2.350  0.650  0.650
assign (segid A and residue 243 and name HN) (segid A and residue 246 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 241 and name HA) (segid A and residue 244 and name HN)        3.350  1.650  1.650
assign (segid A and residue 244 and name HN) (segid A and residue 244 and name HA)        2.350  0.650  0.650
assign (segid A and residue 244 and name HN) (segid A and residue 244 and name HB)        2.350  0.650  0.650
assign (segid A and residue 244 and name HA) (segid A and residue 245 and name HN)        3.350  1.650  1.650
assign (segid A and residue 245 and name HN) (segid A and residue 245 and name HA)        3.350  1.650  1.650
assign (segid A and residue 245 and name HN) (segid A and residue 245 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 245 and name HN) (segid A and residue 245 and name HD#)       3.350  1.650  1.650
assign (segid A and residue 245 and name HN) (segid A and residue 245 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 242 and name HB#) (segid A and residue 246 and name HN)       3.350  1.650  1.650
assign (segid A and residue 243 and name HA) (segid A and residue 246 and name HN)        3.350  1.650  1.650
assign (segid A and residue 244 and name HA) (segid A and residue 246 and name HN)        3.350  1.650  1.650
assign (segid A and residue 246 and name HN) (segid A and residue 246 and name HA)        2.350  0.650  0.650
assign (segid A and residue 246 and name HN) (segid A and residue 246 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 246 and name HN) (segid A and residue 246 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 246 and name HN) (segid A and residue 246 and name HD2#)      3.600  1.900  1.900
assign (segid A and residue 244 and name HA) (segid A and residue 247 and name HN)        3.350  1.650  1.650
assign (segid A and residue 245 and name HA) (segid A and residue 247 and name HN)        3.350  1.650  1.650
assign (segid A and residue 247 and name HN) (segid A and residue 247 and name HA)        2.350  0.650  0.650
assign (segid A and residue 247 and name HN) (segid A and residue 247 and name HD1#)      3.600  1.900  1.900
```

```
assign (segid A and residue 247 and name HN) (segid A and residue 247 and name HD2#)       3.600  1.900  1.900
assign (segid A and residue 247 and name HN) (segid A and residue 247 and name HG)        2.350  0.650  0.650
assign (segid A and residue 244 and name HA) (segid A and residue 248 and name HN)        3.350  1.650  1.650
assign (segid A and residue 245 and name HA) (segid A and residue 248 and name HN)        3.350  1.650  1.650
assign (segid A and residue 247 and name HD1#) (segid A and residue 248 and name HN)      3.600  1.900  1.900
assign (segid A and residue 248 and name HN) (segid A and residue 248 and name HA)        2.350  0.650  0.650
assign (segid A and residue 249 and name HN) (segid A and residue 249 and name HA)        2.350  0.650  0.650
assign (segid A and residue 249 and name HN) (segid A and residue 249 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 248 and name HB) (segid A and residue 250 and name HN)        3.350  1.650  1.650
assign (segid A and residue 248 and name HG1#) (segid A and residue 250 and name HN)      3.600  1.900  1.900
assign (segid A and residue 248 and name HG2#) (segid A and residue 250 and name HN)      3.600  1.900  1.900
assign (segid A and residue 250 and name HN) (segid A and residue 250 and name HA)        3.350  1.650  1.650
assign (segid A and residue 251 and name HN) (segid A and residue 251 and name HA)        2.350  0.650  0.650
assign (segid A and residue 251 and name HN) (segid A and residue 251 and name HB#)       2.350  0.650  0.650
assign (segid A and residue 251 and name HN) (segid A and residue 251 and name HD#)       3.350  1.650  1.650
assign (segid A and residue 251 and name HN) (segid A and residue 251 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 251 and name HD#) (segid A and residue 252 and name HN)       3.350  1.650  1.650
assign (segid A and residue 251 and name HG#) (segid A and residue 252 and name HN)       3.350  1.650  1.650
assign (segid A and residue 252 and name HN) (segid A and residue 252 and name HA)        2.350  0.650  0.650
assign (segid A and residue 252 and name HN) (segid A and residue 253 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 252 and name HN) (segid A and residue 256 and name HG1#)      3.600  1.900  1.900
assign (segid A and residue 252 and name HA) (segid A and residue 253 and name HN)        3.350  1.650  1.650
assign (segid A and residue 252 and name HB#) (segid A and residue 253 and name HN)       3.350  1.650  1.650
assign (segid A and residue 253 and name HN) (segid A and residue 253 and name HA)        2.350  0.650  0.650
assign (segid A and residue 253 and name HN) (segid A and residue 253 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 253 and name HN) (segid A and residue 256 and name HG1#)      3.600  1.900  1.900
assign (segid A and residue 254 and name HG#) (segid A and residue 255 and name HN)       3.350  1.650  1.650
assign (segid A and residue 255 and name HN) (segid A and residue 255 and name HA)        3.350  1.650  1.650
assign (segid A and residue 256 and name HN) (segid A and residue 256 and name HA)        3.350  1.650  1.650
assign (segid A and residue 256 and name HN) (segid A and residue 256 and name HB)        2.350  0.650  0.650
assign (segid A and residue 256 and name HN) (segid A and residue 256 and name HG1#)      3.600  1.900  1.900
assign (segid A and residue 256 and name HA) (segid A and residue 258 and name HN)        3.350  1.650  1.650
assign (segid A and residue 256 and name HG1#) (segid A and residue 258 and name HN)      3.600  1.900  1.900
assign (segid A and residue 257 and name HD#) (segid A and residue 258 and name HN)       3.350  1.650  1.650
assign (segid A and residue 258 and name HN) (segid A and residue 258 and name HA)        3.350  1.650  1.650
assign (segid A and residue 258 and name HN) (segid A and residue 258 and name HB#)       2.600  0.900  0.900
assign (segid A and residue 258 and name HN) (segid A and residue 259 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 256 and name HA) (segid A and residue 259 and name HN)        3.350  1.650  1.650
assign (segid A and residue 257 and name HA) (segid A and residue 259 and name HN)        3.350  1.650  1.650
assign (segid A and residue 257 and name HD#) (segid A and residue 259 and name HN)       3.350  1.650  1.650
assign (segid A and residue 259 and name HN) (segid A and residue 259 and name HA)        3.350  1.650  1.650
assign (segid A and residue 259 and name HN) (segid A and residue 259 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 259 and name HN) (segid A and residue 259 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 259 and name HN) (segid A and residue 259 and name HD2#)      3.600  1.900  1.900
```

```
assign (segid A and residue 257 and name HD#) (segid A and residue 260 and name HN)        3.350  1.650  1.650
assign (segid A and residue 258 and name HA) (segid A and residue 260 and name HN)        3.350  1.650  1.650
assign (segid A and residue 258 and name HB#) (segid A and residue 260 and name HN)       3.600  1.900  1.900
assign (segid A and residue 259 and name HB#) (segid A and residue 260 and name HN)       3.350  1.650  1.650
assign (segid A and residue 259 and name HD1#) (segid A and residue 260 and name HN)      3.600  1.900  1.900
assign (segid A and residue 259 and name HD2#) (segid A and residue 260 and name HN)      3.600  1.900  1.900
assign (segid A and residue 259 and name HG) (segid A and residue 260 and name HN)        3.350  1.650  1.650
assign (segid A and residue 260 and name HN) (segid A and residue 260 and name HA)        3.350  1.650  1.650
assign (segid A and residue 260 and name HN) (segid A and residue 260 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 262 and name HN) (segid A and residue 262 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 262 and name HN) (segid A and residue 262 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 262 and name HN) (segid A and residue 262 and name HG)        3.600  1.900  1.900
assign (segid A and residue 262 and name HN) (segid A and residue 263 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 262 and name HB#) (segid A and residue 263 and name HN)       3.350  1.650  1.650
assign (segid A and residue 263 and name HN) (segid A and residue 263 and name HA)        3.350  1.650  1.650
assign (segid A and residue 263 and name HN) (segid A and residue 263 and name HB)        3.350  1.650  1.650
assign (segid A and residue 263 and name HN) (segid A and residue 263 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 263 and name HN) (segid A and residue 263 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 263 and name HA) (segid A and residue 264 and name HN)        3.350  1.650  1.650
assign (segid A and residue 263 and name HB) (segid A and residue 264 and name HN)        3.350  1.650  1.650
assign (segid A and residue 263 and name HD1#) (segid A and residue 264 and name HN)      3.600  1.900  1.900
assign (segid A and residue 263 and name HG1#) (segid A and residue 264 and name HN)      3.350  1.650  1.650
assign (segid A and residue 264 and name HN) (segid A and residue 264 and name HA)        3.350  1.650  1.650
assign (segid A and residue 264 and name HN) (segid A and residue 264 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 265 and name HN) (segid A and residue 265 and name HA)        3.350  1.650  1.650
assign (segid A and residue 265 and name HN) (segid A and residue 265 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 265 and name HN) (segid A and residue 265 and name HD1#)      3.600  1.900  1.900
assign (segid A and residue 265 and name HN) (segid A and residue 265 and name HD2#)      3.600  1.900  1.900
assign (segid A and residue 265 and name HN) (segid A and residue 265 and name HG)        3.350  1.650  1.650
assign (segid A and residue 265 and name HN) (segid A and residue 266 and name HB)        3.350  1.650  1.650
assign (segid A and residue 263 and name HD1#) (segid A and residue 266 and name HN)      3.600  1.900  1.900
assign (segid A and residue 265 and name HD1#) (segid A and residue 266 and name HN)      3.600  1.900  1.900
assign (segid A and residue 265 and name HD2#) (segid A and residue 266 and name HN)      3.600  1.900  1.900
assign (segid A and residue 266 and name HN) (segid A and residue 266 and name HG2#)      2.600  0.900  0.900
assign (segid A and residue 266 and name HB) (segid A and residue 267 and name HN)        3.350  1.650  1.650
assign (segid A and residue 266 and name HG1#) (segid A and residue 267 and name HN)      3.600  1.900  1.900
assign (segid A and residue 267 and name HN) (segid A and residue 267 and name HA)        3.350  1.650  1.650
assign (segid A and residue 267 and name HN) (segid A and residue 267 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 267 and name HN) (segid A and residue 276 and name HB#)       3.600  1.900  1.900
assign (segid A and residue 265 and name HD2#) (segid A and residue 268 and name HN)      3.600  1.900  1.900
assign (segid A and residue 267 and name HB#) (segid A and residue 268 and name HN)       3.350  1.650  1.650
assign (segid A and residue 268 and name HN) (segid A and residue 268 and name HA)        3.350  1.650  1.650
assign (segid A and residue 268 and name HN) (segid A and residue 268 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 268 and name HN) (segid A and residue 268 and name HD#)       3.350  1.650  1.650
```

```
assign (segid A and residue 268 and name HN) (segid A and residue 268 and name HG#)        3.350  1.650  1.650
assign (segid A and residue 268 and name HN) (segid A and residue 269 and name HA)         3.350  1.650  1.650
assign (segid A and residue 268 and name HN) (segid A and residue 276 and name HB#)        3.600  1.900  1.900
assign (segid A and residue 265 and name HD2#) (segid A and residue 269 and name HN)       3.600  1.900  1.900
assign (segid A and residue 268 and name HA) (segid A and residue 269 and name HN)         3.350  1.650  1.650
assign (segid A and residue 269 and name HN) (segid A and residue 269 and name HA)         3.350  1.650  1.650
assign (segid A and residue 269 and name HN) (segid A and residue 269 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 270 and name HN) (segid A and residue 270 and name HA)         3.350  1.650  1.650
assign (segid A and residue 270 and name HN) (segid A and residue 270 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 270 and name HN) (segid A and residue 271 and name HA)         3.350  1.650  1.650
assign (segid A and residue 269 and name HA) (segid A and residue 271 and name HN)         3.350  1.650  1.650
assign (segid A and residue 270 and name HB#) (segid A and residue 271 and name HN)        3.350  1.650  1.650
assign (segid A and residue 270 and name HD#) (segid A and residue 271 and name HN)        3.350  1.650  1.650
assign (segid A and residue 271 and name HN) (segid A and residue 271 and name HA)         2.350  0.650  0.650
assign (segid A and residue 271 and name HN) (segid A and residue 271 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 234 and name HD1#) (segid A and residue 272 and name HN)       3.600  1.900  1.900
assign (segid A and residue 267 and name HA) (segid A and residue 272 and name HN)         3.350  1.650  1.650
assign (segid A and residue 271 and name HA) (segid A and residue 272 and name HN)         3.350  1.650  1.650
assign (segid A and residue 271 and name HB#) (segid A and residue 272 and name HN)        3.350  1.650  1.650
assign (segid A and residue 272 and name HN) (segid A and residue 272 and name HA)         3.350  1.650  1.650
assign (segid A and residue 272 and name HN) (segid A and residue 272 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 272 and name HA) (segid A and residue 273 and name HN)         3.350  1.650  1.650
assign (segid A and residue 273 and name HN) (segid A and residue 273 and name HA)         3.350  1.650  1.650
assign (segid A and residue 273 and name HN) (segid A and residue 273 and name HB#)        2.350  0.650  0.650
assign (segid A and residue 273 and name HN) (segid A and residue 273 and name HD#)        3.350  1.650  1.650
assign (segid A and residue 273 and name HN) (segid A and residue 273 and name HG#)        2.350  0.650  0.650
assign (segid A and residue 273 and name HD#) (segid A and residue 274 and name HN)        3.350  1.650  1.650
assign (segid A and residue 274 and name HN) (segid A and residue 274 and name HA)         3.350  1.650  1.650
assign (segid A and residue 274 and name HN) (segid A and residue 274 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 274 and name HN) (segid A and residue 275 and name HD1#)       3.600  1.900  1.900
assign (segid A and residue 274 and name HN) (segid A and residue 276 and name HB#)        3.600  1.900  1.900
assign (segid A and residue 275 and name HN) (segid A and residue 275 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 275 and name HN) (segid A and residue 275 and name HG)         3.350  1.650  1.650
assign (segid A and residue 273 and name HA) (segid A and residue 276 and name HN)         3.350  1.650  1.650
assign (segid A and residue 273 and name HD#) (segid A and residue 276 and name HN)        3.350  1.650  1.650
assign (segid A and residue 275 and name HB#) (segid A and residue 276 and name HN)        3.350  1.650  1.650
assign (segid A and residue 275 and name HD2#) (segid A and residue 276 and name HN)       3.600  1.900  1.900
assign (segid A and residue 276 and name HN) (segid A and residue 276 and name HA)         3.350  1.650  1.650
assign (segid A and residue 276 and name HN) (segid A and residue 276 and name HB#)        3.600  1.900  1.900
assign (segid A and residue 263 and name HG2#) (segid A and residue 277 and name HN)       3.600  1.900  1.900
assign (segid A and residue 277 and name HN) (segid A and residue 277 and name HA)         3.350  1.650  1.650
assign (segid A and residue 277 and name HN) (segid A and residue 277 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 277 and name HB#) (segid A and residue 278 and name HN)        3.350  1.650  1.650
assign (segid A and residue 278 and name HN) (segid A and residue 278 and name HB#)        2.350  0.650  0.650
```

```
assign (segid A and residue 278 and name HN) (segid A and residue 278 and name HG#)        2.350  0.650  0.650
assign (segid A and residue 278 and name HN) (segid A and residue 279 and name HD#)        3.350  1.650  1.650
assign (segid A and residue 278 and name HG#) (segid A and residue 280 and name HN)        3.350  1.650  1.650
assign (segid A and residue 280 and name HN) (segid A and residue 280 and name HA)        2.350  0.650  0.650
assign (segid A and residue 280 and name HN) (segid A and residue 281 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 280 and name HN) (segid A and residue 281 and name HG)        3.350  1.650  1.650
assign (segid A and residue 278 and name HB#) (segid A and residue 281 and name HN)        3.350  1.650  1.650
assign (segid A and residue 278 and name HG#) (segid A and residue 281 and name HN)        3.350  1.650  1.650
assign (segid A and residue 279 and name HA) (segid A and residue 281 and name HN)        3.350  1.650  1.650
assign (segid A and residue 281 and name HN) (segid A and residue 281 and name HA)        2.350  0.650  0.650
assign (segid A and residue 281 and name HN) (segid A and residue 281 and name HD1#)       3.600  1.900  1.900
assign (segid A and residue 281 and name HN) (segid A and residue 281 and name HD2#)       3.600  1.900  1.900
assign (segid A and residue 281 and name HN) (segid A and residue 281 and name HG)        2.350  0.650  0.650
assign (segid A and residue 280 and name HB#) (segid A and residue 282 and name HN)        3.350  1.650  1.650
assign (segid A and residue 281 and name HD2#) (segid A and residue 282 and name HN)       3.600  1.900  1.900
assign (segid A and residue 282 and name HN) (segid A and residue 282 and name HA)        2.350  0.650  0.650
assign (segid A and residue 282 and name HN) (segid A and residue 282 and name HG#)        2.350  0.650  0.650
assign (segid A and residue 280 and name HB#) (segid A and residue 283 and name HN)        3.350  1.650  1.650
assign (segid A and residue 281 and name HB#) (segid A and residue 283 and name HN)        3.350  1.650  1.650
assign (segid A and residue 281 and name HD1#) (segid A and residue 283 and name HN)       3.600  1.900  1.900
assign (segid A and residue 281 and name HD2#) (segid A and residue 283 and name HN)       3.600  1.900  1.900
assign (segid A and residue 281 and name HG) (segid A and residue 283 and name HN)        3.350  1.650  1.650
assign (segid A and residue 283 and name HN) (segid A and residue 283 and name HA)        2.350  0.650  0.650
assign (segid A and residue 283 and name HN) (segid A and residue 283 and name HB#)        2.350  0.650  0.650
assign (segid B and residue 31 and name HA) (segid B and residue 32 and name HN)          3.350  1.650  1.650
assign (segid B and residue 33 and name HN) (segid B and residue 33 and name HB)          3.350  1.650  1.650
assign (segid B and residue 33 and name HN) (segid B and residue 33 and name HG1#)        3.350  1.650  1.650
assign (segid B and residue 49 and name HA) (segid B and residue 50 and name HN)          3.350  1.650  1.650
assign (segid B and residue 51 and name HN) (segid B and residue 51 and name HB#)         3.350  1.650  1.650
assign (segid A and residue 108 and name HA) (segid A and residue 109 and name HN)        3.350  1.650  1.650
assign (segid A and residue 109 and name HN) (segid A and residue 109 and name HG#)       3.350  1.650  1.650
assign (segid A and residue 115 and name HA) (segid A and residue 116 and name HN)        3.350  1.650  1.650
assign (segid A and residue 119 and name HN) (segid A and residue 119 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 127 and name HN) (segid A and residue 127 and name HB#)       3.350  1.650  1.650
assign (segid A and residue 136 and name HA) (segid A and residue 137 and name HN)        3.350  1.650  1.650
assign (segid A and residue 152 and name HA) (segid A and residue 153 and name HN)        3.350  1.650  1.650
assign (segid A and residue 156 and name HA) (segid A and residue 157 and name HN)        3.350  1.650  1.650
assign (segid A and residue 159 and name HB) (segid A and residue 160 and name HN)        3.350  1.650  1.650
assign (segid A and residue 161 and name HB#) (segid A and residue 162 and name HN)       3.350  1.650  1.650
assign (segid A and residue 164 and name HA) (segid A and residue 165 and name HN)        3.350  1.650  1.650
assign (segid A and residue 164 and name HB#) (segid A and residue 165 and name HN)       3.350  1.650  1.650
assign (segid A and residue 169 and name HN) (segid A and residue 169 and name HG2#)      3.600  1.900  1.900
assign (segid A and residue 171 and name HA) (segid A and residue 172 and name HN)        3.350  1.650  1.650
assign (segid A and residue 172 and name HN) (segid A and residue 172 and name HG1#)      3.600  1.900  1.900
```

```
assign (segid A and residue 174 and name HA) (segid A and residue 175 and name HN)         3.350  1.650  1.650
assign (segid A and residue 186 and name HA) (segid A and residue 187 and name HN)         3.350  1.650  1.650
assign (segid A and residue 223 and name HN) (segid A and residue 223 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 226 and name HN) (segid A and residue 226 and name HG)         3.350  1.650  1.650
assign (segid A and residue 231 and name HA) (segid A and residue 232 and name HN)         3.350  1.650  1.650
assign (segid A and residue 230 and name HA) (segid A and residue 233 and name HN)         3.350  1.650  1.650
assign (segid A and residue 233 and name HN) (segid A and residue 233 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 242 and name HA) (segid A and residue 243 and name HN)         3.350  1.650  1.650
assign (segid A and residue 246 and name HN) (segid A and residue 246 and name HG)         3.350  1.650  1.650
assign (segid A and residue 248 and name HN) (segid A and residue 248 and name HG1#)       3.600  1.900  1.900
assign (segid A and residue 281 and name HN) (segid A and residue 281 and name HB#)        3.350  1.650  1.650
assign (segid A and residue 282 and name HN) (segid A and residue 282 and name HB#)        3.350  1.650  1.650
```

From the 3D ^{13}C aromatic NOESY-HSQC

```
assign (segid B and residue 33 and name HG2#) (segid B and residue 34 and name HD1) 3.60 1.90 1.90
assign (segid B and residue 34 and name HD1) (segid B and residue 34 and name HA) 3.35 1.65 1.65
assign (segid B and residue 34 and name HD1) (segid B and residue 34 and name HB#) 3.35 1.65 1.65
assign (segid B and residue 34 and name HE3) (segid B and residue 34 and name HZ3) 3.35 1.65 1.65
assign (segid B and residue 33 and name HG1#) (segid B and residue 34 and name HH2) 3.35 1.65 1.65
assign (segid B and residue 34 and name HH2) (segid B and residue 34 and name HZ2) 3.35 1.65 1.65
assign (segid B and residue 34 and name HH2) (segid B and residue 34 and name HZ3) 3.35 1.65 1.65
assign (segid B and residue 33 and name HD1#) (segid B and residue 34 and name HZ2) 3.60 1.90 1.90
assign (segid B and residue 33 and name HG1#) (segid B and residue 34 and name HZ2) 3.35 1.65 1.65
assign (segid B and residue 34 and name HZ2) (segid B and residue 34 and name HH2) 3.35 1.65 1.65
assign (segid B and residue 34 and name HZ2) (segid B and residue 34 and name HZ3) 3.35 1.65 1.65
assign (segid B and residue 34 and name HZ3) (segid B and residue 34 and name HE3) 3.35 1.65 1.65
assign (segid B and residue 34 and name HZ3) (segid B and residue 34 and name HH2) 3.35 1.65 1.65
assign (segid B and residue 43 and name HE#) (segid B and residue 43 and name HA) 3.35 1.65 1.65
assign (segid B and residue 43 and name HE#) (segid B and residue 43 and name HB#) 3.35 1.65 1.65
assign (segid B and residue 43 and name HE#) (segid B and residue 43 and name HD#) 3.35 1.65 1.65
assign (segid B and residue 43 and name HE#) (segid A and residue 212 and name HA2) 3.35 1.65 1.65
assign (segid B and residue 50 and name HD#) (segid B and residue 50 and name HA) 3.35 1.65 1.65
assign (segid B and residue 50 and name HD#) (segid B and residue 50 and name HB#) 3.35 1.65 1.65
assign (segid B and residue 50 and name HD#) (segid B and residue 50 and name HE#) 3.35 1.65 1.65
assign (segid B and residue 50 and name HE#) (segid B and residue 50 and name HB#) 3.35 1.65 1.65
assign (segid B and residue 50 and name HE#) (segid B and residue 50 and name HD#) 3.35 1.65 1.65
assign (segid B and residue 50 and name HZ) (segid B and residue 50 and name HE#) 3.35 1.65 1.65
assign (segid A and residue 96 and name HD#) (segid A and residue 96 and name HA) 3.35 1.65 1.65
assign (segid A and residue 96 and name HD#) (segid A and residue 96 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 96 and name HD#) (segid A and residue 96 and name HE#) 3.35 1.65 1.65
assign (segid A and residue 96 and name HE#) (segid A and residue 96 and name HA) 3.35 1.65 1.65
```

```
assign (segid A and residue 96 and name HE#) (segid A and residue 96 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 101 and name HB#) (segid A and residue 102 and name HD1) 3.35 1.65 1.65
assign (segid A and residue 101 and name HG#) (segid A and residue 102 and name HD1) 3.35 1.65 1.65
assign (segid A and residue 102 and name HD1) (segid A and residue 102 and name HA) 3.35 1.65 1.65
assign (segid A and residue 102 and name HD1) (segid A and residue 102 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 110 and name HD#) (segid A and residue 265 and name HD1#) 3.60 1.90 1.90
assign (segid A and residue 120 and name HD2) (segid A and residue 120 and name HA) 3.35 1.65 1.65
assign (segid A and residue 120 and name HD2) (segid A and residue 120 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 119 and name HB#) (segid A and residue 120 and name HE1) 3.35 1.65 1.65
assign (segid A and residue 119 and name HE#) (segid A and residue 120 and name HE1) 3.35 1.65 1.65
assign (segid A and residue 119 and name HG#) (segid A and residue 120 and name HE1) 3.35 1.65 1.65
assign (segid A and residue 120 and name HE1) (segid A and residue 120 and name HA) 3.35 1.65 1.65
assign (segid A and residue 120 and name HE1) (segid A and residue 120 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 120 and name HE1) (segid A and residue 120 and name HD2) 3.35 1.65 1.65
assign (segid A and residue 123 and name HE1) (segid A and residue 123 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 123 and name HE1) (segid A and residue 123 and name HD2) 3.35 1.65 1.65
assign (segid A and residue 120 and name HA) (segid A and residue 124 and name HD1) 3.35 1.65 1.65
assign (segid A and residue 120 and name HB#) (segid A and residue 124 and name HD1) 3.35 1.65 1.65
assign (segid A and residue 124 and name HD1) (segid A and residue 124 and name HA) 3.35 1.65 1.65
assign (segid A and residue 124 and name HD1) (segid A and residue 124 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 144 and name HD1) (segid A and residue 144 and name HA) 3.35 1.65 1.65
assign (segid A and residue 144 and name HD1) (segid A and residue 144 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 144 and name HZ2) (segid A and residue 144 and name HH2) 3.35 1.65 1.65
assign (segid A and residue 135 and name HE#) (segid A and residue 144 and name HZ2) 3.60 1.90 1.90
assign (segid A and residue 135 and name HE#) (segid A and residue 144 and name HH2) 3.60 1.90 1.90
assign (segid A and residue 147 and name HD#) (segid A and residue 147 and name HA) 3.35 1.65 1.65
assign (segid A and residue 147 and name HD#) (segid A and residue 147 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 147 and name HD#) (segid A and residue 147 and name HE#) 3.35 1.65 1.65
assign (segid A and residue 136 and name HG#) (segid A and residue 147 and name HD#) 3.35 1.65 1.65
assign (segid A and residue 136 and name HD#) (segid A and residue 147 and name HD#) 3.35 1.65 1.65
assign (segid A and residue 147 and name HE#) (segid A and residue 147 and name HA) 3.35 1.65 1.65
assign (segid A and residue 147 and name HE#) (segid A and residue 147 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 147 and name HE#) (segid A and residue 147 and name HD#) 3.35 1.65 1.65
assign (segid A and residue 169 and name HD1#) (segid A and residue 171 and name HD#) 3.35 1.65 1.65
assign (segid A and residue 169 and name HG2#) (segid A and residue 171 and name HD#) 3.35 1.65 1.65
assign (segid A and residue 171 and name HD#) (segid A and residue 171 and name HA) 3.35 1.65 1.65
assign (segid A and residue 171 and name HD#) (segid A and residue 171 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 171 and name HD#) (segid A and residue 171 and name HE#) 3.35 1.65 1.65
assign (segid A and residue 171 and name HE#) (segid A and residue 171 and name HD#) 3.35 1.65 1.65
assign (segid A and residue 133 and name HG1#) (segid A and residue 176 and name HD#) 3.60 1.90 1.90
assign (segid A and residue 133 and name HG2#) (segid A and residue 176 and name HD#) 3.60 1.90 1.90
assign (segid A and residue 176 and name HD#) (segid A and residue 176 and name HA) 3.35 1.65 1.65
assign (segid A and residue 176 and name HD#) (segid A and residue 176 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 176 and name HD#) (segid A and residue 176 and name HE#) 3.35 1.65 1.65
```

```
assign (segid A and residue 176 and name HD#) (segid A and residue 177 and name HD#) 3.35 1.65 1.65
assign (segid A and residue 176 and name HD#) (segid A and residue 182 and name HD1#) 3.60 1.90 1.90
assign (segid A and residue 133 and name HA) (segid A and residue 176 and name HE#) 3.35 1.65 1.65
assign (segid A and residue 133 and name HG1#) (segid A and residue 176 and name HE#) 3.60 1.90 1.90
assign (segid A and residue 133 and name HG2#) (segid A and residue 176 and name HE#) 3.60 1.90 1.90
assign (segid A and residue 134 and name HG2#) (segid A and residue 176 and name HE#) 3.60 1.90 1.90
assign (segid A and residue 176 and name HE#) (segid A and residue 176 and name HA) 3.35 1.65 1.65
assign (segid A and residue 176 and name HE#) (segid A and residue 176 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 176 and name HE#) (segid A and residue 176 and name HD#) 3.35 1.65 1.65
assign (segid A and residue 207 and name HD#) (segid A and residue 207 and name HA) 3.35 1.65 1.65
assign (segid A and residue 207 and name HD#) (segid A and residue 207 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 207 and name HD#) (segid A and residue 207 and name HE#) 3.35 1.65 1.65
assign (segid A and residue 205 and name HB#) (segid A and residue 207 and name HE#) 3.35 1.65 1.65
assign (segid A and residue 207 and name HE#) (segid A and residue 207 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 207 and name HE#) (segid A and residue 207 and name HD#) 3.35 1.65 1.65
assign (segid A and residue 207 and name HE#) (segid A and residue 207 and name HZ) 3.35 1.65 1.65
assign (segid A and residue 207 and name HE#) (segid A and residue 209 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 207 and name HE#) (segid A and residue 244 and name HG#) 3.60 1.90 1.90
assign (segid A and residue 205 and name HB#) (segid A and residue 207 and name HZ) 3.35 1.65 1.65
assign (segid A and residue 207 and name HZ) (segid A and residue 207 and name HE#) 3.35 1.65 1.65
assign (segid A and residue 207 and name HZ) (segid A and residue 244 and name HG#) 3.60 1.90 1.90
assign (segid A and residue 214 and name HD1) (segid A and residue 214 and name HA) 3.35 1.65 1.65
assign (segid A and residue 214 and name HD1) (segid A and residue 214 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 214 and name HD1) (segid A and residue 214 and name HE1) 3.35 1.65 1.65
assign (segid A and residue 214 and name HD1) (segid A and residue 214 and name HE3) 3.35 1.65 1.65
assign (segid A and residue 214 and name HE3) (segid A and residue 214 and name HA) 3.35 1.65 1.65
assign (segid A and residue 214 and name HE3) (segid A and residue 214 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 214 and name HE3) (segid A and residue 214 and name HZ3) 3.35 1.65 1.65
assign (segid A and residue 214 and name HZ2) (segid A and residue 214 and name HZ3) 3.35 1.65 1.65
assign (segid A and residue 214 and name HZ2) (segid A and residue 217 and name HB#) 3.60 1.90 1.90
assign (segid A and residue 214 and name HZ3) (segid A and residue 214 and name HE3) 3.35 1.65 1.65
assign (segid A and residue 214 and name HZ3) (segid A and residue 214 and name HH2) 3.35 1.65 1.65
assign (segid A and residue 214 and name HZ3) (segid A and residue 217 and name HB#) 3.60 1.90 1.90
assign (segid A and residue 216 and name HD#) (segid A and residue 216 and name HA) 3.35 1.65 1.65
assign (segid A and residue 216 and name HD#) (segid A and residue 216 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 216 and name HD#) (segid A and residue 216 and name HE#) 3.35 1.65 1.65
assign (segid A and residue 216 and name HD#) (segid A and residue 216 and name HN) 3.35 1.65 1.65
assign (segid A and residue 216 and name HD#) (segid A and residue 217 and name HN) 3.35 1.65 1.65
assign (segid B and residue 47 and name HG2#) (segid A and residue 216 and name HE#) 3.60 1.90 1.90
assign (segid A and residue 213 and name HG#) (segid A and residue 216 and name HE#) 3.35 1.65 1.65
assign (segid A and residue 216 and name HE#) (segid A and residue 216 and name HA) 3.35 1.65 1.65
assign (segid A and residue 231 and name HD2) (segid A and residue 231 and name HA) 3.35 1.65 1.65
assign (segid A and residue 231 and name HD2) (segid A and residue 231 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 226 and name HD#) (segid A and residue 231 and name HE1) 3.35 1.65 1.65
```

```
assign (segid A and residue 231 and name HE1) (segid A and residue 231 and name HA) 3.35 1.65 1.65
assign (segid A and residue 231 and name HE1) (segid A and residue 231 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 231 and name HE1) (segid A and residue 231 and name HD2) 3.35 1.65 1.65
assign (segid A and residue 231 and name HE1) (segid A and residue 234 and name HD1#) 3.60 1.90 1.90
assign (segid A and residue 231 and name HE1) (segid A and residue 271 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 265 and name HD2#) (segid A and residue 269 and name HD#) 3.60 1.90 1.90
assign (segid A and residue 269 and name HD#) (segid A and residue 269 and name HA) 3.35 1.65 1.65
assign (segid A and residue 269 and name HD#) (segid A and residue 269 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 269 and name HD#) (segid A and residue 269 and name HE#) 3.35 1.65 1.65
assign (segid A and residue 269 and name HD#) (segid A and residue 270 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 269 and name HE#) (segid A and residue 269 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 269 and name HE#) (segid A and residue 269 and name HD#) 3.35 1.65 1.65
assign (segid A and residue 266 and name HG1#) (segid A and residue 270 and name HD#) 3.35 1.65 1.65
assign (segid A and residue 270 and name HD#) (segid A and residue 270 and name HA) 3.35 1.65 1.65
assign (segid A and residue 270 and name HD#) (segid A and residue 270 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 283 and name HD#) (segid A and residue 283 and name HA) 3.35 1.65 1.65
assign (segid A and residue 283 and name HD#) (segid A and residue 283 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 283 and name HD#) (segid A and residue 283 and name HE#) 3.35 1.65 1.65
assign (segid A and residue 283 and name HE#) (segid A and residue 283 and name HB#) 3.35 1.65 1.65
assign (segid A and residue 283 and name HE#) (segid A and residue 283 and name HD#) 3.35 1.65 1.65
assign (segid A and residue 136 and name HB#) (segid A and residue 144 and name HE1) 3.35 1.65 1.65
assign (segid A and residue 124 and name HD1) (segid A and residue 180 and name HD1#) 3.60 1.90 1.90
assign (segid A and residue 124 and name HD1) (segid A and residue 180 and name HD2#) 3.60 1.90 1.90
assign (segid A and residue 124 and name HH2) (segid A and residue 179 and name HD2#) 3.60 1.90 1.90
assign (segid A and residue 124 and name HE1) (segid A and residue 180 and name HD1#) 3.60 1.90 1.90
assign (segid A and residue 123 and name HE1) (segid A and residue 180 and name HD2#) 3.60 1.90 1.90
assign (segid A and residue 123 and name HE1) (segid A and residue 180 and name HD1#) 3.60 1.90 1.90
assign (segid A and residue 144 and name HD1) (segid A and residue 194 and name HG2#) 3.60 1.90 1.90
assign (segid A and residue 144 and name HH2) (segid A and residue 186 and name HE#) 3.60 1.90 1.90
assign (segid A and residue 144 and name HH2) (segid A and residue 190 and name HG2#) 3.60 1.90 1.90
assign (segid A and residue 133 and name HG2#) (segid A and residue 147 and name HE#) 3.60 1.90 1.90
assign (segid A and residue 133 and name HG1#) (segid A and residue 147 and name HE#) 3.60 1.90 1.90
assign (segid A and residue 133 and name HG2#) (segid A and residue 147 and name HD#) 3.60 1.90 1.90
assign (segid A and residue 135 and name HG#) (segid A and residue 147 and name HD#) 3.35 1.65 1.65
assign (segid A and residue 135 and name HB#) (segid A and residue 147 and name HD#) 3.35 1.65 1.65
assign (segid A and residue 149 and name HD2#) (segid A and residue 214 and name HD1) 3.60 1.90 1.90
assign (segid A and residue 149 and name HD1#) (segid A and residue 214 and name HD1) 3.60 1.90 1.90
assign (segid A and residue 149 and name HD1#) (segid A and residue 197 and name HE#) 3.85 2.15 2.15
assign (segid A and residue 197 and name HE#) (segid A and residue 198 and name HD2#) 3.85 2.15 2.15
assign (segid A and residue 197 and name HE#) (segid A and residue 197 and name HA) 3.35 1.65 1.65
assign (segid B and residue 38 and name HB#) (segid A and residue 120 and name HE1) 3.60 1.90 1.90
assign (segid B and residue 36 and name HB#) (segid A and residue 120 and name HE1) 3.35 1.65 1.65
assign (segid B and residue 33 and name HG2#) (segid A and residue 123 and name HD2) 3.60 1.90 1.90
assign (segid B and residue 33 and name HD1#) (segid A and residue 120 and name HB#) 3.60 1.90 1.90
```

Table B2. Backbone φ and ψ angles calculated from chemical shifts using the TALOS+ software program and used in the XPLOR-NIH calculation of the SMN$^{26-51}$-Gemin2$^{95-280}$ ensemble. The value of each dihedral angle is measured in degrees.

φ dihedral angles

residue	angle (°)	error (°)	residue	angle (°)	error (°)
D32	-89	20.2	S131	-65.8	11.6
D36	-63.2	10.9	T134	-102.3	7.9
T37	-97.5	18.4	S138	-60	4.5
A38	-57.3	8.7	E139	-77.8	9.3
L39	-64	5.7	E141	-54.4	7.6
I40	-66.2	5.2	E142	-57.6	6.5
K41	-62	7.1	G143	-65.7	5.5
A42	-64.9	4.1	W144	-63.1	5.2
Y43	-67	9	K145	-63.5	4.2
D44	-64.4	3.2	K146	-66.4	4.5
K45	-67.5	3.2	F147	-66.3	8.3
A46	-65.2	4.2	C148	-67	11.7
V47	-66.4	5.8	L149	-91.1	7.7
A48	-68.2	9.3	G150	-60.5	3.5
L100	-60.8	2.9	L153	-65.2	9.9
Q101	-66.3	10	Y171	-59	7.4
W102	-65.7	5.3	V172	-67.5	10.1
Q103	-62.3	4.4	I174	-100.4	16.1
Q104	-63.1	5	F176	-88.3	17.4
Q105	-65.5	4.2	P177	-69.4	9.5
Q106	-62.4	3.7	P178	-72.2	6.7
Q109	-63.9	6.1	L180	-57.1	6.4
F110	-63.4	4.8	S181	-60.2	6.2
S111	-64.2	4.5	I182	-65.3	11.3
T112	-66.7	4.2	V183	-63.1	3.9
V113	-65.5	7.1	S184	-67.1	10.4
R114	-63.8	6.4	R185	-99	10.2
Q115	-65.3	3.3	N187	-80.6	13.3
N116	-64.7	2.3	Q188	-59.6	3.9
V117	-67.5	11.8	A189	-65	4.8
N118	-65.6	6	T190	-64.5	3
K119	-66.6	7.3	T192	-66.8	6.3
H120	-89.5	19.6	S193	-68.9	20.8
R121	-57.7	8.1	V194	-60.4	7.8
S122	-63.9	5.2	L195	-62.3	3.3
H123	-65.8	3.1	E196	-65.4	3.9
W124	-63.9	4.9	Y197	-63.2	5.1
K125	-67.7	9.3	L198	-61.9	7.7
S126	-93	14.5	S199	-66.7	6.8

residue	angle (°)	error (°)	residue	angle (°)	error (°)
N200	-67.4	5.5	V248	-74.8	17.1
W201	-62.6	8.9	D249	-66.3	12.2
F202	68.3	13.5	S250	-94.7	15.4
D206	-100.3	21.7	V256	-53	7.6
E210	-63	5.5	P257	-58.5	3.7
L211	-64.2	4.9	A258	-73.2	16.4
G212	-64.2	4.9	L259	-63.6	3.6
R213	-61	3.8	N260	-62.5	4.2
W214	-67.4	9.1	L261	-65.2	4.7
L215	-58	7.3	L262	-60.7	5
Y216	-63.2	5.9	I263	-63.7	5.2
A217	-59	2.7	C264	-60.7	5.4
L218	-62.4	3.6	L265	-62.7	3.3
L219	-65.2	9.7	V266	-66.7	4.5
A220	-65.2	6.7	S267	-69.7	13.1
C221	-99.3	14.4	R268	-75.4	14.2
L222	-82.2	18.8	Y269	-65.8	8.3
E223	-73.5	8.2	F270	-76.9	18.6
L226	-100.1	22.9	R273	-66.1	12
L227	-84.8	24.9	D274	-74.2	15.4
P228	-51.7	5.6	L275	-102	11.6
E229	-63.5	5.6	R278	-83.9	18.6
A230	-65.1	5.5			
H231	-60.4	9.3			
S232	-57.7	7.1			
L233	-64.1	3.7			
I234	-63	4.2			
R235	-64.9	6			
Q236	-64.4	3.4			
L237	-62.8	5.6			
A238	-62.4	4.4			
R239	-64.5	7.8			
R240	-66.1	2.7			
C241	-60.2	4.3			
S242	-65.8	5.6			
E243	-66.4	2.7			
V244	-63.4	5.4			
R245	-64.1	6.8			
L246	-67.2	9.9			
L247	-91.5	15.2			

ψ dihedral angles

residue	angle (°)	error (°)	residue	angle (°)	error (°)
D32	-11	21.9	S131	-32.4	11.5
D36	-29.7	13	T134	122.2	10.1
T37	-11.1	19.5	S138	-33.4	7.2
A38	-46.4	7.9	E139	-2.5	10
L39	-38.9	8.7	E141	-38.3	18
I40	-41.9	7.7	G143	-41.5	5.9
K41	-38.2	8.5	W144	-42.4	3.8
A42	-37.4	6.5	K145	-44.3	5.1
Y43	-44.1	7.1	K146	-35.4	9
D44	-40.9	5.5	F147	-44.1	6.9
K45	-39.2	4.6	C148	-32	11.7
A46	-44.5	6	K152	-39.4	10.4
V47	-32.5	9.7	L153	-42.6	8.3
A48	-29.4	19.8	Y171	-32.2	12.2
L100	-37.8	6.1	I174	-0.2	22.7
W102	-43.8	4	F176	122.7	20.4
Q103	-43.7	5	P177	150.5	10.9
Q104	-42.5	6.7	P178	148.4	8.7
Q105	-44.9	4.7	L180	-32.6	15.4
V107	-45.3	4.5	S181	-37	6.2
A108	-46	6.3	I182	-48	11.3
Q109	-41.5	3.6	V183	-35.4	3.9
F110	-43.1	4.2	S184	-30.3	12.4
S111	-39.1	2.8	R185	-4.8	15.8
T112	-42.6	7.2	N187	159.9	20.4
V113	-41.9	5.1	Q188	-34.7	6.7
R114	-43.1	6.3	A189	-37.2	4.8
Q115	-41.9	5.1	T192	-34.7	10.5
N116	-42.5	5.5	S193	-40.4	6.2
V117	-39.5	11.2	L195	-37.4	9.2
N118	-41.3	8.2	E196	-42.5	4
K119	-39	6.1	Y197	-39.3	10
H120	-10	22.1	L198	-40	7.6
R121	-46.5	9.3	S199	-39	8.5
S122	-37.2	7	N200	-41.4	6
H123	-43.6	5.6	W201	-41.4	5.5
W124	-35.4	11.6	G203	24.1	14.7
K125	-28.2	10.2	D206	156.9	10.5
S126	-0.5	20.3	E210	-35.3	5.5

residue	angle (°)	error (°)	residue	angle (°)	error (°)
L211	-40.9	12.3	A258	-29.2	18.8
G212	-42	6.7	L259	-41.9	5.9
R213	-44.5	6.7	N260	-38	6.7
W214	-45.5	6.2	L261	-38.2	13.9
L215	-40.9	12.3	I263	-42.5	6.3
Y216	-43.7	4.1	C264	-42	8
A217	-41.2	7.6	L265	-39.5	5.7
L218	-43.1	4	V266	-41	4.9
L219	-39.6	8.7	S267	-35.5	14.8
A220	-27	16.6	R268	-29.4	15.8
C221	-7.1	9.2	Y269	-37.8	7.4
L222	131	8.4	F270	-26.3	18.3
P225	151.6	9.2	R273	-28	18.1
L226	131.4	16.3	D274	-18	13.1
L227	145.7	23.6	L275	5.5	16.6
P228	-38.2	5.8	R278	133.7	19.9
A230	-40.7	7.3			
H231	-47.4	6.8			
S232	-46.1	5.8			
L233	-41	9.2			
I234	-36.7	5.2			
R235	-40.7	3.4			
Q236	-42.6	4.6			
L237	-39.4	4.7			
A238	-39.2	4.4			
R239	-39.8	8.4			
R240	-43	4.2			
C241	-45.8	7.1			
S242	-39.6	5.4			
E243	-44.1	4.4			
V244	-39.4	7.4			
R245	-39	7.7			
L246	-34.8	9.5			
D249	-27	15.8			
K251	-26.1	10.7			
D252	-7.8	17.1			
E254	-35.3	7.9			
R255	-40.5	8			
V256	-51.8	5.7			
P257	-34	9.2			

Table B3. Hydrogen bonding distance restraints from regions of helical secondary structure used in the XPLOR-NIH calculation of the SMN$^{26-51}$-Gemin2$^{95-280}$ ensemble.

After the two atom selections, the distance is indicated in Angstroms, followed by increments defining its upper and lower bound.

```
assign (residue 38 and name O) (residue 42 and name HN) 3.0 0.5 0.5
assign (residue 39 and name O) (residue 43 and name HN) 3.0 0.5 0.5
assign (residue 40 and name O) (residue 44 and name HN) 3.0 0.5 0.5
assign (residue 41 and name O) (residue 45 and name HN) 3.0 0.5 0.5
assign (residue 42 and name O) (residue 46 and name HN) 3.0 0.5 0.5
assign (residue 43 and name O) (residue 47 and name HN) 3.0 0.5 0.5

assign (residue 101 and name O) (residue 105 and name HN) 3.0 0.5 0.5
assign (residue 102 and name O) (residue 106 and name HN) 3.0 0.5 0.5
assign (residue 103 and name O) (residue 107 and name HN) 3.0 0.5 0.5
assign (residue 105 and name O) (residue 109 and name HN) 3.0 0.5 0.5
assign (residue 106 and name O) (residue 110 and name HN) 3.0 0.5 0.5
assign (residue 108 and name O) (residue 112 and name HN) 3.0 0.5 0.5
assign (residue 110 and name O) (residue 114 and name HN) 3.0 0.5 0.5
assign (residue 111 and name O) (residue 115 and name HN) 3.0 0.5 0.5
assign (residue 112 and name O) (residue 116 and name HN) 3.0 0.5 0.5
assign (residue 113 and name O) (residue 117 and name HN) 3.0 0.5 0.5
assign (residue 114 and name O) (residue 118 and name HN) 3.0 0.5 0.5
assign (residue 115 and name O) (residue 119 and name HN) 3.0 0.5 0.5
assign (residue 116 and name O) (residue 120 and name HN) 3.0 0.5 0.5
assign (residue 117 and name O) (residue 121 and name HN) 3.0 0.5 0.5
assign (residue 118 and name O) (residue 122 and name HN) 3.0 0.5 0.5
assign (residue 119 and name O) (residue 123 and name HN) 3.0 0.5 0.5

assign (residue 189 and name O) (residue 193 and name HN) 3.0 0.5 0.5
assign (residue 190 and name O) (residue 194 and name HN) 3.0 0.5 0.5
assign (residue 191 and name O) (residue 195 and name HN) 3.0 0.5 0.5
assign (residue 192 and name O) (residue 196 and name HN) 3.0 0.5 0.5
assign (residue 193 and name O) (residue 197 and name HN) 3.0 0.5 0.5
assign (residue 194 and name O) (residue 198 and name HN) 3.0 0.5 0.5
assign (residue 195 and name O) (residue 199 and name HN) 3.0 0.5 0.5
assign (residue 196 and name O) (residue 200 and name HN) 3.0 0.5 0.5
assign (residue 197 and name O) (residue 201 and name HN) 3.0 0.5 0.5

assign (residue 212 and name O) (residue 216 and name HN) 3.0 0.5 0.5
assign (residue 213 and name O) (residue 217 and name HN) 3.0 0.5 0.5
assign (residue 214 and name O) (residue 218 and name HN) 3.0 0.5 0.5
assign (residue 215 and name O) (residue 219 and name HN) 3.0 0.5 0.5
assign (residue 216 and name O) (residue 220 and name HN) 3.0 0.5 0.5

assign (residue 230 and name O) (residue 234 and name HN) 3.0 0.5 0.5
assign (residue 231 and name O) (residue 235 and name HN) 3.0 0.5 0.5
assign (residue 232 and name O) (residue 236 and name HN) 3.0 0.5 0.5
assign (residue 233 and name O) (residue 237 and name HN) 3.0 0.5 0.5
assign (residue 234 and name O) (residue 238 and name HN) 3.0 0.5 0.5
assign (residue 235 and name O) (residue 239 and name HN) 3.0 0.5 0.5
```

```
assign (residue 236 and name O) (residue 240 and name HN) 3.0 0.5 0.5
assign (residue 237 and name O) (residue 241 and name HN) 3.0 0.5 0.5
assign (residue 238 and name O) (residue 242 and name HN) 3.0 0.5 0.5
assign (residue 239 and name O) (residue 243 and name HN) 3.0 0.5 0.5
assign (residue 240 and name O) (residue 244 and name HN) 3.0 0.5 0.5
assign (residue 241 and name O) (residue 245 and name HN) 3.0 0.5 0.5
assign (residue 242 and name O) (residue 246 and name HN) 3.0 0.5 0.5

assign (residue 259 and name O) (residue 263 and name HN) 3.0 0.5 0.5
assign (residue 260 and name O) (residue 264 and name HN) 3.0 0.5 0.5
assign (residue 261 and name O) (residue 265 and name HN) 3.0 0.5 0.5
assign (residue 262 and name O) (residue 266 and name HN) 3.0 0.5 0.5
assign (residue 263 and name O) (residue 267 and name HN) 3.0 0.5 0.5
assign (residue 264 and name O) (residue 268 and name HN) 3.0 0.5 0.5
```

Table B4a. The small-angle scattering curve input as an XPLOR-NIH restraint.
The scattering angle q and associated intensity I(q) are indicated; error (σ(q)) represents the standard deviation to the mean.

q (Å^{-1})	I(q)	σ(q)
6.850E-03	7.531E+01	2.739E+01
7.084E-03	6.770E+01	2.112E+01
7.326E-03	6.316E+01	1.903E+01
7.577E-03	6.117E+01	1.435E+01
7.836E-03	5.929E+01	1.536E+01
8.104E-03	5.855E+01	9.980E+00
8.382E-03	5.980E+01	1.010E+01
8.668E-03	5.891E+01	8.473E+00
8.965E-03	6.117E+01	8.519E+00
9.272E-03	5.969E+01	6.667E+00
9.589E-03	6.046E+01	6.369E+00
9.917E-03	5.754E+01	6.459E+00
1.026E-02	5.691E+01	5.517E+00
1.061E-02	5.690E+01	4.815E+00
1.097E-02	5.747E+01	4.678E+00
1.135E-02	5.718E+01	4.409E+00
1.173E-02	5.705E+01	3.967E+00
1.214E-02	5.816E+01	3.638E+00
1.255E-02	5.803E+01	3.244E+00
1.298E-02	5.691E+01	3.164E+00
1.342E-02	5.681E+01	2.784E+00
1.388E-02	5.797E+01	2.689E+00
1.436E-02	5.891E+01	2.366E+00
1.485E-02	5.791E+01	2.231E+00
1.536E-02	5.782E+01	2.069E+00
1.588E-02	5.800E+01	1.880E+00
1.643E-02	5.876E+01	1.833E+00
1.699E-02	5.823E+01	1.629E+00
1.757E-02	5.833E+01	1.539E+00
1.817E-02	5.764E+01	1.459E+00
1.879E-02	5.736E+01	1.343E+00
1.944E-02	5.828E+01	1.267E+00
2.010E-02	5.755E+01	1.179E+00
2.079E-02	5.732E+01	1.104E+00
2.150E-02	5.712E+01	1.082E+00
2.224E-02	5.693E+01	9.762E-01
2.300E-02	5.653E+01	9.591E-01
2.378E-02	5.593E+01	9.051E-01
2.460E-02	5.604E+01	8.575E-01
2.544E-02	5.554E+01	8.080E-01
2.631E-02	5.568E+01	7.715E-01
2.721E-02	5.505E+01	7.396E-01
2.814E-02	5.454E+01	7.145E-01
2.910E-02	5.400E+01	6.835E-01
3.010E-02	5.357E+01	6.538E-01
3.113E-02	5.286E+01	6.465E-01
3.219E-02	5.228E+01	6.243E-01
3.329E-02	5.191E+01	6.198E-01
3.443E-02	5.136E+01	6.291E-01
3.561E-02	5.050E+01	6.104E-01
3.683E-02	5.003E+01	5.996E-01
3.809E-02	4.939E+01	5.825E-01
3.939E-02	4.886E+01	5.722E-01
4.074E-02	4.802E+01	5.554E-01
4.214E-02	4.694E+01	5.440E-01
4.358E-02	4.650E+01	5.274E-01
4.507E-02	4.564E+01	5.150E-01
4.661E-02	4.456E+01	4.996E-01
4.820E-02	4.382E+01	4.871E-01
4.985E-02	4.256E+01	4.736E-01
5.156E-02	4.149E+01	4.605E-01
5.332E-02	4.040E+01	4.470E-01
5.515E-02	3.925E+01	4.339E-01
5.703E-02	3.826E+01	4.228E-01
5.899E-02	3.703E+01	4.094E-01
6.100E-02	3.599E+01	3.989E-01
6.309E-02	3.480E+01	3.864E-01
6.525E-02	3.332E+01	3.761E-01
6.748E-02	3.215E+01	3.630E-01
6.979E-02	3.072E+01	3.549E-01
7.218E-02	2.933E+01	3.420E-01
7.465E-02	2.797E+01	3.322E-01
7.720E-02	2.654E+01	3.229E-01

q (Å^{-1})	I(q)	σ(q)	q (Å^{-1})	I(q)	σ(q)
7.984E-02	2.488E+01	3.124E-01	1.195E-01	8.322E+00	2.156E-01
8.257E-02	2.348E+01	3.030E-01	1.236E-01	7.378E+00	2.088E-01
8.540E-02	2.202E+01	2.942E-01	1.279E-01	6.246E+00	2.144E-01
8.832E-02	2.052E+01	2.842E-01	1.322E-01	5.383E+00	2.249E-01
9.134E-02	1.922E+01	2.757E-01	1.368E-01	4.313E+00	2.294E-01
9.447E-02	1.758E+01	2.671E-01	1.414E-01	3.672E+00	2.332E-01
9.770E-02	1.635E+01	2.600E-01	1.463E-01	2.930E+00	2.368E-01
1.010E-01	1.474E+01	2.513E-01	1.513E-01	2.342E+00	2.413E-01
1.045E-01	1.329E+01	2.437E-01	1.565E-01	1.626E+00	2.434E-01
1.081E-01	1.219E+01	2.363E-01	1.618E-01	1.622E+00	2.401E-01
1.118E-01	1.072E+01	2.293E-01	1.673E-01	1.198E+00	2.370E-01
1.156E-01	9.493E+00	2.219E-01	1.731E-01	8.607E-01	2.309E-01

Table B4b. The sparsened (50 points) small-angle scattering curve data set calculated by XPLOR-NIH and used in calculations. The scattering angle q and associated intensity I(q) are indicated; error (σ(q)) represents the standard deviation to the mean.

q (Å^{-1})	I(q)	σ(q)
6.800E-03	7.531E+01	2.739E+01
1.040E-02	5.690E+01	5.274E+00
1.390E-02	5.794E+01	2.691E+00
1.740E-02	5.830E+01	1.566E+00
2.090E-02	5.729E+01	1.100E+00
2.440E-02	5.601E+01	8.676E-01
2.790E-02	5.466E+01	7.201E-01
3.140E-02	5.269E+01	6.398E-01
3.500E-02	5.098E+01	6.207E-01
3.850E-02	4.924E+01	5.795E-01
4.200E-02	4.706E+01	5.452E-01
4.550E-02	4.534E+01	5.107E-01
4.900E-02	4.321E+01	4.804E-01
5.250E-02	4.090E+01	4.531E-01
5.600E-02	3.879E+01	4.286E-01
5.950E-02	3.674E+01	4.064E-01
6.310E-02	3.482E+01	3.866E-01
6.660E-02	3.263E+01	3.682E-01
7.010E-02	3.055E+01	3.533E-01
7.360E-02	2.855E+01	3.363E-01
7.710E-02	2.660E+01	3.232E-01
8.060E-02	2.448E+01	3.097E-01
8.410E-02	2.268E+01	2.981E-01
8.760E-02	2.087E+01	2.864E-01
9.120E-02	1.930E+01	2.762E-01
9.470E-02	1.750E+01	2.667E-01
9.820E-02	1.611E+01	2.587E-01
1.017E-01	1.447E+01	2.499E-01
1.052E-01	1.307E+01	2.422E-01
1.087E-01	1.193E+01	2.350E-01
1.122E-01	1.057E+01	2.284E-01
1.157E-01	9.448E+00	2.216E-01
1.193E-01	8.408E+00	2.160E-01
1.228E-01	7.578E+00	2.102E-01
1.263E-01	6.669E+00	2.123E-01
1.298E-01	5.865E+00	2.189E-01
1.333E-01	5.130E+00	2.260E-01
1.368E-01	4.305E+00	2.294E-01
1.403E-01	3.824E+00	2.323E-01
1.438E-01	3.302E+00	2.350E-01
1.474E-01	2.802E+00	2.378E-01
1.509E-01	2.390E+00	2.409E-01
1.544E-01	1.912E+00	2.426E-01
1.579E-01	1.625E+00	2.425E-01
1.614E-01	1.623E+00	2.404E-01
1.649E-01	1.383E+00	2.384E-01
1.684E-01	1.133E+00	2.358E-01
1.719E-01	9.260E-01	2.321E-01
1.755E-01	6.810E-01	2.265E-01
1.790E-01	4.180E-01	2.205E-01

Table B5. Observed $^{1}D_{NH}$ backbone amide and tryptophan ε1 residual dipolar couplings used in the XPLOR-NIH calculation of the SMN$^{26-51}$-Gemin2$^{95-280}$ ensemble. The value of each observed RDC is measured in Hz; the experimental values were twice those listed here and were uniformly halved for convenience. The error was approximated from visual inspection of point-to-point resolution of the ^{1}H-^{15}N HSQC IPAP spectra.

Backbone amide RDCs

Residue	RDC (Hz)	Error (Hz)
W34	4.75	2.5
D35	5.36	2.5
T37	10.85	3.5
A38	5.47	2.5
I40	12.46	3.5
D44	13.22	2.5
V47	13.16	2.5
A48	4.07	2.5
S49	7.35	2.5
F50	10.32	2.5
T99	11.03	2.5
Q105	2.82	3.5
A108	8.86	3.5
Q109	3.82	3.5
T112	5.75	2.5
V113	9.32	2.5
V117	11.38	3.5
Q127	15.08	2.5
Q128	9.79	3.5
L129	7.04	2.5
N132	8.17	2.5
V133	1	2.5
M135	-1.37	2.5
K137	-1.22	2.5
S138	-2.46	2.5
E139	8.64	2.5
E141	10.3	2.5
W144	10.79	3.5
G150	-1.43	2.5
L153	2.85	2.5
D170	4.04	2.5
V172	13.04	2.5
Q173	12.99	2.5
F176	-2.4	3.5
L180	12.34	2.5
I182	8.9	2.5
S184	15.89	2.5
M186	10.45	2.5
Q188	-8.02	2.5
T190	-4.3	2.5
T192	-6.18	2.5
S193	1.5	2.5
L198	-8.66	2.5
N200	-0.84	2.5
G203	2.56	2.5
R205	-7.81	2.5
D206	5.85	2.5
F207	8.46	2.5
E210	-9.59	2.5
L211	-5.77	2.5
G212	-6.91	3.5
W214	-9.62	2.5
A220	-5.95	2.5
L227	11.65	3.5
E229	4.69	2.5
A230	-2.35	3.5
S232	4.1	2.5
I234	-7.01	2.5
R235	3.32	5
L247	0.24	2.5
V248	-4.41	2.5
D249	11.87	2.5
S250	6.82	2.5
D252	-1.52	3.5
D252	12.96	2.5
R255	-4.82	2.5
V256	-3.36	3.5
A258	-9.34	2.5
L259	-4.29	3.5
N260	-0.37	3.5
L265	-3.12	3.5
S267	-2.54	3.5
R268	2.03	3.5
R273	-7.07	3.5
D274	8.13	2.5
A276	1.03	2.5
D277	12.44	2.5
E278	0.03	2.5

Tryptophan sidechain ε1 RDCs

Residue	RDC (Hz)	Error (Hz)
W44	6.13	3.5
W102	13.79	3.5
W124	10.75	3.5
W144	4.55	3.5
W201	6.53	3.5
W214	10.62	3.5

Table B6. Distance restraints, based upon observed paramagnetic relaxation enhancement due to an MTSSL moiety covalently coupled to Cys241, used in the XPLOR-NIH calculation of the SMN$^{26-51}$-Gemin2$^{95-280}$ ensemble.
Shown here in XPLOR format. After the two atom selections, the distance is indicated in Angstroms, followed by increments defining its upper and lower bound.

```
assign ( resid 241 and segid A and name OS1 ) ( resid   38 and segid B
and name HN )  20.5 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid   39 and segid B
and name HN )  20.4 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid   40 and segid B
and name HN )  22.8 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid   46 and segid B
and name HN )  21.5 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid   50 and segid B
and name HN )  20.1 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  108 and segid A
and name HN )  20.2 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  112 and segid A
and name HN )  22.1 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  113 and segid A
and name HN )  20.4 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  135 and segid A
and name HN )  22.1 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  138 and segid A
and name HN )  23.6 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  139 and segid A
and name HN )  22.1 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  141 and segid A
and name HN )  21.0 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  144 and segid A
and name HN )  20.0 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  150 and segid A
and name HN )  17.6 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  182 and segid A
and name HN )  22.1 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  183 and segid A
and name HN )  18.4 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  184 and segid A
and name HN )  19.7 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  185 and segid A
and name HN )  23.6 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  186 and segid A
and name HN )  22.8 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  200 and segid A
and name HN )  15.0 15.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  203 and segid A
and name HN )  15.0 15.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  205 and segid A
and name HN )  15.0 15.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  223 and segid A
and name HN )  20.0 3.0 3.0
```

```
assign ( resid 241 and segid A and name OS1 ) ( resid  226 and segid A
and name HN )  20.6 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  229 and segid A
and name HN )  22.1 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  232 and segid A
and name HN )  18.7 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  246 and segid A
and name HN )  18.9 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  249 and segid A
and name HN )  20.3 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  251 and segid A
and name HN )  21.5 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  256 and segid A
and name HN )  15.8 3.0 3.0
assign ( resid 241 and segid A and name OS1 ) ( resid  258 and segid A
and name HN )  21.0 3.0 3.0
```

Appendix C. Sample python XPLOR-NIH refinement script. Run under XPLOR-NIH 2.26.

```
xplor.requireVersion("2.18")

#
#After high-temp dynamics, performs slow cooling protocol in
#torsion angle space, then in Cartesian space for Gemin2(95-
#280)/SMN(26-51). Uses NOE, TALOS backbone dihedral, hbond,
#small-angle x-ray scattering, RDC and PRE restraints.
#
#Each restraint type was added iteratively, in the order listed
#above.
#This is the script for the final refinement, including all
#restraints and starting from the lowest-energy structure in the
#previous iteration.

#Gemin2 in this step only (anneal_rdcs_mtsl.pdb) is the
#C154S/C221S/C264S mutant to accommodate the MTSSL moiety bound
#to C241.

# KLS 2011/10/20
#


(opts,args) = xplor.parseArguments(["quick"]) # check for
command-line typos

quick=False
for opt in opts:
    if opt[0]=="quick":  #specify -quick to just test that the
script runs
        quick=True
        pass
    pass


outFilename = "SCRIPT_STRUCTURE.pdb"
numberOfStructures=92

if quick:
    numberOfStructures=3
    pass

# protocol module has many high-level helper functions.
#
import protocol
protocol.initRandomSeed(326)   #explicitly set random seed
```

```
#
# annealing settings
#

command = xplor.command

protocol.initTopology(('protein'))
protocol.initParams("protein")

# read an existing model
#
protocol.initStruct("gemin2smn_mtsl.psf")
protocol.initCoords("anneal_rdcs_mtsl.pdb")
xplor.simulation.deleteAtoms("not known")

protocol.fixupCovalentGeom(maxIters=100,useVDW=1)


# a PotList contains a list of potential terms. This is used to
# specify which terms are active during refinement.

from potList import PotList
potList = PotList()

# parameters to ramp up during the simulated annealing protocol

from simulationTools import MultRamp, StaticRamp, InitialParams

rampedParams=[]
highTempParams=[]

# set up RDC potential

from varTensorTools import create_VarTensor
media={}
#                        medium  Da   rhombicity
# you could have a whole list here
for (medium,Da,Rh) in [ ('t',   -10.56, 0.35)]:
    oTensor = create_VarTensor(medium)
    oTensor.setDa(Da)
    oTensor.setRh(Rh)
    media[medium] = oTensor
    pass

from rdcPotTools import create_RDCPot, scale_toNH
rdcs = PotList('rdc')
for (medium,expt,file,                  scale) in \
    [('t','NH' ,'gemin2smn_rdc.tbl'       ,1),
```

```
     ]:
    rdc =
create_RDCPot("%s_%s"%(medium,expt),file,media[medium])
    rdc.setScale(scale)
    rdc.setShowAllRestraints(1) #all restraints are printed
during analysis
    rdc.setThreshold(0.5)       # in Hz
    rdc.setPotType("square")
    rdcs.append(rdc)
    pass
potList.append(rdcs)
rampedParams.append(MultRamp(0.03,3.0,"rdcs.setScale(VALUE)") )

from varTensorTools import calcTensorOrientation
for medium in media.values():
    calcTensorOrientation(medium)
    pass


# set up NOE potential
noe=PotList('noe')
potList.append(noe)
from noePotTools import create_NOEPot
for (name,scale,file) in [('all',1,"gemin2smn_noe_clean.tbl"),
                  ('hbond',1,"gemin2smn_hbond.tbl")
                          #add entries for additional tables
                          ]:
    pot = create_NOEPot(name,file)
    # pot.setPotType("soft") - if you think there may be bad
      # NOEs
    pot.setScale(scale)
    noe.append(pot)
rampedParams.append( MultRamp(2,50, "noe.setScale( VALUE )") )

# set up PRE potential
pre=PotList('pre')
potList.append(pre)
from noePotTools import create_NOEPot
for (name,scale,file) in [('c241s',1,"c241stest.tbl"),
                          #add entries for additional tables
                          ]:
    pot = create_NOEPot(name,file)
    pot.setScale(scale)
    pot.setThreshold(0)
    pre.append(pot)
rampedParams.append( MultRamp(2,50, "pre.setScale( VALUE )") )

# set up dihedral angles
from xplorPot import XplorPot
```

```
protocol.initDihedrals("gemin2smn_dihed.tbl",
                       #useDefaults=False
                       # by default, symmetric sidechain
                       # restraints are included
                       )
potList.append( XplorPot('CDIH') )
highTempParams.append(
StaticRamp("potList['CDIH'].setScale(10)") )
rampedParams.append(StaticRamp("potList['CDIH'].setScale(200)")
)
#set custom values of threshold values for violation calculation
#
potList['CDIH'].setThreshold( 5 ) #5 degrees is the default
#value, though


#Rama torsion angle database
#
protocol.initRamaDatabase()
potList.append( XplorPot('RAMA') )
rampedParams.append(
MultRamp(.002,1,"potList['RAMA'].setScale(VALUE)") )

#
# setup parameters for atom-atom repulsive term. (van der Waals-
# like term)
#
potList.append( XplorPot('VDW') )
rampedParams.append( StaticRamp("protocol.initNBond()") )
rampedParams.append( MultRamp(0.9,0.8,
                              "command('param nbonds repel VALUE
end end')") )
rampedParams.append( MultRamp(.004,8,
                              "command('param nbonds rcon VALUE
end end')") )
# nonbonded interaction only between CA atoms
highTempParams.append(
StaticRamp("""protocol.initNBond(cutnb=100,

tolerance=45,

repel=0.5,

onlyCA=0)""") )


potList.append( XplorPot("BOND") )
potList.append( XplorPot("ANGL") )
potList['ANGL'].setThreshold( 5 )
```

```
rampedParams.append(
MultRamp(0.4,1,"potList['ANGL'].setScale(VALUE)") )
potList.append( XplorPot("IMPR") )
potList['IMPR'].setThreshold( 5 )
rampedParams.append(
MultRamp(0.1,1,"potList['IMPR'].setScale(VALUE)") )

#set up SAXS term
from solnXRayPotTools import create_solnXRayPot
import solnXRayPotTools

xray=create_solnXRayPot('xray',experiment='G2SMN_D2O_Hi_t.dat',
                        numPoints=50,
                        normalizeIndex=-3,preweighted=False)

solnXRayPotTools.useGlobs(xray)

xray.setNumAngles(50)
xray.setScale(40)
xray.setCmpType("plain")
potList.append(xray)

print xray.calcEnergy()
from solnScatPotTools import fitParams



# Give atoms uniform weights, except for the anisotropy axis
#
from atomAction import SetProperty
import varTensorTools
AtomSel("not resname ANI").apply( SetProperty("mass",100.) )
varTensorTools.massSetup(media.values(),300)
AtomSel("all            ").apply( SetProperty("fric",10.) )


# IVM setup
#   the IVM is used for performing dynamics and minimization in
# torsion-angle and in Cartesian space.
#
from ivm import IVM
dyn = IVM()
for m in media.values():
    m.setFreedom("fixDa, fixRh")        #fix tensor Rh, Da, vary
# orientation
protocol.torsionTopology(dyn,oTensors=media.values())

# minc used for final cartesian minimization
```

```
#
minc = IVM()
protocol.initMinimize(minc)
for m in media.values():
    m.setFreedom("fixDa, fixRh")    #allow all tensor parameters
# float here
    pass
protocol.cartesianTopology(minc,oTensors=media.values())


# object which performs simulated annealing
#
from simulationTools import AnnealIVM
init_t  = 3000.     # Need high temp and slow annealing to
converge
cool = AnnealIVM(initTemp =init_t,
                 finalTemp=0,
                 tempStep =10,
                 ivm=dyn,
                 rampedParams = rampedParams)
# a round of Cartesian space cooling always following torsion-
# angle space cooling. Not standard, but truly necessary for
# this unwieldy system.
cart_cool = AnnealIVM(initTemp =init_t,
                      finalTemp=0,
                      tempStep =10,
                      ivm=minc,
                      rampedParams = rampedParams)

def accept(potList):
    """
    return True if current structure meets acceptance criteria
    """
    if potList['noe'].violations()>5:
        return False
    if potList['CDIH'].violations()>5:
        return False
    if potList['BOND'].rms()>0.1:
        return False
    if potList['ANGL'].rms()>2:
        return False
    if potList['IMPR'].rms()>4:
        return False

    return True

def calcOneStructure(loopInfo):
    """ this function calculates a single structure, performs
analysis on the
```

```
structure, and then writes out a pdb file, with remarks.
"""

# initialize parameters for high temp dynamics.
InitialParams( rampedParams )
# high-temp dynamics setup - only need to specify parameters
  # which
#   differfrom initial values in rampedParams
InitialParams( highTempParams )

# high temp dynamics
#
protocol.initDynamics(dyn,
                      potList=potList, # potential terms to
                                       # use
                      bathTemp=init_t,
                      initVelocities=1,
                      finalTime=25,    # stops at 10ps or
                                       #5000 steps
                      numSteps=20000,   # whichever comes
                                       # first
                      printInterval=100)

dyn.setETolerance( init_t/100 )  #used to det. stepsize.
                                  # default: t/1000
dyn.run()

# initialize parameters for cooling loop
InitialParams( rampedParams )


# initialize integrator for simulated annealing
#
protocol.initDynamics(dyn,
                      potList=potList,
                      numSteps=1000,       #at each temp:
                                             # 100 steps or
                      finalTime=2 ,       # .2ps, whichever
                                             #is less
                      printInterval=100)

# perform simulated annealing
#
cool.run()


# final torsion angle minimization
#
protocol.initMinimize(dyn,
```

```
                          printInterval=50)
    dyn.run()
    protocol.initDynamics(minc,
                          potList=potList,
                          numSteps=1000,       #at each temp:
                                                 #100 steps or
                          finalTime=2 ,       # .2ps, whichever
                                                 #is less
                          printInterval=100)
    cart_cool.run()

    # final all- atom minimization
    #
    protocol.initMinimize(minc,
                          potList=potList,
                          dEPred=10)
    minc.run()

    #do analysis and write structure
    loopInfo.writeStructure(potList)
    pass



from simulationTools import StructureLoop, FinalParams
StructureLoop(numStructures=numberOfStructures,
              pdbTemplate=outFilename,
              structLoopAction=calcOneStructure,
              genViolationStats=1,
              averagePotList=potList,

averageSortPots=[potList['BOND'],potList['ANGL'],potList['IMPR']
,

noe,xray,rdcs,pre,potList['CDIH']],
              averageTopFraction=1, #report only on best 50% of
                                    #structs
              averageAccept=accept,   #only use structures which
                                         #pass accept()
              averageContext=FinalParams(rampedParams),
              averageFilename="SCRIPT_ave.pdb",    #generate
                                # regularized ave structure
              averageFitSel="name CA",
              averageCompSel="not hydro"     ).run()
```

References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389-3402.

Alías L, Bernal S, Fuentes-Prior P, Barceló MJ, Also E et al. (2009) Mutation update of spinal muscular atrophy in Spain: molecular characterization of 745 unrelated patients and identification of four novel mutations in the SMN1 gene. Hum Genet 125: 29-39.

Ast G (2004) How did alternative splicing evolve?. Nat Rev Genet 5: 773-782.

Baccon J, Pellizzoni L, Rappsilber J, Mann M, Dreyfuss G (2002) Identification and characterization of Gemin7, a novel component of the survival of motor neuron complex. J Biol Chem 277: 31957-31962.

Battiste JL, Wagner G (2000) Utilization of site-directed spin labeling and high-resolution heteronuclear nuclear magnetic resonance for global fold determination of large proteins with limited nuclear overhauser effect data. Biochemistry 39: 5355-5365.

Battle DJ, Kasim M, Wang J, Dreyfuss G (2007) SMN-independent subunits of the SMN complex. Identification of a small nuclear ribonucleoprotein assembly intermediate. J Biol Chem 282: 27953-27959.

Battle DJ, Lau C, Wan L, Deng H, Lotti F et al. (2006) The Gemin5 protein of the SMN complex identifies snRNAs. Mol Cell 23: 273-279.

Beadle GW, Tatum EL (1941) Genetic Control of Biochemical Reactions in Neurospora. Proc Natl Acad Sci U S A 27: 499-506.

Berget SM (1995) Exon recognition in vertebrate splicing. J Biol Chem 270: 2411-2414.

Berget SM, Moore C, Sharp PA (1977) Spliced segments at the 5' terminus of adenovirus 2 late mRNA. Proc Natl Acad Sci U S A 74: 3171-3175.

Bouveret E, Rigaut G, Shevchenko A, Wilm M, Séraphin B (2000) A Sm-like protein complex that participates in mRNA degradation. EMBO J 19: 1661-1671.

Brahms H, Meheus L, de Brabandere V, Fischer U, Lührmann R (2001) Symmetrical dimethylation of arginine residues in spliceosomal Sm protein B/B' and the Sm-like protein LSm4, and their interaction with the SMN protein. RNA 7: 1531-1542.

Briese M, Esmaeili B, Fraboulet S, Burt EC, Christodoulou S et al. (2009) Deletion of smn-1, the Caenorhabditis elegans ortholog of the spinal muscular atrophy gene, results in locomotor dysfunction and reduced lifespan. Hum Mol Genet 18: 97-104.

Briese M, Esmaeili B, Sattelle DB (2005) Is spinal muscular atrophy the result of defects in motor neuron processes?. Bioessays 27: 946-957.

Brzustowicz LM, Kleyn PW, Boyce FM, Lien LL, Monaco AP et al. (1992) Fine-mapping of the spinal muscular atrophy locus to a region flanked by MAP1B and D5S6. Genomics 13: 991-998.

Brzustowicz LM, Lehner T, Castilla LH, Penchaszadeh GK, Wilhelmsen KC et al. (1990) Genetic mapping of chronic childhood-onset spinal muscular atrophy to chromosome 5q11.2-13.3. Nature 344: 540-541.

Brünger AT, Adams PD, Clore GM, DeLano WL, Gros P et al. (1998) Crystallography & NMR system: A new software suite for macromolecular structure determination. Acta Crystallogr D Biol Crystallogr 54: 905-921.

Burge CB, Tuschi T, Sharp PA (1999) Splicing of precursors to mrnas by the spliceosomes. In: Gesteland RF, Cech TR, Atkins J F, editors. The rna world, 2nd edn.. Cold Spring Harbor Laboratory Press. pp. 525-560.

Burghes AHM, Beattie CE (2009) Spinal muscular atrophy: why do low levels of survival motor neuron protein make motor neurons sick?. Nat Rev Neurosci 10: 597-609.

Béchade C, Rostaing P, Cisterni C, Kalisch R, La Bella V et al. (1999) Subcellular distribution of survival motor neuron (SMN) protein: possible involvement in nucleocytoplasmic and dendritic transport. Eur J Neurosci 11: 293-304.

Bühler D, Raker V, Lührmann R, Fischer U (1999) Essential role for the tudor domain of SMN in spliceosomal U snRNP assembly: implications for spinal muscular atrophy. Hum Mol Genet 8: 2351-2357.

Camasses A, Bragado-Nilsson E, Martin R, Séraphin B, Bordonné R (1998) Interactions within the yeast Sm core complex: from proteins to amino acids. Mol Cell Biol 18: 1956-1966.

Campion Y, Neel H, Gostan T, Soret J, Bordonné R (2010) Specific splicing defects in S. pombe carrying a degron allele of the Survival of Motor Neuron gene. EMBO J 29: 1817-1829.

Canaves JM, Page R, Wilson IA, Stevens RC (2004) Protein biophysical properties that correlate with crystallization success in Thermotoga maritima: maximum clustering strategy for structural genomics. J Mol Biol 344: 977-991.

Carissimi C, Baccon J, Straccia M, Chiarella P, Maiolica A et al. (2005) Unrip is a component of SMN complexes active in snRNP assembly. FEBS Lett 579: 2348-2354.

Carissimi C, Saieva L, Baccon J, Chiarella P, Maiolica A et al. (2006a) Gemin8 is a novel component of the survival motor neuron complex and functions in small nuclear ribonucleoprotein assembly. J Biol Chem 281: 8126-8134.

Carissimi C, Saieva L, Gabanella F, Pellizzoni L (2006b) Gemin8 is required for the architecture and function of the survival motor neuron complex. J Biol Chem 281: 37009-37016.

Carrel TL, McWhorter ML, Workman E, Zhang H, Wolstencroft EC et al. (2006) Survival motor neuron function in motor axons is independent of functions required for small nuclear ribonucleoprotein biogenesis. J Neurosci 26: 11014-11022.

Cartegni L, Krainer AR (2002) Disruption of an SF2/ASF-dependent exonic splicing enhancer in SMN2 causes spinal muscular atrophy in the absence of SMN1. Nat Genet 30: 377-384.

Chan YB, Miguel-Aliaga I, Franks C, Thomas N, Trülzsch B et al. (2003) Neuromuscular defects in a Drosophila survival motor neuron gene mutant. Hum Mol Genet 12: 1367-1376.

Chang HC, Dimlich DN, Yokokura T, Mukherjee A, Kankel MW et al. (2008) Modeling spinal muscular atrophy in Drosophila. PLoS One 3: e3209.

Chari A, Golas MM, Klingenhäger M, Neuenkirchen N, Sander B et al. (2008) An assembly chaperone collaborates with the SMN complex to generate spliceosomal SnRNPs. Cell 135: 497-509.

Charroux B, Pellizzoni L, Perkinson RA, Shevchenko A, Mann M et al. (1999) Gemin3: A novel DEAD box protein that interacts with SMN, the spinal muscular atrophy gene product, and is a component of gems. J Cell Biol 147: 1181-1194.

Charroux B, Pellizzoni L, Perkinson RA, Yong J, Shevchenko A et al. (2000) Gemin4. A novel component of the SMN complex that is found in both gems and nucleoli. J Cell Biol 148: 1177-1186.

Cherepanov P, Maertens G, Proost P, Devreese B, Van Beeumen J et al. (2003) HIV-1 integrase forms stable tetramers and associates with LEDGF/p75 protein in human cells. J Biol Chem 278: 372-381.

Cho S, Dreyfuss G (2010) A degron created by SMN2 exon 7 skipping is a principal contributor to spinal muscular atrophy severity. Genes Dev 24: 438-442.

Chow LT, Gelinas RE, Broker TR, Roberts RJ (1977) An amazing sequence arrangement at the 5' ends of adenovirus 2 messenger RNA. Cell 12: 1-8.

Clore GM, Gronenborn AM, Bax A (1998) A robust method for determining the magnitude of the fully asymmetric alignment tensor of oriented macromolecules in the absence of structural information. J Magn Reson 133: 216-221.

Clore GM, Gronenborn AM, Nilges M, Ryan CA (1987) Three-dimensional structure of potato carboxypeptidase inhibitor in solution. A study using nuclear magnetic resonance, distance geometry, and restrained molecular dynamics. Biochemistry 26: 8012-8023.

Coovert DD, Le TT, McAndrew PE, Strasswimmer J, Crawford TO et al. (1997) The survival motor neuron protein in spinal muscular atrophy. Hum Mol Genet 6: 1205-1214.

Crick FH (1958) On protein synthesis. Symp Soc Exp Biol 12: 138-163.

Cuscó I, Barceló MJ, del Río E, Baiget M, Tizzano EF (2004) Detection of novel mutations in the SMN Tudor domain in type I SMA patients. Neurology 63: 146-149.

D'Amico A, Mercuri E, Tiziano FD, Bertini E (2011) Spinal muscular atrophy. Orphanet J Rare Dis 6: 71.

Delano W (2002) The pymol molecular graphics system. . p.

Deutsch M, Long M (1999) Intron-exon structures of eukaryotic model organisms. Nucleic Acids Res 27: 3219-3228.

Diercks T, Coles M, Kessler H (1999) An efficient strategy for assignment of cross-peaks in 3D heternuclear NOESY experiments. J. Biomol. NMR 15: 177-180.

Dmitrova M, Younès-Cauet G, Oertel-Buchheit P, Porte D, Schnarr M et al. (1998) A new LexA-based genetic system for monitoring and analyzing protein heterodimerization in Escherichia coli. Mol Gen Genet 257: 205-212.

Doniach S (2001) Changes in biomolecular conformation seen by small angle X-ray scattering. Chem Rev 101: 1763-1778.

Early P, Rogers J, Davis M, Calame K, Bond M et al. (1980) Two mRNAs can be produced from a single immunoglobulin mu gene by alternative RNA processing pathways. Cell 20: 313-319.

Eggert C, Chari A, Laggerbauer B, Fischer U (2006) Spinal muscular atrophy: the RNP connection. Trends Mol Med 12: 113-121.

Erickson HP (2009) Size and shape of protein molecules at the nanometer level determined by sedimentation, gel filtration, and electron microscopy. Biol Proced Online 11: 32-51.

Fan L, Simard LR (2002) Survival motor neuron (SMN) protein: role in neurite outgrowth and neuromuscular maturation during neuronal differentiation and development. Hum Mol Genet 11: 1605-1614.

Farrow NA, Muhandiram R, Singer AU, Pascal SM, Kay CM et al. (1994) Backbone dynamics of a free and phosphopeptide-complexed Src homology 2 domain studied by 15N NMR relaxation. Biochemistry 33: 5984-6003.

Feng W, Gubitz AK, Wan L, Battle DJ, Dostie J et al. (2005) Gemins modulate the expression and activity of the SMN complex. Hum Mol Genet 14: 1605-1611.

Fernandez CF, Pannone BK, Chen X, Fuchs G, Wolin SL (2004) An Lsm2-Lsm7 complex in Saccharomyces cerevisiae associates with the small nucleolar RNA snR5. Mol Biol Cell 15: 2842-2852.

Fischer U, Liu Q, Dreyfuss G (1997) The SMN-SIP1 complex has an essential role in spliceosomal snRNP biogenesis. Cell 90: 1023-1029.

Fox-Walsh KL, Dou Y, Lam BJ, Hung S, Baldi PF et al. (2005) The architecture of pre-mRNAs affects mechanisms of splice-site pairing. Proc Natl Acad Sci U S A 102: 16176-16181.

Friesen WJ, Massenet S, Paushkin S, Wyce A, Dreyfuss G (2001a) SMN, the product of the spinal muscular atrophy gene, binds preferentially to dimethylarginine-containing protein targets. Mol Cell 7: 1111-1117.

Friesen WJ, Paushkin S, Wyce A, Massenet S, Pesiridis GS et al. (2001b) The methylosome, a 20S complex containing JBP1 and pICln, produces dimethylarginine-modified Sm proteins. Mol Cell Biol 21: 8289-8300.

Gabanella F, Butchbach MER, Saieva L, Carissimi C, Burghes AHM et al. (2007) Ribonucleoprotein assembly defects correlate with spinal muscular atrophy severity and preferentially affect a subset of spliceosomal snRNPs. PLoS One 2: e921.

Gabanella F, Carissimi C, Usiello A, Pellizzoni L (2005) The activity of the spinal muscular atrophy protein is regulated during development and cellular differentiation. Hum Mol Genet 14: 3629-3642.

Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD et al. (2003) ExPASy: The proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Res 31: 3784-3788.

Gavrilina TO, McGovern VL, Workman E, Crawford TO, Gogliotti RG et al. (2008) Neuronal SMN expression corrects spinal muscular atrophy in severe SMA mice while muscle-specific SMN expression has no phenotypic effect. Hum Mol Genet 17: 1063-1075.

Goddard T, Kneller D (2008) Sparky 3. : .

Grimmler M, Bauer L, Nousiainen M, Körner R, Meister G et al. (2005b) Phosphorylation regulates the activity of the SMN complex during assembly of spliceosomal U snRNPs. EMBO Rep 6: 70-76.

Grimmler M, Otter S, Peter C, Müller F, Chari A et al. (2005a) Unrip, a factor implicated in cap-independent translation, associates with the cytosolic SMN complex and influences its intracellular localization. Hum Mol Genet 14: 3099-3111.

Gubitz AK, Feng W, Dreyfuss G (2004) The SMN complex. Exp Cell Res 296: 51-56.

Gubitz AK, Mourelatos Z, Abel L, Rappsilber J, Mann M et al. (2002) Gemin5, a novel WD repeat protein component of the SMN complex that binds Sm proteins. J Biol Chem 277: 5631-5636.

Hamamoto S, Nishitsuji H, Amagasa T, Kannagi M, Masuda T (2006) Identification of a novel human immunodeficiency virus type 1 integrase interactor, Gemin2, that facilitates efficient viral cDNA synthesis in vivo. J Virol 80: 5670-5677.

Hannus S, Bühler D, Romano M, Seraphin B, Fischer U (2000) The Schizosaccharomyces pombe protein Yab8p and a novel factor, Yip1p, share structural and functional similarity with the spinal muscular atrophy-associated proteins SMN and SIP1. Hum Mol Genet 9: 663-674.

Hao LT, Fuller HR, Lam LT, Le TT, Burghes AHM et al. (2007) Absence of gemin5 from SMN complexes in nuclear Cajal bodies. BMC Cell Biol 8: 28.

Henry SA, Patton-Vogt JL (1998) Progress in nucleic acid research and molecular biology. Academic Press. p.

Hirokawa N (1998) Kinesin and dynein superfamily proteins and the mechanism of organelle transport. Science 279: 519-526.

Hochleitner EO, Sondermann P, Lottspeich F (2004) Determination of the stoichiometry of protein complexes using liquid chromatography with fluorescence and mass spectrometric detection of fluorescently labeled proteolytic peptides. Proteomics 4: 669-676.

Holm L, Sander C (1995) Dali: a network tool for protein structure comparison. Trends Biochem Sci 20: 478-480.

Hsieh-Li HM, Chang JG, Jong YJ, Wu MH, Wang NM et al. (2000) A mouse model for spinal muscular atrophy. Nat Genet 24: 66-70.

Hura GL, Menon AL, Hammel M, Rambo RP, Poole FL2 et al. (2009) Robust, high-throughput solution structural analyses by small angle X-ray scattering (SAXS). Nat Methods 6: 606-612.

Jablonka S, Beck M, Lechner BD, Mayer C, Sendtner M (2007) Defective Ca2+ channel clustering in axon terminals disturbs excitability in motoneurons in spinal muscular atrophy. J Cell Biol 179: 139-149.

Jablonka S, Holtmann B, Meister G, Bandilla M, Rossoll W et al. (2002) Gene targeting of Gemin2 in mice reveals a correlation between defects in the biogenesis of U snRNPs and motoneuron cell death. Proc Natl Acad Sci U S A 99: 10126-10131.

Janson J (2011) Protein purification: principles, high resolution methods, and applications. John Wiley & Sons. 61 p.

Kambach C, Walke S, Young R, Avis JM, de la Fortelle E et al. (1999) Crystal structures of two Sm protein complexes and their implications for the assembly of the spliceosomal snRNPs. Cell 96: 375-387.

Kashima T, Manley JL (2003) A negative element in SMN2 exon 7 inhibits splicing in spinal muscular atrophy. Nat Genet 34: 460-463.

Kenakin TP (1993) Pharmacologic analysis of drug-receptor interaction. Raven Press. 483 p.

Konarev PV, Volkov VV, Sokolova AV, Koch MHJ, Svergun DI (2003) PRIMUS - a Windows-PC based system for small-angle scattering data analaysis. J Appl Cryst 36: 1277-1282.

Kroiss M, Schultz J, Wiesner J, Chari A, Sickmann A et al. (2008) Evolution of an RNP assembly system: a minimal SMN complex facilitates formation of UsnRNPs in Drosophila melanogaster. Proc Natl Acad Sci U S A 105: 10045-10050.

Krummel DAP, Nagai K, Oubridge C (2010) Structure of spliceosomal ribonucleoproteins. F1000 Biol Rep 2: .

Kyte J, Doolittle RF (1982) A simple method for displaying the hydropathic character of a protein. J Mol Biol 157: 105-132.

Lau C, Bachorik JL, Dreyfuss G (2009) Gemin5-snRNA interaction reveals an RNA binding function for WD repeat domains. Nat Struct Mol Biol 16: 486-491.

Lebowitz J, Lewis MS, Schuck P (2002) Modern analytical ultracentrifugation in protein science: a tutorial review. Protein Sci 11: 2067-2079.

Lefebvre S, Burlet P, Liu Q, Bertrandy S, Clermont O et al. (1997) Correlation between severity and SMN protein level in spinal muscular atrophy. Nat Genet 16: 265-269.

Lefebvre S, Bürglen L, Reboullet S, Clermont O, Burlet P et al. (1995) Identification and characterization of a spinal muscular atrophy-determining gene. Cell 80: 155-165.

Lehmeier T, Raker V, Hermann H, Lührmann R (1994) cDNA cloning of the Sm proteins D2 and D3 from human small nuclear ribonucleoproteins: evidence for a direct D1-D2 interaction. Proc Natl Acad Sci U S A 91: 12317-12321.

Lipari G, Szabo A (1982) Model-free approach to the interpretation of nuclear magnetic resonance relaxation in macromolecules. 1. Theory and range of validity.. J. Am. Chem. Soc. 104: 4546-4559.

Liu Q, Dreyfuss G (1996) A novel nuclear structure containing the survival of motor neurons protein. EMBO J 15: 3555-3565.

Liu Q, Fischer U, Wang F, Dreyfuss G (1997) The spinal muscular atrophy disease gene product, SMN, and its associated protein SIP1 are in a complex with spliceosomal snRNP proteins. Cell 90: 1013-1021.

Lorson CL, Androphy EJ (1998) The domain encoded by exon 2 of the survival motor neuron protein mediates nucleic acid binding. Hum Mol Genet 7: 1269-1275.

Lorson CL, Androphy EJ (2000) An exonic enhancer is required for inclusion of an essential exon in the SMA-determining gene SMN. Hum Mol Genet 9: 259-265.

Lorson CL, Strasswimmer J, Yao JM, Baleja JD, Hahnen E et al. (1998) SMN oligomerization defect correlates with spinal muscular atrophy severity. Nat Genet 19: 63-66.

Ma Y, Dostie J, Dreyfuss G, Van Duyne GD (2005) The Gemin6-Gemin7 heterodimer from the survival of motor neurons complex has an Sm protein-like structure. Structure 13: 883-892.

Martin R (2011) Structural and biochemical studies of the SMN oligomerization domain: a novel glycine zipper. : .

Matera AG, Terns RM, Terns MP (2007) Non-coding RNAs: lessons from the small nuclear and small nucleolar RNAs. Nat Rev Mol Cell Biol 8: 209-220.

McWhorter ML, Boon K, Horan ES, Burghes AHM, Beattie CE (2008) The SMN binding protein Gemin2 is not involved in motor axon outgrowth. Dev Neurobiol 68: 182-194.

Meister G, Bühler D, Pillai R, Lottspeich F, Fischer U (2001a) A multiprotein complex mediates the ATP-dependent assembly of spliceosomal U snRNPs. Nat Cell Biol 3: 945-949.

Meister G, Eggert C, Bühler D, Brahms H, Kambach C et al. (2001b) Methylation of Sm proteins by a complex containing PRMT5 and the putative U snRNP assembly factor pICln. Curr Biol 11: 1990-1994.

Neidhardt FC, Bloch PL, Smith DF (1974) Culture medium for enterobacteria. J Bacteriol 119: 736-747.

Neri D, Szyperski T, Otting G, Senn H, Wüthrich K (1989) Stereospecific nuclear magnetic resonance assignments of the methyl groups of valine and leucine in the DNA-binding domain of the 434 repressor by biosynthetically directed fractional 13C labeling. Biochemistry 28: 7510-7516.

Nilsen TW, Graveley BR (2010) Expansion of the eukaryotic proteome by alternative splicing. Nature 463: 457-463.

Nishitsuji H, Hayashi T, Takahashi T, Miyano M, Kannagi M et al. (2009) Augmentation of reverse transcription by integrase through an interaction with host factor, SIP1/Gemin2 Is critical for HIV-1 infection. PLoS One 4: e7825.

Nizami ZF, Deryusheva S, Gall JG (2010) Cajal bodies and histone locus bodies in Drosophila and Xenopus. Cold Spring Harb Symp Quant Biol 75: 313-320.

Noble SM, Guthrie C (1996a) Identification of novel genes required for yeast pre-mRNA splicing by means of cold-sensitive mutations. Genetics 143: 67-80.

Noble SM, Guthrie C (1996b) Transcriptional pulse-chase analysis reveals a role for a novel snRNP-associated protein in the manufacture of spliceosomal snRNPs. EMBO J 15: 4368-4379.

Ogawa C, Usui K, Aoki M, Ito F, Itoh M et al. (2007) Gemin2 plays an important role in stabilizing the survival of motor neuron complex. J Biol Chem 282: 11122-11134.

Ogawa C, Usui K, Ito F, Itoh M, Hayashizaki Y et al. (2009) Role of survival motor neuron complex components in small nuclear ribonucleoprotein assembly. J Biol Chem 284: 14609-14617.

Oprea GE, Kröber S, McWhorter ML, Rossoll W, Müller S et al. (2008) Plastin 3 is a protective modifier of autosomal recessive spinal muscular atrophy. Science 320: 524-527.

Otter S, Grimmler M, Neuenkirchen N, Chari A, Sickmann A et al. (2007) A comprehensive interaction map of the human survival of motor neuron (SMN) complex. J Biol Chem 282: 5825-5833.

Ottiger M, Delaglio F, Bax A (1998) Measurement of J and dipolar couplings from simplified two-dimensional NMR spectra. J Magn Reson 131: 373-378.

Owen N, Doe CL, Mellor J, Davies KE (2000) Characterization of the Schizosaccharomyces pombe orthologue of the human survival motor neuron (SMN) protein. Hum Mol Genet 9: 675-684.

Pagliardini S, Giavazzi A, Setola V, Lizier C, Di Luca M et al. (2000) Subcellular localization and axonal transport of the survival motor neuron (SMN) protein in the developing rat spinal cord. Hum Mol Genet 9: 47-56.

Palfi Z, Jaé N, Preusser C, Kaminska KH, Bujnicki JM et al. (2009) SMN-assisted assembly of snRNP-specific Sm cores in trypanosomes. Genes Dev 23: 1650-1664.

Pan Q, Shai O, Lee LJ, Frey BJ, Blencowe BJ (2008) Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. Nat Genet 40: 1413-1415.

Patel AA, Steitz JA (2003) Splicing double: insights from the second spliceosome. Nat Rev Mol Cell Biol 4: 960-970.

Paushkin S, Charroux B, Abel L, Perkinson RA, Pellizzoni L et al. (2000) The survival motor neuron protein of Schizosacharomyces pombe. Conservation of survival motor neuron interaction domains in divergent organisms. J Biol Chem 275: 23841-23846.

Paushkin S, Gubitz AK, Massenet S, Dreyfuss G (2002) The SMN complex, an assemblyosome of ribonucleoproteins. Curr Opin Cell Biol 14: 305-312.

Pellizzoni L (2007) Chaperoning ribonucleoprotein biogenesis in health and disease. EMBO Rep 8: 340-345.

Pellizzoni L, Baccon J, Rappsilber J, Mann M, Dreyfuss G (2002a) Purification of native survival of motor neurons complexes and identification of Gemin6 as a novel component. J Biol Chem 277: 7540-7545.

Pellizzoni L, Charroux B, Dreyfuss G (1999) SMN mutants of spinal muscular atrophy patients are defective in binding to snRNP proteins. Proc Natl Acad Sci U S A 96: 11167-11172.

Pellizzoni L, Yong J, Dreyfuss G (2002b) Essential role for the SMN complex in the specificity of snRNP assembly. Science 298: 1775-1779.

Pesiridis GS, Diamond E, Van Duyne GD (2009) Role of pICLn in methylation of Sm proteins by PRMT5. J Biol Chem 284: 21347-21359.

Pu WT, Krapivinsky GB, Krapivinsky L, Clapham DE (1999) pICln inhibits snRNP biogenesis by binding core spliceosomal proteins. Mol Cell Biol 19: 4113-4120.

Rajendra TK, Gonsalvez GB, Walker MP, Shpargel KB, Salz HK et al. (2007) A Drosophila melanogaster model of spinal muscular atrophy reveals a function for SMN in striated muscle. J Cell Biol 176: 831-841.

Raker VA, Plessel G, Lührmann R (1996) The snRNP core assembly pathway: identification of stable core protein heteromeric complexes and an snRNP subcore particle in vitro. EMBO J 15: 2256-2269.

Reed R (2000) Mechanisms of fidelity in pre-mRNA splicing. Curr Opin Cell Biol 12: 340-345.

Ritchie DB, Schellenberg MJ, MacMillan AM (2009) Spliceosome structure: piece by piece. Biochim Biophys Acta 1789: 624-633.

Ross A, Czisch M, Cieslar C, Holak T (1993) Efficient methods for obtaining phase-sensitive, gradient-enhanced HMQC data. J. Biol. NMR 3: 215-224.

Rossoll W, Jablonka S, Andreassi C, Kröning A, Karle K et al. (2003) Smn, the spinal muscular atrophy-determining gene product, modulates axon growth and localization of beta-actin mRNA in growth cones of motoneurons. J Cell Biol 163: 801-812.

Rossoll W, Kröning A, Ohndorf U, Steegborn C, Jablonka S et al. (2002) Specific interaction of Smn, the spinal muscular atrophy determining gene product, with hnRNP-R and gry-rbp/hnRNP-Q: a role for Smn in RNA processing in motor axons?. Hum Mol Genet 11: 93-105.

Sattler M, Schleucher J, Griesinger C (1999) Heteronuclear multidimensional NMR experiments for the structure determination of proteins in solution employing pulsed field gradients. Prog. Nuc. Mag. Res. Spectroscopy 34: 93-158.

Schrank B, Götz R, Gunnersen JM, Ure JM, Toyka KV et al. (1997) Inactivation of the survival motor neuron gene, a candidate gene for human spinal muscular atrophy, leads to massive cell death in early mouse embryos. Proc Natl Acad Sci U S A 94: 9920-9925.

Schuck P, Rossmanith P (2000) Determination of the sedimentation coefficient distribution by least-squares boundary modeling. Biopolymers 54: 328-341.

Schwieters CD, Kuszewski JJ, Clore GM (2006) Using Xplor-NIH for NMR molecular structure determination. Progress in Nuclear Magnetic Resonance Spectroscopy 48: 47-62.

Schwieters CD, Kuszewski JJ, Tjandra N, Clore GM (2003) The Xplor-NIH NMR molecular structure determination package. J Magn Reson 160: 65-73.

Selenko P, Sprangers R, Stier G, Bühler D, Fischer U et al. (2001) SMN tudor domain structure and its interaction with the Sm proteins. Nat Struct Biol 8: 27-31.

Semenyuk A, Svergun D (1991) GNOM - A program package for small-angle scattering data processing. J. Appl. Cryst. 24: 537-540.

Seyfang A, Jin JH (2004) Multiple site-directed mutagenesis of more than 10 sites simultaneously and in a single round. Anal Biochem 324: 285-291.

Sharma A, Lambrechts A, Hao LT, Le TT, Sewry CA et al. (2005) A role for complexes of survival of motor neurons (SMN) protein with gemins and profilin in neurite-like cytoplasmic extensions of cultured nerve cells. Exp Cell Res 309: 185-197.

Shen Y, Delaglio F, Cornilescu G, Bax A (2009) TALOS+: a hybrid method for predicting protein backbone torsion angles from NMR chemical shifts. J Biomol NMR 44: 213-223.

Shpargel KB, Matera AG (2005) Gemin proteins are required for efficient assembly of Sm-class ribonucleoproteins. Proc Natl Acad Sci U S A 102: 17372-17377.

Spiller MP, Reijns MAM, Beggs JD (2007) Requirements for nuclear localization of the Lsm2-8p complex and competition between nuclear and cytoplasmic Lsm complexes. J Cell Sci 120: 4310-4320.

Sprangers R, Groves MR, Sinning I, Sattler M (2003) High-resolution X-ray and NMR structures of the SMN Tudor domain: conformational variation in the binding site for symmetrically dimethylated arginine residues. J Mol Biol 327: 507-520.

Staley JP, Guthrie C (1998) Mechanical devices of the spliceosome: motors, clocks, springs, and things. Cell 92: 315-326.

Staley JP, Woolford JLJ (2009) Assembly of ribosomes and spliceosomes: complex ribonucleoprotein machines. Curr Opin Cell Biol 21: 109-118.

Stark H, Dube P, Lührmann R, Kastner B (2001) Arrangement of RNA and proteins in the spliceosomal U1 small nuclear ribonucleoprotein particle. Nature 409: 539-542.

Sterner DA, Carlo T, Berget SM (1996) Architectural limits on split genes. Proc Natl Acad Sci U S A 93: 15081-15085.

Sun Y, Grimmler M, Schwarzer V, Schoenen F, Fischer U et al. (2005) Molecular and functional analysis of intragenic SMN1 mutations in patients with spinal muscular atrophy. Hum Mutat 25: 64-71.

Svergun DI, Petoukhov MV, Koch MH (2001) Determination of domain structure of proteins from X-ray solution scattering. Biophys J 80: 2946-2953.

Takaku M, Tsujita T, Horikoshi N, Takizawa Y, Qing Y et al. (2011) Purification of the human SMN-GEMIN2 complex and assessment of its stimulation of RAD51-mediated DNA recombination reactions. Biochemistry 50: 6797-6805.

Takizawa Y, Qing Y, Takaku M, Ishida T, Morozumi Y et al. (2010) GEMIN2 promotes accumulation of RAD51 at double-strand breaks in homologous recombination. Nucleic Acids Res 38: 5059-5074.

Talbot K, Miguel-Aliaga I, Mohaghegh P, Ponting CP, Davies KE (1998) Characterization of a gene encoding survival motor neuron (SMN)-related protein, a constituent of the spliceosome complex. Hum Mol Genet 7: 2149-2156.

Tharun S, He W, Mayes AE, Lennertz P, Beggs JD et al. (2000) Yeast Sm-like proteins function in mRNA decapping and decay. Nature 404: 515-518.

Uversky VN, Gillespie JR, Fink AL (2000) Why are "natively unfolded" proteins unstructured under physiologic conditions?. Proteins 41: 415-427.

Vistica J, Dam J, Balbo A, Yikilmaz E, Mariuzza RA et al. (2004) Sedimentation equilibrium analysis of protein interactions with global implicit mass conservation constraints and systematic noise decomposition. Anal Biochem 326: 234-256.

Volkov VV, Svergun DI (2003) Uniqueness of ab initio shape determination in small-angle scattering. J. Appl. Crystallogr. 36: 860-864.

Vuister G, Clore G, Gronenborn A, Powers R, Garrett D et al. (1993) Increased resolution and improved spectral quality in four-dimensional 13C/13C separated HMQC-NOESY-HMQC spectra using pulsed field gradients. J. Mag. Res. B 101: 210-213.

Walker MP, Rajendra TK, Saieva L, Fuentes JL, Pellizzoni L et al. (2008) SMN complex localizes to the sarcomeric Z-disc and is a proteolytic target of calpain. Hum Mol Genet 17: 3399-3410.

Ward JJ, Sodhi JS, McGuffin LJ, Buxton BF, Jones DT (2004) Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. J Mol Biol 337: 635-645.

Whipple FW (1998) Genetic analysis of prokaryotic and eukaryotic DNA-binding proteins in Escherichia coli. Nucleic Acids Res 26: 3700-3706.

Will CL, Lührmann R (2001) Spliceosomal UsnRNP biogenesis, structure and function. Curr Opin Cell Biol 13: 290-301.

Will CL, Lührmann R (2005) Splicing of a rare class of introns by the U12-dependent spliceosome. Biol Chem 386: 713-724.

Will CL, Lührmann R (2011) Spliceosome structure and function. Cold Spring Harb Perspect Biol 3: .

Winkler C, Eggert C, Gradl D, Meister G, Giegerich M et al. (2005) Reduced U snRNP assembly causes motor axon degeneration in an animal model for spinal muscular atrophy. Genes Dev 19: 2320-2330.

Wüthrich K (1986) Nmr of proteins and nucleic acids. Wiley-Interscience. p.

Yamashita M, Nishio H, Harada Y, Matsuo M, Yamamoto T (2004) Significant increase in the number of the SMN2 gene copies in an adult-onset Type III spinal muscular atrophy patient with homozygous deletion of the NAIP gene. Eur Neurol 52: 101-106.

Yong J, Kasim M, Bachorik JL, Wan L, Dreyfuss G (2010) Gemin5 delivers snRNA precursors to the SMN complex for snRNP biogenesis. Mol Cell 38: 551-562.

Young PJ, Man NT, Lorson CL, Le TT, Androphy EJ et al. (2000) The exon 2b region of the spinal muscular atrophy protein, SMN, is involved in self-association and SIP1 binding. Hum Mol Genet 9: 2869-2877.

Zerres K, Davies KE (1999) 59th ENMC International Workshop: Spinal Muscular Atrophies: recent progress and revised diagnostic criteria 17-19 April 1998, Soestduinen, The Netherlands. Neuromuscul Disord 9: 272-278.

Zhang H, Xing L, Rossoll W, Wichterle H, Singer RH et al. (2006) Multiprotein complexes of the survival of motor neuron protein SMN with Gemins traffic to neuronal processes and growth cones of motor neurons. J Neurosci 26: 8622-8632.

Zhang HL, Pan F, Hong D, Shenoy SM, Singer RH et al. (2003) Active transport of the survival motor neuron protein and the role of exon-7 in cytoplasmic localization. J Neurosci 23: 6627-6637.

Zhang R, So BR, Li P, Yong J, Glisovic T et al. (2011) Structure of a key intermediate of the SMN complex reveals Gemin2's crucial function in snRNP assembly. Cell 146: 384-395.

Zhang Z, Lotti F, Dittmar K, Younis I, Wan L et al. (2008) SMN deficiency causes tissue-specific perturbations in the repertoire of snRNAs and widespread defects in splicing. Cell 133: 585-600.