

10-16-2012

# $(1)H$ , $(15)N$ and $(13)C$ backbone resonance assignments of the TPR1 and TPR2A domains of mouse STI1.

Andrzej Maciejewski

Marco A Prado

Wing-Yiu Choy

Follow this and additional works at: <https://ir.lib.uwo.ca/biochempub>

 Part of the [Biochemistry Commons](#)

---

## Citation of this paper:

Maciejewski, Andrzej; Prado, Marco A; and Choy, Wing-Yiu, "(1)H, (15)N and (13)C backbone resonance assignments of the TPR1 and TPR2A domains of mouse STI1." (2012). *Biochemistry Publications*. 155.  
<https://ir.lib.uwo.ca/biochempub/155>

# **$^1\text{H}$ , $^{15}\text{N}$ and $^{13}\text{C}$ backbone resonance assignments of the TPR1 and TPR2A domains of mouse STI1**

Andrzej Maciejewski<sup>1</sup>, Marco A. Prado<sup>2</sup> and Wing-Yiu Choy<sup>1\*</sup>

*<sup>1</sup>Department of Biochemistry, The University of Western Ontario, London, Ontario, Canada N6A 5C1*

*<sup>2</sup>Robarts Research Institute, Department of Physiology and Pharmacology and Department of Anatomy & Cell Biology, The University of Western Ontario, London, Ontario, Canada N6A 5K8*

\*Corresponding author

E-mail: [jchoy4@uwo.ca](mailto:jchoy4@uwo.ca)

Tel: 519-661-3161

Fax: 519-661-3175

## **Abstract**

Hop/STI1 (Hsp-organizing protein/stress-induced-phosphoprotein 1) is a molecular co-chaperone, which coordinates Hsp70 and Hsp90 activity during client protein folding through interactions with its TPR1 and TPR2A domains. Hsp90 substrates include a diverse set of proteins, many of which have been implicated in tumorigenesis. Over-expression of Hsp90 in cancer cells stabilizes mutant oncoproteins promoting cancer cell survival. Disruption of Hsp90 and its co-chaperone machinery has become a promising strategy for the treatment of cancer. STI1 has also been described as a neurotrophic signaling molecule through its interactions with the prion protein (PrP<sup>C</sup>). Here, we report the <sup>1</sup>H, <sup>13</sup>C and <sup>15</sup>N backbone assignments of the TPR1 and TPR2A domains of mouse STI1, which interact with Hsp70 and Hsp90, respectively. <sup>1</sup>H-<sup>15</sup>N HSQC spectra of TPR2A domain in the presence of a peptide encoding the C-terminal Hsp90 binding site revealed significant chemical shift changes indicating complex formation. These results will facilitate the screening of potential molecules that inhibit STI1 complex formation with Hsp70 and/or Hsp90 for the treatment of cancer and detailed structural studies of the STI1-PrP<sup>C</sup> complex.

## **Keywords**

Hop/STI1; TPR domain; co-chaperone; Hsp90; protein-protein interaction

## Biological context

Heat-shock protein (Hsp)70 and Hsp90 are chaperones which mediate folding of key cellular client proteins involved in processes such as proliferation, differentiation and apoptosis (Taipale et al. 2010; Young et al. 2004). Hsp90 targets numerous proteins implicated in tumor growth and survival including steroid hormone receptors and proto-oncogenic kinases such as Akt (Sato et al. 2000), Raf-1 (Stancato et al. 1993) and Her2/neu (Xu et al. 2001). Hsp90 over-expression correlates with tumor invasiveness and poor prognosis, thus pharmacological inhibition of the chaperone response has become an alluring target for therapeutic intervention in multiple cancer types (Trepel et al. 2010).

Client maturation is facilitated by a number of co-chaperone proteins that regulate Hsp70 and Hsp90 activity. Hop/STI1 (Hsp-organizing protein/stress-induced-phosphoprotein 1) is a key scaffold protein, which mediates client transfer from Hsp70 to Hsp90 in the later stages of client maturation (Smith et al. 1993; Chen and Smith 1998). Initially, client proteins complex with Hsp40 and Hsp70 (Hernandez et al. 2002; Pratt and Toft 2003). Then Hop/STI1 promotes client transfer from Hsp70 to Hsp90 by simultaneously binding the two chaperones through distinct tetracopeptide repeat (TPR) domains (Scheufler et al. 2000).

Hop/STI1 encodes three TPR domains, TPR1, TPR2A and TPR2B and two aspartate-proline motif regions (DP1 and DP2) (Odunuga et al. 2003). TPR domains are composed of multiple degenerate 34 amino acid repeats forming anti-parallel helix-turn-helix motifs. These serve as protein-protein interaction modules in multi-protein complexes (Cortajarena and Regan 2006). TPR1 and TPR2A preferentially bind the conserved C-terminal residue 'EEVD' motifs of Hsp70 and Hsp90, respectively. X-ray crystallographic data of human TPR1 and TPR2A domain in complex with Hsp70 and Hsp90 C-terminal peptides indicate specificity is determined by hydrophobic contacts directly N-terminal of the 'EEVD' motif (Scheufler et al. 2000).

Studies have demonstrated that STI1 promotes proliferation and migration in glioblastoma and pancreatic cancer cell lines (Fonseca et al. 2012; Walsh et al. 2011). Down-regulation of STI1 by RNAi resulted in decreased pancreatic cell line invasiveness and down-regulation of numerous oncogenic Hsp90 client proteins (Walsh et al. 2011). Inhibition of Hsp90 interaction with the TPR2A domain of STI1 using a novel hybrid TPR peptide has demonstrated selective cancer-cell cytotoxicity (Horibe et al. 2011). Secreted STI1 also functions as a cell-signaling molecule promoting neuriteogenesis and neuroprotection to hippocampal neurons through its interaction with the cellular prion protein (PrP<sup>C</sup>) (Zanata et al. 2002; Lopes et al. 2005). The PrP<sup>C</sup> binding site of STI1 has been localized to the TPR2A domain of STI1. The neurotrophic signaling induced by PrP<sup>C</sup>-STI1 binding may have a role in the pathology of neurodegenerative diseases. Backbone resonance assignment of the TPR1 and TPR2A domains will facilitate screening of further selective inhibitors of Hsp complex formation that may prove effective in cancer treatment and for future studies involving the STI1-PrP<sup>C</sup> signaling complex.

## Methods and experiments

### Plasmid generations

The expression vector pTrcHis containing mouse STI1 cDNA (GenBank# NM\_016737) was graciously provided by Dr. Vilma Martins (A.C. Camargo Hospital, Sao Paulo, Brazil). Sequence encoding the TPR1 domain, residues 2-118, were amplified by PCR using the two primers:

5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTCTGAAAACCTGTATTTTCAGG GAGAGCAGGTGAATGAGCTAAAGG-3' and

5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCTCACCTGGCCTCCATGTTCT G-3'.

TPR2A domain, residues 217-352, were amplified by two successive rounds of PCR using primers 5'-GAAAACCTGTATTTTCAGGGAGATCTTCCAGAGAATAA-3' and 5'-AAGAAAGCTGGGTCTCACAAGCGCTCCTG-3' followed by amplification using 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTCTGAAAACCTGTATTTTC-3' and 5'-GGGGACCACTTTGTACAAGAAAGCTGGG-3'. The resultant cDNA was cloned into separate pDEST17 (Invitrogen) expression vectors with an additional tobacco etch virus (TEV) cleavage recognition site 'ENLYFQG' between the 6xHis tag and the insert.

### Sample preparation

The expression vectors containing mouse TPR1 and TPR2A were transformed in *E. coli* BL21 (DE3) pLysS. Uniformly  $^{15}\text{N}$ ,  $^{13}\text{C}$ - labeled protein was over-expressed by growing *E. coli* in 1 L M9 media supplemented with 1 g of  $\text{NH}_4\text{Cl}$  and 3 g of  $^{13}\text{C}$ -glucose at  $37^\circ\text{C}$  to an  $\text{OD}_{600}$  of 0.9, at which point protein over-expression was induced with 1 mM IPTG. Cultures grew for 6 hours at  $37^\circ\text{C}$  and centrifuged pellets were frozen. N-terminally His-tagged fusion proteins were purified by affinity chromatography using Ni Sepharose<sup>TM</sup> 6 Fast Flow beads (Amersham Biosciences). The N-terminal His-tag was removed by incubation with His-tagged TEV protease overnight at  $22^\circ\text{C}$ . TEV protease was removed by an additional affinity chromatography step using Ni Sepharose<sup>TM</sup> 6 Fast Flow beads (Amersham Biosciences). Proteins were dialyzed into 50 mM sodium phosphate buffer, 50 mM NaCl, 1mM DTT at pH 7 and subsequently concentrated to  $\sim 500 \mu\text{M}$ . NMR samples contained 10 %  $\text{D}_2\text{O}$  and 100  $\mu\text{M}$  2,2-dimethyl-2-sila-pentane-5-sulfonic acid (DSS) for  $^1\text{H}$  and  $^{13}\text{C}$  chemical shift referencing.

### NMR spectroscopy

NMR experiments for backbone resonance assignments of TPR1 and TPR2A were conducted at  $25^\circ\text{C}$  on a Varian INOVA 600 MHz spectrometer equipped with either a cryogenic probe or a regular triple resonance probe (UWO Biomolecular NMR Facility). Sequential assignments were obtained for the TPR1 domain using the following experiments: HNCACB and CBCA(CO)NH and C(CO)NH spectra. The TPR2A domain was assigned using HNCACB and CBCA(CO)NH experiments. All spectra were

processed using NMRPipe (Delaglio et al. 1995) and analyzed using CARA (Keller 2004).

### Ligand Binding experiment

Binding of the TPR2A domain to the peptide encoding the C-terminal Hsp90 residues (Ac-MEEVD-NH<sub>2</sub> ordered from GenScript Inc. USA) was confirmed by <sup>1</sup>H-<sup>15</sup>N HSQC spectra collected in the presence and absence of equimolar concentrations of Hsp90 peptide (~250 μM). Experiments were collected on a Varian INOVA 600 MHz spectrometer equipped with a cryogenic probe (UWO Biomolecular NMR Facility).

### Assignments and data deposition

Backbone resonance assignments for <sup>1</sup>H<sup>N</sup>, <sup>15</sup>N, <sup>13</sup>C $\alpha$  and <sup>13</sup>C $\beta$  were obtained for the TPR1 and TPR2A domains of STI1. For the TPR1 domain, 97% of the <sup>1</sup>H<sup>N</sup> and <sup>15</sup>N resonances of non-proline residues, 94% <sup>13</sup>C $\alpha$  and 87% <sup>13</sup>C $\beta$  of all residues were assigned (Fig. 1). For the TPR2A domain, 99% of <sup>1</sup>H<sup>N</sup> and <sup>15</sup>N resonances of non-proline residues, 95% <sup>13</sup>C $\alpha$  and 96% <sup>13</sup>C $\beta$  of all residues were assigned (Fig. 2). Chemical shift assignments have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu>), under accession numbers 18691 and 18689 for the TPR1 and TPR2A domains, respectively.

Crystal structures of TPR1 and TPR2A in complex with C-terminal peptides of Hsp70 and Hsp90 indicate each domain to be composed of seven  $\alpha$ -helices arranged in anti-parallel helix-turn-helix motifs (Scheufler et al. 2000) (PDB: 1ELW for TPR1 and PDB: 1ELR for TPR2A). Secondary structure propensity (SSP) scores (Marsh et al. 2006) using <sup>13</sup>C  $\alpha/\beta$  chemical shifts indicate no  $\beta$ -strand propensity in TPR1 or TPR2A domains and agree with the secondary structure diagram (Laskowski 2009) generated from the TPR1 and TPR2A crystal structures (Fig. 3) (Scheufler et al. 2000).

<sup>1</sup>H-<sup>15</sup>N HSQC of the TPR2A domain collected in the absence and presence of equimolar concentrations of Hsp90 C-terminal peptide produced large chemical shift changes indicative of binding (Fig. 4a). The magnitude of chemical shift perturbations for traceable residues was calculated from the combined chemical shift changes in <sup>1</sup>H and <sup>15</sup>N dimensions ( $\Delta\omega = |0.2 * \Delta^{15}\text{N}| + |\Delta^{1}\text{H}^{\text{N}}|$ ) in ppm. Residues demonstrating the largest combined chemical shift changes (> 0.1 ppm) clustered to the binding interface of the TPR2A-Hsp90 C-terminal peptide complex (Fig. 4b) (Scheufler et al. 2000) further confirming the assignment.

### Acknowledgements

This work was supported by grants from the Canadian Institutes of Health Research (CIHR; MOP# 74679) and PrioNet-Canada. An Ontario Graduate Scholarship (OGS) was awarded to AM. The authors would like to thank Anne Brickenden for her help in the plasmid preparations.

## Figure Legends

**Fig. 1 a**  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectrum and backbone resonance assignment of  $^{15}\text{N}/^{13}\text{C}$  labeled TPR1 domain of STI1. Spectra were generated using Sparky (Goddard and Kneller). **b** Amino acid sequence of TPR1 domain with unassigned residues *colored red*. The N-terminal glycine is a non-native residue from the TEV protease recognition site.

**Fig. 2 a**  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectrum and backbone resonance assignment of  $^{15}\text{N}/^{13}\text{C}$  labeled TPR2A domain of STI1. Spectra were generated using Sparky (Goddard and Kneller). **b** Amino acid sequence of TPR2A domain with unassigned residues *colored red*. The N-terminal glycine is a non-native residue from the TEV protease recognition site.

**Fig. 3** Secondary structure propensity (SSP) scores and DSSP analysis of **a** the TPR1 and **b** TPR2A domains of STI1.  $^{13}\text{C}$   $\alpha/\beta$  chemical shifts were used to calculate SSP scores for each domain (Marsh et al. 2006). Crystal structures of TPR1 in complex with Hsp70 C-terminal peptide (PDB: 1ELW) and TPR2A in complex with C-terminal Hsp90 peptide (PDB: 1ELR) (Scheufler et al. 2000) were used to generate the secondary structure diagrams of each domain (Laskowski 2009).

**Fig. 4 a** Overlay of  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of TPR2A in the absence (black) and presence (red) of equimolar concentrations of Hsp90 C-terminal peptide. **b** Crystal structure of TPR2A in complex with Hsp90 peptide (PDB: 1ELR; Scheufler et al. 2000) with traceable chemical shift changes upon addition of Hsp90 peptide colored based on the magnitude of the combined chemical shift changes in  $^1\text{H}$  and  $^{15}\text{N}$  dimensions.

## References

- Chen S, Smith DF (1998) Hop as an adaptor in the heat shock protein 70 (Hsp70) and Hsp90 chaperone machinery. *J Biol Chem* 273:35194-35200.
- Cortajarena AL, Regan L (2006) Ligand binding by TPR domains. *Protein Sci* 15:1193-1198.
- Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeifer J, Bax A (1995) NMRPipe: a multidimensional spectral processing system based on UNIX pipes. *J Biomol NMR* 6:277-293.
- Fonseca AC, Romao L, Amaral RF, Assad Kahn S, Lobo D, Martins S, Marcondes de Souza J, Moura-Neto V, Lima FR (2012) Microglial stress inducible protein 1 promotes proliferation and migration in human glioblastoma. *Neuroscience* 200:130-141.
- Goddard TD, Kneller DG, SPARKY 3, University of California, San Francisco. <http://www.cgl.ucsf.edu/home/sparky/>.
- Hernandez MP, Chadli AC, Toft DO (2002) Hsp40 binding is the first step in the Hsp90 chaperoning pathway for the progesterone receptor. *J Biol Chem* 277:11873-11881.
- Horibe T, Kohno M, Haramoto M, Ohara K, Kawakami K (2011) Designed hybrid TPR peptide targeting Hsp90 as a novel anticancer agent. *J Transl Med* 9:8.
- Keller R (2004) The computer aided resonance assignment tutorial. Verlag Goldau, Cantina, Switzerland.
- Laskowski RA (2009) PDBsum new things. *Nucleic Acids Res* 37:D355-D359.
- Lopes MH, Hajj GN, Muras AG, Mancini GL, Castro RM, Ribeiro KC, Brentani RR, Linden R, Martins VR (2005) Interaction of cellular prion and stress-inducible protein 1 promotes neuritogenesis and neuroprotection by distinct signaling pathways. *J Neurosci* 25:11330-11339.
- Marsh JA, Singh VK, Jia Z, Forman-Kay JD (2006) Sensitivity of secondary structure propensities to sequence differences between alpha- and gamma-synuclein: implications for fibrillation. *Protein Sci* 15:2795-2804.
- Odunuga OO, Longshaw VM, Blatch GL (2004) Hop: more than an Hsp70/Hsp90 adaptor protein. *Bioessays* 26:1058-1068.
- Pratt WB, Toft DO (2003) Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Exp Biol Med (Maywood)* 228:111-133.



Sato S, Fujita N, Tsuruo T (2000) Modulation of Akt kinase activity by binding to Hsp90. *Proc Natl Acad Sci U S A* 97:10832-10837.

Scheufler C, Brinker A, Bourenkov G, Pegoraro S, Moroder L, Bartunik H, Hartl FU, Moarefi I (2000) Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. *Cell* 101:199-210.

Smith DF, Sullivan WP, Marion TN, Zaitsev K, Madden B, McCormick DJ, Toft DO (1993) Identification of a 60-kilodalton stress-related protein, p60, which interacts with Hsp90 and Hsp70. *Mol Cell Biol* 13:869-876.

Stancato LF, Chow YH, Hutchinson KA, Perdew GH, Jove R, Pratt WB (1993) Raf exists in a native heterocomplex with hsp90 and p53 that can be reconstituted in a cell-free system. *J Biol Chem* 268:21711-21716.

Taipale M, Jarosz DF, Lindquist S (2010) Hsp90 at the hub of protein homeostasis: emerging mechanistic insights. *Nat Rev Mol Cell Biol* 11:515-528.

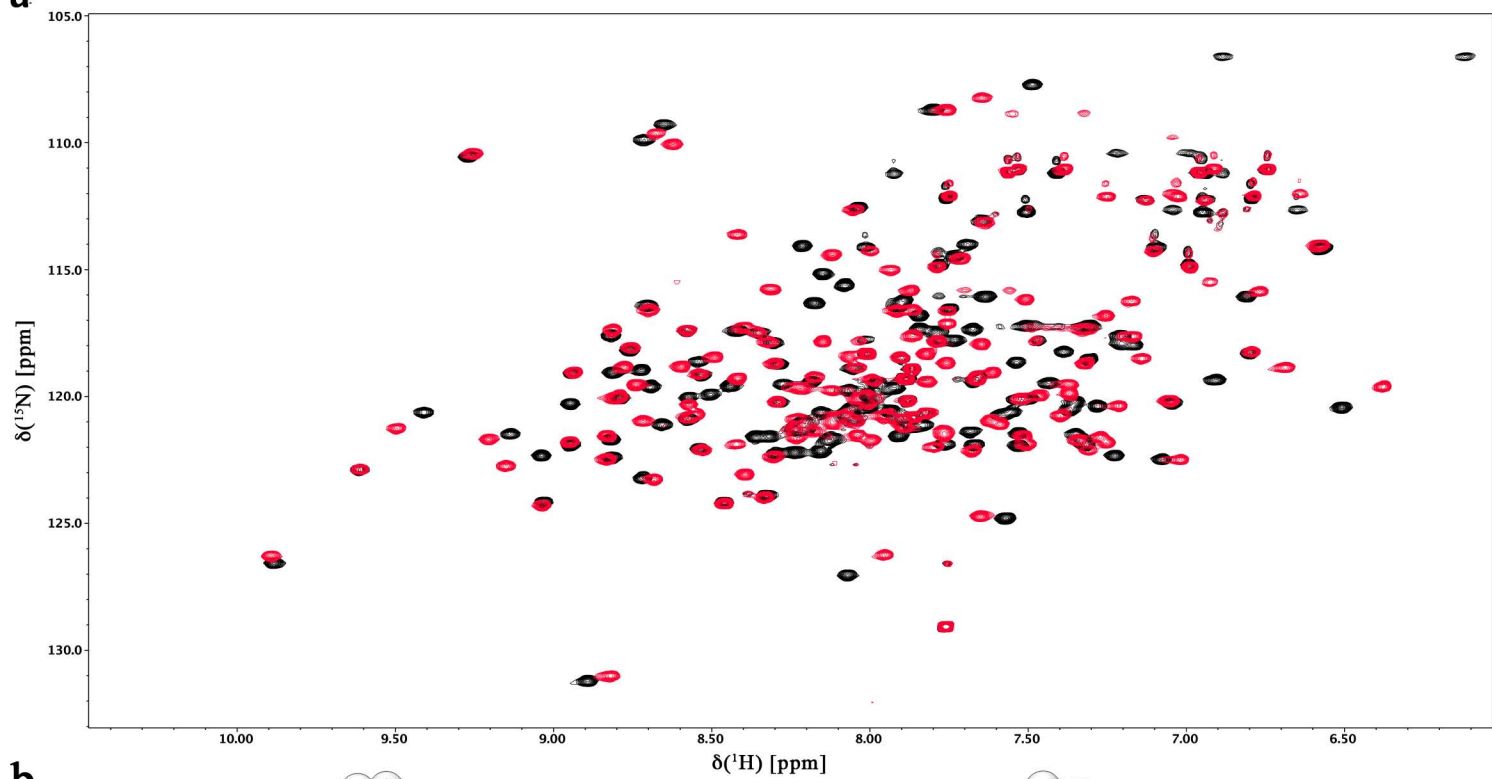
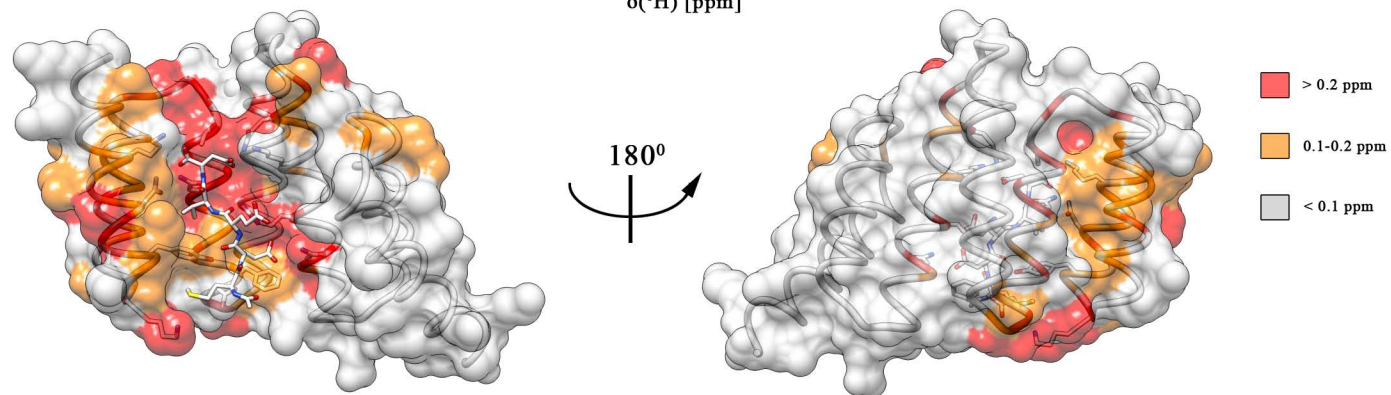
Trepel J, Mollapour M, Giaccone G, Neckers L (2010) Targeting the dynamic HSP90 complex in cancer. *Nat Rev Cancer* 10:537-549.

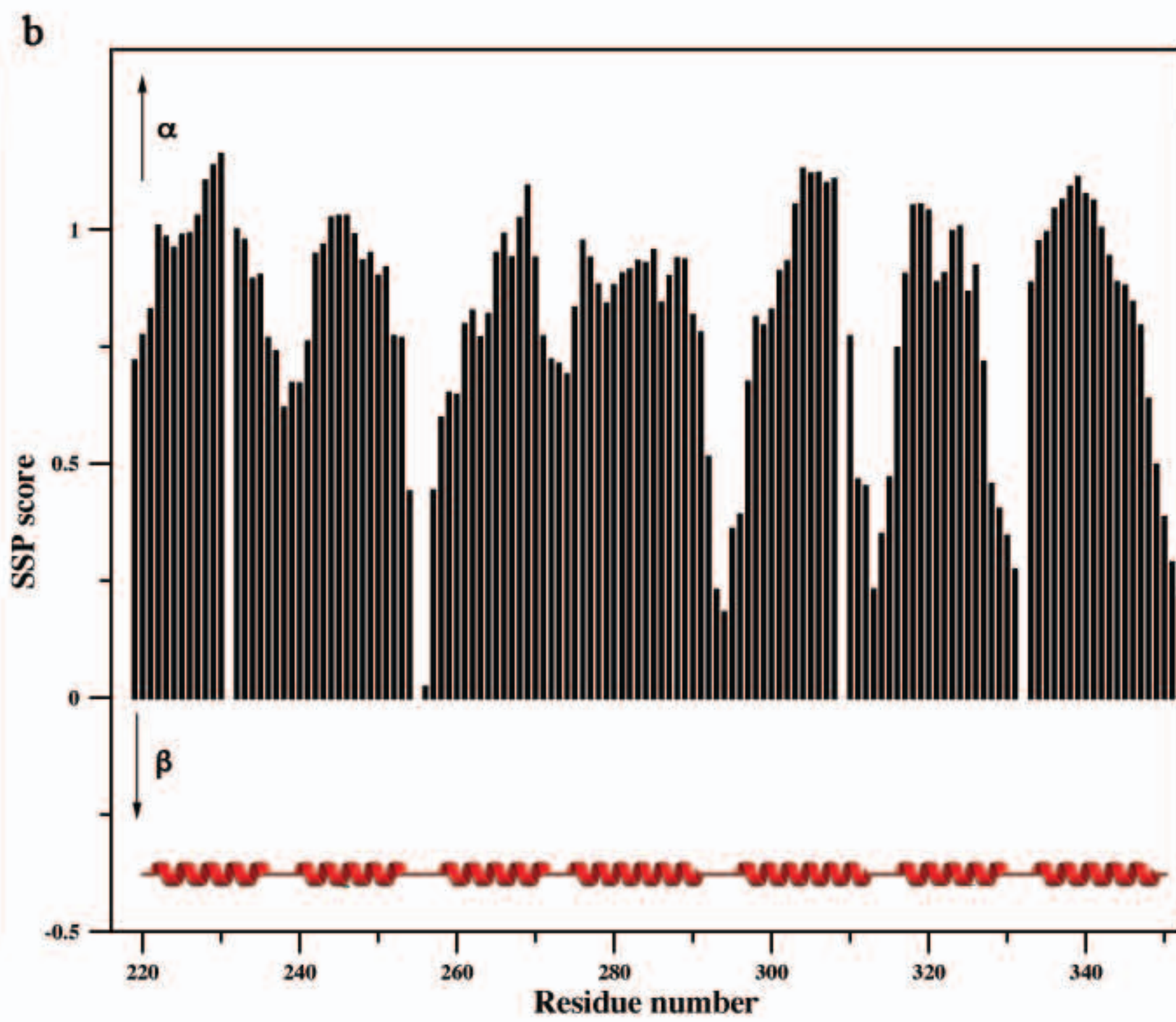
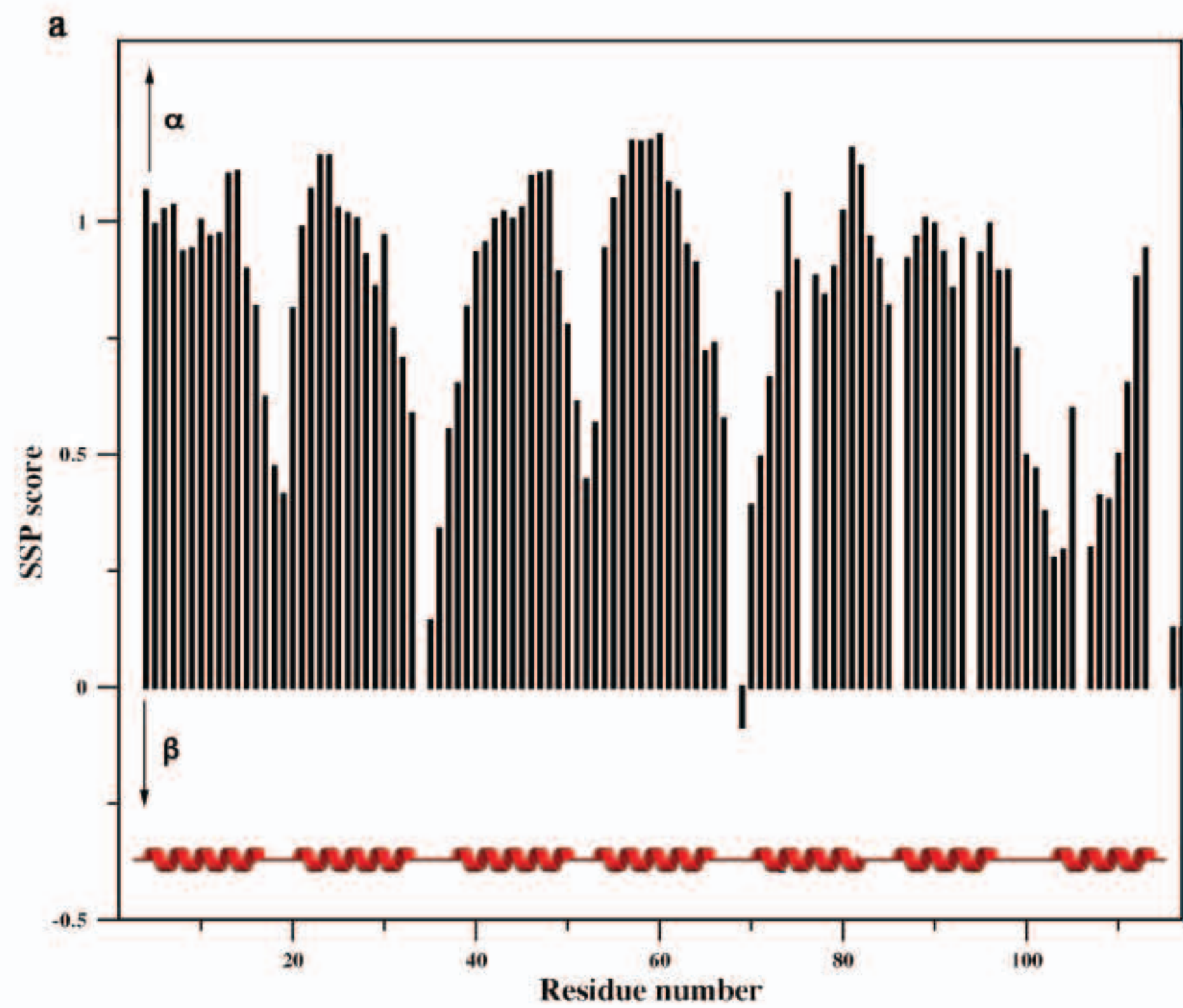
Walsh N, Larkin A, Swan N, Conlon K, Dowling P, McDermott R, Clynes M (2011) RNAi knockdown of Hop (Hsp70/Hsp90 organizing protein) decreases invasion via MMP-2 down regulation. *Cancer Lett* 306:180-189.

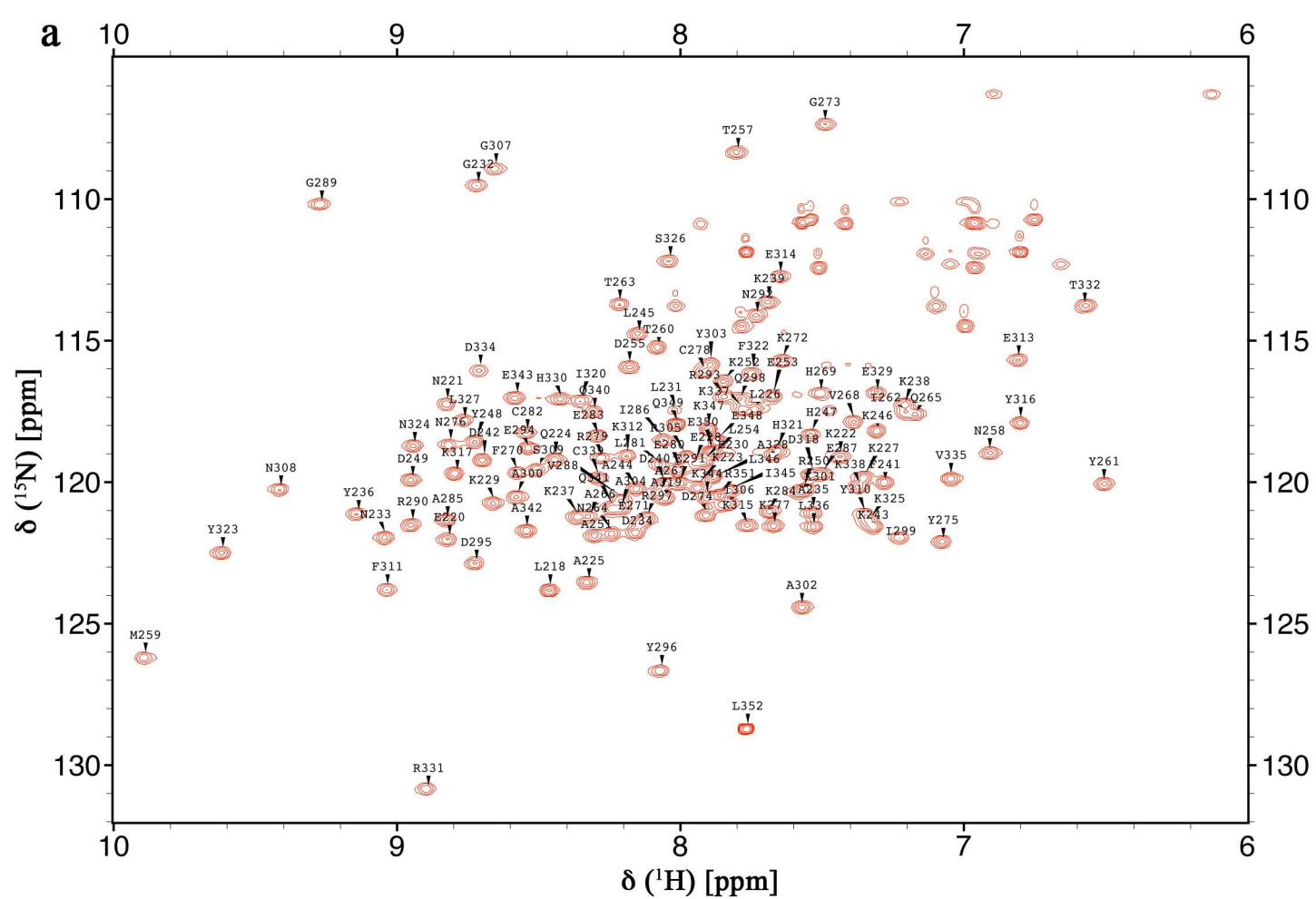
Xu W, Mimnaugh E, Rosser MF, Nicchitta C, Marcu M, Yarden Y, Neckers L (2001) Sensitivity of mature ErbB2 to geldanamycin is conferred by its kinase domain and is mediated by the chaperone protein Hsp90. *J Biol Chem* 276:3702-3708.

Young JC, Agashe VR, Siegers K, Hartl FU (2004) Pathways of chaperone mediated protein folding in the cytosol. *Nat Rev Mol Cell Biol* 5:781-791.

Zanata SM, Lopes MH, Mercadante AF, Hajj GN, Chiarini LB, Nomizo R, Freitas AR, Cabral AL, Lee KS, Juliano MA, de Oliveira E, Jachieri SG, Burlingame A, Huang L, Linden R, Brentani RR, Martins VR (2002) Stress-inducible protein 1 is a cell surface ligand for cellular prion that triggers neuroprotection. *EMBO J* 21:3307-3316.

**a****b**





**b**

217 240

**G****D**L**P****E****N****K****K****Q****A****L****K****E****K****E****L****G****N****D****A****Y****K****K****K****D****F****D****K****A****L****K****H****Y****D****R****A****K****E****L****D**

260 280

**P****T****N****M****T****Y****I****T****N****Q****A****A****V****H****F****E****K****G****D****Y****N****K****C****R****E****L****C****E****K****A****I****E****V****G****R****E****N****R****E****D****Y**

300 320

**R****Q****I****A****K****A****Y****A****R****I****G****N****S****Y****F****K****E****E****K****Y****K****D****A****I****H****F****Y****N****K****S****L****A****E****H****R****T****P****D****V****L****K****K**

340 352

**C****Q****Q****A****E****K****I****L****K****E****Q****E****R****L**

