236-Pos Board B16
Characterizing a New Metal Binding Site in S100B
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S100B and other members of the S100 family of proteins have a conserved N-terminal ''pseudo'' EF-hand and a C-terminal ''typical'' EF-hand Ca^{2+}-binding motif. Upon Ca^{2+} binding, most dimeric S100 proteins undergo a conformational change that regulates binding to target proteins necessary for modulating biological processes including cell proliferation. In addition to Ca^{2+}, S100B also binds Mg^{2+} and Zn^{2+}, which affect Ca^{2+} and target protein binding. In the case of Mg^{2+}, which is isoelectronic with water, it is often difficult to identify its binding site unambiguously using x-ray crystallography. For this, we have characterized Mn^{2+} binding to S100B and initiated studies using NMR to monitor the paramagnetic effects of Mn^{2+} binding. Using X-ray crystallography, we have confirmed that S100B binds Mn^{2+} in the typical EF-hand (EF2). This site exhibits very distinct electron density and square pyramidal coordination geometry with coordination by aspartates 63 and 69, two water molecules, and the backbone carbonyl atom from another monomer; no conformational change was observed when compared to crystals collected in the absence of Mn^{2+}. Given that Mn^{2+} also binds to the Zn^{2+} site and is used to mimic Mg^{2+}, we turned our attention to these metal ion sites. For this, modifications were made to a 2D HCACO experiment to optimize it for detecting beta carbons of Asn, Gln, Asp, and Glu residues. In this regard, we monitored paramagnetic effects on resonances that are involved in coordinated Mn^{2+} in solution, which will be described here. Also to be discussed are subsequent competition studies completed with diamagnetic metals that bind S100B, including Ca^{2+} Mg^{2+}, and Zn^{2+}.

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NMR Characterization of an Unusual 37 kDa Epimerization Domain of Yersinabactin Synthetase
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Nonribosomal Peptide Synthetases (NRPSs) are endogenous microbial supramolecular (1-4 MDa) assemblies that are responsible for the biosynthesis of important natural products, including antibiotics. Genetic reprogramming of NRPS machinery for the generation of novel-or perhaps ''designer''-antibiotics (or other therapeutics) represents the apogee of NRPS research. Our model NRPS system is yersiniabactin synthetase (YS), which produces the virulence factor yersiniabactin. Heterocycles (e.g. thiazoline rings) in natural products, such as yersiniabactin, exhibit unique medicinal properties (antibiotic, anticancer, immunosuppressant). An unusual epimerization domain (EA) in YS catalyzes the stereoconversion of a chiral heterocyclic center. Interestingly, EA itself is embedded within the primary sequence of a so-called adenylation domain (A, recognizes and activates substrates). To understand the overall choreography of catalysis, we require structural models of each catalytic domain in its monomeric state, bound to its substrate(s), and in complex with other relevant domains. NRPS domains can be difficult to crystallize in the biologically relevant conformations and complexes, so we take advantage of NMR spectroscopy, which can offer structural, kinetic, thermodynamic, and dynamical data. However, conventional bimolecular NMR experiments will fail on domains such as EA (37 kDa) largely because of relaxation losses but also spectral crowding. This work discusses how nonuniform sampling allowed us to rescue sensitivity and collect preliminary data on a partially deuterated sample. We also exploit covariance NMR spectroscopy to ease the challenging backbone assignment procedure. Using such data we report ~70% backbone assignment. We also present a chemical biology strategy that will allow us to synthesize otherwise challenging substrates, which will facilitate measurement of steady state kinetic parameters and inform our structural models.

238-Pos Board B18
Fibrinogen Hydrodynamic Properties from NMR-Diffusion Studies
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Fibrinogen is a blood plasma protein with crystallographically determined rod-like structure with globular domains, but it is unclear if this conformation is kept in blood. We studied fibrinogen in solution using NMR diffusometry. Pulsed-field gradient NMR self-diffusion measurements were made on a Bruker AVANCE-III-600 NMR-spectrometer equipped with a z-gradient inverse probehead (TBI, 5-mm-tube). Diffusion experiments were performed using a stimulated-echo sequence incorporating bipolar gradient pulses and a longitudinal eddy current delay (BPP-LED). The water signal was suppressed by presaturation. Data processing was performed using Topspin 2.1 software. The self-diffusion coefficient (SDC) was measured within T=278-315K for bovine fibrinogen (97% pure) at 0.3-43 mg/ml in a physiological buffer. By applying the Stokes-Einstein equation, we calculated an effective hydrodynamic radius, RH, of the fibrinogen molecule, corresponding to the radius of a sphere having the same mobility. At 37C in a highly dilute solution RH=6.7 nm. However, a concentration dependence of the fibrinogen SDC revealed a dramatic deviation from the spherical particle-like behavior. The SDC-[fibrinogen] plot was fitted well with a stretched exponential, characteristic of the sub-diffusion or hindered diffusion observed in crowded molecular systems with direct intermolecular interactions. Accordingly, the activation energy of the protein diffusive motion increased from 20 kJ/mol at 1.4 mg/ml (close to the activation energy of water) to 32 kJ/mol at 36 mg/ml, suggesting a marked fibrinogen self-interaction at higher concentrations, likely mediated by the αC regions. The mean value of RH=6.7 nm fits with a model for hydrodynamic motion of an elongated cylinder 45 nm in length with the cylinder radius of ~1.70 nm, close to the known crystallographic dimensions. The results suggest that fibrinogen molecules in solution maintain a rod-like shape and undergo direct intermolecular interactions/collisions during diffusive motion. (Supported by the Program of Competitive Growth of Kazan Federal University)

239-Pos Board B19
Site-Resolved Measurements of Protein Hydration Dynamics
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The interactions of biological macromolecules with water are fundamental to their structure, dynamics and function. Protein hydration has historically been quite difficult to measure experimentally. Confinement of a solvated protein within the protective nanoscale interior of a reverse micelle permits comprehensive measurement of protein-water interactions through the nuclear Overhauser effect. This method was first developed using the 8.5 kDa globular protein ubiquitin. We have now applied this approach to the 14.4 kDa hen egg white lysozyme (HEWL) and the 16.1 kDa Staphylococcal nuclease (SNase). The hydration dynamics of the free and inhibitor liganded states of HEWL were studied. In the free state, fast waters were observed within the peptidoglycan-binding region and within a buried hydrophillic cavity. Two differences were observed in the inhibitor-bound state: slow waters were now observed between the inhibitor and the protein and faster water dynamics were observed at some remote areas of the protein. For SNase we find that the surface hydration dynamics are heterogeneous, and have little correlation with surface chemistry such as residue type, hydrophobicity, or charge. Furthermore, we also find that there is a correlation between surfaces with slow water and whether that surface participates in a binding interface. These general observations are consistent with initial studies that measured hydration dynamics of ubiquitin in bis(2-ethylhexyl) sulfosuccinate (AOT) reverse micelles. Supported by NSF grant MCB 0842814, NIH predoctoral training grants GM071339 (BSM) and GM008275 (CJ), and NIH postdoctoral fellowship GM087099 to NVN.

240-Pos Board B20
Internal Cavities and their Role as Determinants of Pressure Unfolding of Proteins
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The molecular mechanisms of chemical and heat denaturation of proteins are relatively well established; those of pressure unfolding are not. Volume is the conjugate variable of pressure; it is the fundamental thermodynamic variable governing the pressure sensitivity of proteins. By generating artificial cavities by substitution of internal hydrophobic residues in staphylococcal nuclease (SNase) with Ala we have shown previously that cavities that are present in the native state and absent in the unfolded state contribute significantly to the change in volume upon unfolding (ΔV). We have extended these studies to variants of SNase with very large cavities achieved with multiple Ala substitutions, variants with substitutions of small residues with large ones that eliminate the