2894-Pos Board B49
Azido Groups as Site Specific IR Probes of Hydration Around Model Helical Peptides
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Previous work from our group and others indicates that the NNN asymmetric stretching peak of aliphatic azido groups is sensitive to the hydrogen bond density of the azido group's local environment, but not to the local electrostatics. Thus, the azido group is a sensitive indicator of local hydration of a chosen site. The behavior of the probe in a model helical peptide that displays a cooperative helix/coil transition was investigated using solutions composed of varying ratios of TFE and water. Far-UV circular dichroism was used to determine whether the azido group disrupted the secondary structure of the peptide. A series of peptides labeled with azido groups on increasingly extended side chains provided information about the hydration around the backbone of the peptide through multiple solvation layers. Local desolvation by trimethylamine N-oxide and urea was investigated using the same system.

2895-Pos Board B50
Effect of Dimethyl Sulfoxide on the Stability of Macromolecular Structures
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Dimethyl sulfoxide (DMSO) is best known for its use in cryoprotection of biological cells and as a co-solvent in molecular formulations. In our study we investigate how DMSO affects the stability of two very different macromolecular aggregates: one represented by multilamellar lipid bilayers and the other by fibrin networks. Although chemically different, both macromolecular structures are influenced by interactions at the water interface which in turn depend on the presence of perturbants such as DMSO. We use x-ray scattering as well as confocal and differential interference microscopy to characterize structural changes in multilamellar lipid bilayers and in fibrin networks. We find that although DMSO effects have similarities to the osmotic action of standard osmolytes such as polyethylene glycol (PEG), chemical specificity also play a significant role.

2896-Pos Board B51
Thermodynamic Fingerprints Reveal Variability in Cosolute Effect on Peptide Folding
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On the molecular level, life is established through the specific interaction between and within macromolecules in an aqueous environment. It is increasingly realized, however, that even modest changes in solution conditions, brought on by the presence of many cellular cosolutes, can profoundly affect the balance of forces underlying the specificity of interaction, with consequences that can be severe if not fatal. We have followed the thermodynamic effect of several cosolute classes, including polymers, cellular osmolytes, and inorganic salts, on the stability of peptide folding. By comparing changes in free energy, enthalpy, and entropy upon cosolute addition for this process, we identify several thermodynamically distinct mechanisms. Surprisingly, even while many cosolutes display similar linear scaling of the free energy with cosolute concentration, a breakdown of this free energy into enthalpic and entropic contributions distinguishes between different families of cosolutes. We show how these ''thermodynamic fingerprints'' can be used to group distinct cosolute families, and to suggest the existence of different mechanisms by which members of these families act.
Sukenik S, Sapir L, Gilman-Politi R, Harries D (2012) Diversity in the mechanisms of cosolute action on biomolecular processes Faraday Disc. 106 doi: 10.1039/C2FD20101A

2897-Pos Board B52
Towards the Design of Metamorphic Proteins using Ensemble-Based Energetic Information
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The discovery of natural and engineered proteins that adopt more than one functionally relevant structure represents an emerging paradigm shift in the field of protein folding and stability. This work explores a preliminary approach to understanding how a single amino acid sequence may adopt more than one stable structure. Key to the approach is information derived from a previously reported (and experimentally validated) statistical mechanical ensemble description of globular protein thermodynamics. Such energetic information is demonstrated to correctly match and align amino acid sequences to the corresponding known structures in large databases. As a first step, an algorithm was developed to generate novel amino acid sequences energetically compatible with a single target structure, the SH3 domain. Information about both the native and denatured state energetics of the target was taken into account, as we hypothesize that the denatured state in particular cryptically encodes necessary negative design information. These designed sequences indeed demonstrated primary, secondary, and tertiary structure properties similar to known SH3 domains in silico. Experimental characterization of one designed sequence revealed reversible cooperative two-state denaturation, consistent with the expected biophysical properties of an SH3 domain. Encouraged by these results, we are proceeding to discover naturally occurring sequences that are energetically compatible with more than one structure. Initial analysis suggests that natural sequences exhibit a surprisingly large range of compatibility with non-self structures, with some so-called ''promiscuous'' sequences potentially compatible with many structures. Statistically enriched regions of sequence containing glycine, matching with low-stability (e.g. turn) regions in the non-self structure, appears to be one general mechanism mediating this energetic ''promiscuity''. Such insight, only possible because of our unique thermodynamic approach to protein design, may become part of a future experimental strategy to engineer metamorphic proteins.

2898-Pos Board B53
Investigation of the Structural Stability of cpSRP43 Chromodomain2 by Hydrogen-Deuterium Exchange
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The essential pathway by which light-harvesting chlorophyll binding proteins are inserted into the thylakoid of the chloroplast is mediated by a unique signal recognition particle (cpSRP). Its novel 43kDa subunit (cpSRP43) contains three chromodomains (CDs). CD2 has been shown to be significant in various aspects of the cpSRP-mediated pathway. While structural information about CD2 is known, this study aims to gain a more comprehensive understanding of the structural stability through analysis of hydrogen-deuterium exchange. 15N-labeled CD2 samples will be produced from over-expression of CD2 in E. coli cultured in labeled media. Subsequently, the readily-occurring exchange between amide hydrogen and solvent deuterium will be monitored by NMR spectroscopy. The kinetics of the exchange can provide useful information about the free energy of exchange and thus the stability.

2899-Pos Board B54
Protein Unfolding Pathway Examined by Pulse Proteolysis using a Super-Stable Protease
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We examined the slow unfolding pathway of Tk-RNase H2 by pulse proteolysis using a super-stable protease, Tk-subtilisin. Tk-subtilisin has its enzymatic activity in highly concentrated GdnHCl where Tk-RNase H2 unfolds slowly.The native state of Tk-RNase H2 is completely resistant to Tk-subtilisin, whereas the unfolded state of Tk-RNase H2 (induced by 4 M GdnHCl) can be degraded by Tk-subtilisin. In pulse proteolysis, some degradation products of Tk-RNase H2 were detected by Tricine-SDS-PAGE. We identified the cleavage sites in Tk-RNase H2 by N-terminal sequencing and MALDI-TOF mass spectrometry, and constructed mimics of Tk-RNase H2 unfolding intermediate by protein engineering. The mimics were biophysically characterized. We found that the native state of Tk-RNase H2 (N-state) shifted to two intermediate forms, IB-state and IC-state. The IB-state was digested by Tk-subtilisin in the C-terminal region, but the IC-state was a Tk-subtilisin resistant form. The results represent that pulse proteolysis by a super-stable protease was a suitable strategy and effective tool for analyzing intermediate structures of proteins with slow unfolding properties.

2900-Pos Board B55
Structural Reorganization Triggered by the Ionization of Lys Residues buried in Hydrophobic Environments
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A study of the structural consequences of ionization of amino acid side chains buried in the hydrophobic interior of proteins was performed by systematic introduction of lysine at 25 internal positions in a highly stable form of staphylococcal nuclease. Crystal structures for 12 of the variants at pH values where the Lys was expected to be uncharged revealed nearly identical backbones with the reference protein and ordered internal Lys side chains at the substitution site. The structures demonstrate that neutral Lys can be buried in the