properties of water and solvent mediated effects play a crucial major role in protein stabilisation also under high hydrostatic pressure conditions, where, for example, TMAO and pressure have counteracting effects on the water structural properties. These findings may help to understand the upregulation of TMAO under high pressure stress conditions and the compensatory effect of urea-TMAO mixtures in deep-sea organisms. Also the effect of confinement on the solvational properties and intermolecular interaction of proteins was studied, including the effects of self-crowding and macromolecular crowders (such as dextran) on the temperature-pressure stability diagram of proteins. Moreover, we also discuss the effect of pressure on the second virial coefficient B_{22} and how pressure can be used to control and fine-tune protein crystallization.

970-Plat
Molecular Mechanism for the Preferential Exclusion of Osmolytes from Protein Surfaces
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Trimethylamine N-oxide (TMAO) is a naturally occurring protecting osmolyte that stabilizes the folded state of proteins and also counteracts the destabilizing effect of urea on protein stability. Experimentally, it has been inferred that TMAO is preferentially excluded from the vicinity of protein surfaces. Here, we combine computer modeling and experimental measurements to gain an understanding of the mechanism of the protecting effect of TMAO on proteins. We have developed an all-atom molecular model for TMAO that captures the exclusion of TMAO from model compounds and protein surfaces, as a consequence of incorporating realistic TMAO-water interactions through osmotic pressure measurements. Osmotic pressure measurements also suggest no significant attraction between urea and TMAO molecules in solution. To obtain an accurate potential for molecular simulations of protein stability in TMAO solutions, we have explored different ways of parameterizing the protein/osmolyte and osmolyte/osmolyte interactions by scaling charges and the strength of Lennard-Jones interactions and carried out equilibrium folding experiments of Trp-cage miniprotein in presence of TMAO to guide the parameterization. Our calculations suggest a general principle for preferential interaction behavior of cosolvents with protein surfaces - preferentially excluded osmolytes have repulsive self-interaction given by osmotic coefficient larger than one, while denaturants, in addition to having attractive interactions with the proteins, have favorable self-interaction given by osmotic coefficient less than one to enable preferential accumulation in the vicinity of proteins.
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971-Plat
From Model Compounds to Proteins: A Volumetric Study of the Stabilizing Action of Glycine Betaine
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Glycine betaine is a strong protecting osmolyte capable of stabilizing the folded conformations of proteins. A lingering problem has been to determine the molecular mechanisms underlying the enhancement of protein stability. One approach to tackle this problem is to examine the differential solvation properties of proteins and their components in water and glycine betaine. We present a new statistical thermodynamic formalism to quantify the individual binding affinities of glycine betaine for the polar and nonpolar moieties of globular proteins. This approach enables one to rationalize partial molar volume, V°, and adiabatic compressibility, K_s°, measurements in terms of interactions of glycine betaine with polar and nonpolar protein groups. We characterize in this way glycine betaine interactions with ribonuclease A, lysozyme, cytochrome C, and ovalbumin. To this end, we use our previous volumetric data on N-acetyl amino acid amides (models for amino acid side chains) and oligoglycines (models for peptide backbone) in solutions of glycine betaine at concentrations ranging from 0 to 4 M. We determine the average binding affinity, k, of glycine betaine for the proteins as well as changes in the volume, ΔV_o, and adiabatic compressibility, ΔK_{so}, associated with the binding of a glycine betaine molecule to the proteins. We find good agreement between these characteristics determined for proteins and their low molecular weight model compounds. These results suggest little or no cooperativity for glycine betaine interactions with proteins. Our experimental results in conjunction with the statistical thermodynamic formalism that we developed offer a new paradigm for investigating weak protein-ligand interactions.

972-Plat
Effect of Ionizable Residues on the Folding Energy Landscape of Globular Proteins: Linking Experiment and Computation
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Ionizable residues play important role in protein structure, stability and function. In globular proteins, majority of ionizable residues are located on the protein surface. We have previously used computational approach to optimized surface charge-charge interactions in several globular proteins: ubiquitin, the activation domain of human procarboxypeptidase A2 (ADA2), the fibronectin typeIII domain (TnfIII), acylphosphatase (ACP), the N-terminal domain of human U1A protein (U1A), Cdc42 GTPase, Fyn SH3 domain, and cold shock protein CspB(1-3). Biophysical studies have shown that all the designed variants have increased thermodynamic stability. Here we have measured experimentally the folding kinetics of the wild-type and designed variants of four proteins: U1A, Ten, ACP and ADA2. We have also performed native topology-based model simulations that correspond to a funneled energy landscape, but explicitly include non-native charge-charge interactions. Analysis of computer simulations provided molecular details for the experimentally observed changes in the folding rates of charge-charge optimized variants.
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Platform: Intrinsically Disordered Proteins

973-Plat
Folding Upon Binding - Not a Simple Protein Folding Problem
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Although there have been many thermodynamic studies of the association of intrinsically disordered proteins (IDPs) with their binding partners, there have been relatively few kinetic studies.
We have analysed the association of two different IDP systems, which form helical structure upon binding. These systems have markedly different behaviour. One associates very slowly and weakly, and the other very rapidly and strongly. The kinetic data therefore need to be analysed in a different manner to each other, and to the folding of single domain proteins. We discuss why standard approaches developed for folded protein-protein interactions may not be appropriate for reactions where one or both proteins are disordered. In addition, we show how protein engineering can be used to provide insights into the pathways/mechanisms of folding.
Finally we have analysed the kinetics of the folding of an intrinsically disordered region (IDR) within a protein - a domain that only folds when attached to a neighbouring folded domain. This multidomain protein folds in a very different manner compared to those formed from adjacent domains which can fold in isolation.

974-Plat
Regulation of Protein-Protein Binding through the Coupling Between Phosphorylation and Intrinsic Disorder
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Phosphorylation offers a dynamic way to regulate protein activity, subcellular localization, and stability. At the same time phosphorylation sites are often