Ice-binding proteins (IBPs) depress the freezing point of body fluids below the melting point, resulting in a thermal hysteresis (TH) that prevents freezing of the organism. The potential of these proteins in the medical sector, in cryopreservation, in the frozen food industry, and in agriculture is enormous. We are investigating the mechanism by which IBPs interact with ice surfaces and inhibit ice growth and recrystallization. We have developed novel methods for these studies, including fluorescence microscopy techniques combined with temperature-controlled microfluidic devices. These techniques have enabled the replacement of the IBP solution surrounding an IBP-bound ice crystal by buffer, without losing the bound IBP or the TH activity. Our results show the irreversibility of the protein:ice interactions and the indirect dependence of TH activity on the protein concentration in solution. We found that the dynamics of the interactions with ice vary dramatically between different types of IBPs. From our results and other recent developments a new understanding of the mechanisms by which various IBPs act is emerging. This understanding is critical for the successful use of IBPs in cryopreservation applications. Supported by the European-Research-Council (ERC), the National-Science-Foundation (NSF), and the Israel-Science-Foundation (ISF), Canadian Institutes of Health (CIHR), The Lady Davis Foundation, and the Canada Research Chair program.

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A Two-Color Non-Muscle SERCA FRET Sensor for Diabetes Drug Discovery Using Fluorescence Lifetime Detection

Tory Schaar1, Li Li1, Rocio Fonseca1, Simon Gruber1, Kurt Peterson2, Karl Petersen3, Cornea Razvan4, Greg Gillispie5,6, David Bernlohr,7 David Thomas1
1Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN, USA, 2Fluorescence Innovations, Inc., Minneapolis, MN, USA.

We have developed intramolecular FRET sensors capable of detecting cytoplasmic headpiece movements of human SERCA (sarco/endo-plasmic reticulum calcium ATPase) in fluorescent assays, including a cytoplasmic domain (SERCA2b) isoform to be used in drug discovery for treatment of diabetes. Two fluorescent proteins, clover (green) and mRuby2 (red) were directly fused to selected locations on human SERCA1a (skeletal muscle), 2a (cardiac muscle), and 2b (non muscle), based on a previously reported SERCA2a construct (Gruber et al., 2014), and expressed stably in HEK cells. We have used these cells in a novel fluorescence lifetime plate reader (FLT-PR) to screen small-molecule libraries, to discover modulators of SERCA structure and function. The present study focuses on SERCA2b, with the goal of obtaining small molecules that activate SERCA in non-muscle cells. Since recent reports indicate that SERCA overexpression in non-muscle cells can alleviate Type II diabetes, we seek small-molecule activators of SERCA for the same purpose. The small-molecule modulators identified in the high-throughput FRET screen were examined for their ability to affect SERCA’s function, through assays of ATPase and calcium pumping activities. In order to obtain functional data more directly related to Type II diabetes, we tested the compound’s alleviation of endoplasmic reticulum stress in 3T3-L1 adipocytes, using an XF24 Extracellular Flux Analyzer to measure mitochondrial function after inducing ER stress with the inflammatory cytokine TNF-z. While this study is designed to find activators of SERCA2b for treatment of diabetes, constructs based on other SERCA isoforms show promise in targeted therapeutics for muscular dystrophy (SERCA1a) and heart failure (SERCA2a).

Putting on the Squeeze: Solution NMR Investigations of Protein Structure and Hydration under High Pressure

Nathaniel V. Nucci1, Brian Fuglestad2, Connie Liao1, Evangelia A. Athanassoul2, A. Joshua Wand2
1Physics & Astronomy and Biomedical & Translational Sciences, Rowan University, Glassboro, NJ, USA, 2Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA, USA.

It is well known that high hydrostatic pressures can induce the unfolding of proteins. The physical underpinnings of this phenomenon have been investigated extensively but remain controversial. Changes in solvation energetics due to applied hydrostatic pressure have been a commonly proposed mechanism for unfolding, but recent studies have provided strong evidence that elimination of void volumes in the native folded state is a principal determinant. Here we use the cavity-containing L99A mutant of T4 lysozyme to examine the pressure unfolding of a multi-domain protein using solution NMR. The cavity-containing C-terminal domain completely unfolds at moderate pressures while the N-terminal domain remains largely structured to high pressures. This pressure response is completely suppressed by benzene binding to the hydrophobic cavity. These results contrast to the pseudo wild type protein, which has a residual cavity volume very similar to that of the L99A-hexene complex but shows extensive subglobal reorganizations with pressure. Encapsulation of the L99A mutant in the aqueous nanoscale core of a reverse micelle suppresses the pressure-induced unfolding transition due to the volume restriction and promotes high-pressure filling of the cavity with water. This result indicates that hydration of the hydrophobic cavity is more energetically unfavorable than global unfolding. Overall these observations point to a range of cooperativity and energetics in the pressure response of proteins and illuminate the fact
that small changes in physical parameters can significantly alter this response. Supported by NSF grant MCB-115803 and by NIH postdoctoral fellowship GM087099 to V.N.V.

1742-Plat
Effects of Crowding, Osmolytes, Temperature and Pressure on the Interaction Potential of Dense Protein Solutions
Roland Winter
TU Dortmund University, Dortmund, Germany.
We studied the effect of pressure on the structure and intermolecular interactions of dense lysozyme solutions in various cosolvent mixtures and upon addition of various Hofmeister anions using small-angle X-ray scattering in combination with liquid-state theoretical approaches [1-3]. Supplementary thermodynamic information was obtained by employing calorimetric techniques, densitometry and ultrasound velocimetry. We show that the particular structural properties of water and specific ion effects play a crucial major role in protein stabilisation, notably under high hydrostatic pressure conditions. 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 on the temperature-pressure stability diagram of proteins [4]. We also discuss the effect of pressure on the second virial coefficient and how pressure can be used to control and fine-tune protein crystallization. Moreover, we present results on the phase behavior of dense lysozyme solutions in the liquid-liquid phase separation region. A re-entrant liquid-liquid phase separation region has been discovered at elevated pressures, which originates in the pressure dependence of the solvent-mediated protein-protein interactions [3].


1743-Plat
A Multiscale Model for pH-Dependent Folding and Binding of a Conditionally Disordered Chaperone
Logan S. Ahlstrom, Sean M. Law, Alex Dickson, Charles L. Brooks III, Department of Chemistry, University of Michigan, Ann Arbor, MI, USA.
The bacterial acid stress-sensing chaperone HdeA loses structure to gain function. As enteropathogenic E. coli pass through the severely acidic environment of the mammalian stomach, HdeA transitions from an inactive, folded dimer to chaperone-active, unfolded monomers to protect against the acid-induced aggregation of periplasmic proteins. Toward achieving an atomic-level mechanistic understanding of the acid stress response of HdeA, we develop a multiscale modeling approach to capture its pH-dependent thermodynamics. Our approach utilizes pKa calculations from all-atom constant pH molecular dynamics simulations to alter the coarse-grained model for representing different pH environments. Changes in the thermodynamics of binding as a function of pH are explored using the efficient “Hamiltonian mapping” reweighting formalism. We propose new features of the pH-sensing mechanism of HdeA that can be directly tested by experiment. Namely, our model predicts that HdeA is maximally stable under mildly acidic conditions and that a partially unfolded dimeric intermediate may contribute to substrate binding. Our multiscale approach is general such that it can be applied toward understanding pH-dependent functional transitions in other systems and sets a foundation from which to construct models of HdeA-substrate interaction.

1744-Plat
Structural Origin of Landscape Roughness in Protein Folding from Single-Molecule FRET and All-Atom Molecular Dynamics Simulations
Hoi Sang Chung1, Stefano Piana-Agostinetti1, David E. Shaw2, William A. Eaton1
1Laboratory of Chemical Physics, NIDDK/NIH, Bethesda, MD, USA, 2D. E. Shaw Research, New York, NY, USA.
Folding of most single-domain proteins has been successfully described by diffusion on a one-dimensional (1D) free energy surface. Although the 1D surface is smooth, there are many local minima in the underlying energy landscape, giving rise to landscape “roughness”. According to Kramers’ reaction-rate theory, roughness slows folding kinetics by reducing the diffusion coefficient at the top of the free energy barrier that separates folded and unfolded states. By measuring the transition-path time (\(t_{\text{TP}}\)) from a maximum likelihood analysis of photon trajectories in single molecule FRET experiments, we have recently shown that the Kramers diffusion coefficient for a designed z-helical protein, \(\zeta_{\text{D}}\), is markedly reduced (Chung and Eaton, Nature, 2013). To discover the structural origin of this slow diffusion, we have combined additional single-molecule FRET measurements with all-atom molecular dynamics (MD) calculations. \(\zeta_{\text{D}}\) contains 12 negatively-charged and 10 positively-charged side-chains. Analysis of the transition paths in the MD simulations shows that many non-native salt-bridges form during the folding transition path, suggesting them as the structural origin of the decrease in \(\zeta_{\text{D}}\). To test this idea, we lowered the pH to neutralize the carboxylates and eliminate salt-bridges, which increased the folding rate by about 10-fold and significantly reduced \(t_{\text{TP}}\). Although it was only possible to determine an upper bound for \(t_{\text{TP}}\), even at the highest possible solvent viscosity (15 cp), simulations of photon trajectories suggested that most, if not all, of the increase in folding rate could be accounted for by a decreased \(t_{\text{TP}}\) and an increased Kramers diffusion coefficient. Neutralizing the carboxylates in MD simulations also increases the folding rate and diffusion coefficient and decreases \(t_{\text{TP}}\). These results provide the first quantitative glimpse of the effect of specific intra-molecular interactions on barrier crossing dynamics in protein folding.