Protein Conformation I

215-Pos Board B1
A Tale of Two Dimers: GFP Proteins under Macromolecular Crowding Studied by Small Angle Neutron Scattering
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Proteins usually fulfill their natural functions in the crowded cellular environment where they interact with a dense mixture of other biological macromolecules such as proteins. The concentration is very high, for example, up to several hundreds of mg/ml in cytosol of some E. coli, which is in contrast to the very dilute solutions used in most studies of function and structure. Here, we used Small-Angle Neutron Scattering (SANS) with the contrast matching technique to study the structure and oligomerization state of green fluorescent protein (GFP) in solutions containing various concentrations of the protein human serum albumin (HSA) as a crowding agent. By using HSA, a common protein in blood serum, we were creating a more biologically relevant crowding condition than other crowders usually used such as polyethylene glycol or Ficoll. GFP protein is a relatively stable protein with a beta-barrel structure, usually forming a dimer in dilute solution. By using perdeuterated GFP and hydrogenated HSA, we were able to probe only the GFP in the solutions by contrast matching HSA with an appropriate D2O/H2O buffer mixture. A series of HSA concentrations from 5mg/ml to 200 mg/ml were used. Analysis of the data indicates that GFP undergoes an HSA concentration-dependent transition that alters the way in which GFP oligomerizes in the solution. The dimer present in HSA-free solution remains unaltered by low concentrations of HSA. As the concentration of the crowder increases past 100 mg/ml, GFP adopts an ensemble of states with the transition to another dimeric configuration.

216-Pos Board B2
Evaluating the Consequences of Adsorption-Induced Structural Perturbations of Proteins
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Maintaining the stability of a biologically-active therapeutic agent throughout its lifecycle is a critical parameter in successful pharmaceutical formulation. Interfacial effects such as surface adsorption, which can occur during drug storage and delivery, may culminate in various forms of instabilities: protein unfolding, activity loss and population of non-native, aggregate-prone states. A central paradigm that underpins our understanding of proteins with solid surfaces is that protein adsorption leads to changes in secondary structure. Bound proteins tend to denature and these non-native, adsorbed structures are likely stabilized by loss of alpha-helices and concomitant formation of intermolecular beta-sheets. This research seeks to critically assess the impact this behavior has on protein desorption, where irreversible conformational change may lead to aggregation or other forms of instability. We employ a robust study design to examine the kinetics of adsorption, desorption, and structural transitions of lysozyme on fumed silica nanoparticles as a function of surface coverage. We use circular dichroism (CD) spectroscopy to monitor structural transitions on the surface, in situ. The results show that despite significant adsorption-induced structural loss, surprisingly, adsorption is reversible, and protein desorption is predictable in a coverage-dependent manner. We find evidence of a two-state model, involving exchange between a native-like dissolved and highly perturbed adsorbed state. Since the in situ circular dichroism (CD) derived secondary structure of the adsorbed proteins are essentially unaffected by changes in surface coverage, these results are not consistent with previous claims that surface-induced denaturation is coverage dependent. Inspired by results from homopolymer adsorption experiments, we speculate that more local descriptors, such as the number of amino acids per chain that are physically adsorbed on the surface, likely control the desorption process.

217-Pos Board B3
Using Pressure Perturbation for Studying Conformational and Functional Substates of Proteins
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Regulation of protein function is often linked to a conformational switch triggered by chemical or physical signals. To evaluate such conformational changes and to elucidate the underlying molecular mechanisms of subsequent protein function, experimental identification of conformational substates and characterization of conformational equilibria are mandatory. We applied pressure in combination with spectroscopic and scattering techniques to reveal equilibria between substates of various proteins. Pressure has the advantage that its thermodynamic conjugate is volume, a parameter that is directly related to structure. The conformational dynamics of a signaling protein, Ras, in the absence and presence of a model biomembrane was probed by pressure perturbation. We show that not only the nucleotide binding state, but also the presence of the lipid bilayer membrane has a drastic effect on the conformational dynamics and selection of conformational substates of the protein. These results provide also insights into the influence of pressure on Ras-controlled signaling events in organisms living under extreme environmental conditions as they are met in the deep sea where pressures reach the kbar range. Furthermore, the pressure perturbation approach has been used to disentangle aggregation pathways of amyloidogenic proteins, such as insulin, IAPP, and the prion protein. This approach reveals new insights into the pre-aggregated regime as well as mechanistic details about concurrent aggregation pathways and the differential stability of the protein aggregates formed.

218-Pos Board B4
Effect of Hydrostatic Pressure on Allosteric Couplings in a Tryptophan-Shifted Variant of Phosphofructokinase from Bacillus Stearothermophilus
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Phosphofructokinase from Bacillus stearothermophilus is an allosteric, homotetrameric enzyme containing one tryptophan per subunit. Unlike its homolog from E. coli, the fluorescence of the native tryptophan is unresponsive to ligand binding. This study utilizes a tryptophan-shifted mutant (W179F/F240W) that is functionally similar to wild-type, however a decrease in fluorescence intensity of about 6.5% is associated with substrate fructose 6-phosphate (Fru-6-P), binding and a decrease of approximately 20% is associated with inhibitor, phosphoenol pyruvate (PEP), binding. Dissociation constants equal 1.9 ± 0.3 μM for Fru-6-P and 107 ± 13 μM for PEP. This dissociation constant for PEP agrees with that determined kinetically (128 ± 5 μM). Due to MgATP antagonism, the dissociation constant for Fru-6-P in the absence of MgATP is lower than at saturating MgATP (36 ± 1 μM) as determined by steady-state kinetics. The coupling between PEP and Fru-6-P increases with temperature and results from compensating enthalpy and entropy components. The sign of the coupling free energy is opposite that of the enthalpy and is therefore determined by the larger absolute value of the entropy term. This relationship is opposite that for the allosteric response in EcPFK, where the sign is established by the enthalpy component. Fluorescence intensity of the BsPFK variant increases by about 15% linearly as a function of pressure from 0 to 2 kbar. Pressure induced changes to the fluorescence intensity in this pressure range are completely reversible and the enzyme retains 100% of its activity. This variant is being used to determine how the thermodynamic components of the coupling free energy change as a function of pressure with the goal of better understanding the thermodynamic forces involved in the allosteric effect. Initial results indicate that the coupling in BsPFK is relatively pressure insensitive at 25°C. Funding: NIH-GM33261, NIH-CBI, Welch-A1543.

219-Pos Board B5
How Proteins Unfold
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A lot of research has been performed in the area of protein folding and denaturation. Although different denaturing salts are commonly used to induce the unfolding process, salt-specific effects on protein structure remain controversial despite decades of research. In this work, we combine accurate multi-parameter single-molecule Förster Resonance Energy Transfer (smFRET) experiments, Circular Dichroism (CD) measurements and explicit-water Molecular Dynamics (MD) simulations to investigate salt-specific effects on a short charged α-helix peptide.
Using CD and MD, we explored the structural influence of guanidinium chloride (GdnCl), sodium perchlorate (NaClO4) and potassium chloride (KCl). NaClO4 behaved as a folding aid in CD, contradictory to what is observed in the MD simulations.