The pH and Concentration Dependence of Protein–Protein Interactions, Conformation, and Viscosity in Crowded Protein Solutions

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3364-Pos Board B92
Solvent Dependent Shift of Fluorescence Properties of Fluorescent Proteins
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Fluorescence properties of fluorophores are known to be affected by various environmental factors (e.g. pH, temperature and permittivity of solvent). By utilizing these properties, we think various fluorescent probes been developed and utilized to monitor the intracellular environment of living cells. The properties of fluorescent proteins (FPs) are also affected by the environment but in a more complicated manner due to their unique chromophore structures and the surrounding β-barrel region. In this study, we investigated how the fluorescent properties of FPs are affected by solvents, and explored the possibility of using them as specific probes to characterize the local environment of a target protein. When purified FPs (BFP, ECFP, EGFP, Venus, mCherry and mRFP) were exposed to various alchols (methanol, 1-propanol, 2-propanol, ethanol, 2,2,2-trifluoroethanol (TFE)), fluorescent intensities were significantly affected. The fluorescence intensities of ECFP, EGFP and BFP, but not Venus, mRFP and mCherry, were dramatically decreased in a solution of more than 30% TFE, whereas a lower concentration of alcohol had almost no effect. The circular dichroism (CD) spectrum of ECFP demonstrated that β-sheets are significantly collapsed in 30% TFE. Molecular dynamics (MD) simulations also demonstrated significant changes in the β-barrel structure and in the accessibility of solvents to the chromophore. The fluorescence life-time measurements demonstrated that the life-time of ECFP in 30% TFE was drastically decreased, suggesting that solvent relaxation occurs. These results suggest that hydrophobic solvent such as TFE first attacks the β-barrel structure of FPs, which then allows solvent molecules to access the chromophore. By combining environment-sensitive and -intensive FPs, we successfully developed FP probes to monitor the local environment of target proteins.

3365-Pos Board B93
Influence of Hofmeister Salts on the Structure, Aggregation, and Unfolding of RECA
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RecA is an *Escherichia coli* protein that catalyzes the strand exchange process involved in DNA repair. Previous circular dichroism (CD) studies in our lab have shown that high salt concentrations stabilize RecA in a reverse anionic Hofmeister series. Here we utilize infrared spectroscopy to further investigate how various Hofmeister salts alter RecA structure, aggregation, and solvation. Infrared studies were performed in water and deuterium oxide. Spectroscopic evidence shows that salts alter the water OH stretch and amide I and amide II vibrations arising from the protein backbone. Our data suggests salt specific influences on RecA aggregation, secondary structure, and unfolding. Additional experiments were performed under various solution conditions known to influence ion-protein and possibly water-protein interactions. These data are compared to those obtained in different Hofmeister salts in efforts to identify changes in solvation and RecA structure.

3366-Pos Board B94
Modeling the Effects of Hydrogen-Bond Disrupting Solvents on the Structure of Model Peptide Antibiotics
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The increase in antibiotic resistant infections is a serious threat to public health. Peptide antibiotics, which can perturb the cell membrane, offer one promising solution. We are investigating the structural properties of peptide antibiotic models composed of the hydrophobic dialkylated amino acid Aib (α-aminoisobutyric acid), which imparts a strong 310-helical bias due to steric hindrance at the α-carbon. Previous studies have shown that insertion of adjacent neutral monoalkylated amino acids into an Aib sequence creates a region of the helix that is highly sensitive to disruption of hydrogen bonds by strongly hydrogen-bonding solvents. In particular, the chemical shift of the amide hydrogen at position six of the octameric peptide AA45, which has two adjacent alanines in the center of the helix, is highly sensitive to the concentration of DMSO. Smaller changes in chemical shift are also observed at position seven on the helix. In this study, we have developed a thermodynamic model that describes the solvent-enabled disruption of internal hydrogen bonds within the helix as a function of DMSO concentration. We observe that the unusual concavity of the titration curve for the amide hydrogen at position six can be modeled as the result of DMSO-driven disruption of the hydrogen bond in the presence of competing hydrogen bonds with the surrounding solvent (chloroform). In addition, we compare the results of this model with NMR structures for the corresponding Aib helix and find that the behavior of the amide hydrogen at both positions six and seven is consistent with the formation of a kink in the helix at that position.

3367-Pos Board B95
Spin-Label ESR Study of the Protein Domain Motion and Stability in the Presence of Crowding Effects
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Proteins fold and function in cellular environments that are crowded with other macromolecules. The cellular crowding effects on protein structure and stability is a key issue in molecular and structural biology. Here we report a spin-label ESR study of the T4 lysozyme protein at varying temperatures (280 ~ 338 K) as well as crowding effects (300 ~ 500 g/L of crowding agents, including glycerol, ficoll, and PVP). A doubly labeled T4 lysozyme (T4L-127/155), of which the two sites belong to two different but spatially-closed helical domains, is used as a model for the stability study. The corresponding cw-ESR spectra of the T4 lysozyme exhibit a substantial dipolar broadening at room temperature and confirm that the conformation is not disrupted by the mutations. By extracting the dipolar broadening from the spectra, this study shows a gradual decrease in the broadening with increasing temperature as glycerol or ficoll is used as a molecular crowder. Whereas, a drastic change is observed at temperatures around 330 K, which is about 10 K higher than the melting temperature of T4 lysozyme at a regular buffer, as the crowding agent is changed to PVP. The protein stability is found to increase in the presence of the PVP crowding; namely, as a consequence of the greater excluded volume effects of PVP (as opposed to those of glycerol and ficoll). Besides, an important mechanism underlying the crowding effects observed in this study probably is the glycerol/ficoll-mediated increase in solution viscosity that leads to protein domain motion inhibition.

3368-Pos Board B96
The Effect of Molecular Crowding on the Stability of Peptides
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We use Replica Exchange Statistical Temperature Molecular Dynamics (RESTMD) to investigate the effects of molecular crowding on the structure and thermodynamics of several test peptides. The test peptides are small, biologically relevant peptide that have a dominant secondary structure (either all helical or a β-barrel) and are represented via a coarse-grained computational model where the side chain - side chain interactions are based on a statistical analysis of the Protein Data Bank. The crowding agents are also peptides and are represented by the same coarse-grained model. The RESTMD algorithm naturally calculates the density of states of the system enabling us to analyze the entropic and enthalpic effects of crowding separately. The entropic effects of crowding lead to a stabilization of the test peptide relative to dilute solution. Of particular interest are the enthalpic effects, which can be either stabilizing or destabilizing. The destabilization can be large enough to overcome the entropic stabilization, resulting is a peptide that is destabilized in a crowded environment. We investigate the effect of different crowder hydrophobicities on the enthalpic contribution to the (de)stabilization of the test peptide. We show that there is a crossover temperature below which crowding agents destabilize a peptide and above which the same crowding agents stabilize it and relate this crossover temperature to the hydrophobic nature of the crowders. In addition, we will discuss the effect that crowding has on secondary structure content of the test peptides.

3369-Pos Board B97
The pH and Concentration Dependence of Protein-Protein Interactions, Conformation, and Viscosity in Crowded Protein Solutions
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Proteins are complex macromolecules with dynamic conformations that are pH- and concentration-dependent. These attributes have marked effects on solution thermodynamics and hydrodynamics (i.e. intermolecular interactions, diffusivity, and viscosity). While significant progress has been made in dilute solution hydrodynamics and thermodynamics of proteins, there is a considerable gap in our understanding of how they are altered at high concentrations. In this talk, we attempt to bridge this gap, where we present comprehensive studies of the pH and concentration dependence of conformation and viscosity of a globular protein, Bovine Serum Albumin (BSA), and an IgG1 using small-angle neutron scattering (SANS) and microfluidic rheometry, respectively. We

investigate a wide protein concentration and pH range of 2 mg/mL $\leq$ [Protein] $\leq$ 500 mg/mL and 3.0 $\leq$ pH $\leq$ 11 and find clear evidence for pH and concentration dependencies of conformation from our SANS data. The data are successfully modeled using the random-phase approximation (RPA), where we use a phenomenological model for the form factor that is able to capture contributions from both monomers and clusters in solution. Owing to the separability of the form factor into contributions from monomers and clusters, we are able to obtain structure factors that reflect monomer-monomer, monomer-cluster, and cluster-cluster correlations in solution, which allows us to gain realistic insights into packing and intermolecular interactions at high concentrations. We use these data as inputs into a modification of the model developed by Minton for protein mixtures, which can accurately capture the contributions of monomer and clustered species to model the concentration and pH dependent viscosity of BSA and the IgG1 solution.

3370-Pos Board B98

All-Atom Molecular Dynamic Simulations of Pulmonary Surfactant Protein Sp-B Interacting with Lipid Bilayers

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Pulmonary lung surfactant protein B (SP-B) is a hydrophobic protein with 79 residues, from the saposin superfamily. The saposin superfamily proteins have a conserved pattern of 3 intra-chain disulfide bonds. SP-B associates with phospholipids at the air/water interface of the lungs and plays an essential role in respiration. Its mechanism appears to relate to SP-B's ability to modify the structures of phospholipid bilayers and monolayers. An experimental 3D structure of SP-B has not yet been determined. It is possible to produce homology models of SP-B based on other saposin superfamily proteins such as NK-Lysin, Saposin B, and Saposin C. However, the homology is relatively low and it is not known exactly which segments of SP-B are helical. More importantly, it is not clear if SP-B forms a closed structure, as in NK-lysin, or a more open structure, as in Saposin C. Whether SP-B is open or closed vastly modifies the hydrophobic surface that is exposed and the consequent mechanisms for interacting with the phospholipids. In this work, we start with different homology model structures for SP-B and different initial topologies for SP-B interacting with POPC lipid bilayers. Molecular dynamics simulations are carried out using GROMACS with the all-atom force field OPLS-AA.

3371-Pos Board B99

Membrane Properties Affect Opening Behaviors of the Bacterial Mechanosensitive Channel MscL: Molecular Dynamics Study

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Mechanosensitive channel MscL is constituted of homopentamer of a subunit with two transmembrane inner (TM1) and outer (TM2) helices and its 3D structure of the closed state has been resolved. TM1s line an ion permeable pore and cross each other near cytoplasmic side, forming the most constricted part of the pore called gate. TM2s face lipids and some amino acids in TM2 act as a tension sensor. MscL is activated by sensing membrane tension and the major issue of MscL is to understand the gating mechanism driven by membrane tension. Previous studies propose that MscL embedded in thin membrane can activate with a lower threshold than that embedded in thick membrane. However, it remains unclear how MscL activation (opening of the gate) depends on membrane thickness in detail. In this study, we performed molecular dynamics (MD) simulations of MscL embedded in four types of lipid bilayer with different membrane thickness (DLPC, DMPC, DPPC and DSPC) to get insight into the dependency at an atomic level. As a result, it was shown also in our results that MscL in a thin lipid bilayer DLPC opened more widely than that embedded in a thick lipid bilayer DSPC. Furthermore, it was found that the thinner membrane tended to make the transmembrane helices of MscL tilt more largely. In order to check MscL-lipid interactions, we calculated the interaction energy between MscL and lipids and found that the interaction energy between Phe93 and lipids, located at the cytoplasmic side, was smaller as the membrane was thinner. This seems to be due to a hydrophobic mismatch between MscL and lipids, which affect the tilting of transmembrane helices followed by expanding of the gate during opening.

3372-Pos Board B100

Molecular Dynamics Analysis on the Role of the N-Terminal Domain in Mechano-Gating of E-Coli Mechanosensitive Channel MscL

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The bacterial mechanosensitive channel MscL is constituted of homopentamer of a subunit with two transmembrane inner and outer (TM1, TM2) α-helices, and its 3D structure of the closed state has been resolved. The major issue of MscL is to understand the gating mechanism driven by tension in the membrane. To address this question, molecular dynamics (MD) studies have been performed, however, as they do not include MscL-lipid interactions, it remains unclear which amino acids sense membrane tension and how the sensed force induces channel opening. Thus we performed MD simulations for the opening of MscL embedded in the lipid bilayer. Among amino acids in TM2 facing the bilayer, Phe78 showed exceptionally strong interaction with lipids. Upon membrane stretch, Phe78 was dragged by lipids, leading to an opening of MscL. Thus Phe78 was concluded to be the major tension sensor. Neighboring TM1s cross and interact with each other near the cytoplasmic side through hydrophobic interaction between Leu19-Val23 in one TM1 and Gly22 in the neighboring TM1, forming the most constricted hydrophobic part of the pore called gate. Upon membrane stretch, the helices are dragged by lipids at Phe78 and tilted, accompanied by the outward sliding of the crossings, leading to expanding of the gate. In this study, we newly modeled the Eco-MscL with the N-terminal (S1) helices running parallel to the cytoplasmic membrane instead of forming the tight bundle proposed previously and determined the role of the S1 helices in channel opening. As a result, RMSD of the newly modeled MscL was smaller and the channel opened faster than the previous one, suggesting that the newly modeled S1 helices play a role of stabilizing the channel in closed and accelerating the opening.

3373-Pos Board B101

Continuum Electrostatic Approach for Evaluating Membrane Protein Positions in Membrane

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The spatial orientation of membrane proteins within the lipid environment has been evaluated based on Poisson-Boltzmann (PB) solvent continuum approach. The strategy involves in the calculation of the electrostatic free energy of the protein solvation at various orientations in the lipid bilayer. The solvation free energy is obtained by computing the difference in electrostatic energies of the protein immerged in water and lipid environments treated as implicit solvent models. In the evaluation carried out for a number of ion channel membrane proteins and soluble proteins, the results showed a distinguish pattern of the solvation energy landscapes between transmembrane proteins and soluble proteins. The study also showed that the electrostatic contribution for transferring proteins from the high-dielectric aqueous phase to a lower-dielectric membrane environment is always unfavourable. Furthermore, detailed energy analysis of five types of membrane proteins provides two distinct patterns of the solvation energy that are useful for discriminating between transmembrane and non-transmembrane proteins. Finally, the evaluation of the position of membrane proteins available from Orientations of Proteins in Membranes (OPM) database has been achieved for a total of 1060 proteins. In the case of transmembrane proteins, most of the tested proteins are in good agreement with those of the OPM database. The results for non-transmembrane α-helical and peripheral/monotopic proteins appear to contradict that there is no apparent minimum. It is expected that the present approach is of great assistance for constructing protein-lipid structure systems suitable for experimental and computational studies.

3374-Pos Board B102

Protein-Protein and Protein-Membrane Interactions Regarding the Erbb2/Trastuzumab-Fab Complexes. A Coarse-Grained Molecular Dynamics Description

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ErbB2 is a member of epidermal growth factor receptor (EGFR) family and is overexpressed in many cancers. Specifically, Trastuzumab, which is a monoclonal antibody, is used against ErbB2, but its action mechanism is still unknown. ErbB2 can exist as both monomers and Homodimers, suggesting that Trastuzumab mechanims may be subtle. On the other hand, the membrane plays a role in the action mechanism of Trastuzumab but generates difficulties for structural studies. Coarse-Grained Molecular Dynamics has been used to study the influence of the Trastuzumab on the protein-protein and protein-membrane interactions of the full ErbB2 receptor. Our simulations start from conformations which both extracelullar and intracelullar domains are extended. The results show in both monomers and homodimers systems a folded conformation on the membrane: several experimental results, mainly obtained on ErbB1 support them. The protein-protein interaction on transmembrane and juxtamembrane domains are disrupted on the dimer and disordered on the monomer by the Trastuzumab effect, therefore, the dimerization-driven activation are unfavourable. We present a detailed description