1660-Pos Board B504
Reverse Micelles as a Tool to Probe the Synergy Between Confinement and Osmolytes with Respect to Protein Hydration Properties
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There is a rapidly growing awareness not only that dynamics are essential for protein function but that functionally important protein motions are tightly coupled to hydration shell dynamics. The dynamics of hydration waters are in turn a function of both confinement conditions and the presence of surrounding co-solutes/osmolytes. Mimicking the combination of confinement and osmolyte levels for proteins in the *in vivo* cell environment is challenging. Here we present results obtained through the use of reverse micelles as a unique medium chosen to represent this crowded environment of proteins. The effects on the hydration properties of proteins encapsulated within reverse micelles are presented, and compared with confinement within Sol-gels. The addition of biologically relevant osmolytes to the reverse micelles and Sol-gels is used to expose potential synergy between confinement and osmolyte with respect to impact on hydration.

1661-Pos Board B505
Extracellular pH and Regulation of Integrin Activation
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It is well established that chemomechanical properties of the extracellular matrix (ECM) can have a profound effect on cell processes such as adhesion, migration, and differentiation. To understand and modulate such complex processes, it is crucial to have a detailed understanding of the feedback between a cell and the adjacent microenvironment. This is particularly important in the tumor and wound environments, where the ECM often exhibits altered characteristics such as acidic extracellular pH. This microenvironmental property could significantly alter the interactions between cell surface integrin receptors and ECM ligands, which are critical to downstream cell behaviors such as adhesion, migration, and signaling. Here, we use molecular dynamics simulations to examine the role of acidic extracellular pH in regulating integrin activation. The simulation system is the headpiece domains of integrin $\alpha v\beta 3$ in complex with a cyclic RGD peptide. Multi Conformation Continuum Electrostatics was used to predict pKa values for all titratable residues in the system, and results were used to select residues for protonation in order to represent an effective acidic extracellular pH. Molecular dynamics simulations at acidic and physiological pH were compared to examine the effect of pH on $\alpha v\beta 3$-RGD conformational states. Our results suggest that acidic pH promotes integrin headpiece opening, which is one of the steps in integrin activation, and we propose a possible mechanism for this effect. This finding is consistent with experimental data from the literature, and has important implications for cell adhesion and migration in cancer and wound healing.

1662-Pos Board B506
A Discrete Generalized Model for Dynamic Turnover in Molecular Protein Complexes
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Recent new evidence indicates that components of functional molecular complexes in living cells may turnover relatively rapidly over timescales of seconds. The bacterial flagellar motor is one such complex. It is a highly intricate molecular machine formed by several key proteins, and is ultimately responsible for the generation of filament torque enabling bacteria to swim. Here we have developed a robust Monte Carlo simulation to account for observed molecular turnover in experimental observations using real-time high-contrast single-molecule in vivo fluorescence microscopy on fluorescent genetic fusions of a protein FliM which is implicated in a switching complex for the motor. From this we obtain reliable estimates for the range of dwell times of the FliM subunit at the molecular switching complex. We show how our methodology can be extended into general cases for reaction-diffusion kinetics of molecular complexes in living cells.

1663-Pos Board B507
The Effect of Complex Solvents on the Structure and Dynamics of Protein Solutions
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Trehalose-water binary mixtures have been implicated in the anhydrobiosys of tradigrades. Although, there exists extensive experimental data to explain the mechanism of protection offered by trehalose, a study elucidating the microscopic details is missing. We have employed molecular dynamics simulations for this purpose. Studies were conducted with a protein in 0, 10, 20, 30 and 100% trehalose by weight solutions. We observe no appreciable change in the protein structure in water-containing solutions but a shrinkage is seen in pure trehalose. This has been correlated to bending of angles and dihedral angles in the protein in a way that compresses the protein evenly. This manifests in the different dynamics of protein observed in water-containing and pure trehalose solutions. The reason behind similar dynamics in water-containing solutions is preferential hydration of protein. The dynamics have further been found to be coupled to the behavior of hydrogen bonds.

1664-Pos Board B508
Exploring Protein Conformational Change Using The Double Well Network Model
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Conformational changes in proteins are often crucial to their function. However understanding the mechanisms underlying these movements remains a significant challenge. Because of the long time-scales on which conformational change occurs, coarse graining methods are needed to provide a realistic approximation to detailed atomistic interactions while reducing the computational cost. Elastic network models (ENMs),which approximate protein structure as a network of alpha carbons connected by harmonic potentials, predict the direction of large-scale motion in many systems. However, since ENMs stabilize only one conformation, effective modeling of the transition pathways between two local minima is precluded. Rather than using uniform spring constants as in conventional ENM, we systematically obtain spring constants for each of two stable conformations using the heteroENM approach recently reported by our group. The potentials describing pairwise interactions from these two states are then individually mixed using a combination of harmonic, double well, and Morse potentials to allow for transitions between minima. Langevin dynamics simulations using the new methodology display multiple energy minima and intermediates between states, suggesting that this approach can realistically model a frustrated energy landscape. We evaluate equilibrium dynamics trajectories and minimum energy paths for the transition between the two local minima obtained using this approach for several model proteins.

Membrane Protein Function I

1665-Pos Board B509
Physical Mechanism of SERCA2a Inhibition by Peroxynitrite
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In the heart, age-based nitration of the sarco(endo)plasmic reticulum Ca-ATPase (SERCA2a), specifically at tyrosine residues Y294, Y295 and Y753 inhibits Ca^{2+} transport activity. However, the physical mechanism by which tyrosine nitration inhibits SERCA2a activity is not understood. Likewise, the effects of nitration on SERCA2a regulation by phospholamban (PLB) are not known. Therefore, we are combining kinetics assays with spectroscopic experiments to determine the effects of nitration on specific steps in the SERCA2a enzyme cycle, as affected by PLB. For these studies, we are using expressed SERCA2a either alone or coexpressed with PLB in High Five insect cell microsomes, and peroxynitrite ($ONOO^-$) as the nitrating agent. As observed previously with native cardiac sarcoplasmic reticulum (SR) vesicles, treatment of the expressed samples with increasing $ONOO^-$ inhibited SERCA2a activity. $ONOO^-$ inhibition of SERCA2a was more potent in the presence of PLB than in its absence, but $ONOO^-$ did not affect the $[Ca^{2+}]$-dependence of SERCA2a activity either in the absence or presence of PLB. Conventional and saturation transfer EPR studies of maleimide spin-labeled SERCA2a were used to assess the effect of $ONOO^-$ on SERCA2a protein-protein interactions, as affected by PLB. $ONOO^-$ treatment had no effect on SERCA2a rotational mobility either in the absence or presence of PLB. Our data indicate that SERCA2a nitration does not affect the apparent Ca^{2+} affinity of the enzyme, suggesting nitration does not inhibit the enzyme by affecting Ca^{2+} binding and activation for ATP-dependent phosphorylation. In contrast, nitration may inhibit enzyme turnover by affecting Ca^{2+} release and enzyme dephosphorylation steps of the enzyme cycle. Specific kinetic and fluorescence experiments will test this proposal.

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Structure-Function Studies of the XIP Regions of the Na^+-Ca^{2+} Exchangers NCX1 and NCX2
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The Na^+-Ca^{2+} exchanger (NCX) is an integral protein essential for cellular Ca^{2+} homeostasis. Previous structure-function studies have identified regulatory