1660-Pos Board B504
Reverse Micelles as a Tool to Probe the Synergy Between Confinement and Osmolytes with Respect to Protein Hydration Properties
Camille J. Roche, David Dantsker, Feng Guo, Joel M. Friedman.
Albert Einstein College of Medicine, Bronx, NY, USA.
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
Ranjani Krishnan, Douglas A. Lauffenburger, Krystyn J. Van Vliet.
Massachusetts Institute of Technology, Cambridge, MA, USA.
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
Gabriel Rosser, Nicolas Delalez, George H. Wadhams, Judith P. Armitage, Mark C. Leake.
University of Oxford, Oxford, United Kingdom.
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
Pavan K. Ghatty.
University of Akron, Akron, OH, USA.
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
Marissa G. Saunders, Gregory A. Voth.
University of Utah, SLC, UT, USA.
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
Vidhya Sivakumaran[1], Lesly De Arras[2], Johnathan Bermudez[2], Diana J. Bigelow[3], James E. Mahaney[2,1].
[1]Virginia Polytechnic Institute and State University, Blacksburg, VA, USA, [2]Virginia College of Osteopathic Medicine, Blacksburg, VA, USA, [3]Pacific Northwest National Laboratory, Richlands, WA, USA.
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.

1666-Pos Board B510
Structure-Function Studies of the XIP Regions of the Na^+-Ca^{2+} Exchangers NCX1 and NCX2
Danielle L. de Moissac, Hoa D. Le, Kristy D. Ranson, Daron Baxter, Vladimir Yurkov, Alexander Omelchenko, Mark Hnatowich, Larry V. Hryshko.
University of Manitoba, Winnipeg, MB, Canada.
The Na^+-Ca^{2+} exchanger (NCX) is an integral protein essential for cellular Ca^{2+} homeostasis. Previous structure-function studies have identified regulatory

regions within the exchanger's cytoplasmic domain, of which the XIP region shown to be involved in the rate and extent of Na^+-dependent inactivation (I_1). Although the XIP region is highly conserved amongst NCX isoforms, distinct I_1 phenotypes exist. To better define the role of this region, we constructed chimaeric NCX1:NCX2.1 proteins with their respective XIP regions interchanged as well as amino acid substitutions within the XIP region to examine the more subtle aspects of phenotypic differences between NCX1.3 and NCX2.1. Mutant exchangers were expressed in *Xenopus* oocytes, and outward Na^+-Ca^{2+} exchange activity was assessed using the giant, excised patch clamp technique. Substitution of the XIP region of NCX1.4 with the corresponding region from NCX2.1 caused an apparent loss of I_1 whereas a reduction in the extent of inactivation and a 15-fold increase in the rate of recovery from I_1 were observed in the NCX1.3 - XIP2 chimaera. Similarly, substitution of charged amino acids within the XIP region in NCX1.3 caused a slight increase in the rate of recovery, equivalent to that observed for NCX2.1. Thus, non-conserved residues in the XIP region may be essential in maintaining the structural stability of the Na^+-dependent inactive state of NCX1. Furthermore, the XIP region must interact with other regulatory domains of the protein, such as the mutually exclusive exon, thereby contributing to the structure-function relationship as well as the regulatory phenotype of each Na^+-Ca^{2+} exchanger variant and isoform.

1667-Pos Board B511

The Role of Phospholamban Cysteines in the Activation of the Cardiac Sarcoplasmic Reticulum Calcium Pump by Nitroxyl

Chevon Thorpe[1], Lesly De Arras[2], John P. Toscano[3], Gizem Keceli[3], Christopher Pavlos[3], Nazareno Paolocci[4], Jeffrey P. Froehlich[4], James E. Mahaney[2,1].
[1]Virginia Polytechnic Institute and State University, Blacksburg, VA, USA, [2]Virginia College of Osteopathic Medicine, Blacksburg, VA, USA, [3]Johns Hopkins University, Baltimore, MD, USA, [4]Johns Hopkins Medical Institutions, Baltimore, MD, USA.

Phospholamban (PLN) is an integral membrane protein that regulates the Ca^{2+} pump (SERCA2a) in cardiac sarcoplasmic reticulum (CSR). Phosphorylation of PLN in response to β-adrenergic stimulation enhances cardiac inotropy by increasing CSR Ca^{2+} uptake. Nitroxyl (HNO), a new candidate drug therapy for congestive heart failure, improves overall cardiovascular function by increasing Ca^{2+} release and re-uptake in CSR through a direct interaction with RyR2 and SERCA2a, respectively. Using insect cell ER microsomes expressing SERCA2a +/− PLN (WT and Cys → Ala mutant) we have shown that activation of SERCA2a by HNO is PLN-dependent and entails covalent modification of PLN cysteines. Although HNO stimulates SERCA2a activity by uncoupling PLN from SERCA2a, the role of the cysteine residues in the activation mechanism is not completely understood. We propose that HNO, a thiol oxidant, modifies one or more of the three PLN cysteine residues (C36, C41, C46), affecting the regulatory potency of PLN toward SERCA2a. Examples include intra-molecular disulfide cross-links within single PLN molecules or inter-molecular disulfide cross-links between PLN molecules or PLN and SERCA2a. To test this hypothesis, we have constructed a series of PLN mutants containing single, double and triple cysteine substitutions (alanine replacing cysteine). Each of these mutant PLNs will be co-expressed with SERCA2a in insect cells and cell microsomes will be treated with Angeli's salt (an HNO donor) to determine which cysteine residue(s) are essential for activation monitored by enzyme assay and fluorescence spectroscopy of SERCA2a. The results show that intermolecular PLN disulfides play a minor role in activation by HNO. Studies with the Cys → Ala mutations will be useful in determining which cysteine pairs in PLN contribute to intramolecular disulfide cross-links leading to the relief of PLN inhibition and SERCA2a activation.

1668-Pos Board B512

Site Directed Mutagenesis of Human GLTP: Role of Tryptophan Residues

Ravi Kanth Kamlekar[1], Yongguang Gao[1], Helen Pike[1], Roopa Kenoth[1], Franklyn G. Prendergast[2], Sergei Yu. Venyaminov[2], Rhoderick E. Brown[1].
[1]University of Minnesota, Austin, MN, USA, [2]Mayo Clinic College of Medicine, Rochester, MN, USA.

Glycolipid transfer proteins (GLTPs) are small, soluble, ubiquitously expressed proteins that selectively accelerate the intermembrane transfer of glycolipids in vitro. Mammalian GLTPs (209 aa) are intrinsically fluorescent by virtue of having three tryptophans and ten tyrosines. The crystal structure of human GLTP (glycolipid-bound form) reveals the importance of W96 in the glycolipid liganding site where its aromatic indole ring acts as a stacking platform that facilitates hydrogen bonding of the initial ceramide-linked sugar with Asp48, Asn52, and Lys55. To gain insights into W96 functionality and to define the role of the other two Trp residues (i.e., W85 & W142), three GLTP Trp mutants (W96Y, W85Y-W96F, W96F-W142Y) were constructed by QuikChange™ site-directed mutagenesis, overexpressed (pET-30) in E. coli, purified by metal ion affinity and FPLC size exclusion chromatography, and characterized by glycolipid transfer activity measurements and by fluorescence and CD spectroscopy. Compared to wtGLTP, the single Trp mutant, W96Y, retained 65% activity; whereas the double Trp mutants, W85Y-W96F & W96F-W142Y, retained 22% and 110% activities. Quenching with acrylamide and potassium iodide at physiological ionic strength resulted in linear Stern-Volmer plots, suggesting accessibility of emitting Trp residues to soluble quenchers and consistent with wtGLTP native folding. However, CD measurements revealed significant differences in the secondary structure of W85Y-W96F-GLTP compared to wtGLTP; whereas W96F-W142Y-GLTP and W96Y-GLTP retained native secondary structure. We conclude that the negative consequences of conservative mutation of Trp 85 suggest a crucial role in proper folding of GLTP; whereas, the tolerance of Trp96 and Trp142 for conservative, but not radical, mutation is consistent with specialized roles in GLTP function, i.e. glycolipid liganding and membrane interaction. [Support: NIH/NIGMS GM45928 & GM34847, NIH/NCI CA121493, The Hormel & Mayo Foundations].

1669-Pos Board B513

Effects Of Gap Junction Blockers On The $P2x_7$ Receptor

Anael V.P. Alberto, Robson Xavier Faria, Maira M. Fróes, Luiz Anastacio Alves.
Oswaldo Cruz Institute, Rio de Janeiro, Brazil.

Peritoneal macrophages express the $P2X_7$ receptor, which opens a pore in the membrane after long exposure with ATP, allowing passage of molecules up to 900 Daltons. It has been argued that activation of $P2X_7$ receptor leads to opening of an independent pore entity, not structurally related to the $P2X_7$ receptor. Based on results with connexin knock outs and pharmacological manipulation with known gap junction blockers, some groups have included connexins and pannexins, the gap junction-forming proteins in vertebrates, as reliable candidates to provide for the large permeation pores associated with $P2X_7$ activation. In the present study we performed electrophysiological (whole cell patch clamping recordings) and permeabilization assays (optical analysis and FACS analysis) in which both efficacy and specificity of some gap junction blockers were tested at conditions of putative $P2X_7R$ activation by ATP. ATP generated a current in a nA levels that was blocked by well know P2X blockers as BBG, KN-62 and oxidized ATP, in contrast the junction blockers did not interfere with these effect. More than that, the up take assays showed similar results to the patch clamp experiments, none of the junction blockers was able to block the up take of ethidium bromide or propidium iodide. Our results indicate that well-known gap junction pharmacological blockers do not interfere with current generation or dye uptake after activation of $P2X_7$ receptor. Taken together, our data strongly suggest that the high permeability pore evident at prolonged $P2X_7$ activation does not correspond to connexin or pannexin hemichannels in peritoneal macrophages.

1670-Pos Board B514

Qm Simulation Of Binding Site In P-type Atpases

Per J. Greisen.
Institute of Physics, Copenhagen, Denmark.

P-type ATPases are enzymes that stablish cation gradients across biologcal membranes where ions are transported using the energy from hydrolysis of ATP. The Na,K-ATPase and SERCA1a transport 3 Na+/ 2 K+ and Ca2+ , respectively, against a concentration gradient for each ATP molecule hydrolyzed. The two enzymes have very high sequence homology and show structural similarities in the binding site of the ions. This raises questions concerning the selectivity of the different structural basis and how each protein select its specificion. The present study aims at characterising this selectivety.

In order to understand the specificity of the enzymes, we investigate the coordination site in the two crystal structure of SERCA1a(PDB ID: 1SU4) and Na+/K+ ATPase(PDB ID: 3B8) by constructing models of the active site. The models are constructed using analogs of the amino acids from the first and second solvation shell. We optimise the geometry of the models by constraining the C-alpha atoms using semi-empirical methods such as PM6 and AM1. The energies and geometries are investigated further using ab initio, restricted Hartree Fock, and density functional theory with the B3LYP functional using the basis set 6-31G(d,p)*. We substitute Ca2 ± ions into the binding pocket of the Na+/K+ ATPase model to investigate how the specificity is related to the coordination environment. Furthermore, the protonation state of side chains coordinating the ions are investigated in order to estimate the influence of pH changes on the binding of ions in the two pumps.

1671-Pos Board B515

Substrate Specificity And Peptide Bond Selectivity In Rhomboid Intramembrane Proteases

Jose L. Chavez, Imane Djemil, Davide Provasi, Celia M. Torres, Marta Filizola, Iban Ubarretxena.
Mount Sinai School of Medicine, New York, NY, USA.