adsorption of wild-typed HD5 (WT) and T7E21R on a bacterial membrane were conducted. A bacterial membrane models with O-antigen of lipopolysaccharides (LPS) mimicking *E. coli* membrane was used. Atomistic molecular simulations were performed to obtain an insight into the adsorption of these peptides on the bacterial membrane. Our data highlight the different dimeric conformation between WT and T7E21R. It is found that the parallel β-strands of T7E21R is formed instead of antiparallel in HD5. This conformation of change results in different degrees of binding affinities. Key interactions are also extracted here. However, it is shown that WT can penetrate deeper into the O-antigen than T7E21R. The uncharged residues such as Thr, Ser, Gly, and Tyr are dominated in contacts with O-antigen of LPS as much as the charged residues. An insight obtained here can serve as a guideline for further improvement of defensin-derived antibiotic design.

1404-Pos

Brave New Surfactant World Revisited by Lipases: Activation and Unfolding in SDS

Mohamed Shehata[1], Aise Unlu[2], Emel Timucin[3].
[1]Medical Biotechnoloy, Acibadem University, Istanbul, Turkey, [2]Gebze Technical University, Gebze, Turkey, [3]Department of Biostatistics and Medical Informatics, Acibadem University, Istanbul, Turkey.

Lipases are activated at the water-lipid interface in a process known as interfacial activation which involves a large conformational change of the lid domain. Surfactants, as they mimic lipase substrates, can be utilized in crystallization studies to induce lipase activation. Nevertheless, lipase-surfactant interactions and how these interactions induce interfacial activation are unclear. To this end, we have performed μs-scale molecular dynamic (MD) simulations to delineate the interactions between lipase and sodium dodecyl sulfate (SDS) which is arguably the most widely used surfactant in wet-lab. In our study, two distinct lipase conformers, open and closed, were analyzed at 2 distinct temperatures Analysis of the trajectories showed that at ambient temperature SDS micelles were immediately formed after 1-2 ns and largely interacted with the lid domain. These interactions ultimately triggered a conformational change in the closed lid, consistent with the macroscopic observation of interfacial activation by surfactants. Other than these lid movements, neither of the lipase conformers unfolded in 12% (w/v) SDS. On the other hand, at 373 K, SDS induced unfolding of both conformations. Notably, unfolding was initiated from the same region of two conformations which corresponds to the zinc binding domain. Overall our study provides insights into *in silico* lipase activation by an ionic surfactant for the first time, reflecting the potential of SDS to capture the active form of lipases.

1405-Pos

Single Particle Analysis Reveals the Organization of the Membrane Remodeling Protein Caveolin-1 within Disc-Shaped Complexes

Bing Han[1,2], Jason C. Porta[3], Jessica L. Hanks[3], Yelena Peskova[1,2], Elad Binshtein[4], Kelly A. Dryden[2], Derek P. Claxton[5], Hassane S. Mchaourab[5], Erkan Karakas[5], Melanie D. Ohi[3], Anne K. Kenworthy[1,2].
[1]Center for Membrane and Cell Physiology, University of Virginia, Charlottesville, VA, USA, [2]Department of Molecular Physiology and Biological Physics, University of Virginia School of Medicine, Charlottesville, VA, USA, [3]Life Sciences Institute, University of Michigan, Ann Arbor, MI, USA, [4]Center for Structural Biology, Vanderbilt University, Nashville, TN, USA, [5]Dept Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, USA.

Highly stable oligomeric complexes of the monotopic membrane protein caveolin serve as fundamental building blocks of caveolae. Current evidence suggests these complexes are disc shaped, but the details of their structural organization and how they assemble are poorly understood. Here, we address these questions using single particle electron microscopy (EM) of negatively-stained recombinant 8S complexes of human caveolin-1 (CAV1). We show that 8S complexes are toroidal structures ∼15 nm in diameter that consist of an outer ring composed of distinct globular domains, an inner ring, and a central protruding stalk. Based on the stain distribution along the outer ring, each 8S complex appears to contain approximately 10 CAV1 monomers. To determine the orientation of monomers within the complex, we labeled the N- and C-terminus with EM-recognizable tags. We found that the C-terminus of CAV1 monomers are positioned in the center of the complex whereas the N-terminus is orientated toward the edge of the toroidal structure. Next, we performed sequence truncation analysis to investigate the role of the N-and C-terminus in disc formation. These studies revealed both the N- and C-termini are required to maintain the size and disc shape of 8S complexes. Finally, we used negative staining to visualize 8S complexes in heterologous caveolae (h-caveolae) induced by the expression of CAV1 in *E. coli*. We found that h-caveolae do in fact appear to be composed of multiple copies of 8S complexes and the complexes can partially retain an arc-like organization when h-caveolae were disassembled by detergent. Taken together, these findings provide critical insights into the structural features of 8S complexes and allow us to propose a new model for how these highly stable membrane-embedded complexes are generated.

1406-Pos

Mechanism of Cholesterol Translocation in Mycobacteria

Tian Chen, Fei Peng, Xiyao Cheng, Yongqi Huang, Zhengding Su.
Protein Engineering and Biopharmaceutical Sciences Laboratory, Hubei University of Technology, Wuhan, China.

Oxidization of cholesterol by cholesterol oxidase (ChOx) is thought to the initiation step in cholesterol translocation and degradation. However, the mechanism of cholesterol translocation in mycobacteria have not been thoroughly investigated. Previously, we analyzed mycobacteria phytosterol metabolism pathway in Mycobacterium HGMS2 strain we identified three enzymes, i.e., ChoM1, ChoM2 and HsD, which could oxidized cholesterol oxidization. In this work, we evaluated the activities of the three enzymes in vitro. The three enzymes exhibited different abilities to oxidize cholesterol as ranking by ChoM1 ≫ HsD > ChoM2. Furthermore, we employed gene knockout approach to verify the functional role of the three enzymes. Unexpectedly, a triple mutant that deleted these three genes still enabled to oxidized cholesterol as the wild-type HGMS2 strain, implying that the mycobacterial stain contained unexplored enzymes for cholesterol oxidization. As such, we used structural biology, bioinformatic and enzymatic strategies to search for new cholesterol oxidases. The results and discussion on mechanism of cholesterol translocation in mycobacteria will be presented in this report.

1407-Pos

Investigation of Cyclic AMP Binding Interactions with Isolated Cyclic Nucleotide Binding Domain of HCN1 Channel using Atomistic Molecular Dynamics Simulations at Microsecond Timescale

Matthew G. Brownd, Adithya Polasa, Mahmoud Moradi.
Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR, USA.

Hyperpolarization-activated cyclic nucleotide-gated channel 1 (HCN1) is a homotetrameric, voltage-gated potassium/sodium channel whose activity is modulated by cyclic AMP. The binding of cAMP to an intracellular cyclic nucleotide binding domain (CNBD) promotes an increase in channel activity through a depolarizing shift in the voltage threshold necessary for channel activation, as well as kinetically favoring the active channel conformation, leading to improved conductance. While the precise mechanisms of this change are still being investigated, the shift in the CNDB region, which is induced by the binding of cAMP, is able to be propagated to the remainder of the channel through an adjacent C-linker, causing an alteration in the channel that leads to increased conductance. Although static structures of both the apo and holo channel states have been solved and compared, there is still a need to understand the precise binding interactions which occur between the cyclic nucleotide and the binding pocket, as well as the dynamic changes to the local protein structure that might lead to the aforementioned changes in activity. Using atomistic molecular dynamics simulations, we have simulated both the apo and holo forms of the isolated CNBD region at a microsecond timescale, and have performed a variety of analyses to relate the two conformations and investigate the interactions of the ligand with the binding pocket. We have also used enhanced sampling techniques to calculate the binding free energy of cAMP. This study provides a computational framework for the study of HCN1-ligand interactions.

1408-Pos

EPR Studies of KCNE3 in Proteoliposomes using Electron Paramagnetic Resonance (EPR) Spectroscopy

Indra D. Sahu[1,2], Alberto Perez[1], Samuel Haralu[1], Matthew Scheyer[1], Gary A. Lorigan[2].
[1]Natural Science Division, Campbellsville University, Campbellsville, KY, USA, [2]Dept Chemistry and Biochemistry, Miami Univ, Oxford, OH, USA.

KCNE3 (E3 or MiRP2) is a single transmembrane protein of the KCNE family that modulates the function and trafficking of several voltage gated potassium channels, including KCNQ1 (Q1), and KCNQ4. Voltage gated K^+ channels are critical for the function of cardiac, nervous and auditory systems and represent promising targets for various therapeutic agents. Recently, a solution NMR structure of KCNE3 in isotropic DHPC/DMPG bicelles by the Sanders group indicated a curved α-helical transmembrane domain (TMD) with bending being most pronounced near the C-terminal end of the helical TMD, and an extracellular surface associated amphipathic helix in N-terminal, and a short juxtamembrane helix followed by a disordered region in C-terminus. The structural topology and dynamics of KCNE3 are not fully understood in its native like