ion-releasing state and the modeled ion-binding state, confirming that it is more difficult to release the substrate in the presence of the Na^+ ion in its binding site.
Furthermore, local transition of the Na^+ binding site from an ion-releasing state to an ion-binding state in our constrained simulation induced significantly global conformational change in the protein, specifically, partial opening of the periplasmic side and closing of the cytoplasmic side, thus, capturing for the first time large-scale conformational changes between the inward-facing and outward-facing states in the transport cycle of this secondary transporter.

3579-Pos
Modeling of the Inward-Facing State of LeuT and Dynamics of the Outward-To-Inward Transition
Saher A. Shaikh, Emad Tajkhorshid.
University of Illinois Urbana Champaign, Urbana, IL, USA.
Leucine transporter (LeuT) is a bacterial aminoacid transporter belonging to the neurotransmitter:sodium symporter family. An 'alternating-access model'is proposed for LeuT function, wherein the transporter alternates between outward-facing and inward-facing states. While multiple crystal structures of LeuT bound with substrate and inhibitors have been reported, only the outward-facing state is known. The inward facing state, and the dynamics of the outward-to-inward transition remain undescribed.
Several transporters from different families report a structural fold similar to the basic 'LeuT fold'indicating the significance of this fold in transporter function. These structures include some in the inward-facing state. Exploiting this information, we have generated a model for the inward-facing state of LeuT. Since the inward-facing structure employed for modeling had very low sequence similarity, and moderate structural similarity to LeuT, a combination of several techniques was required for model generation. A detailed modeling approach was adopted, including sequence- and structure-based approaches, combined with molecular dynamics techniques, such as targeted MD. The final model retains the secondary structural features of LeuT and the substrate/ion binding sites, while adopting an inward-facing state.
We have also employed this model to study the dynamics of outward-to-inward transition of LeuT. The behavior of bound substrate and ions during this transition was recorded and shows interesting features relevant to the transport mechanism. Water permeation was monitored with the progress in transition. The main structural elements of LeuT involved in this transition are described. This study thus presents a model of the inward-facing state of LeuT and a description of a possibly general transport mechanism in transporters adopting the LeuT fold.

3580-Pos
Molecular Determinants of the Stoichiometry of Transport in GlpT
Giray Enkavi, Emad Tajkhorshid.
Department of Biochemistry, Center for Biophysics and Computational Biology, Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, IL, USA.
The glycerol-3-phosphate/phosphate antiporter GlpT belongs to the Major facilitator superfamily (MFS), the largest group of secondary active membrane transporters. Stoichiometry of transport in organophosphate/phosphate antiporters has been a long-standing question. Experimental studies performed on hexose-6-phosphate transporter (UhpT), a close homolog of GlpT, suggested that the exchange stoichiometry might be regulated by pH in these antiporters. The crystal structure of GlpT, although devoid of a bound substrate, seemed to contradict with ''variable stoichiometry'' hypothesis, featuring a ''single'' putative binding site with two arginines (R45 and R269). The ''putative'' binding site also involves a histidine residue (H165) whose titration state have been suggested to play a key role in transport. We have previously identified one of the arginines (R45) as the binding site residue, but our simulations showed no indication of binding to the other arginine (R269). In order to examine the capacity of GlpT for binding two substrates simultaneously, and to investigate the molecular basis of the ''variable stoichiometry'' model, we have performed an exhaustive set of MD simulations in which binding of a second substrate to GlpT was simulated. The simulations, performed at different titration states of the substrates (Pi & G3P) and of H165, indicate that the GlpT binding site can, indeed, accommodate two substrates simultaneously upon protonation of H165 and R269 is the preferential binding residue for the second substrate. Moreover, combining of the trajectories of MD simulations with pKa calculations based on continuum electrostatics, we also analyzed the substrate-induced changes of the titration state in the binding site. Our results further indicate that H165 might act as a ''pH sensor / stoichiometric switch'' in addition to coordinating the substrate. Our findings might represent a general mechanism for transporters with ''variable stoichiometry''.

3581-Pos
Conformational Changes in the ApcT Amino Acid Transporter: Monte Carlo Normal Mode Following
Gennady V. Miloshevsky[1,2], Ahmed Hassanein[1], **Peter C. Jordan**[2].
[1]Purdue University, West Lafayette, IN, USA, [2]Brandeis University, Waltham, MA, USA.
Amino acid/polyamine/organocation (APC) transporters belong to a large family (~250) of secondary transport proteins that catalyze bilayer translocation of a broad range of substrates. Monte Carlo Normal Mode Following [Miloshevsky & Jordan, *Structure* **14**, 1241 (2006); **15**, 1654 (2007)] is used to explore possible conformational change mechanisms in a proton-dependent APC transporter, ApcT, a bacterial homologue from *Methanocaldococcus jannaschii* [Shaffer *et al.*, *Science* **325**, 1010 (2009)]. ApcT was captured in an inward-facing apo state. Gating is initiated by global counter-torsions of the intracellular and extracellular domains of ApcT around the pore axis, with the extracellular half rotating clockwise and the intracellular half anticlockwise, and vice versa. The domain motions are highly concerted and cooperative. The stationary plane relative to which counter-torsion occurs passes through the center of ApcT parallel to the membrane. Intracellularly, overall rotation of the peripheral helices (TM7, TM5, TM8, TM3, TM4, TM9, TM10, TM11 and TM12) reconfigures TM6a significantly and TM1b slightly. These helices alternately approach and separate from the opposed peripheral TM10 and TM11, affecting the intracellular mouth. TM6a and TM1b move toward the protein's perimeter and become buried inside the protein. Loops and small helices on the intracellular surface of ApcT undergo large-scale rotations. Extracellular motion is similar. Overall peripheral helix rotation affects TM1a significantly and TM6b slightly, displacing them from and collapsing them onto TM8 and TM5. TM8 and TM5 alternately undergo large-scale bending near their midpoints. Normal mode following along the lowest-frequency eigenvector(s) reveals details of the gating transition in the ApcT transporter.

3582-Pos
The Allosteric Role of Ion Binding in the Functional Mechanisms of Transporters With LeuT Fold
Lei Shi, Harel Weinstein.
Weill Cornell Medical College, New York, NY, USA.
Recent crystallographic studies revealed that five transporter families without much sequence similarities among them have similar structure folds to LeuT, a bacterial neurotransmitter:sodium symporter (NSS). The LeuT fold is characterized by an internal two-fold structural pseudosymmetry. Interestingly, the transport cycle of at least some members of each of these families is dependent on a sodium gradient across the membrane. Remarkably, the role of sodium is mimicked by a proton in others. We report our computational findings focusing on the LeuT conformations with various combinations of bound substrate and ions, performed in the context of on-going collaborative studies utilizing electron paramagnetic resonance (EPR) spectroscopy and single-molecule fluorescence (smFRET) to identify dynamic details of the mechanism. The resulting mechanistic implications from the study of LeuT are generalized to two other transporter families, the sodium:solute symporter (SSS) and amino acid-polyamine-organocation (APC) transporter, using comparative molecular dynamics simulations. These comparative studies lead to the proposal of a set of common structure-function-dynamic elements recognizable in the conformational transitions of the transporters with LeuT-fold.

Membrane Domains & Lipid Dynamics

3583-Pos
Neuroligin-1 Oligomerization Induces Cell Morphology Changes Via Lipid Domain Nucleation
Nicole Mende, **Sophie Pautot**.
CRTD, Dresden, Germany.
Thus far, neuroligin-1 (Nlg-1) has been known as a post-synaptic adhesion-signaling membrane protein involved in initiating synaptic contact, and in triggering presynaptic differentiation in a neurexin-expressing axon. Here, we are reporting that nlg-1 might also play a role in neuron morphology changes. Indeed, when nlg-1 was coexpressed in HEK-293 with psd-95, a scaffolding protein which binds nlg-1 PDZ domain, we have observed extensive cell morphology changes. Co-transfected cells exhibited long expansions resembling dendritic branches, as well as a significant increase in cell surface area. However, nlg-1 dimerization mutant did not lead to any major changes in morphology suggesting that nlg-1 multimerization was required.

These expansions could be destabilized by adding a PI3K inhibitor, or by depleting the membrane cholesterol suggesting that the formation of these branches is due to membrane lipid domain formation around nlg-1 clusters. To confirm the role nlg-1 clustering, we have resorted to polarization fluorescence lifetime imaging microscopy to spatially resolve the nlg-1 oligomerization state at different location through out these expansions. We have exploited the photophysical properties of cerulean, a fluorescent protein, to assess the interneuroligin distances and decipher nlg-1 molecular interactions. These measurements confirmed that neuroligin tight clustering was involved in the formation of membrane cholesterol rich domains enabling the recruitment of PI3K, which in turns promotes the growth and the maintenance of these expansions.

3584-Pos

Don't Fence Me in: Evidence for a 'fence' that Impedes the Diffusion of PIP_2 Into and Out of Nascent Phagosomes in Macrophages

Urszula P. Golebiewska[1,2], Jason Kay[3], Sergio Grinstein[3], Wonpil Im[4], Richard Pastor[5], Suzanne Scarlata[1], Stuart McLaughlin[1].
[1]Stony Brook University, Stony Brook, NY, USA, [2]Queensborough Community College, Bayside, NY, USA, [3]Hospital for Sick Children, Toronto, ON, Canada, [4]University of Kansas, Lawrence, KS, USA, [5]National Heart, Lung and Blood Institute, NIH, Bethesda, MD, USA.

To account for the many roles that phosphatidylinositol 4,5-bisphosphate (PIP_2) plays (e.g., in phagocytosis, exocytosis, activation of ion channels) a number of investigators have suggested there are separate pools of PIP_2 in the plasma membrane. Recent experiments show that the free concentration of PIP_2 is indeed enhanced in nascent phagosomes, syntaxin clusters, and the furrows of dividing cells. Kinases that produce PIP_2 (PIPkins) are also concentrated in these regions. But how is the PIP_2 produced by these PIPkins prevented from diffusing rapidly away? *First*, proteins could act as 'fences/corrals'around the perimeter of these regions. *Second*, some factor (e.g., a protein that acted as a PIP_2 buffer) could decrease significantly the diffusion coefficient, D, of PIP_2 within these regions. We used FCS and FRAP to investigate these two possibilities in the nascent phagosomes of J774 macrophages injected with fluorescent PIP_2. FCS measurements show PIP_2 diffuses with similar fast diffusion coefficients in the nascent phagosomes and in the bulk (unengaged) plasma membrane: $D = 0.6 \pm 0.3\ \mu m^2/s$ and $0.8 \pm 0.2\ \mu m^2/s$, respectively. FRAP measurements show the fluorescence from PIP_2 recovers slowly (>100 s) after photobleaching the entire nascent phagosome but recovers rapidly (<10 s) in a comparable area of the plasma membrane outside the cup. These results support the first hypothesis: a 'fence'impedes the diffusion of PIP_2 into and out of nascent phagosomes. The nature of the PIP_2 fence remains an enigma: although actin filaments are concentrated at the perimeter of the forming phagosomes, electrostatic and Brownian Dynamics calculations suggest individual negatively charged actin filaments near the membrane do not significantly impede the diffusion of PIP_2 into or out of the cup.

3585-Pos

Hard to Fence You in: Computational Approaches to Explore the Hypothesis that Actin Filaments Impede PIP2 Diffusion in Membranes

Wonpil Im[1], Stuart McLaughlin[2], Richard W. Pastor[3].
[1]The University of Kansas, Lawrence, KS, USA, [2]Stony Brook University, Stony Brook, NY, USA, [3]NIH, Bethesda, MD, USA.

Experiments described by Golebiewska et al. at this meeting suggest the existence of a 'fence'that impedes the diffusion of phosphatidylinositol 4,5-bisphosphate (PIP2) into and out of nascent phagosomes in macrophages. Although the nature of the fence remains an enigma, actin filaments are plausible components. They are highly negatively charged (as is PIP2), are swept away from the central region and are concentrated at the perimeter of the forming phagosome. To explore the actin fence hypothesis, we have used (1) Poisson-Boltzmann continuum electrostatics and a grid-based repulsive potential to describe a fence model made of a single layer of actin filaments, and (2) Brownian dynamics to describe the diffusion of PIP2 molecules modeled as single spheres. The simulations with actin filaments positioned parallel to the membrane indicate that a single filament without attached proteins does not significantly impede the diffusion of PIP2. A helical stripe of basic residues on the acidic actin filament provides a hole in the putative fence through which PIP2 can diffuse, no matter how close the filament is positioned to the membrane. Results from simulations of PIP2 diffusion out of corrals formed of multiple layers of actin filaments, and mazes of non-electrostatic barriers will also be presented.

3586-Pos

Regions of Correlated Fluctuations in Membrane Lipid Concentrations as a Consequence of Charged Cytoplasmic Lipids

Michael Schick, Gregory G. Putzel.
University of Washington, Seattle, WA, USA.

The cytoplasmic leaflet of the mammalian plasma membrane is characterized by charged lipids such as phosphatidylserine and phosphatidylinositol and its phosphorylated derivatives PIP and PIP2. Their concentrations vary from about ten to less than one percent. The charges are balanced by counter ions in the cytosol. As a consequence there are electric dipole moments in the cytoplasmic leaflet. The extra-cellular leaflet has essentially no charged lipids. In contrast to the electric dipole moments of the lipid head groups in both leaves which, due to their opposite orientation, essentially cancel one another at large distances, the dipole moments of the charged lipids in the cytoplasmic leaf interact via a long-ranged force. As a consequence, the fluctuations in density of these dipole moments are characterized by a non-zero length which depends upon the dipole density and temperature. Because the tails of these lipids couple them to the lipids of the extra-cellular leaflet, composition fluctuations in the outer leaflet will also display characteristic sizes. Thus the presence of charged lipids in the cytoplasmic leaflet results in coupled fluctuations in both leaves of a characteristic size. Such coupled regions could be important in the signaling processes which are associated with the charged lipids.

3587-Pos

Probing Spatial Organization in Cell Membrane at the Immunological Synapse

Yan Yu, Jay T. Groves.
Univ of California-Berkeley, Berkeley, CA, USA.

The large-scale spatial arrangement of cell surface molecules has been gradually realized to regulate specific cellular outcomes in many cellular processes. This phenomenon is particularly striking in the antigen recognition by T cells. Signaling through discrete T cell receptors (TCRs) in the context of immunological synapse, involves the orchestrated movement and reorganization of TCRs on multi length scales. Microcluster movement is believed to be associated with centripetal actin flow, but the underlying physical mechanism remains unclear. By using the hybrid live T cells-supported membrane system, our study to probe the membrane spatial organization of T cells at their immunological synapse and its connection with TCRs movement will be discussed.

3588-Pos

The Effects of High Voltages on the Morphology of a Dppc Lipid Bilayer

Kasper E. Feld.
University of Copenhagen, København S, Denmark.

Biological membranes are often subject to large voltages compared to their small thickness, especially true in nerves where fluxes of ions and corresponding voltage changes are thought to be the main mechanism behind the nerve signal. Yet the effects that these voltages have on the phospholipids that makes up the membrane are largely unknown. Lipids of biological membranes are often charged or swittterionic, high electrical fields should be expected to have a large effect on their organization and thermodynamical properties. Fluorescence microscopy is utilized to image the effects of high voltage fields on the domain structure of a model system consisting of a Langmuir-Blodgett monolayer of phospholipids.

3589-Pos

Lysophosphatidic Acid Interactions with Model Membranes: a Novel Cell Signaling Regulatory Mechanism?

Evan Mintzer.
Stern College for Women, New York, NY, USA.

Lysophosphatidic acid (LPA), the structurally simplest of the glycerophospholipids, is a potent second messenger whose functional diversity makes it a compelling target in lipid research. LPA, the effects of which include cell motility and proliferation, platelet activation, fertility and development, and neuropathic pain, is believed to act through a family of G protein-coupled receptors (GPCR). Since some members of this family of proteins are localized in ordered lipid domains (membrane rafts), a role for LPA in altering and re-ordering membranes as part of regulation of the signaling pathway cannot be discounted.
As part of a series of efforts to obtain biophysical information about the effects of LPA on membranes, we have employed the Langmuir monolayer technique and isothermal titration calorimetry (ITC) to measure the kinetics and thermodynamics of LPA intercalation into lipid films and bilayers of various compositions representing different physical phases known to exist in biomembranes.