518-Pos Board B273
Role of M2 Influenza Protein on Viral Budding and Scission
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M2 is a pH-dependent matrix protein from influenza virus widely known for its role in viral uncoating and the target of the amantadine flu drug that prevents proton transport. An additional role played by M2 relies on collective effects where clusters of M2-homotetramer proteins have been hypothesized to induce local membrane curvature effects (Rossman et al., Cell 142, pp. 902-913, 2010).
We use molecular dynamics simulations with the MARTINI coarse grained parametrization to study a system comprised by two model membranes linked by an hour-glass shaped structure. We study the role played by M2 homotetramers in the scission step, leading to lipid-bilayer separation. The geometry of the model and the membrane-lipid interaction details are further used to adjust parameters on a Helfrich-like functional in order to propose a minimal model for viral scission at the mesoscopic scale.

519-Pos Board B274
Comparative Analysis of Inorganic Phosphate Binding in a Synthetic and a Native P-Loop Peptide Using Molecular Dynamics Simulations
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Binding of anions by proteins is a topic of great interest, the binding being due to interactions either with the protein's positively charged side chains and/or with the main chain. In particular the phosphate anion is essential for a large number of functions and pathways within cells, and the specific binding of phosphate anion has been widely studied. However, investigation of the structural features in phosphate binding sites and the mechanisms for phosphate binding is still a field that warrants more research.
One of the most common ways in which proteins bind to phosphate is via a highly conserved consensus sequence that folds into what is known as a P-loop. Here, we use molecular mechanics and quantum mechanical calculations to characterize interactions of the phosphate anion with 1) a native P-loop whose structure available in the PDB database (1MAB), and 2) a synthetic peptide designed to bind phosphate by mimicking the P-loop function. Whereas the structure of the native P-loop is fairly well characterized and restricted within the 1MAB structure, the synthetic peptide explores a larger conformational space - yet the synthetic peptide is able to bind phosphate.
Specifically we investigate the structural properties and the conformational space of both the peptides, we characterize how they interact with phosphate, and we examine the mechanism by which the peptides fold into the P-loop structure and bind phosphate.

520-Pos Board B275
Computational Study of Transmembrane Helix-Helix Interactions in Model Peptides Derived from the DesK Minimal Sensor
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DesK is a bacterial transmembrane protein that acts as a molecular switch to regulate membrane fluidity as a function of temperature change. The full function of DesK has been experimentally modeled by a chimeric construct, denominated minimal sensor (MS), consisting of a single transmembrane (TM) helix. Multiple mutants based on the TM part of DesK-MS have been reported, suggesting that DesK is sensitive to changes in membrane thickness as a result of changes in temperature. The current view of DesK-MS signaling mechanism points towards formation of a dimer capable of switching its conformation depending on the temperature.
In this study we investigate the molecular details of the functioning of the DesK-MS using experimentally developed model peptides of the TM part only, both the wild type and some of its mutants. We employ a method recently developed in our group for exploring the energy landscape of helix-helix interactions in the membrane environment, which allows high throughput screening of trans-membrane helix dimers. In order to gain more insight into the interactions between the dimers and their lipid environment, a multiscale approach is used. The results presented here are compared to the available experimental data and provide basis for further exploration of the molecular basis of the switch mechanism in DesK-MS.

521-Pos Board B276
Charged Protein-Lipid Interactions in Bilayers with Wide-Ranging Thickness
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Charged amino acids play important roles in membrane protein structure and function. All-atom molecular dynamics (MD) studies have previously been applied to investigate the mechanisms by which charged groups move through lipid bilayers, but have not fully explored the wide range of membrane topologies that may influence charge transport. Here we have performed simulations of the arginine (Arg) side chain analog, $MguanH^+$, moving across bilayers of mono-unsaturated phosphatidylcholine (PC) with 14-24 carbon tails, with and without cholesterol (in a 2:1 lipid:cholesterol ratio), generating hydrophobic thickness increasing systematically from 24 to 42 Angstrom. We demonstrate that free energies grow in proportion to membrane thickness due to an ion-induced defect mechanism, where the presence of the ion leads to membrane deformations and sharp free energy barriers ranging from 14 kcal/mol in D14:1PC to 40 kcal/mol in D24:1PC+cholesterol. Our findings provide a deeper understanding of membrane charge transport phenomena, including uncatalyzed ion permeation and the actions of membrane-active charged peptides for a range of membrane compositions.

522-Pos Board B277
Capturing Spontaneous Binding of Human Islet Amyloid Polypeptide to Anionic Membranes using a Highly Mobile Membrane Mimetic Model
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Human islet amyloid polypeptide (hIAPP) aggregates and forms amyloid fibrils in the pancreas of patients with type2 diabetes. Anionic membranes bind hIAPP and accelerate the fibril formation. During this process, hIAPP disrupts the membrane, a toxic process that has been associated with the N-terminal 19 residues and proposed to lead to the destruction of the insulin-producing β-cells in the pancreas. The mechanism by which the membrane is disrupted is therefore key to our understanding of toxicity of hIAPP, and to design of effective disease modifying treatments.
Employing molecular dynamics (MD) simulations, we have investigated the molecular events key to hIAPP binding to anionic phospholipid bilayers. The diffusion of lipids in conventional membrane models is too slow to allow for hIAPP-membrane binding on the time-scale feasible with conventional MD simulations. In order to overcome the slow diffusion of lipids in conventional membranes which might not allow for adequate sampling of the binding of hIAPP binding to the membrane, we have employed a highly mobile membrane mimetic (HMMM) model.
Multiple independent MD simulations, amounting to 1.2 μs, both for the N-terminal 19-residue peptide, and for the full (37-residue) hIAPP, resulted in spontaneous binding to mixed anionic/zwitterionic HMMM membranes. Initial contacts between the peptide and lipids, which form rapidly (within the first 10 ns of the simulations), are mediated by a group of positively charged residues, Lys1, Arg11, and His18, indicating that the N-terminal part of hIAPP is responsible for its interaction with anionic membranes. These simulations have provided an unprecedented level of statistic on lipid-protein interaction for hIAPP peptides, characterizing residues and lipid chemical groups key for bindig and interaction, as well as the structure and dynamics of the peptide in its membrane-bound form.

523-Pos Board B278
Molecular Dynamics Studies of PEGylated Antimicrobial Peptides with Lipid Bilayers
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We performed all-atom molecular dynamics (MD) simulations of polyethylene glycol (PEG)-grafted magainin 2 and tachyplesin I with lipid bilayers. In the simulations of PEGylated magainin 2 and tachyplesin I in water, both peptides are wrapped by PEG chains because of the interaction between oxygens of PEGs and the cationic residues of peptides. The α-helical structure of PEGylated magainin 2 is broken, while β-sheet of PEGylated tachyplesin I keeps stable, similar to the structural behavior of unPEGylated peptides, in agreement with experiments. Simulations of PEGylated peptides with lipid bilayers show that PEG chains block the electrostatic interaction between cationic residues of peptides and anionic phosphates of lipids, leading to the less binding of the peptide to the bilayer surface, which is observed more significantly for magainin 2 than

for tachyplesin I. Since the random-coiled magainin 2 can be more completely covered by PEGs than does the β-sheet tachyplesin I, the PEGylation effect on the decreased binding is larger for magainin 2, showing the dependence of PEGylation on the peptide structure. These results qualitatively support that PEGylated magainin 2 and tachyplesin I have the different extents of the membrane-permeabilizing activity on lipid bilayer surface.

524-Pos Board B279

Double-Belt a Novel Structure of Membrane Pore

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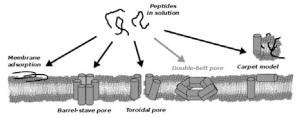
Amphiphilic proteins and peptides can induce formation of stable and metastable pores in phospholipid membranes, which has been associated with toxicity or antimicrobial activity. Using coarse-grained simulations we have studied peptide orientation within the pores and have found that peptides can be oriented perpendicular, parallel, or tilted with respect to the membrane plane. The orientation depends on the length of the peptide and its hydrophobicity distribution, which we rationalized in terms of the hydrophobic mismatch. Apart from well-known barrel-stave or toroidal pores our simulations suggest a novel 'double-belt' pore structure, where peptides within the membrane pore are oriented parallel to the membrane plane. This result was verified using more detailed simulations with the MARTINI force field, where the double-belt structure was stable in microsecond time scale of our simulation.



525-Pos Board B280

Interaction of the Inward Rectifier Potassium Channel Kir 2.2 with Phosphatidylserine

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Inward-rectifier K^+ (Kir) channels are ion channels that transport potassium into the cell. They are essential to maintain the resting membrane potential and to regulate the action potential duration in excitable cells *[1]*. As a consequence, Kir mutations result in several diseases, such as periodic paralysis or cardiac arrhythmia *[1, 2]*.

The activity of Kir channels is regulated by phosphatidylinositol-(4,5)-bisphosphate (PIP_2), a negatively charged phospholipid that has been recognized as one of the major regulators of membrane excitability *[2]*. Moreover, it has been suggested that Kir activation is not only regulated by PIP_2, but it has a secondary, non-specific requirement for other anionic phospholipids *[3]*.

Here we have investigated the interaction of phosphatidylserine (PS) with Kir 2.2 by means of all-atom molecular dynamics (MD). Simulations have been performed on both the apo and PIP_2-bound states of the channel *[4]*, embedded in a POPS membrane in the presence of KCl. These trajectories reveal the key protein residues interacting with POPS, complementing previous docking *[3]* and coarse-grain *[5]* studies. Furthermore, they suggest that the Kir 2.2-POPS interactions drive the cytoplasmic domain closer to the membrane and help to pre-assemble the PIP_2 binding site. In other words, our simulations provide a molecular picture of the sensitized state proposed by Nichols and coworkers to explain the synergistic effect of anionic phospholipids *[3]*.

[1] Physiol. Rev. 90:291-366 (2010).
[2] Pflugers Arch. 460:321-341 (2010).
[3] (a) Biophys. J. 100:620-628 (2011). (b) J. Biol. Chem. 288:16726-16737 (2013). (c) Biophys. J., 104:433a (2013).
[4] (a) Science, 326:1668-1674 (2009). (b) Nature (Letter), 477:495-499 (2011)
[5] Biochemistry, 52:279-281 (2013).

526-Pos Board B281

Permeation of Lipidated Protein in Bilayer using Unbiased Simulations Reveals Signature Motif for Protein-Membrane Binding

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Structural and dynamic reorganization of membrane domains by proteins is a central theme in many biological processes. Eukaryotic cells possess potential regulatory mechanisms to mediate many of its protein-membrane interactions in form of lipid-modified proteins. Two forms of these proteins exist; cytosolic conformers and functional membrane bound complexes. At present, there are many questions that remain to be answered with regard to the molecular mechanism of interaction of lipidated proteins with membrane that generates a trigger for physiological response, only upon membrane binding.

A particularly exciting area of membrane trafficking, autophagy, has been receiving increasing attention. It is a cellular degradative process that involves the formation of autophagosome, a specialized vesicle to deliver the dispensable cellular cargo to the lysosome. During initiation of autophagy, key regulator protein, LC3-I (cytosolic form) is conjugated reversibly to phosphotidylethanolamine (PE) resulting in a nonsoluble form of LC3 that stably associates with the autophagosomal membrane. The present work reports partitioning of PE chain of LC3 in bilayer using MARTINI force field based coarse grain molecular dynamics simulations.

Spontaneous insertion of PE chain at microseconds timescale is observed in fourteen out of fifteen trajectories. This unbiased insertion of lipidated protein reveals a novel insertion pathway through an arginine rich patch that drives the insertion of PE chain. Interestingly, membrane curvature properties reveal variations in spontaneous curvature of the membrane, confirming the role of LC3 in forming a vesicle during autophagy. This first unbiased study of active insertion of a lipid chain provides future avenues to investigate detailed regulatory aspects of this phenomenon using experimental techniques.

527-Pos Board B282

Structural Basis of Lipid Exchange in the Oxysterol-Binding Protein Homologue (OSH) Family

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Oxysterol-binding proteins, long known to be involved in the non-vesicular transport of sterol, have recently been shown to also transfer glycerophospholipids, such as phosphatidyl-inositol-4-phosphate (PI4P) and phosphatidylserine (PS) between membranes.

Using a combination of atomistic molecular dynamics simulations and in vitro lipid transport assays, we characterized the structural determinants that regulate the extraction and release of different lipids by the various Osh proteins.

Our results define how distinct lipid-binding modes govern the ability of Osh proteins to transport or exchange lipids between organelles.

528-Pos Board B283

Study of the Mecanism of Action of a Hybrid Peptide in POPG:POPC Bilayers

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A hybrid peptide sequence has been synthesized from the antimicrobial peptides pediocin A and plantaricin 149A. Previous studies of circular dichroism and fluorescence spectroscopic studies have shown a disordered to ordered conformational transition of the peptide upon binding to POPG but not to POPC. Further, single vesicle experiments under optical microscopy observation have indicated that at low concentrations the peptide causes the disruption of POPG membrane and formation of small, heterogeneous complexes of phospholipids and peptides. In order to analyze those interactions, concentration effects and to propose a disruption mechanism, molecular dynamics simulations were carried out using the GROMOS parameter set 54A7 and MARTINI. The systems were analyzed with respect to time-dependent (peptide secondary structure and lipid tilt angle), and average properties (density profiles and deuterium order parameters).

It was found that the peptides adsorb on both PG and PC membranes via electrostatic interactions. Only upon binding to the PG surface there is an increase of helical content compared to the peptide in solution. Higher helical content is also observed for the single peptide embedded in PG compared to PC membranes. The density of the membrane medium makes conformational transition of the peptide embedded slower than on the surface of the membrane. Our simulations indicate disruption of the membrane without deep penetration of the peptides. Evidence for that comes from increased disorder of the membrane and persistent interactions between the peptide and membrane headgroups throughout the membrane disruption process. Our findings suggest that the hybrid peptide disrupt the membrane via a carpet-like mechanism which has also been postulated for the action of Pediocin A and Plantaricin 149A [1,2].

[1] Gaussier et al. App. Environm. MicroBiol, 69, 6777 (2003);
[2] Lopes et al., BBA, 1788, 2252 (2009).

529-Pos Board B284

Oligomerization of Huntingtin N-Terminal Fragment on a Phospholipid Bilayer Revealed by Molecular Dynamics Simulations

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The Huntingtin protein is characterized by a consecutive segment of glutamines that leads, when the number of repeats exceeds a certain length, to fibrillation. Misfolding of this amyloid protein is related to the Huntington's disease through pathways that could involve interactions with phospholipid membranes. For instance,