2720-Pos Board B706
Exchange of Gramicidin between Lipid Bilayers: Implications for the Mechanism of Channel Formation and Gating
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The canonical mechanism of gramicidin (gA) channel formation is a transmembrane dimerization of non-conducting subunits that reside in opposite bilayer leaflets. The channels do not open and close per se; rather they appear and disappear. This basic monomer-dimer mechanism is supported by conductance-concentration studies, fluorescence measurements, conductance relaxation experiments using voltage, and pressure and light-inactivation experiments to perturb the monomer-dimer equilibrium. Recently this mechanism was challenged by Jones et al. (BJ 98:1486, 2010). Using lipid vesicle-incorporated gA under conditions where vesicle fusion could be controlled, Jones et al. proposed that gA single-channel current transitions result from closed-open transitions in long-lived bilayer-spanning dimers. A key assumption in these experiments is that gA monomers do not partition between vesicles; see Kemp and Wenner (ABB 176:547, 1976) and Bruggemann and Kayalar (PNAS 83:4276, 1986). To explore this issue further, we added gA-containing 1,2-dierucoyl-sn-glycero-3-phosphocholine (DC22:1PC) vesicles to one or both sides of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) planar bilayers. This protocol produced gA activity that was 10-fold higher when gA-containing vesicles were added to both sides of the bilayer, as compared to one side. We also found that when gA-free DC22:1PC vesicles were added to both sides of planar DOPC bilayers pre-incubated with gA, channel activity would reduce about 10-fold. We conclude that gA subunits can exchange between lipid vesicles and planar bilayers. We similarly could demonstrate the exchange of gA between lipid vesicles using a fluorescence assay for gA channel activity (Ingólfsson and Andersen, ADDT 4:427, 2010). We conclude that the parsimonious interpretation of the results of Jones et al. is that their channel activity is due to gA exchange between the vesicles and the planar bilayer, and that gA channels form by the canonical mechanism- transbilayer association of non-conducting subunits.

2721-Pos Board B707
Exploring Hydrophobic Mismatch using Molecular Dynamics Simulations of Gramicidin A in Lipid Bilayers
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Gramicidin A (gA) is a 15-amino acid peptide antibiotic with an alternating L-D sequence, which forms bilayer-spanning, monovalent cation permeable channels in biological membranes and synthetic bilayers. Due to its relatively small size and extensive documentation of its function, it is a perfect candidate for molecular dynamics (MD) simulations to understand not only ion permeation but also channel-bilayer interactions. In this study, we have performed MD simulations of gA in all-atom, explicit dilauroylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), dioleoyl-phosphatidylcholine (DOPC), and 1-palmitoyl,2-oleoylphosphatidylcholine (POPC) bilayers. The variation in acyl chain length in these different phospholipids provides a way to alter protein-membrane interactions as a function of bilayer hydrophobic thickness. A particular goal of the study is to determine whether the predicted and observed local membrane thinning and thickening occurs adjacent to the channel. Furthermore, what is the profile of the bilayer deformation, how far does the perturbation extend radially, and does the extent of the perturbation vary as a function of the unperturbed bilayer thickness. In addition, does the channel distort in order to minimize the hydrophobic restraints imposed by the channel-bilayer hydrophobic mismatch. All of the work will be categorized based on energetic favorability, building a model for the prediction of protein-membrane interactions. Currently each system has been simulated for 10 ns.

2722-Pos Board B708
Move, Dither, Move, Dither. On the Structure of Random Walks and Single-Particle Trajectories
Michael J. Saxton.
The fundamental principle in interpreting single-particle trajectories is that a pure random walk is the control and null hypothesis. In order to make any claim about a putative physical or biological event in an observed single-particle trajectory, one must evaluate the probability that the event could have occurred by chance in the corresponding pure random walk. By eye, a random walk often shows alternating periods of "moving" and "dithering." We quantify this apparent structure by Monte Carlo calculations of pure random walks in which various measures of apparent directed motion ("moving") and apparent confinement ("dithering") are compared. A key point is that long dithering or moving events are rare in themselves. But in examining a trajectory for these events, we do not specify the starting point of the event, and all that we specify about the duration is that it be above some minimum threshold of detectability. The large number of potential starting points and durations of these events compensates for their rarity. An essential feature of the approach is to separate three distinct aspects of the problem. First, we separate characterization from trajectory segmentation, and treat only characterization here. Second, we separate characterization from the effect of noise, and assume that the particle positions are exactly known. In practical applications to experimental single-particle trajectories, the random noise in the position measurement must be taken into account. The move-dither analysis will ultimately form the basis for new tests to identify directed and confined motion in single-particle trajectories, and to distinguish anomalous from normal diffusion. Supported by NIH grant GM038133.

2723-Pos Board B709
Membrane Diffusion of Tethered Dimer and Trimer Systems
Michael G. Lerner, Richard W. Pastor.
We have used molecular dynamics simulations to investigate the diffusion of tethered dimer and trimer systems in lipid bilayers. Coarse-grained (CG) models of DPPC dimers were simulated in a DPPC bilayer with the MARTINI model, and single-lipid diffusion constants compared to those obtained for dimers and trimers at various tether lengths. The ratio of diffusion constants matches well with theoretical predictions of a simple bead model. A full theoretical model of the translational and rotational diffusion of tethered dimers and trimers was developed at the Saffman-Delbruck level, and results were compared to the simple bead model. The ratios of diffusion constants from the theoretical and CG models were then compared to experimental diffusion constants of pleckstrin homology (PH) domains bound to lipids with a PIP3 (phosphatidylinositol (3,4,5)-trisphosphate) head group. Excellent agreement between all systems was found, indicating that the frictional contributions of multiple, coupled but well-separated lipids are additive, analogous to the free-draining limit for isotropic fluids.

2724-Pos Board B710
Effects of Water Molecules on Lipid Bilayer Dynamics in Aqueous Salt Solutions:Analyses of Molecular Dynamics Simulation
Rina Kagawa, Yoshinori Hirano, Kenji Yasuoka, Masato Yasui.
The cell membrane that consists of lipid bilayer plays a critical role in physiology. Lipid molecules are directly contacted with water molecules and ions (i.e. Na+, K+, Ca2+, and Cl-). Fluidity of the lipid bilayer decrease in aqueous salt solutions compared with pure water, which is affected by bonding of ions to lipids. However, its mechanism has not been clarified yet, and decrease in membrane fluidity may be influenced by other factors.
Lipid, water and ions interact with each other, which contribute to the membrane dynamics. Water molecules construct "hydrogen bond network". Some ions change static and dynamic properties of this network. In addition, water molecules directly affect dynamic properties of lipid bilayers. Therefore, we hypothesized that the hydrogen bond network of water, which is modified by ions, may play a role in dynamic behavior of lipid bilayer.
To analyze the effect of ions and water molecules on membrane dynamics, we constructed four model systems, POPE (1-palmitoyl-2-oleoyl-phosphatidylethanolamine (18:1)) lipid bilayer and water molecules without (WATER) or with ions (KCl, CaCl2 and NaCl). We performed 100ns MD simulations and compared each system. Each system consists of 282 lipids and about 22000 water molecules, and the salt concentration is about 0.09 M, which is similar with biophysical conditions. MD simulations were carried out using Amber 8 program on HITACHI HA8000 cluster system at the University of Tokyo.
We found that the self-diffusion-coefficient of water molecules in each system away from lipid bilayer were essentially the same as those in experimental data (KCl>WATER>NaCl>CaCl2). However, self-diffusion-coefficient of water molecules adjacent the bilayer are different from those in bulk solutions (NaCl>KCl>CaCl2>WATER). Our study suggests that the increase of self-diffusion-coefficient of water is related to the decrease in membrane fluidity.

2725-Pos Board B711
Anomalous Diffusion of Water Molecules in Hydrated Lipid Bilayers
Jhuma Das, Elijah Flenner, Ioan Kosztin.
We use all-atom molecular dynamics (MD) simulation to study the structure and dynamics of water molecules in a hydrated lipid membrane. By using a 0.1 microsecond long MD trajectory of a fully solvated DMPC phospholipid bilayer we identify (by means of Voronoi tessellation) four dynamically connected water regions termed: buried, hydration, intermediate and bulk, respectively. Due to their proximity to the polar lipid headgroups, buried and hydration waters have qualitatively different dynamical properties from bulk water. To identify and quantify these differences we investigate the time evolution of the lateral mean square displacement (MSD) of water molecules and the lifetime of hydrogen bonds between water and lipid molecules. We find that before entering the linear diffusion regime (t>10ns), on sub-nanosecond time scale buried and hydration waters undergo anomalous diffusion characterized by well separated