2676-Pos Board B662

Ions at Dipolar Membrane Surfaces
Or Szekely, Ariel Stein, Pablo Szekely, Roi Asor, Uri Raviv.

In pure water, zwitterionic (dipolar) lipids form lamellar phases with equilibrium water gap of order 2 nm. This is attributed to the dominating van der Waals attraction between dipolar membranes. If divalent ions are adsorbed onto charge-neutral membranes, the membranes may become charged and behave as charged membranes. Using solution X-ray scattering we studied in detail the interaction of divalent ions with dipolar lipids. We found that the interaction is determined solely by the lipid tail structure. Divalent ions can adsorb only onto lipids with saturated tails. A single double bond in the lipid tail is sufficient to prevent divalent ion adsorption. This is attributed to the relatively loose packing of lipids with unsaturated tail/s that enables free rotation of the headgroups. Divalent ion adsorption links with both lipids and limits their free rotation. The ion-dipole interaction gained by the adsorption of the ions onto the membranes is insufficient to compensate for the loss of lipid-headgroup free-rotation entropy.

This study provides insight into the interactions between cell membranes and various charged ions and how lipid composition controls those interactions. It also forms the basis for controlling the interactions between membranes and charged proteins or peptides for protein encapsulation and drug delivery applications.

2677-Pos Board B663

Computational Characterization of DMPI and DMPI(4P) Head Groups
Michael Rowland, Richard Pastor, Wonpil Im.

Results from Molecular Dynamics simulations of dimyristoyl-phosphatidyl-inositol (DMPI) using the C36 CHARMM lipid force field are compared with those from neutron-diffraction and 2H-NMR studies. Calculated and experimental quadrupolar splitting ratios and distances from the bilayer center to inositol deuteration sites are in good agreement. The simulations compared with those from neutron-diffraction and 2H-NMR studies. Calculated and experimental quadrupolar splitting ratios and distances from the bilayer center to inositol deuteration sites are in good agreement. The simulations are in agreement with those from neutron-diffraction and 2H-NMR studies. Calculated and experimental quadrupolar splitting ratios and distances from the bilayer center to inositol deuteration sites are in good agreement. The simulations compared with those from neutron-diffraction and 2H-NMR studies. Calculated and experimental quadrupolar splitting ratios and distances from the bilayer center to inositol deuteration sites are in good agreement. The simulations compared with those from neutron-diffraction and 2H-NMR studies. Calculated and experimental quadrupolar splitting ratios and distances from the bilayer center to inositol deuteration sites are in good agreement.

Phase separation of lipid bilayers into liquid-ordered and liquid-disordered regions is reflective of the inter-domain boundary of the surface tension at the hydrophilic-hydrophobic interface. If the LC and LE lipids differ only by the flexural rigidities ratio, A- and B- mark LC and LE lipids respectively. Fluctuating single chain statistically swipes a cylindrical volume. In a mono-component bilayer these cylinders form a close pack. But at the LC/LE boundary the cylinders of the two different diameters can’t form a close pack. Hence, an area per lipid is greater at the boundary than in the bulk of domains. In our model this excessive area leads to a local decrease within the inter-domain boundary of the surface tension at the hydrophilic-hydrophobic interface. If the LC and LE lipids differ only by the flexural rigidity of hydrocarbon chains, then the (flat) boundary energy is negative, Fig. 1, with consequences for domains size distribution.

2679-Pos Board B665

Enthality Drives Phase Separation in Model Membranes: Evidence from Molecular Dynamics Simulations of Non-Lipid Amphiphiles Vitamin-E, triton-X, and Benzyl Alcohol
Peter J. Butler, Hari S. Muddana, Homer Chiang.

Phase separation of lipid bilayers into liquid-ordered and liquid-disordered regions facilitates compartmentalization of membrane proteins, an important prerequisite for cellular biochemical signaling. In this study, we investigated the phase behavior of binary and ternary lipid mixtures under the influence of three non-lipid amphiphiles, Vitamin-E, Triton-X 100, and benzyl alcohol. Molecular dynamics (MD) simulations of these additives in fluid-phase lipid bilayers were performed to investigate their effect on membrane thickness and fluidity as read-outs of enthalpic and entropic changes, respectively. Simulation results indicate that Vitamin-E increases bilayer thickness and lowers the fluidity, while Triton-X and benzyl alcohol each decrease bilayer thickness and increase fluidity. Experimentally, in both binary and ternary lipid mixtures, Vitamin-E induced ideal mixing of the lipids whereas Triton-X and benzyl alcohol either resulted in sustained phase separation or induced large-scale phase separation. Because changes in bilayer thickness determined by MD correlate better than fluidity with phase separation observed in model membranes we conclude that hydrophobic mismatch of different lipid types (enthalpic effect) dominate the effect of fluidity (entropic effect) as the key driving force for the phase separation behavior observed in lipid bilayers. Moreover, these non-lipid amphiphiles provide new tools to tune raft formation/disruption and to study raft-related cell membrane functions through modulation of non-raft membrane thickness.

2680-Pos Board B666

Vitamin E Forms Domains with Pufa in Membranes: An EPR Spin Trapping Study of Lipid Peroxidation in Model Membranes
Cynthia D. Wassall, William Stillwell, Marvin D. Kemple, Stephen R. Wassall.

Spin trapping in conjunction with EPR spectroscopy is a widely used technique for measurement of short-lived free radical species of biological interest due to its high sensitivity and specificity. In spin trapping a molecule (the spin trap) reacts with the free radical producing a spin adduct that is sufficiently stable to be detected by EPR. In this study, we employ lipid-soluble N-tetra-butyl-phenyl-hydrinolate (PBN) as our spin trap. PBN spin adducts detected by EPR are believed to be lipid hydroperoxides that are secondary species resulting from free radical attack on cell membranes. Thus the presence of the spin-adducts is indicative of the presence of ROS levels. Lipid auto oxidation will be monitored in model membranes containing 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (PDPC) with various concentrations of α-tocopherol, the major lipid soluble antioxidant in membranes and a primary component of vitamin E. Preliminary data of PDPC without α-tocopherol show auto oxidation that leads to a free radical cascade. By measuring the reduction in lipid peroxidation due to the presence of α-tocopherol, we test the hypothesis that the vitamin co-localizes with polyunsaturated lipids in membrane domains to ensure its close proximity to the most vulnerable lipid species.

2681-Pos Board B667

Vitamin E Responds to its Lipid Environment
Justin A. Williams, Drew Marquardt, William Stillwell, Jeffrey Atkinson, Thad A. Harroun, Stephen R. Wassall.

Vitamin E (α-tocopherol) is recognized as the premier lipid soluble antioxidant in biological membranes, but many questions persist of how the vitamin actually functions. We have hypothesized that α-tocopherol preferentially locates in domains that are enriched in polyunsaturated phospholipids, increasing the concentration of the vitamin in the vicinity of the lipid species most vulnerable to oxidation. These highly disordered domains depleted in cholesterol are analogous, although organizationally the antithesis, of the well-studied lipid rafts which are highly ordered and rich in cholesterol. Here we investigate the molecular organization of α-tocopherol in model membranes as a function of unsaturation. Using solid state 2H NMR and ESR, we see that there is less restriction to the motion of α-tocopherol and that this has less impact on chain order in more unsaturated membranes. We also have now measured the location of the chromanol ring of α-tocopherol using neutron diffraction. The results show that the vitamin sits lower in the membrane and is more intimately tied to the lipid chains when they are more unsaturated (where its antioxidant role is needed), and generally sits higher in the membrane and is excluded vertically from the chains when they are more saturated (where its antioxidant role is not needed). Together these findings support α-tocopherol’s putative structure/function role as a free-radical scavenger by locating itself in the right place at the right time.

2682-Pos Board B668

Laser-Triggered Release of Entrapped Photo-Reactive Solutes from Liposomes Containing Diacytlenic Phosphatidylethanolamine

We have previously reported on a novel class of light-triggerable liposomes prepared from a photoactivatable phospholipid DClαPC (1,2-bis (tricosa-10,12-diyonyl)-sn-glycero-3-phosphocholine) and DPPC (1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine)1-2. UV radiation (254 nm) exposure on the liposomes resulted in photoactivation of DClαPC which subsequently resulted in the release of contents (see abstract at this meeting by Puri et al). Treatment of these liposomes by 514 nm laser light is also found to promote the release of encapsulated calcine (Ex/Em 490/517 nm) or (an anticancer drug) Doxorubicin (Ex/Em 490/590 nm); however the onset of this release is dramatically rapid and is found to occur within 1-2 minutes of laser treatment.

Tuesday, March 8, 2011 493a
in contrast to greater than 10 minutes after the 254 nm UV exposure. When a different dye: calcine-blue (Ex/Em 360/460 nm) was encapsulated in the liposomes, green fluorescent exposure to the laser blue dye suggesting the release mechanism to be wavelength specific. The 514 nm laser treatment on calcine loaded liposomes did not result in any measurable chemical changes in the lipids as determined by LC-MS and morphological changes in the liposomes as determined by Cryo electron microscopy. The 514 nm treatment of calcine-loaded liposomes also resulted in the production of reactive oxygen species as determined through ROS assays. Moreover, the laser-induced release of calcine was inhibited in the presence of oxygen radical scavengers. Based on these observations, we propose that visible-light triggered release of entrapped solutes from DPPC/DC4aPC liposomes occurs via a novel mechanism that involves photo-sensitizer-generated reactive oxygen species to perturb DC4aPC in the lipidosome membrane. These formulations are currently under investigations for drug delivery/release applications.

**Membrane Active Peptides II**

### 2683-Pos Board B609

**On the Origin of Multi-Exponential Kinetics in Peptide Binding to Phospholipid Vesicles**

**Alex Kreutzberger, Antje Pokorny.**

Binding kinetics of amphipathic peptides to phospholipid bilayer vesicles are generally well described by a single exponential function. However, closer inspection of the data sometimes reveals the presence of additional exponential phases. These usually depend on the exact experimental conditions and on the peptide studied, but are indicative of other processes that contribute to the binding kinetics. We investigated the binding of a model amphipathic peptide, Lysette-26, to unilamellar lipid vesicles composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) as a function of lipid and peptide concentration. Lysette-26 is a 22 residue peptide with the sequence IISTIGDLVK WIDTVKWIIDTVNKFHTK. The peptide binds well to zwitterionic lipid membranes and forms an amphipathic α-helix when bound at the membrane-water interface. Peptide binding was measured through fluorescence energy transfer from the intrinsic tryptophan residue in the peptide to an acceptor fluorophore embedded in the membrane at low concentrations. A series of exact kinetic models was developed to describe the experimental data. We found that the kinetics of peptide binding to POPC vesicles depended strongly on peptide concentration in solution. The model that best described the experimental data included the presence of peptide monomers and aggregates in solution that bind independently and with different rate constants to the lipid vesicles. Peptide aggregation is favored at higher peptide concentrations, leading to the appearance of an additional exponential phase. Based on the kinetic models, other processes, such as peptide insertion or translocation across the bilayer, could be excluded as the source of the second exponential phase in the binding kinetics.

### 2684-Pos Board B670

**Peptides with Multiple Arginines Can Spontaneously Translocate across Lipid Vesicle Membranes and across Living Cell Membranes**

**Jessica R. Marks, Kalina Hristova, William C. Wimley.**

Using orthogonal high-throughput screening and rational combinatorial peptide libraries, we have identified a family of peptides that appear to spontaneously translocate across membranes without any membrane permeabilization. The conserved 9-residue translocation motif is mostly hydrophobic, composed of leucine, isoleucine and proline, but also has several conserved arginine or lysine residues and an open terminal amino group. Using confocal fluorescence microscopy, we tested the ability of the selected peptides to transport a large polar dye, TAMRA, across anionic PC/PG (90:10) vesicles and across zwitterionic PC vesicles. To increase the stringency of the experiment, multilamellar vesicles were used such that the peptides had to sequentially pass through 10-15 bilayers in order to equilibrate into the interior of the vesicles. The peptides identified in the screen rapidly and completely equilibrated across vesicles of either composition within about 15 minutes of addition. Binding of the peptides to the vesicles was weak, suggesting that the small number of peptides bound at any moment in time translocate extremely efficiently. A 3000 Da fluorescent dextran was excluded from the vesicles, even in the presence of rapidly translocating peptides. The same dye-labeled peptides also rapidly translocate across the plasma membranes of living cells, filling the cytoplasm with a uniform fluorescence. Translocation was rapid, and no evidence of oxygen radic.

### 2685-Pos Board B671

**Characterizing the Antibacterial Mechanism of Three Novel Histone-Derived Antimicrobial Peptides**

**Sara A. Spinella, SeiEun Chun, Kathryn E. Pavia, Andrew C. Webb, Donald E. Elmore.**

The rise in antibiotic resistant pathogens has sparked interest in antimicrobial AMPs (APMS), especially those that are active against a wide variety of bacterial strains. Cell penetrating AMPs are of particular importance for their potential as both drug delivery systems and antimicrobial agents. Buforin II (BF2) is a well-characterized antimicrobial peptide derived from histone subunit H2A that kills bacteria by translocating across cell membranes and binding to nucleic acids. In this study, we compared the mechanisms of action of three novel histone-derived antimicrobial peptides (HDAPs), termed DesHDAP1-3, to that of BF2. DesHDAP1’s antibacterial potency is similar to that of BF2 across several bacterial species, while DesHDAP2 and DesHDAP3 are generally weaker antibacterial agents. Our current data also implies that DesHDAP1 shows increased cytotoxicity against cancerous cells lines compared to BF2. Lipid vesicle studies measuring the translocation of all three designed peptides showed that DesHDAP2 does not cross lipid membranes as readily as DesHDAP1, DesHDAP3, or BF2, which may explain its poor antibacterial activity. For comparison, we have also considered the membrane permeabilization caused by the designed peptides using a propidium iodide uptake assay. Finally, we have considered the role of nucleic acid binding in the mechanism of the designed peptides by measuring the DNA binding and antimicrobial activity of mutant versions of the designed peptides. This data shows that all three peptides may not show the same correlation between DNA binding and activity observed for BF2. An understanding of how the designed peptides function is an important step in assessing their therapeutic potential and considering future design strategies.

### 2686-Pos Board B672

**Cell Penetrating Peptides. How do they Cross Membranes?**

**Joachim Seelig, André Ziegler, Gabriela Kloock.**

Cell penetrating peptides (CPPs) are cationic peptides which, when linked to proteins, genes, or nanoparticles, facilitate the transport of these entities across the cell membrane. Different models have been suggested for their mode of action. This talk will describe our findings on the binding and translocation mechanism of these amphipathic peptides and the formation of non-bilayer structures. We have studied the binding of two CPPs (Rn, HIV-1 TAT) to model membranes with isothermal titration calorimetry and observed indeed a strong binding to the membrane. However, the bilayer remains intact and non-bilayer structures can be excluded based on H+/P-NMR spectroscopy. A second mechanism is the binding of CPPs to sulfated sugars at the membrane surface, followed by endocytosis. We have characterized the binding of a large variety of CPPs to heparin sulfate (HS), heparin, and related sulfated glycosaminoglycans (GAGs) and found binding constants in the order of 10^4 - 10^6 M^-1 per binding site. An even stronger interaction is found between CPPs and DNA with binding constants in 10^9 - 10^11 M^-1. We have further synthesized a fluorescent derivative of the HIV-1 TAT protein transduction domain (Fg-CPP1AT10F10) and have observed its uptake into non-fixed living fibroblasts and the time-lapse confocal microscopy. We find that Fg-CPP1AT10F10 enters the cytoplasm and nucleus of non-fixed fibroblasts within seconds. With differential interference contrast microscopy we furthermore detect dense aggregates on the cell surface. Several observations suggest that these aggregates consist of Fg-CPP1AT10F10 bound to membrane-associated heparan sulfate (HS). Finally a pore-forming peptide, melittin, also binds to sulfated GAGs but not magainin 2 or nisin Z. The binding of the melittin to lipid bilayers is furthermore an excellent model system to elucidate the thermodynamic parameters of membrane-induced α-helix and β-sheet formation.

### 2687-Pos Board B673

**AFM Force Spectroscopy on TAT Membrane Penetration**

**Elizabeth A. Hager-Barnard, Benjamin D. Alnquist, Nicholas A. Melosh.**

We present a combined experimental and theoretical study of the interactions between cell-penetrating peptides (CPPs) and lipid bilayers using dynamic AFM measurements. Understanding how CPPs can pass through cell membranes is critical for designing drug delivery agents. While CPPs like HIV-TAT have been widely studied, their ability to penetrate membranes directly, without active transport, is still a matter of considerable debate. Here, we directly measure TAT-lipid mechanics during the actual membrane translocation event using TAT-functionalized AFM probes to penetrate through a stack of lipid bilayers. Dynamic force spectroscopy revealed that both the bilayer breakthrough force and bilayer thickness depended on the TAT-bilayer contact time. The results provide a detailed view of how TAT interacts with the bilayer. Upon contact, TAT inserts into the bilayer headgroup to a depth of ~1nm in