squared displacement (MSD) of single molecule tracks obtained from live cell measurements. Exploiting the photo-switching of PA fluorescent proteins and blinking organic fluorophores, we detect an ensemble of single molecules in each cell investigated, and can analyze populations of diffusers with incredible statistics. By comparing theoretical predictions with quantitative experimental observations, we aim to test our working hypothesis that critical composition fluctuations provide the physical basis of raft heterogeneity.

1. Machta, B.B., S. Papanikolaou, J.P. Sethna, and S.L. Veatch, 2011. *Minimal model of plasma membrane heterogeneity requires coupling cortical actin to criticality.* Biophys J. 100: 1668-77.

423-Pos Board B209

Phosphoinositides Alter Lipid Bilayer Properties

Radda Rusinova, E. Ashley Hobart, Olaf S. Andersen.
Weill Medical College of Cornell University, New York, NY, USA.

Phosphoinositides are involved in cell-signaling pathways that regulate vital cell functions such as membrane excitability and trafficking, and cell metabolism, motility and proliferation. At the plasma membrane, phosphatidylinositol-4,5-bisphosphate (PIP_2), which constitutes approximately 0.25% of cell phospholipid, is a key messenger in membrane-delimited signaling. PIP_2 regulates structurally and functionally diverse membrane proteins including voltage- and ligand-gated ion channels, inwardly rectifying ion channels, transporters and receptors. The mechanism(s) by which PIP_2 regulates many of its various ''receptors'' remain to be elucidated. Here we explore the notion that the amphiphilic phosphoinositides, by adsorbing to the bilayer/solution interface, alter bilayer properties such as curvature and elasticity. Such changes in bilayer properties can alter the equilibrium between membrane protein conformational states and thereby alter function. Taking advantage of the gramicidin channels' sensitivity to changes in the lipid bilayer properties, we used fluorescence-based and single-channel gA assays to examine the effects of (diC8) phosphoinositides -PI, $PI(4,5)P_2$, $PI(3,5)P_2$, $PI(3,4)P_2$ $PI(3,4,5)P_3$ as well as long-chain $PI(4,5)P_2$ on the lipid bilayer. The diC8 phosphoinositides, except for $PI(3,5)P_2$, alter lipid bilayer properties with potency that decreases with increasing charge. Among the long-chain $PI(4,5)P_2$s, the naturally occurring 1-stearyl-2-arachidonyl-$PI(4,5)P_2$ is a more potent bilayer modifier than di-oleoyl-$PI(4,5)P_2$. The diC8 and the naturally occurring $PI(4,5)P_2$ have similar effects on short and long gA channels, indicating that changes in bilayer curvature dominate over those on bilayer elasticity. In contrast, diC8PI, which was more bilayer-active than diC8PIP_2 altered bilayer elasticity. Our results show that application of exogenous PIP_2 and its structural analogues (with changes in acyl chain length or phosphorylation state) alters lipid bilayer properties. These PIP_2 lipid bilayer effects may be important for some of the many different effects on membrane protein function.

424-Pos Board B210

Direct Observation of Plasma Membrane Domains using Super Resolution Microscopy

Rolf Harkes, Franziska Zosel, Anna Pezzarossa, Thomas Schmidt.
Leiden universiteit, Leiden, Netherlands.

The composition of the plasma membrane has long been modeled as a mosaic fluid. However, in the last few years there has been evidence that suggests the plasma membrane to be a dynamic and highly compartmentalized structure. This organization in domains results in a differential spatial distribution of signaling proteins on both leaflets of the plasma membrane. It is still debated whether inner and outer leaflet domains are linked. The lateral segregation of membrane proteins plays a role in cell signaling and protein-protein interaction. Thus, it is of high scientific interest to further investigate these domains.

The Ras protein resides on the inner leaflet of the plasma membrane. Here we used super-resolution microscopy to study the compartmentalization of H-Ras and its membrane anchor CAAX, fused to the photo convertible dye Dendra2. The signal of single Dendra2 molecules is recorded and statistical analysis is applied to localize these molecules. On the apical membrane of 3T3 fibroblast, domains of 150nm were detected for both the full protein and its membrane anchor. To investigate a possible link between inner and outer leaflet domains, cells where treated with Choleratoxin B (CtxB). This leads to clustering of the outer-leaflet ganglioside GM1. Neither size nor the amount of domains were dependent on incubation with CtxB. However, incubation with CtxB did lead to an increase in H-Ras density inside the domains, indicating a connection between lipid organization on the outside and protein distribution on the inside of the plasmamembrane.

425-Pos Board B211

Statins Modify Lipid Bilayer Properties

R. Lea Sanford[1], Subhi J. Al'Aref[1], Rodger E. Koeppe, II[2], Olaf S. Andersen[1].
[1]Weill Cornell Medical College, New York, NY, USA,
[2]University of Arkansas, Fayettville, AR, USA.

Statins are drugs that are widely prescribed to manage hypercholesterolemia. Statins exert their primary mechanism of action by inhibiting the HMG-CoA reductase, thus preventing cholesterol synthesis. In addition to this canonical action they also alter the function of diverse membrane proteins. Because statins are amphiphiles that modulate the function of different, structurally unrelated membrane proteins, we investigated whether statins could alter lipid bilayer properties at concentrations where they alter membrane protein function. To this end, we used the gramicidin-based fluorescence assay (GBFA) as well as single-channel electrophysiology. We found that atorvastatin, fluvastatin, lovastatin, mevastatin, pravastatin, and simvastatin all increased the rate of fluorescence quenching, meaning that they shifted the gramicidin (gA) monomer dimer equilibrium toward the formation of conducting dimers. Statins thus alter lipid bilayer properties, with fluvastatin being the most active and rosuvastatin the least active. When examined using single-channel electrophysiology, simvastatin, pravastatin, and fluvastatin increased the lifetime and appearance rate of gA channels with fluvastatin being the most active and pravastain being the least active. We observe larger effects on the shorter channels; the hydrophobic mismatch dependant effects indicate a change in bilayer elasticity. We conclude that statins alter lipid bilayer properties by a common mechanism, through an increase in bilayer elasticity, and that specific channel-statin interactions are not the sole mechanism of action for statins.

426-Pos Board B212

Heterogeneity of Water Dynamics of Hydrated Lipid Bilayers in Atomistic MD Simulations

Timothy C. Young, Matthias Heyden, Juan A. Freites, Douglas J. Tobias.
UC Irvine, Irvine, CA, USA.

Probing dynamics of water molecules interacting with polar headgroups of hydrated lipid membranes is vital in understanding general properties of membrane systems. Recent terahertz spectroscopy experiments provided new insights into dipolar relaxation and dynamics of water molecule reorientation in lipid bilayers with decreasing hydration level[1]. We perform molecular dynamics simulations of DOPC with varied levels of hydration. Our simulation models reproduce the experimental therahertz spectroscopy results with reasonable accuracy. Previously, three different types of water molecules were proposed that were described as irrotational water, bulk water, and fast water with distinct relaxation dynamics. We analyze single molecule dipole correlations in detail to study reorientational dynamics of water molecules in our simulated systems. Our results provide us with distributions of relaxation properties as a function of hydration level. We identify a population of water molecules which are tightly bound to lipid headgroups and exhibit relatively very slow relaxation dynamics. The remaining water molecules in the simulated systems, whose reorientational dynamics can be probed on the timescale of our simulations exhibit a broad heterogenous distribution of dynamical properties. This result suggests that models used to interpret experiments probing the reorientational dynamics of water molecules in a hydrated lipid bilayer should be based on a proper description of this distribution instead of isolated populations of water molecules with distinct properties.

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427-Pos Board B213

Understanding Plasma Memebrane Organization and Cellular Homeostasis Relationship

Susana Sanchez[1,2], María S. Jaureguiberry[3], Omar J. Rimoldi[3], Gabriela S. Finarelli[3], Maria A. Tricerri[3].
[1]Microscopy and Dynamic Imaging Unit. Vascular Biology and Inflammation Department. Fundación CNIC- Carlos III., Madrid, Spain, [2]Laboratory for Fluorescence Dynamics. UCI, Irvine, CA, USA, [3]Instituto de Investigaciones Bioquímicas La Plata (INIBIOLP), Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina.

Plasma membrane (PM) is a dynamic barrier which translates the outside signals to the cell. We are interested in how changes in the PM (composition, organization etc) influence cell homeostasis and vice versa. For this purpose we constructed two permanent Chinese Hamster Ovary (CHO KI) cell lines with permanent modifications in the PM lipid composition. The first line (SCD), over expressing Stearoyl CoA desaturase resulted in an increase of monounsaturated fatty acids at the PM phospholipids. The second cell line over expressing both Δ5 and Δ 6 desaturases (Δ 5/ Δ 6-cells), turned into a rise of poly unsaturated fatty acids and lower ratio cholesterol/ phospholipids.
Laurdan Generalized Polarization (Laurdan GP) microscopy and two-photon excitation, used to measure membrane packing (fluidity) in the PM of alive cells, showed for the SCD similar fluidity than control cells, indicating a metabolic compensation due to a relative increase in cholesterol content induced by the mutation. For the second cell line (Δ5/Δ6-cells) this compensation process did not occurred and the membrane fluidity increased compared with the control.
The MTT (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) reduction assay showed an increased viability in the two mutant cells as compared with the control. Cholesterol removal mediated by apolipoprotein A-I was measured by scintillation counting and showed to be reduced both cell lines. Together, our results indicate that PM lipid composition determines cholesterol partition, the feasibility of cholesterol to be exported and thus regulates its toxicity and homeostasis. Acknowledgements: This work was supported by US grant P41-RRO3155 to SAS and Argentinean grants ANPCyT (PICT 2106-2008) and CONICET (PIP 112-200801-00953) to MAT.

428-Pos Board B214

A Possible Mechanism on Pressure Reversal of General Anesthesia in Membrane

Eiji Yamamoto[1], Takuma Akimoto[1], Yoshinori Hirano[2], Kenji Yasuoka[1], Masato Yasui[3].
[1]Department of Mechanical Engineering, Keio University, yokohama, Japan, [2]RIKEN, Kobe, Japan, [3]Department of Pharmacology, School of Medicine, Keio University, Tokyo, Japan.

We propose a possible mechanism about pressure reversal of general anesthesia. The effect of general anesthetic can be controllable by the ambient pressure. To elucidate the mechanism of pressure reversal of general anesthesia, we perform molecular dynamics (MD) simulations for a membrane with anesthetics (xenon) by changing the lateral pressure. With the effect of xenon molecules in the membrane, decreasing of orientational order of lipid tails, increasing in area and volume per lipid, increasing in fluidity of membrane, and changing in electric potential of membrane are caused. We found that the properties of the lipid bilayer at high pressure are returned to that without xenon molecules at 0.1 MPa. Furthermore, we found that xenon molecules are distributed in the middle of the membrane at high pressures by the pushing effect, and the diffusivity of a xenon molecule is suppressed. These results suggest that the pressure reversal originates from a jamming of xenon molecules in the lipid bilayer.

429-Pos Board B215

The Action of Alpha Hederin, a Triperpenoid Saponin, on Membranes

Joseph Lorent.
Université catholique de Louvain, Brussels, Belgium.

α-hederin is a triterpenoid saponin which has shown hemolytic and apoptotic properties. (Chwalek et al. 2006, Gauthier et al. 2009). Many saponins are known to interact with biological membranes on a cholesterol dependent or independent manner (Francis et al. 2002) but the underlying mechanisms need to be further explored. We have studied the effects of α-hederin on size, membrane permeability and fluidity in a LUV model composed of DMPC including 25% (mol/mol) of cholesterol or not. Size was determined by quasi-elastic light scattering spectroscopy. Permeability was evaluated by the release of calcein, entrapped at self-quenching concentrations in liposomes. Membrane dynamics at the hydrophobic core was evaluated by fluorescence anisotropy of diphenylhexatriene, upon increasing temperatures.
α-hederin increased the size of cholesterol-rich liposomes and induced release of calcein entrapped within liposomes, on a time- and concentration-dependent manner. It was without effect when cholesterol was absent. α-hederin reduced the transition temperature on a concentration-dependent manner only in liposomes containing cholesterol but increased anisotropy beyond the transition temperature in both types of liposomes.
Our data suggest that cholesterol is required for α-hederin's effects on liposome's size, permeability and transition temperature but probably not for the effect on membrane fluidity, when lipids are in fluid phase. The role of these effects for apoptosis induced by α-hederin is under investigation.

430-Pos Board B216

Interaction of Novel Anticancer Drug Erastin with Lipid Bilayers Probed by Gramicidin A

Tatiana K. Rostovtseva[1], **Philip A. Gurnev**[1], Daniel Jacobs[1], Michael Weinrich[2], Michael Weinrich[2], Sergey M. Bezrukov[1].
[1]Program in Physical Biology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD, USA, [2]National Center for Medical Rehabilitation Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD, USA.

The novel anticancer agent erastin selectively targets tumor cells bearing oncogenic RAS. It is suggested that erastin induces cell death in human tumor cells through oxidative, non-apoptotic pathways by affecting the mitochondrial voltage-dependent anion channel (VDAC) and changing the permeability of the mitochondria outer membrane (Yagoda et al., 2007). Motivated by our findings on VDAC modulation by membrane lipids (ROStovtseva et al., 2006), we studied interactions of erastin and its physiologically inactive analog, A8, with planar lipid bilayers of different lipid composition. We used gramicidin A (gA) channel as a sensitive probe of membrane mechanical properties (Andersen, Koeppe, 2007). We found that erastin in micromolar range did not affect ion conductance of gA channel, but substantially increased the life-time of these channels. In contrast, addition of A8 produced a decrease in the channel life-time with a slight increase in its conductance. Thus, the two low-molecular-weight compounds whose structures are different by only one chlorophenoxy-group, have opposite effects on membrane mechanics, as monitored by the gA channel. These results may offer a new insight into the mechanism(s) of erastin physiological action. In addition, they may shed light on the inhibition of the VDAC-tubulin interaction by this agent (Maldonado et al., 2011). We have found that dimeric tubulin also modifies lipid bilayer properties as observed by changes in gA lifetime and conductance, and that these effects strongly depend on the membrane lipid composition. In contrast to the action of erastin that was similar in DOPC and DOPE/DOPC membranes, tubulin increased gA channel lifetime in DOPE but not in DOPC bilayers. Moreover, tubulin reduced the sensitivity of gA lifetime to erastin, thus suggesting that the inhibitory effect of erastin on VDAC-tubulin interaction is mediated through alterations in lipid bilayer mechanics.

431-Pos Board B217

Laurdan Fluorescence Lifetime as a Biosensor to Detect Differences and Changes in Membrane of Living Cells

Ottavia Golfetto, Elizabeth Hinde, Enrico Gratton.
University of California, Irvine, Irvine, CA, USA.

The cellular membrane directs and influences cell function through its structural and dynamical properties. It is generally recognized that the presence of cholesterol and lipid rafts in the phospholipid bilayers not only determines the physical rigidity of the membrane but also modulates the membrane fluidity and the capability for the cell to exchange lipid molecules. These are two fundamental factors which can explain the different ability of cells to move as well as reproduce and therefore play an important role in pathogenesis.
The lipophilic probe Laurdan has been used extensively to study synthetic and natural membranes since Laurdan is sensitive to membrane packing. The emission spectrum of Laurdan is shifted toward blue in the ordered lipid phase of the membrane (more rigid) and toward the green in the disordered lipid phase, the shift can be quantified by calculating the generalized polarization (GP).
Here we explore the fluorescence lifetime of Laurdan at different emission wavelengths in addition to the GP function. The basic idea is that the lifetime of Laurdan could be sensitive to the packing and also the average composition of the membrane. We observe changes in cell membrane packing after stimulation of COS7 cells with epidermal growth factor (EGF), and use Laurdan as a biosensor to monitor specific changes occurring in the membrane while the cell is migrating.