spreading response to TCR ligand. These data point to competing influences of TCR and ICAM-1 on immune synapse force generation and cytoskeletal fluidization. These effects may dictate the dynamics of T cell - antigen presenting cell interaction, modulating T cell activation.

PLATFORM P: Membrane Dynamics & Bilayer Probes

180-Plat
pH Dependence of the Adhesion Property of Lipid Bilayers and a Method of Measuring the Adhesion Energy
Yen Sun, Chang-Chun Lee, Huey W. Huang.
Rice University, Houston, TX, USA.
We found that phospholipid bilayers are adhesive to each other at pH values lower than 5, while they are not adhesive at pH values higher than 6. This is significant to membrane fusion occurring at low pH, and to membrane experiments using lipid vesicles. We used the experimental method invented by Evans and collaborators in which one flaccid GUV was released to adhere to one tensed GUV. We developed a new analysis method to measure the adhesion energy per unit area. This new method is independent of how the adhesion state was reached. The order of magnitude of the adhesion energy is ~0.01 to ~0.02 erg/cm2 for SOPC. The addition of SOPE slightly decreases the adhesion energy of pure SOPC, while the addition of cholesterol has little effect. The same method of measurement was applied to a case where two lipid bilayers underwent the first step of membrane fusion, called hemifusion. Hemifusion was induced by injecting 5 wt % PEG8000 solution at pH 4. The PEG injection was used to produce a transient osmotic depletion attraction between the two GUVs. The energy of hemifusion is one order of magnitude larger than the adhesion energy, about ~0.3 erg/cm2 for DOPC/DOPE/cholesterol (4:4:2). This is the first time the free energy of the membrane fusion intermediate state was experimentally measured.

181-Plat
Inter-Leaflet Coupling and Domain Formation in Asymmetric Giant Unilamellar Vesicles
Salvatore Chiantia, Erwin London.
State University of New York, Stony Brook, NY, USA.
Cell membranes exhibit an asymmetric distribution of lipids across the two leaflets of the bilayer. While the sphingolipid-rich outer (exoplasmic) leaflet has a lipid composition prone to lateral segregation and domain formation, the situation in the sphingolipid-poor inner (cytosolic) leaflet is unclear. The interaction between the two leaflets, although involved in several biological processes, is not well understood.
Although both supported and free-standing asymmetric bilayers have been previously produced, such approaches were based on a leaflet-by-leaflet assembly of the bilayer and, therefore, possibly incompatible with the reconstitution of transmembrane proteins. A highly reproducible solvent-free method that is fast, has a high yield and can be used with a wide variety of lipids and membrane proteins, is still needed.
Here, we introduce a simple lipid exchange method to obtain stable asymmetric giant unilamellar vesicles (GUV) that satisfies the above-mentioned requirements. The asymmetry of the GUVs is investigated using fluorescence correlation spectroscopy (FCS) in order to probe dynamics of lipids in the two leaflets. It is shown that introduction of up to 50% sphingomyelin into the outer leaflet of GUV composed of an unsaturated phosphatidylcholine (PC) in the inner leaflet results in a larger decrease in outer leaflet lateral diffusion than inner leaflet lateral diffusion. The degree to which the inner leaflet later diffusion decreases depends on the identity of the inner leaflet PC. We also show that a transmembrane protein can be incorporated into the asymmetric GUV and its diffusion probed by FCS. Thus, this approach can be used to investigate the molecular mechanisms behind inter-leaflet coupling and the effect of membrane asymmetry on transmembrane proteins.

182-Plat
Alcohol Effects on Lipid Bilayer Properties as Measured using a Gramicidin-Based Fluorescence Assay
Helgi I. Ingólfsson, Olaf S. Andersen.
Weill Cornell Medical College, New York, NY, USA.
Alcohols are known modulators of lipid bilayer properties and their anesthetic action was for long sole attributed to their bilayer modifying effects. More recently, it has been shown that alcohols can alter protein function through direct alcohol-membrane protein interactions. This raises the question to what extent the anesthetic/intoxicating action(s) of alcohols result from direct protein-alcohol interactions or from general changes in membrane properties. The efficacy of alcohols of varying chain lengths (different number of carbon atoms) studied in very different assays tend to exhibit a so-called ''cut off'' effect - increased potency with increased chain length, which then levels off and maybe declines above a certain chain length. The ''cut off'' chain length varies depending on the assay in question and numerous mechanisms have been proposed, e.g.: limited size of the alcohol-protein interaction site (binding pocket), limited alcohol solubility, and chain length-dependent lipid bilayer-alcohol interaction. To address these questions, and to explore the general chemical structure-activity relationship for amphiphiles' bilayer perturbing effects, we determined the membrane-modifying potential for a series of aliphatic alcohols using a gramicidin-based fluorescence assay. All the alcohols tested (chain-lengths varying between 1 and 10 carbons) alter bilayer properties, as sensed by a bilayer-spanning channel. The concentration at which they start affecting bilayer properties is higher or equal than the concentrations where they cause intoxication, equal or lower than the concentrations reported for direct alcohol-membrane protein interaction, and lower than the concentrations where they cause anesthesia. For short alcohol chain lengths, the alcohol's bilayer modifying potency scales linearly with alcohol bilayer partitioning, which then tapers off at higher chain lengths, reminiscent of a ''cut off'' effect - in a system where there is no alcohol binding pocket.

183-Plat
Electrical Response of Planar Lipid Bilayers to Ultrasound
Martin L. Prieto, Merritt C. Maduke.
Stanford University, Stanford, CA, USA.
Recent results *in vitro* and *in vivo* demonstrate that application of low-frequency ultrasound to neurons leads to activation of voltage-gated sodium channels (Na_v channels), thereby increasing the rate of action potential firing. These results suggest that modulation of brain activity by transcranial ultrasound may facilitate radical new approaches to behavioral neuroscience and treatment of neurological disorders. However, it is unclear what the mechanistic basis of this effect is, or even whether it represents a direct action of ultrasound on Na_v channels. To help answer these questions, and to better understand the interactions of ultrasound with membrane proteins in general, we have studied the electrical response of planar lipid bilayers under voltage-clamp to ultrasound (1 MHz). The response to sufficiently long ultrasound pulses consists of distinct dynamic ''on'' and ''off'' components with no apparent steady-state effects. The on and off responses are exponentially decaying sinusoidal current oscillations about the baseline level, and are identical except for the polarity of the current. For bilayers made of POPE and POPG (3:1 by weight), the frequency and decay constant of the on and off responses are approximately 1070 Hz and 610 s^{-1}. The current oscillation is dependent on the lipid composition of the bilayer, with bilayers made from cholesterol having higher frequency and larger decay constants. The electrical response is probably a capacitive current (under voltage-clamp, $I_C = V(dC/dt)$) due to distortions of bilayer structure by ultrasonic radiation force. We hypothesize that ultrasound-induced changes in bilayer structure may alter the activity of membrane proteins by changing the contribution of protein/bilayer hydrophobic mismatch to the free energy difference between protein conformations. This mechanism could underlie the activation of neuronal Na_v channels by ultrasound. The effects of ultrasound on hydrophobic mismatch can be investigated using gramicidin channels.

184-Plat
Static and Dynamic Disorder Observed in the Phase Transition Behavior of Individual Small Unilamellar Vesicles
Dimitrios G. Stamou, Poul Martin Bendix, Peter P. Wibroe,
Nikos S. Hatzakis.
Univ Copenhagen, Copenhagen, Denmark.
The first experiments able to record the activity of single molecules revealed that single molecules do not work constantly in time (a property termed dynamic disorder) and that not all molecules work with the same efficiency (static disorder). However very little is known about the stochastic behavior of individual biologically active functional units that comprise large molecular assemblies e.g. synaptic vesicles or signaling complexes. Here we extended our previous work on surface immobilized liposomes (see references) to investigate the phase transition properties of individual small unilamellar vesicles as a prototypic demonstration of the existence of static and dynamic disorder in the collective functional behavior of an integrated biomolecular assembly comprising several thousands lipid molecules.
Selected references:
Nature Chemical Biology, 2009. 5 (11); PNAS. 2009. 106 (30); JACS. 2008. 130 (44); Biophysical J. 2008. 95 (3).