Primary cilium function as specialized compartments for signal transduction in many tissues. The stereotyped structure and signaling function of cilia are inextricably dependent on the selective segregation of molecules in cilia, demonstrated by the finding that damage to ciliary protein transport mechanisms lead to a family of human genetic diseases known as ciliopathies. Despite its importance, the fundamental principles governing the access of soluble proteins to primary cilia remain unresolved, primarily because it has not been possible to monitor the kinetics of protein movement into these organelles in unperturbed live cells. We have developed a methodology termed Chemically-Inducible Diffusion Trap (C-IDT) based on chemically-inducible protein dimerization and live-cell microscopy to visualize the diffusion process of a series of fluorescent proteins ranging in size from 32 to 79 kDa into primary cilia. Contrary to previous reports equating ciliary import to nuclear import, we did not find evidence for a fixed diffusion barrier that excludes proteins above a particular size, the interior of the cilium was accessible to proteins as large as 79 kDa (~650 kDa). However, the kinetics of ciliary accumulation of this panel of proteins was exponentially limited by their Stokes radii. Quantitative modeling from our kinetic data suggests that the diffusion barrier operates as a molecular sieve. In addition to elucidating key physical properties, our study presents a set of powerful, generally applicable tools for the quantitative monitoring of ciliary protein diffusion under both physiological and pathological conditions. (Current under review at Nature Cell Biology)

164-Plat
Transmembrane Helix Association of ErbB Receptors Investigated by
Coarse-Grained Metadynamics Simulations
Mickael Lelimousin1, Vittorio Limongelli1, Mark S.P. Sansom1, 2
1University of Oxford, Oxford, United Kingdom, 2University of Naples “Federico II”, Naples, Italy.

Receptor tyrosine kinases (RTKs) are essential components of signaling at cell membranes. Members of the epidermal growth factor receptor family (ErbB receptors) play critical roles in regulating cell proliferation, differentiation and migration. Their activation involves the dimerization of alpha helices in the transmembrane (TM) domain of the receptors. Single point mutations in this region can modulate efficiency of helix dimerization and abnormal activation leads to human cancers. Therefore, it is important to determine the mode and the strength of the association in functional receptors and oncogenic variants. Various experimental and computational techniques have been recently used in this respect, which have provided substantial results. The development of innovative approaches might contribute to improve our understanding of the mechanisms of association.

Here, we present a new computational protocol that combines coarse-grained molecular dynamics and metadynamics, to investigate the dimerization of TM helices of ErbB receptors. In such a way the sampling of the dimerization process is highly enhanced and the free energy profile of the association computed. First, different coarse-grained models are assessed by comparison to experimental structures of homo and heterodimers. Then, metadynamics simulations are tested using different set of parameters to analyze the reliability and the reproducibility of the results. The final results are examined in reference to the literature data. This study provides a promising protocol for systematic investigation of RTK transmembrane domains in general. Possible extensions of the methodology are finally considered to treat larger part of RTK dimers as well as more complex clustering in membrane, representing a tool of general interest in biophysical research.

165-Plat
A Zoom on Membrane Fusion through Coarse-Grained, Atomistic
and Hybrid Molecular Dynamics of SNARE Proteins
Alex Tek1, Leonardo Darre2, Peter J. Bond3, Mark S.P. Sansom4, Sergio Pantoano5, Marc Baaden6.
1IBPC, CNRS UPR9080, Paris, France, 2Institut Pasteur de Montevideo, Montevideo, Uruguay, 3University of Cambridge, Cambridge, United Kingdom, 4SBCB Unit, Department of Biochemistry, University of Oxford, Oxford, United Kingdom.

The SNARE protein complex is central to membrane fusion. Modeling this system in order to better understand its guiding principles is a challenging task and requires the combination of original methods. We developed a hybrid representation mixing the SPC water model with a coarse grained (CG) model for solvation that can effectively mimic the hydration, structure, and dynamics of the SNARE membrane fusion complex, a trimeric protein-protein bundle embedded in a double phospholipid bilayer [1]. Comparison with a fully atomistic reference simulation illustrates the equivalence between both approaches. In this hybrid approach, bulk regions are treated at a CG level, while keeping the atomistic details around the solute. Since water represents about 80% of any biological system, this approach may offer a significant reduction in the computational cost of simulations without compromising atomistic details. In addition, we modeled the SNARE system at both CG level [2] and at full atomic detail [3]. By comparing a series of simulations where amino acid, membrane and electrolyte compositions are varied, we observe marked effects on bilayer curvature and deformations around the transmembrane domains, leading to a decreasing distance between them. If the link between membrane and bundle is severed, both membranes go back to a flat state.

[1] Darre et al. Mixing atomistic and coarse grained solvation models for MD simulations; let WT4 handle the bulk. JCTC, 2012. DOI: 10.1021/ci3001816

Computing time was provided by the French supercomputer centers IDRIS and CINES (Projects Nos 201207174 and LBT2411).

Platform: Membrane Physical Chemistry I

166-Plat
Physical Models for Early Membrane Growth and Evolution
Itay Budin1, Jack W. Szostak2.
1UC Berkeley, Berkeley, CA, USA, 2Massachusetts General Hospital, Boston, MA, USA.

Lipid membranes are a ubiquitous structure in biology and would have been an essential component of the first cells. In the absence of evolved cellular machinery, early membranes had to be capable of growth, division, and solute transport by intrinsic physical processes. We have used a combination of spectroscopic and physical methods to characterize the phase properties of fatty acids and related single-chain lipids, which have been proposed to serve as a role as primitive membrane lipids. We found that low concentration vesicle solutions have structure significant amounts of coexisting micelles, while high concentration solutions were predominantly lamellar (vesicles). This concentration-dependent equilibrium allowed us to drive the growth of pre-existing vesicles by gentle solution evaporation, which raises the lipid concentration thus favoring lamellar incorporation. This phenomenon could have provided a mechanism for early cell membrane growth driven entirely by environmental fluctuations. We have also characterized a very different growth mechanism that relies on the inter-vesicle exchange of monomers, which occurs rapidly for single chain lipids. Small amounts of double-chain lipids, such as phospholipids, drive the growth of fatty acid vesicles and the concurrent shrinkage of neighboring vesicles with fewer or no phospholipids. This phenomenon would have provided a direct selective advantage of the adoption of phospholipid-based membranes, a critical step in early cellular evolution. We have found that such a putative transition in lipid composition is accompanied by changes in the physical properties of the membrane, most notably a decrease in membrane fluidity and permeability. These findings support a model in which early cell membranes, composed of single-chain lipids, had the intrinsic permeability to allow for passive solute transport. The subsequent evolution of less permeable, phospholipid membranes would have then allowed for the adoption of protein-based transport machinery and internalized metabolite synthesis.

167-Plat
Mechanism of Solubilization of Phospholipid Liposomes, Revisited
Dov A. Lichtenberg1, 2.
1Tel Aviv University, Tel Aviv, Israel, 2Sackler School of Medicine, Tel Aviv University, Israel.

Solubilization of membranes by detergents, commonly described by phase diagrams, is an essential tool in membrane biochemistry and biophysics. In spite of its importance in assuring that the states of aggregation in lipid-detergent mixtures are at equilibrium, the mechanism(s) of solubilization proposed thus far are based on ambiguous analyses of indirect, not necessarily relevant evidence. Our recent analysis of the available data, particularly kinetic studies yielded better understanding of the mechanism. Specifically, at sub-solubilizing detergent concentrations, partitioning of detergent into the bilayers of small vesicles induces size growth via a disproportionation mechanism, at higher detergent concentration, in the range of coexistence of detergent-saturated vesicles and lipid-saturated mixed micelles (between the onset and completion of solubilization) electron micrographs show thread-like micelles and vesicles with pores of increasing size, in agreement with the leakage of entrapped solutes of increasing size. In several electron micrographs, thread-like micelles have been observed attached to perforated...
bilayers. These probably transient microstructures indicate that the micellization of detergent super-saturated vesicles occurs via a series of three stages: (i) bending of detergent-rich monolayers into curved thread-like cover of the perimeter of the holes, (ii) formation of thread-like micelles attached to the vesicles due to the line tension of the holes and (iii) detachment of the (most stable) mixed micelles from the vesicles. All the available data (spectroscopic, microscopic and calorimetric) are consistent with this mechanistic model.

168-Plat
Measuring Lipid Bilayer Bending Energy in a Dual-Beam Optical Trap
Mehmet E. Solmaz, Shalene Sankhagovit, Roshni Biswas, James R. Thompson, Camilo A. Mejía, Michelle L. Povinelli, Noah Malmstadt.
University of Southern California, Los Angeles, CA, USA.
While cell membrane bending is central to many physiological processes, techniques for measuring the bending energy of lipid bilayers have reported widely divergent results. Here, we show that a dual-beam optical trap (DBOT) can be used to apply finely controlled tensions to lipid bilayer membranes in a giant unilamellar vesicle (GUV) format. Optical force from the trap stretches the GUV; video microscopy is used to measure the change in membrane area during stretching. As laser power is increased, the surface area of the GUV also increases. Laser power is translated to membrane tension using a ray optics approach. The resulting tension-area relationship is fit to a model of membrane mechanics to yield a bending modulus.

The entire DBOT system is integrated with a microfluidic flow channel in such a manner as to facilitate the high-throughput analysis of large populations of GUVs. Performing such an analysis, we have shown that the presence of cholesterol affects the bending modulus of bilayers made from the unsaturated lipid 1-palmitoyl2-oleoyl-sn-glycero-3-phosphocholine (POPC). Both pure POPC bilayers and bilayers consisting of 80% POPC, 20% cholesterol have a bending modulus around 8 kT.

This technique is a promising route to detailed, accurate data relating lipid bilayer composition to membrane mechanical properties.

169-Plat
DNA-Based Patterning of Tethered Membrane Patches
Laura D. Hughes, Steven G. Boxer.
Stanford University, Stanford, CA, USA.
Solid-supported lipid bilayers are useful model systems for mimicking cellular membranes; however, the interaction of the bilayer with the surface can disrupt the function of integral membrane proteins. As a result, many groups have introduced tethered lipid bilayers, which retain the proximity to the surface, enabling surface-sensitive techniques, but physically distance the bilayer from the surface. We have recently developed a method for spatially separating a lipid bilayer from a solid support using DNA lipids (Chung and Boxer et al., J. Struct. Biol., 2009). In this system, a DNA strand is covalently attached to a silane-modified glass slide or SiO2 wafer. The complementary DNA strand conjugated to a lipid moiety is inserted into giant unilamellar vesicles (GUVs), and the DNA-modified GUVs hybridize to the strands on the surface, inducing flattening and rupture of the GUV to a planar tethered lipid bilayer. However, the location of the patch is random, determined by where the DNA-GUV initially encountered the membrane.

In this study, we present a method for creating spatially discrete tethered membrane patches on a glass slide using microarray printing. Surface-reactive DNA sequences are spotted onto the slide, incubated to covalently link the DNA to the surface, and DNA-GUVs patches are formed selectively on the printed DNA. Different DNA sequences can be printed on the same slide, creating a unique handle on each GUV patch. This handle enables the creation of patches of different lipid compositions, dyes, and/or DNA-lipid sequences in adjacent but distinct areas, and the control over the placement of the tethered lipid bilayer potentially allows interfacing with devices. This approach would also enable rapid screening of different patches in protein binding assays and as targets for membrane fusion.

Sleeping Bubbles: Effects of Volatile Anesthetics in the Lateral Structure of Giant Unilamellar Vesicles
Leonel S. Malacrida1, Arturo Briva1, Luis A. Bagatolli2, Pablo Aguilar3, Ana Denicola4.
1Área de Investigación Respiratoria, Departamento de Fisiopatología, Hospital de Clínicas, Universidad de la República, Montevideo, Uruguay, 2Laboratorio de Biología Celular de Membranas, Instituto Pasteur de Montevideo, Montevideo, Uruguay, 3Laboratorio de Fisicoquímica Biológica, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.
Volatile anesthetics have been widely used for more than 170 years. However, the mechanisms underlying the effects of anesthetics on membrane dynamics and structure are still under debate. Herein we study this problem for the first time using fluorescence confocal microscopy experiments of giant Unilamellar Vesicles (GUVs). This approach allows obtaining spatially resolved information on membrane structure at a microscopic level. GUVs were prepared using DLPC/DPPC 3:7 mol. This mixture showed a characteristic gel (Lbeta)/liquid disordered (Lalpha) phase coexistence at room temperature, with line-shape domains (Lbeta) of variable width depending on the temperature[1]. The volatile anesthetic used was Sevoflurane. The administration strategies were two: high concentration with reduced exposure time; or low concentration (clinically relevant) with long term exposition. A dramatic alteration of the phase coexistence was observed, with a marked effect on the morphology of the gel phase domains. For the experiments with high anesthetic concentration, the boundaries of the domains became diffuse with an increment of the domain’s perimeter/area ratio. When low anesthetic concentrations were used, a complete loss of the domains structure was observed with appearance of small circular shaped domains. Sevoflurane dramatically affected the lateral structure of the studied membranes, suggesting that similar mechanisms may occur in biological relevant membranes. Particularly, at low concentration of the anesthetic our results show some structural characteristic to that observed for cholesterol in canonical raft ternary lipid mixtures (formation of round domains). More research is underway to better understand the mechanisms underlying the sevoflurane effects on membranes.


The Influence of Noble Gases in Protein-Free Membranes and the Pressure Reversal Effect
Francisco J. Sierra Valdez, Cinvestav, Apodaca, N.L., Mexico.
The knowledge gathered so far reports a surprising variety of different chemical substances to induce anesthesia. The questions about how the noble gases modify the excitability of nerve cells and even how such excitability can be recovered under hyperbaric pressure remain open [1]. In the literature, one finds competing theories relating anesthesia to their effect on lipid membranes or on their effect on proteins, but the origin of the anesthetic effect is still not understood. Currently, the fashion of the biological mechanisms make us resort to proteins, however, in anesthesia, we must not neglect that anesthetics produce a melting point depression in pure lipid systems [2]. Furthermore, the pressure reversal in the effect produced by some anesthetics is well-known in animals, so that it seems hardly explainable regarding the protein receptors theory. Whereby, the intrinsic physical properties of the noble gases may give us hints to understand the general anesthetics mechanism. In the present work we show, for the first time, calorimetric results of the melting point depression phenomenon in protein-free membranes induced by noble gases, followed by a reversal effect of such depression with hydrostatic pressure. We finally correlate the electric polarizability of noble gases with the shift in the melting transition of the lipid membranes. Our results, in a pure lipid system, concur with other findings to underline the idea that anesthesia does not need a specific binding site in a protein and allow us to speculate that anesthesia only depends on the ability of certain atom or molecule to solubilized in lipids increasing the disorder of the membrane.

References

Restructuring of Membrane Bilayers due to Osmotic Pressure: Deuterium Solid-State NMR Study
K.J. Mallikarjunaiah1, Muwei Zheng, Emma P. Myers, Jacob J. Kinnun2, Horia I. Petre1, Michael F. Brown2.
1Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ, USA, 2Department of Physics, University of Arizona, Tucson, AZ, USA, 3Department of Physics, Indiana University-Purdue University, Indianapolis, IN, USA.
Lipid membrane composition and biophysical properties have substantial influences on cellular functions. Studies of environmental effects on membrane bilayers are a prerequisite for understanding membrane protein functions. Experimental measures of structural parameters like cross-sectional area/lipid of membrane bilayers are vital for molecular dynamics simulations [1,2]. We