We recently reported a new method for radial sizing of membrane nanotubes. The technique is based on imaging of membrane translocation from a lipid reservoir (multilamellar vesicle or cell with attached plasma membrane bleb) through the nanotube walls to a newly formed expanding vesicle. Since the nanotube radius is defined by the bilayer stiffness, the membrane bending rigidity modulus can be studied with this technique.
Here we have observed changes in bending rigidity of model membranes upon varying cholesterol content of the membrane (0, 5, 10 and 20 mol % cholesterol), exchanging type of membrane sterol (10 mol % of cholesterol, lathosterol, sitosterol, 7-dehydrocholesterol or lanosterol) and increasing salt concentration in solution (0, 100, 200 and 500 mM sodium chloride). We also have applied the method to analyze cell plasma membrane and detected changes induced by MβCD extrusion of cholesterol. The method proved to be quite sensitive for both artificial and cell plasma membranes and all obtained results are in agreement with previous studies.

222-Plat
Measurement of Lipid Bilayer Viscosity by Microfluidic Shear Transmission
Aurelia R. Honerkamp-Smith, Francis G. Woodhouse, Vasily Kantsler, Raymond E. Goldstein.
University of Cambridge, Cambridge, United Kingdom.
We use an experimental method for measuring shear transmission through an anchored hemispherical vesicle to estimate the viscosity for several different lipid membranes. We also validate predicted flow patterns and confirm a recently calculated universal ratio [1] for the flow geometry. Comparing experimental with calculated results provides insight about how membrane viscosity contributes to flow patterns in biological configurations, such as characean algae cells, where a lipid membrane experiences strong shear flow [2]. We apply a similar flow to vesicles containing an electrostatically coupled actin cortex in order to observe the effects of external flow on reorganization of proteins inside the vesicle.
References
1. F.G. Woodhouse and R.E. Goldstein, Shear driven circulation patterns in lipid membrane vesicles, Journal of Fluid Mechanics, 705, 165-175 (2012)
2. K. Wolff, D. Marenduzzo, and M.E. Cates. Cytoplasmic streaming in plant cells: the role of wall slip. Journal of the Royal Society Interface, v. 9 n. 71 p. 1398 (2012).

223-Plat
Effective Charge and Membrane Binding, Mixing, and Permeation of Lipopeptides Characterized by Zeta Potential Measurements
Mozhgan Nazari, Gaurav Raval, Zubeir Khan, Hiren Patel, **Heiko Heerklotz**.
UofT, Toronto, ON, Canada.
We demonstrate the use of zeta potential measurements of liposomes to address membrane binding of peptides and surfactants, membrane-induced protonation and counterion binding effects, membrane asymmetry and permeation, and membrane domain formation.
Instead of estimating membrane binding from the surface charge density by guessing the effective charge per molecule, we used what we refer to as an equi-activity evaluation to correct for binding and, hence, measure the effective charge. To this end, zeta potentials were recorded for an array of different lipid and peptide concentrations. It turns that the effective charge of a membrane-bound peptide is not straightforward to be guessed, because it may depend sensitively on membrane-induced (de)protonation and counterion-specific neutralization effects. The importance of the effective charge for trans-membrane flip-flop and interactions with other membrane components underlines the value of its direct measurement as explained here.
Another interesting feature of the zeta potential is that it specifically reflects the charge density in the outer leaflet of the liposome. This allows for addressing the asymmetric binding of a peptide and detecting its threshold for transmembrane equilibration due to bilayer asymmetry stress or pore formation. Finally, composition-dependent changes of the apparent charge already at low membrane content may indicate the formation of peptide-rich domains.
These approaches are demonstrated for the Bacillus lipopeptides surfactin and fengycin, as well as for SDS in different buffers.

224-Plat
A New Biomimetic Phase of Surfactant Bilayers Maintains Membrane Protein Activity
Vladimir Adrien[1,2], Gamal Rayan[1], Myriam Reffay[1], Martin Picard[2], Arnaud Ducruix[2], Amir Maldonado[3], Lionel Porcar[4], Nicolas Taulier[1], Wladimir Urbach[1].
[1]Laboratoire de Physique Statistique, Paris, France, [2]Laboratoire de Cristallographie et RMN Biologiques, Paris, France, [3]Departamento de Física, Universidad de Sonora, Hermosillo, Mexico, [4]Institut Laue-Langevin, Grenoble, France.
For several years lipidic cubic (Q) mesophases have been used to crystallize membrane proteins. Because they have the rheology of a thick paste, working with Q-phases remain a challenge. We will present a new fluid L_3 phase in which transmembrane proteins such as Bacteriodopsin, SERCA1a, and Cytochrome oxidase maintain their activity. Macroscopically a L_3 phase can be viewed as a sponge made of a surfactant whereas the holes in the sponge are filled with solvent. Topologically it consists of a single bilayer surrounded on either side by a solvent forming a continuous network of channels. It is comparable to a molten cubic phase, but it possesses water-like viscosity. Locally, it is similar to a lamellar phase but it is isotropic and optically transparent and thus suitable for spectroscopic studies. The L_3 phases presented here were characterized by polarized light microscopy, diffusion of a fluorescent probe by fluorescence recovery after pattern photobleaching (FRAPP) and freeze fracture electron microscopy (FFEM).
Tuning the distance between adjacent bilayers from 3 to 40 nm is an asset for the study of interactions between proteins. This is obtained by varying the water content of the phase. Characteristic distances (d_b) of the phase were obtained from small angle scattering spectra (SAXS/SANS) as well as from FFEM, which yielded similar d_b values: the L_3 phase preserves its structure when a transmembrane protein is incorporated into the bilayers, when the non-ionic co-surfactant is replaced by another one and when the temperature varies from 6°C to 30°C. These findings illustrate that a biomimetic surfactant sponge phase can be obtained in the presence of detergents widely used to solubilize membrane proteins and thus make it a versatile medium for membrane protein studies.

225-Plat
Modulating the Physical Properties of Micelles for Membrane Protein Investigations
Ryan C. Oliver[1], Jan Lipfert[2,3], Linda Columbus[1].
[1]University of Virginia, Charlottesville, VA, USA, [2]Kavli Institute of Nanoscience, Delft, Netherlands, [3]Delft University of Technology, Delft, Netherlands.
Micelle-forming detergents are used to solubilize integral membrane proteins for biochemical and physical characterizations. However, the unique properties of each membrane protein require exhaustive, and currently empirical, screening to optimize the detergent conditions which yield a stable protein-detergent complex (PDC). Detergent mixtures provide a means of expanding the available micellar environments, while also allowing select properties of micelles to be engineered. The properties of detergent mixtures must be well understood to correlate these properties with the stability of the protein fold and function. Using small-angle X-ray scattering, we determined the sizes and shapes of micelles formed by a comprehensive set of commercially available detergents commonly used with membrane proteins, and systematically assessed binary mixtures of these detergents. Micelle size and shape were determined directly from a Guinier analysis of the low angle data, the position of the second maxima at intermediate angles, and a core-shell model fit to the micelle scattering profiles. Many micelle properties, such as hydrophobic thickness, have a linear dependence on the micelle mole fraction. In addition to modulating the size of the micelle, other properties such as surface charge and fluidity can also be engineered. The results of this investigation can now be used to rationally design micelles for membrane protein investigations.

Workshop 1: Signaling Dynamics of Membrane Proteins in Living Cells

226-Wkshp
Probing G Protein-Coupled Receptor Signalling Complex Dynamics and Cellular Outcomes in Living Cells using Bioluminescence Resonance Energy Transfer (BRET)
Michel Bouvier.
University of Montreal, Canada, Montréal, QC, Canada.
G protein-coupled receptors (GPCRs) represent the largest family of proteins involved in signals transduction across biological membranes. In recent years, it has become clear that GPCRs are not uni-dimensional switches that turn 'on' or 'off' a single signalling pathway. Instead, each receptor can engage multiple signalling partners to form dynamic signalling complexes that can engage various downstream effector systems. Individual ligands can have differential efficacies toward specific subsets of the signalling effectors that can be regulated by a given receptor. This phenomenon, known as ligand-biased signalling,