Studying the fundamental physical interactions of supported lipid bi-layers with incorporated transmembrane proteins

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For many years, supported lipid membranes on solid surfaces have been used as models of biological membranes and as a physiological matrix for the study of the structure and function of membrane proteins and receptors.[1] These primitive models of the cell membrane surfaces do not allow for biologically applicable lateral diffusion of both lipid membrane and trans-membrane proteins, or membrane deformation during fusion. In this investigation, we are developing model surfaces in which, a tethered polymer will act as a "spacer" between the solid substrate and the lipid membrane, providing a hydrated cushion for bio-chemically active membrane proteins.

Using self-assembly chemistry, we deposit a thin film (~3-10 nm) of hydrophilic Polyethylene Glycol (PEG) polymer layer on mica substrate, to cushion a lipid membrane. Through vesicle fusion, a lipid composition of 85% 1-Palmitoyl-2-Oleoyl-sn-glycerol-3-Phosphocholine (POPC) and 15% 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (DOPS), was injected onto the polymer within an aqueous buffer to form the cushioned membrane. We monitored the lateral diffusion of the lipids in the bi-layer using confocal fluorescence recovery after bleaching (FRAP). We have measured a lateral diffusion coefficient for our system of ~ 6 $\mu m^2/s$, which for solid surface supported lipids is ~10^{-3} $\mu m^2/s$. Therefore, we have developed a mobile lipid bi-layer surface suitable to incorporate trans-membrane proteins, without denaturing them.

The next step will be to incorporate membrane proteins in the cushioned membranes, followed by force measurements with the Surface Forces Apparatus (SFA). We aim to specifically examine Synaptotagmin 1 (syt1) protein, which is a synaptic vesicle anchored membrane protein that acts as the calcium sensor for neuro-transmission, to observe how binding is affected by the curvature, fluidity and deformity of the lipid bi-layer.[2]

[1] Michael L. Wagner and Lukas K. Tamm 2000. "Tethered Polymer-Supported Planar Lipid Bilayers for Reconstitution of Integral Membrane Proteins: Silane-Polyethyleneglycol-Lipid as a Cushion and Covalent Linker". *Biophysical Journal Volume 79 1400–1414*

[2] Weiwei Kuo, Dawn Z. Herrick, and David S. Cafiso 2011. "Phosphatidylinositol 4,5-Bisphosphate Alters Synaptotagmin 1 Membrane Docking and Drives Opposing Bilayers Closer Together". *Biochemistry 2011, 50, 2633–2641*