Experimental Report

Proposal: **9-13-511** **Council:** 10/2012

Title: Lipid flip-flop on supported silica particles: surface and curvature effects

This proposal is a new proposal

Researh Area: Biology

Main proposer: **PEREZ-SALAS Ursula**

Experimental Team: PEREZ-SALAS Ursula
PORCAR Lionel
BREIDIGAN Jeffrey

Local Contact: PORCAR Lionel

Samples: Silica -SiOx
DMPC - C36H72NO8P
POPC -C42H82NO8P
D2O and H2O
ethanol - C2H6O

Instrument	Req. Days	All. Days	From	To
D22	3	2	23/07/2013	25/07/2013

Abstract:

In biological membranes compositional asymmetry across organelles as well as between two contiguous leaflets belonging to the same membrane is essential for function. The passive movement of lipids tends to uniformly distribute and homogenize membranes. This is energetically helpful during the distribution of lipids across the cell but it is not helpful when gradients have to be maintained. Gradients are maintained by the active work of proteins that act against homogenization. The work needed depends on the transport characteristics of these lipids. The energetics of the passive movement of lipids in membranes gives the protein-assistance burden necessary to keep the lipid compositional gradients required for function. Using TR-SANS we were able to measure the flipping and exchange rates of cholesterol and lipids and found them to be slow. In order to decouple exchange and flipping rates we used TR-NR to find that the flipping rates, however these were fast. We believe the surface and curvature are having a strong effect. We propose to use membrane coated silica nano particles to confirm these effects.

Proposal #: 9-13-511

Experimental report:

In biological membranes compositional asymmetry across organelles as well as between two contiguous leaflets belonging to the same membrane is essential for function. The passive movement of lipids tends to uniformly distribute and homogenize membranes. This is energetically helpful during the distribution of lipids across the cell but it is not helpful when gradients have to be maintained. Gradients are maintained by the active work of proteins that act against homogenization. The energetics of the passive movement of lipids across membranes and the membrane's bilayered structure determines the energetic load on these proteins.

Using TR-SANS it is possible to measure the rates of the movement of lipids across leaflets in a membrane as well as the rates of exchange between membranes [1, 2, 3]. By measuring these rates at different temperatures one can extract the energetic barrier for these processes [1]. Using TR-SANS we found that the flipping rates for cholesterol were much slower than was suggested by previous work [4] while the exchange rates, were consistent with those reported earlier. To explain this apparent discrepancy we argued that the structure of the molecule has a significant impact on its kinetics characteristics and therefore other techniques studying DHE, for example, which is a naturally fluorescent analogue of cholesterol has very different transport and energetic characteristics [1]. Our measurements of cholesterol versus DHE show DHE moving across membranes significantly faster than cholesterol. However, even though our argument (and data) explains this basic discrepancy, in our measurements the effect of flipping and exchange are coupled. In order to decouple the contribution of exchange and flipping we used time resolved neutron reflectivity (TR- NR). For long chain fatty acid chain lipids, however, it was found that the flipping rates were fast rather than slow [5]. To explain this apparent contradiction, we suspect that the surface and/or the curvature of the membrane are having a strong effect on the movement of the lipids. We therefore have been working on studying the movement of lipids on membrane coated silica nano particles to determine what is source of these very different results.

During our experiment 9-13-511 we verified that making supported lipid membranes on silica particles was possible for both hydrogenated and deuterated DMPC on 28nm (in diameter) silica particles through a solvent exchange procedure [6]. SANS data on bilayers of self-assembled lipids onto 28 nm in diameter silica nanoparticles are shown in figure 1. Lipid self-assembly on silica nanoparticles is done at 40-50% ethanol. Once a bilayer of lipid coats the silica particles, the solution is diluted with water to reduce the amount of ethanol in the sample. However, because it is known that ethanol increases the permeability of membranes significantly which would also affect flipping and exchange rates, it is important to remove it. Dialysis was used to remove ethanol from the solvent, however this caused the coated particles to aggregate. The onset of aggregation can be seen in figure 2 where excess scattering is observed in the low-Q region as a result of ethanol removal (while keeping the concentration of lipid coated silica constant). Notwithstanding, we compared the exchange and flipping lipid rates of DMPC (hDMPC and dDMPC) in supported and free membranes on 28 nm silica particles (figure 3). The

presence of the surface appears to slow down the movement of lipids, which is a surprising and unexpected result.

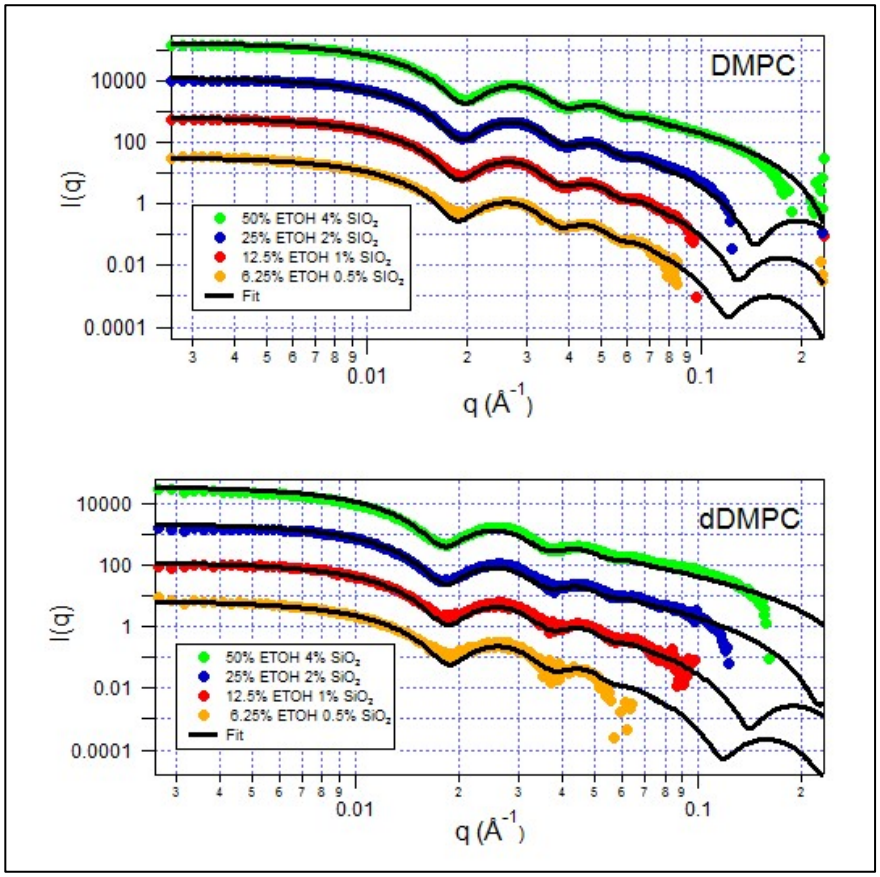

Figure 1: DMPC and dDMPC supported lipid bilayers (SLB) on 28 nm silica particles (CM to the solvent) at different ethanol concentrations. A polycore one shell model suggest that there may be solvent between the inner leaflet and surface of the silica particle. During the process of dilution the bilayer increases in thickness and ethanol leaves the bilayer. This agrees with the μDSC results which also shows ethanol leaving the bilayer upon water dilution.

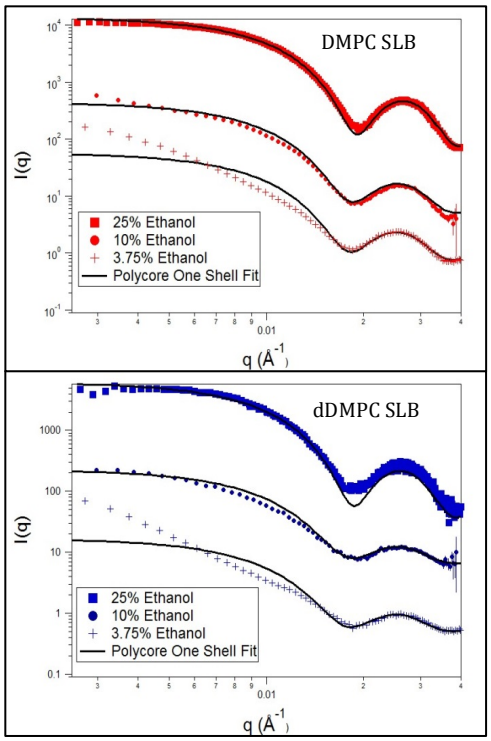

Figure 2: DMPC and dDMPC SLB on 28 nm silica at different ethanol concentrations. The silica particles remained, for all ethanol concentrations, constant at 1%. A polycore one shell model suggest that there may be solvent between the inner leaflet and surface of the silica particle. During the process of dilution the bilayer increases in thickness and ethanol leaves the bilayer. This agrees with the μDSC results which also shows ethanol leaving the bilayer upon water dilution.

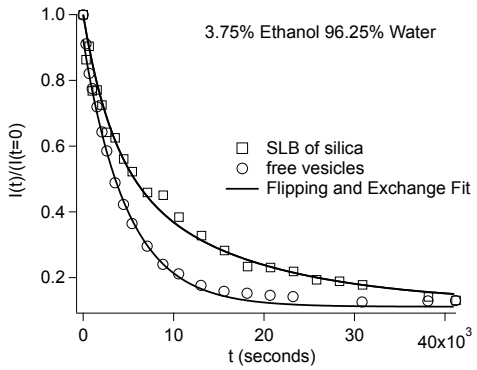

Figure 3: Total normalized intensity curves as a function of time for 3.75% ethanol 1% silica lipid coated 28nm nanoparticles – squares – and free 30nm (in diameter) vesicles – circles. The q-integrated form factors excluded the aggregation region shown in **Figure 2** (T= 35ºC)

We are working on strategies to completely remove ethanol from the system – by doping the supported membranes with charged lipids. We are also working on suspensions of large 100 nm silica particles from different companies to study both the contribution of curvature and the variability of the silica surface. In this way we will verify if the kinetic results from 9-13-511 still hold. A proposal is being submitted to continue this work.

1. S. Garg S., Porcar L. Woodka A.C.,Butler P.D. , Perez-Salas U. 2011. Biophys. J. 101:370–377
2. S. Garg S., Porcar L. Woodka A.C., Butler P.D. , Perez-Salas U. 2012. Biophys. J. 102:947–949
3. Nakano M., Fukuda M., Kudo T., Matsuzaki N., Azuma T., Sekine K., Endo H., Handa T. 2009. J. Phys. Chem. B 113: 6745–6748
4. T. L. Steck, J. Ye, Y. Lange. 2002. Biophys. J. 83(4): 2118–2125
5. Y. Gerelli, *et al*. 2013 Langmuir 29:12762-12769
6. A.O. Hohner *et.al*. 2010. Biointerphases (1)1-8