Experimental report

19/11/2016

Proposal: 9-13-650 **Council:** 4/2016

Title: STRUCTURE OF POLYMER-SUPPORTED LIPID BILAYERS DERIVED FROM NATIVE CELL-MEMBRANE VESICLES

Research area: Biology

This proposal is a new proposal

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Samples: Native membrane extract from Spodoptera frugiperda
synthetic lipids

Instrument	Requested days	Allocated days	From	To
FIGARO	2	0		
D17	2	2	26/09/2016	28/09/2016

Abstract:

Recently a generic method for producing polymer-supported lipid bilayers (pSLBs) directly from cell-derived native membrane vesicles (NMVs) was discovered. These pSLBs contain essentially all the naturally occurring cell-membrane components of the donor cell line or organelle while still retaining transmembrane protein mobility and activity. These surfaces offer a new paradigm in SLB-based biomimetic surfaces and bioanalytical sensor design. While fluorescence microscopy studies have indicated that there is at minimum a 5 nm hydration layer between the pSLB and the underlying substrate, the use of neutron reflectivity is expected to provide better insight into the architecture of these complex hybrid pSLBs.

EXPERIMENTAL REPORT FOR PROPOSAL# 9-13-650:

STRUCTURE OF POLYMER-SUPPORTED LIPID BILAYERS DERIVED FROM NATIVE CELL-MEMBRANE VESICLES

We recently published a procedure on how to form polymer-supported lipid bilayers (pSLBs) from native membrane vesicles (NMVs) to produce a new class of cell surface mimic. [1] Results in our previous publication indicate that there is a hydration cushion between the pSLB and the substrate that is ~5 nm. We have employed neutron reflectivity (NR) to provide an independent measure of the hydration cushion and to characterize the structure of the NMV-derived pSLB.

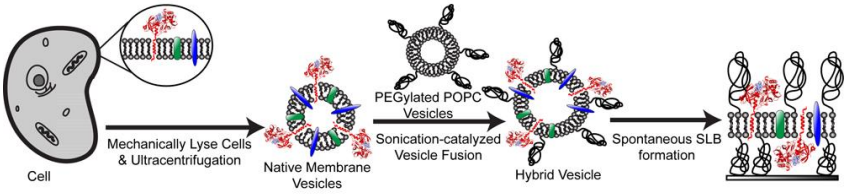

Figure 1: Schematic representation of the method to create polymer supported lipid bilayers from native membrane vesicles. Reprinted with permission from Pace et al.[1]

In order to model the complexity of the NMV-derived hybrid pSLB a simpler system was first measured and modeled; a purely synthetic, 0.5 mol% PEG5000cerimide_99.5 mol% POPC (0.5%PEG_POPC) pSLB. In all systems studied deuterated POPC (16:0-d31-18:1 PC) was used together with 4 different contrast variations (100% D_2O, 3MW, SiMW, and 100% H_2O) to enhance the ability to resolve the structure of the pSLBs. NR data analysis was done using the Aurore program.[2]

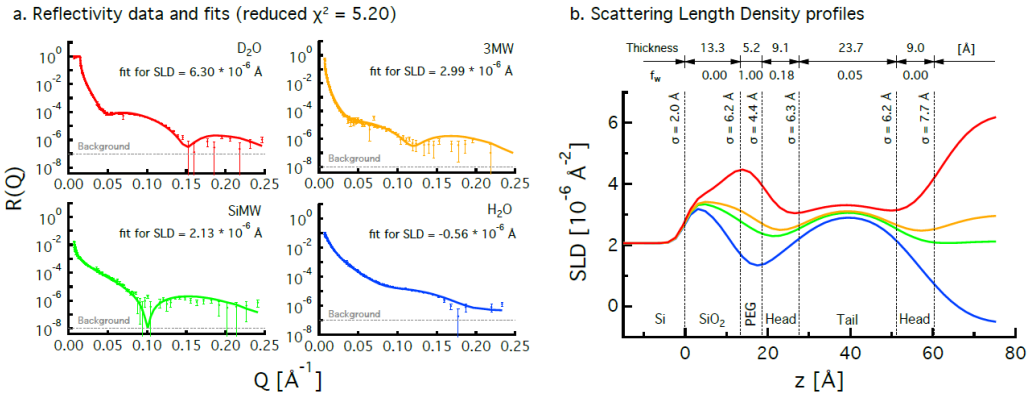

Figure 2: Neutron reflectivity data from a 0.5%PEG_POPC pSLB on a Si (111) substrate. (a) Experimental (symbols) and simulated (lines) for reflectivity versus Q [$Å^{-1}$]. (b) Scattering length density profiles corresponding to the fits reported in (a). The thickness and water fraction (f_w) of each modeled layer, as well as, the roughness (σ) at each interface have been annotated. The same color code is used in (a) and (b).

Figure 2a shows the fits of the four contrasts for a 0.5%PEG_POPC pSLB on Si(111). The corresponding SLD profiles are shown in Figure 2b. The 0.5%PEG_POPC pSLB has a bilayer thickness of 41.8 Å (9.1 + 23.7 + 9.0 Å) with ~5% of the lipid tail region's volume containing defects. The hydration cushion between the pSLB and the native oxide of the Si (111) layer was estimated to be 5.2 Å and ~100% solvent. While this thickness is not consistent with our previous FM results[1], SLBs without PEG tend to rarely be modeled as having more than ~1 Å, if at all[3,4]. This indicates that the PEG is indeed having a measurable effect on the thickness of the hydrated cushion.

The model derived from the 0.5%PEG_POPC pSLB was used as a starting point for modeling the NMV-derived pSLB (0.5%PEG_NMV_POPC). The hybrid vesicles used to make this pSLB were a mixture of 0.5%PEG_POPC and NMVs that were merged via sonication. Thus the resulting pSLB contains native lipids and proteins which alters the reflectivity profiles of the pSLB, as shown in Figure 3. However, the preliminary fitting of the reflectivity profiles also show that many aspects of the pSLB are conserved. The thickness of the pSLB remains the same (41.8 Å) and the volume fraction of "defects" in the lipid tail region has a modest increases from 5% to 6%. The biggest contrast in comparison to the synthetic 0.5%PEG_POPC system is the decrease in the SLD of the tail region from 3.16 to 2.63×10^{-6} $Å^{-2}$. Based on the calculated SLD of the tail region, it can be estimated that the native lipids and proteins make up ~20-25% of this region, a value which agrees with estimations based on the mass ratio of NMV to 0.5%PEG_POPC mixed together to form the hybrid vesicles[1]. Additionally the NMV-derived pSLB had a higher roughness at the interfaces of both the head and tail regions, presumably due to the added diversity of lipids with different tail lengths and head groups and presence of transmembrane proteins.

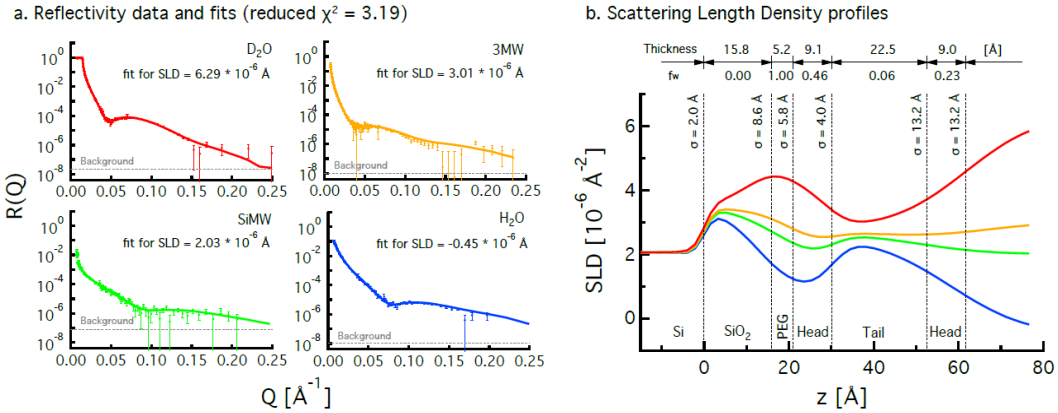

Figure 3: Neutron reflectivity data from 0.5%PEG_NMV_POPC pSLB on a Si (111) substrate. (a) Experimental (symbols) and simulated (lines) reflectivity for reflectivity versus Q [Å^{-1}]. (b) Scattering length density profiles corresponding to the fits reported in (a). The thickness and water fraction (f_w) of each modeled layer and the roughness (σ) at each interface have been annotated. The same color code is used in (a) and (b).

We also measured the effect on the thickness of the hydration cushion when the concentration of PEG5000-conjugated lipids in the NMV-derived pSLBs was increased from 0.5 to 1.0 mole %. Previous reports have shown that the thickness of the polymer cushion below the pSLBs can be tailored through the incorporation of different lengths of PEG polymer or different surface concentrations.[5] Additionally, different combinations of PEG length and surface density have been reported to affect transmembrane protein mobility.[6] As shown in Figure 4a, the increase in PEG concentration within the pSLB has an effect on its structure; however, further modelling is required before we can conclusively state that there is an increase in the thickness of the hydration cushion beneath the pSLB.

In preparation for our beam time we also investigated SLBs on Si (111) substrates using fluorescence microscopy (FM) to determine which cleaning protocol was preferred for that specific substrate. For these experiments SLBs were formed via vesicle fusion from vesicles that were 1 mol % rhodamine-DOPE and 99 mol% POPC (Rho_POPC). Fluorescence recovery after photo-bleaching (FRAP), where the diffusion of fluorescent lipids filling in a bleached spot as a function of time, is a commonly used method to determine both the presence and quality of a SLB on a surface. As shown in Figure 5, the quality of the SLBs observed on Si (111) was hard to asses with FRAP due to a combination of fluorescence quenching[7] and a large number of mobile, adsorbed vesicles on top of the SLBs (middle row) in comparison to SLBs formed on the usual FM substrate (top row). SLB experiments done with FM are usually done on borosilicate substrates (RMS ~3Å), whereas QCM-D, in which we observe formation of prober SLBs with no detectable vesicles on top, utilizes a 50 nm sputtered silica oxide coating (RMS ~1.2 nm). We thus sputtered a 20 nm SiO_2 layer onto the Si (111) wafer to test its effect (bottom row). The addition of the sputtered SiO_2 resulted in a bilayer free from any noticeable adsorbed vesicles on the top. It is plausible that the sputtered SiO_2 attenuates the attraction forces between the vesicles and the Si (111) substrate. If this explanation is true, it can also explain our NR data in Figures 2 and 3, which suggest a smaller hydration cushion than expected based on our previous work[1].

While the use of Si (111) substrates for SLB studies using NR is well established, previous studies have shown that quartz is amenable to both NR and FM experiments with SLBs. Thus, we investigated if there was any structural differences in the pSLB when formed on top of a quartz NR block. As Figure 4b shows, there is a pronounced difference in the reflectivity profiles of a 0.5%PEG_NMV_POPC pSLB on either a Si (111) (red) or quartz (orange) substrate. While the modeling is still being investigated, there appears to be a big effect on the structure of the SLB. The change in profile suggests that the pSLB is much thicker, very likely due to a thicker cushion, and is also much rougher. The increased roughness (σ) could be arising from an increase in z-dimensional fluctuations caused by decoupling the surface from the underlying substrate.

Additional data about the effect of vesicle concentration on the thickness of the hydration cushion between the pSLB and the substrate is still to be processed. It will appear in the next update of the experimental report.

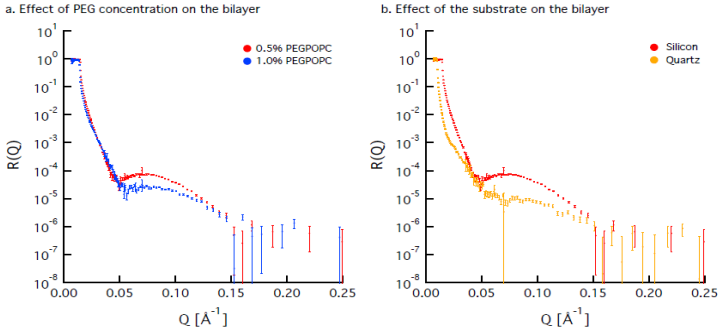

Figure 4: Non-modeled raw neutron reflectivity profiles in 100% D_2O (a) comparing 0.5%PEG_NMV_POPC (red) vs 1.0%PEG_NMV_POPC (blue) pSLBs on Si (111) substrates and (b) comparing 0.5%PEG_NMV_POPC pSLBs on either a Si (111) (red) or quartz (orange) substrates.

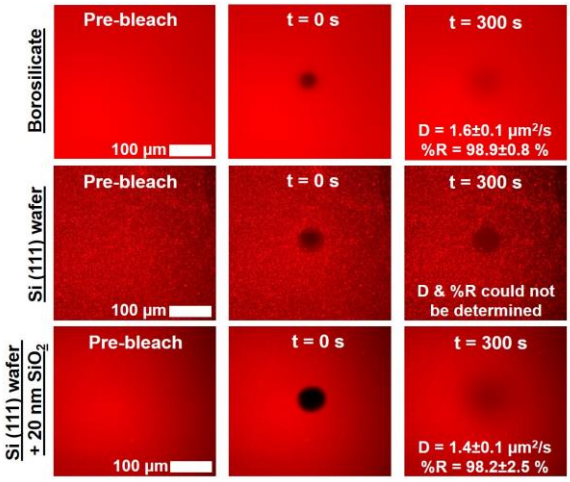

Figure 5: Representative epifluorescence microscopy images of Rho-POPC SLBs on various substrates: Borosilicate (top row), Si (111) wafer (middle row), and Si (111) wafer with a 20 nm sputter coated SiO_2 layer (bottom row). The columns show the dissipation of a bleach spot as a function of time (FRAP). Calculated diffusion coefficient (D) and percentage recovery (%R) values for the rhodamine-conjugated lipids is annotated. Scale bars are 100 µm. The size of the bleach spots differs due to distance between the substrate and the objective.

Conclusions

The aim of this proposed beam time was to study the structure of the NMV-derived pSLB formed under conditions used in our previous publication and also to investigate how this structure changed if either the concentration of PEG-lipids in the pSLB was increased or if the pSLB was formed at higher vesicle concentration. We managed to attain the data needed to achieve the aims of our proposal. In particular, we have models (still being refined) of both 0.5%PEG_POPC and 0.5%PEG_NMV_POPC pSLBs which indicate that the presence of PEG does increase the hydration cushion underneath the pSLB, although not to the expected degree. We have also estimated that the NMV-derived pSLB contains ~20-25% native membrane material (lipids and proteins). While not fully modelled yet, it also appears that increasing the concentration of the PEG in the pSLB might result in a thicker hydration cushion.

In order to understand the effect of the underlying substrate (silicon vs. quartz) on the structure of the pSLB and how this is related to our FM results, pSLBs were investigated on both Si (111) and quartz blocks with NR. Our preliminary investigation indicate that the pSLBs formed on the Si (111) substrates showed a reduced thickness of the hydration layer below the pSLB and the substrate in comparison to pSLBs formed on the quartz (data still to be further modelled). This, along with our FM measurements tentatively suggest that the Si (111) substrate exhibits an attractive force on lipids that draws them closer to the substrate when compared to either sputtered silica or quartz substrates.

Our next proposal focuses on how to optimize the amount of native membrane material that can be incorporated in the pSLB. These two proposals taken together with complementary fluorescent and QCM-D measurements will provide a clear correlation between the native membrane content, architecture, and quality of our NMV-derived pSLBs as a function of preparation protocol. Thereby, we will contribute the foundation for a plethora of future experiments studying phenomena that occur at the cell surface via this new class of NR-compatible biomimetic surfaces. Additionally, the experiments focusing on the effect of substrate on the structure of the pSLB should produce a publication that is of high impact for the SLB-NR community by clearly showing the disconnect between SLB studies on Si (111) and other substrates, as well as the existence of electrostatic / dispersion forces that might be sufficiently large to influence the adsorption properties of SLBs on Si (111).

References

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