Phosphatidyl serine containing liposomes on titania: phase behaviour, bilayer formation, and lipid asymmetry.

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Interactions between surfaces of inorganic materials and biological systems are important in numerous technological contexts (implant integration, biosensor development). They also present basic challenges. For example, the role of surface ion equilibrium in the biological response to the material is not well understood, although a casual link between the two has been proposed.[1] Here, we investigate the behavior of phosphatidyl serine (PS)-containing liposomes on TiO_2. Our interest in the PS is due to its crucial role in blood coagulation: platelets (small anuclear cell fragments circulating in the blood responsible for maintaining haemostasis) expose PS upon activation, converting an inert outer membrane surface into a reactive, pro-coagulant one.[2] Blood coagulation, on the other hand, is one of the first responses of the body to the introduction of a foreign implant material. As a model biomaterial surface we focus on titania, because of its wide range of applications in implants.[3] Unraveling its interactions with biological systems, in particular various blood components,[1,4,5] is crucial for understanding implant integration and rejection processes.
It is known that Ca ions bind to both TiO_2[6] and to PS.[7] Therefore, we studied the behavior of PS-containing liposomes on TiO_2 as a function of PS content and Ca concentration (Figure 1).[8, 9] We determine a “phase diagram”, where a percolation-type transition between adsorbed liposomes and supported bilayers is observed, describe the driving force for this transition, and identify the role of surface heterogeneities in this process. Finally, we quantify the distribution of PS in the resulting supported bilayers by neutron reflectometry (Figure 2).

References

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Figures

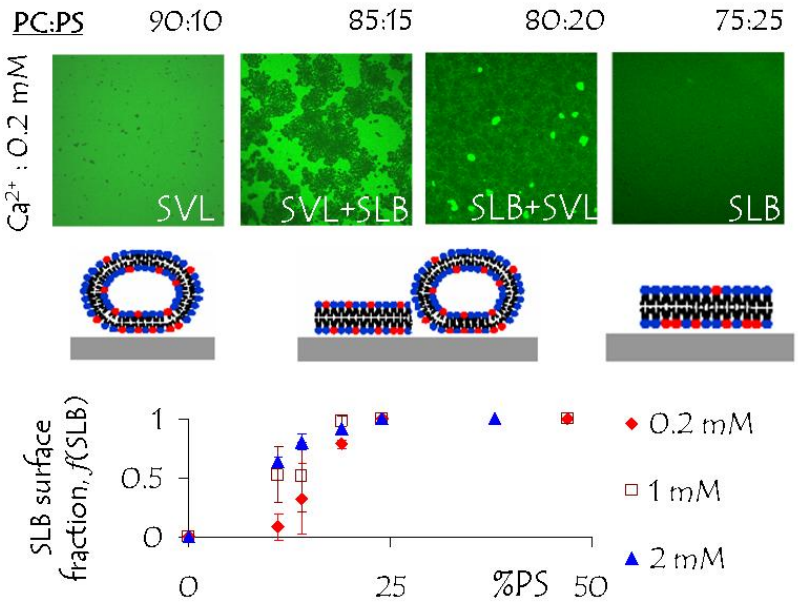

Figure 1: Studying "phase behavior" of PS-containing liposomes on TiO_2. Top row: Phosphatidyl serine containing liposomes of various compositions were incubated with TiO_2-coated glass slides at a Ca^{2+} concentration of 0.2 mM. Three types of structures were observed, as indicated with the labels and diagrams below the images. SVL, or supported vesicular layer, consists of liposomes that adsorb to the surface and remain intact. At higher PS contents, the adsorbing liposomes open and form a continuous SLB, or supported lipid bilayer. Intermediate PS contents lead to intermediate structures, where both non-ruptured liposomes and continuous bilayers are present on the surface. Identification of the structures was based on the fluorescence images, as well as atomic force microscopy and quartz crystal microbalance data (now shown). Fluorescence images are 149 x 149 μm^2 **Bottom row:** The fraction of the surface occupied by the supported lipid bilayers was quantified from the fluorescence images such as the ones shown above, and plotted as a function of PS content in the liposomes for three different Ca concentrations.

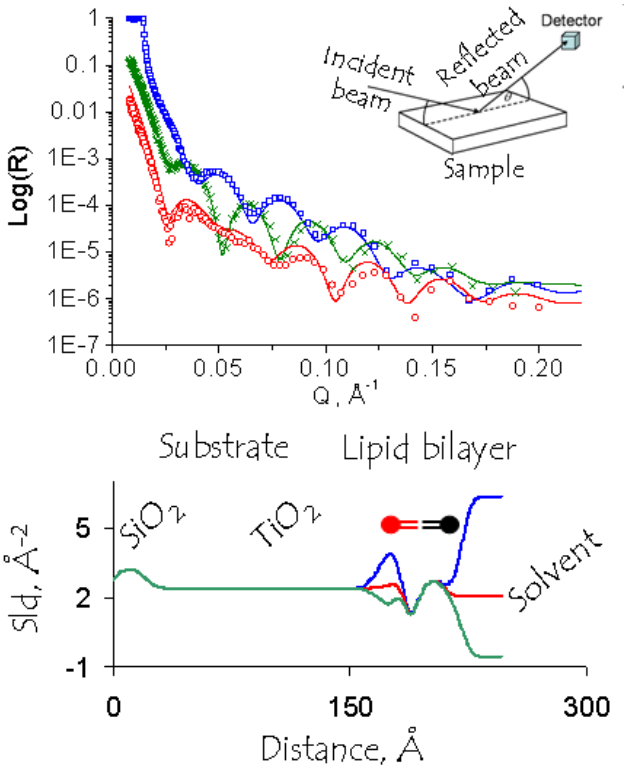

Figure 2: Quantifying bilayer composition by neutron reflectometry. Top: Experimental reflectometry profiles acquired from a supported lipid bilayer prepared on TiO_2 from liposomes containing deuterated POPC and hydrogenated DOPS in a ratio 65 : 35. Experimental geometry is shown in the inset. The measurement probes the distribution of material along the direction normal to the surface. The results are plotted as the ratio of reflected beam intensity to that of the incident beam as a function of inverse spacing. This inverse spacing can be thought of as the size of the "window" probing the interface. The smaller the window (the larger the inverse spacing), the more fine details can be resolved. Lines are calculated reflectometry profiles based on the model shown below. **Bottom:** The substrate consists of an SiO_2 layer and a TiO_2 layer. The bilayer is modeled as four layers: two leaflets, one facing the surface (red) and one facing the solvent (black), with each leaflet divided into the headgroup region of the lipids (circles) and the chains region of the lipids (lines). Scattering length density (sld), plotted on the Y-axis, is analogous to refractive index in optics and is a function of layer composition. It is used to calculate the composition of each layer in terms of %dPOPC and %DOPS. In this case, the surface-facing layer is found to contain ~ 70% DOPS, while the solution facing layer – only ~ 10%; the bilayer is highly asymmetric. Three colors in both plots correspond to measurements performed in solvents that have different sld's. If a measurement is done in one solvent only, it may be impossible to find a unique model.[10, 11]