ESRF	**Experiment title:** Interaction of solid-bound lipid membranes with proteins at the solid-liquid interface under high hydrostatic pressure	**Experiment number**: SC-4148
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Report:

In a former experiment at beamline ID15A (see experimental report SC3806), we investigated the phase behavior of solid-supported multilayers of the phospholipid DMPC (1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine) at high hydrostatic pressure. We were able to determine phase boundaries between the liquid phase und different gel phases and, moreover, observed specific effects like a pressure-induced multilayer formation and a pressure-dependent filling of the water layers between the separate lipid bilayers in a multilayer system [1]. Extending the model, we now went one step forward to a more biologically relevant structure and added cholesterol to the investigated lipid layers, as in nature, membranes are highly complex systems that are interstratified by cholesterol and proteins.

In order to obtain information about the vertical structure of solid-supported DMPC membranes with cholesterol, we conducted high energy x-ray reflectivity measurements at the solid-liquid interface between silicon and an aqueous buffer solution. These measurements were performed in a custom-made high hydrostatic pressure cell [2] and at a photon energy of 70 keV. The beam size was approximately 5 µm (vertical) $\times$ 40 µm (horizontal) with a photon flux of about 10^{12} photons per second and square millimeter. Pressures up to 5 kbar and temperatures of 20 °C and 37 °C were applied. Our samples were prepared on silicon wafers by a spin coating process. For this purpose, 2 mg DMPC and different amounts of cholesterol up to 28 mol% were dissolved in 2 ml 2-propanol.

At 37 °C, we were able to investigate the effects of cholesterol on the pressure-dependent main phase transition of DMPC membranes from the liquid phase into a gel phase. We observed a decrease of the critical pressure

and an expansion of the transition area. At 20 °C, the lipid layers show an approximately linear modification of their thickness with increasing pressure, so that it is possible to describe their behavior by a linear compressibility. Notably, we observed a fundamental difference between the compressibilities of bi- and multilayers. The compressibility is monotonically decreasing with increasing amount of cholesterol undergoing a change of sign at about 25 mol% in the bilayer case, while it is non-monotonic and permanently negative in the multilayer case. At both temperatures, bi- and multilayers were studied in order to obtain information on the influence of the substrate by comparing these different layer structures. [publication in preparation]
In figure 1, some of the reflectivity curves and corresponding density profiles are shown. The data of a pure DMPC bilayer and a multilayer containing a high amount of cholesterol, each at different pressures, are depicted. The obtained knowledge on the behavior of solid-supported lipid membranes enables further planned measurements regarding lipid membrane/protein interactions.

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References

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[2] F.J. Wirkert, M. Paulus, J. Nase, J. Möller, S. Kujawski, C. Sternemann, and M. Tolan, *X-ray reflectivity measurements of liquid/solid interfaces under high hydrostatic pressure*, Journal of Synchrotron Radiation 21, 76 (2014).

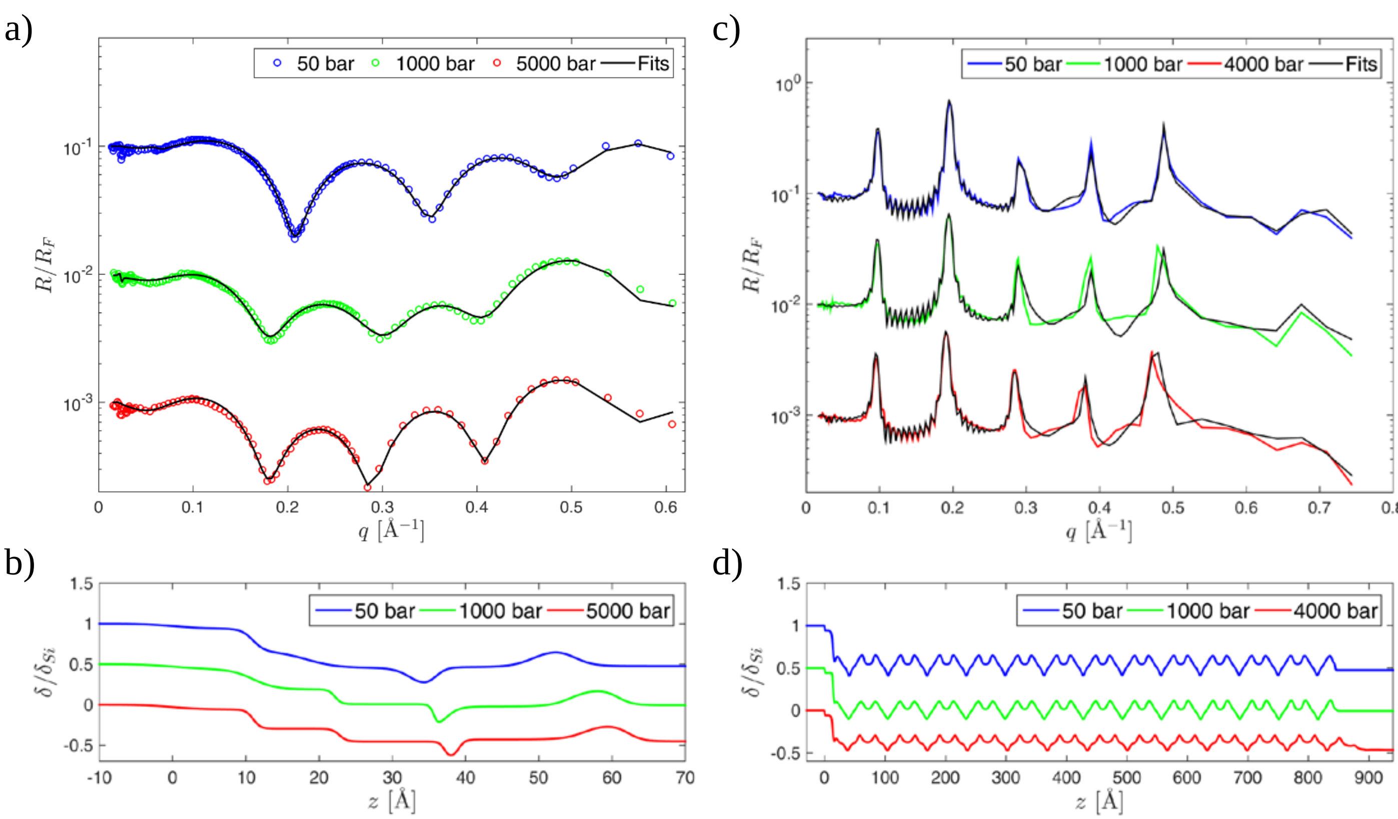

Fig. 3: a) Bilayer reflectivity data of pure DMPC at 37°C and b) the corresponding electron density profiles. c) Reflectivity data of DMPC multilayers containing 28 mol% cholesterol at 20°C and d) the corresponding electron density profiles.