ESRF	**Experiment title:** Specific binding of proteins on solid supported lipid membranes.	**Experiment number:** SC-1922
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Report:

Our research focuses on structural properties of single supported lipid bilayers and their interactions with proteins. We use synchrotron radiation and fluorescence microscopy as complementary tools on a compact experimental setup [1], see Fig. 1. Lipid bilayers composed of 90% SOPC and 10% biotin-X-DPPE and very small amounts of the fluorescence label Texas-Red-DPPE were spread on SiO_2-substrates by the method of spin-coating [2]. Streptavidin and avidin are proteins which are well known to bind specifically to biotin receptors. Such protein mixtures were added to the supported lipid bilayers in different compositions. Streptavidin is known to form 2D protein crystals on the surface of lipid bilayers, while avidin does not.

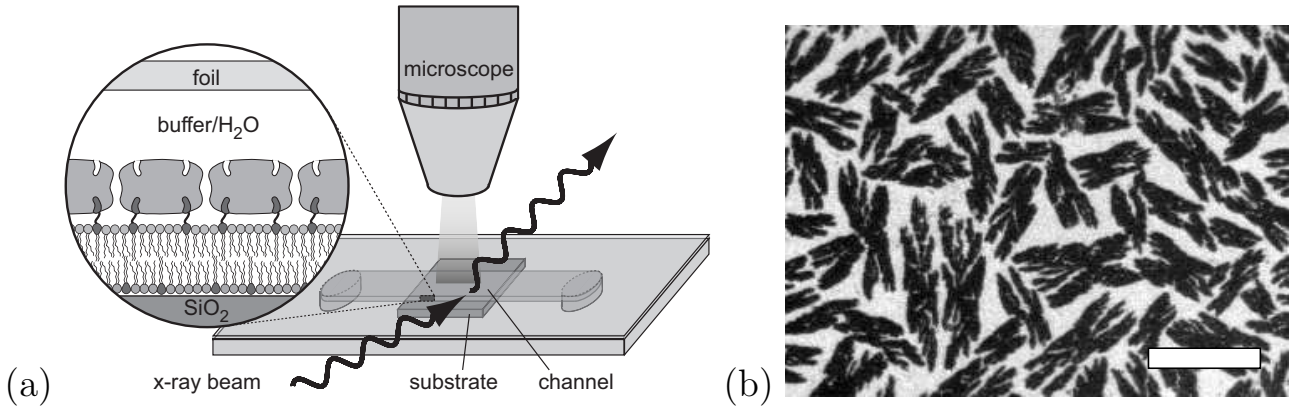

Fig. 1(a): Setup for complementary X-ray reflectivity and fluorescence microscopy studies. (b) Fluorescence micrograph of streptavidin crystals formed on top of the supported lipid bilayer. The microscopy option allows to characterize sample quality and homogeneity before and after X-ray experiments. Scale bar is 20 μm.

The samples were freshly prepared on-site at the ESRF chemistry lab prior to the experiments. Reflectivity scans were performed at a fixed energy of 19.5 keV with a pre-sample beam aperture of 20×200 μm^2 (vertical x horizontal). The high energy is necessary in order to minimize radiation damage. The size of the illuminated interface area at low incidence angles is comparable to the area that can be typically investigated by fluorescence microscopy (Fig. 1). Great care was taken to avoid over-exposure of the sample during the synchrotron experiments, using automatic attenuators and short exposure times.

Our results show that streptavidin/avidin binding to the supported lipid bilayer is an entirely peripheral process. A small water layer ($\sim$ 8 Å) separates the crystallized protein complex from the lipid bilayer (Fig. 2). Further, the lipid bilayer structure is not affected by the presence of the proteins. This is in clear contrast to previous studies of lipid monolayers where a collapse of membrane structure was observed upon streptavidin/avidin binding [3-5]. We propose that a lipid bilayer is a more stable and biologically relevant model system for investigations of protein binding [6].

GISAXS measurements on the lateral structure of the protein layers were so far not successful due to a lack of scattering contrast. For future experiments, contrast may be improved by using ion-labeled proteins with a significantly higher electron density.

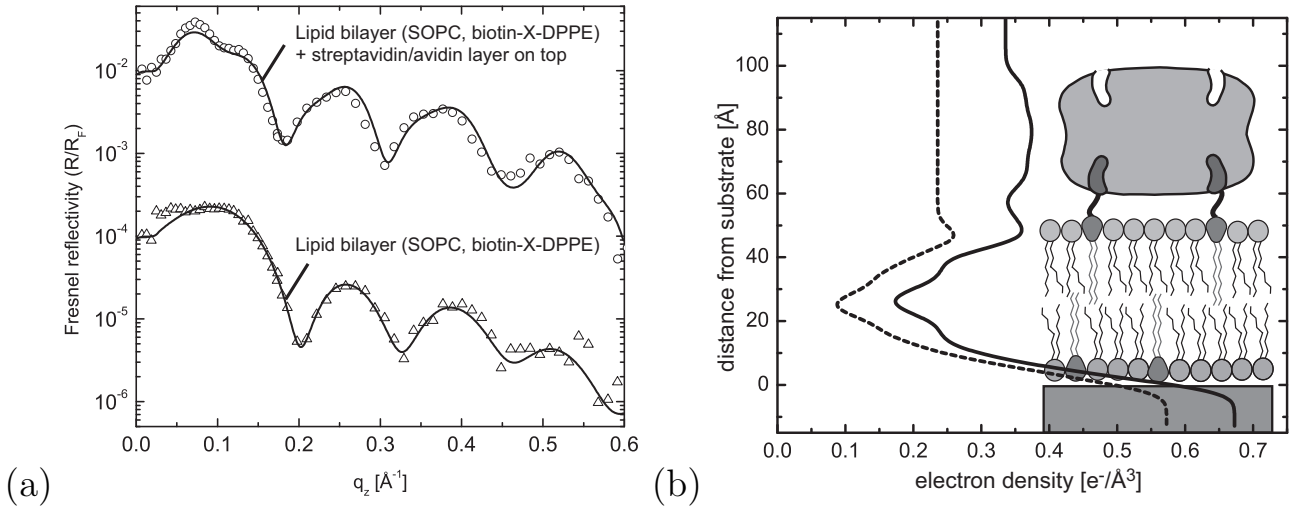

Fig. 2(a): Reflectivity (normalized data and fits) of a lipid bilayer (SOPC, biotin-X-DPPE) with and without a streptavidin/avidin layer. The signal characteristics change significantly upon protein binding. (b) Electron density corresponding to the fits in (a), showing the profile of a lipid bilayer with protein layer (solid line) and without (dotted line, shifted by 0.1 $e^-/Å^{-1}$ for clarity).

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