Experimental Report: ESRF Proposal BM05-SC2105 (Salditt)

Introduction - We report a x-ray reflectivity and phase contrast imaging experiment on free standing bilayer lipid membranes (BLMs). We have developed an experimental setup allowing for freely suspended bilayers in aqueous solution spanning a controlled aperture between two compartments (differing in pH, ion concentrations etc.), and amenable to collimated and/or focused synchrotron beams for reflectivity experiments as well as for coherent x-ray phase contrast imaging. To combine electrophysiological measurements with a in-situ structural probe, the membrane spanned aperture has to be adapted in size and geometry to the beam size. In a first experiment, the vertical density profile $\rho(z)$ of the membrane was to be studied by x-ray reflectivity. The main experimental challenges here are related to (i) the asymmetric footprint of the x-ray beam under grazing incidence, (ii) thermal fluctuations and planarity, and (iii) the relatively low contrast of water/lipid/water interfaces. In the second setup, a partially coherent x-ray beam is used to image the bilayer in phase contrast mode by grazing incidence illumination (membrane normal vector perpendicular to the beam).

Materials and Methods – Three different types of large apertures for enhanced membrane stability were used as a support. The first one is based on thin teflon foils with a thickness in the range from 10 µm to 25 µm, which were cut by means of a focused ion beam (FIB; FEI, Germany)). The second attempt is based on ultra thin etched silicon foils with thickness between 100 nm and 250 nm which were also machined by FIB. The third one used standard silicon wafers in which subsequently sharp and well defined edge apertures were etched. Silicon surfaces needed to be hydrophobized in a silanization process to let lipid assemble to a planar membrane structure in an aqueous environment. Membranes were prepared by following the procedure of Müller and Rudin [1]. Using a thin hair brush a lipid solution of DPhyPC (Diphytanoyl-Phosphatidylcholine; Avanti Polar Lipids, USA) soluted in decane (20 mg/ml) was painted in situ over the aperture of the substrates. After a thinning process of about half an hour a solvent containing BLM remained, whose electrophysiological properties, such as capacity and conductivity, were observed with a patch clamp amplifier NPC-1 Port-a-Patch (Nanion, Germany). The samples were prepared in a sealed teflon cell equipped with Kapton windows and a 10 mm water path. Reflectivity and phase contrast imaging experiments have been performed with a CRL-focused beam (77Be lenses) using bending magnet radiation at an energy of 19.76 keV at BM05. To compensate the relatively low brilliance of the bending magnet, multilayer monochromators with a broad bandpass were used. Reflectivity was recorded with a fast scintillation counter (Cyberstar), phase contrast images by a CCD camera (Sensicam QE 12-bit CCD camera, PCO Imaging, Germany, with 1376 (h) x 1040 (v) pixels, pixel size of 6.54 µm x 6.54 µm).

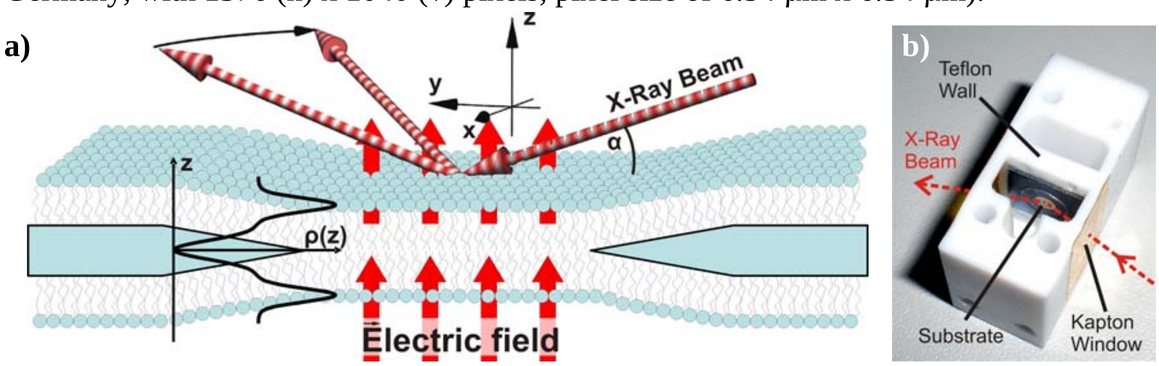

Fig. 1: a) X-ray analysis of freely suspended lipid membrane with its normal vector along the *z*-axis. Reflectivity is measured as a function of incidence angle α in the *x*-*y*-plane. From the measured $R(\alpha)$ curve, the vertical density profile $\rho(z)$ can be inferred. **b)** Image of the teflon chamber with a silicon substrate inside.

Results (reflectivity) : In contrast to calculations, the X-ray-beam could not be focussed down to the necessary size below 5 µm in horizontal direction (necessary because of footprint elongation in reflectivity). Instead only 16 µm were achieved, so that the apertures of about 1.3 mm were constantly over-illuminated at gracing incident angles. In addition, deviations from planarity of the thin (empty) teflon foil led to poor reflectivity signals. Thus, a proper reflectivity measurement under these conditions was not possible for 2 **problems:** 1.) The experiment is impossible using focussed bending magnet radiation, but needs highly collimated undulator radiation (unfocussed, for better control of the beam size in front of the sample by high precision slits). 2.) The planarity of the supporting foils has to be improved. **Solutions:** 1.) Test experiments at ID1 have shown in the meantime that the experiment will be possible at ID01 (see continuation proposal of SC2105). 2.) Foil planarity has been improved in the meantime by apertures fabricated in standard silicon wafers in which subsequently sharp and well defined edge apertures were etched, and which were rendered hydrophobic by silanization.

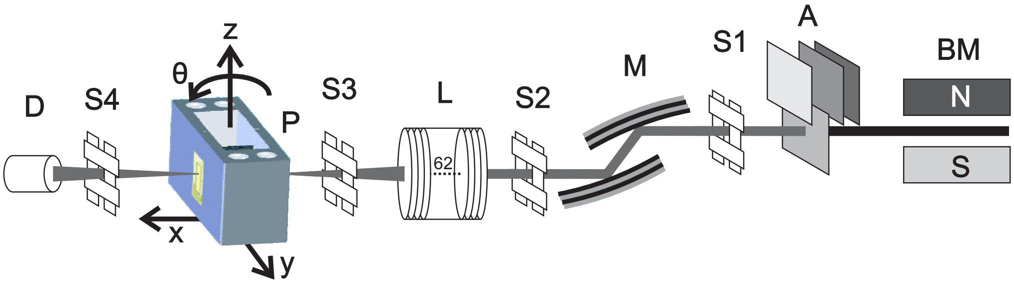

Fig. 2: Experimental scheme used at BM05. The Be lenses were taken out for phase contrast imaging in transmission (successful), but were inserted to provide the small footprint necessary for reflectivity from a small membrane patch so far (unsuccessful).

Results (phase contrast) : By imaging the transmitted beam (unfocused parallel beam) one can directly observe the bilayer in differential phase contrast. The accumulated phase shift accumulated by tangential propagation of the x-rays was sufficient to image a 5 nm thick curved lipid membrane spanning a hole in a silicon substrate, see Fig. 3. The membrane was bent by increasing the hydrostatic pressure on one side of the membrane. After the thinning process this membrane is in the range of 5 nm and its single bilayer character was verified by electrophysio-logical measurement.

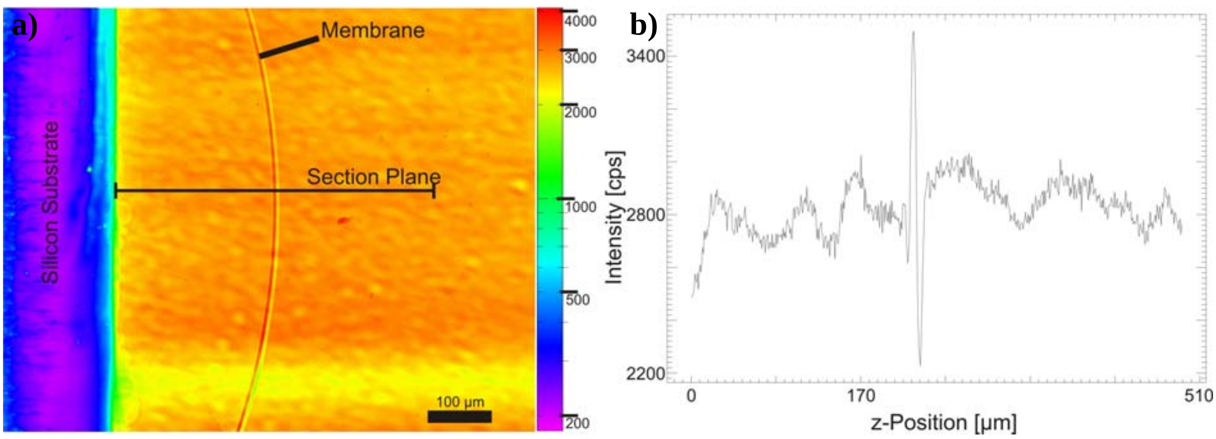

Fig. 3: a) Phase contrast image of a curved, single freestanding bilayer lipid membrane spanning a 1.3mm hole in a silicon substrate. b) Cut through the membrane showing an unexpected high phase shift in the outer rim of the membrane

Calculation on the phase contrast effects are the next step to extract quantitative details of the profile and to establish the sensitivity of this measure applied to lipid membranes.