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We report a novel approach tested successfully at ESRF (ID01, SC-935 beam time) for studying the structure, fluctuations, and interaction forces in highly charged lamellar phases by means of X-ray reflectivity and grazing incidence diffraction techniques. A few thousands bilayers of DDABr (synthetic surfactant) and POPS (phospholipid) were aligned on a flat silicon wafer and swollen under controlled osmotic pressure (Π) by direct contact with an osmotic stressor reservoir. Then, specular and non-specular X-ray reflectivity of these multilayers were conducted through the reservoir (**Fig.1**).

Following this approach, the Equations Of State (Pressure-Distance diagram) of these molecules have been constructed over lamellar periodicities (*d*) extending from *d*~29Å (at $\Pi \sim 10^{6.63}$ Pa, by immersion in vapour of controlled relative humidity) up to *d*~342Å (at $\Pi \sim 10^{3.82}$ Pa, by immersion in a high mass polyelectrolyte solution that don't penetrate the sample) **[1]**. Under these conditions, thermally excited undulations and periodicity fluctuations can be studied by Bragg-peak line shape analysis. It revealed that these solid supported lamellar phases are to be considered has perfect smectic-A crystals since their mosaic spread is less than a hundredth of a degree (~60μrad measured with rocking scans) allowing to perform high resolution scans along different symmetry axes of the lamellar phases **[1,2]**.

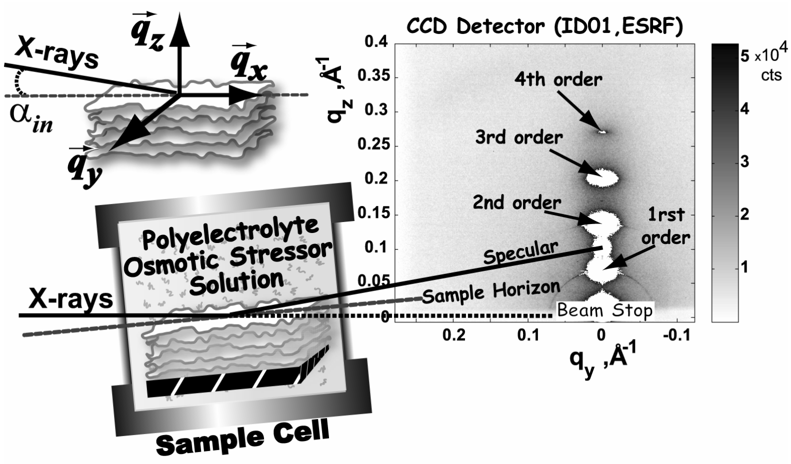


**Figure 1.** *Sketch of the experimental geometry used for applying osmotic stress by immersion of the solid supported and charged lamellar phase in a polyelectrolyte solution of the same sign and counter-ions than the sample. A typical out-of-plane diffuse scattering pattern (measured with the ID01 CCD camera) is shown for a DDACl sample at P~105.49 Pa.*

We have mainly focused this first experiment on the combination of perfectly aligned charged lamellar phases and the extension of the osmotic stress technique that uses vapour phases as well as polyelectrolytes solutions in direct contact with the sample [1].

Our aim was also to address the counterion distribution profile in the water layer, since one of the most interesting issues on highly charged membranes concern the role of counterions in membrane rigidity and in membrane interactions (both dominated by electrostatic repulsion between the charged head-groups). For this purpose we performed anomalous reflectivity measurements at the absorption edges of Br- (13.47 keV) for the DDABr samples, and Rb+ (15.2 keV) for the POPS. We report that the specular reflectivity curves are strongly dependent on the beam energy and on the angle of illumination, owing to the high degree of alignment which leads to ultra-sharp and intense Bragg-peaks which are usually smeared out by the power averaging for isotropic bulk samples composed of multilamellar vesicles. Two specular reflectivity curves measured at different energies (13.43keV and 13.47keV) on a partially hydrated DDABr multilayer are plotted in **Fig.2**. They show a very strong variation around the first Bragg peak that indicates a counterion profile which is a weakly varying function (continuous curve) and can be described by low Fourier components. Note the strong energy dependence of the reflectivity in-between the two first Bragg-peaks that indicates a good sensitivity of our measurements to the bilayer electron density profile including the counterions distribution. About 90% of the $Br^-$ counterions can be considered as bounded to the head-group surface at this swelling which corresponds to water layers of less than a nanometer separating the surfactant bilayers.

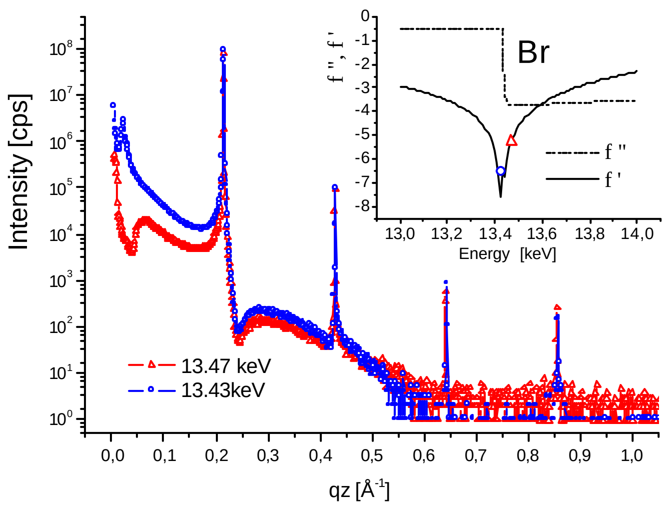


**Figure 2.** *Anomalous specular reflectivity of a DDABr multilayer immersed in a water vapour phase at P~$10^{5.97}$Pa. The specular reflectivity shows strong changes due to the varied contrast of the $Br^-$ counterions distributed in between the $DDA^+$ bilayers. The edge has been scanned in fine steps, yielding data sets of typically 50 curves which have to be fitted to the same profile. Hence a high degree of statistical significance is reached (Analysis under progress).*

Few samples in full hydration obtained by immersion in a polyelectrolyte solution with same counterion (Br-) were measured succesfully and exhibit similar features. Nevertheless, the absorption of the polyelectrolyte solution is varying with the energy and makes the analysis very complicated. We now propose to conduct these measurements from the solid interface using transparent substrates (such as thin Berylium weafers) in order to obtain the data in full hydration (high swellings) that are needed for improving our understanding of the counterion role in charged bilayers assemblies.

[1] G. Brotons, T. Salditt, M. Dubois, and Th. Zemb, ***Highly Oriented, Charged Multilamellar Membranes Osmotically Stressed by a Polyelectrolyte of the Same Sign***, Langmuir *In Press* (Web Release Date: 22-Aug-2003; DOI: 10.1021/la034733j).

[2] U. Mennicke, PhD Dissertation, Georg-August-Universität zu Göttingen, August 2003.