



	Experiment title: Membrane protein conformation by anomalous scattering / Counterion distribution by anomalous reflectivity	Experiment number: SC 1797 SC 1376
Beamline: ID01	Date of experiment: from: 25/10/2005 to: 01/11/2005	Date of report: 31/08/2006
Shifts:	Local contact(s): Peter Bösecke	<i>Received at ESRF:</i>
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Report:

SC 1797: We have carried out an anomalous X-ray reflectivity study to locate a labelled residue of a membrane protein with respect to the lipid bilayer. From such experiments, important constraints on the protein or peptide conformation can be derived. Specifically, we have localized an iodine-labelled phenylalanine in the SARS E protein, incorporated in DMPC phospholipid bilayers, which was deposited in form of thick multilamellar stacks on silicon surfaces. The label position at the hydrophilic/hydrophobic interface helped to confirm a conformational model (helical hairpin) of SARS E. Since SARS E was not available for most of the allocated beamtime (problems with formalities of safety issues and planning), most of the allocated beamtime was used for an extension of the previous proposal SX 1376. However, from the available shifts including a previous test run at ID01, enough data was collected, so that the work was carried out

successful [1]. Since the result of this work is already well published, we report here primarily on the counterion density, which successfully completed the efforts of SC1376 (previously unreported and yet unpublished).

SC 1376: The main experimental goal was a resonant X-ray reflectivity study of the counterion distribution near a planar charged interface. This distribution is described classically by the Poisson-Boltzmann equation, yielding an algebraic decay of the counterion distribution in the direction perpendicular to the surface for the case, when there are just counterions in the liquid face near the interface, so that the total system is electrically neutral. This salt-free case has only poorly been investigated experimentally so far.

A flat charged wall of controlled charge density σ_s in salt-free ultrapure water was realized by deposition of a solid-supported monolayer composed of the cationic double-chain surfactant DODAB (Di-Octadecyl-Dimethyl-Ammonium-Bromide) with Bromide counterions and the neutral co-lipid DPPC (Di-Phosphatidyl-Choline) on top of a silanized silicon wafer. The surface charge density σ_s was varied by using different molar fractions α_{DAB} of DODAB in the mixed surface monolayer. More specifically, systems with

$\alpha_{\text{DAB}} = 1, 0.5, 0.25$ and 0 (i.e. pure DPPC) were prepared by the vesicle fusion technique. To verify the independence of the results on the experimental preparation pathway an alternative protocol based on thermal desorption of a thick multilamellar stack of fully charged DODAB layers was applied, yielding essentially the same structural results.

The sample deposition was performed in a sealed and temperature controlled Teflon cell equipped with Kapton windows and a 10 mm water path for the X-ray reflectivity experiment. Reflectivities were recorded at the Br K_α absorption edge ($E_e = 13.475$ keV) yielding high resolution (~ 4 Å) electron density profiles obtained by the standard box model analysis technique with an additional contribution for the Bromide counterions (cf. Fig. 1).

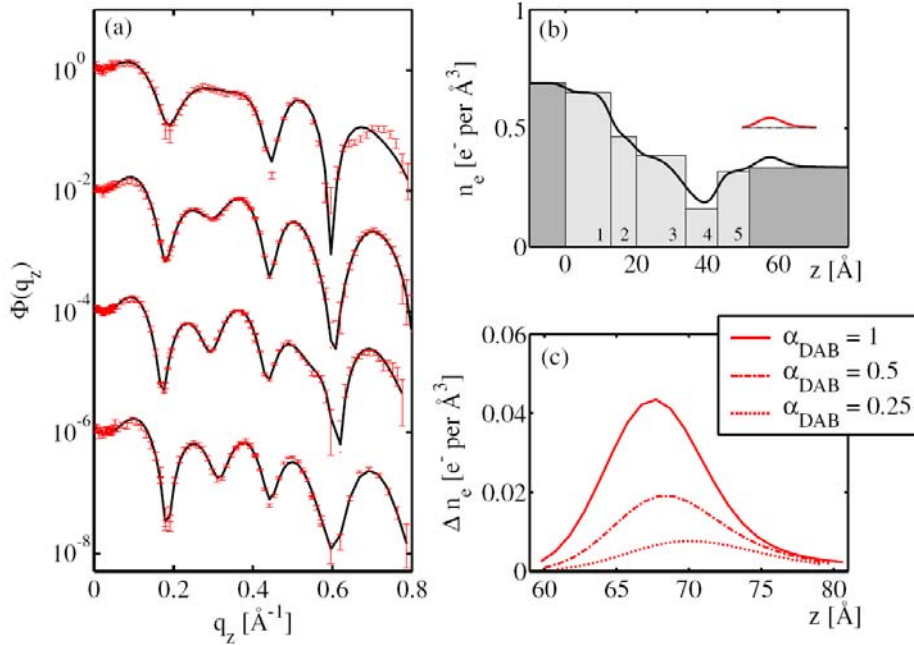


Figure 1: (a) Normalized reflectivity curves, vertically shifted for clarity, for the dilution series ($\alpha_{\text{DAB}} = 1, 0.5, 0.25, 0$ from top to bottom) along with best fits of a box model with an additional counterion part. (b) Example for a box model fit for the case of pure DODAB. (c) Resulting counterion profiles (freely translated in z -direction) for different surface charge densities.

The data was consistent with an algebraic Poisson-Boltzmann counterion profile, convoluted by a Gaussian function of width $\xi \approx 3$ Å. Within the experimental uncertainty, reflectivity curves obtained for the same samples at a photon energy significantly below the Br K_α edge ($E_0 = 13.228$ keV) did not show systematic differences to the scans obtained at E_e . Among possible counterion distributions this served as a major restriction, excluding very narrow, highly condensed distributions with $\xi < 2$ Å. For the samples with $\alpha_{\text{DAB}} = 1, 0.5, 0.25$ we were able to obtain values for the Gouy-Chapman length μ_{GC} , well in agreement with the expected ratio of one counterion per DODAB molecule and with an enlargement of the area per charge according to the respective reduction of α_{DAB} [2].

As an independent analysis procedure an unbiased free-form model based on the representation of the laterally averaged density profile as a sum of Heaviside step functions was performed. Within experimental uncertainties the resulting counterion distributions were also in agreement with the Poisson-Boltzmann solution. Furthermore, it was found that the determination of the counterion distributions was not limited by the experimental resolution, but the spacial fluctuations of the charged interface.

In an additional section of the beamtime high resolution single-energy reflectivities from phospholipid membranes deposited on silicon by vesicle fusion were performed. From the analysis based on a modeling of the bilayer density profile as a Fourier series we were able to distinguish two headgroup maxima in the profile, if the sample was in the phase coexistence regime. Furthermore, the thickness of the water layer between the bilayer and the silicon was found to be dependent on the lipid surface charge density [3].

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