

# Charge Inversion near Modulated Interfaces by Multivalent Counterions Studied in Anomalous Reflectivity Measurements

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## Introduction

The screening of charged interfaces by counterions from electrolytic solutions is key for the understanding of a broad range of phenomena in molecular physiology, colloid and polymer science, and microfluidics. It has recently been intensively investigated by theory [1–3]. On a molecular scale, the discreteness of charges leads to fundamental deviations from predictions based on the continuum Poisson-Boltzmann approach, *i.e.* Debye-Hückel theory. In emerging theories that take account of charge discreteness, the adsorption of multivalent ions to charge-modulated interfaces has been predicted to lead to counter-intuitive phenomena, such as charge inversion, that may lead to attraction between macromolecules of the same net surface charge [1–3]. These phenomena are critical for the understanding of important biological effects, such as the compaction of DNA, the interaction between cell membranes in fusion or the folding of peptides near membrane surfaces. A recent AFM study has produced evidence that charge inversion at such interfaces may be a universal electrostatic phenomenon [4]. We recently reported the feasibility of quantitative studies of charge inversion with x-ray reflectivity measurements that utilize the anomalous dispersion of multivalent cations [5]. Here, we expand this work into a systematic study of the concentration dependence of cation adsorption to charge-modulated model interfaces.

## Methods and Materials

### Samples

Surface monolayers of a charged phospholipid, dimyristoylphosphatidic acid (DMPA; source: Avanti), were used as a model system to prepare interfaces of a controlled density of discrete anionic charges in contact with an aqueous solution. They were spread in a Langmuir film balance on electrolytic subphases prepared from ultrapure water (Millipore Milli-Q) and p.a. grade  $\text{LaCl}_3$  (Sigma). To minimize ion contamination, the subphases were handled and transferred to the Langmuir film balance in bottles made from Teflon (PTFE). After solvent evaporation, the monolayers were slowly ( $\sim 1 \text{ \AA}^2/(\text{molecule} \times \text{min})$ ) compressed and measured at surface pressures  $\pi = 15$ , 30 and 40 mN/m. At high ion concentrations, monolayers were less stable, which prevented some measurements at high  $\pi$ .

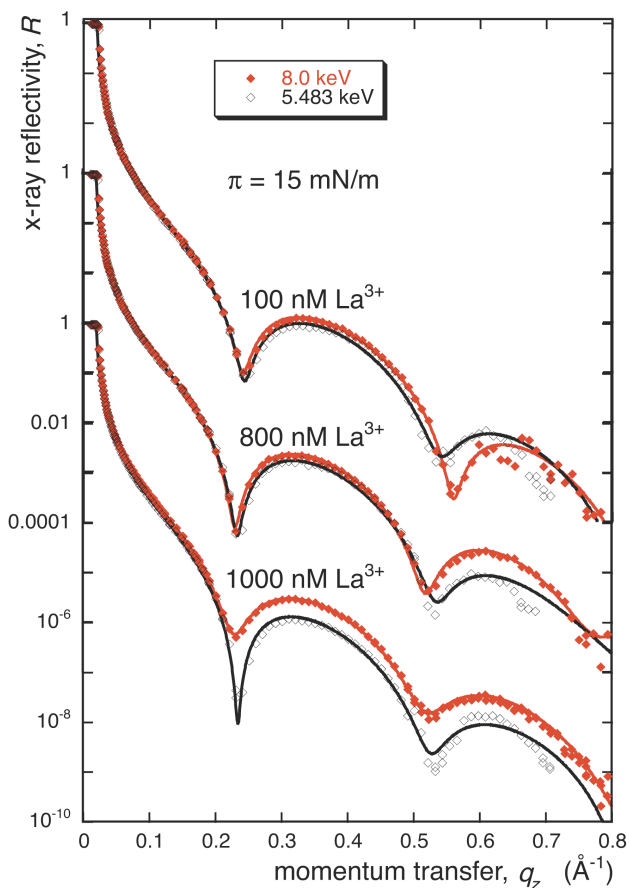
### Scattering Experiments

Reflectivity experiments were conducted at the horizontal surface diffractometer with a highly monochromatic beam ( $\Delta E \sim 1 \text{ eV}$ ) on beamline 6-ID-B at 8.0 (off-resonance) and 5.486 keV (La  $L_{\text{III}}$  absorption edge). The instrument [6], sample preparation and energy calibration have been previously described [5]. While in our previous feasibility study we measured the condensation of  $\text{Ba}^{2+}$  to  $\text{DMPA}^-$  at large (10 mM) subphase concentration, we have in this work systematically varied the cation ( $\text{La}^{3+}$ ) concentration from approx. 1 nM to 10  $\mu\text{M}$ . The choice of  $\text{La}^{3+}$  permits direct comparison of the results with recent AFM work [4].

## Results

### Experimental

Figure 1 shows a comparison of the on-resonance and off-resonance x-ray reflectivities of DMPA monolayers on (a) 100 nM, (b) 800 nM and (c) 1  $\mu\text{M}$   $\text{LaCl}_3$  measured at  $\pi = 15 \text{ mN/m}$  and 22 °C. Results at 10  $\mu\text{M}$   $\text{LaCl}_3$  (not shown) resembled those at 1  $\mu\text{M}$  concentration. The area per DMPA molecule in the surface films is virtually identical in all cases,  $A_{\text{lipid}} \sim 42 \text{ \AA}^2$ .



**Figure 1:** On-resonance and off-resonance x-ray reflectivities of DMPA monolayers on different  $\text{LaCl}_3$  subphases as indicated

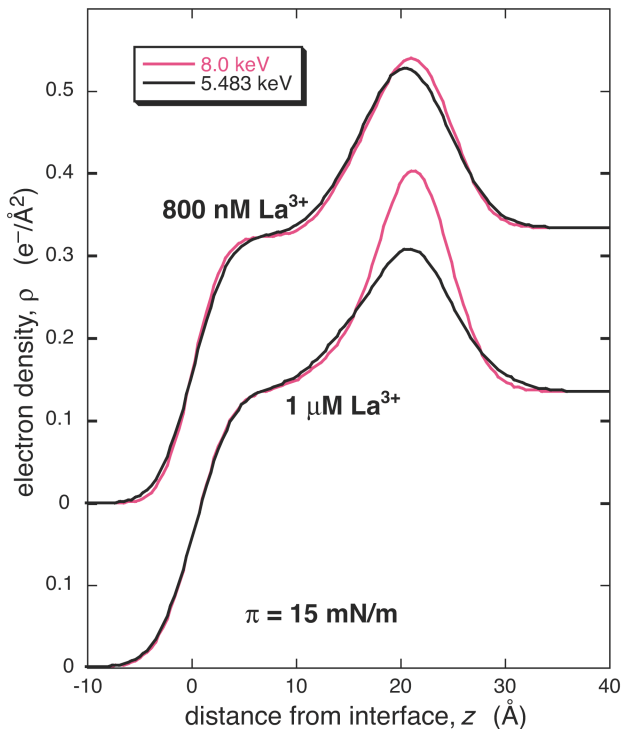
### Data Interpretation

To arrive at a model-independent quantification of the  $\text{La}^{3+}$  concentration at the charge-modulated interface, we have inverted the reflectivity data using a model-free procedure [7] as well as box models and an approach based on volume-restricted distribution functions (VRDF) of molecular subfragments [5, 8]. Off-resonance and on-resonance data were treated without reference to each other, and only the derived overall electron density profiles have been evaluated thus far. Under the

assumption that differences in the reflection data at the two distinct photon energies are exclusively due to the index changes of La with the energy, the integrated electron density differences between off-resonance and on-resonance profiles provide a direct and model-independent measure of the total  $\text{La}^{3+}$  concentration at the interface. Relating this to the surface density of the lipid, we use as a first approximation

$$n_{\text{La}} = \frac{\int \Delta\rho \, dz}{\Delta z_{\text{eff}}} \times A_{\text{lipid}} \quad (1)$$

to determine the number of  $\text{La}^{3+}$  ions per lipid bound to the interface. In Eq. (1),  $z$  is the direction of the surface normal,  $\Delta\rho(z)$  is the difference in off-resonance and on-resonance electron densities and  $\Delta z_{\text{eff}} \sim 28.1$  [9] is the difference in the effective number of Thomson scatterers on the  $\text{La}^{3+}$  ions at 8.0 and 5.483 keV.



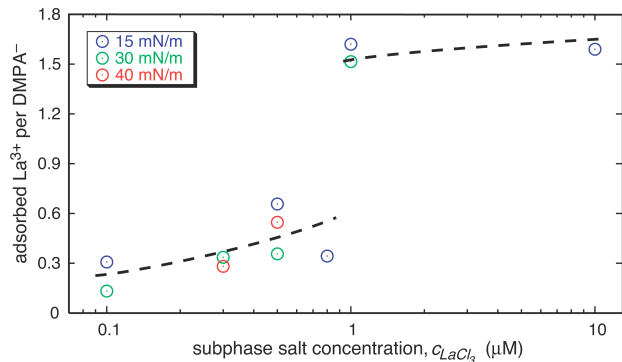
**Figure 2:** Effective electron density profiles that were used to create the fits to the data shown in Figure 1. The models have been derived using VRDF approach [8]. Free-form fits [7] or box models yield quantitatively similar results for the effective electron density differences at different photon energies.

In Figure 2, we compare such on-resonance and off-resonance electron density profiles at  $\pi = 15$  mN/m for  $\text{LaCl}_3$  subphase concentrations of 800 nM and 1000 nM. It is evident that these profiles differ less at 800 nM  $\text{LaCl}_3$  than at 1  $\mu\text{M}$   $\text{LaCl}_3$  – in accordance with the raw data. A preliminary quantification according to Eq. (1) indicates approximately stoichiometric (1:3) adsorption of  $\text{La}^{3+}$  to  $\text{DMPA}^-$  at  $c_{\text{La}}$  between 100 and 800 nM, but an excess of  $\text{La}^{3+}$  (ratio to  $\text{DMPA}^- \sim 1:1$ ) at 1  $\mu\text{M}$  and above (10  $\mu\text{M}$   $\text{LaCl}_3$  was the highest subphase concentration studied).

## Discussion

A preliminary data evaluation suggests a distinct cross-over with subphase salt concentration between stoichiometric cation binding and cation excess at the interface. Stoichiometric binding is observed even at exceedingly low concentrations, as small as 100 nM. Above 800 nM, the amount of surface-bound

cations jumps to more than 3 times the stoichiometric ratio for charge compensation, implying charge inversion. This jump is visible in the raw data (Fig. 1) and does not depend on the method of deriving the electron density profiles. Unlike reports on charge inversion phenomena, such as the analysis of electrophoretic drift velocities that hinge on hydrodynamic modeling, the results presented here represent thus direct evidence for the multivalent ion condensation on a structured, charged model surface.



**Figure 3:** Number of adsorbed  $\text{La}^{3+}$  cations per  $\text{DMPA}^-$  in surface monolayers as derived from effective electron density profiles by using Eq. (1)

A quantitative discrepancy with respect to the cross-over behavior exists, however, with the recently published AFM experiment [4]. In this work, charge inversion was reported for  $\text{La}^{3+}$  concentrations of  $\sim 100$   $\mu\text{M}$  salt solution. This is  $\sim 2$  orders of magnitude higher in concentration than where we observe the cross-over. This large discrepancy is unlikely to be due to differences in the molecular or geometric details of the studied systems. Quasi-molecular modeling [8] of our data will allow us to refine the positions and amounts of adsorbed  $\text{La}^{3+}$  and coadsorbed anions, if any, to investigate this discrepancy.

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