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## X-ray Reflectivity Studies of Adsorbed Proteins on Langmuir Layers

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Beamline(s): X19C

**Introduction:** The mechanism of protein membrane binding is of great interest in both biology and physics and was extensively studied using biochemical methods (e.g. [1, 2, 3]). X-ray reflectivity from the protein-phospholipid monolayer system provides an opportunity to better understand the binding mechanism and addresses the issue of protein orientation with respect to the membrane and the extent of its penetration into the membrane.

**Methods and Materials:** X-ray reflectivity was used to study the interaction of the C2 domain of cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>-C2) with a phospholipid membrane. SOPC (1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) monolayer has been chosen as our model membrane. SOPC monolayer was supported on a buffered Ca<sup>2+</sup> containing aqueous solution. The specular reflectivity from each phospholipid monolayer was measured prior to injecting protein solution into the subphase.

**Results:** Fig. 1 is the example of the reflectivity from SOPC monolayer (black) and from the SOPC monolayer with protein adsorbed to it (red). Fig.2 shows the real space profiles received from the fits to the reflectivity curves on Fig. 1. The original phospholipid layer, which can be described by a two layer model roughened by capillary wave theory is modified and a three layer model is necessary to fit the data after the protein is injected. The data analysis indicates that a third layer attached to the phospholipids headgroup region is formed. This additional layer corresponds to the proteins bound to the phospholipids. Buffer containing no Ca<sup>2+</sup> was used as the control experiment. No evidence of protein adsorption to the phospholipid monolayer was found in this case. Our results thus support the idea, that the presence of Ca<sup>2+</sup> ions in the buffer is essential for the membrane binding of this protein.

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### References:

- [1] L. Bittova, M. Sumandea, and W. Cho, "A Structure-Function Study of the C2 Domain of Cytosolic Phospholipase A<sub>2</sub>," J. Biol. Chem. 274, 9665-9672 (1999).
- [2] M. Medkova, and W. Cho, "Mutagenesis of the C2 Domain of Protein Kinase C- $\alpha$ ," J. Biol. Chem. 273, 17544-17552 (1998).
- [3] E. Bitto, M. Li, A. Tikhonov, M. Schlossman, and W. Cho, "Mechanism of Annexin I-Mediated Membrane Aggregation," Biochemistry 39, 13469-13477 (2000).

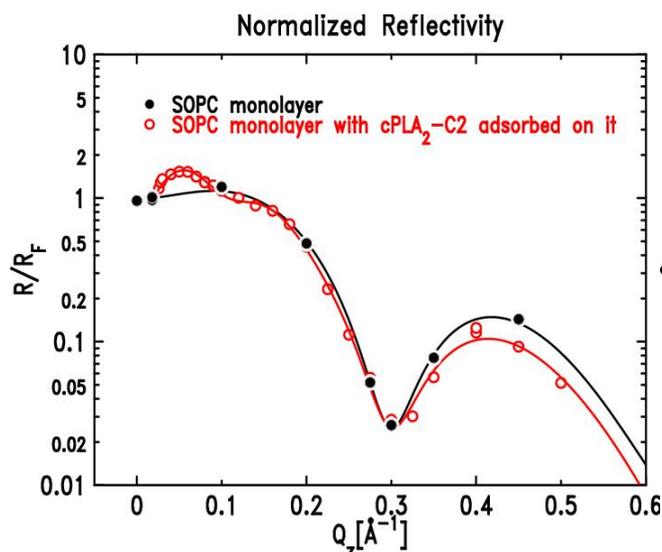


Figure 1

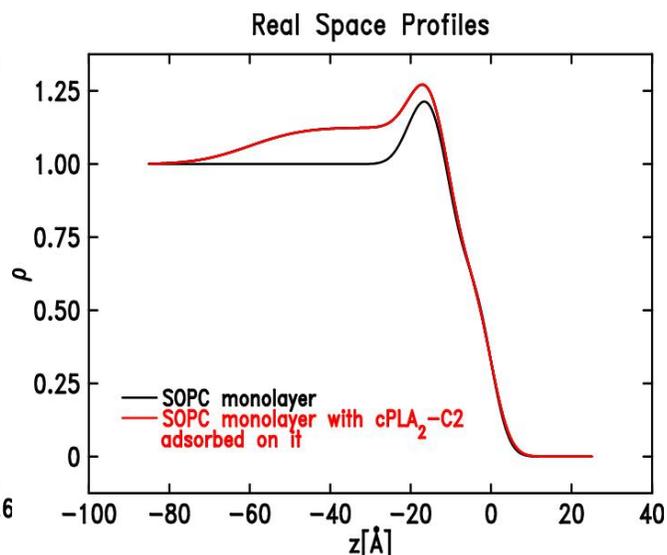


Figure 2