Report to the beam time:

Impact of Phospholipid Oxidation on Biophysical Properties of Membranes and their interaction with membrane active peptide

The primary aim of the proposed project is to quantitatively determine the impact of oxidized phospholipids on the fine structure of lipid monolayers at the air/water interface as well as on the interaction with bioactive peptide. The combination of specular X-ray reflectivity (XRR) and grazing-incidence X-ray fluorescence (GIXF) is used to extract the electron density profiles perpendicular to the membrane plane, structure and form factor, and lateral concentration of ions coupled with the membrane surface. In this work two oxidized phospholipids have been used PoxnoPC and PazePC which are two stable lipid oxidation products originated from POPC oxidization. PazePC and PoxnoPC bear carboxyl and carbonyl groups respectively at the end of their truncated sn-2 chains.

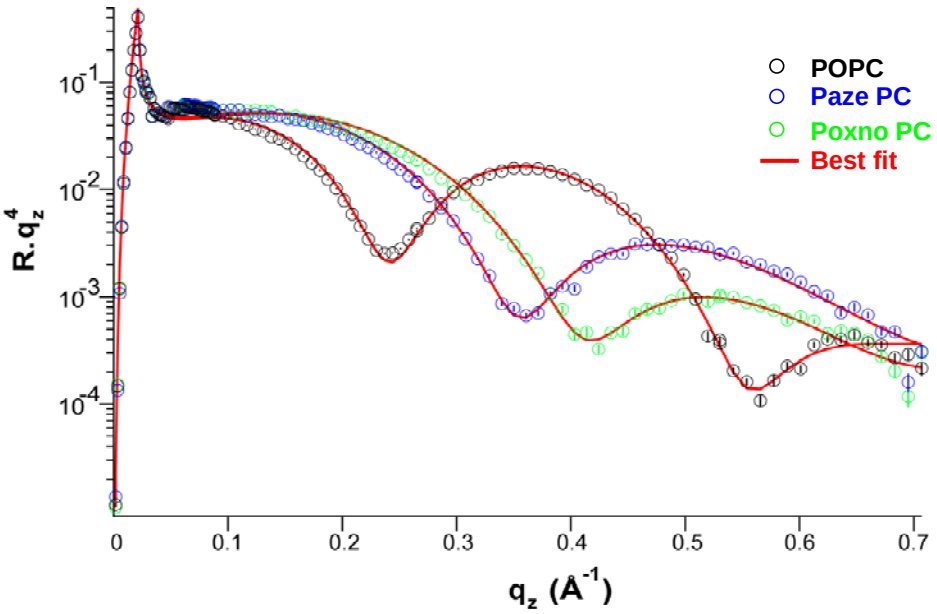

Figure 1: XRR data from POPC (black), PazePC (blue) and PoxnoPC monolayer (green) at SP~ 21mN/m on Hepes buffer together with the best fits (solid red line).

Figure 1 represents the recorded XRR measurements for POPC, PazePC and PoxnoPC monolayers with the best fits. As shown in this figure, oxidized monolayers exhibit a lower thickness (PazePC ~ 17 Å, PoxnoPC ~ 15Å) compared to pure POPC (~23 Å). In addition, oxidized phospholipids showed a higher electron density at the polar headgroups region (ρ_H = 0.507 e Å^{-3} for PazePC and 0.523 e Å^{-3} for PoxnoPC) than that of POPC monolayer (ρ_H =

0.496 e Å^{-3}). This suggests together with the chemical structure of oxidized phospholipids the presence of oxidized moieties (carbonyl or carboxyl) in the vicinity of polar headgroups. This result is in accordance with a molecular dynamics simulation by Mouritsen Group (consortium member of EUFP7 OxPL) which suggests that a large free energy penalty of embedding a charged carboxyl group of PazePC in the hydrophobic core of a lipid bilayer induces the reorientation of the oxidized chain into the aqueous phase.

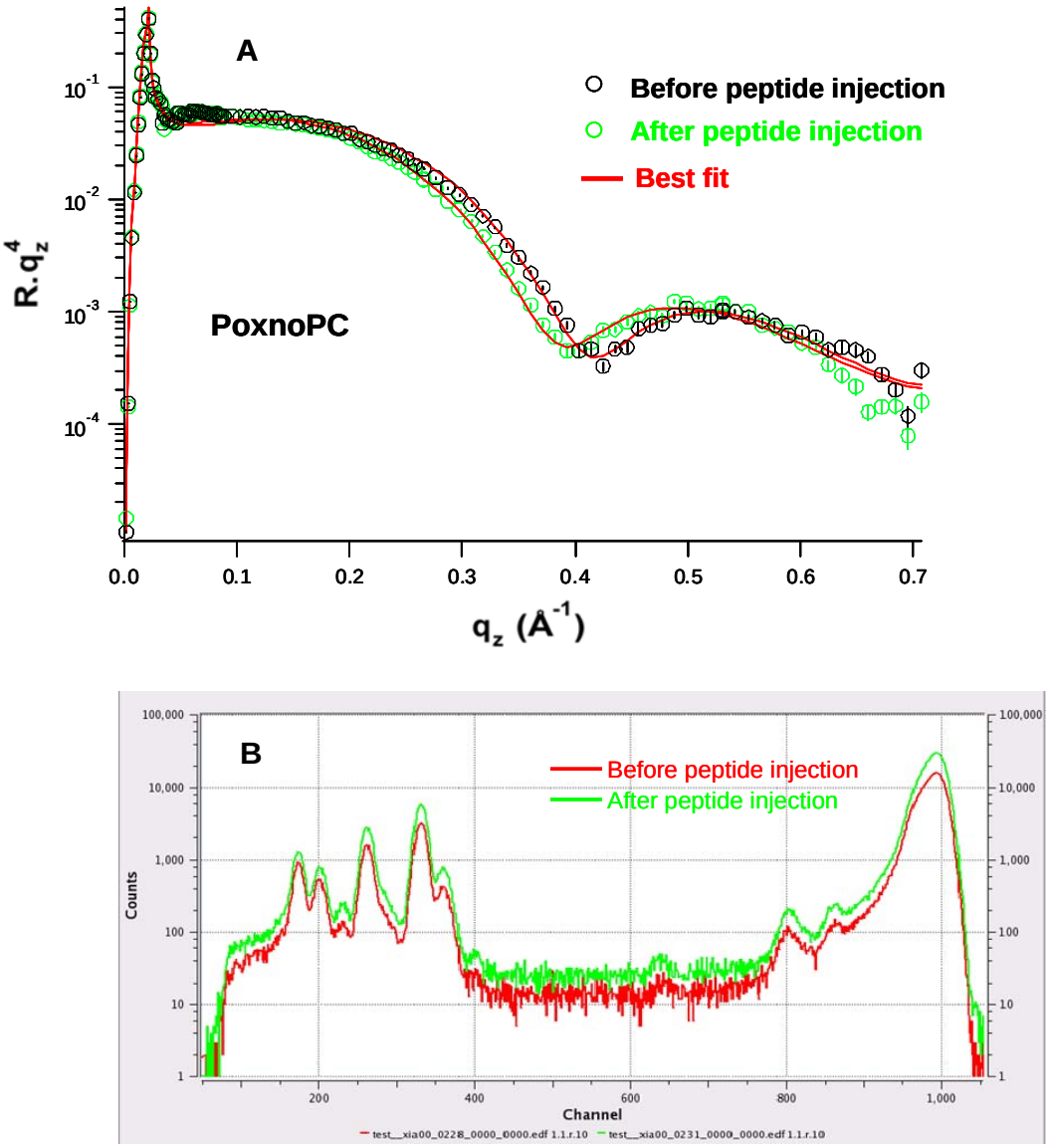

Figure 2: A. XRR data from PoxnoPC before (black) and after peptide injection (green) on Hepes buffer together with the best fits (solid red line). B. GIXF measurements for the same monolayers.

Figure 2 A represents the XRR measurements of PoxnoPC monolayer before (black curve) and after 1 hour of the injection of Peptide-4F (anti-inflammatory apoA-I mimetic peptide). As shown in this figure the injection of peptide-4F induced an increased of the monolayer thickness. This could be due to the formation of a peptide layer underneath of the PoxnoPC monolayer. However, our preliminary GIXF measurements (figure 2C) do not show a significant increase of sulfur (contained in sulphide amino acids within the peptide) peak at the air/water interface. This discrepancy could be related to the short time of incubation of the peptide in the subphase.

In order to finalize the results into publishable form an optimization the experimental procedure is required. For example, adjusting the initial surface pressure of the oxidized phospholipids monolayer is a crucial factor that determines the excess area required for peptide penetration. In addition, a longer incubation time of the peptide with the monolayer should allow the formation of a complete peptide monolayer and thus would give higher chances for a clear GIXF signal for sulfur peak from the peptide.