ESRF	**Experiment title:** Interaction of synaptic vesicles with lipid monolayers: A structural investigation by x-ray reflectivity and GID	**Experiment number**: SC2713
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Names and affiliations of applicants (* indicates experimentalists):

Sajal Kumar Ghosh* & Simon Castorph*, Tim Salditt*,*Institute for X-ray Physics, University of Goettingen, Goettingen, Germany*

Reinhard Jahn & Matthew Holt, *Max Planck Institute for Biophysical Chemistry, Goettingen, Germany*

Oleg Konovalov, *European Synchrotron Radiation Facility, Grenoble, France*

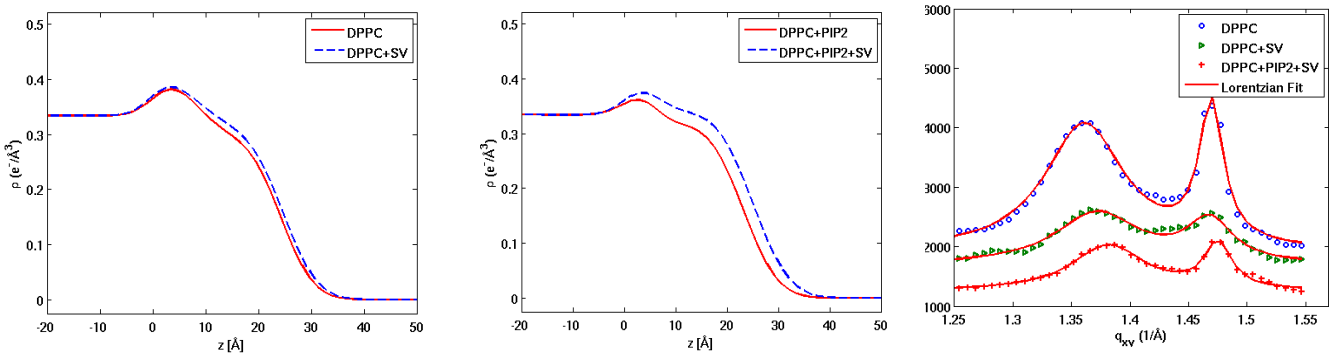
Report:

Synaptic vesicles (SVs) are trafficking organelles in the presynaptic nerve terminals responsible for transport and release of neurotransmitters into the synaptic cleft [1]. The adhesion and fusion of these vesicles with the plasma membranes are essential steps of the SNARE mediated and Ca^{2+} dependent exocytosis process. For a detailed and high resolution structural analysis of this process, we have performed an advanced interface sensitive x-ray scattering experiment on a system where the inner leaflet of the synaptic membrane has been modeled by a controlled lipid monolayer at the air-water interface.

A custom-made shallow, single movable teflon barrier Langmuir trough was used for the experiment. About 125 µl of chloroform solution of lipid (0.2mg/ml) was spread to form the monolayer at the air-water interface. Synaptic vesicles were purified from subcellular fraction of rat brain by controlled pore size exclusion chromatography [2]. The lipid monolayer was compressed to surface pressure of 30mN/m and then the SVs were injected into the subphase. The structural investigations were performed using a monochromatized x-ray beam of energy 8 keV.

The x-ray reflectivity data are fitted with two-box model: one box for the lipid head group region and the other for the hydrocarbon chain region. This analysis provides us the in-sample-depth electron density profile and hence the structural information. The interaction of synaptic vesicles with 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) lipid film is manifested by the changes of the structural parameters of lipid molecules (*Fig. 1, left*). The changes become pronounced at the presence of 5mole% of phosphatidylinositol-4,5-biphosphate (PIP_2) in the lipid mixtures (*Fig. 1, middle*). The increase of electron density across the monolayer after the injection of synaptic vesicles clearly indicates the insertion of some portion of the synaptic vesicles into the monolayer. The GID data provide the information about the molecular packing in the monolayer and the corresponding changes brought out due to the presence of SVs into the subphase. The in-plane component (q_{xy}) of the diffraction vector shows two peaks indicating a centered rectangular packing of the hydrocarbon chain [3]. The chains are tilted to nearest neighbors. Although the presence of synaptic vesicles does not change the lattice type, the peaks are shifted towards the higher q_{xy} values indicating a

smaller surface area per chain and thus a reduced tilt angle (*Fig. 1, right*). The presence of a few mole% of PIP_2 is found to decrease the tilt angle further.



Fig(1): (Left & middle) Electron density profiles at the interface along the axis normal to the interface. The interaction between the lipid films and the SVs increases at the presence of PIP_2. (Right) GID measurements and corresponding Lorentzian fit.

Recent small angle x-ray scattering (SAXS) studies of synaptic vesicles reveal that the proteins surrounded the synaptic vesicles have higher electron density than that of lipid head and chain regions [4]. The increase of electron density across the lipid monolayer, of chain area and the tilt angle at the presence of synaptic vesicles constitute a consistent structural response of the monolayer upon interaction with the SVs and the associated proteins. In vivo studies have shown that the local concentration of PIP_2 in the lipid bilayer is higher at the release site of large dense core vesicles (LDCV) [5]. In this study, the formation of PIP_2 - rich microdomains might be responsible for the enhancement of the interaction of SV with the model lipid membrane. In particular, the SV-associated protein synaptotagmin may specifically bind to the PIP_2 - rich microdomains. In a second step these binding events may then cause an increase in lateral pressure and a collective structural rearrangement of the monolayer, (*Fig. 2*).

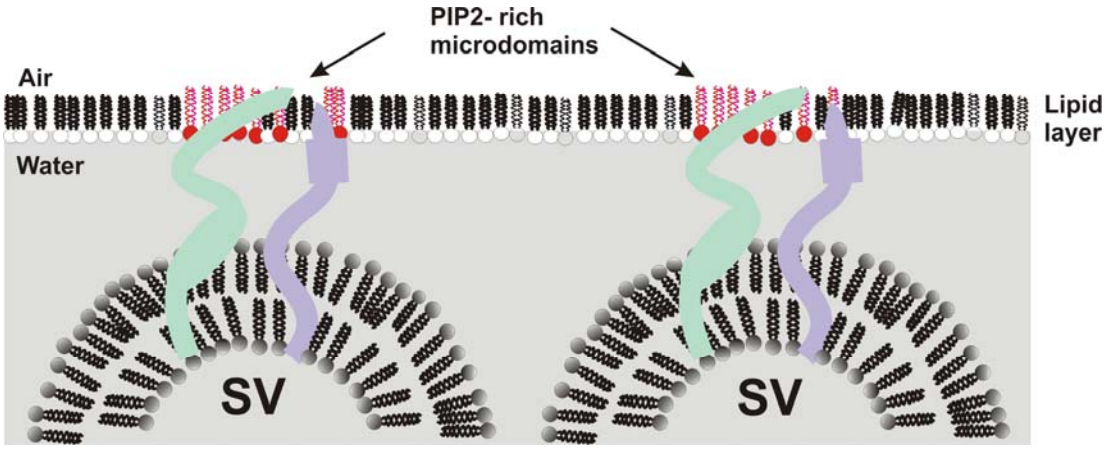


Fig. 2: Schematic of the monolayer with synaptic vesicles associated protein inserted into the PIP2-rich microdomain.

References:

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