ESRF	**Interaction of synaptic vesicles with lipid membranes modulated by synapsin: a structural investigation by x-ray reflectivity and GISAXS**	**Experiment number**: SC-5361
Beamline: **ID 10b**	**Date of experiment**: from: 01 Sep 2022 to: 05 Sep 2022	**Date of report**: 28.02.2023
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Names and affiliations of applicants (* indicates experimentalists): Hendrik Bruns*, Charlotte Neuhaus*, Titus Czajka*, Jette Alfken, Tim Salditt*, University of Göttingen

Report: We have studied the interaction of synaptic proteins [1] and lipid model vesicles with different lipid monolayers modulated by the synaptic protein synapsin [2], using a Langmuir trough (film balance). Structural changes of the monolayer were monitored by X-ray reflectivity (XRR), grazing incidence diffraction (GID), and grazing incidence small-angle X-ray scattering (GISAXS), while injecting proteins and vesicles into the subphase, and at controlled (and varied) lateral pressure.
As in our previous studies [5-7], we used DPPC/DPPS (1:1) and DPPC/PIP_2 (19:1) monolayers, interacting with synapsin 1a, synaptic vesicles and artificial liposomes at different concentrations. The monolayers serve as models for the for the inner leaflet of the presynaptic membrane. We then measured the response of adding several potential effectors in different sequences to the subphase. The effectors include intact and fully functional SV, isolated from CNS tissue, artificial lipid vesicles, and the presynaptic protein synapsin 1a, which is associated with forming synaptic vesicle clusters in the presynapse. The goal of this experiment is to shed light on the extend of its interaction with the presynaptic membrane. The measured interaction with the charged monolayers serves as a proxy for the interaction of a presynaptic bilayer.

The 22keV beam of three undulators operated in the third harmonic was collimated to 20x30 μm^2 with a flux of approximately 10^{12} ph/s. Vesicles were injected into the subphase to test their interaction with the monolayer-synapsin complex. Pure lipid model vesicles LV4 (55% DOPC, 20% DOPS, 15% DOPE, 10% cholesterol) served as a control for SVs from rat brain. As a control for the role of synapsin, the vesicles were added first into the subphase, followed by synapsin. After monolayer and effector(s) were prepared, $CaCl_2$ was added in addition. For each run (new trough), XRR and GISAX and GID scans were recorded, sequentially after each preparation step. The minimum filter wheel position was set to 3 (attenuation factor 0.0347), acquisition time per scan point was 1s (XRR, low q), or 5s (XRR, high q), respectively. The trough was translated after each measurement set (XRR+GID). Resulting curves were checked for radiation damage. One measurement set took 25 minutes; 80 sets were recorded in total. During acquisition and the injection, the surface pressure was also recorded. Two Langmuir troughs where used for the experiment. The beamline specific 400 ml with a single Teflon barrier compressing the monolayer, allowing for a pressure control during the experiment. In this configuration each experimental step was measured at three different surface pressures Π = (30, 20, 10) mN/m. The second half of the experiment a smaller trough with 25 ml subphase volume was placed in the larger one, allowing for higher concentrations of the limited synapsin protein and SVs. This configuration does not allow pressure control, as it has no barrier to change the monolayer area. Lipids where added in order to reach an initial surface pressure of approx. Π =20 mN/m. Evaporation from the subphase and the parasitic scattering was

circumvented or reduced by closing a hood over the trough and flushing with water-saturated helium.

The smaller trough allowed for a synapsin concentration of up to 9.6 nM in the subphase. Fig.1 shows (left) a schematic, and (right), the measured tilt angle τ and area per lipid chain A of the DPPC/DPPS (1:1) monolayer, for different subsequent injections, as labelled by numbers: (1) pure monolayer, (2) synapsin, (3) lipid vesicles (LV4), (4) $CaCl_2$ 10µM, and (5) $CaCl_2$ 1mM. Synapsin, which was added first, caused a significant decrease in τ and A, followed by further changes when adding the vesicles.

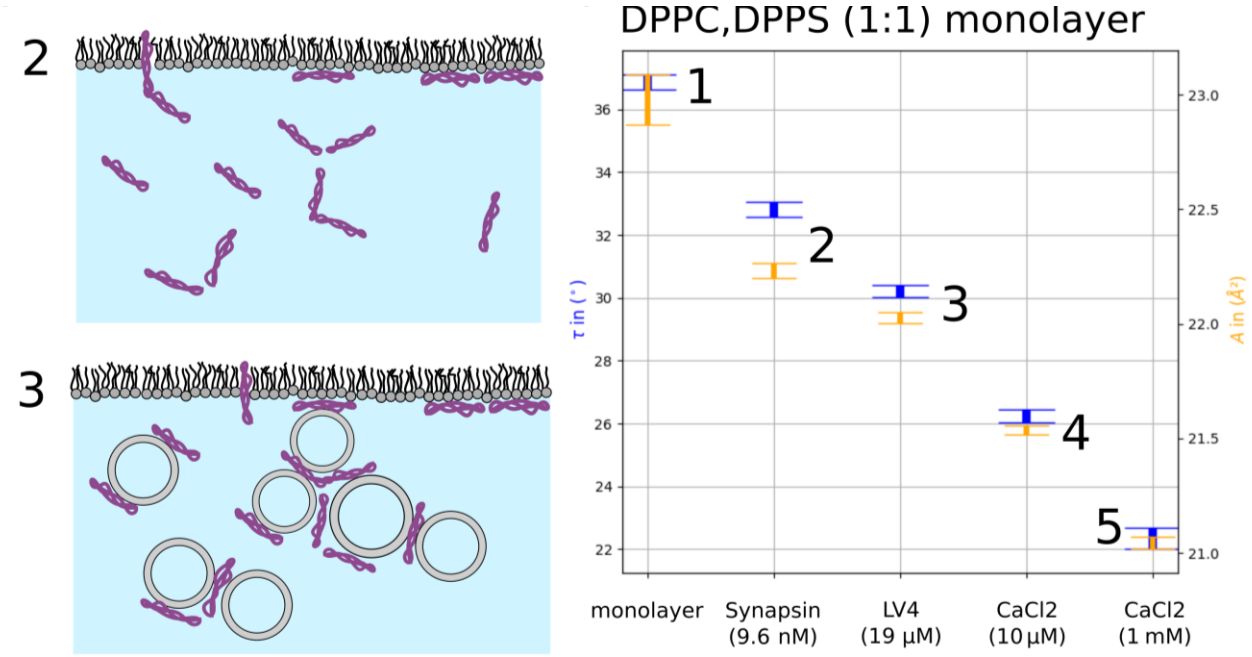

Fig. (1): (left) Schematic of the monolayer on the subphase(1), the synapsin (purple) added in step (2) and the added LV4 Vesicles added in (3). (right) Measured tilt angle τ (blue) and measured area per lipid chain A (orange), for each additive injection into the subphase.

Combining the information from XRR, GISAXS (based on the Eiger detector) and GID measurements, and the pressure-area isotherms in addition, a refined structural model of the interaction of synaptic vesicles with a lipid film and its modulation by the protein synapsin, is the goal of the ongoing analysis. For each sample, the quantitative fitting will provide structural parameters such as the electron density profile, interfacial disorder (e.g. roughness), as well as the structural response of the monolayer (rearrangement of lipid chain packing). This structural information can be extracted for different synapsin concentrations, lipid compositions, and for different buffer conditions ([Ca^{++}]). Analysis is ongoing. The work is embedded in the research network SFB1286 Quantitative Synaptology of the German science foundation (DFG), and carried out in collaboration with C. Hoffmann and D. Milovanovic (DZNE & Charité Berlin)

References

[1] S. Takamori, …, R. Jahn.. Cell. 127, 831 (2006); S.O. Rizzoli, W.J. Betz, Nat.Rev.Neur. 6, 57 (2005).
[2] D. Milovanovic, Y. Wu,Y. Bian, P. De Camilli, Science 361, 604 (2018).
[4] J. Lautenschläger et al., Nat.Comm, 9, 712 (2018); S. Ray et al., Nat. Chemistry 12, 705 (2020).
[5] S. Castorph,…, T. Salditt.. Biophys.J. 102:1200-1208 (2010); S. Castorph, …,T. Salditt. Eur. Phys. J. E 34: 6 (2011);.K. Ghosh, …, T. Salditt New J. Phys. 12, 105004 (2010) ; K. Ghosh, …, T. Salditt.. Biophys.J. 102: 1394-1402 (2012).
[6] T. Salditt, K. Komorowski and K. Frank , X-ray structure analysis of lipid membrane systems: solid-supported bilayers, bilayer stacks, and vesicles in Charact. of Biol. Membr.. De Gruyter STEM (2019).
[7] K. Komorowski,…,T. Salditt . Biophys. J. 114, 1908 (2018); Soft Matter 16, 4142 (2020).