ESRF	**Dynamics of vesicles in dense pools studied by XPCS**	**Experiment number**: SC-5277
Beamline: ID10A	**Date of experiment**: from: 02/17/2022 to: 02/21/2022	**Date of report**: 02/27/2023 revised 09/12/2023
Shifts: 12	**Local contact(s)**: Federico Zontone	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Titus Czajka*, Charlotte Neuhaus*, Jette Alfken*, Moritz Stammer*, Tim Salditt*

Report:

We have carried out a study of the dynamics of artificial lipid vesicles in dense vesicle pools by XPCS. Pools are aggregates of vesicles which are clustered together by synapsin Ia protein causing an attractive interaction. Synapsin Ia induced vesicle pools have previously been reported to display liquid-liquid phase separation [1,2,3]. Additionally, as a control sample with well-defined interactions, lipid vesicle clustering induced by Ca^{2+} ions dissolved in ultrapure water was investigated. The experiments were performed at the EH2 endstation of ID10 to profit from the XPCS-optimised setup at intermediate q-values.

XPCS scattering data was recorded with an Eiger500k detector positioned 7 m downstream from the sample, located in a quartz capillary (1 mm diameter). The 8.09 keV beam was collimated to a beam size of about 40x40 µm². As in our experiments assessing the static structure of synapsin clusters (e.g. at ID02, SC-5112 and [4,5]), lipid model vesicles LV4 (55% DOPC, 20% DOPS, 15% DOPE, 10% cholesterol) and lipid model vesicles LV2 (50% DOPC, 50% DOPS) were used and were found to yield good ID10 SAXS contrast at concentrations of 24-36 mM and 50 mM, respectively. LV2-type vesicles suspended in water were extruded at the PSCM laboratories and mixed with either $CaCl_2$ (0.5, 1, 2, 4, 8, 10, 40 mM) or $MgCl_2$ (4, 10 mM) to form the clusters used as a control system. LV4-type vesicles in a TRIS buffer (pH 7.4) were prepared in a similar way and mixed with synapsin at a 1:500 and 1:10,800 ratio.

Single XPCS runs usually comprised 4,000 diffraction images taken successively at exposure times of 5, 25, and 100 ms at different attenuator values, adjusted to minimise beam damage to the sample (0-3 silicon attenuators, 80 µm thick). At beam intensities that were strong enough to achieve good correlation signals, beam damage was usually visible. To limit the influence of beam induced changes and assuming a homogenous sample, multiple XPCS runs were carried out at different positions along the capillary and individual correlation signals were later averaged to find a mean correlation function. This simple setup, however, did not yield the desired results, due to either low signal strength or strong beam induced effects.

To increase signal strength, we decided to measure the correlation of passive tracer particles (DUKE silica colloids, 490 nm diameter, 2.05 $\%_{CV}$) suspended in the sample solutions. Using this passive XPCS microrheology approach, the static SAXS signal was dominated by the DUKE tracer particles. This made measurements at a much lower dose possible, but some beamdamage was still observable in the averaged intensity, especially near the minima of the silica particles. Results showing the amount of beam damage to a calcium and synapsin sample at different dose rates (0.27 kGy/s and 0.82 kGy/s, resp.) are shown in Fig. 1b and Fig. 1d, respectively. The relative change of signal strength is compared against the dose received by the sample or, equivalently, to the time after the start of the measurement. The correlation functions obtained from these datasets have good contrast and reflect different effects. At low $CaCl_2$ concentrations, the loss of correlation is dominated by the diffusion of tracer particles at very small lag times (below 10

ms), requiring very short exposure times of 35 µs (10,000 frames). At high calcium concentrations, the signal correlation is lost only at much larger lag times (about 1 s). The q-dependence of the relaxation rate Γ shows a non-linear behaviour with two different regimes. A slow decorrelation at low q and a fast decorrelation at high q were observed, as shown in Fig. 1a. Monte-Carlo simulations of colloidal systems with superimposed rotational flow show qualitatively similar results, suggesting that viscoelastic effects within the vesicle cluster could be responsible for the observed q-dependence of the relaxation rates. The synapsin sample displayed a similar q-dependence (not shown here). However, the two-time correlation function (TTCF) of the sample, shown in Fig. 1c, is very heterogeneous even where beamdamage is presumed to be negligible (inset Fig. 1c), implying highly complex dynamics within the sample. Further (e.g. microrheological, XPCS) experiments investigating the interaction between vesicle clusters and silica colloidal particles will be carried out to continue the development of a XPCS-microrheology protocol for complex fluids at low sample concentrations. The work is embedded in the research network SFB 1286 Quantitative Synaptology of the German science foundation (DFG) and carried out in collaboration with C. Hoffmann and D. Milovanovic (DZNE & Charité Berlin) as well as D. Pontoni and Y. Chushkin (ESRF).

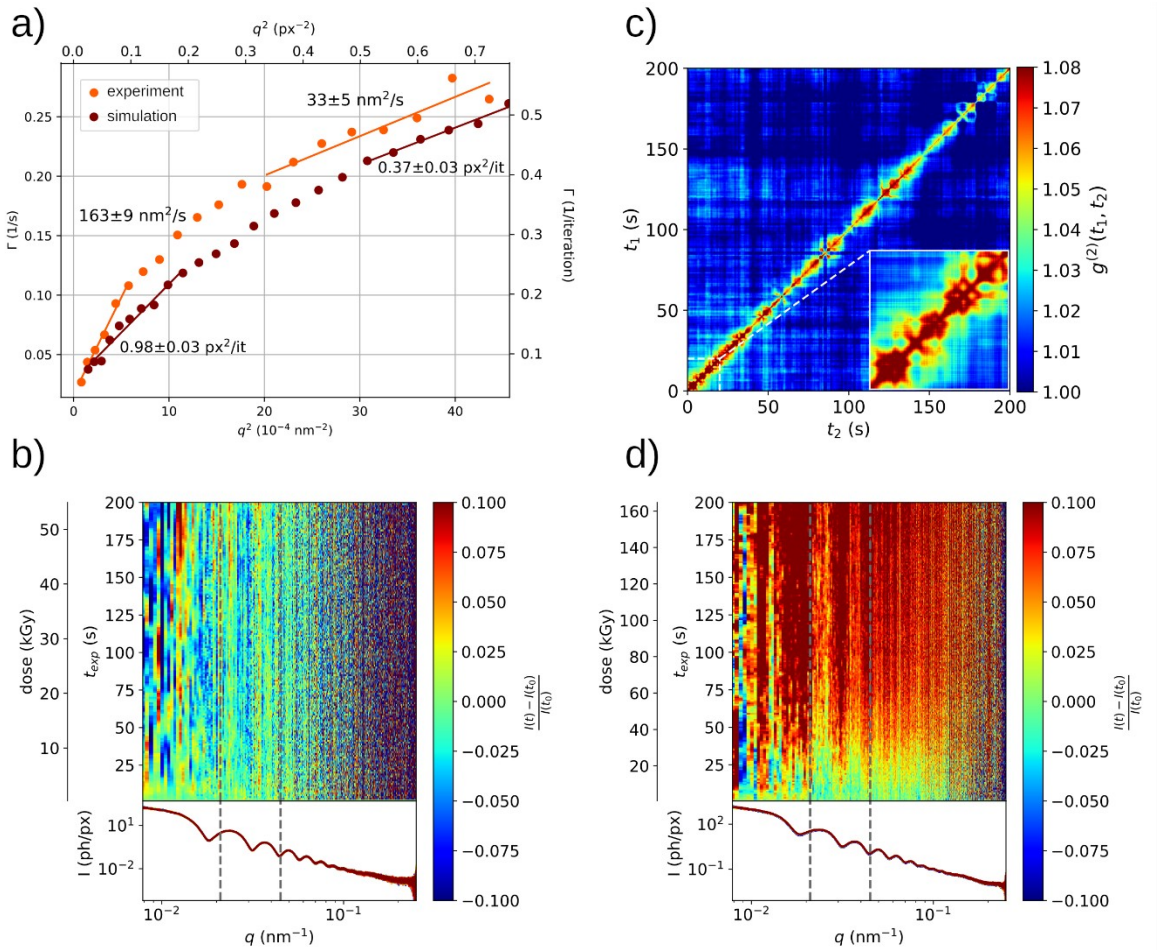

Figure 1: *q-dependence of the relaxation rate Γ of a mix of DUKE particles with 10 mM LV2 and 8 mM $CaCl_2$, displaying two distinct regimes (a) and the beam damage plot corresponding to that measurement (b). 4,000 images of the calcium sample were taken, each with 50 ms exposure time. The two-time correlation function (at q =0.0165 nm^{-1}) of a 1:500 mix of synapsin protein (5.4 µM) and LV4 (3.3 mM) mixed with an equal amount of silica particles displays very heterogeneous dynamics (c), even where beamdamage is presumed negligible (inset). The corresponding beam damage plot is shown in (d). 5,000 images of the synapsin sample were taken, each with 40 ms exposure time.*

References:

[1] D. Milovanovic, Y. Wu,Y. Bian, P. De Camilli, Science 361, 604 (2018).
[2] S. Albert, A. Gladfelter, T. Mittag, Cell 176, 419 (2019).
[3] J. Lautenschläger et al., Nat.Comm, 9, 712 (2018); S. Ray et al., Nat. Chemistry 12, 705 (2020).
[4] 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).
[5] K. Komorowski,…,T. Salditt, Biophys. J. 114, 1908 (2018); Soft Matter 16, 4142 (2020).