	Experiment title: Vesicle fusion studied by SAXS in microfluidic channels	**Experiment number**: sc4390
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Shifts:	**Local contact(s)**: Michael Sztucki	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Karlo Komorowski*,Jannis Schaeper,Yihui Xu*, Gerrit Brehm, Sarah Köster, Tim Salditt*

Institut für Röntgenphysik, Göttingen, Germany

Report:

The goal of the experiment was to investigate the structure of vesicle adhesion and fusion using both pure lipid model systems and lipid vesicles reconstituted with SNAREs (proteins that mediate fusion, e.g. in synaptic exocytosis). Fusion of pure lipid vesicles was induced 'physically' by $CaCl_2$. SNARE-mediated docking and fusion was performed by using vesicles reconstituted with sybΔ84 and sybWT, respectively, and vesicles reconstituted with the ΔN-complex as the acceptor complex [1,2]. SAXS and GISAXS are used in this project as unique tools to probe lipid bilayer density profiles and distances under physiological conditions [3,4,5]. Apart from high resolution SAXS measurements in capillaries, a particular goal of the present experiment was to study the structural dynamics of the adhesion and fusion of lipid vesicles in microfluidic channels by small-angle x-ray scattering (SAXS).

For all experiments, the photon energy was set to 12.56 keV and the Rayonix MX-170HS CCD pixel detector (3840 x 3840 pixels) was used at a sample-to-detector distance of 1.5 m to cover a *q*-range of ~ 0.056 - 5.13 nm^{-1}. For all microfluidic experiments, the beam size at the sample plane was 30 x 30 µm². We have used self-fabricated microfluidic devices made of Topas with four inlets (Fig. 1A,C) [6,7], where the two diagonal inlets were used for the delivery of the lipid vesicle suspension and the CaCl2 buffer, and the two vertical inlets were used for focusing of the "reaction flow" along the outlet with ultra pure water (MQ).

Fig. 1 shows the results of two microfluidic experiments, mixing of 10 mg/ml DOPC:DOPS (1:1) vesicles with 2 mM CaCl2 (A,B) and of 10 mg/ml DOPC:DOPS (10:1) with 10 mM CaCl2 (C-E), as an illustrative example. The darkfields in (A,C) were obtained by scanning SAXS of the microfluidic channels. (B) displays a selection of SAXS profiles along the reaction flow in (A), where a transition from unilamellar vesicles (ULVs) to multilamellar (MLVs) can be observed. (D,E) display a background subtracted SAXS profile (correspoding to the darkfield in (C)) showing characteristic structure factor modulations of adhering vesicles and a least-squares fit using the docking model as reported in [5]. From the fit, we have obtained structural bilayer parameters and the distance between two adjacent bilayers (2.39 nm) in the docking (adhesion) state.

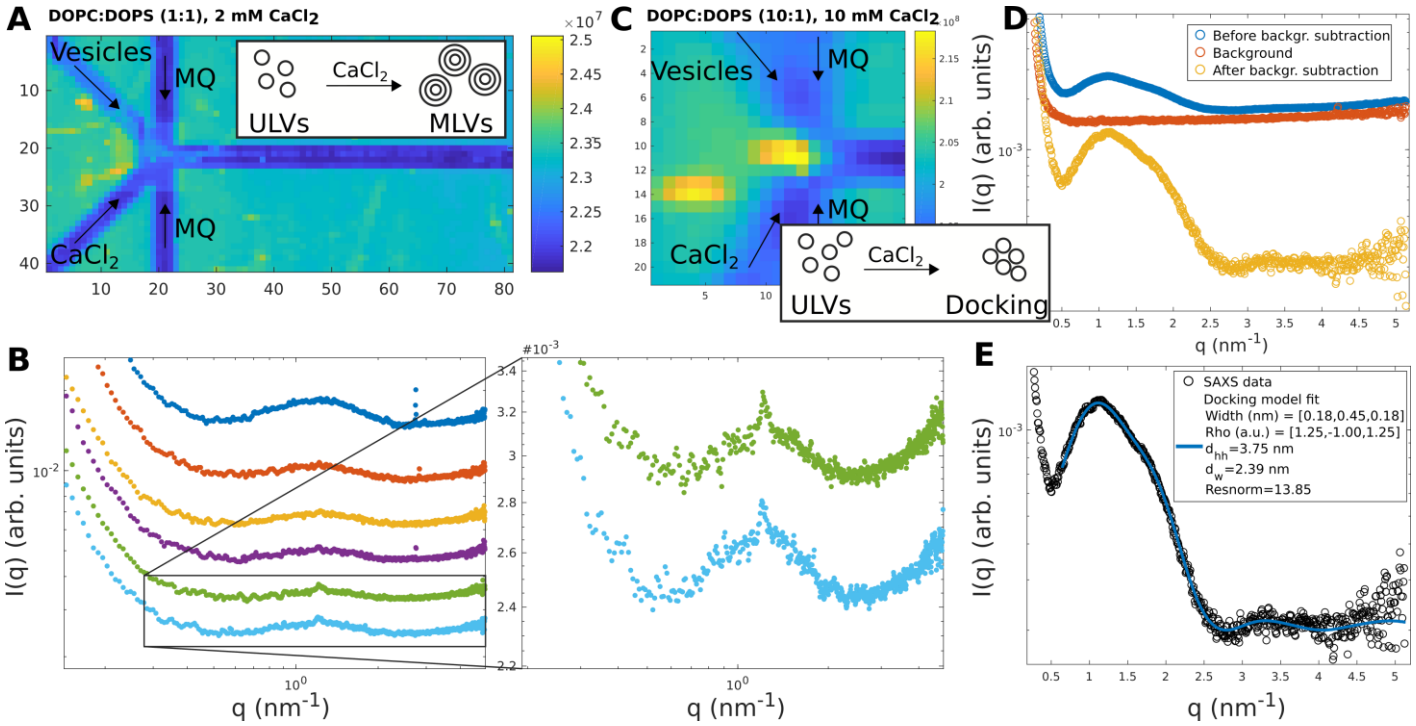

Figure 1: (A) Darkfield obtained by scanning-SAXS of the microfluidic flow for the mixing of 10 mg/ml DOPC:DOPS (1:1) with 2 mM CaCl2. The volume flow rates in the inlets are 25 µl/h for the vesicle suspension and for the $CaCl_2$ buffer, and 50 µl/h for both MQ water inlets. **(B)** Selection of SAXS profiles corresponding to the "reaction flow" in (A). **(C)** Darkfield obtained by scanning-SAXS of the microfluidic flow for the mixing of 10 mg/ml DOPC:DOPS (10:1) with 10 mM CaCl2. **(D)** Background subtracted SAXS profile (corresponding to (C)) showing characteristic structure factor modulations of docked vesicles. **(E)** Least-squares analysis of the SAXS profile in (D) using the docking model [4] yields a water spacing of 2.39 nm.

Hence, the experiment has successfully demonstrated that structural dynamics of lipid vesicle fusion can be probed by the experimental approach, and that important parameters such as the inter-bilayer distance in the docking state can quantified. The data is now further analyzed, using SAXS models developed in [5], and in additional tools presented in [8], and results are interpreted in view of different fusion mechanisms (K. Komorowski et al. manuscript in preparation).

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