ESRF	**Experiment title:** "Using pressure-jump induced TRSAXS to reveal the effect of membrane fusion promoting domains on the bilayer to cubic phase transition of monoolein"	**Experiment number**: SC-2802
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Report:

In the present study, time-resolved small-angle X-ray scattering (TRSAXS) experiments were carried out to evaluate the effect of membrane fusion promoting domains on the fluid lamellar to inverse bicontinuous cubic phase (L_α-to-Q_{II}) transition of monoolein, which serves as a well established model system for studying the final steps of the fusion process of bilayer membranes. Recently, we succeeded already in studying the L_α-to-Q_{II} transition of monoacylglycerides in the absence of fusion promoting domains, which revealed the existence of a stalk intermediate. Here, the influence of the fusion peptide of influenza virus haemagglutinin A (HA2) on the thermotropic and barotropic ordered phases of monoolein was studied exemplarily for membrane fusion promoting domains at T = 3 to 64 °C and in a pressure range from ambient pressure up to 4.0 kbar. As a reverence, a non-fusogenic, artificial control peptide (L16 H/G) has been studied as well.

A common method of measuring the intrinsic monolayer curvature in lipid systems is the determination of the repeat distances, i.e. lattice spacings, of the inverse cubic phase assemblies by small-angle X-ray scattering. For the present experiments, the diffraction patterns had been taken with very short exposure times (5-100 ms), which is only possible at synchrotron sources with high flux (in this case the ID02 beamline with ~$1 \cdot 10^{13}$ photons s^{-1}). The experiments were performed by using a home-built thermostated high pressure-jump equipment which has been successfully used before for studies of membrane phase transitions. The X-ray sample cell with flat diamond windows of 1 mm thickness, which is specified for pressures up to 4 kbar and temperatures up to 65 °C, has a sample volume of 25 µL. Pressure-jumps could be achieved in ~5 ms using pneumatic high-pressure valves. The beamline shutter triggers the electronics controlling the valves so that the pressure-jump and data acquisition occur simultaneously, thus facilitating time-resolved series of SAXS diffraction patterns with high time resolution. The use of the high pressure cell with strongly absorbing diamond windows required a high X-ray intensity of 12 keV which could only be obtained at the synchrotron source. The samples were placed in the cell with teflon (PTFE) rings and mylar foils to separate the sample from the pressurizing medium (water).

All three different samples have been investigated in a temperature range between ~6 °C and 65 °C at ambient pressure. The temperature and pressure dependent phase behavior of monoolein with and without incorporated peptides was revealed by applying pressures up to 4 kbar at selected temperatures. Finally, several pressure jumps were conducted for all samples each crossing a phase boundary between different lipid mesophases. First, the pure lipid sample (monoolein (MO) at a hydration level of 17 wt.%) was investigated. Figure 1 shows the temperature dependent scattering patterns revealing the phase behavior of monoolein at ambient pressure (1 bar). At low temperatures ($T \leq 28$ °C), a lamellar lipid phase with a d-spacing of 4.5 nm is observed. Between 28 °C and 34 °C, a phase transition to a lamellar-cubic coexistance region can be found, which is stable up to the final temperature of 64 °C. In figure 3, the scattering patterns

of MO are shown in a pressure range up to 3 kbar at 64 °C. It should be noted that – in this case – an increase in pressure drives the lipid phase transition in the same direction as a decrease in temperature. Thus, a similar phase transition behavior is observed, going from a lamellar-cubic phase coexisting region at $p \leq 1.5$ kbar to the lamellar phase at higher pressures.

Second, a sample with monoolein at the same hydration level (17 wt.%) including the viral fusion peptide HA2 (2 wt.%) was investigated in the temperature range between 10 °C and 64 °C at ambient pressure. The scattering patterns are shown in figure 2. In contrast to the pure MO sample, two phase transitions can be determined: First, a transition from the pure lamellar phase to a lamellar-cubic two-phase region at around 25 °C, and second from the coexisting phases to a pure cubic phase at higher temperatures. This shift of the phase transition temperatures in comparison to those of the pure lipid phase reveals the capability of the fusion peptide to destabilize lamellar lipid phases relative to nonlamellar lipid phases and thus to facilitate the formation of bicontinuous cubic lipid phases. In figure 4, the pressure dependent scattering patterns of this lipid phase are given at 64 °C in a pressure range up to 3.5 kbar and compared to MO, a similar effect on the lipid phase stabilities is observed, i.e., a shift of the phase transition of more than 500 bar. To ensure that the observed effect is indeed a characteristic property of the viral fusion peptide, the influence of a non-fusogenic control peptide (so called L16 H/G) on the phase behavior of monoolein was studied as well. In fact, the data of the control peptide L16 H/G were nearly identical to the data of the pure lipid sample.

Taken together, after completion of the data analysis we will be able to 1) establish the *p,T*-phase diagram for monoolein at a hydration level of 17 wt.% with and without the viral fusion peptide HA2 as well as for MO including the control peptide L16 H/G, and 2) reveal the kinetics and mechanism of the phase transitions in these systems. The data will help us to evaluate the effects of different membrane fusion promoting domains, i.e., viral fusion peptides, on the curvature properties of the membrane by determining how these domains influence the L_α-to-Q_{II} transition of monoolein and will thus provide new insights into the transient structural, energetic as well as kinetic properties of the membrane fusion events.

Figures 1-4: