ESRF	**Experiment title:** "Effect of viral fusion peptides and transmembrane domains on the lipid phase behavior of monoolein studied by pressure-jump induced TRSAXS"	**Experiment number**: SC-2905
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Shifts: 9	**Local contact(s)**: Dr. Theyencheri Narayanan, Dr. Michael Sztucki	*Received at ESRF:*

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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 a class I viral fusion peptide (influenza virus haemagglutinin A, HA2) and a class II fusion peptide (tick-borne encephalitis virus, TBEV) on the thermotropic and barotropic ordered phases of monoolein was studied exemplarily for membrane fusion promoting domains of different virus classes at T = 4 to 70 °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 70 °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).

A sample with monoolein (MO) at a hydration level of 17 wt.% including the fusion peptide TBEV revealed a completely different temperature dependent phase behavior than all other peptides investigated so far. All previously analyzed systems showed temperature and pressure dependent phase transitions between lamellar and cubic phases. For the sample containing TBEV, two coexisting hexagonal phases could be detected at low temperature which were dominant over the whole temperature range covered (4 –70 °C) at ambient pressure (Fig. 1). Additionally, a lamellar phase with low intensity could be observed that coexists with the hexagonal phases at temperatures above 18 °C. To ensure that the observed effect is indeed a

characteristic property of the viral fusion peptide, the influence of a non-fusogenic control peptide (L16 H/G) on the phase behavior of MO was studied. A sample with MO at the same hydration level (17 wt.%) including the control peptide L16 was investigated in the temperature range between 6 and 68 °C at ambient pressure. The corresponding scattering patterns are shown in Figure 2. A distinct transition from a lamellar phase to a cubic (Ia3d) phase can be found from 25 to 35 °C, including a two-phase coexisting region in the respective temperature range. Additionally, pressure dependent measurements between ambient pressure and 4 kbar were conducted at various temperatures. Accordingly, the data of the control peptide L16 H/G were found to be nearly identical to the data of the pure lipid sample.

Finally, numerous pressure jumps were conducted across the lamellar to non-lamellar phase boundary on a sample consisiting of MO at a hydration level of 17 wt.% and the fusion peptide HA2. Figure 3 shows the scattering patterns at 58.2 °C of a jump from a cubic phase at 0.7 kbar into a lamellar phase region at 1.7 kbar. On the right-hand side, the time dependent peak intensities of the Bragg reflections of all involved phases are shown. The blue line indicates the moment of the pressure jump at t = 0 s. Shortly after the jump, a decrease of the intenisty of the cubic phase can be observed. Simultaneously, the intensity of the new emerging lamellar phase increases significantly within the first 5 s after the jump. Afterwards, both phases coexist for approximately 3 s and finally the cubic phase vanishes completely ~20 s after the pressure jump.

Taken together, the static and kinetic measurements performed so far will allow us to evaluate the correlation between the distinct structural properties and thus different target membrane interactions of the fusion peptides from different viral fusion protein classes and their ability to modulate membrane curvature. From the experimental results, *p,T*-phase diagrams are established and the kinetics and mechanism of the phase transitions are revealed. The data will help us to assess 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.

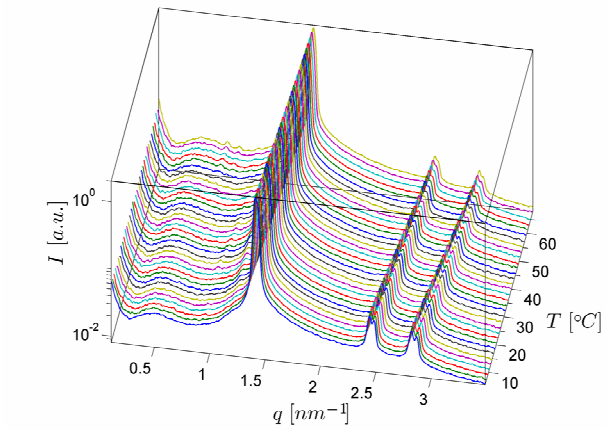


Fig. 1: SAXS patterns for MO+TBEV at ambient pressure

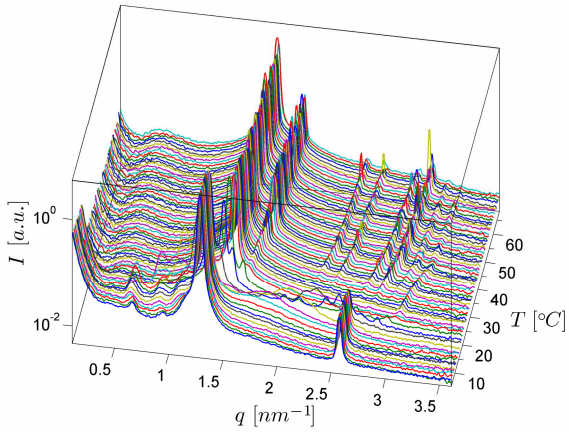
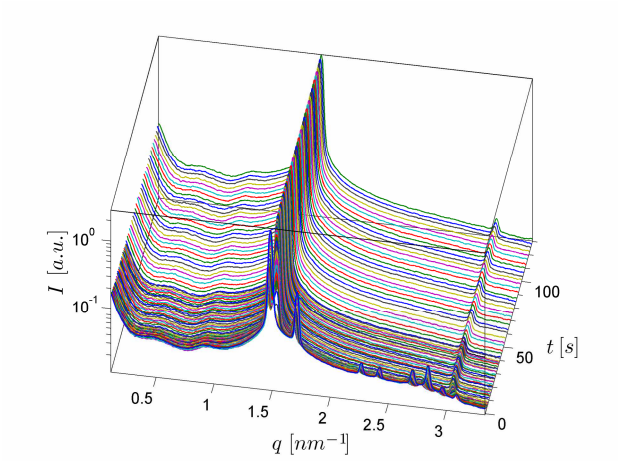


Fig. 2: SAXS patterns for MO+L16 H/G at ambient pressure



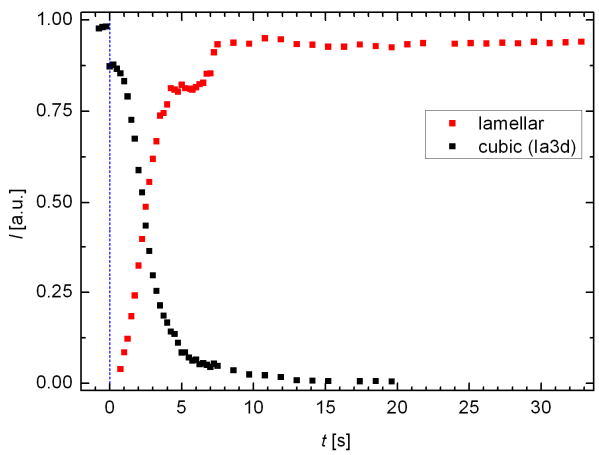


Fig. 3: Time-evolution of the SAXS patterns for a pressure jump from 0.7 kbar to 1.7 kbar of the system MO+HA2 (left) and the corresponding time-dependency of the Bragg peak intensity of the different phases (right).