ESRF	**Experiment title:** "Studying the effect of conformational flexibility and membrane-fusogenicity of de novo designed transmembrane peptides on the lipid phase behavior of monoolein by TRSAXS"	**Experiment number**: SC-3003
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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. Previous experiments revealed the existence of a stalk intermediate in the L_α-to-Q_{II} transition of monoacylglycerides in the absence of fusion promoting domains. Here, we continued the study of the previous proposal in comparing the influence of viral fusion peptides on the thermotropic and barotropic ordered phases of monoolein with that of viral transmembrane domains and de novo designed peptides. Therefore, the fusion peptide (FP) and transmembrane domain (TMD) of a class III fusion protein (vesicular stomatitus virus, VSV) as well as the de novo designed, artificial control peptide L16 H/G have been investigated in a pressure range from ambient pressure up to 4.0 kbar and at temperatures between 4 and 70 °C.

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 have been taken with very short exposure times (0.05 – 1 s), which is only possible at synchrotron sources with high flux (in this case the ID02 beamline with ~$1 \cdot 10^{14}$ 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 0.7 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).

The measurements of a sample consisting of monoolein (MO) at a hydration level of 17 wt.% and 2 wt.% VSV-FP revealed a completely different membrane phase behavior than the previously analyzed samples containing the fusion peptides of class I and II viral fusion proteins (cf. report SC-2905). Whereas the latter showed a temperature and pressure dependent phase transition between lamellar, the cubic Ia3d, and hexagonal phases, the appearence of two new cubic phases can be demonstrated for the class III VSV-FP (Fig. 1). At 26 °C, a phase transition from two coexisting cubic phases, Im3m and Pn3m, to a three-phase region with

an additional hexagonal (H_{II}) phase can be observed. The complete *p,T*-phase diagram for MO+VSV-FP has been conducted and revealed a complex phase behavior changing between lamellar, the two cubic Im3m and Pn3m, as well as hexagonal phases. The *p,T*-phase diagram accomplished for the sample containing MO and the TMD of VSV at the same hydration level also revealed the existence of the cubic Pn3m phase with increasing temperature. Whereas the cubic Im3m phase cannot be observed for this sample, a phase transition from a cubic (Ia3d+Pn3m) coexistence region to a two-phase-lamellar (L) region can be detected with increasing pressure at 24.5 °C (Fig. 2). Numerous pressure jumps conducted across the lamellar to non-lamellar phase boundaries completed the study of the membrane phase behavior of MO containing the FP and TMD of the class III fusion protein VSV by revealing also the kinetics of the different phase transitions. In contrast, the data of the non-fusogenic, de novo designed control peptide L16 H/G were found to be nearly identical to the data of the pure lipid sample in showing the same L_{α}-to-Q_{II} transition as monoolein.

Taken together, the complete *p,T*-phase diagrams of MO containing the FP and TMD of a class III viral fusion protein as well as the control peptide L16 H/G were established from the experimental results of this study and can now be compared to the ones set up previously for class I and II viral fusion peptides (cf. SC-2905). From that a correlation between distinct structural properties and thus different target membrane interactions of the FPs from different viral fusion protein classes and their ability to modulate membrane curvature can be revealed. In addition, we are able to show that transmembrane domains are also able to lower the bilayer to hexagonal phase transition temperature and thus promote negative curvature of the membrane; a feature that has been thought to be a unique characteristic for viral fusion peptides. Finally, the kinetic measurements performed allow further insights into the kinetics of the phase transitions and thus the kinetic properties of the membrane fusion event.

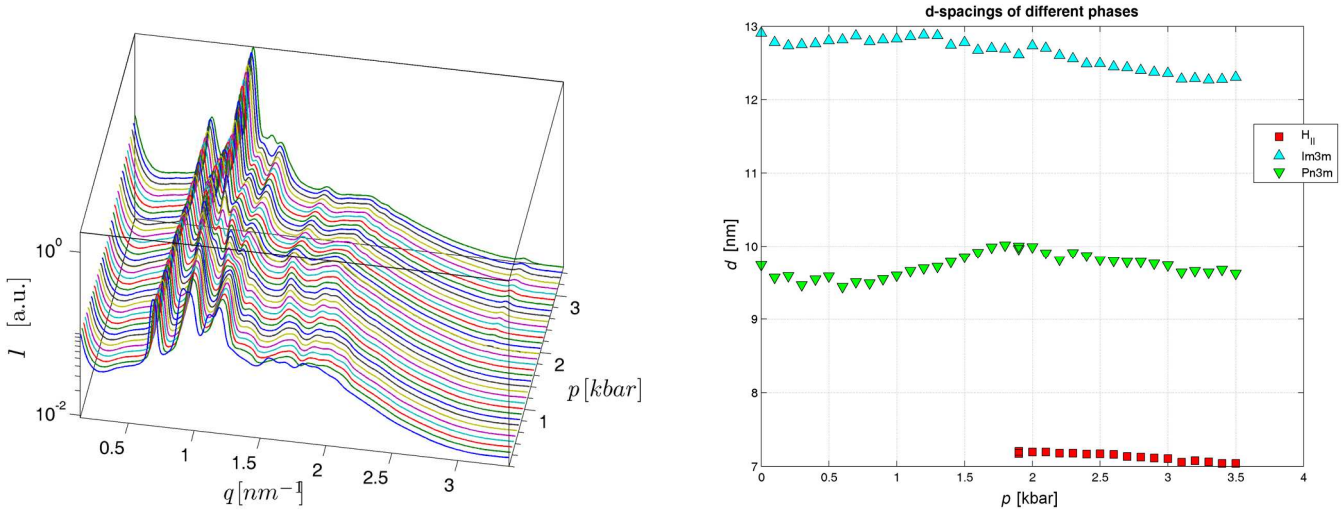

Fig. 1: SAXS patterns (left) and the concomitant change in *d* spacings (right) for a pressure dependency measurement at *T* = 26.0°C for the system MO+VSV-FP.

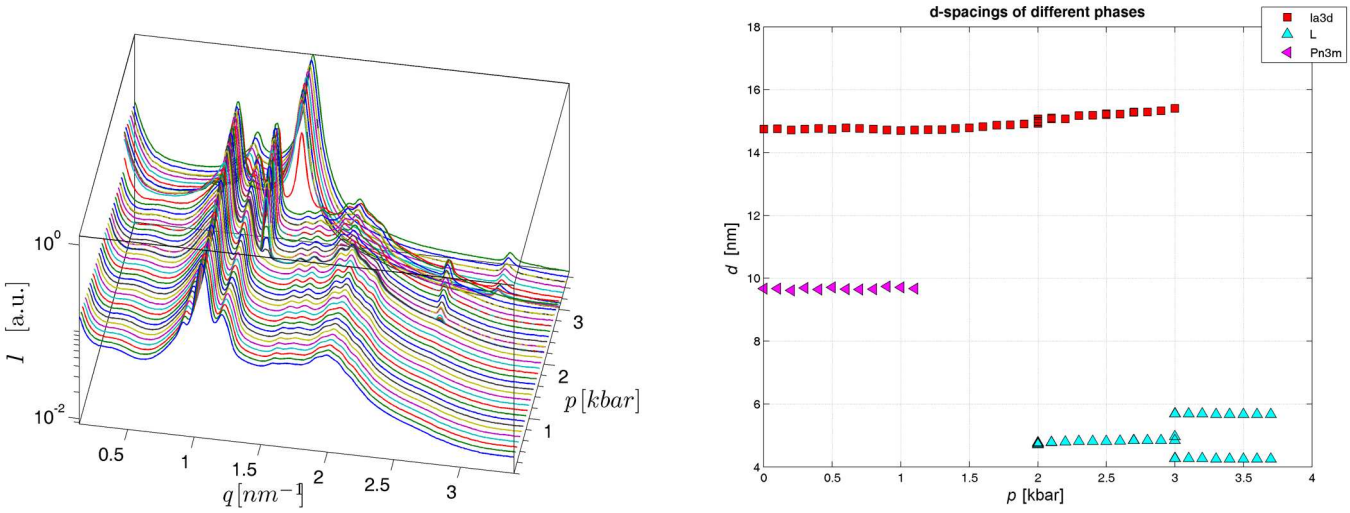

Fig. 2: SAXS patterns (left) and the concomitant change in *d* spacings (right) for a pressure dependency measurement at *T* = 24.5°C for the system MO+VSV-TMD.