ESRF	**Experiment title:** The interaction of viral fusion peptides with model lipid membranes	**Experiment number**: SC-4460
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Report:

We conducted a high energy XRR study on the interaction of viral fusion peptides with model lipid membranes at the solid-liquid interface in order to contribute to the basic understanding of the underlying mechanisms of membrane fusion. More precisely, we investigated the pressure-dependent phase behavior of monoolein/water mixtures that form various hexagonal, cubic and lamellar phases in absence and presence of different viral fusion peptides. As shifts of the phase boundaries correspond to changes of membrane properties induced by the viral fusion peptides, this approach provides information about the peptide-membrane interaction.

The XRR technique enables to resolve the vertical electron density profile of the sample system with sub-Ångström resolution, thus, it provides direct insight in the structure of monoolein layers that form at a solid substrate. Additionally, we recorded SAXS patterns before and after every reflectivity scan to capture the volume phase structure.

The measurements were performed with a custom-made high hydrostatic pressure cell [1] and at a photon energy of 70 keV. The beam size was approximately 5 µm (vertical) × 40 µm (horizontal). Pressures between 50 and 5000 bar and a temperature of 25 °C were applied. A hydrophilic siliconwafer was introduced into the cell and the cell volume was filled with a mixture of monoolein and 40 %wt water. The fusion peptides were added in concentrations of 2 %wt. We investigated the behavior of monoolein membranes interacting with the class I fusion peptide of Hemagglutinin 2 (HA2-FP), the class II fusion peptide of Tick-borne encephalitis virus (TBEV-FP) and a control peptide (L16) [2].

The SAXS data show for every composition that the monoolein lipids form cubic phases at 50 bar. For pure monoolein/water mixtures, the Pn3m phase is dominant at low pressures. With increasing pressure, the Im3m phase forms and a coexistence of both phases is observed. Between 2 and 3 kbar, a phase transition into a lamellar phase occurs. This transition is shifted to pressures of 1 to 1.5 kbar when TBEV-FP is added. In contrast, in presence of HA2-FP cubic and lamellar phases coexist between 1.5 and 3 kbar, before the system

ends up in the lamellar phase at high pressures. On the basis of the reflectivity data, the near-surface behavior of monoolein/water mixtures at a hydrophilic interface can be determined. In case of pure monoolein/water mixtures, we observe a formation of strong *Bragg* reflections with a spacing that corresponds to the lamellar phase, when a pressure of 1 kbar is applied. The *Bragg* reflections become wider and flatter as soon as the volume phase transition into the lamellar phase occurs, since the volume scattering overlies the reflectivity signal. With HA2-FP, the multilayer formation at the surface appears also at 1 kbar, thus approximately 0.5 kbar before the coexistence of cubic and lamellar phases is observed in the volume. In *Figure 1*, reflectivity data and the corresponding electron density profiles of monoolein multilayers are depicted. In presence of TBEV-FP, the multilayer formation occurs at the same pressure as the volume phase transition into the lamellar phase. However, it takes place much faster, as the volume phase needs approximately 2 hours to reach a steady state, while the *Bragg* peaks are already visible in the reflectivity after a few minutes. *Figure 2* shows reflectivity data of monoolein/water mixtures in presence and absence of TBEV-FP (data fitting in progress).
The conducted measurements demonstrate the capability of our approach to study the role of fusion peptides in viral cell fusion as we were able to study the effect of HA2-FP and TBEV-FP on the phase behavior and the structural properties of monoolein model membranes. Therefore, we would like to perform a continuation of this experiment to complete the dataset, including measurements on class III fusion peptides and transmembrane domains.

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References: [1] F.J. Wirkert, M. Paulus, J. Nase, J. Möller, S. Kujawski, C. Sternemann, and M. Tolan, *Journal of Synchrotron Radiation* **21** (2014) 76. [2] K. Weise, and J. Reed, *ChemBioChem* **9** (2008) 934.

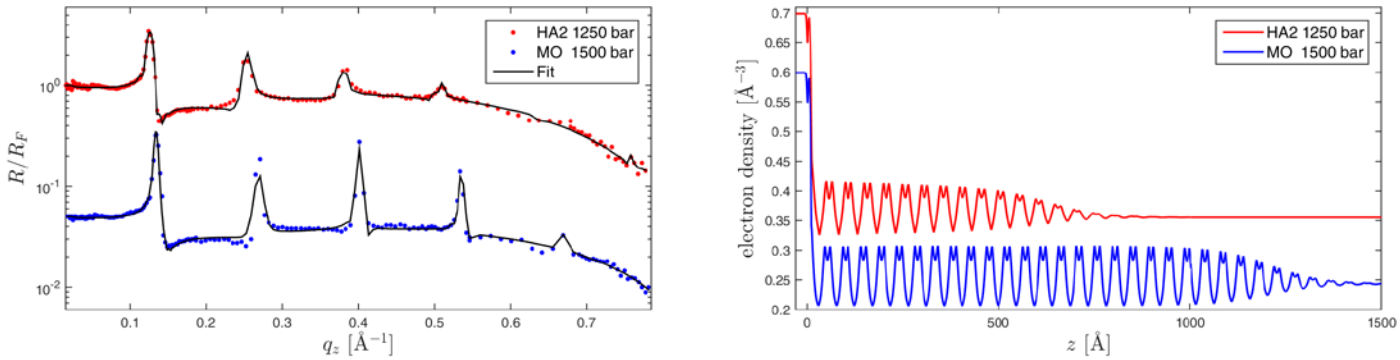

Figure 1: Reflectivity data of a monoolein/water mixture in presence and absence of HA2-FP shown with fits and the corresponding electron density profiles.

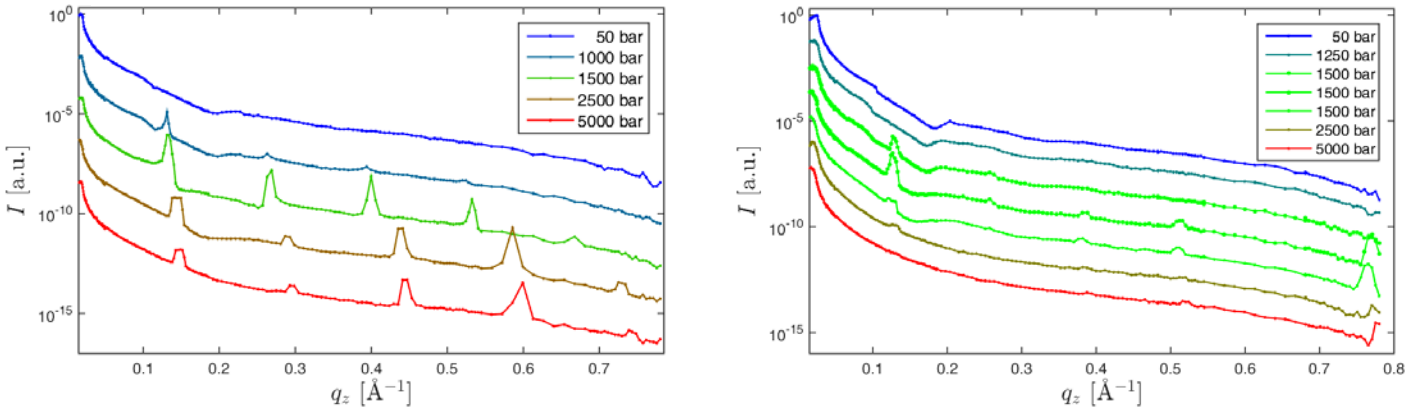

Figure 2: Reflectivity curves of a monoolein/water mixture in absence (left) and presence (right) of TBEV-FP at selected pressures.