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Report:

We investigated the behavior of monoolein model membranes interacting with viral fusion peptides (FP) in the bulk with small angle X-ray scattering (SAXS) and in proximity of a hydrophilic silicon dioxide surface with X-ray reflectometry (XRR) at pressurization. FPs are segments of viral envelope proteins located in the ectodomain and play an important role in the membrane fusion process that occurs when an enveloped virus enters a host cell. Their insertion into the target membrane leads to destabilization and initiates the formation of fusion pores. The membrane perturbing properties of FPs are reflected in a modified p - T -diagram of mesophases formed by lipids in an aqueous environment [1]. In our study, we applied liquid crystalline structures of monoolein as model membranes and studied how they are affected by the addition of viral fusion peptides. Monoolein forms various hexagonal, cubic and lamellar phases in an accessible temperature and pressure range. We determined changes of phase boundaries and the lattice constant a induced by viral fusion peptides by SAXS. Moreover, we conducted XRR at the solid/liquid interface to obtain a direct structural resolution of ordered lipid layers that form at a hydrophilic silicon dioxide surface. Additionally, we performed SAXS under grazing incidence. This geometry provides surface-sensitivity and enables to study the alignment of monoolein mesophases in proximity of a solid surface.

The measurements were performed in a custom-made high hydrostatic pressure cell [2] at a photon energy of 70 keV. The beam size was approximately 5 μm (vertical) \times 40 μm (horizontal). Pressures between 50 bar and 3500 bar and a temperature of 25 $^{\circ}\text{C}$ were applied. A hydrophilic silicon wafer was introduced into the cell and the cell volume was filled with a mixture of monoolein and 80 %wt water. The FPs were added in concentrations of 2 %wt. Viral fusion proteins are categorized in three different classes based on structural features. We investigated the behavior of monoolein membranes interacting with the class I FP of Hemagglutinin 2 (HA2-FP), the class II FP of Tick-borne encephalitis virus (TBEV-FP), and the class III FP of vesicular stomatitis virus (VSV-FP) [3].

The phase behavior of the monoolein/water mixtures was determined from the scattering angles of diffraction fringes that we observed in the SAXS experiments. The scattering angle α is connected to a momentum transfer $q = \frac{4\pi}{\lambda} \sin \frac{\alpha}{2}$. The fringes occur at specific multiples of the reciprocal lattice constant $\frac{2\pi}{a}$ that are characteristic for every given phase, e.g., $\sqrt{2}$, $\sqrt{3}$, $\sqrt{4}$, $\sqrt{6}$... for the Pn3m phase, $\sqrt{2}$, $\sqrt{4}$, $\sqrt{6}$, $\sqrt{8}$... for the Im3m phase, and 1, 2, 3, 4 ... for lamellar phases. Figure 1 shows that pure monoolein adopted the cubic Pn3m phase at low pressures (different colors indicate different samples). At pressurization, the lattice constant slightly increased. Between 2000 and 2500 bar, a phase transition into a lamellar phase occurred. Once HA2-FP was added, we observed the cubic Im3m phase at low pressures (Figure 2). In Systems with TBEV-FP, the sample likewise adopted the Pn3m phase at low pressures but with a by about 10 \AA increased lattice constant compared to pure

monoolein (Figure 3). With VSV-FP we observed the Pn3m phase as well as the Im3m phase (Figure 4). In all three cases, lamellar phases occurred at lower pressures compared to pure monoolein.

Figure 5 shows the diffraction pattern of monoolein in presence of VSV-FP at 50 bar obtained in grazing incidence geometry, which illustrates the typical orientation of the Pn3m phase at the silicon dioxide surface. Figure 6 depicts the scattered intensities integrated in small regions around the first two diffraction fringes as a function of the azimuthal angle φ . The strong maximum at $q = \sqrt{3} \cdot \frac{2\pi}{a}$ and $\varphi = 0^\circ$ indicates that the Pn3m phase was ordered with a $\{111\}$ -plane parallel to the surface. Therefore, the $\{110\}$ - and the other $\{111\}$ -planes included defined angles with the sample surface. These angles are marked by the dashed ($\{110\}$) and solid ($\{111\}$) lines in figure 5. At the corresponding scattering angles, weak Bragg reflections occurred. A detailed analysis of the surface measurements including the XRR data is still in progress.

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References: [1] Artem Levin, Christoph Jeworrek, Roland Winter, Katrin Weise, and Claus Czeslik, *J. Phys. Chem. B* **121** (2017) 8492. [2] F.J. Wirkert, M. Paulus, J. Nase, J. Möller, S. Kujawski, C. Sternemann, and M. Tolan, *Journal of Synchrotron Radiation* **21** (2014) 76. [3] K. Weise, and J. Reed, *ChemBioChem* **9** (2008) 934.

