ESRF	**Experiment title:** Vesicle-to-nanotube transformation of diyne phospholipid aggregates	**Experiment number:** SC-5249
Beamline: ID02	**Date of experiment:** from: 27 May 2022 to: 29 May 2022	**Date of report:** August 15, 2022
Shifts: 6	**Local contact(s):** Lauren Matthews	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):
Hörmann, Anja Franziska[*,a], Gradzielski, Michael, Prof.[a], Chamchoum, Matteo[*,a,b], Alvarado Galindo, Fernanda[*,b]
a: Stranski-Laboratory for Physical and Theoretical Chemistry, Technische Universität Berlin, Berlin, Germany
b: Institut Laue-Langevin, Grenoble, France

Report:

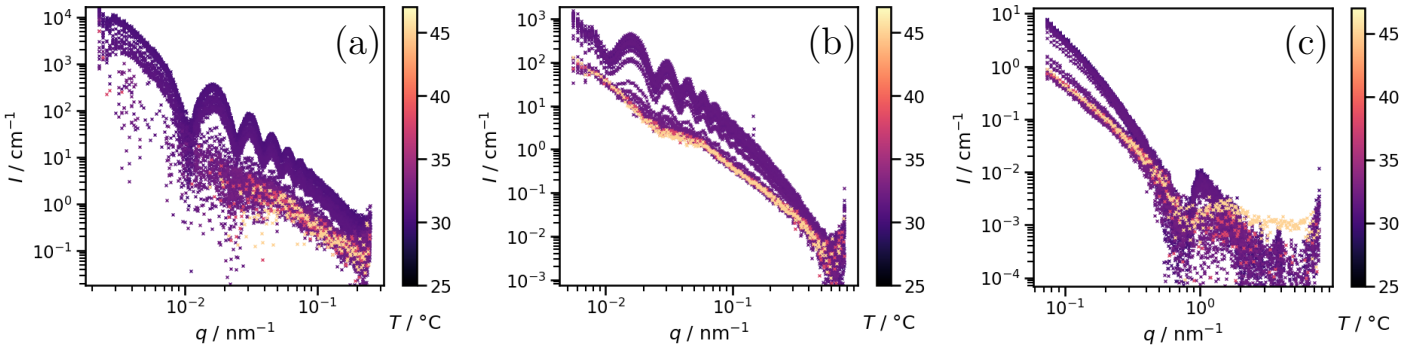
Experiment

We measured 0.5 g/L, 1 g/L, 2 g/L and 4 g/L of the diyne phospholipid $DC_{8,9}PC$ in a solvent consisting of 70%$_{v/v}$ methanol and 30%$_{v/v}$ water. The sample environment consisted of a flow-through quartz capillary of 2 mm diameter connected to a syringe pump (`sfd`) and temperature controlled using a lfi controller with a water bath and a Peltier element. Photon energy was 12.3 keV and measurements were performed at three sample-to-detector distances, 30.0 m, 10.0 m and 1.0 m, yielding a q-range of 0.003 nm^{-1} to 7 nm^{-1}. For each sample, we measured three cooling runs at 30 m, one at 10 and one or two at 1 m. The solvent was measured once at higher temperature resolution and all three configurations, all in the same spot of the capillary.

Temperature Protocol After preheating at 50 °C in an oven, the sample was measured at 45 °C and cooled quickly (80 K/h to 90 K/h) cooled down to a temperature safely above the transition, 31.5 °C to 33 °C depending on concentration, with one measurement in between. Then, the sample was cooled at 4 K/h in steps of 0.011 °C, measuring every 0.066 °C for about 48 min.

Beam Damage Mitigation We employed a combination of attenuation (50 µm Mo), slow movement of the sample and relaxation time between exposures to avoid beam damage. We achieved a time resolution of 72 to 73 s. The exposure time at 30 m was 10 ms, at 10 and 1 m, 100 ms could be used without polymerizing the sample.

Results



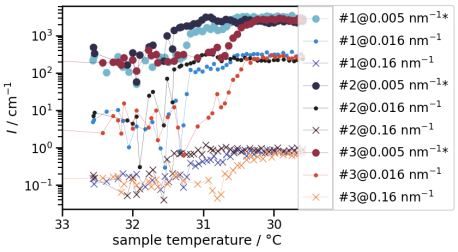


Figure 1 (top): Data minus solvent at the same temperature for the sample at 2 g/L. *Exposure was* 10 ms *in subfigure a (SD:* 30 m*) and* 100 ms *in subfigures b (SD:* 10 m*) and c (SD:* 1 m*).*
Figure 2 (left): Intensity at selected q values vs. sample temperature for 2 g/L *at* 30 m. *#1-#3 indicates the number of the cooling run. The data marked with ∗ were oversubtracted and shifted by* 500 cm^{-1} *for these plots.*

In Fig. 1, data are plotted per cooling run, color-coded by temperature. The sample at 2 g/L was chosen as an example, here, changes are most evident. In the following discussion, we will note if some of the other samples differ from the sample shown in a particular feature. All data shown result from the first data treatment, done by subtracting the background measurement closest to the data in temperature from the data. First, a clear difference between initial and final state is visible at all configurations. The mesoscopic transition from vesicular to tubular structures is observed at 30 and 10 m with several intermediate scattering patterns between initial and final state. Due to issues with this initial background subtraction at 30 m, the vesicle form factor is clearly visible at 10 m only and we observe a change in oscillation position and mid-q slope. At low temperature, up to 9 characteristic oscillations of tubular structures ($R \approx 210\,\mathrm{nm}$) are discernible by eye. 1 m measurements show the onset of the tubular scattering as well as a change in bilayer form factor. Upon crystallization, a multilamellar peak appears at about $1\,\mathrm{nm}^{-1}$ ($d \approx 6.3\,\mathrm{nm}$) at 2 g/L and 4 g/L lipid concentration.

Fig. 2 shows intensity at characteristic q-values from data at 30 m and the sample at 2 g/L. We chose the lowest q available, the position of the maximum after the first oscillation, and a value at mid-q that is also contained in the 10 m and 1 m configurations. Importantly, initial and final state seem to be consistent over different cooling runs. The transition, however, sometimes starts at different temperatures or does not proceed steadily in one direction. This is probably a sign of imperfect sample homogeneity, as trials without moving the sample (not shown) showed more discrepancy in that respect. Since nucleation of liquid-crystalline domains is a random process and there are only between 13 and 108 vesicles in the beam at any given time, randomness may become observable in the average, especially since contrast increases by a factor of 3.5 due to freezing and collisions may influence or propagate the process. Quantitative analysis will follow an improved background subtraction (in progress).