EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:
https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report")*,
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Mechanism of detergent induced formation of striated structures in lipid vesicles	**Experiment number**: SC-5163
Beamline: ID02	**Date of experiment**: from: 16.9.2021 to: 19.9.2021	**Date of report**: 4.3.2021
Shifts: 9	**Local contact(s)**: Thomas Zinn	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Victoria Ariel Bjørnestad, University of Oslo

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Report:

The plan was to study the time-evolution of both Triton X-100 and DDM on DPPC and DMPC bilayer (4 different systems), using mixing ratios where striated structures are observed with SAXS and WAXS. We had many problems occurring during the beamtime that both prevented us from completing this plan and also affected the reliability and usability of the experimental data. *The stopped-flow apparatus was leaking between the chambers during the majority of the experiment, causing the reservoirs to be contaminated over time and in addition leaking a large amount of air*. Consequently, most of the kinetic measurements were ruined by the presence of bubbles occurring at the time of measurements. The beamline scientist attempted to fix the stopped flow twice, meaning that we could not use it for large amounts of time and eventually the hard-stop was removed which did not work well with our samples. In addition, near the end of the experiment the beam also went down for several hours. All this has led to little and mostly inconclusive data, which took a very long time to sort through since each dataset has to be checked frame by frame to see if any kinetics could be observed in frames that did not have bubbles. Due to the problems, we only had time and, in the end only, enough of the lipid sample to do experiments on Triton X-100 on DPPC and DMPC bilayer. We performed stopped-flow experiments at 20, 25 and 30 °C for DPPC before

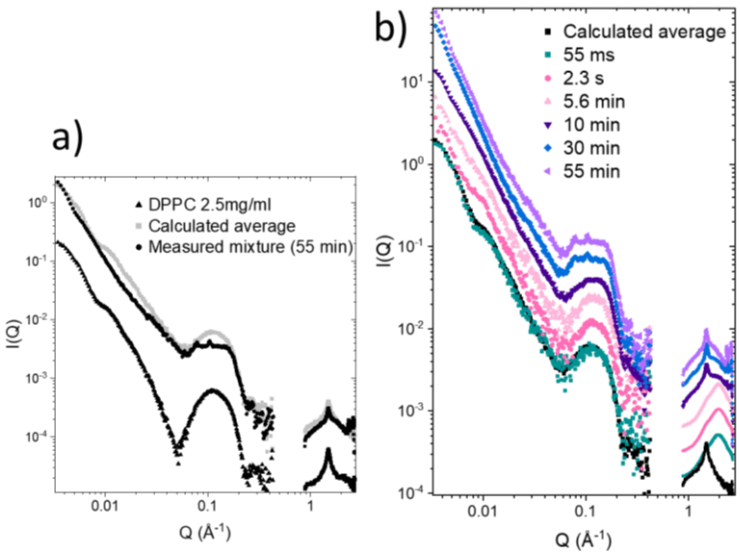

Figure 1: a) Comparison between data of a pure DPPC sample, calculated average (sum of DPPC and TX-100 meausurement) and the 1:1 mixture (2 curves at the top are scaled). b) Measurement of 1:1 mixture over time

running out of sample due to the number of attempts to get measurements without bubbles (what was left was also contaminated by the surfactant) and at 10 °C DMPC.
For DPPC at 20 °C the main interest was to see whether we could observe changes in the WAXS pattern due to a possible change in the lipid lipid packing and other signatures of the striated structures at this temperature. For pure DPPC we observe a peak at 1.5 $Å^{-1}$ (figure 1a), which can also clearly be seen from the plot of the sum of DPPC and Triton X-100 (calculated average). When looking at the mixture of DPPC and Triton X-100 that yields the peaks associated with the striation 55 minutes after mixing, there does not seem to be any changes from the calculated average. Looking at the SAXS data right after mixing, it seems that the process is relatively slow (Figure 1b): the data detected at 55 ms after mixing overlaps perfectly with the calculated average of the two components, which is then followed by a with a faster change in the low Q-region (seconds timescale) where we see the disappearance of the spherical form factor oscillation and a very slow change in the bilayer scattering (minutes to hours timescale). In the WAXS pattern however, it could seem that the typical DPPC gel peak actually is smeared out and shifted right after mixing, but then shifts back after several minutes (Figure 1b). We also tried to measure the end state after several hours incubation using the flow through cell, but because the flow through cell was not temperature regulated and the room temperature exceeded 25 °C in the beginning of the experiment, these incubated samples dissolved and were thereby destroyed each time we attempted this and we unfortunately ran out of uncontaminated sample before we could attempt again.
Examples of measurements of DPPC:TX-100 at 30 °C are displayed in Figure 2. It was very difficult to get frames in the stopped-flow mixing without bubbles at this temperature due to the beforementioned leakage. The most successful was the 1:1 mixture (Figure 2b) where we can see the complete solubilisation within approximately 2 minutes. For the 1:2 mixture we detect frames without bubbles between the start and end of the solubilisation (Figure 2a), while for the 2:1 mixture we only have a slight change in the low Q region followed by the slow appearance of small peaks in the bilayer scattering region after many minutes.

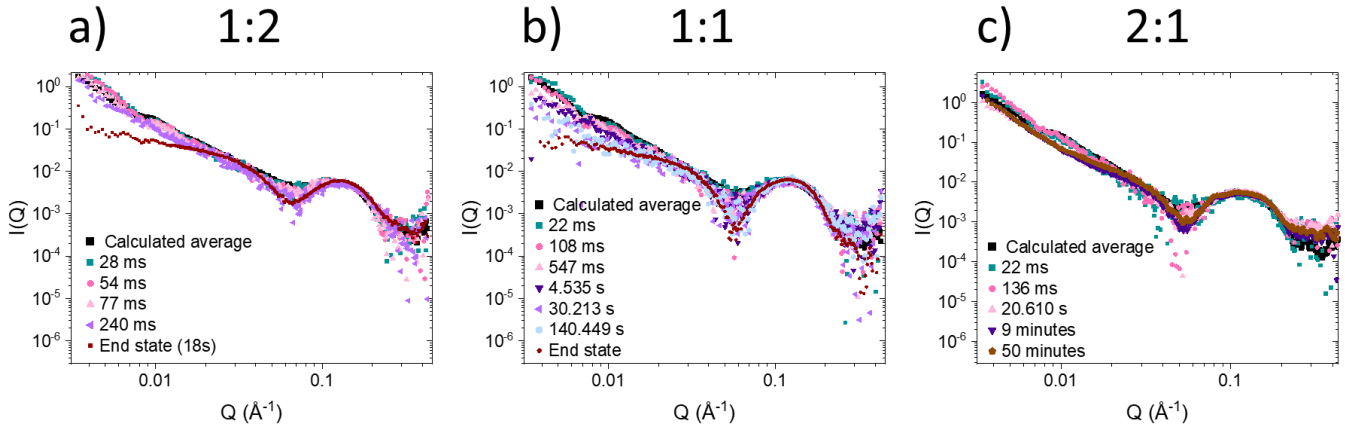

Figure 2: Examples of measurements of DPPC:TX-100 mixtures at 30 °C. a) Measurement of 1:2 mixture over time, b) Measurement of 1:1 mixture over time, c) Measurement of 2:1 mixture over time

For the DPPC:TX measurements at 25 °C, which was done the last, we can see the first sign of the DPPC resovoirs being contaminated by the appearance of a small peak in the bilayer scattering of the pure DPPC measurements. The data collection at this temperature was also affected in a large degree by the amount of bubbles. Examples of measurements at this temperature can be seen in figure 3. For the 1:2 and 2:1 mixtures (Figure 2b and c) we could only see a change in the low Q region with the disappearance of the oscillation at a seconds timescale followed by the slow appearance of peaks in the bilayer scattering at the minutes timescale, seemingly faster than for the mixtures at 20 °C however. For the 1:2 mixture, there were much more drastic changes, however, it is difficult to deduce which changes were real or due to bubbles. In this case, the bilayer does appear to partially solubilise in addition to forming larger striated aggregates.

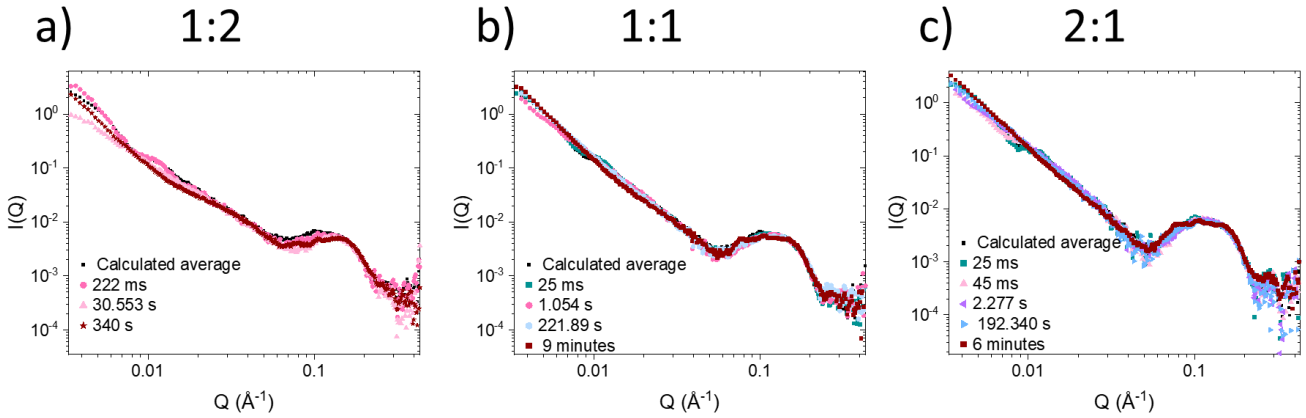

Figure 3: Examples of measurements of DPPC:TX-100 mixtures at 25 °C. a) Measurement of 1:2 mixture over time, b) Measurement of 1:1 mixture over time, c) Measurement of 2:1 mixture over time

For DMPC at 10°C we could actually observe the full solubilisation into bicelles for the 2:1 and 1:1 ratios that were measured (Figure 3 a and b), which occurs within the first 80 s for the 2:1 ratio and within the first second for the 1:1 ratio. These were however the only ratios that there was time to measure. Attempts were made to also measure at 15°C, where the kinetics seemed to be faster, but the measurements proved to be quite erratic at this point and therefore not included.

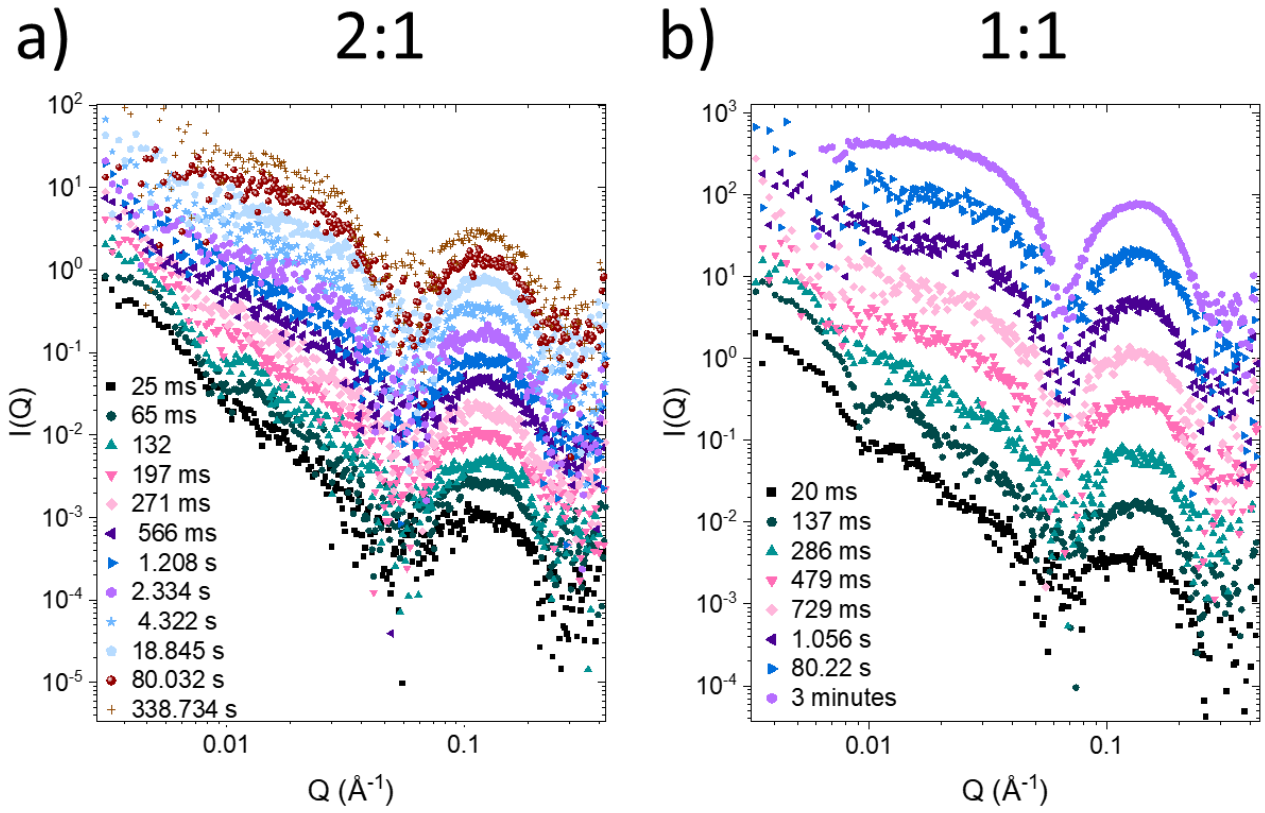

Figure 4: Examples of measurements of DMPC:TX-100 at 10 °C. a) Measurement of 2:1 mixture over time, b) Measurement of 1:1 mixture over time