Experimental report

06/09/2022

Proposal: 9-13-1026 **Council:** 4/2021

Title: Probing photoswitching in lipid vesicles using small-angle neutron scattering

Research area: Soft condensed matter

This proposal is a resubmission of 9-13-988

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Samples: DPPC - C40H80NO8P
deuterated- DPPC C40D62H18NO8P
Azobenzene glycolipid

Instrument	Requested days	Allocated days	From	To
D11	2	1	14/09/2021	15/09/2021

Abstract:

As with cell membranes where the transport of materials in and out is controlled by specific channels, changing the porosity of vesicles can be exploited for controlled release of drugs in medical treatments. This proposal concerns a photoswitchable system. SANS with hydrogen/deuterium contrast variation will be used to study a model system consisting of an azobenzene-glycolipid in phospholipid (DPPC) vesicles. We will first determine the localization of the amphiphilic glycolipid within the phospholipid bilayer, and then probe whether the structural changes upon photo-switching with UV and blue light, which induce changes in the orientation azobenzene-glycolipid, cause reorientation of the DPPC head or tail-group, or a combination of both. The fundamental understanding gained in this work will guide future practical developments.

Actual Experiment:

The aim of this beamtime was to study the static structural changes induced by photoswitchable molecules, azobenzene-glycolipids (AZ), in unilamellar vesicle (ULV) of Dipalmitoylphosphatdylcholin (DPPC). The AZ can be switched between the thermal relaxed trans isomer to the cis isomer with UV light and back with visible light. Based on Langmuir monolayer X-ray studies of the mixed AZ:DPPC systems, a ratio of 1:10 was used to prepare the ULVs by extrusion. In the monolayer studies a reproducible change in the layer thickness upon switching was observed. Yet, the exact position of the AZ and especially the switch element could not be determined with the X-ray studies. Therefore, in addition to the aim to study the switching effect, multiple contrasts were prepared to determine the localisation of the AZ withing the phospholipid bilayer. These studies will help to understand inner membrane dynamics and to develop applications for controlled modification of membrane structure.

Experimental Equipment:

We used our own illumination device, consisting of 2 sets of LEDs with a wavelength of 365 nm and 455 nm, to illuminate the samples at the sample stage. Each sample was illuminated for 5 minutes.

Achievement of Aims:

As this was the first neutron scattering experiment on this system, we decided on one specific AZ:DPPC ratio but to measure multiple contrasts by using both DPPC and d62-DPPC and changing the D2O:H2O ratio. In addition, pure DPPC and d62-DPPC ULV were measured for comparison. In total 6 contrasts were chosen to find the best match for the tail and head group for the AZ within the ULVs. The AZ:DPPC ULVs were measured first without any illumination to get the full-trans isomer. After illumination with UV light the cis state was measured before switching back to trans with visible light to check for reproducibility.

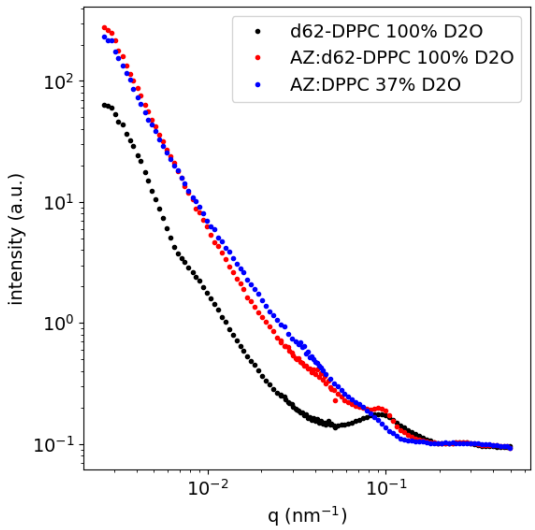

Figure 1 Scattering data of different unilamellar vesicles measured in different D2O:H2O ratios to vary the contrast.

The samples were prepared freshly in the chemistry lab the day before and brought to the beamline. The measurements ran smoothly without interruption. Two detector distances (2.5 m and 24 m) were used to measure the high and low q scattering region. At 24 m distance the transmission was also measured.

Upon switching no visible difference was observed for neither contrast. This could have multiple reasons, as for one the used setup of the illumination device in combination with the small opening on the sample holder could lead to a poor and ineffective illumination of the sample. Further, some samples sedimented slightly over time. Due to the limited time of one day, no new samples could be prepared.

Yet, the different contrast measurements show great differences in the scattering data. In figure 1, the d62-DPPC ULV in 100% D2O (to match the tail group roughly) data is shown in comparison to the data of the mixed AZ:DPPC 1:10 ULV also in 100% D2O but also in 37% D2O (match to DPPC head). The analysis is ongoing, but we are positive to get a better understanding of the localisation of the AZ within the bilayer with this data.