Experimental report

26/03/2017

Proposal: 9-13-591 **Council:** 10/2014

Title: Transport of Water Across LiposomeMembranes Studied by Contrast Variation Stopped-Flow Experiments

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: DOPC
DPPC, D2O, cholesterol

Instrument	Requested days	Allocated days	From	To
D33	3	0		
D22	3	2	08/07/2015	10/07/2015

Abstract:

The transport of water across liposome membranes is essential to life and death of cells. In our experiment we want to address that question by rapidly mixing identical liposomes prepared once with deuterated, once with normal water in the stopped-flow cell and then following the subsequent water exchange from the interior of the liposomes by SANS. This experiment uses the unique detecting properties of neutrons and will allow us to cover the kinetics of this process in the 30 ms to 100 min range. These experiments will be done for pure DOPC liposomes and mixtures with either cholesterol or DPPC to see how these alternative membrane builders affect the membrane permeability. From these experiments we will then be able to deduce a comprehensive physico-chemical picture of how the water exchange kinetics is related to the molecular composition of the phospholipid membrane and how it can be controlled by an appropriate choice of lipids.

Experimental report for experiment 9-13-591 at D22 (July 8-10, 2015)

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Berlin, March 26, 2017

Introduction

We studied the kinetics of solvent exchange in phospholipid vesicles, the most simple model system for biological cells. Harvesting the H/D isotopic sensitivity of neutrons, we followed the corresponding change in intensity as a function of the phospholipid concentration, addition of cholesterol, flow rate and temperature via stopped-flow SANS.

Samples and Experiment

Phospholipid vesicles were prepared by extrusion in shell contrast (D_2O) and at the contrast matching point (85% H_2O, see fig. 1) using membranes with a pore size of 100 nm. We used different concentrations of DOPC vesicles (0.1 wt%, 0.2 wt%, 0.5 wt%) as well as DOPC with either DPPC (0.5 wt% phospholipid, 5 mol% DPPC w.r.t. total phospholipid), Cholesterol (0.5 wt% total, 40 mol% Cholesterol w.r.t. total) or silica nanoparticles (NP) (0.2 wt% total, 30 NP/vesicle) as an additive. With the exception of the nanoparticles which were added after extrusion and have been shown to decorate the vesicles [1], all additives were included prior to extrusion to ensure their incorporation into the membrane.

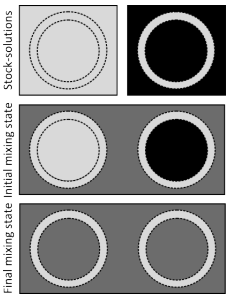

Fig. 1: Contrast conditions of the stock solutions and upon mixing equal volumes.

To be able to follow the expected relatively fast solvent exchange, we used one configuration at low q ($0.03\,\mathrm{nm}^{-1} \leq q \leq 0.4\,\mathrm{nm}^{-1}$, where the scattering is expected to decrease the most: see fig. 2) and a white beam to increase the flux, allowing for a higher time resolution from binning after the experiment.

Results

Surprisingly, the changes in scattering intensity were much less than the expected order of magnitude in decrease and heavily flow-rate dependant. For most samples, the intensity was basically constant at a flow rate of 4 mL/s (the highest flow rate used in this experiment and a commonly used value for this type of experiment), while changes including the process of filling the cuvette could be followed nicely at the lowest flow rate, 0.07 mL/s. An example is shown in figure 5. We further find a much higher intensity for the low flow rate, which may be a further hint towards the influence of shear during the stopped-flow mixing process on the kinetics of the solvent exchange. Speculatively, the vesicles may have been ruptured due to the shear acting on them, thereby finishing the solvent exchange process before the sample cell was even reached.

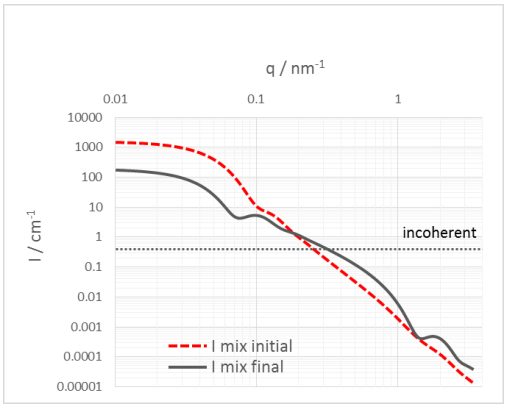

Fig. 2: Simulation of spectra for DOPC, $R = 50$ nm. Dashed: initial, continuous: final.

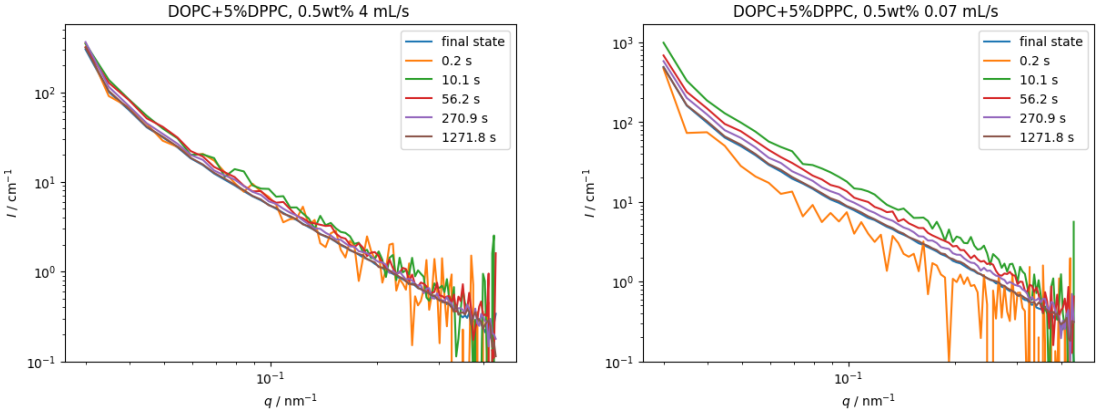

Fig. 3: Flow-rate dependance of the kinetics for the example of DOPC+5% DPPC, total concentration 0.5wt%.

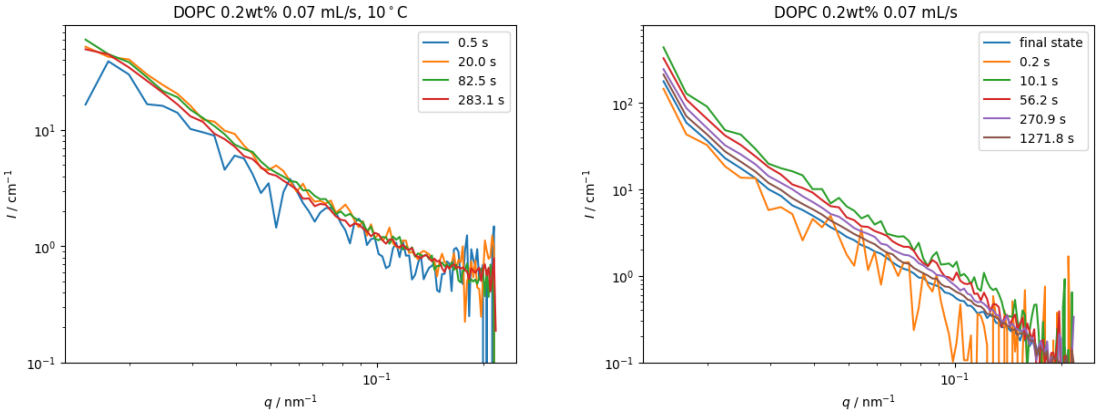

Fig. 4: Temperature dependance of the kinetics for the example of DOPC, total concentration 0.2wt% and a flow rate of 0.07 mL/s. Left: measurement at 10 °C, right: measurement at room temperature.

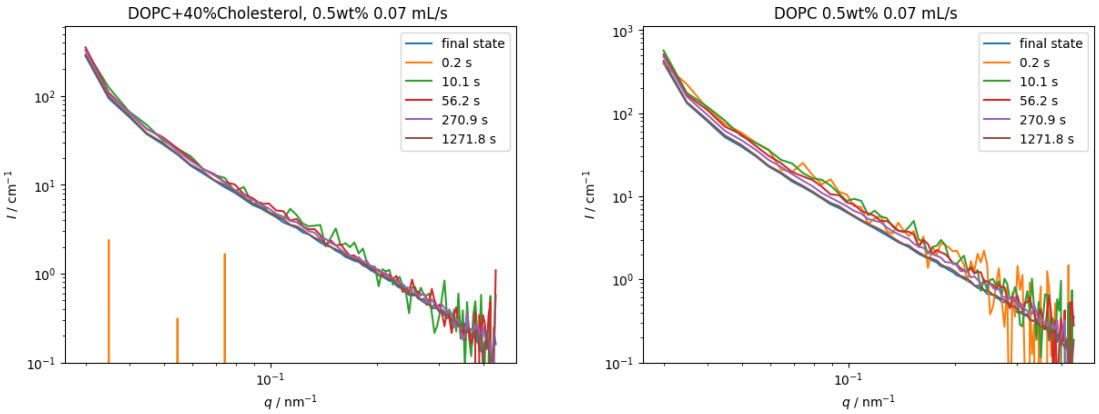

Fig. 5: Influence of Cholesterol on the kinetics for a total concentration of 0.5wt% and a flow rate of 0.07 mL/s. Left: 40 mol% Cholesterol w.r.t. total, right: pure DOPC.

Of course, at lower flow rate also the filling of the cuvette then took correspondingly longer thereby limiting the time resolution. At the same time at lower flow rate the effectiveness of mixing will also be substantially reduced. This effectiveness of mixing is currently

evaluated by studying other mixing processes in our lab stopped-flow instrument. The effects observed there will then be employed to further analyse the SANS-sf results. At the same time we are comparing these results to the mixing effects observed when mixing not by the stopped-flow equipment, but by simple external solution mixing. Due to the amount of open questions and the inconclusiveness of the qualitative SANS analysis, by taking into account these additional experiments a careful quantitative analysis of this SANS-sf experiment will be carried out. From it we will then hopefully not only be able to gain information regarding the exchange inside/outside of the vesicles but also about their mechanical stability in the flow field employed here.

References

[1] Raphael Michel, Tobias Plostica, Ludmila Abezgauz, Dganit Danino, and Michael Gradzielski. Control of the stability and structure of liposomes by means of nanoparticles. *Soft Matter*, 9:4167–4177, 2013.