Experimental report

05/09/2022

Proposal: 9-12-644 **Council:** 10/2020

Title: Enzyme Diffusion through a Crosslinked Polymersome Membrane

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: PEO-copolymers

Instrument	Requested days	Allocated days	From	To
D11	3	1	05/10/2021	06/10/2021

Abstract:

Polymersomes are fabricated using self-assembly of amphiphilic block copolymers to multicompartment-capsules, and hold great promise as synthetic cells to mimic cell functions and in the diagnostics and therapeutics of different diseases. However, complete knowledge of the molecular processes for the encapsulation and release of nanometer-sized biomolecules from tunable multiresponsive polymersomes within the physiological pH range of 4-8 is still required. Current studies on the encapsulation processes using dynamic and static light scattering or TEM deliver inly information on global size dimensions. These techniques are limited when it comes to an in depth analysis of membrane properties at the sub-nanometer scale. Thus, an essential breakthrough in the understanding the diffusion properties through the membrane depending on pH, size and charge of the cargo, is still lacking due to the very small sizes of the membrane and its amphiphilic structure. In this project, we aim at systematical SANS experiments on model polymersome-protein hybrids to understand the location of cargo, permeability of the polymersome membrane and conformation of the amphiphilic polymers in the membrane.

Report for ILL proposal 9-12-644

Enzyme Diffusion through a Crosslinked Polymersome Membrane

SANS is an important analytical tool in the analysis of biological or soft materials, as it offers a non-destructive way to determine the R_g and conformation of structures down to 1 nm. The contrast of SANS is given by the scattering length b of the analyte, allowing to highlight specific segments of biomolecules or polymers by changing their scattering length. The most common approach lies within deuterium labelling. Hydrogen and deuterium differ in their scattering length, thus using D_2O instead of H_2O would provide the necessary contrast between solvent and polymeric vesicle membrane. In order to allow structural analysis of the post Mb-Psomes´ membrane with minimal influence of loaded Mb on the scattering signal, deuterated Mb was required. For this purpose, Mb was first deuterated by hydrogen-deuterium-exchange (HDX) in pure D_2O. HDX describes a reversible chemical reaction, during which covalently bond acidic hydrogen atoms are replaced by deuterium atoms.[1] It should be considered that only 22 % of all hydrogen atoms within Mb were exchangeable by incubation in D_2O.[2] Nevertheless, this method increases the contrast between Psomes and Mb sufficiently for analysis in D_2O.

The scattering intensities of empty-Psomes were tested at different concentrations to determine optimal measurement parameters (Figure 1). The pH was set to 8, ensuring collapsed Psomes. At low q range ($q < 10^{-2}$ 1/Å) the comparison between samples with 0.3 and 0.1 mg mL^{-1} demonstrates different slopes. Only the 0.1 mg mL^{-1} concentration indicates sufficient dilution of the samples and thus the onset of the Guinier plateau, also described as I(0), while the sample at 0.3 mg mL^{-1} indicates an increased particle size which is most probably due to aggregation.

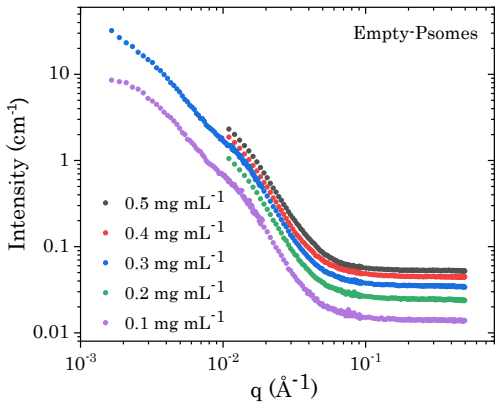

Figure 1: SANS scattering curves of empty-Psomes in the collapsed state after background subtraction.

At low q, the scattering curve contains information about the total particle size, such as R_g and M_W. The calculation is performed using the Zimm- or Guinier-plot. The data in the mid q range of the scattering curve follows the term $q \cdot R_g > \sqrt{3}$.[3] This range of the scattering curve involves information about the particle shape and conformation. For vesicle like structures, e.g. polymeric vesicles, this q-range also contains information about the membrane thickness and the general Psomes compactness.[4] Higher q values enable interpretation at the lower scales such as segment size or even information on the atomic scale.

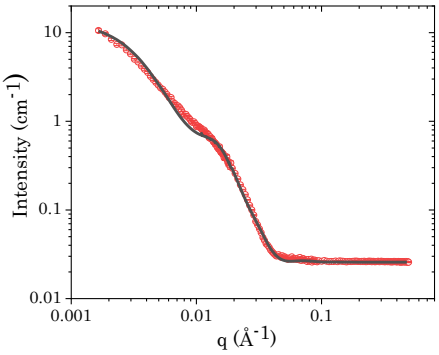

Figure 2: SANS scattering curves of post Mb-Psomes in the collapsed state at 0.1 mg mL^{-1}. Solid line represents fitting curve based on the vesicle model.

At high q, the Mb-Psomes exhibit higher intensities compared to empty-Psomes (Figure 3A). During data processing a background subtraction for D_2O scattering was performed. Yet, for scattering caused by Mb this subtraction was not applied as the fraction of free Mb is not known. Scattering curves of Mb-Psomes exhibit higher background intensity than the empty-Psomes, due to higher hydrogen concentration from the added Mb.

The scattering curve of the Mb-Psomes and empty-Psomes at low q are almost identical, however, in the mid q range, a slight increase in intensity for post Mb-Psomes is visible (Figure 3A). The similar curve shape suggests an identical to very similar particle shape of Mb-Psomes and empty-Psomes. Yet,

the differences in the mid q range should be related to changes in the membrane after loading. A detailed analysis of this q-range can be performed by a model fit of the scattering curves. Due to the assumed spherical structure based on TEM and AF4 measurements, two vesicle model fits were used in comparison.

In the first model, Psomes are assumed to be vesicles with a uniform membrane. The form factor can be calculated by a modified hollow sphere model:[5]

The polydisperse character of the Psomes was taken into account within these calculations considering Gaussian size distribution of particles. Calculations were performed based on the calculated SLDs, the polymersome size polydispersity of 0.3 from DLS measurements as well as the regular distribution of the membrane thickness due to the nature of the self-assembly. For the multilayer vesicle method, the number of shells was fixed to three. Each shell refers to one of the main components of the BCP: PEG, DEAEMA and DMAEMA. Sufficient fit quality was obtained when also taking into account a hard sphere structure factor (see Figure 3B).[6]

Figure 3B shows the calculated fits for empty-Psomes by both described models. The fits of the post loeaded Mb-Psomes are shown in Figure 2. The resulting fits offer a good match to the experimental data, showing χ^2 values of 5.6 for the vesicle fit and 7.6 for the multilayer vesicle fit. Both models deliver a R_g of 29 to 30 nm which is in accordance with the results obtained from multidetector AF4 measurements and are comparable to data from DLS and cryo-TEM (Table 4). Due to the similarity of both models and the better χ^2 value of the vesicle fit, the multilayer vesicle fit was not applied for the Mb-Psomes samples. Furthermore, the vesicle fit contains fewer fit parameters. The vesicle fit yields R_{core} of 20 nm for both the empty and the loaded polymersome, while the membrane thickness increases after post-loading from 8.7 to 11.5 nm, most likely due to incorporation of Mb into it.

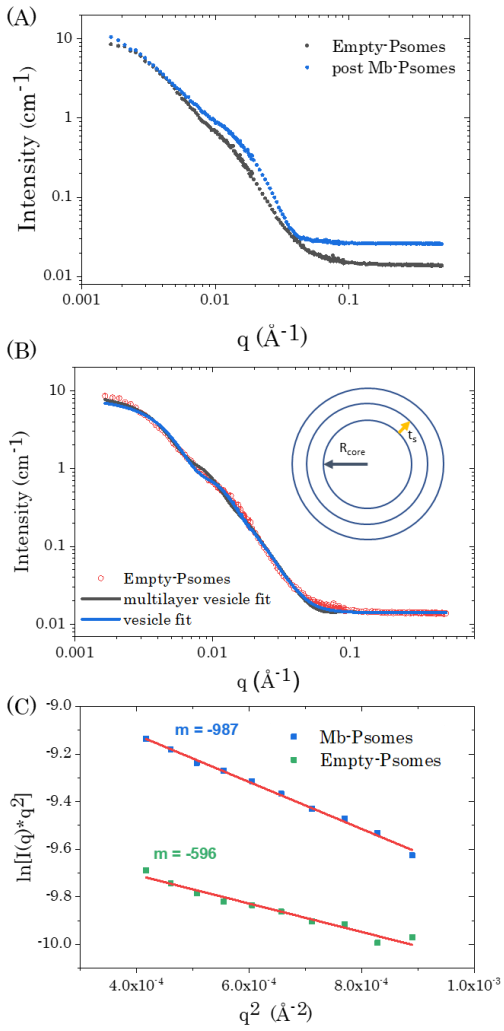

Figure 3: (A) SANS scattering curves of empty-Psomes and Mb-Psomes (BCP-AS109) in the collapsed state; (B) Model fits of the empty-Psomes using two different models. (C) Kratky-Porod plot for empty and loaded polymersomes in D_2O. Conditions: c_{BCP} = 0.1 mg mL^{-1}.

Another helpful approach for the analysis of vesicle like structures is the Kratky-Porod plot. It is obtained by plotting $\ln[I(q) \times q^2]$ vs q^2 and provides valuable information on the membrane thickness of vesicle like structures.[7]This method can be applied in the q range $1/R < q < 1/d$, where d is the membrane thickness and R the radius of the particle. If those conditions are met.The Kratky-Porod plots for empty-Psomes and post Mb-Psomes are show in Figure 4C. The resulting membrane thicknesses for empty (d = 8.5 nm ± 2.3 nm) and post Mb-Psomes (d =10.9 nm ± 1.8 nm) are in the range of the ones calculated by the vesicle fit (Table 1).

Table 1: SANS of membrane structure parameters as measured for empty-Psomes and post Mb-Psomes. Different fitting models as well as Kratky-Porod plot have been applied. For multilayer vesicles only, the total membrane thickness is reported.

Sample	Fit Model	R_{core} [nm]	t_s [nm]	Kratky-Porod d [nm]
Empty-Psomes	vesicle	20.9 ± 0.2	8.7 ± 0.04	8.5 ± 2.3
	multilayer vesicle	19.8 ± 0.2	8.7 ± 0.03	
post Mb-Psomes	vesicle	20.0 ± 0.2	11.5 ± 0.09	10.9 ± 1.8

The SANS measurements and applied calculation procedures confirm the results from DLS, AF4 or cryo-TEM regarding size and shape of the empty-Psomes. In addition to R_g the membrane thickness was successfully calculated based on received scattering curves. A slight increase in membrane thickness compared to empty-Psomes shows possible loading and incorporation of Mb inside or on the outer surface of the membrane. To clarify the exact location of the protein further synthetic procedures and extended SANS analysis is required. On the one hand, more permanent method for deuteration of Mb has to be applied, for example by synthesis through bacteria.[8] On the other hand, selective deuteration of the BCP has the potential to give a detailed information about the position of cargo in the Psomes membrane,

Experimental details

SANS measurements were carried out at the instrument D11 of the Institut Laue-Langevin (ILL, Grenoble) at a constant temperature of 293 K. The neutron wavelength was set to 6 Å. A broad q-range from 0.0016 Å-1 – 0.49 Å-1 was covered using three sample-detector distances of 1.7 m, 8 m and 38 m (with collimation distances of 4 m, 8 m and 40.5 m) for empty and loaded Psomes at c_{BCP} = 0.1 mg mL^{-1}. For all other samples only the two shorter sample-detector distances have been used, yielding a q-range of 0.01 Å-1 – 0.49 Å-1. Quartz cuvettes with a pathlength of 2 cm have been used. The scattering intensities were recorded with a 3He Reuter-Stokes multi-tube detector consisting of three panels with a total of 256 tubes with 256 pixels each. The pixel size is 4mm along a tube and 8mm due to the inner tube diameter. Received scattering curves were fitted using standard functions within SASview (https://www.sasview.org/).

The investigated samples were produced based on the procedures described in the next section "Sample preparation". All used solvents have been replaced by their deuterated analogue, resulting in a D_2O solution containing Psomes and deuterated myoglobin. Empty-Psomes were measured in the dilute solution regime with concentrations of 0.5, 0.4, 0.3, 0.2, and 0.1 mg mL^{-1} BCP. Mb-Psomes were diluted and measured at a concentration of 0.1 mg mL^{-1}. The preparation of samples was performed a day prior to the measurements.

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