Experimental report

16/11/2021

Proposal: 8-03-1039 **Council:** 10/2020

Title: Kinetics of the Liquid-Liquid Phase Separation of Myelin Basic Protein Investigated by Time-Resolved USANS and SANS

Research area: Biology

This proposal is a new proposal

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Samples: myelin basic protein (MBP)
partially deuterated polyethyleneoxide

Instrument	Requested days	Allocated days	From	To
D11	3	2	24/06/2021	26/06/2021

Abstract:

The myelin sheath of the central nervous system is formed by membranes that wrap concentrically around nerve fibers ensuring correct signal transmission. Myelin basic protein (MBP) is an integral part of the myelin sheath. We discovered recently a liquid-liquid phase separation (LLPS) of MBP on top of biomimetic membranes and under crowding conditions. Although the LLPS of MBP is essential for its biological function, it has received little attention yet. In this proposal, we suggest to investigate the growth kinetics of phase separated condensates that form during the LLPS of MBP by means of USANS/ SANS on D11 using a stopped-flow device in the sub-min to min time-range. Partially deuterated PEG will be used as crowding agent that is contrast matched in D2O. Our hypothesis is that at in the sub min time range formation of small droplets by Ostwald ripening might occur, while at later times we expect coalescence of larger droplets to prevail. Those mechanisms are expected to be relevant for the formation and stability of the myelin sheath in vivo. Our study would help to identify molecular mechanism that are fundamental for human health and myelin related neurological diseases.

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During 2 days of granted beamtime at D11, our group has examined the kinetics of the so-called Liquid-Liquid Phase Separation (LLPS) of the Myelin Basic Protein (MBP). Our experimental procedure based on using a Stopped-Flow device, which allowed us to irradiate our sample immediately after the LLPS was initiated. We considered the starting time (t=0 sec) by mixing of appropriate solutions in the stopped flow cuvette. At three different detector distances and two different neutron wavelengths used (1.7m @ 4.6Å, 16m @ 4.6Å, and 38m @ 13Å), we followed the neutron scattering intensity in a broad *q*-range.

By using several detector settings, we were able to follow both protein structure changes as well as LLPS droplet nucleation and growth from early stages onwards. Analysis of the droplet's radius of gyration (R_g) in Fig. 1 shows the expected time dependence starting at around 10 seconds. For earlier times, however, the data suggest a very fast nucleation followed by shrinkage of the droplets. Currently, we believe this data are probably incorrect due to

- Air bubbles which are injected during a shot and falsify the experiment
- Extremely low binning time of approximately 1 second in the time range of the first few seconds (we chose a logarithmic binning type, i.e. the time intervals steadily increase by a factor of 1.2)
- Large uncertainties of the values (large error bars)
- Possible fusion/ripening processes that take place starting from 10 seconds

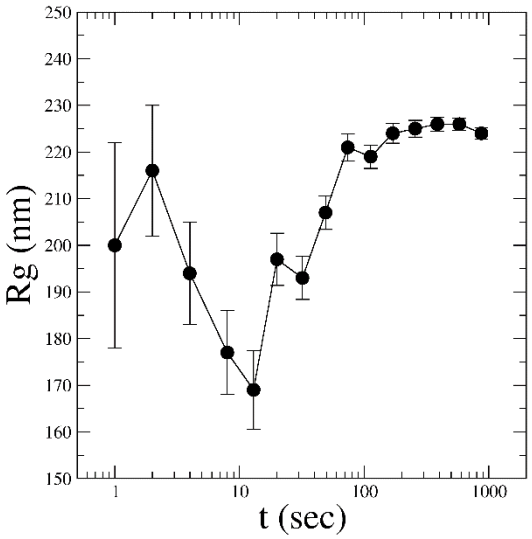

Figure 1: Plot of droplet radius of gyration versus time. Binning time was chosen such that the intervals become larger by a constant time factor of 1.2, meaning they appear equally distant in a logarithmic plot.
When neglecting the data earlier than 10 seconds, we can observe the expected growth that yields an equilibrium size after roughly 60 seconds. This finding is in great accordance to preliminary experiments. However, the reason for deviations from the expected behaviour in early time scales is yet unclear.

In order to confirm or disprove the mentioned findings, we aim to conduct further experiments. For those experiments, fast data acquisition is essential, as we want to get a deeper insight into the early droplet nucleation stages. Here, the use of synchrotron X-ray radiation might be a promising approach.
Nevertheless, during our experiments we performed close to 300 Stopped-Flow injections and followed the neutron scattering evolution for about 15 minutes. Hence, the acquired data

provide a huge set of fundamental information, in particular with regard to longer time scales. Owing to those experiments, we were able to confirm our earlier hypothesis and already collect crucial data for further analysis.

Besides kinetic investigations, we also measured solutions in their phase separated state after an equilibrium was reached. In those steady-state experiments, we could further characterise properties of LLPS droplets in terms of their average size and probable structure. This information is complementary to preliminary measurements at KWS-3 at MLZ, Garching, and now allows us to analyse combined scattering profiles from $q = 8 \cdot 10^{-5} - 7 \cdot 10^{-1}$ Å^{-1} (Fig. 2). Considering the entire q-range, we aim to extract information about droplet structure as well as the protein conformation which we can subsequently compare to the MBP structure in a non-LLPS state.

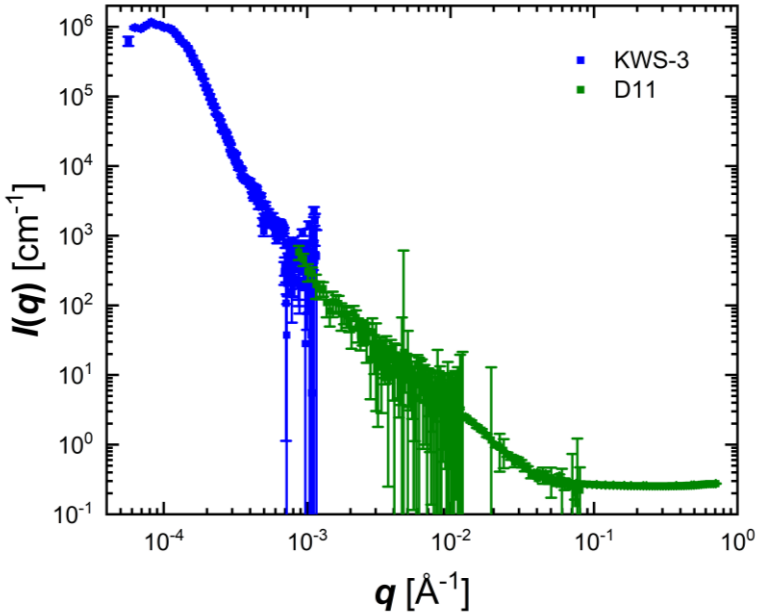

Figure 2: Merged SANS data of phase separated MBP droplets in the presence of contrast matched d-PEG at 100% D2O. The scattering data of performed experiments at D11 fits well to previous data taken at KWS-3, MLZ Garching.

All in all, we are very grateful for the beamtime that was granted to us, as well as for the scientific support that was mainly provided by our local contact and instrument scientist at D11, Olga Matsarskaia.