Experimental report

16/03/2021

Proposal: 9-13-829 **Council:** 4/2019

Title: Determination of the short-time self-diffusion of antibodies approaching an arrested state

Research area: Soft condensed matter

This proposal is a resubmission of 9-13-794

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Samples: Bovine Immunoglobulines and PEG

Instrument	Requested days	Allocated days	From	To
IN16B	5	4	22/02/2020	26/02/2020

Abstract:

We submit this proposal to college 9 due to the fundamental soft-matter nature of our approach. We replied in the text to the comments from the previous round.
An important part of the immune response of mammals relies on proteins, immunoglobulins (Igs), serving as antibodies. Igs are also used at high doses, and sometimes formulated at high concentrations to treat immunodeficiencies, autoimmune diseases and other conditions. At high concentrations, however, the viscosity of Igs increases significantly, posing a challenge to clinical administration. Modifying protein-protein interactions, influencing the viscosity, may also render the solution unstable at low storage temperatures and cause aggregation and liquid-liquid phase-separation (LLPS).
We propose to profit from the unique capability of IN16B to record inelastic fixed window scans to perform a real-time study of the dynamics of Igs in aqueous solution with polyethylene glycol (PEG), the presence of which modifies protein-protein interactions and can lead to an arrested LLPS state upon cooling to common storage temperatures. We will combine the neutron spectroscopy data with complementary small-angle scattering data.

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1 Sample preparation

Bovine γ-globulin (purity $\geq$ 99%, Sigma-Aldrich, SLCB1603), PEG1000 (Merck), NaCl (Sigma-Aldrich, STBH6613), HEPES (Sigma-Aldrich, SLBV3740) and NaN_3 (Sigma-Aldrich, BCBX7565) were used as received. All solutions were prepared in a buffer of composition 20 mM HEPES pH = 7.0, 2 mM NaN_3 in D_2O. All samples were prepared using buffer and stock solutions from γ-globulin, PEG 1000 36 % (w/v) and NaCl 4 M, to a final NaCl concentration of 150 mM. The concentration of the γ-globulin stock solutions was determined by UV-Vis absorption with a V-630 Jasco at 280 nm and an extinction coefficient $E_{280} = 1.4\,mg^{-1}\,mL\,cm^{-1}$.

Samples were prepared starting from compositions in the LLPS region of the phase diagram. After equilibration over night at 21°C all phase separated samples were centrifuged for an average of 15min at 6500rpm until both phases appeared clear.

2 Measurements

An overview about the samples measured is shown in Table 1.

Table 1: Summary of all samples measured at IN16b in the experiment 9-13-829. The title of the proposal was 'Determination of the short-time self-diffusion of antibodies approaching an arrested state'.

Date	Sample	Remarks
22-25.02.20	100 mg/ml Ig + 6 % PEG	dense phase full QENS measured at 37°C, 18°C, 14°C, 10°C, 4°C, 0°C quenches from 37°C to 18°C, 14°C, 10°C, 4°C, 0°C
24.02.20	2.5 % PEG	estimated % of PEG in the dense phase measured at 6°C
24.02.20	5 % PEG	measured at 18°C
25-26.02.20	100 mg/ml Ig + 8 % PEG	dense phase full QENS measured at 6°C quench measured with FWS from 37°C to 6°C

All of the data gathered in this experiment will be analyzed together with experiment 9-13-879. The conditions investigated here are supplementary to the results that were yielded before.

3 Preliminary results

First results taken from the Elastic Fixed Window Scans are shown in Figure 1 and Figure 2.

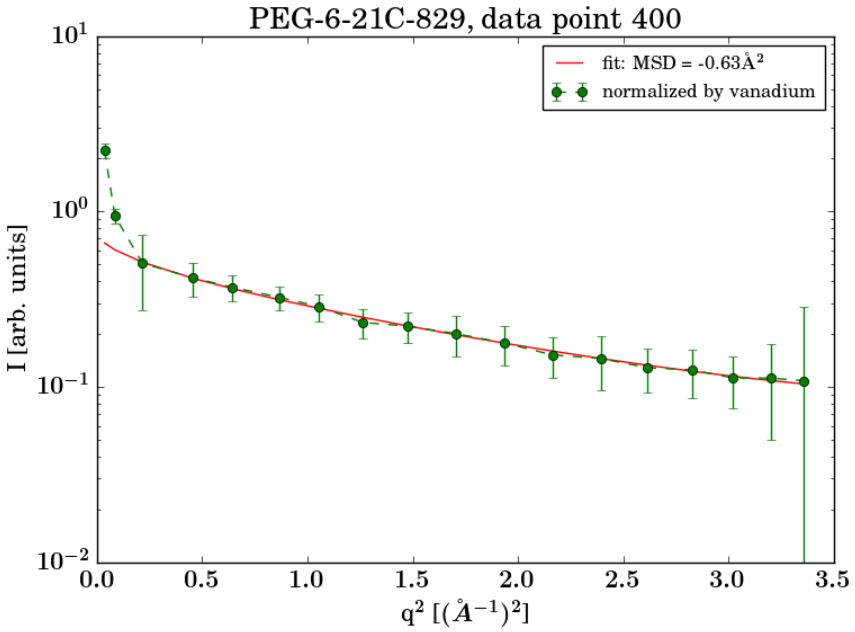

Figure 1: Normalized data fitted with $a + b \cdot x + c \cdot x^2$. Here a corresponds to I_0, b is the mean-squared displacement (MSD) and c is the variance of the MSD. The value of the MSD (parameter b) for this data point is given in the legend.

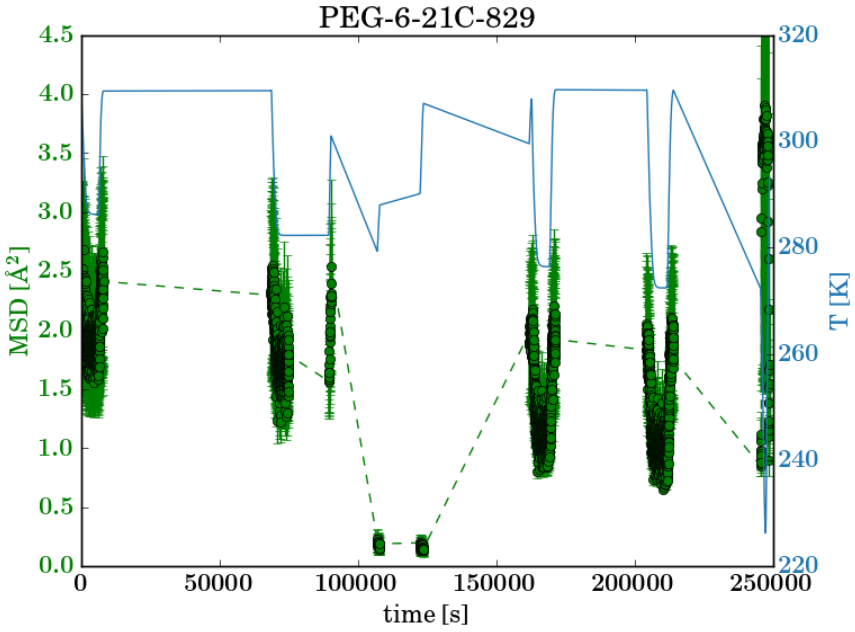

Figure 2: The MSD plotted over time (green) in comparison to changes in temperature (blue). The errorbars correspond to the variance of the MSD. It is visible that changes in MSD correlate with changes in temperature.