Experimental report

03/03/2020

Proposal: 9-13-879 **Council:** 10/2019

Title: Monitoring the short-time diffusion upon approaching the arrested state

Research area: Physics

This proposal is a new proposal

Main proposer: Christian BECK

Experimental team: Famke BAEUERLE
Olga MATSARSKAIA
Christian BECK
Anita GIRELLI

Local contacts: Tilo SEYDEL
Sylvain PREVOST
Orsolya CZAKKEL

Samples: IgG + PEG + D2O

Instrument	Requested days	Allocated days	From	To
IN16B	2	2	15/01/2020	17/01/2020
D11	1	1	12/01/2020	13/01/2020
IN15	2	2	21/01/2020	23/01/2020

Abstract:

Dissolved proteins can feature rich phase diagrams in the presence of additives. The addition of PEG to antibody solutions lead to liquid-liquid phase separations due to depletion interactions. This LLPS is characterized by a lower critical solution temperature. If the phase boundary is passed fast enough by quenching the temperature, the system can be driven into an arrested state. We use this model system of antibodies and PEG which we have characterized previously with static methods such as SANS and kinetic methods as XPCS. Previous measured elastic fixed window scans on neutron back scattering instruments showed time dependent changes in the scattering signal after the quench. To be able to separate the global and internal dynamics of the proteins, we aim for measuring full QENS spectra using a floating average to capture time dependent changes next to the elastic and inelastic measurements and is planned as continuation proposal of experiment 9-13-829. We aim to close the gap in the different time scales of the dynamics by measuring also NSE on IN15. The expected results might help in the future for pharmaceutical applications and are relevant for colloidal theory.

Experimental Report 9-13-879

doi:10.5291/ILL-DATA.9-13-879

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 until both phases appeared clear.

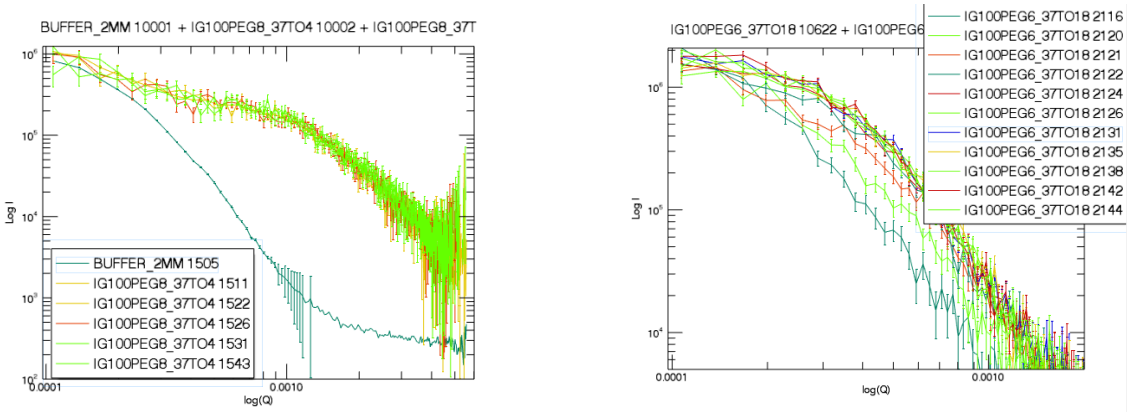
2 Measurements

An overview about the samples measured is shown in Table 1. The measurements at D11 and at IN15 were conducted using the same samples in the same cuvettes. For the IN16b samples a sample of 100 mg/ml Ig + 6 % PEG was equilibrated at 21°C over night, centrifuged and separated. A part of the dense phase was measured and a part of the dense phase and the dilute phase was equilibrated at 6°C and at 18°C. All four samples were then centrifuged at 6°C, resp. 18°C and separated.

Table 1: Summary of all samples measured at D11, IN16b and IN15 in the experiment 9-13-879. The title of the proposal was 'Monitoring the short-time diffusion upon approaching the arrested state'.

Date	Inst	Sample	Remarks
12.01.20	D11	buffer	
12-13.01.20	D11	100 mg/ml Ig + 8 % PEG	dense phase, measured at 37°C quenches from 37°C to 4°C, 18°C, 20°C, 12°C, 8°C
12-13.01.20	D11	100 mg/ml Ig + 7 % PEG	dense phase, quenches from 37°C to 18°C, 6°C, 10°C, 4°C, 8°C
12-13.01.20	D11	150 mg/ml Ig + 3 % PEG	not separated at 21°C, measured at 4°C & 6°C quenches from 37°C to 12°C, 8°C, 4°C, 6°C
12.01.20	D11	100 mg/ml Ig + 8 % PEG	dilute phase, quench from 37°C to 5°C
13.01.20	D11	100 mg/ml Ig + 6 % PEG	dense phase, measured at 37°C quenches from 37°C to 6°C, 18°C, 12°C
15.01.20	IN16b	empty Al cylinder	
15-16.01.20	IN16b	100 mg/ml Ig + 6 % PEG	equilibrated at 21°C, dense phase
16.01.20	IN16b	100 mg/ml Ig + 6 % PEG	dense phase (6°C) of dense phase (21°C)
17.01.20	IN16b	100 mg/ml Ig + 6 % PEG	dilute phase (6°C) of dilute phase (21°C)
17.01.20	IN16b	100 mg/ml Ig + 6 % PEG	dense phase (18°C) of dense phase (21°C)
21-23.01.20	IN15	buffer	
21-23.01.20	IN15	buffer + 2.5 % PEG	estimated % of PEG in the dense phase
21-23.01.20	IN15	100 mg/ml Ig + 6 % PEG	dense phase, quenches from 37°C to 6°C, 18°C
21-23.01.20	IN15	100 mg/ml Ig + 8 % PEG	dense phase, quenches from 37°C to 6°C, 18°C

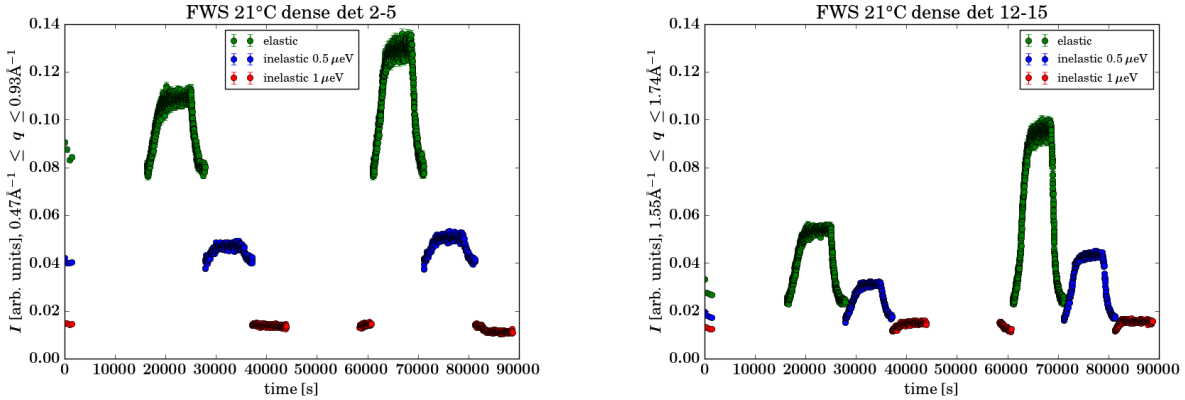
3 Preliminary results



(a) 100 mg/ml Ig + 8 % PEG, quench to 4°C

(b) 100 mg/ml Ig + 6 % PEG, quench to 18°C

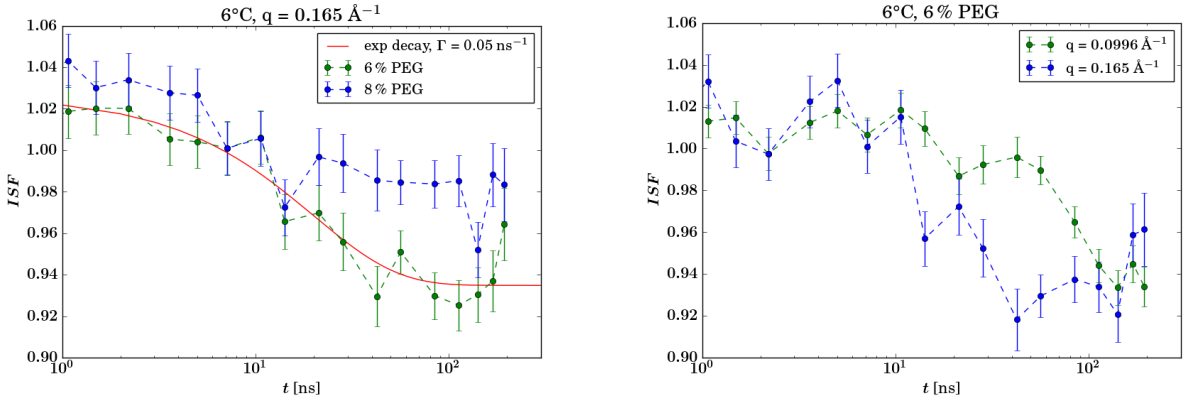
Figure 1: SANS measurements conclusion: 7% and 8% are arrested at all temperature, instead 6% is on the way to arrest at 18C and for low temperature arrested.



(a) Sum of detectors 2-5

(b) Sum of detectors 12-15

Figure 2: EFWS and IFWS measurements (see legend): Both Figures show the dense phase of 100 mg/ml Ig + 6 % PEG equilibrated at 21°C. The changes in intensity correspond to changes in temperature (quenches to 18°C and 6°C).



(a) q = 0.165 Å^{-1}, different PEG values. The solid line denotes a fit of a single exponential.

(b) 6% PEG, different q-values

Figure 3: NSE measurements at 6°C with 100 mg/ml Ig show q-dependance.