Experimental Report

Proposal: **8-04-724** **Council:** 4/2014

Title: Static versus dynamic protein clusters in solutions of bovine beta-lactoglobulin

This proposal is a new proposal

Researh Area: Soft condensed matter

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Samples: aqueous betalactoglobulin (BLG) protein solutions

Instrument	Req. Days	All. Days	From	To
IN16B	2	2	01/12/2014	03/12/2014
IN11	7	6	25/11/2014	01/12/2014

Abstract:

The issue of the presence of dynamic or transient clusters in protein solutions is of great current interest due its relevance for understanding pathological pathways as well as for drug design [K.P.Johnston et al., ACS Nano Vol.6, p.1357 (2012)]. Earlier studies combining SAXS and neutron spin-echo spectroscopy have addressed this issue using the model protein lysozyme [L.Porcar et al., J. Phys. Chem. Lett. Vol.1, p.126 (2010)]. Here we propose a systematic study of transient or dynamic protein clusters in aqueous solutions to a new model protein beta-lactoglobulin. We propose to measure the diffusion function for several values of q below and above q_c, and, using neutron spin-echo (IN11) and the new spectrometer IN16B, measure the self-diffusion for several protein concentrations. In combination with the accumulated results of SAXS and DLS by our group on BLG and also other proteins, we expect to gain quantitative knowledge on BLG clusters.

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Proposal number:	8-04-724
Experiment title:	**Static versus dynamic protein clusters in solutions of bovine beta-lactoglobulin**
Instrument	IN16B, IN11
Dates	IN11 Nov.25-Dec.1, 2014; IN16B Dec.1-3, 2014
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During experiment 8-04-724 we have collected a data set on the diffusion of the globular protein beta-lactoglobulin (BLG) in aqueous (D_2O) solutions.

Sample Preparation: IN16B - The solutions at different concentrations of BLG in D_2O (see table below) were filled in double-walled cylindrical aluminum sample holders with an outer diameter 23mm and a gap between the two walls of 0.25mm (capacity of about 1.2 ml). The containers where sealed hermetically with indium wire to avoid evaporation in the cryofurnace.
IN11 – Two solutions (72 and 144 mg/ml) were filled in quartz cuvettes, which were sealed with parafilm to avoid evaporation and D-H exchange.

Nominal concentration of BLG [mg/ml]	Temperature (set point) [K]
72*	295
110	295
144*	295
161	295
200	295
250	295
300	295
Pure D_2O*	295

* measured also on IN11

Measurements: IN16B - In addition to the samples listed in the table above, the following measurements were done to allow the calibration and the subtraction of the various contributions from the sample spectra in later analysis: (i) vanadium foil, (ii) empty sample holder, (iii) empty cryofurnace.
IN11 – In addition to the samples marked with an asterisk in the table above, graphite was measured for calibration.
Data Treatment:
IN16B - The following steps were done with MATLAB code:

- Subtraction of the empty sample holder
- Calibration, correction for detector efficiency and determination of the resolution function using the vanadium spectrum. The resolution function was described by a combination of five Gaussians and a flat background.
- To accurately take into account the contribution of D_2O, at every scattering vector Q a fixed term $\beta_{D_2O} L_{\gamma_{D_2O}}(\omega)$ with the Lorentzian function $L_{\gamma D2O}$ and scalar β (convoluted with the resolution function) was added directly to the model used for the fit of the spectra [1]:

$$S(Q,\omega) = R(\omega) * [\beta_1 L(\omega, \gamma) + \beta_2 L(\omega, \gamma + \Gamma) + \beta_{D_2O} L_{\gamma_{D_2O}}(\omega)] + B$$

  Therein the width Γ accounts for the internal modes, while γ describes the global center-of mass diffusion of the proteins consisting of contributions from the translational and rotational diffusion. The width of the Lorentzian describing the diffusion of D_2O was determined from a measurement done with IN5.
- By the slope of the HWHM γ as a function of q^2, an apparent diffusion coefficient D can be determined, from which a translational diffusion coefficient D_t is calculated [1].

IN11 – The reduced data corrected for the resolution function and the contribution due to D_2O was fitted by an exponential function $f(q,t) = a_0 \exp(-\gamma t)$, where $\gamma \equiv Dq^2$.

Outcome of the Experiment :

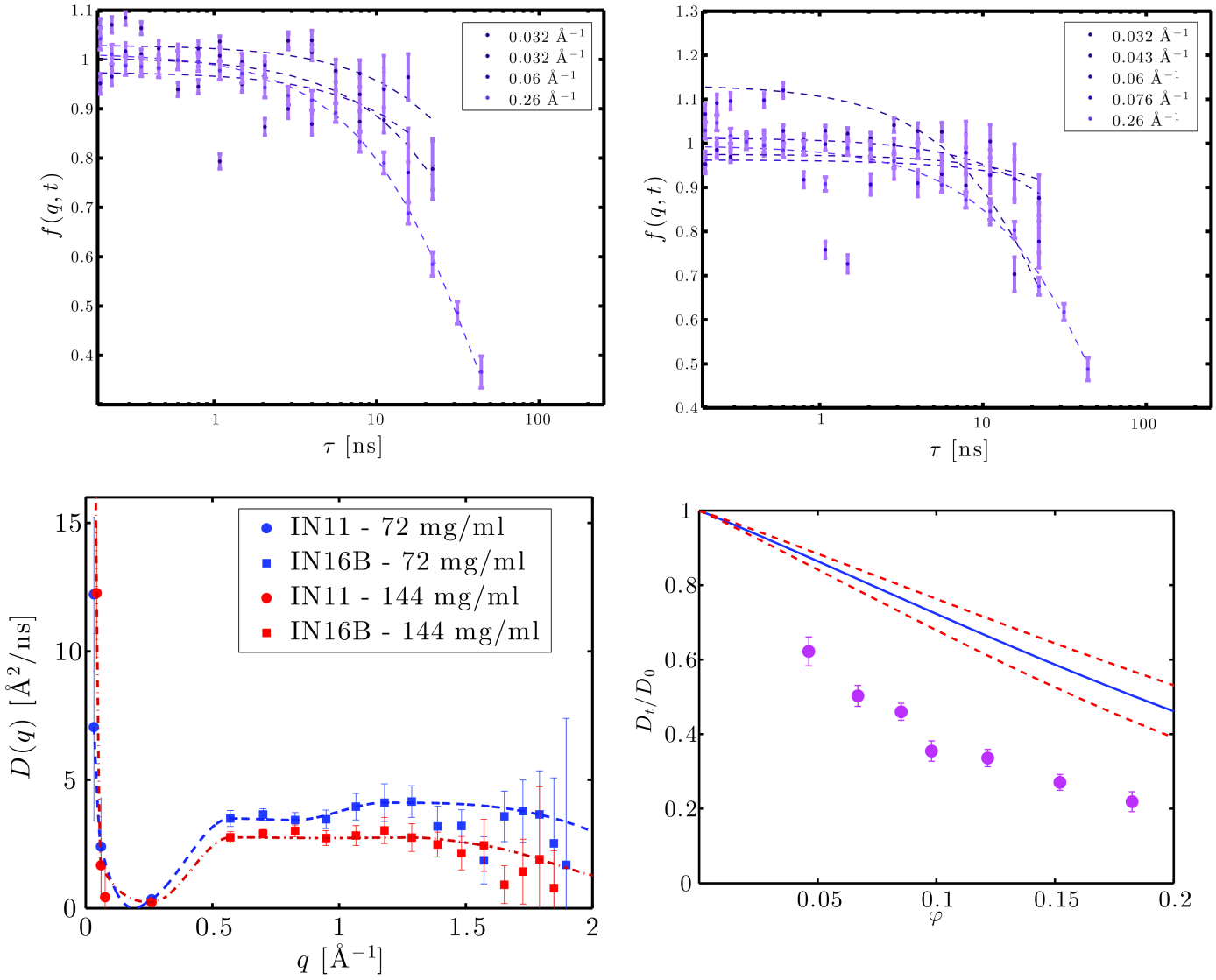

Figure1: Top: IN11 correlation functions of BLG 72 mg/ml (left) and BLG 144 mg/ml (right). The dashed lines are the fits with an exponential function. Bottom left: Diffusion coefficients as a function of q from IN11 and IN16B on BLG 72 and 144 mg/ml. The dashed lines are guides to the eye. Bottom right: Normalized short-time translational self-diffusion coefficient of BLG in aqueous solution as a function of the volume fraction φ (IN16B data). The blue solid line depicts the theoretical reduced short-time diffusion coefficient assuming BLG dimers, and the dashed lines denote the theory assuming 5% error on the determination of the effective volume fraction [1].

A preliminary analysis of the recorded data indicates that the average diffusion coefficient of BLG in solution is slower than theoretically expected for dimers (cf. Figure 1, bottom right), suggesting the presence of larger oligomers. Spin-echo data show only the beginning of a decay for most of the q-values.

Reference:

[1] Roosen-Runge, F. *et al.* Protein self-diffusion in crowded solutions. *Proc. Natl. Acad. Sci. U.S.A.***108,** 11815–20 (2011).