Experimental report

02/09/2022

Proposal: 9-13-952 **Council:** 10/2020

Title: Diffusive dynamics in crowded solutions containing two types of proteins

Research area: Soft condensed matter

This proposal is a resubmission of 9-13-920

Main proposer: Louise COLIN

Experimental team: Christian BECK
Kevin POUNOT
Louise COLIN

Local contacts: Tilo SEYDEL
Markus APPEL

Samples: aqueous (D2O) solutions of bovine serum album (BSA) and bovine polyclonal gamma globulin (Ig) proteins with different protein concentrations

Instrument	Requested days	Allocated days	From	To
IN16B Si 111 BATS	6	5	03/02/2021	08/02/2021

Abstract:

We have successfully investigated "binary" mixtures of proteins, namely bovine serum albumin (BSA) and polyclonal bovine gamma-globulin (Ig) in aqueous (D2O) solutions using IN16B in its high-resolution setup employing the Doppler drive. The center-of-mass diffusion of the proteins observed in these mixtures can be understood in terms of the total volume fraction of the mixture.

We wish to add data using BATS

(1) to access lower total volume fractions corresponding to overall faster motions better probed by the wider energy range of BATS;

(2) to focus on the protein internal dynamics. By global fits describing the energy and momentum transfers due to the internal dynamics simultaneously, even minute changes in the internal dynamics of the different proteins will be accessible.

Previous panel comment: "Very nice proposal, improved since last submission, taking into account all comments. Should be done."

It will benefit from the world-leading signal-to-noise ratio of BATS and our experience with QENS data from complex mixtures such as tracer proteins in cellular lysate [M.Grimaldo et al., J.Phys.Chem.Lett. vol.10, p.1709 (2019)], which we studied by experiment and simulations.

Experimental Report Experiment 9-13-952

Christian Beck, Felix Roosen-Runge, Tilo Seydel, Marco Grimaldo, Olga Matsarskaia, Fajun Zhang, Frank Schreiber, Lena Bühl, Michal Feustel

Motivation: The aim of this beamtime was to investigate the influence of binary suspensions on the internal diffusive processes of the two components involved, namely bovine serum albumin (BSA) and polyclonal immunoglobulin (Ig).
Performed Measurements: Several calibration measurements were necessary for a future successfully data analysis. The calibration measurements are shown in the upper part of Table 1. The vanadium and empty can measurements were shared with experiment "DIR-211". The samples of interest were prepared to have two different overall volume fractions φ and the mixing ratio y between the proteins was varied. This approach allows to reduce the influence of the volume fraction on the global diffusion, which was studied previously [1]. A detailed list of the samples are given in the bottom part of Table 1. All measurements had an acquisition time between 5 and 6 hours and were performed using the resolution lres-4.
Preliminary analysis: As a first analysis, the spectra were analyzed using an averaging approach for both the center of mass diffusion as well as the internal diffusion averaging over the BSA and Ig contained in the solution. The fit model thus can be described by:

$$S(q,\omega)\mathscr{R} \otimes [\beta\left(A_0\mathscr{L}_\gamma(\omega) + (1-A_0)\mathscr{L}_{\gamma+\Gamma}(\omega)\right) + \beta_{D_2O}S_{D_2O}(q,\omega)] \tag{1}$$

An Example spectrum is shown in Figure 1. The q dependence of the widths Γ of the second Lorentzian function $\mathscr{L}$ describing the internal diffusive processes averaged over both proteins were analyzed using a jump diffusion model:

$$\Gamma = \frac{D_{int}q^2}{1 + D_{int}q^2\tau} \tag{2}$$

Example fits are shown in Figure 2. A clear deviation from the jump diffusion model is seen already in the raw-data. Given the energy transfer range investigated, this is already expected [2]. However, the pronounced oscillations point out the possibility to

Table 1: Performed measurements during beamtime 9-13-952

Sample name	Temperature
D_2O	280K 295K 310K
empty cryofurnace	310K

Sample name	Temperature	Sample name	Temperature
Ig $\varphi = 0.1$	280K 295K 310K	Ig $\varphi = 0.05$	310K
Ig BSA $\varphi = 0.1; y = 0.25$	280K 295K 310K	Ig BSA $\varphi = 0.05; y = 0.25$	280 K 310K
Ig BSA $\varphi = 0.1; y = 0.5$	280K 310K		
Ig BSA $\varphi = 0.1; y = 0.75$	280K 295K 310K	Ig BSA $\varphi = 0.05; y = 0.75$	310K
BSA$\varphi = 0.1$	280K 310K	BSA $\varphi = 0.05$	310K

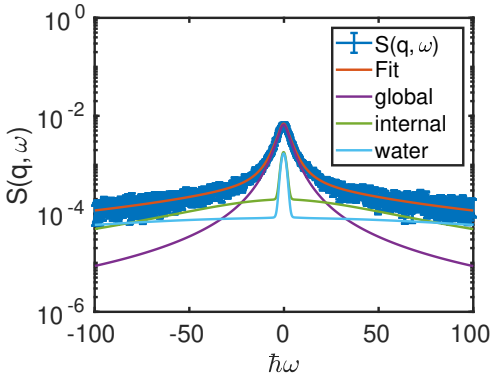

Figure 1: Example spectrum measured during the beamtime. $\varphi = 0.1$; $y = 0.5$; T=310K; q=1Å^{-1}. The empty can contribution is not shown additionally.

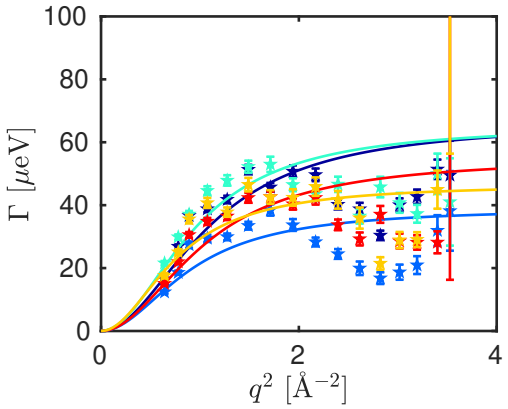

Figure 2: Internal widths Γ as a function of q^2. the spectra were fitted with Equation 2

separate the contributions with advanced corresponding fit models.

Future analysis: In a next step, the spectra will be analyzed separating the two different protein signals. The solvent background will be rescaled using the diffraction data collected simultaneously. By applying global fits taking the q dependence into account as well as considering the known concentrations, the global contributions can be fixed based on previous experiments and the focus can be on the internal diffusive contributions of the proteins:

$$S(q,\omega) = \mathscr{R} \otimes \left\{ \sum_{i=\{\mathrm{BSA,Ig}\}} \left(s_i \mathscr{L}_{\gamma^i} \otimes \left(A_0^i + (1 - A_0^i) S_{int}^i(q,\omega) \right) \right) \right\} + \beta_{D_2O} S_{D_2O}(q,\omega) \quad (3)$$

with s_i, β_{D_2O} being fixed based on the protein concentrations. It still has to be determined, if the different internal contributions can be modeled with a two-state model for each protein [2] or if only two single Lorentzian functions can be used.

References

[1] C. Beck, M. Grimaldo, H. Lopez, S. da Vela, B. Sohmen, F. Zhang, M. Oettel, J.-L. Barrat, F. Roosen-Runge, F. Schreiber, and T. Seydel, "Short-time Transport Properties of Bidisperse Suspensions of Immunoglobulins and Serum Albumins Consistent with a Colloid Physics Picture," *JPCB, under review*, 2022.

[2] M. Grimaldo, F. Roosen-Runge, M. Hennig, F. Zanini, F. Zhang, N. Jalarvo, M. Zamponi, F. Schreiber, and T. Seydel, "Hierarchical molecular dynamics of bovine serum albumin in concentrated aqueous solution below and above thermal denaturation," *Phys. Chem. Chem. Phys.*, vol. 17, no. 6, pp. 4645–4655, 2015.