Experimental report

04/03/2020

Proposal: 8-04-855 **Council:** 10/2018

Title: Towards a realistic model of the cellular environment: Dynamics in "naturally" crowded protein solutions

Research area: Biology

This proposal is a resubmission of 8-04-832

Main proposer: Marco GRIMALDO

Experimental team: Olga MATSARSKAIA
Christian BECK
Frank SCHREIBER
Trevor FORSYTH

Local contacts: Tilo SEYDEL
Markus APPEL

Samples: Bovine immunoglobulins and E. Coli lysate

Instrument	Requested days	Allocated days	From	To
IN16B Si 111 BATS	7	4	03/07/2019	07/07/2019

Abstract:

Using deuterated cellular lysate obtained from living cells, we will establish a natural crowding environment for protonated "tracer" or "target" immunoglobulin (Ig) proteins. In a previous experiment, we were able to identify protein diffusion and an internal dynamical process in the QENS signal of IN16B in standard configuration. Given the rather low Ig concentrations and the presence of lysate, IN16B in the standard configuration probes mainly the global diffusion and the slow part of the internal dynamics. To obtain a comprehensive picture of both the slow and fast internal dynamics in such a "naturally" crowded environment, a broader dynamic range is needed. We plan to systematically investigate the influence of the presence of lysate on the internal diffusive motions of the target proteins using BATS. In this way, we will maintain a good overlap with existing IN16B data, while having a dynamic range sufficiently large to better determine the antibody fast internal dynamics. The study will be also an essential methodological step to establish a reliable access to protein dynamics under native-like crowding conditions.

Experimental report for beamtime 8-04-855

Experimenters: BECK Christian (ILL), GRABITZ Lara (ILL), MATSARSKAIA Olga (ILL), SCHREIBER Frank (University of Tübingen), SEYDEL Tilo (ILL)

The interior of living cells is occupied by macromolecules such as proteins, which occur at a high total volume fraction φ on the order of 30% in the aqueous solution of the intracellular fluid. The issue of macromolecular crowding is therefore of primordial importance for the function of living cells [1]. In particular diffusion processes, which constitute a principal biological transport mechanism, are affected by the situation of crowding. In vitro, the simplest approximation to model the situation of crowding is achieved by self-crowding. In this case, the same macromolecule is used both as tracer particle and as crowding agent in aqueous solution. Previously, we have studied this situation of self-crowding using the model proteins bovine serum albumin (BSA) [2], representing a nearly spherical shape, and bovine gamma-globulin (or: immunoglobulin) (IgG) [3], representing a strongly branched shape, in aqueous (D_2O) solutions. We have established a method to separate the rotational (D_r) and translational (D_t) contributions to the observed diffusion coefficient $D(\varphi)=D(D_r(\varphi),D_t(\varphi))$ employing the knowledge of the exact shape of the protein. We were able to show that the volume fraction dependence of the translational short-time self-diffusion $D_t(\varphi)$ of BSA and IgG can each be described quantitatively with predictions from colloid theory for hard spheres [2-4]. In addition, we investigated the internal dynamics [5,6] of pure solutions of both proteins, using the spectrometers IN16B and BASIS. Advanced models allow to access the diffusive dynamics of the protein backbone and the side chains separately [5,8]. These internal dynamics of proteins are relevant for biological processes, such as enzymatic activities or antibody-triggered immune responses. For the situation of self-crowding, employing these novel approaches, significant differences in the internal dynamics have been confirmed for IgG and BSA proteins [6]. Recently, we investigated the global dynamics of IgG in naturally crowded environments induced by deuterated cellular lysate [7]. With this work, we have further developed the analysis of QENS data for very complex suspensions. We have found that the global short-time diffusion of IgG, which has a radius close to the lysate ensemble effective radius, can be described as a function of the total volume fraction occupied by all macromolecules present in the solution [7]. A similar observation was confirmed by measurements on the binary system of Ig-BSA mixtures in D_2O performed on IN16B.

In its standard setup, IN16B focuses mainly on the global center-of-mass diffusion of the proteins and offers only a comparatively limited access to the internal dynamics due to the limited energy transfer of ΔE_{max} = 30μeV. By exploiting the large energy range (ΔE_{max} = ±150μeV) [9], the experiment described here aimed at extending the data obtained for the IgG-lysate system [7] to proteins with a radius markedly below or above that of the lysate ensemble effective radius. To this end, we employed the proteins myoglobin from equine heart (Mb), BSA, ovalbumin (OVA) and β-lactoglobulin (BLG). The samples measured are given in Table 1. We measured BATS spectra of pure protein solutions in D_2O and of the respective proteins in the presence of deuterated lysate provided by the D-lab (proposal no. DL-03-185/DL-04-45). An example spectrum is shown in Fig. 1. The excellent signal-to-noise ratio and energy range become apparent.

Preliminary analysis of both samples and the resolution function (Figs. 2 and 3) shows that the data can be very well fitted using approaches established earlier (cf. Refs. 2-7). The global center-of-mass diffusion is in perfect agreement with simple Fickian diffusion for all tracer proteins. Due to the wide energy range accessed by BATS, a more complex model will be required for the internal diffusion. When taking only a single Lorentzian (squares in Fig. 2), the q-dependence of its linewidths shows that an additional Lorentzian would be required, as shown by Grimaldo et al. [5]. Detailed data analysis is currently ongoing.

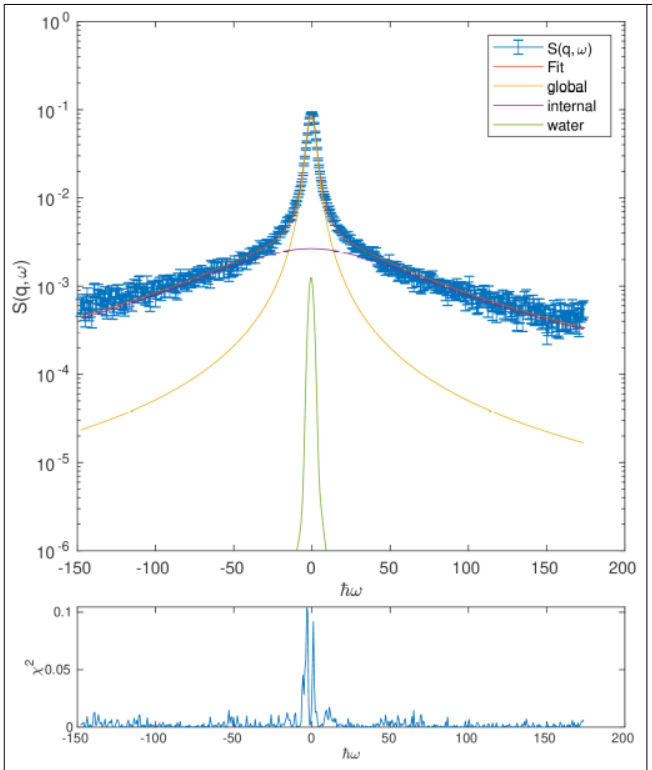

Fig.1: Example spectrum: Top: Myoglobin 241mg/mL in D_2O without additive, q≈1Å^{-1}; bottom: residuals from the fit

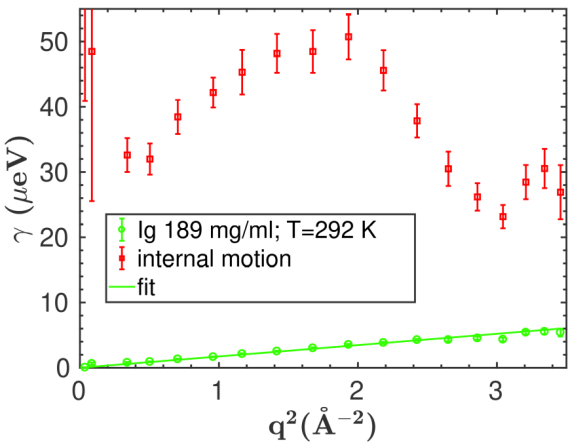

Fig. 2: Example fit result for Ig (189 mg/mL) in D_2O without additive. The red square symbols account for the internal diffusion, the green circles represent the center-of-mass diffusion which is in perfect agreement with simple Fickian diffusion.

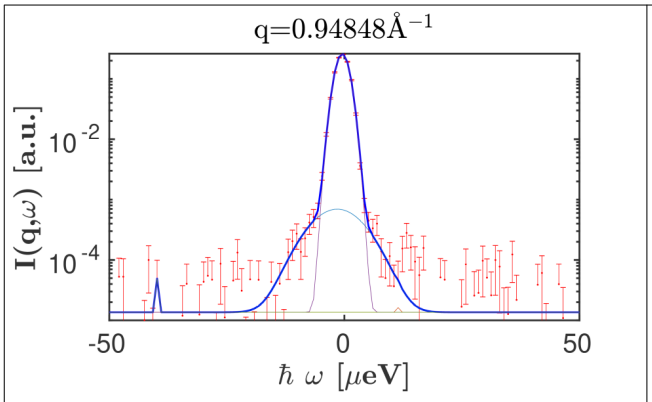

Fig. 3: Example resolution function recorded on a Vanadium standard. The BATS resolution function can be very well fitted by a single Gaussian function over three orders of magnitude in intensity. (The deviations at low intensity arise from the fact of using single crystal analyzers and from noise.)

Table 1: List of measured samples

Sample	**Tracer protein concentration (mg/mL)**	**Deuterated lysate concentration (mg/mL)**
Lysate	0	212
Lysate	0	100
Mb	241	0
BSA	216	0

Ig	189	0
Ig	100	100
Mb	100	100
BSA	100	100
OVA	100	100
OVA	228	0
Mb	390	0
Mb	200	100

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