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Neutron spectroscopy on protein solutions employing backscattering with an increased energy range

Christian Beck^{a,b}, Markus Appel^{a,c}, Marco Grimaldo^a, Felix Roosen-Runge^d, Fajun Zhang^b, Bernhard Frick^a, Frank Schreiber^b, Tilo Seydel^{a,*}

^a*Institut Max von Laue - Paul Langevin (ILL), 71 Avenue des Martyrs, CS20156, F-38042 Grenoble, France*

^b*Institut für Angewandte Physik, Eberhard-Karls-Universität Tübingen, Auf der Morgenstelle 10, D-72076 Tübingen, Germany*

^c*Center for Medical Physics and Technology, Friedrich-Alexander Universität Erlangen-Nürnberg, Henkestrasse 91, D-91052, Erlangen, Germany*

^d*Physical Chemistry - Kemicentrum, Lund University, PO Box 124, SE-221 00 Lund, Sweden*

Abstract

Novel cold neutron backscattering spectrometers contribute substantially to the understanding of the diffusive dynamics of proteins in dense aqueous suspensions. Such suspensions are fundamentally interesting for instance in terms of the so-called macromolecular crowding, protein cluster formation, gelation, and self-assembly. Notably, backscattering spectrometers with the highest flux can simultaneously access the center-of-mass diffusion of the proteins and the superimposed internal molecular diffusive motions. The nearly complete absence of protein-protein collisions on the accessible nanosecond observation time scale even in dense protein suspensions implies that neutron backscattering accesses the so-called short-time limit for the center-of-mass diffusion. This limit is particularly interesting in terms of a theoretical un-

*Corresponding author, e-mail: seydel@ill.eu, phone ++33 476 20 75 79

derstanding by concepts from colloid physics. Here we briefly review recent progress in studying protein dynamics achieved with the latest generation of backscattering spectrometers. We illustrate this progress by the first data from a protein solution using the backscattering-and-time-of-flight option BATS on IN16B at the ILL and we outline future perspectives.

Keywords: cold neutron backscattering, aqueous protein solutions, self-diffusion

1. Introduction

Proteins in aqueous liquid suspensions are subject to a superposition of their center-of-mass diffusion and their internal diffusive molecular motions. These diffusive motions on the nanometer length scale can generally be well accessed by neutron backscattering spectrometers [1, 2, 3, 4, 5]. When such a backscattering experiment probes protonated tracer proteins dissolved in heavy water (D_2O), the recorded signal from the incoherent scattering of the proteins informs on their self-diffusive dynamics. The complexity of this hierarchically superimposed center-of-mass and internal dynamics in dense aqueous protein solutions has been subject to numerous studies using neutron spectroscopy [2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18]. On the nanosecond time scale observed in a backscattering experiment, the measured protein center-of-mass diffusion corresponds to the colloidal short-time limit, on which protein-protein collisions are negligible and hydrodynamic interactions are dominant [19], providing one of the aspects of the useful complementarity of quasi-elastic neutron spectroscopy (QENS) to nuclear magnetic resonance (NMR) measurements on protein solutions [19, 20]. The center-of-

mass diffusion is represented by an observable apparent diffusion coefficient D which is an implicit function $D = D(D_t, D_r)$ of the translational D_t and rotational D_r diffusion coefficients [19, 21]. In the short-time limit, colloid physics models describing the diffusion of hard spheres [22] can be applied to quantitatively understand the dependence of $D_t = D_t(\varphi)$ and $D_r = D_r(\varphi)$ on the volume fraction φ occupied by the proteins in the sample solution [19]. Neutron backscattering experiments have already explored protein solutions in a wide range of protein concentrations from below 50 mg/ml up to above 500 mg/ml [12, 19]. Current topics of interest include for instance the investigation of protein cluster formation [11, 15, 17, 23, 24, 25, 26, 27, 28], the dynamics of intrinsically disordered proteins [8, 29, 30, 31, 32], the effect of crowding under pressure [33, 34], and thermal unfolding [4, 5, 35, 36, 37]. Moreover, the effect of ion-induced charges in protein solutions has moved into the focus of current research, driven by a mutual inspiration of experiments on the phase behavior of protein solutions tuned by the presence of multivalent salts [11, 38, 39] and the theory of so-called patchy colloids (Refs. [40, 41] and references therein).

The quasi-elastic scattering signal $S(q, \omega)$ from a protein solution depending on the energy transfer ω and magnitude of the scattering vector q can be modeled by [42]

$$\begin{aligned}
S(q, \omega) = & \mathcal{R}(q, \omega) \otimes \{ \beta(q) [A_0(q) \mathcal{L}(\gamma(q), \omega) + \\
& (1 - A_0(q)) \mathcal{L}(\Gamma(q) + \gamma(q), \omega)] \\
& + \beta_{\text{D}_2\text{O}}(q) \mathcal{L}(\gamma_{\text{D}_2\text{O}}(q), \omega) \} ,
\end{aligned} \tag{1}$$

where \mathcal{R} is the spectrometer resolution function, β , $\beta_{\text{D}_2\text{O}}$ and A_0 are scalars,

and \mathcal{L} Lorentzian functions. The symbol \otimes represents the convolution. The Lorentzian widths γ , Γ , and $\gamma_{\text{D}_2\text{O}}$ are associated with the center-of-mass and internal diffusion of the proteins and with the D_2O solvent contribution, respectively. $A_0(q)$ is the Elastic Incoherent Structure Factor (EISF). $\mathcal{L}(\Gamma(q) + \gamma(q), \omega)$ may be replaced by a more general function such as a generalized model of internal diffusivity [5, 43], the Fourier transform of a Kohlrausch [4, 16, 44, 45] or Mittag-Leffler [46, 47, 48] function, or the result of a molecular dynamics simulation, if applicable. In reported backscattering experiments on protein solutions, the apparent center-of-mass diffusion was consistent with simple Brownian diffusion, $\gamma(q) = D q^2$ [9, 15, 17, 19].

To best measure $S(q, \omega)$ (equation 1), a good energy resolution to best capture $\gamma(q)$ as well as sufficient ranges in q and ω to access $A_0(q)$ and $\Gamma(q)$ are required, as discussed in the following section.

2. Progress in backscattering instrumentation

Cold neutron backscattering spectrometers outfitted with Silicon(111) analyzer crystals associated with the elastic wavelength $\lambda \approx 6.27 \text{ \AA}$ are ideally suited to observe the short-time center-of-mass self-diffusion and the superimposed internal diffusion. For a good comparison of the experimental EISF to models, a high maximum q of nearly 2 \AA^{-1} as obtained with Si(111) analyzer crystals is very useful. An energy resolution on the order of $1 \mu\text{eV}$ is required to measure the center-of-mass diffusion. To also access the internal dynamics, a sufficient energy range is additionally needed. Finally, the possibility to simultaneously measure both the center-of-mass diffusion of proteins in rather dilute aqueous solutions and the internal molecular mo-

tions of these proteins requires the high flux and good signal-to-noise ratios of the most recent backscattering spectrometers [49, 50, 51, 52, 53, 54]. These spectrometers are located at the brightest cold neutron sources [55, 56] and are based on advances in neutron optics such as the phase space transformation in the case of exact backscattering [57] and modern neutron guide layouts [58].

So far, backscattering spectrometers have followed two complementary design concepts: (1) the concept of exact backscattering, where both the monochromator and analyzer single crystal reciprocal lattice vectors are oriented precisely parallel to the neutron optical axis and, thus, achieving the best energy resolution. These instruments are typically located at neutron sources that are continuous in time [50, 53, 59, 60]. The excellent energy resolution in this design comes at the cost of a limited maximum energy transfer range given by the requirement that this transfer can with the current state-of-the-art only be reasonably achieved by carrying out the monochromatizing Bragg reflection in a mechanically moving reference frame. (2) The alternative design concept obtains the wavelength definition of the incident beam at the sample position by the spread with flight time of an initially very brief but polychromatic neutron pulse [50, 51, 61, 62, 63]. This design increases the energy range and comes at the expense of a lower energy resolution compared to concept (1).

The spectrometer IN16B [50, 64] combined with its optional configuration denoted BATS (Backscattering-And-Time-of-flight Spectrometer) [52, 65] for the first time aims at providing both these concepts in one single instrument, where the BATS option represents concept (2). BATS notably provides the

possibility to tune the energy range and resolution within certain ranges by changing the chopper settings [65]. For more detailed information we refer to Refs. [52, 65] and the dedicated article on BATS within this proceedings volume [66]. In the following section we report on the first application of the BATS option to record a QENS spectrum on a protein solution sample.

3. First QENS signal from a protein solution using BATS

During the first ILL reactor cycle with IN16B operating in the BATS configuration (March/April 2018), we have recorded QENS data on a solution of *bovine serum albumin* (BSA) proteins dissolved in heavy water (D_2O). The sample was prepared by dissolving $m_p = 300$ mg of BSA powder as purchased from Sigma-Aldrich (catalog number A3059, batch number SLBR6762V) without further purification in $V = 1.5$ mL D_2O , such that the dry protein volume fraction in the sample solution amounted to $\varphi = m_p v_p / (V + m_p v_p) \approx 0.13$ with the specific volume of BSA $v_p = 0.735$ mL/g [17, 19]. We remark that for a conclusive interpretation of crowding effects on protein diffusion, an effective hydrodynamic volume fraction taking into account effects of nonsphericity and hydration has to be used, as explained in Ref. [19]. Part of the prepared sample solution was filled in a cylindrical Al sample holder with 7 mm outer diameter and 0.2 mm difference between the inner and outer cylinder walls. Pure D_2O reference data and empty can data were recorded using equivalent sample holders. The QENS data were measured at the temperature $T = 295$ K thermostated by a cryofurnace. The energy resolution was measured with a cylindrical Vanadium foil with the slightly smaller diameter of ≈ 6 mm than the sample cell, thus resulting in a slightly

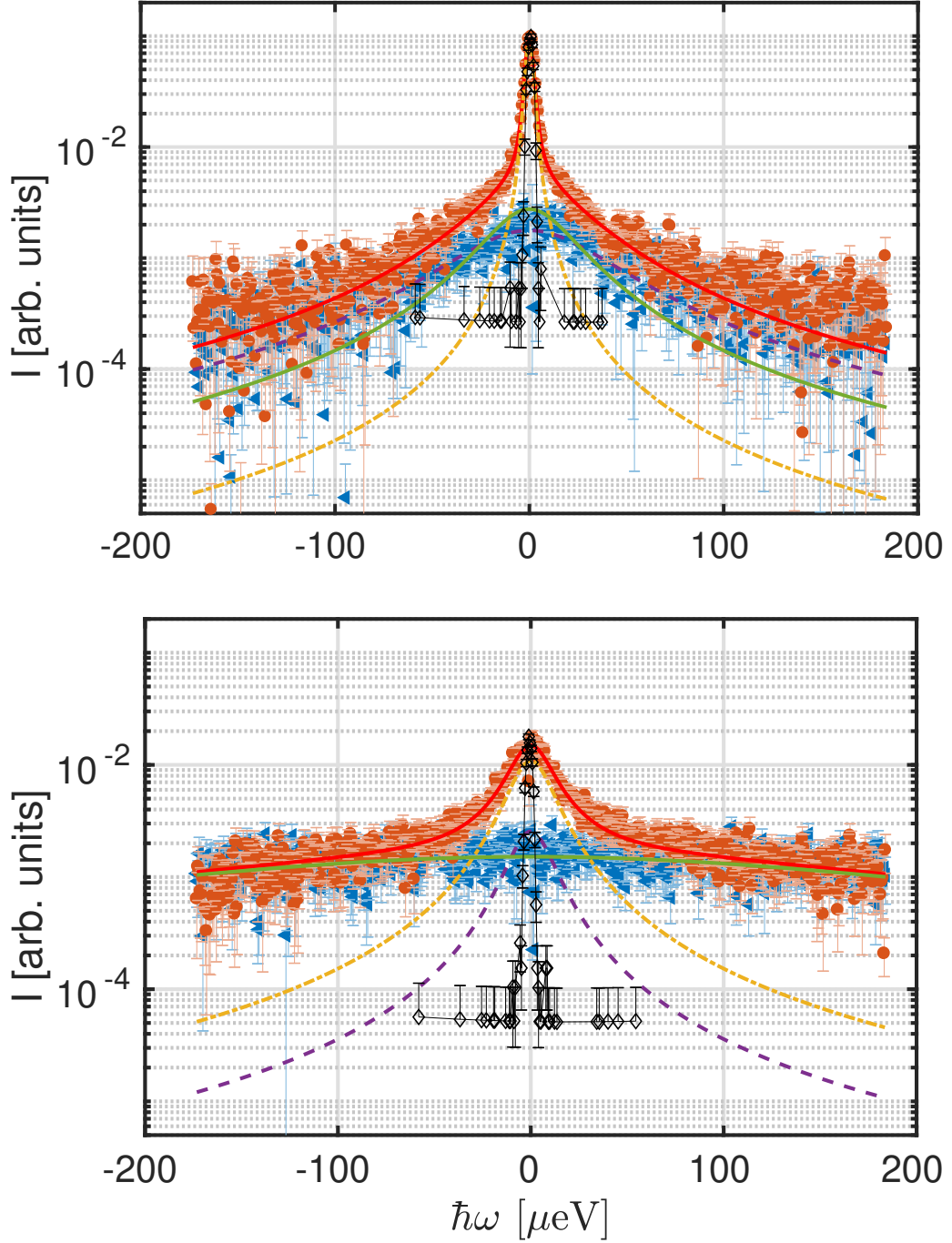


Figure 1: First spectrum (circle symbols) from a BSA protein solution in D₂O recorded using the new BATS option of IN16B in a single tube of the position-sensitive detector (PSD); top: PSD tube number 1, $q = 0.44 \text{ \AA}^{-1}$; bottom: PSD tube 14, $q = 1.79 \text{ \AA}^{-1}$. The square symbols denote the corresponding pure D₂O solvent reference spectrum. The red solid line superimposed on the BSA/D₂O spectrum denotes the fit of equation 1 consisting of the Lorentzian contributions with the widths γ , Γ , and $\gamma_{\text{D}_2\text{O}}$, respectively, assigned to the global diffusion (dashed line), internal diffusion (dash-dotted line) and water contribution (solid line superimposed on the D₂O spectrum). The diamond symbols represent the resolution function measured using Vanadium.

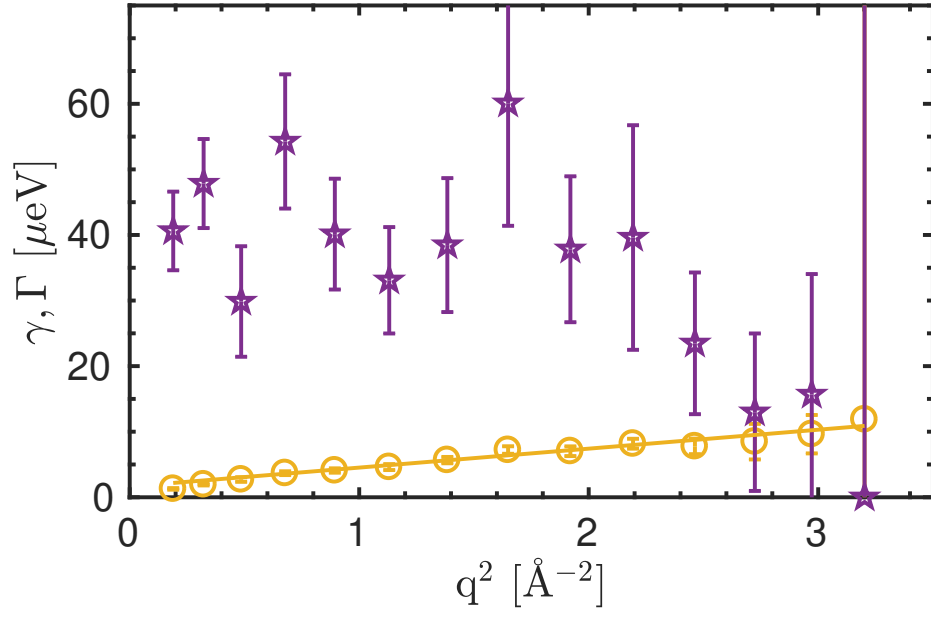


Figure 2: Fit result for the Lorentzian widths γ (circle symbols) and Γ (star symbols) associated with the global and internal diffusive motions of the proteins, respectively, to the spectrum recorded on BSA in D_2O (figure 1) according to equation 1. The line superimposed on the circles is a fit of an affine function $\gamma = Dq^2 + \text{const.}$ (see text).

better resolution than actually achieved on the sample itself. The resolution linewidth was found to be $(3.1 \pm 0.2) \mu\text{eV}$ Gaussian FWHM when fitting Vanadium spectra recorded in individual detectors. When summing the Vanadium spectra of all detectors, the thus obtained integrated resolution amounted to $\approx 3.3 \mu\text{eV}$ FWHM.

BATS was used in the “low repetition rate configuration” [65] with the first counter-rotating chopper-pair using the 20.5° slits and spinning at 79 Hz, i.e. serving as suppressor choppers, and the second pair set to 8° slits and 315 Hz, i.e. serving as the resolution-defining choppers. It is emphasized that during the reported test experiment the specific neutron optics for BATS, namely focus optics at the choppers and before the sample, was not yet installed. Therefore, the flux at the sample was substantially below the expected final flux. The neutrons were detected by a vertically position-sensitive detector (PSD) consisting of 16 vertical tubes used to obtain 16 discrete q -values in the horizontal scattering plane.

The thus obtained first spectrum from a protein solution is depicted in figure 1 (circle symbols) for two q -values, along with the corresponding pure D_2O signal (square symbols) and fit result using equation 1 (lines). The counting time for the BSA and the D_2O spectrum amounted to ≈ 6.5 hours each. The empty can contribution has been subtracted from the BSA/ D_2O and pure D_2O signals. The Vanadium spectrum (diamond symbols in figure 1) was measured without a can. The figure 1 illustrates the measured range in energy transfer of $-180 \mu\text{eV} \leq \omega \leq +180 \mu\text{eV}$, the good visibility of the solvent D_2O contribution on this range, and the convergence of the protein solution and solvent reference spectra for large absolute values of ω .

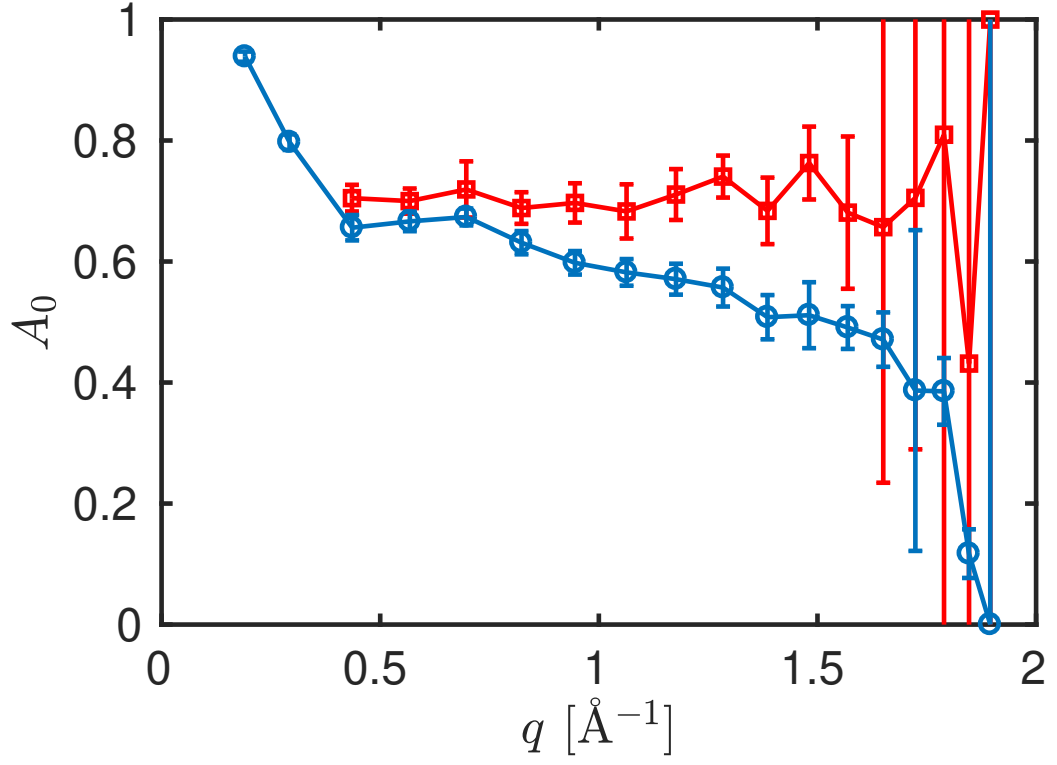


Figure 3: Elastic incoherent structure factor (EISF) $A_0(q)$ of BSA in D_2O resulting from the fit of equation 1 to the spectra displayed in figure 1 (red square symbols). The EISF obtained from a fit of a spectrum recorded on IN16B in the exact-backscattering setup (energy resolution $\approx 0.8 \mu\text{eV}$) is shown for reference (blue circle symbols). (Note that the detectors at the two smallest q -values visible in the IN16B results were not installed on BATS. The lines connecting the symbols are guides to the eye.)

Specific IN16B/BATS software implemented in GNU Octave was used for the initial data reduction. The data analysis was carried out using software implemented in MATLAB (The MathWorks, Inc.) [42]. The fit of equation 1 (lines in figure 1) demonstrates the analysis of the first spectrum from a protein solution measured on BATS. This fit was carried out for the spectra at each q -value individually, i.e. without imposing any q -dependence in the fit. The width γ_{D_2O} of the Lorentzian describing the water contribution was fixed using tabled values for D_2O [42]. β_{D_2O} was fixed using the rescaled pure solvent signal to account for the volume occupied by the proteins [42]. (Within the present accuracy, we cannot model any separate protein hydration water contribution.)

The width $\gamma(q)$ (circle symbols in figure 2) accounting for the global diffusion can be fitted by a straight line (superimposed on the circle symbols in figure 2). The slope of this line results in an apparent diffusion coefficient $D = \gamma(q)/q^2 = (4.39 \pm 0.72) \text{ \AA}^2/\text{ns}$ consistent with earlier IN16B results ($D = (4.14 \pm 0.40) \text{ \AA}^2/\text{ns}$ [17]) on an equivalent sample. However, the offset $\gamma = Dq^2 + (1.6 \pm 0.6) \mu\text{eV}$ of this line is not yet understood. It might arise from a partial cross-talking of contributions from the global and internal diffusion due to the broader energy resolution compared to IN16B, from an insufficient description of the internal motions by only a single Lorentzian [5], from a possibly energy-dependent background contribution, and from the resolution data measured in a slightly different geometry.

The fit result for the linewidth Γ (square symbols in figure 2) is consistent within the errors with previous results from IN16B in the intermediate q -range. The expected improved signal-to-noise ratio with the full implemen-

tation of the BATS neutron optics will substantially increase the accuracy of this information and allow for fitting more complex models. The EISF $A_0(q)$ (figure 3) is reasonably close to a previous IN16B result. In view of the broader energy resolution of BATS, it is consistently slightly above the IN16B result by sampling more motions as “static” within the resolution.

Some uncertainties remain regarding this first test spectrum on a single sample and its interpretation, since the resolution was measured with a slightly different geometry and since an old batch of BSA proteins was used. Moreover, an energy-dependent background may be present in the measured data, which was not considered in the analysis. For these reasons, the present data and analysis cannot serve as quantitative reference for future studies. Nevertheless, these first data outline the promising possibilities with the large ω -range and the good energy resolution in a large q -range.

4. Conclusions and outlook

In this proceedings article we have discussed the use of cold neutron backscattering spectrometers to study the superimposed center-of-mass and internal molecular diffusivities of proteins in aqueous solutions. We have shown the first commissioning data recorded employing the new instrument option BATS at IN16B on a liquid suspension of BSA proteins in D_2O . These data provide a proof of concept prior to the full implementation of all neutron optics in the BATS configuration and, thus, prior to the implementation of the full design neutron flux in the BATS configuration. Moreover, these data can already be quantitatively analyzed using established frameworks, thus pointing to the future possibilities when the full flux and optimized

signal-to-noise ratio will be achieved on BATS. By modeling our data using equation 1, we separate the different contributions to the spectrum by the protein and by the solvent, respectively, via the associated signal intensities and linewidths, as well as by evaluating the additional pure solvent data. A polarization option may be of interest for the future to better experimentally separate the contributions.

We have highlighted the need for versatile backscattering options with complementary energy ranges and resolutions to study the dynamics of proteins in water. Notably, IN16B and its BATS option will provide a choice of different energy resolutions and ranges that would otherwise require the use of several instruments. These new backscattering options open up perspectives for a systematic study of various topics such as the crowding effect in protein solutions, the formation of static or transient protein clusters, the self-assembly of protein aggregates, the thermal unfolding and denaturing of proteins, and the effect of salt-induced charges in protein solutions. These experiments will profit from the large and well-suited range in q – to access the EISF and the q -dependence of the diffusion processes – as well as ω – to access both the center-of-mass and internal molecular diffusion. The increased range in ω will benefit amongst other topics the experiments on more dilute protein suspensions as well as at elevated temperatures where the overall dynamics becomes too fast for the energy range covered by the exact backscattering configuration.

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