Experimental report

17/06/2024

Proposal: 9-13-1046 **Council:** 10/2022

Title: Casein as a model intrinsically disordered protein: Impact of salt onthe internal diffusive dynamics insolution

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: beta-casein from bovine milk in D2O (up to 500 mg/ml), CaCl2 (up to 15 mM)

Instrument	Requested days	Allocated days	From	To
IN16B Si 111 BATS	4	4	08/04/2023	12/04/2023

Abstract:

We aim to investigate fundamental properties of intrinsically disordered proteins (IDPs) in aqueous solution from a polymer physics perspective as part of a collaborative project jointly funded by the French ANR and German DFG agencies and involving both experimental and theory groups. Caseins represent a model system for IDPs that is abundant, safe, non-toxic, well soluble, commercially available, and technologically relevant.

Here, we specifically aim to investigate the influence of calcium Ca2+ ions on the internal diffusive dynamics of ß-casein in aqueous solution. This systematic dependence on the Ca2+ concentration constitutes a clearly missing important piece of information for the physiologically relevant aqueous solution setting of casein micelles. This study will prepare the ground for future studies of the phase behavior of casein in aqueous solution, addressing their association and assembly, involving processes linked to liquid-liquid phase separation.

We will use BATS that has proven to be ideally suited to access the internal diffusive dynamics of proteins in solution.

This work will be part of a future PhD project (student appointed, but not yet arrived).

Experimental Report Experiment 9-13-1046

Casein as a model intrinsically disordered protein: Impact of salt on the internal diffusive dynamics in solution

Experimental Team: Christian Beck, Anna Grundel, Laura Mateo Miñarro, Ilaria Mosca, Roody Nasro, Tilo Seydel

Motivation: The main motivation is to investigate the influence of calcium Ca^{2+} ions on the internal diffusive dynamics of β-casein in aqueous solution. Since a systematic study of the protein in solution was lacking for comparison, the second motivation is to characterize the system in the absence of salt, with which we will also be able to study the effects of self-crowding.

Performed Measurements: Several calibration measurements were necessary for a future successful data analysis. The calibration measurements are shown in the upper part of Table 1. The vanadium and empty can measurements were shared with experiment "9-13-1044". A detailed list of the samples are given in the bottom part of Table 1. All measurements had an acquisition time between 4 and 5 hours and were performed using the resolution *lres-4*.

Table 1: Performed measurements during beamtime 9-13-1046

Sample name	Temperature
Vanadium	280K
Sodium phosphate buffer 25 mM	280K 330K 350K
β-Casein 25 mg/ml (NaPh)	280K 295K 330K
β-Casein 50 mg/ml (NaPh)	280K 295K 330K
β-Casein 75 mg/ml (NaPh)	280K 295K 330K 350K 330K 280K
β-Casein 100 mg/ml (NaPh)	280K 295K 330K 350K
β-Casein 200 mg/ml (NaPh)	280K 295K 330K
β-Casein 75 mg/ml (D_2O)	280K
β-Casein 75 mg/ml + 2.5 mM $CaCl_2$ (D_2O)	280K
β-Casein 75 mg/ml + 3.75 mM $CaCl_2$ (D_2O)	280K
β-Casein 75 mg/ml + 5 mM $CaCl_2$ (D_2O)	280K

Preliminary analysis: As a first approximation, the spectra were analyzed using the quasi-elastic scattering function that has been shown to be a good model for well-folded proteins [1]:

$$S(q,\omega) = \mathscr{R} \otimes [\beta\,(A_0\mathscr{L}_\gamma(\omega) + (1-A_0)\mathscr{L}_{\gamma+\Gamma}(\omega)) + \beta_{D_2O}S_{D_2O}(q,\omega) + \beta_c\mathscr{D}(q)] \quad (1)$$

where $\mathscr{R}$ denotes the instrumental resolution function, modeled by a combination of Gaussian functions, β is a scalar, and $A_0(q)$ represents the elastic incoherent structure factor (EISF). The two Lorentzians $\mathscr{L}_\gamma$ and $\mathscr{L}_{\gamma+\Gamma}$ account for two processes occurring at distinct time scales that could be associated to the global protein self-diffusion and its internal dynamics. The fixed term $\beta_{D_2O}S_{D_2O}(q,\omega)$ models the solvent contribution.Finally, the containers used during the experiment were sufficiently different from each other, such that subtracting the contribution directly from the data (with an initial attempt to take into account the self-shielding) was not accurate enough yet. Instead, we have accounted for the container contribution directly in the model by adding a Dirac delta, $\beta_c\mathscr{D}(q)$.
Example spectra are shown in Figure 2. The spectrum represented with yellow symbols corresponds to the solvent, in which the contribution of the container can be clearly appreciated. The second spectrum, with gray symbols, corresponds to the spectrum of the

sample. The good separation of the protein solution signal and solvent baseline is clearly noticeable even at a relatively low protein concentration of 100 mg/ml. Both the solvent and the sample signal are successfully fitted; however, the resulting fit parameters indicate that the model cannot be final, since the width of the second Lorentzian can not be related with a diffusive process yet. It is not surprising that the fit according to Eq. 1 does not result in a satisfactory physical picture, for instance because it does not account for the micelle assembly of the Caseins in solution and also because it is doubtful if a clear separation of global and internal motions is possible for intrinsically disordered proteins.

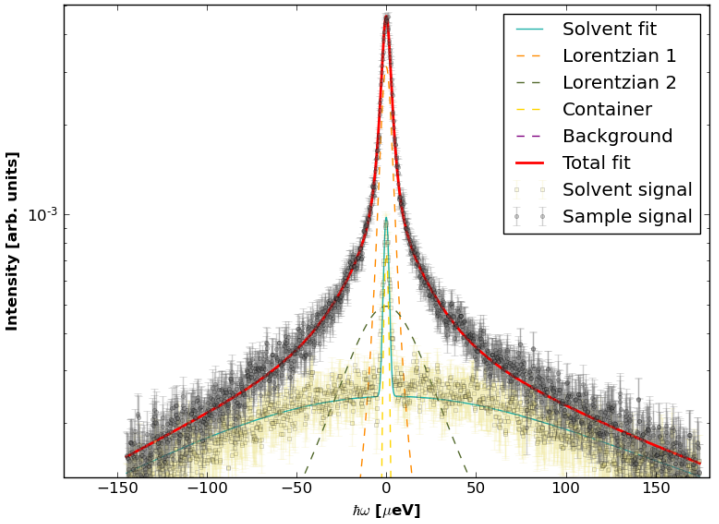

Figure 1: QENS spectra obtained from β-Casein (grey symbols) at a concentration of 100 mg/mL and from D_2O-NaPh solvent (yellow symbols), both at T = 280 K with different dynamic contributions (lines) at $q = 1.57$ Å^{-1}. The red and blue solid lines represent the fit of the sample and of the solvent, respectively. The dashed lines correspond to the different dynamic contributions within Eq. 1 (see the legend). The Dirac accounts for the sample container contribution that was not subtracted in this case, but fitted. All contributions are convoluted with the resolution function.

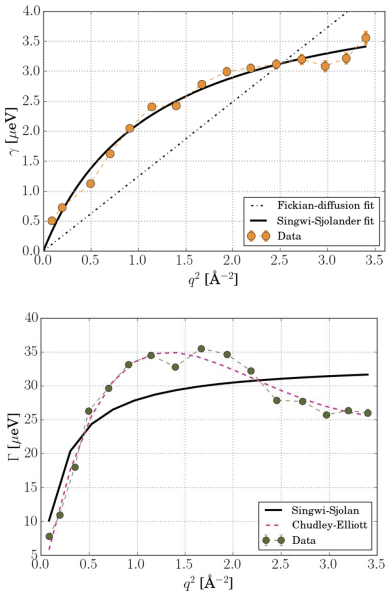

Figure 2: Top: Width γ of the first Lorentzian (orange) related with the slower process (center-of-mass) versus q^2 and shows non-Fickian diffusive behavior. A jump-diffusion fit with the equation from that inset is plotted over the data. Bottom: Width $\gamma + \Gamma$ of the second Lorentzian (grey) versus q^2.

Regarding the samples containing salt, they have not yet been properly analyzed given the need to search for a more accurate model to describe the dynamics of the protein alone. However, the spectra of a salt concentration series with a fixed concentration of β-Casein at the same temperature are shown in Figure 3. It is possible to observe that the increase in salt concentration causes a slight decrease in the width of the spectrum. This is in agreement with the expectation for which the salt promotes the formation of micelles, which results in a lower amount of free monomers and therefore a slower dynamics.

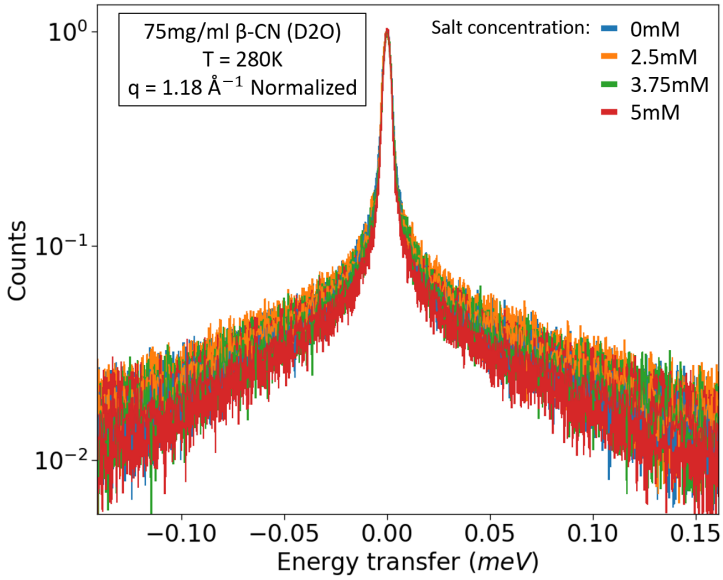

Figure 3: Model-free comparison of spectra of 75 mg/ml β-Casein solution in pure D_2O ($T = 280$K, $q = 0.44$ Å^{-1}) with different salt concentrations: 0 mM (blue), 2.5 mM (orange), 3.75 mM (green) and 5 mM (red).

References

[1] M. Grimaldo, F. Roosen-Runge, F. Zhang, F. Schreiber, and T. Seydel, "Dynamics of proteins in solution," *Quarterly Reviews of Biophysics*, vol. 52, p. e7, 2019.