Experimental report

19/07/2024

Proposal: 8-04-950 **Council:** 4/2023

Title: The effect of chain length on the internal diffusive dynamics of intrinsically disordered proteins

Research area: Chemistry

This proposal is a resubmission of 8-04-946

Main proposer: Marie SKEPO

Experimental team: Oskar SVENSSON
Henrik Vinther SORENSEN

Local contacts: Tilo SEYDEL

Samples: Histatin 5
Double length Histatin 5
Half length Histatin 5
2.5 length of Histatin 5

Instrument	Requested days	Allocated days	From	To
IN16B Si 111 BATS	6	2	26/06/2023	28/06/2023

Abstract:

The goal of this project is to understand how the chain length of intrinsically disordered proteins affects the internal diffusive dynamics. For that purpose we will use our model system, the antimicrobial saliva protein Histatin 5 and variants thereof, varying in length between 12 - 60. Combining IN16B, BATS, and IN5 quasi-elastic neutron scattering would cover a very complete dynamic range. Combining modern data analysis frameworks and simulations, separating the different hierarchical levels of dynamics, unprecedented insights will be allowed. The focus will be on access to (sub)nanosecond internal diffusive motions of the protein in solution for a range of protein concentrations and different temperatures. This choice is based on previous scattering experiments and simulations, which together would yield a molecular understanding of the system of both medical and academic relevance. Here, we specifically apply for BATS to profit from its world-leading signal-to-noise and to fill the gap inaccessible by IN5 and IN16B.

Histatin5 IN16B Data Experiment 8-04-950

The effect of chain length on the internal diffusive dynamics of intrinsically disordered proteins

Goal of the experiment: The goal of this project is to understand how the chain length of intrinsically disordered proteins affects the internal diffusive dynamics. ,For that purpose we will use our model system, the antimicrobial saliva protein Histatin 5 and variants thereof, varying in length between 12 - 60.
Performed measurements: The calibration measurements and A detailed list of the samples are given in Table 1. It is important to mention that since the solvent spectra are unusually broad (perhaps due to H_2O contamination by exchange), the pure D_2O spectra measured during another experiment have been used instead for fitting the solvent contribution.

Table 1: Performed measurements during beamtime 8-04-950

Sample name	Temperature
Vanadium	300K
Buffer: 150 mM NaCl, 20 mM Tris-HCl D2O, pD 7	280K 295K
Histatin 5 Half-Chain, 55 mg/mL (Buffer)	295K
Histatin 5 Half-Chain, 27 mg/mL (Buffer)	280K 295K 310K
Histatin 5 Double-Chain, 55 mg/mL (Buffer)	280K 295K 310K
Histatin 5 Wild Type, 55 mg/mL (Buffer)	295K

Preliminary analysis: As a first approximation, the spectra were analyzed fitting the scattering function $S(q,\omega)$ with the same model used to describe the self-diffusive properties of Histatin 5 [1], but using instead equation 1, which is fundamentally the same as equation **??** but with a slight technical addition marked in bold,

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

where $\mathscr{R}$ denotes the instrumental resolution function, modelled by a Gaussian function, 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 with 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 empty containers were not measured, so it is not possible to subtract the contribution directly from the data; instead, we have accounted for the container contribution directly in the model by adding a Dirac delta function, $\beta_c\mathscr{D}(q)$.

First, we have fitted the spectra for each value of q separately without imposing any dependence on the momentum transfer q. Figure 1 shows an example spectrum obtained from *Histatin 5 wild type*, the sample that has the same chain length as those measured by Fagerberg et al. [1] at a comparable concentration and temperatures. The spectrum represented with yellow symbols corresponds to the solvent (pure D_2O), in which the contribution of the container can be clearly appreciated. The second spectrum, with grey 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 55 mg/ml. Both the solvent and the sample signal are successfully fitted. Regarding the fitting parameters, Figure 2 shows the results of $\gamma(q)$ (top), and

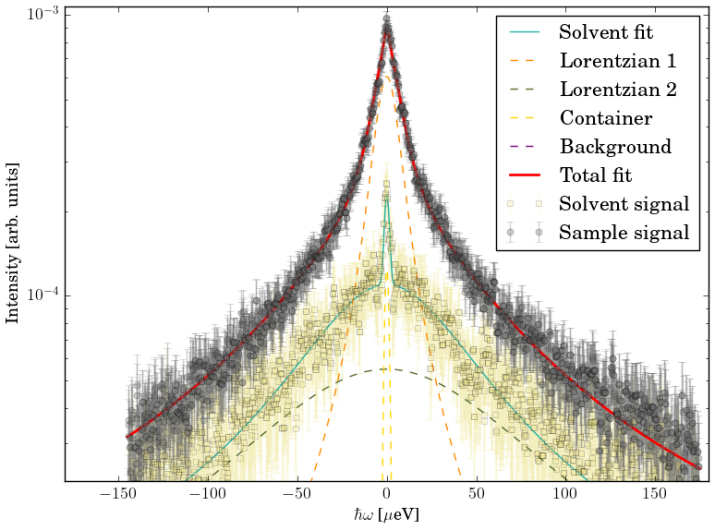

Figure 1: QENS spectra obtained from Histatin 5 wild type (grey symbols) at a concentration of 55 mg/mL and from D_2O solvent (yellow symbols), both at T = 293 K with different dynamic contributions (lines) at $q = 0.84$ Å^{-1}. The red and blue solid lines represent the fit of the sample and the solvent, 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 but fitted. All contributions are convoluted with the resolution function.

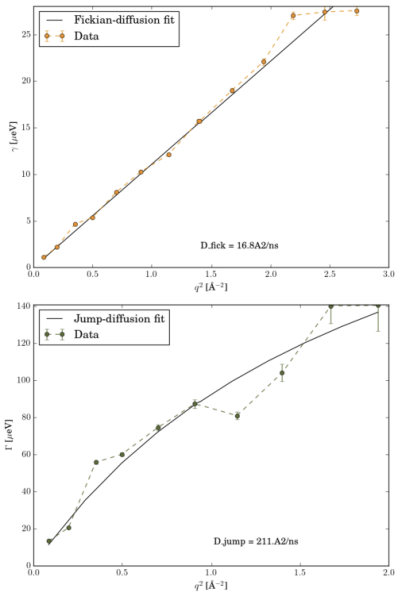

Figure 2: Top: Width γ of the first Lorentzian (orange) related with the slower process (center-of-mass) versus q^2. A jump-diffusion fit with the equation from that inset is plotted over the data.
Bottom: Width $\gamma + \Gamma$ of the second Lorentzian (grey) versus q^2.

$\Gamma(q)$ (bottom) as a function of q^2 of the *Histatin 5 wild type* sample. It is found that γ, associated with the center of mass diffusion, presents a Fickian-type diffusion given by

$$\gamma = Dq^2 \tag{2}$$

(Equation 3) where D is the apparent diffusion coefficient of the center of mass. While Γ, related to internal diffusive motions, can be described in terms of the simplistic jump-diffusion model according to

$$\Gamma = \frac{D_{int}q^2}{1 + D_{int}q^2\tau} \tag{3}$$

where D_{int} is the internal diffusion coefficient and τ is the residence time between jumps. The diffusion coefficient for the center of mass found by fitting the data using equation 3 is $16.8\mathring{A}/ns$ for the sample mentioned before. It is within what was expected when looking at Figure , taking into account that the samples, conditions and instrument setup are significantly different. Regarding internal diffusion, a diffusion coefficient of $211\mathring{A}/ns$ and residence time of $\tau = 2.37ps$ have been found. Both the diffusion coefficient (center of mass and internal) and the internal residence time found for the rest of the samples (Table 1) are shown in Figures 3, 4, and 5 respectively. It has been observed that the relationship between the diffusion coefficient of the center of mass, D, and temperature (Figure 3) is qualitatively similar and consistent with what is observed in Figure . When considering parameters related to the internal dynamics of proteins, such as D_{int} and τ

(Figures 4, 5), it appears that the protein's chain length influences them. However, these findings require further discussion. Additionally, alternative fit models for QENS spectra are currently being evaluated.

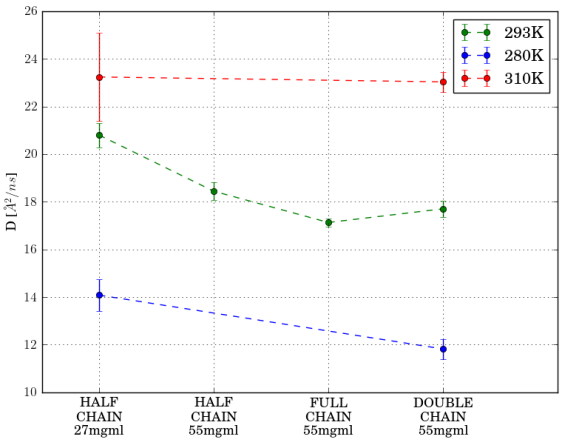

Figure 3: Apparent center of mass diffusion coefficient D versus the different samples (chain length and concentration on the x-axis) at different temperatures (colour-coded dashed lines, see legend).

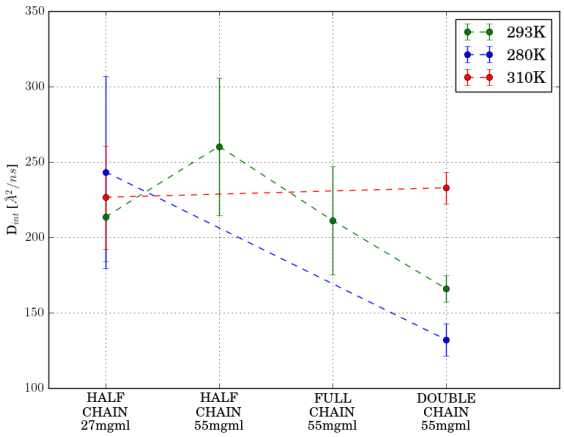

Figure 4: Internal diffusion coefficient D_int versus the different samples (chain length and concentration on the x-axis) at different temperatures (colour-coded dashed lines, see legend).

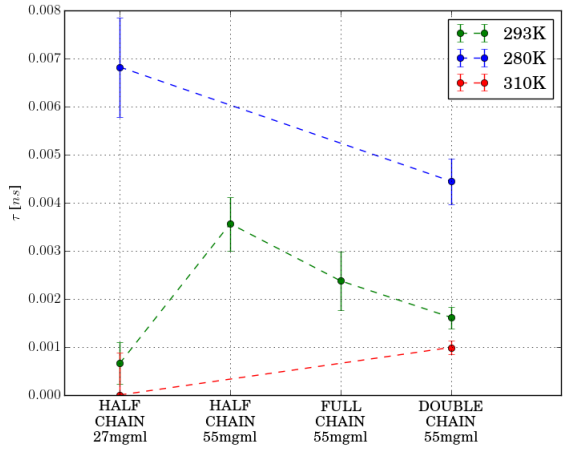

Figure 5: Residence time τ versus versus the different samples (chain legth and concentration on the x-axis) at different temperatures (color coded dashed lines, see legend).

References

[1] E. Fagerberg, S. Lenton, T. Nylander, T. Seydel, and M. Skepo, "Self-diffusive properties of the intrinsically disordered protein histatin 5 and the impact of crowding thereon: a combined neutron spectroscopy and molecular dynamics simulation study," *The Journal of Physical Chemistry B*, vol. 126, no. 4, pp. 789–801, 2022.