

Experimental report

10/09/2024

Proposal: 8-04-964

Council: 4/2023

Title: Internal friction in IDP: Comparison of ζ -helical building domain and intrinsically disordered domain

Research area: Biology

This proposal is a new proposal

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Samples: protein solution 20mg/ml PBS buffer

Instrument	Requested days	Allocated days	From	To
IN15	5	5	26/10/2023	31/10/2023

Abstract:

The inherent flexibility is a central element to the fast response of IDPs to environmental changes and the ability to bind to different binding partners or have varying binding sites.

In the current NSE experiment we want to compare the chain dynamics, respectively internal friction and activation energy of a domains building alpha helices and an acidic disordered domain to get insight into the relation between sequence and internal friction respectively activation energy.

Experimental Report : antibody domain dynamics

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The experiment was performed with prepared samples as described in the main proposal. During initial measurements (up-down scans) we recognized already that the sample was partly contaminated with H₂O. The aSyn wild type sample was only contaminated to a small part that the measurement was possible. Unfortunately, the highest Q at 2 nm⁻¹ was not possible as it vanished in the background. The second sample was tried but the contamination was so strong that after background subtraction the signal was too noisy to be evaluated.

We plan a continuation proposal to repeat the measurements. Here we present a first evaluation of the wild type (WT) aSyn.

The intermediate scattering function after background subtraction is presented in Figure 1 for T=20°C with a fit to a Zimm with internal friction (ZIF) model that also describes the dynamics of intrinsically unfolded proteins [1,2]. Other measurements at different temperatures look similar. To overview the result, we use an effective diffusion plot as presented in Figure 2 [3] using D_{eff} from a fit $\sim \exp(-Q^2 D_{\text{eff}} t)$ for fit functions and data. Original $I(Q,t)/I(Q,0)$ are fitted with the full model.

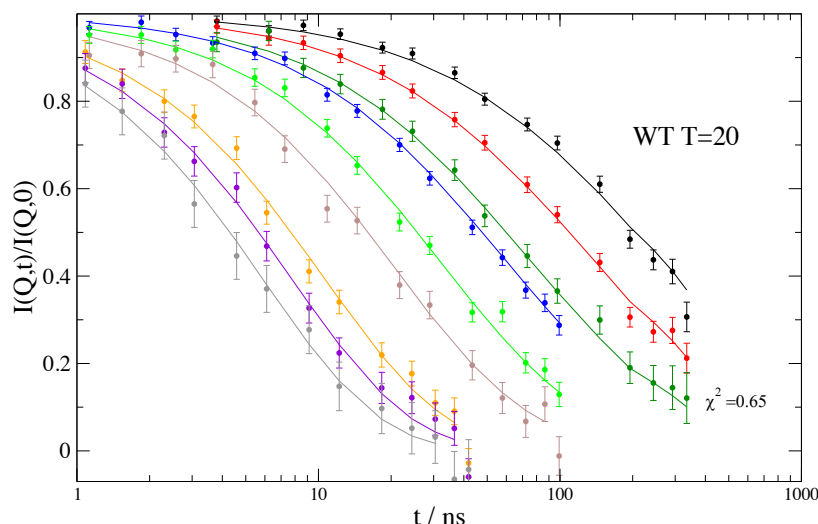


Figure 1 Intermediate scattering function of wild type aSyn

Figure 2 left shows D_{eff} for 20°C for data, ZIF fit and for comparison ZIF with internal friction neglected (pure Zimm model). To get reasonable fits it was necessary to assume rigid segments of 10 amino acid length which led in the Zimm model to a curved line at larger Q. For pure Zimm $D \sim Q$ is expected. The internal friction time with 43 ns is reasonable smaller than the Zimm time with 196 ns. Still there is a strong discrepancy to DLS data at the same concentration which cannot be explained by the $H(Q)/S(Q)$ correction which is also valid for IDP or polyelectrolytes [1,3]. PFG-NMR measurements for the long time self-diffusion will be done in December.

The viscosity scaling $T/\eta(T)$ in figure 2 right shows that the Zimm dynamics mainly visible at lower Q scales as expected with viscosity. The scaling changes at larger Q when internal friction becomes dominant. Unfortunately, higher Q are missing to get more reliable fits which allow to determine the activation energy of the internal friction in aSyn.

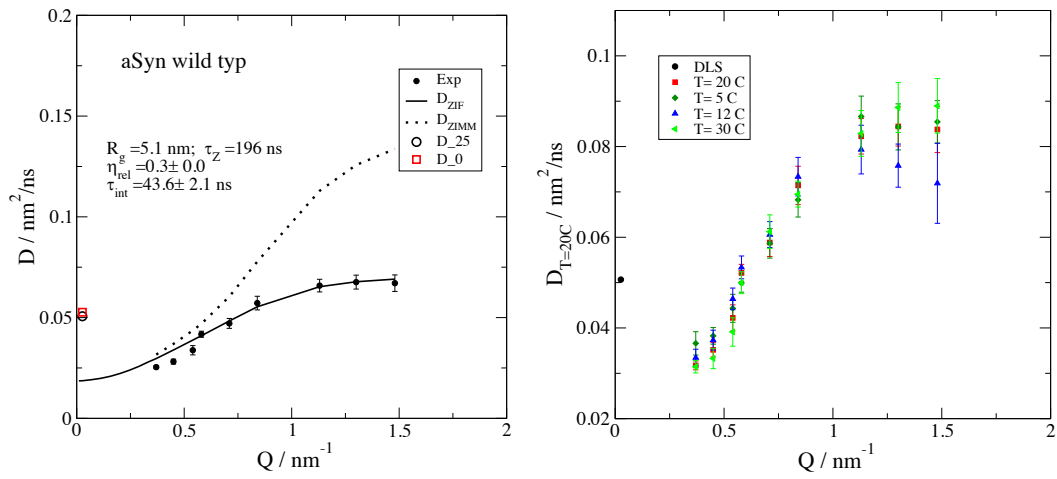


Figure 2 Left: Comparison of effective diffusion extracted from the data and from the fit with a ZIF model complemented by structure factor effects and hydrodynamic interactions as $D=D_0H(Q)/S(Q)$. Right: Effective diffusion scaled to a reference temperature of 20°C in comparison to DLS measured diffusion coefficient for low concentration.

- [1] A. M. Stadler, L. Stingaciu, A. Radulescu, O. Holderer, M. Monkenbusch, R. Biehl, and D. Richter, Internal Nanosecond Dynamics in the Intrinsically Disordered Myelin Basic Protein, *J Am Chem Soc* **136**, 6987 (2014).
- [2] R. R. Cheng, A. T. Hawk, and D. E. Makarov, Exploring the role of internal friction in the dynamics of unfolded proteins using simple polymer models., *J Chem Phys* **138**, 074112 (2013).
- [3] E. Buvalaia, M. Kruteva, I. Hoffmann, A. Radulescu, S. Förster, and R. Biehl, Interchain Hydrodynamic Interaction and Internal Friction of Polyelectrolytes, *ACS Macro Lett* **22**, 1218 (2023).