Experimental report

18/12/2020

Proposal: 8-04-868 **Council:** 4/2019

Title: To understand the antimicrobial activity of the salivary protein Histatin 5

Research area: Chemistry

This proposal is a continuation of 8-04-790

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Samples: Histatin 5, Saliva protein

Instrument	Requested days	Allocated days	From	To
IN5	6	0		
IN16B	4	3	17/01/2020	20/01/2020

Abstract:

The goal of this project is to understand the antimicrobial activity of the intrinsically disordered salivary protein Histatin 5 (His5), which acts in defence against oral candidiasis caused by Candida Albicans. Experiments and simulations have indicated the importance of the dynamic properties of the protein. Combining IN16B and IN5 quasi-elastic neutron scattering would cover a very complete dynamics range. Together with novel data analysis frameworks, separating the different hierarchical levels of dynamics, new insights not previously accessible will be allowed. Focus will be access to (sub) nanosecond internal diffusive motions of the protein in solution for a range of protein concentrations and different temperatures. The choice of focus is based on previous scattering experiments and simulations, which together would yield molecular understanding of the system of both medical and academic relevance.

Measurements performed

Using the backscattering neutron spectrometer IN16B, having an energy resolution of about 0.75 μeV, self-crowded solutions of the intrinsically disordered protein Histatin 5 were measured at different temperatures (280, 298 and 310 K, and one measurement at 290 K at 150 mg/ml protein concentration) and levels of self-crowding. All measurements were performed in D_2O solvent, with 20 mM Tris and 150 mM salt. All samples had been dialyzed before use. A full list of measurements is found in Table 1.

Table 1: All measurements made

Sample	Temperature	Concentration
D2O buffer	280 K	-
D2O buffer	290 K	-
D2O buffer	298 K	-
D2O buffer	310 K	-
Vanadium (for resolution function)	-	-
Histatin 5	280 K	50 mg/ml
Histatin 5	298 K	50 mg/ml
Histatin 5	280 K	100 mg/ml
Histatin 5	298 K	100 mg/ml
Histatin 5	310 K	100 mg/ml
Histatin 5	280 K	150 mg/ml
Histatin 5	290 K	150 mg/ml
Histatin 5	298 K	150 mg/ml
Histatin 5	310 K	150 mg/ml

Initial analysis

An initial analysis during the beam-time of the lowest protein concentration measured (50 mg/ml) showed fast dynamics, increasing with temperature to such an extent that, together with the lower signal-to-noise ratio of this low protein concentration, protein dynamics were hard to distinguish from solvent dynamics, why a measurement at this protein concentration at a temperature of 310 K was deemed fruitless and not performed. The data constituting the basis for this decision is shown in Figure 1.

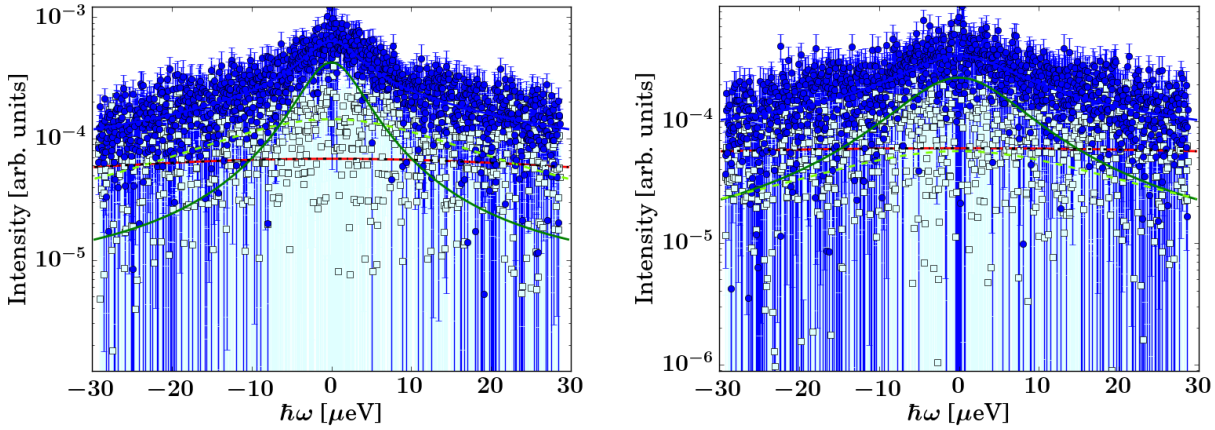

Figure 1: QENS spectra of Histatin 5 at protein concentration of 50 mg/ml. Light blue squares is solvent signal, blue circles is protein+solvent signal. Red line is fit to solvent, green lines are the different components of the Lorentzian fitted, while the blue line is the total fit. To the left: Temperature of 280 K. To the right: Temperature of 298 K.

This shows that IN16B cannot probe dynamics at the faster timescales found with Histatin 5 at more dilute, higher temperatures. For these to be captured, the IN16B BATS mode might be suitable.
This data is to be used in conjunction with the data collected at beam-time proposal 8-04-813.