Experimental report

18/12/2020

Proposal: 8-04-813 **Council:** 4/2017

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

Instrument	Requested days	Allocated days	From	To
IN16B	4	4	25/06/2018	29/06/2018
IN5	7	2	28/05/2018	30/05/2018

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 computer simulations have indicated the importance of the dynamic properties of the protein, and it is hypothesized the binding of ions, for example, Zn(+2) and Fe(+3), might be of importance. We aim to combine IN16B and IN5 to record the quasi-elastic neutron scattering spectra to cover a very complete dynamic range. The use of IN16B and IN15 in combination with novel data analysis frameworks to separate the different hierarchical levels of dynamics, it will allow for new insights that have not previously been accessible. The main goal will be to access the (sub) nanosecond internal diffusive motions of the proteins in solution, with emphasize on the parameters salt (zinc), protein concentration, and temperature. The model system, His5, is well established on both the experimental and simulation side, and by combining neutron scattering with simulations, we will obtain a molecular understanding of system that is both of medical and academic relevance.

Measurements performed

Histatin 5 at high protein concentration was measured at the time-of-flight spectrometer IN5, with an energy resolution of about 100 µeV, for one protein concentration at both 5 Å and 10 Å. Measurements at backscattering spectrometer IN16B (having an energy resolution of about 0.75 µeV) encompassed a re-measure of an older sample measured, acting as a reproducibility check. Samples with no preprocessing ("straight out of the can") at very high protein concentration and sample having been preprocessed (purified through dialysis) were acquired. All measurements performed in D_2O.

Fitting of data

Using Paalman-Pings corrections and a Singwi-Sjölander jump-diffusion model convoluted with a resolution model for IN16B, well-fitted data could be achieved; example is given in left Figure 1. For IN5, a two-Lorentzian model was fitted to the data. An example fit is given in right Figure 1.

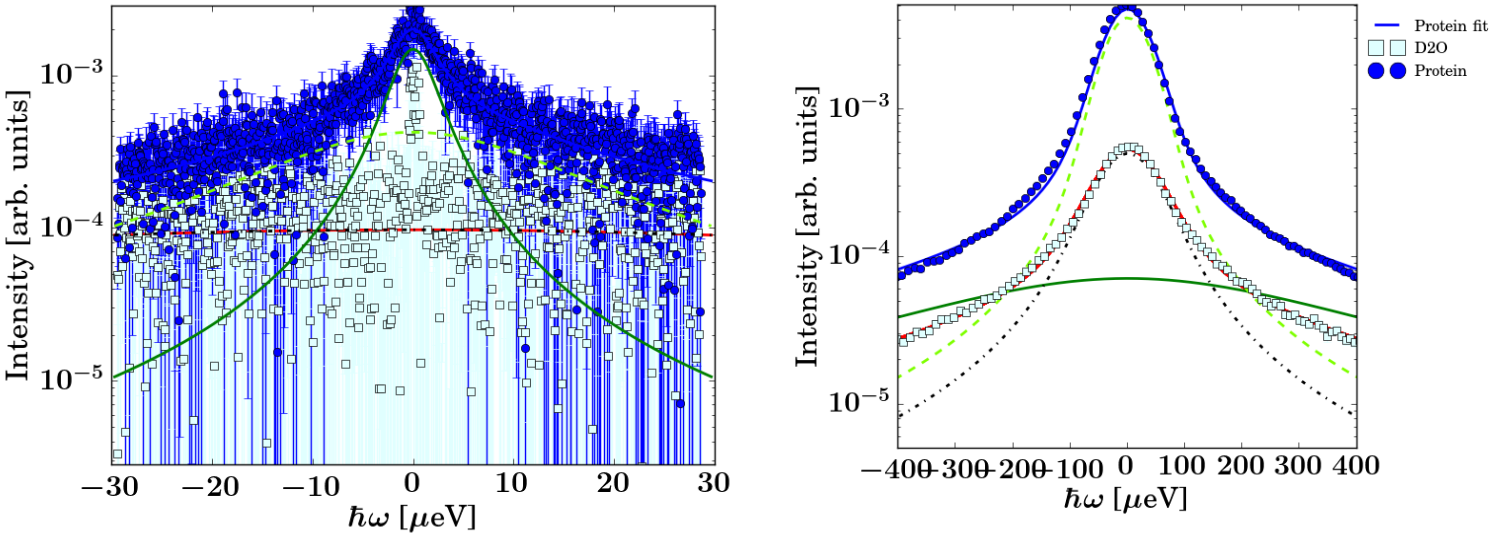

Figure 1: Left: Example spectra and fit of Histatin 5 sample using IN16B, at 200 mg/ml, temperature of 298 K, salt content of 150 mM. Light blue fit is solvent-only spectra, deep blue protein sample. Red line is solvent fit, blue line is overall protein fit, green lines are different Lorentzian components of the overall protein fit. Right: Example spectra and fit of Histatin 5 using IN5 (5A), same sample conditions. Lines and markers as left figure, black dots is Lorentzian component. Error bars may be smaller than markers.

From the fits, apparent diffusion was given, visualized in Figure 2.

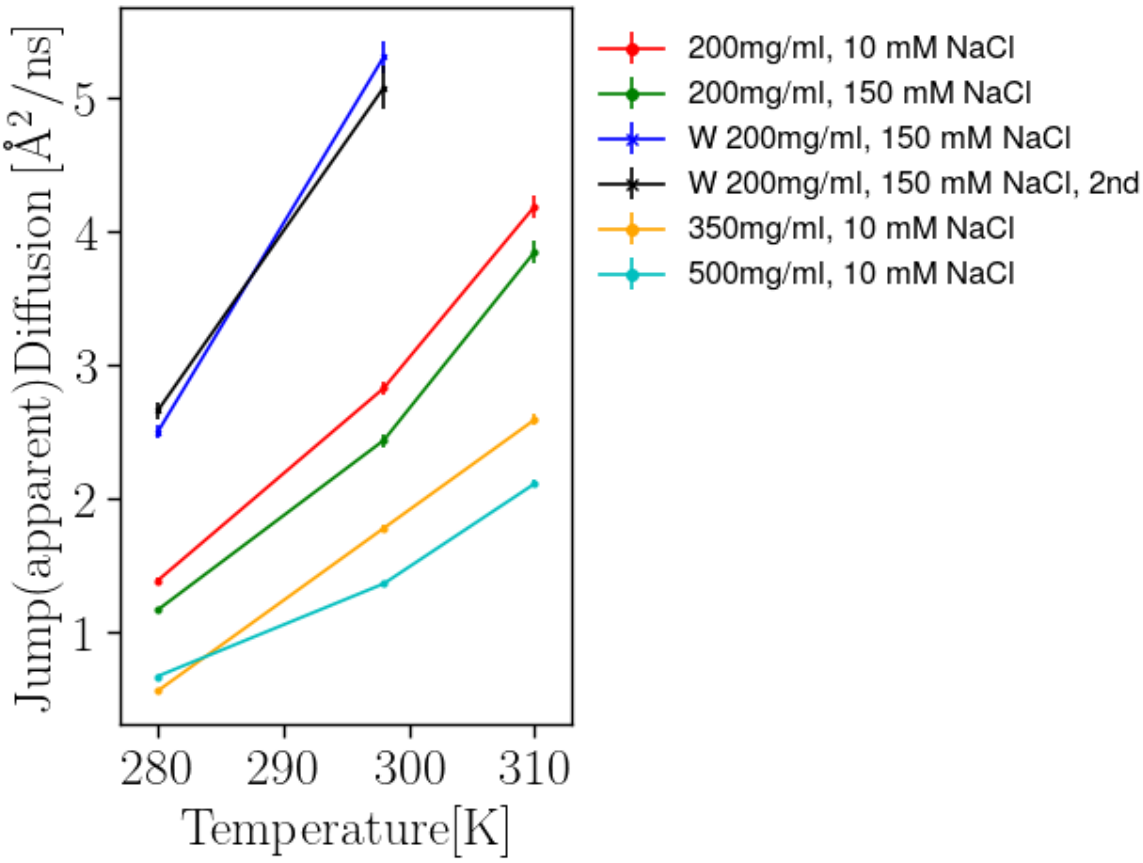

Figure 2: Apparent diffusion derived from jump-diffusion model for different samples. A 'W' indicates samples to have been preprocessed.

Unfortunately, it is shown that non-preprocessed samples and pre-processed samples do not yield the same results. This shows the importance of sample preparation. Disregarding the sample-preparation, some trends can be seen. A clear and expected temperature dependence is shown, as well as a slowing of the dynamics with increasing protein concentration.
For IN5, the fits achieved so far were not completely satisfactory, with an average goodness-of-fit of 5.65 (1 is optimal, below 1 is overfitting, above 1 is underfitting). This error is attributed to an issue of lining up the data correctly, so the peak maximum of the signal aligns at an energy transfer of 0.

The data obtained here is to be used in conjunction with experiment 8-04-868, where additional experiments were made to investigate crowding effects at other levels of crowding.