	Experiment title: To understand the antimicrobial activity of the salivary protein Histatin 5	**Experiment number**: MX2307
Beamline: BM29	**Date of experiment**: from: 23 Jan 2021 to: 24 Jan 2021	**Date of report**:
Shifts: 1	**Local contact(s)**: Petra Pernot	*Received at ESRF:*

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Report:

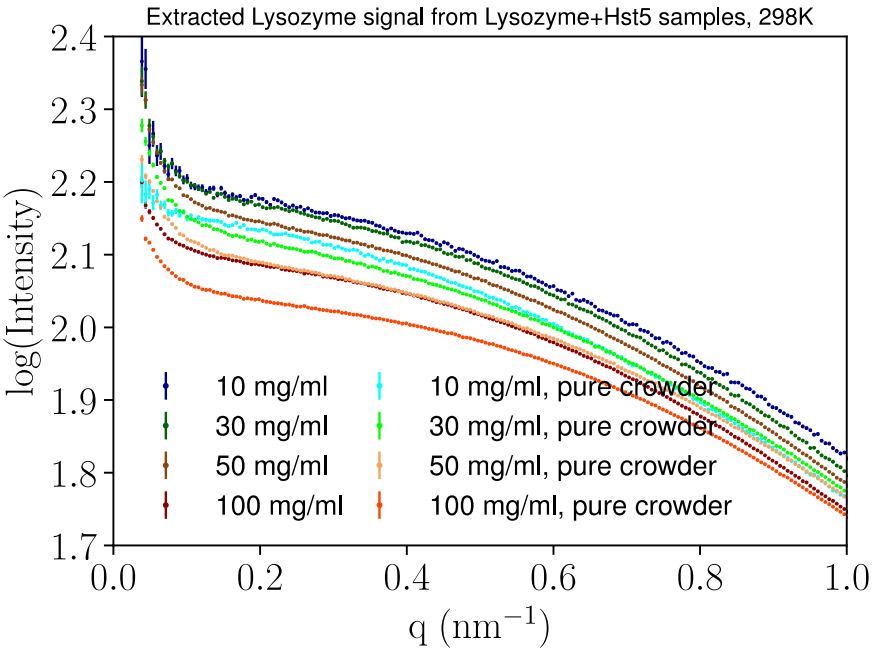
Six solutions of "pure crowders" (BSA, Lysozyme, Ficoll70, PEG2K, PEG4K, PEG6K) were measured at concentrations of 10, 30, 50, and 100 mg/ml crowder concentration, at three different temperatures. Companion solutions, with the same crowder concentration but with 10 mg/ml of Histatin 5 were also measured at the same conditions as well as a pure sample of Histatin 5 at 10 mg/ml. After initial data reduction, frames were inspected for radiation damage and "empty frames" (i.e. no signal from sample), which were removed from the data set before averaging frames. Thereafter buffer spectra were subtracted to attain the sample signal. The radius of gyration and the scattering curves of the Histatin 5 sample were found to be in line with previous measurements. The Histatin 5 measurement was thus used as reference for molecular-weight determination, due to its well-behaving nature.

At the lowest crowder concentration (10 mg/ml), the molecular weight was determined for pure crowders using I(0), however, agreement with literature was not found for any of the samples. The PEG-samples all showed molecular weights at half of expected values, while other samples showed approximately twice the expected molecular weight. This would imply, in the latter case, dimerisation and/or other types of aggregation to different extent, but for Lysozyme, the radius of gyration (via Guinier approximation) was found to be in line with values previously reported in literature.

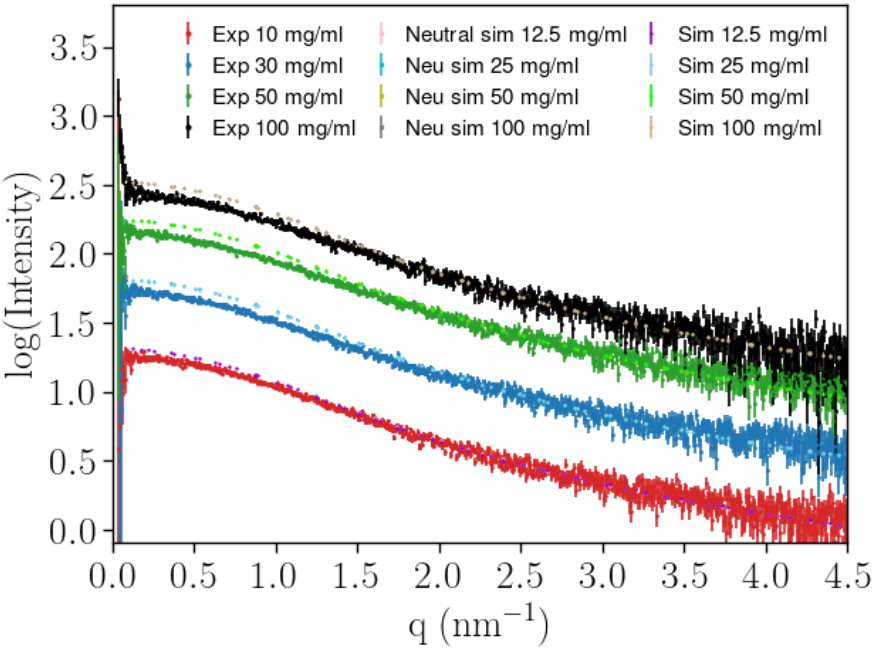
Considering the PEG-data, the scattering density difference between sample and solvent is in the range 1.355-1.372 $*10^{10}$ cm^{-2} obtained from MULCh [1], which is comparably small (corresponding value for Histatin5 is 3.253 $*10^{10}$ cm^{-2} , and it should be remebered that the scattering intensity is related to the square in scattering length density difference), why the lower-than expected I(0) can be attributed to the low scattering of PEG. Despite the potential issues of dimerization/aggregation and scattering contrast, a comparative analysis can still be performed with the samples containing Histatin 5. There are two different assumptions that can be made:

1. The crowder is not affected by the addition of Histatin 5, whereas Histatin 5 is.
2. Histatin 5 is not affected by the crowder, but the crowder is affected by Histatin 5

When using these assumptions, one can subtract either the pure crowder or the pure Histatin 5 data from the solution data, and acquire the spectra for the specie of interest, with the effect of crowder/addition of Histatin 5 incorporated. It should be noted that it can be the case that neither of these assumptions hold, i.e. that the crowder is affected by Histatin 5 and Histatin 5 is affected by the crowder, simultaneously. An example of this procedure is found in Figure 1, where the spectrum of the mix Histatin5+Lysozyme was subtracted with the pure Histatin 5 spectrum, with the result being compared with the corresponding "pure" Lysozyme spectrum. Note that all spectra (including "pure crowder") in Figure 1 has been scaled to the high-q region, with the extracted 10 mg/ml spectrum used as reference.



It is observed that all spectra, which were "extracted" from the Histatin 5 + Lysozyme mixed spectra are shifted to be at higher intensity, indicating an attractive effect from the addition of Histatin 5. The data acquired here will be compared with simulations. Preliminary simulations performed use a coarse-grained treatment of Histatin 5 (as found in [2]) and treating PEG 2K as a spherical particle have been performed. By using the first approximation of the crowder not being affected, but only Histatin 5, Histatin 5 signal was extracted from the PEG 2K+Histatin 5 measurement and compared with the simulation, see results in Figure 2.



In these simulations, there is no clear difference between treating Histatin 5 as a neutral chain or a charged-bead chain. The crowding by PEG 2K seems to have a small impact on Histatin 5, though a minor difference from simulations are present at higher crowder concentrations, which does not seem to be dependent on increased crowding *per se*. This may be related to the fact that the experimental data do not vary much with crowder concentration, apart from a difference at the lowest crowder concentration of 10 mg/ml. The simulation seemingly mirror this behaviour, except from the difference at 10 mg/ml crowder concentration. It is therefore tempting to suggest that the 10 mg/ml experiment measurement is, to minor extent, an outlier in this case.

Future work will further compare the experimental data to simulations, which will evaluate the impact of the aggregation. Some samples may require re-measurement, after more careful preparation to assure samples do not aggregate due to other means.

References

[1] A.E. Whitten, S. Cai and J. Trewhella. "MULCh: modules for the analysis of small-angle neutron contrast variation data from biomolecular assemblies" (2008) *Journal of Applied Crystallography* Vol. 41, No. 1, p. 222-226

[2] C. Cragnell, D. Durand, B. Cabane and M. Skepö. "Coarse-grained modeling of the intrinsically disordered protein Histatin 5 in solution: Monte Carlo simulations in combination with SAXS" (2016) *Proteins* Vol. 84, p. 777-791