# Report on SAXS measurements of Histatin 5 at BM29 (MX-2035)

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## Introduction

Intrinsically disordered proteins (IDPs) are well-known for possessing no tertiary structure, as well as little or no secondary structure. Nevertheless, temperature-induced structural changes are expected, although IDPs do not necessarily change in the same way as globular proteins do. Because of their large conformational ensemble, IDPs are difficult to study experimentally, and while it is possible to obtain average structures from e.g. small-angle X-ray scattering (SAXS) and nuclear magnetic resonance (NMR) experiments, complementary simulations are often needed to obtain a holistic understanding of the conformational properties of IDPs. Unfortunately, simulations are far from perfect and are often parameterized for specific purposes, and are thus not suitable for more general usage. For example, molecular dynamics (MD) force fields used for simulations of globular proteins have been shown to sample too compact conformations when used for simulating IDPs. Similarly, investigations performed by our group has shown that different simulation methods give different trends when performing simulations of the same IDP, Histatin 5 (Hst5), at different temperatures.

Hst5 is a short cationic intrinsically disordered saliva protein. In the saliva, Hst5 is

known for its candidacidal properties that are useful for protecting against yeast infections (thrush) in the mouth. Additionally, Hst5 is known to behave as a typical polyampholytic IDP at room temperature, and is thus often used as a model IDP in both experiments and simulations.

Because of the inconsistencies observed when attempting to simulate Hst5 at different temperatures, we have performed SAXS experiments, in conjunction with NMR and circular dichroism (CD) experiments, to establish the true conformational properties of Hst5 at different temperature. The results were used to compare the accuracy and validity of modern simulation methods used for simulating IDPs, and resulted in our paper "Temperature Dependence of Intrinsically Disordered Proteins in Simulations: What are We Missing?", *Journal of Chemical Theory and Computation*, 2019.[1]

# Results and discussion

SAXS measurements of Hst5 were performed at beamline BM29 in February 2018. The measurements were performed at pH 7 and an ionic strength of 140 mM. Four different temperatures were selected for the measurements: 10 °C, 20 °C, 37 °C, and 50 °C. The resulting SAXS curves, compared to the corresponding results from four different simulation methods, are depicted in Figure 1. Visual inspection of the form factors (top row in Figure 1) showed that the simulations were most accurate at 20 °C, but did not fit very well at any other temperature. The observed plateau in the Kratky plots (bottom row in Figure 1) indicated that Hst5 possesses a high degree of flexibility, which did not seem to change significantly with the temperature. Further analysis of the data in the Kratky plots showed even larger discrepancies between experiments and simulations in the high $qR_g$ region at temperatures other than 20 °C. The significance of these discrepancies were however unclear because of the high sensitivity of the Kratky plot in this region. All SAXS results at 20 °C were in agreement with previous studies.[2,3]

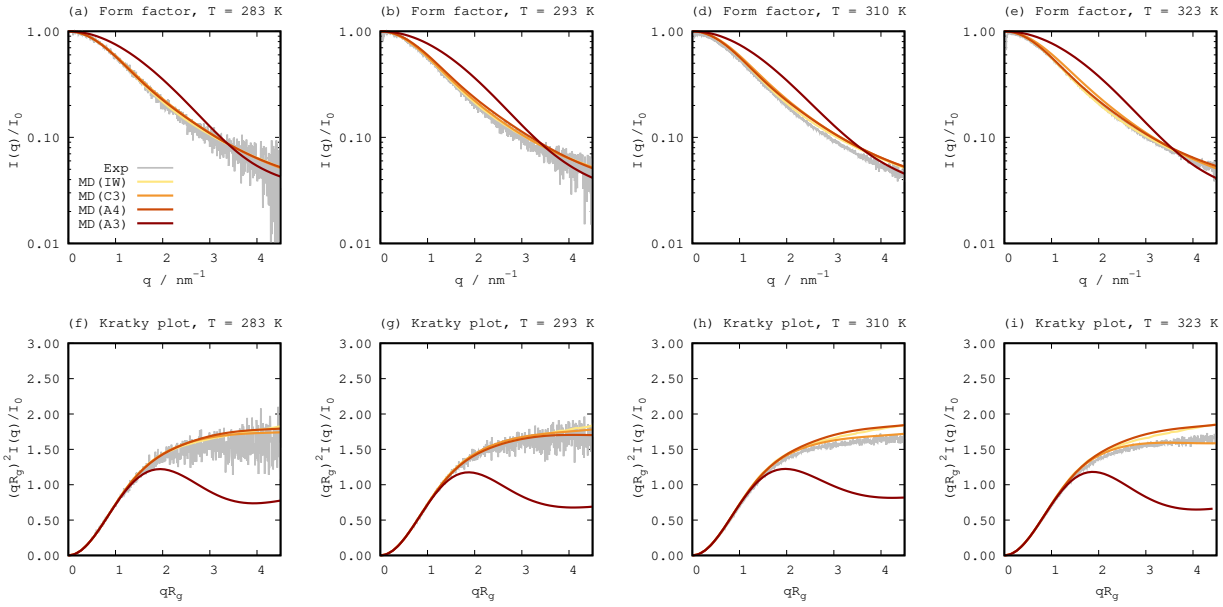


Figure 1: Form factors (a-e) and normalized Kratky plots (f-i) of Hst5 from SAXS experiments (gray) compared to the corresponding curves from simulations at four different temperatures.

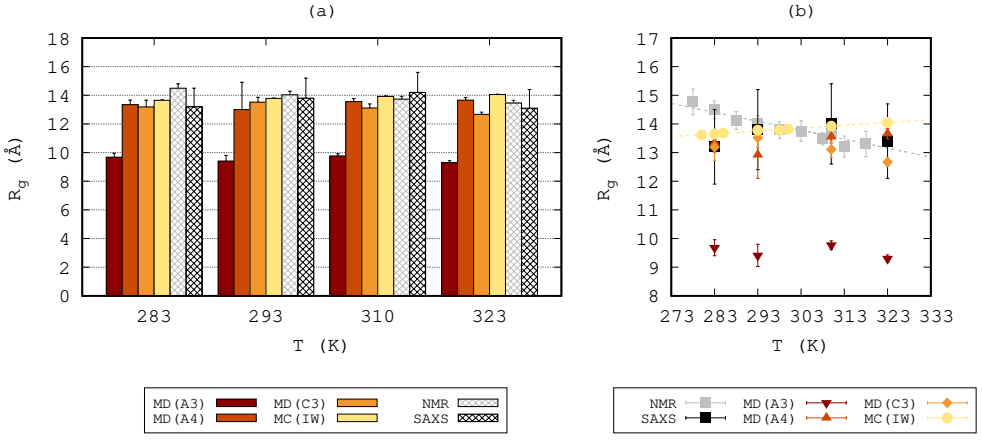


Figure 2: The radius of gyration as a function of the temperature for Hst5 from six different methods. (a) Histograms to emphasize differences between the methods. (b) Trends in radius of gyration with temperature for the six methods.

The radius of gyration of Hst5 as a function of temperature was obtained from SAXS, NMR, and simulations, and is shown in Figure 2. To obtain the radius of gyration from the SAXS measurements, the curves were analyzed using PRIMUS and the Guinier approximation with $qR_g(\max) < 0.8$. As seen in the figure, the SAXS results were inconclusive, but the NMR results suggested a slight collapse of the conformational average with increasing temperature. Although all methods (save `MD(A3)`) provided similar values of the radius of gyration at 20 °C, they did not agree at any other temperature. In the end, the entire study concluded that the currently available simulation methods used for simulating IDPs are *not* suitable for studying temperature effects in IDPs.

# References


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