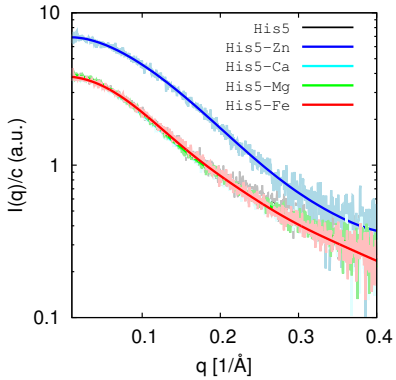
Structural and thermodynamical properties of the saliva protein Histatin 5 - SAXS measurements at BM29.

Carolina Cragnell,* Dominique Durand,# Marie Skepö*
*Chemical department, Theoretical Chemistry, Lund University, Lund, Sweden
#I2BC, Université Paris-Sud, ORSAY Cédex, France

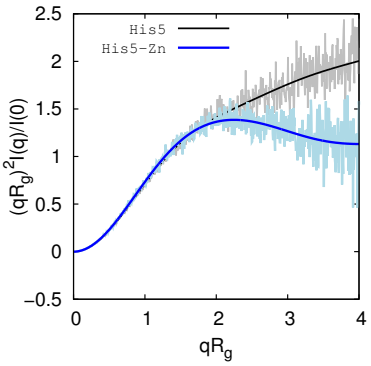
The aim of this project is two-fold: (i) to study structural and thermodynamic properties of the salivary protein Histatin 5 to provide a deeper understanding of its antifungal mechanism, and (ii) to further develop a coarse-grained model for intrinsically disordered proteins (IDPS). This study is a part of a larger project where the goal is to study intrinsically disordered proteins, and relate their structure and function in solution with the adsorbed state. For this purpose a combined theoretical and experimental approach is used where atomistic molecular dynamics simulations and coarse-grained-modeling and Monte Carlo simulations are employed to analyze the experimental results[1, 2, 3].

Within the histatin family, Histatin 5 is the most potent with respect to its antifungal activity. The mechanism behind the Histatin 5 antifungal action is debated. However, there is evidence in the literature that the target of Histatin 5 is intracellular and that conformational changes may be important. Histatin 5 metal binding abilities have been suggested to be important for antifungal mechanism, and studies have established that various transitional metals, such as Zn^{2+}, Ni^{2+}, Cu^{2+}, and Fe^{3+}, are intrinsically present in the saliva. Histatin 5 binds Zn^{2+} and Cu^{2+} and possesses definitive metal binding motifs for Cu^{2+} and Ni^{2+} as well as for Zn^{2+}. It has been shown that Cu^{2+}, Zn^{2+}, or Ni^{2+} do not induce stabilization of the α-helical structure of Histatin 5, nevertheless, there might be changes in conformational properties that are important for its antifungal properties and transport through the cell membrane. Determination of the average conformations and association state of Histatin 5 in presence of multivalent ions is therefore of interest. It is suggested in the literature that Histatin 5 binds up to ten equivalents of Fe and four of Zn^{2+}[4] leading to a total net charge increase of 30 and 8 e, respectively. In addition, there is a strong correlation between peptide cationicity and antimicrobial activity.

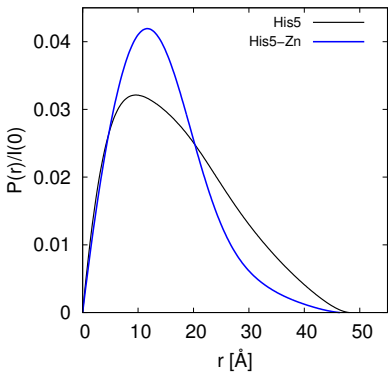
In April 2016 we performed SAXS-mesurements at BM29 for Histatin 5 in solutions with varying protein concentration, salt concentration, and salt valency. Figure 1 shows the scattering intensity, Kratky representation and P(r) with Mg^{2+}, Fe^{3+}, $Ca,^{2+}$ and Zn^{2+} present, pH = 7, ionic strength = 150 mM. The results are very promising and clearly show that Histatin 5 associates in presence of Zn^{2+}. This was not observed with neither



(a)



(b)



(c)

Figure 1: (a) Scattering curves obtained by SAXS 1 mg/mL Histatin 5 at pH = 7 with ionic strength of 150 mM set by NaCl, with various multivalent ions. (b) Kratky representation. (c) Pair distance distribution function obtained from the scattering curves of Histatin 5 and in presence of Zn^{2+} respectively.

Mg^{2+}, Ca^{2+}, nor Fe^{3+}. From the SAXS measurements we further conclude that the association of Histatin 5 in presence of Zn^{2+}, is dependent on the protein concentration, Figure 2. The oligomerisation of Histatin 5 could be of importance for its antifungal

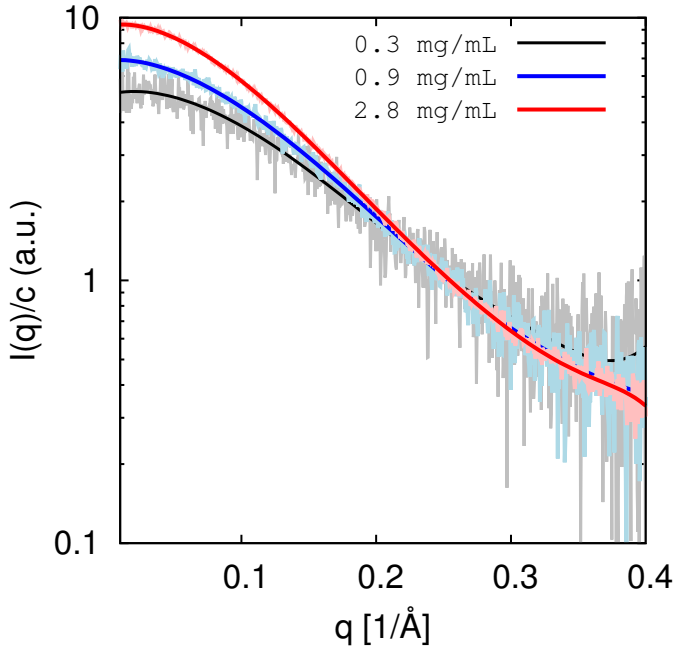


Figure 2: Scattering curves obatained by SAXS with altering Histatin 5 concentration in presence of Zn^{2+}.

properties.

In order to achieve a molecular understanding and a physico-chemical insight of the obtained SAXS results, a model for Monte Carlos simulations has previously been developed[1]. Figure 3 shows the scattering intensity calculated from simulations, utilizing the developed model, for monomers and dimers with Zn^{2+} bound. For comparison the scattering curves for Histatin 5 with and without Zn are also present. This is preliminary results and both coarse-grained and atomistic simulations are on-going and a manuscript for publication is under preparation.

References

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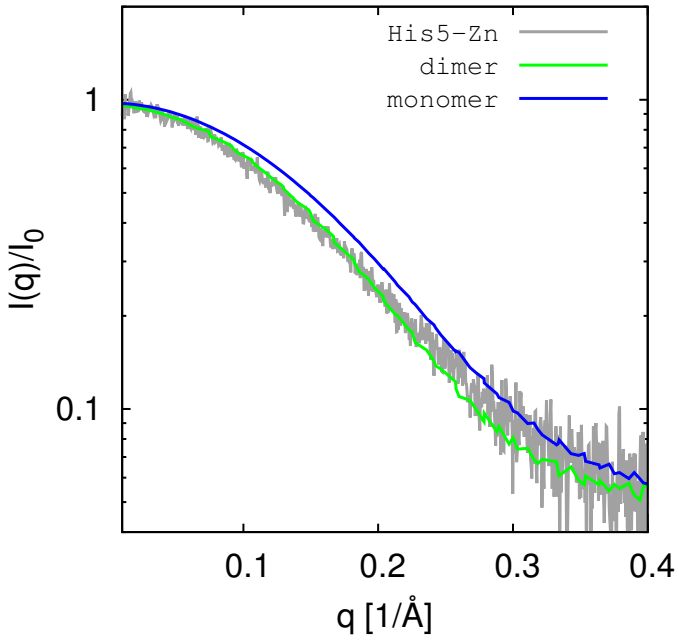

Figure 3: Scattering curves obatained by SAXS Histatin 5 and in presence of Zn^{2+} as well as calculated scattering curves obtained from simulations for monomers and dimers respectively, with Zn^{2+} bound.

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