Self-Assembly of Intrinsically Disordered Proteins. The influence of intermolecular and electrostatic Interactions - SAXS measurements at BM29.

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The aim of this project is twofold: (i) to study the self-assembly of intrinsically disordered proteins (IDPs), and how it is determined by intermolecular, and more specifically, electrostatic interactions, and (ii) to develop a coarse-grained model for IDPs which can be used for analysing complex liquids as saliva and milk. This study is a part of a larger project where the goal is to study IDPs, 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 modelling and Monte Carlo simulations are employed to analyse the experimental results[2, 4, 5].

The bovine milk-protein, β-casein, which belongs to this category[3, 8], has been used as model protein. The primary structure of β-casein is proline-rich, and the protein consists of 209 amino acid residues, including five phosphorylated serines. The molecular mass is approx. 24 kD, and the isoelectric point 5.2. β-casein has an amphiphilic character[7] and exhibits a tendency to associate at a critical concentration of 0.5 mg/ml in aqueous solution.

Throughout the years the self-assembly of β-casein has attracted a lot of attention, and a number of very interesting and solid articles have been published. Many of those studies have been devoted to the structure of the micelle, as the size and the number of proteins, as well as how the shapes are affected by the physico-chemical properties of the solution. In the literature there are reported association numbers between 20 and 70[6] depending on the physico-chemical properties of the solution and the methodology used. Generally it is assumed that β-casein forms a monodisperse solution of micelles.

In these experiments we have measured/determined the association number, the size, and the shape of the self-assemblies as a function of protein concentration. HPLC SAXS and asymmetrical flow field-flow fractionation (AF4) has been utilized in order to determine the distribution of association numbers and sizes of the assemblies.

In January 2015 we performed HPLC SAXS and ordinary SAXS measurements at BM29.

Figure 1 shows the scattering intensities of β-casein at pH=8.5 and ionic strength of 60

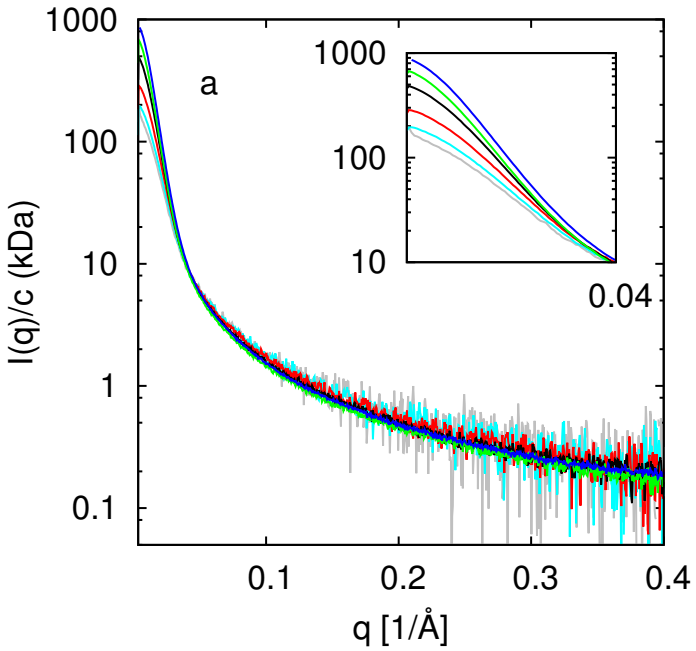

Figure 1: Scattering functions from SAXS, normalized by concentration, of β-casein samples with varying concentration.

mM for varying protein concentrations. At low q it is clearly shown that a concentration trend is detected, and that I(0) is increasing with increasing concentration. For all studied protein concentrations, the scattering functions from SAXS at low q indicate that ellipsoidal assemblies are formed, inline with previous reported shape. Figure 2 shows the corresponding data converted to association numbers (see blue squares) as well as association numbers obtained from osmometry (red circles). In order to further investigate the apparent distribution of assemblies, HPLC and AF4 (data not shown), were employed. Figure 3 shows the radius of gyration and the distribution of *I(0)* present in the solution. Our results from the SAXS, Osmometry, and AF4 confirm that, indeed, β-casein forms a polydisperse distribution of micelles, ranging over almost two orders of magnitude, and more importantly, that the smaller micelles are considerably more numerous in amount.
The results were published in *Food Hydrocolloids* in March 2017[1].

References

[1] C. Cragnell, J. Choi, M. Segad, S. Lee, L. Nilsson, and M. Skepö. Bovine -casein has a polydisperse distribution of equilibrium micelles. *Food Hydrocolloids*, 70:65–68, 9 2017.

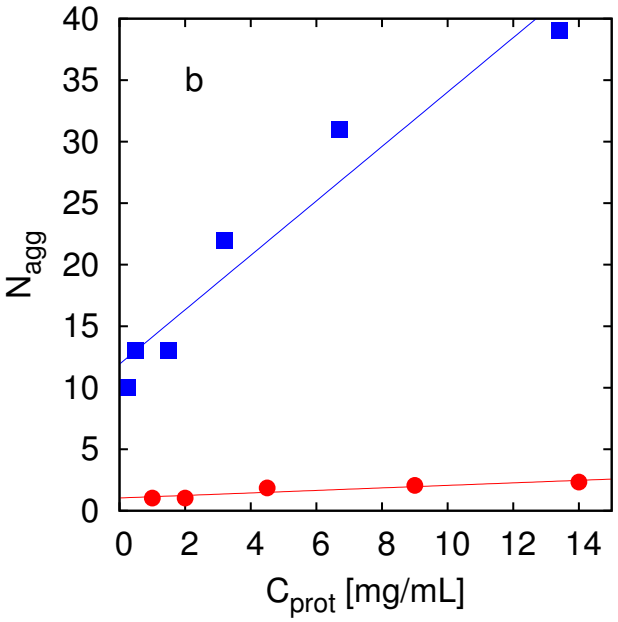

Figure 2: Association numbers obtained from SAXS (blue squares) as well as from the osmotic pressures (red circles) measurements, as a function of β-casein concentration.

[2] C. Cragnell, D. Durand, B. Cabane, and M. Skepö. Coarse-grained modelling of the intrinsically disordered protein Histatin 5 in solution. Monte Carlo simulations in combination with SAXS. *Proteins: Structure, Function, and Bioinformatics*, 2016.

[3] A. K. Dunker, J. D. Lawson, C. J. Brown, R. M. Williams, P. Romero, J. S. Oh, C. J. Oldfield, A. M. Campen, C. M. Ratliff, K. W. Hipps, J. Ausio, M. S. Nissen, R. Reeves, C. Kang, C. R. Kissinger, R. W. Bailey, M. D. Griswold, W. Chiu, E. C. Garner, and Z. Obradovic. Intrinsically disordered protein. *J Mol Graph Model*, 19(1):26–59, 2001.

[4] C. H. Evers, T. Andersson, M. Lund, and M. Skepö. Adsorption of unstructured protein β-casein to hydrophobic and charged surfaces. *Langmuir*, 28(32):11843–11849, 2012. PMID: 22783871.

[5] J. Henriques, C. Cragnell, and M. Skepö. Molecular dynamics simulations of intrinsically disordered proteins: force field evaluation and comparison with experiment. *J. Chem. Theory Comput.*, 11(7):3420–3431, 2015.

[6] J. O'Connell, V. Y. Grinberg, and C. G. de Kruif. Association behavior of beta-casein. *J Colloid Interf Sci*, 258:33–39, 2003.

[7] H. E. Swaisgood. Chemistry of milk protein. *Developments in dairy chemistry*, 1:1–59, 1982.

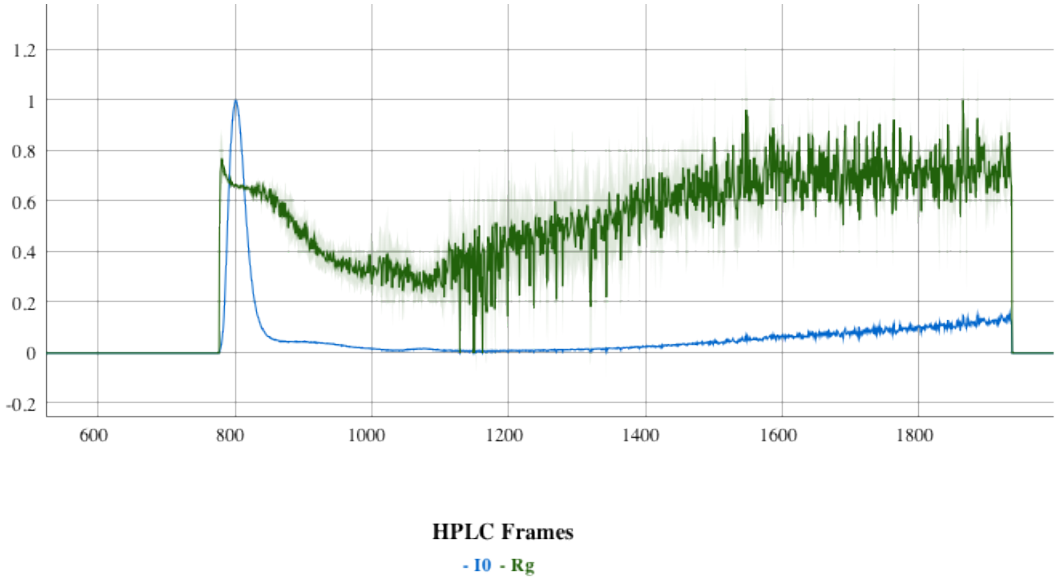

Figure 3: The distribution of radius of gyration and forward scattering obtained from HPLC SAXS.

[8] P. Tompa. Intrinsically unstructured proteins. *Trends Biochem Sci.*, 10(27):527–533, 2002.