Experimental report

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Proposal: 8-04-752 **Council:** 10/2014

Title: Denaturation process in protein solutions studied by inelastic fixed window scans

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: protein solutions of bovine serum albumin and NaCl

Instrument	Requested days	Allocated days	From	To
IN16B	5	3	27/06/2015	30/06/2015

Abstract:

Denaturation in protein solutions combines (partial) unfolding and subsequent aggregation of proteins and depends strongly on the solution conditions. We aim for a systematic investigation of hierarchical dynamics during the thermal denaturation process of bovine serum albumin (BSA) using inelastic fixed window scans at IN16B. The experiment will elucidate the process of unfolding and cross-linking during thermal denaturation. Using different protein and salt concentrations, the interplay between steric and Coulomb repulsion and emerging attractions during denaturation will be explored. Importantly, neutron backscattering at IN16B extends the picture of hierarchical dynamics during denaturation down to the otherwise unaccessible nanoscale. Furthermore, the experiment will present a first showcase for protein condensation under physiological conditions, which opens the field towards a more detailed characterization of nanoscopic dynamical changes during self-assembly processes.

Experimental Report — Exp. 8-04-752

Denaturation process in protein solutions studied by inelastic fixed window scans

Scientific aim and context

Denaturation in protein solutions combines (partial) unfolding and subsequent aggregation of proteins and depends strongly on the solution conditions. The experiment aims for a systematic investigation of hierarchical dynamics during the thermal denaturation process of bovine serum albumin (BSA) using inelastic fixed window scans at IN16B to elucidate the process of unfolding and cross-linking during thermal denaturation. The experiment presents a showcase for protein condensation under quasi-physiological conditions, which opens the field towards a more detailed characterization of nanoscopic dynamical changes during self-assembly processes.

Description of experiment

We have collected elastic and inelastic fixed window scans ($\omega = 0, 1.3, 3, 6, 10\,\mu$eV) for three protein concentrations (150, 250, 500 mg/ml bovine serum albumin (BSA)) and two salt concentrations (0 and 150 mM NaCl) on temperature ramps heating from 295 to 370 K, and fast cooling back to 295 K.

The protein solutions were prepared in D_2O and filled in double-walled cylindrical aluminum sample holders with an outer diameter 23mm and a gap between the two walls of 0.25mm (capacity of about 1.2 ml). The containers where sealed hermetically with indium wire to avoid evaporation in the cryofurnace.

As calibration samples, vanadium, D_2O and empty cell have been measured.

Data analysis

- From the elastic scans, the apparent mean-squared displacement $\langle u^2 \rangle$ has been determined (see Fig. 1).
- We used the inelastic fixed window scan with energy transfer $\omega = 1.3\,\mu$eV to calculate the generalized mean-squared displacement $\langle u^2 \rangle_\omega$, which had been suggested as a model-free indicator for anomalous diffusion.[1]
- The analysis of the full set of elastic and inelastic fixed window scans by using a model of $S(q, \omega)$ is under work.

First results and outlook

The apparent mean-squared displacements $\langle u^2 \rangle$ are shown in Fig. 1. The results are consistent with a previous scan at IN16 on the same experimental conditions (BSA 500 mg/ml) [2], proving the robustness of the approach. We emphasize that $\langle u^2 \rangle$ include contributions of the diffusion of the entire protein molecule and internal motions [2]. Below the denaturation temperature, internal motions and diffusion are stronger and faster, respectively, resulting in an increase of $\langle u^2 \rangle$. Upon denaturation, the proteins unfold and cross-link, thereby suppressing the contribution of diffusion.

From a comparison of the three protein concentrations and salt conditions, trends can be observed: First, the addition of salt seems to enhance the effect of denaturation on the overall dynamics. This can maybe be understood by a varying network structure of the cross-linked molecules due to

electrostatics. Second, crowding effects due to varying protein concentrations seem to have a drastic effect on the absolute value of $\langle u^2 \rangle$. Consistently with intuitive expectations, motions are faster in the less dense solutions.

At this point, it has to be noted that the approach presented here has to be validated further for different reasons: in contrast to the usual fixed window scans on hydrated protein powders, samples in solutions cannot be cooled down to obtain normalization. Thus, we have to critically evaluate effects of the normalization procedure on our results. To this end, a combined analysis of mean-squared displacement and the full set of elastic and inelastic scans might proove helpful.

Furthermore, the hypothesis of differing network structure of the cross-linked, denatured state will be investigated using small-angle scattering on similar samples and temperature protocols.

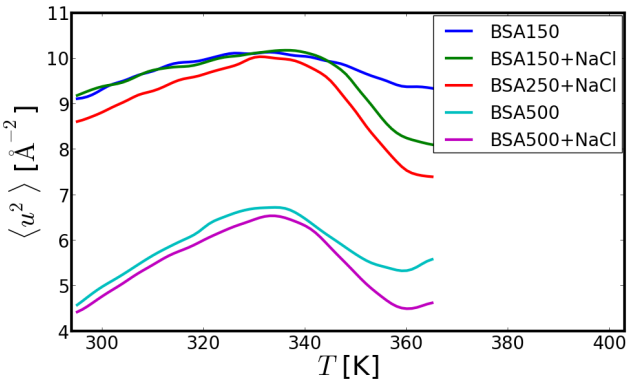

Figure 1: Apparent mean-squared displacement from elastic fixed window scans of BSA solutions (150, 250 and 500 mg/ml) with and without NaCl (150 mM), proving the applicability of fixed window scans to monitor dynamics during thermal denaturation in protein solutions.

References

[1] Felix Roosen-Runge and Tilo Seydel. A generalized mean-squared displacement from inelastic fixed window scans of incoherent neutron scattering as a model-free indicator of anomalous diffusion confinement. *EPJ Web of Conferences*, 83:02015, 2015.

[2] Marcus Hennig, Felix Roosen-Runge, Fajun Zhang, Stefan Zorn, Maximilian W. A. Skoda, Robert M. J. Jacobs, Tilo Seydel, and Frank Schreiber. Dynamics of highly concentrated protein solutions around the denaturing transition. *Soft Matter*, 8:1628–1633, 2012.