

Supplementary Material: Dynamics of Highly Concentrated Protein Solutions around the Denaturing Transition

Marcus Hennig,^{a,b} Felix Roosen-Runge,^b Fajun Zhang,^b Stefan Zorn,^b Maximilian W.A. Skoda,^c Robert M.J. Jacobs,^d Tilo Seydel,^{*a} and Frank Schreiber^b

First published on the web Xth XXXXXXXXXXXX 200X

Here we provide supplementary material for the paper by Hennig *et al.*¹. We briefly discuss the temperature dependence of $\langle u^2 \rangle$ upon cooling of a denatured protein solution and provide a table of the apparent diffusion coefficient used to determine the mean-squared displacement of the internal dynamics $\langle u_{\text{vib}}^2 \rangle + \langle u_{\text{sub}}^2 \rangle$.

1 Cooling of a denatured protein solution

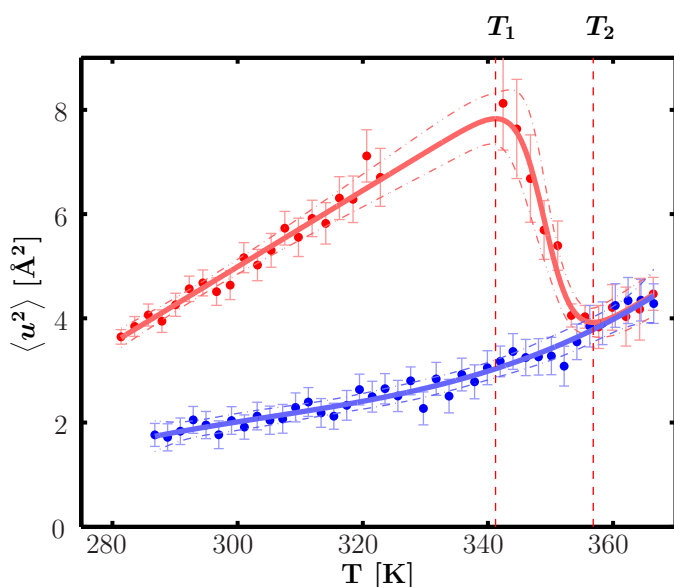


Fig. 1 Total mean-squared displacement $\langle u^2 \rangle$ for a 500 mg/ml BSA aqueous solution with 1 M NaCl upon heating from 280 to 370 K with $8.4 \cdot 10^{-2}$ K/min (red solid line) and cooling back to 280 K with $12.4 \cdot 10^{-2}$ K/min (blue solid line). The dashed dotted lines denote the 95% confidence intervals of the corresponding fits. The transition regime is denoted by the vertical dashed lines. Note that for the heating process between 324 and 342 K no data was recorded due to an unexpected neutron beam stop during the temperature ramp.

Fig. 1 shows $\langle u^2 \rangle$ for a 500 mg/ml BSA aqueous solution with 1 M NaCl for two different processes: heating from 280 to 370 K (red solid line) and cooling from 370 back to 280 K (blue solid line). During the heating process we encountered an unexpected loss of the neutron beam between the temperatures 324 and 342 K, hence no data was recorded. Upon heating $\langle u^2 \rangle$ displays the behavior described in the main paper. Cooling reveals the irreversibility of the denaturing process,

since for $T < T_2$ the curve progression of $\langle u^2 \rangle$ is evidently different from the heating curve. Note that at T_1 there is a weak kink indicating a change in the flexibility of the cross-linked network. For cooling we observe a significantly lower $\langle u^2 \rangle$ which we attribute to the hindered global motion of the proteins due to entanglement and cross-linking. We assume that upon cooling the cross-linked network remains and that the denatured proteins are not ‘disentangled’.

2 Temperature Dependent Apparent Diffusion Coefficient

| T[K] | D [Å²/ns] | ΔD [Å²/ns] |
|------|-------------|--------------------|
| 279 | 0.47 | ± 0.26 |
| 290 | 0.76 | ± 0.01 |
| 300 | 1.23 | ± 0.27 |
| 310 | 1.47 | ± 0.39 |
| 316 | 1.41 | ± 0.33 |
| 319 | 1.81 | ± 0.25 |

Table 1 Temperature dependence of the apparent diffusion coefficient of BSA in 500 mg/ml aqueous (D_2O) solution derived from QENS data recorded at IN10 and IN16.

References

- 1 M. Hennig, F. Roosen-Runge, F. Zhang, S. Zorn, M. Skoda, R. Jacobs and F. Schreiber, *Soft Matter*, 2011, submitted.

^a Institut Laue-Langevin, 6 Rue Jules Horowitz B.P. 156, F-38042 Grenoble, France. Tel: +33 476 20 75 79; E-mail: seydel@ill.eu

^b Universität Tübingen, Institut für Angewandte Physik, Auf der Morgenstelle 10, D-72076 Tübingen, Germany

^c ISIS, Rutherford Appleton Laboratory, Chilton, Didcot OX11 0QX, UK

^d Chemistry Research Laboratory, University of Oxford, Oxford OX1 3QZ, UK