Experimental Report

Proposal: **9-13-477** **Council:** 10/2012

Title: Protein self-diffusion as a function of charge-screening in Ovalbuminsolutions

This proposal is continuation of: 9-13-437

Researh Area: Soft condensed matter

Main proposer: **GLENISSON Vincent**

Experimental Team: ROOSEN-RUNGE Felix
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Local Contact: SEYDEL Tilo

Samples: Ovalbumin protein from the chicken egg white, D2O, YCl3 (up to 0.1 M)

Instrument	Req. Days	All. Days	From	To
IN16B	10	7	05/07/2013	12/07/2013

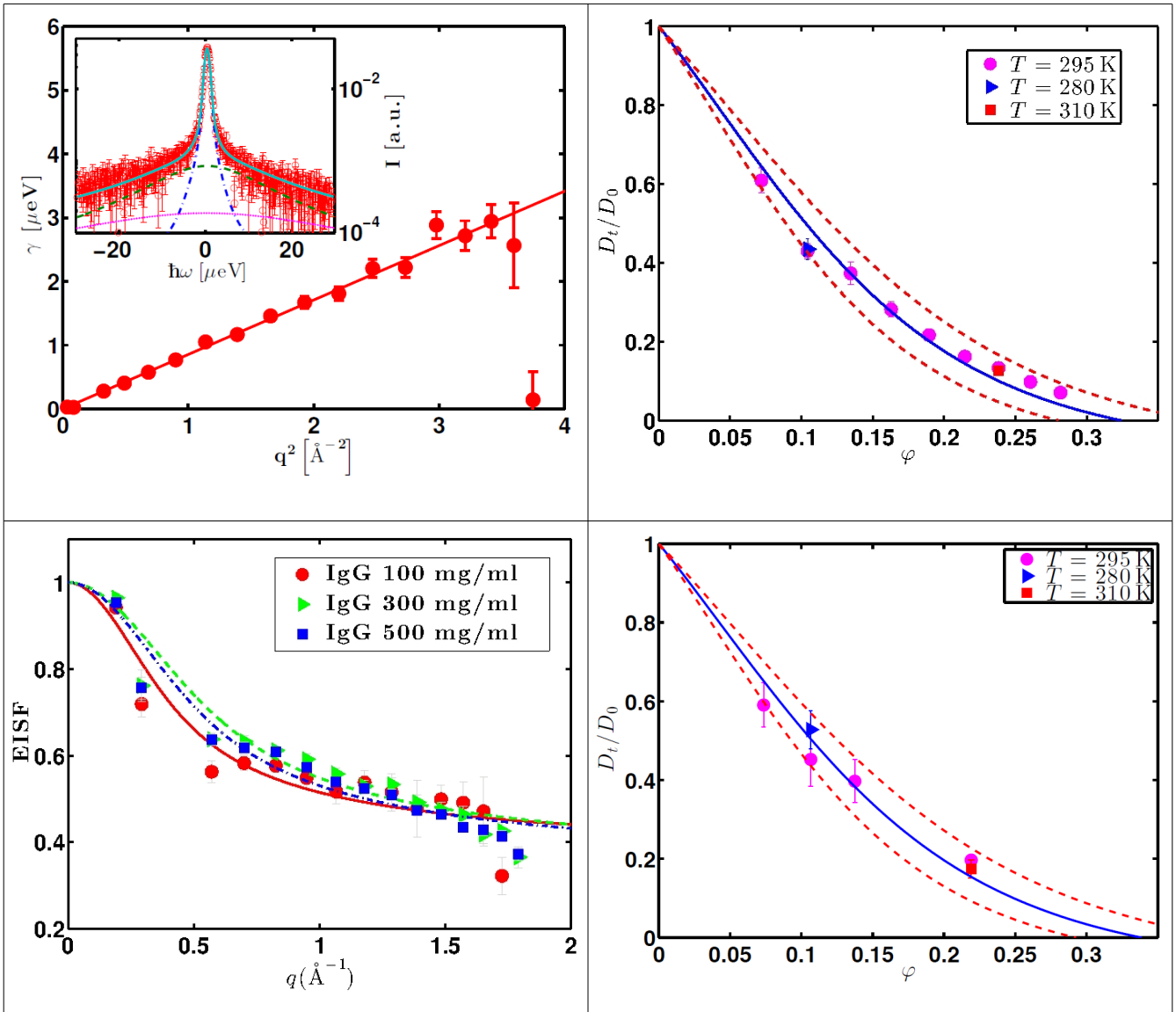
Abstract:

Using neutron backscattering spectroscopy and complementary NSE, SAXS, and DLS experiments, we have very systematically explored the model protein Bovine Serum Albumin (BSA) in aqueous solutions. From these studies, we have derived the necessary experimental and analytical frameworks to obtain the translational diffusion of the dissolved proteins [F.Roosen-Runge et al., PNAS 108, 11815 (2011); M.Hennig et al., Soft Matter 8, 1628 (2012); M. Heinen et al., Soft Matter 8, 1404 (2012)]. We have also developed models for the influence of charge screening on the diffusion in BSA protein solutions [M. Hennig, PhD thesis, University of Tuebingen 2011]. These models point towards possible transient or dynamic clusters at suitable trivalent salt concentrations in the protein solutions. However, to fully develop and test our models, we have to carry out a comparative study on a different protein model system. We therefore propose to extend our study of the dependence of the diffusion on the ionic strength of the solutions to the new protein Ovalbumin (OVA). OVA differs in shape, size, and charge pattern from BSA. These differences can be expected to influence cluster formation.

Report on experiment 9-13-477: Protein short-time self-diffusion in aqueous (D_2O) solutions
Experiment team: Marco Grimaldo, Felix Roosen-Runge, Tilo Seydel
Co-Proposers: Fajun Zhang, Frank Schreiber (University of Tübingen)

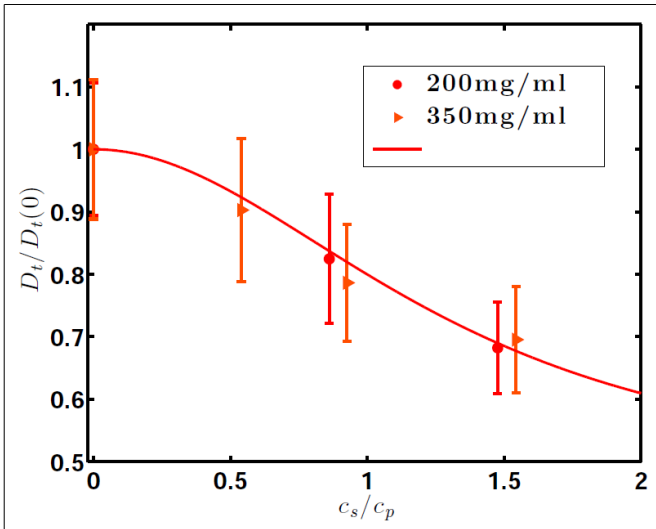
We report on one of the first IN16B user experiments. This experiment has originally been accepted for IN16. Due to the context of the commissioning of IN16B, some samples previously measured on IN16 where measured again on IN16B. In addition, the originally proposed program could be extended due to the very high flux of IN16B.

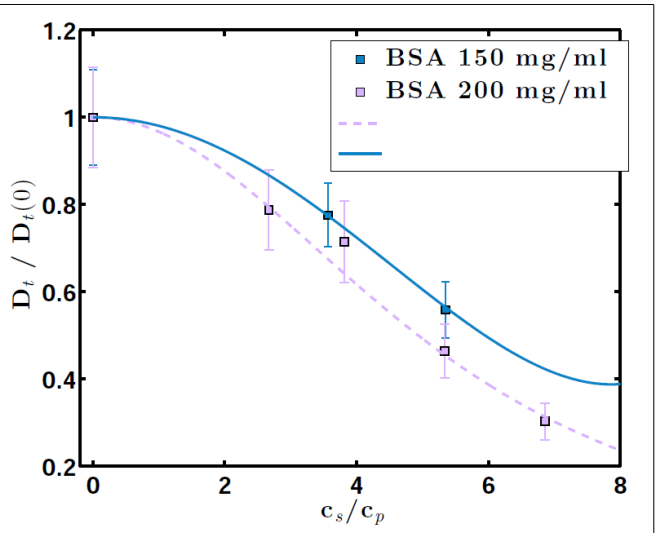
Here we show results on the proteins *bovine serum albumin* (BSA), chicken egg-white *ovalbumin* (OVA) and bovine *gamma-globulin* (IgG) in aqueous (D_2O) solutions.



Panel 1: Left column: Top: Example spectrum (insert, symbols) and fit components (lines) recorded on IgG (300mg/ml) at T=295K and Q=0.81/Å, and line width γ accounting for the global diffusion of the proteins with fit to $\gamma=Dq^2$. Bottom: Elastic incoherent structure factor of the internal modes of IgG for different concentrations.

Right column: Top: Translational diffusion D_t of IgG over the dilute limit diffusion D_0 versus the protein volume fraction φ (symbols) and comparison with the colloid model for hard spheres (solid line). Bottom: Equivalent plot for OVA (symbols) and guide to the eye (solid line). The dashed lines denote confidence intervals.





Panel 2: Left: Translational diffusion $D_t(c_s/c_p)$ of OVA proteins in D_2O normalized by the translational diffusion at zero salt concentration $D_t(0)$ as a function of the YCl_3 salt concentration c_s normalized by the protein concentration c_p for two different protein concentrations (symbols). The lines is a guide to the eye.

Right: Equivalent plot as in the left column for BSA proteins D_2O (symbols). The lines are guides to the eye.

We have recorded very high quality data on all protein solutions (panels 1 and 2). We have addressed both the global diffusion and the internal modes of the different proteins as a function of volume fraction (panel 1) as well as the global diffusion as a function of the concentration of added salt YCl_3 (panel 2). We have been able to extract the internal modes self-consistently by letting the Lorentzian linewidths accounting for the global and internal modes free in the fits. With the exploration of new protein systems we confirm that our analytical framework previously established for BSA [1-3] can be successfully applied to other proteins. This result has not been obvious beforehand, because the new proteins OVA and IgG differ considerably in shape and size from BSA. With the salt-series (panel 2) we test models addressing dynamic clusters and density fluctuations in protein solutions, and for these systems we also carry out complementary light scattering experiments in the PSCM soft-matter lab [4].

We are presently drafting the first manuscript on IgG, and more are to follow based the the results from this experiment. This work is part of the PhD project of Marco Grimaldo (ILL-Tübingen PhD student).

References:

[1] F. Roosen-Runge, M. Hennig, F. Zhang, R. M. J. Jacobs, M. Sztucki, H. Schober, T. Seydel and F. Schreiber, Proc.Natl. Acad. Sci. U. S. A., 2011, 108, 11815–11820.
[2] F. Roosen-Runge, M. Hennig, T. Seydel, F. Zhang, M. W. Skoda, S. Zorn, R. M. Jacobs, M. Maccarini, P. Fouquet and F. Schreiber, Biochim. Biophys. Acta, Proteins Proteomics, 2010, 1804, 68–75.
[3] M. Hennig, F. Roosen-Runge, F. Zhang, S. Zorn, M.W. A. Skoda, R.M. J. Jacobs, T. Seydel, F. Schreiber, Soft Matter, 2012, 8, 1628-1633.
[4] D. Soraruf, F. Roosen-Runge, M. Grimaldo, F. Zanini, R. Schweins, T. Seydel, F. Zhang, R. Roth, M. Oettel and F. Schreiber, Soft Matter, 2014, advance article DOI 10.1039/C3SM52447G .