Experimental report

03/02/2016

Proposal: 8-04-759 **Council:** 4/2015

Title: External crowding in protein solutions

Research area: Biology

This proposal is a continuation of 9-13-526

Main proposer: Marco GRIMALDO

Experimental team: Benedikt SOHMEN
Marco GRIMALDO
Christian BECK

Local contacts: Tilo SEYDEL

Samples: Bovine Gamma-Globulin (IgG), Bovine Serum Albumin (BSA), d-(poly)ethylene glycol, D2O

Instrument	Requested days	Allocated days	From	To
IN16B	3	3	26/10/2015	30/10/2015

Abstract:

The interior of living cells is occupied by macromolecules such as proteins, which occur at a high volume fraction on the order of 30% in the aqueous solution of the cellular fluid. The issue of macromolecular crowding is therefore of primordial importance for the function of living cells [R.J. Ellis, Curr. Opin. Struct. Biol. 2001, 11, 114]. To model the situation of crowding in vitro, we propose to investigate the diffusion in

(1) protein (BSA) - protein (IgG) - water (D2O) ternary mixtures, where the proteins BSA (bovine serum albumin) and IgG (immunoglobulin) are important constituents of blood;

(2) protein(IgG)-crowding agent (deuterated PEG) - water (D2O) ternary mixtures.

Using our previous large QENS data sets of BSA-water and IgG-water binary mixtures and consistent modeling, we expect to be able to separate the different contributions to the signal in the QENS spectra in the ternary mixture. Moreover, we have comprehensively characterized the involved samples using complementary techniques such as SAXS/SANS and SLS/DLS.

“External” crowding in protein solutions

experiment 8-04-759 on IN16B

experimentalists M.Grimaldo, B.Sohmen, C.Beck, F.Roosen-Runge, T.Seydel;
co-proposers M.Braun, S.daVela, F.Zhang, F.Schreiber;

This experiment served to complete a series of measurements on solutions of protein mixtures of bovine gamma-globulin (IgG) and bovine serum albumin (BSA) in D_2O, in continuation of the proposal 9-13-526. The resulting data were analyzed according to the methods described in the previous report on 9-13-526. The resulting apparent diffusion coefficients associated with the global center-of-mass diffusion of the proteins in the solutions are summarized in figure 1. In a second step, even more complex mixtures with a deuterated “crowder” were explored.

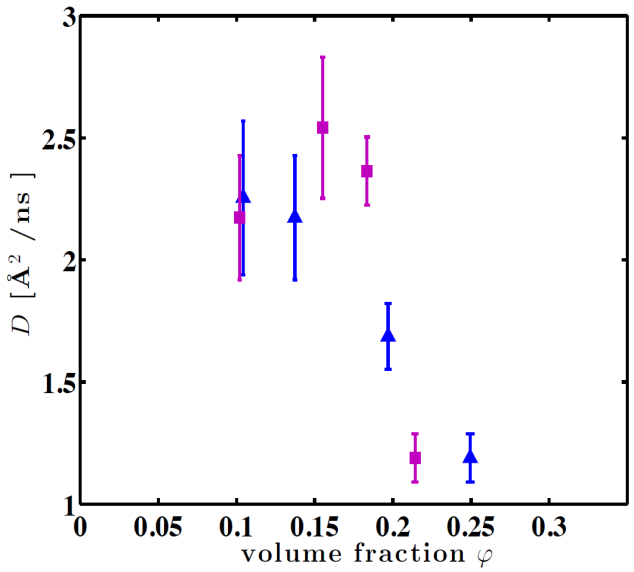

Figure 1: Summary of the apparent ensemble-averaged center-of-mass diffusion coefficients of the proteins in mixtures of BSA and IgG proteins in D_2O. The plot combines the results from the previous proposal 9-13-526 and from the present continuation proposal. The triangles mark solutions with a fixed IgG concentration of 100mg/ml and varying BSA concentration, and φ denotes the total volume fraction of BSA and IgG. The squares mark solutions with a constant IgG concentration of 47mg/ml. (T=295K)

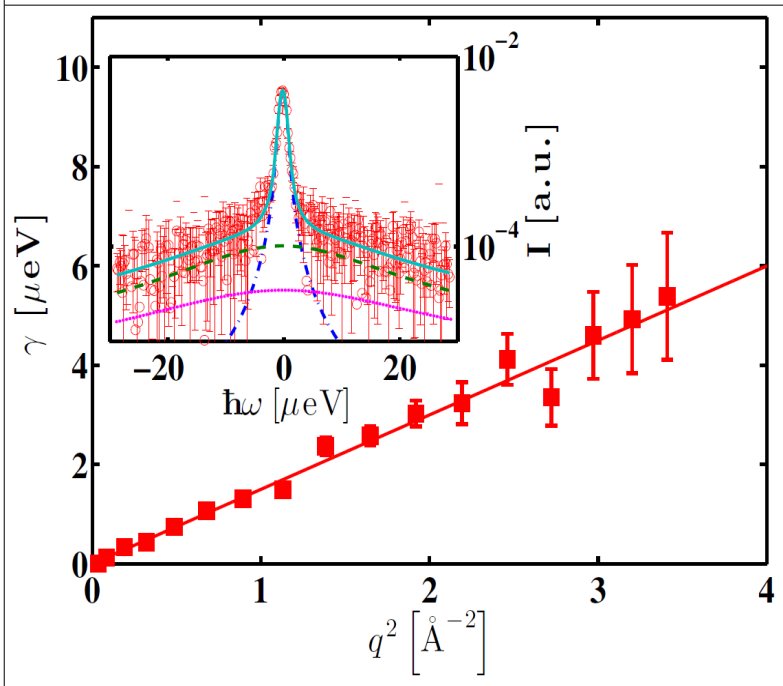

Figure 2: Inset: Example spectrum (symbols) at an IgG protein concentration of 67.7mg/ml and a deuterated lysate concentration of of 56mg/ml in D_2O, recorded at the scattering vector q=0.81Å^{-1} and temperature T=295K, subsequent to the subtraction of the scattering signal of the pure lysate, scaled to the effective volume. The solid line superimposed on the symbols denotes the fit consisting of one Lorentzian associated with the global diffusion of the IgG proteins (dash-dotted), one Lorentzian describing the internal diffusion of the proteins (dashed), and the D_2O contribution (dotted).
The main part of the figure denoted the linewidth of the Lorentzian associate with the global diffusion as a function of q^2.

In the second part of the experiment we have been able to test the effect of an external crowder constituted of fully deuterated cellular lysate provided by the ILL deuteration laboratory (figure 2). This lysate consists of macromolecules from the intracellular fluid of *E.coli* cells and therefore

represents a rather realistic model of a natural crowding agent close to the *in vivo* situation. We have analyzed these data using the methods established by our group [1-3]. Remarkably, we find that in the presence of this external crowding agent, the native, i.e. protonated, “tracer” IgG proteins display a Brownian center-of-mass diffusion (figure 2, main part) in the same way as in the case of self-crowding [1]. This opens up the possibility for systematic studies of the effect of a deuterated “natural” crowding agent, i.e. a crowding agent obtained from a living organism, as a function of both the tracer protein and the crowding agent concentration in D_2O. In this way, colloid models for the diffusion of the tracer proteins as well as the effects of the deuterated crowder on the internal diffusion may be tested [1,3].

References:

[1] M. Grimaldo, F. Roosen-Runge, F. Zhang, T. Seydel, and F. Schreiber. *Diffusion and dynamics of γ-globulin in crowded aqueous solutions;* J.Phys.Chem.B **118**, 7203 (2014).
[2] M. Grimaldo, F Roosen-Runge, N. Jalarvo, M. Zamponi, F. Zanini, M. Hennig, F. Zhang, F. Schreiber, and T. Seydel. *High-resolution neutron spectroscopy on protein solution samples;* EPJ Web of Conferences **83**, 02005 (2015).
[3] F. Roosen-Runge, M. Hennig, F. Zhang, R. M.J. Jacobs, M. Sztucki, H. Schober, T. Seydel, and F. Schreiber. *Protein self-diffusion in crowded solutions;* Proc.Nat'l.Acad.Sci.(USA) **108**, 11815 (2011).