Experimental Report

Proposal: **9-13-526** **Council:** 4/2014

Title: Crowding in ternary protein solutions

This proposal is a new proposal

Researh Area: Soft condensed matter

Main proposer: **SEYDEL Tilo**

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Local Contact: SEYDEL Tilo

Samples: Bovine Serum Albumin - Bovine gamma-globulin - D2O ternary mixtures; commercial proteins from Sigma-Aldrich

Instrument	Req. Days	All. Days	From	To
IN16B	2	2	16/10/2014	18/10/2014

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 of protein(BSA)-protein(IgG)-water(D2O) ternary mixtures, where the proteins BSA (bovine serum albumin) and IgG (immunoglobulin) are important constituents of blood. 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 contributions of the two proteins to the QENS spectra in the ternary mixture.

Experimental Report

Proposal number:	9-13-526		
Experiment title:	**Crowding in ternary protein solutions**		
Instrument	IN16B		
Dates of experiment:	Oct.16-18, 2014	**Date of report:** Feb.10, 2015	
Team: **Names (* marks experimentalists)**	**Addresses**		
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During experiment 9-13-526, we have collected a data set on the diffusion of the mixture of the proteins bovine serum albumin (BSA) and gammaglobulin (Ig) in aqueous (D_2O) solutions.

Sample Preparation: The solutions at different concentrations of BSA and Ig in D_2O (see table below) were 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.

Nominal concentration of BSA [mg/ml]	Nominal concentration of Ig [mg/ml]	Temperature (set point) [K]	Symbol (Figure 1)
50	100	295	Circle
100	100	295	Right-pointing triangle
200	100	295	Square
300	100	295	Red diamond
300	100	310	Yellow diamond
188	47	295	Green up-pointing triangle
188	47	310	Orange up-pointing triangle
Pure D_2O		295	

Measurements: In addition to the samples listed in the table above, vanadium foil and empty sample holder were measured to allow the calibration and the subtraction of the various contributions from the sample spectra in later analysis.

Data Treatment: The following steps were done with MATLAB code:

- Subtraction of the empty sample holder
- Calibration, correction for detector efficiency and determination of the resolution function using the vanadium spectrum. The resolution function was described by a combination of five Gaussians and a flat background.
- To accurately take into account the contribution of D_2O, at every scattering vector Q a fixed term $\beta_{D_2O} L_{\gamma_{D_2O}}(\omega)$ with the Lorentzian function $L_{\gamma D2O}$ and scalar β (convoluted with the resolution function) was added directly to the model used for the fit of the spectra [1]:

$$S(Q,\omega) = R(\omega) * [\beta_1 L(\omega, \gamma) + \beta_2 L(\omega, \gamma + \Gamma) + \beta_{D_2O} L_{\gamma_{D_2O}}(\omega)] + B$$

  Therein the width Γ accounts for the internal modes, while γ describes the global center-of mass diffusion of the proteins consisting of contributions from the translational and rotational diffusion. The width of the Lorentzian describing the diffusion of D_2O was determined from a measurement done with IN5.
- By the slope of the HWHM γ as a function of q^2, an apparent diffusion coefficient D can be determined, from which a translational diffusion coefficient D_t is calculated [1].

Outcome of the Experiment :

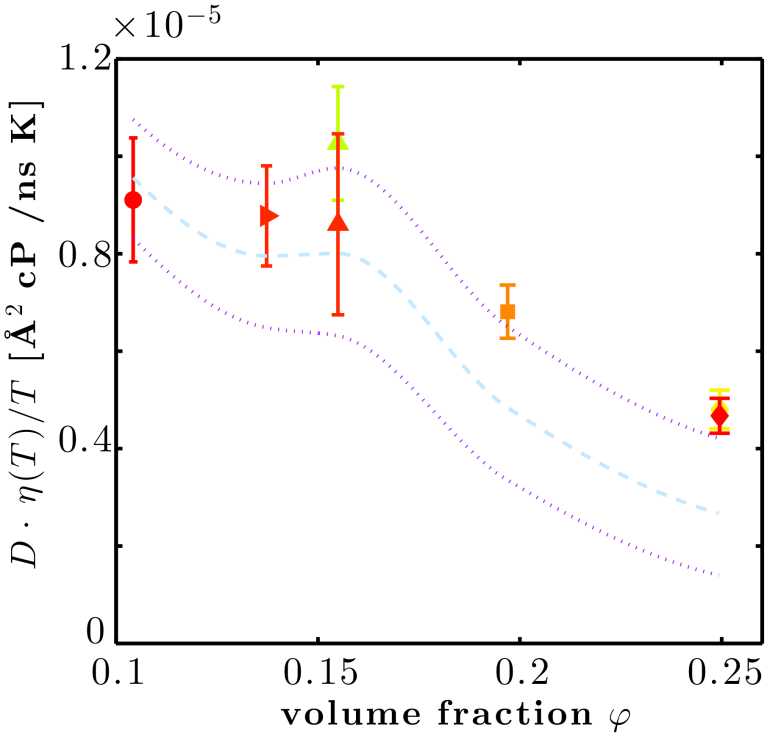

Figure1: Apparent diffusion coefficient D normalized by T / $\eta(T)$ as a function of the total volume fraction $\phi = \phi^{(Ig)} + \phi^{(BSA)}$ (symbols – cf. Table). The dashed blue line represents the values expected from colloid theory, and the dotted magenta lines assume 5% error on the determination of the effective volume fraction. Note that the theoretical line is not strictly monotonic because of the different relative amounts of BSA and Ig.

A preliminary analysis of the recorded data indicates that the average diffusion coefficient of the mixture of BSA and Ig in solution can be semiquantitatively described by the weighted sum $D = \frac{m_{Ig} D_{Ig} + m_{BSA} D_{BSA}}{m_{Ig} + m_{BSA}}$ of the contributions of the two species at the effective volume fraction $\phi_t = \phi_t^{(Ig)} + \phi_t^{(BSA)}$ (Figure 1).