Experimental report

18/08/2017

Proposal: 8-04-774 **Council:** 4/2016

Title: Towards a realistic model of the cellular environment: Dynamics in "naturally" crowded protein solutions

Research area: Biology

This proposal is a new proposal

Main proposer: Christian BECK

Experimental team: Trevor FORSYTH
Christian BECK
Benedikt SOHMEN
Michal BRAUN

Local contacts: Tilo SEYDEL

Samples: bovine gamma globulin proteins in deutrated cellular lysate from E.Coli

Instrument	Requested days	Allocated days	From	To
IN16B	2	2	15/11/2016	17/11/2016

Abstract:

Using deuterated cellular lysate obtained from living cells, we will establish a natural crowding environment for protonated "tracer" or "target" proteins. We will systematically investigate the influence of this natural crowding environment on both the global center-of-mass diffusion as well as the internal diffusive motions of the target proteins using IN16B. We have previously tested the feasibility of this experiment, and we have been allocated the required preparation of the lysate by the ILL deuteration laboratory through a successful peer-reviewed D-lab proposal. We will analyze the data using the frameworks successfully established by our group [cf. M.Grimaldo et al., EPJ Web of Conferences 83, 02005 (2015) and references therein] .

Experimental Report
Experiment Number: 8-04-774
Experimental Team: M. Braun, B. Sohmen, C. Beck

The aim of the experiment was to see the influence of external crowding due to lysate from deuterated e coli bacteria. As a tracer protein, bovine gamma-globulin (IgG) is used. Therefore, samples were measured at different protein and lysate concentrations and at different temperatures:

Protein concentration	Lysate concentration	Temperature
0 mg/ml	56 mg/ml	295 K
0 mg/ml	100 mg/ml	295 K
0 mg/ml	100 mg/ml	280 K
40 mg/ml	56 mg/ml	295 K
100 mg/ml	56 mg/ml	295 K
100 mg/ml	100 mg/ml	295 K
100 mg/ml	100 mg/ml	280 K

Samples were prepared without further purification of the protein obtained from Sigma Aldrich. The deuterated lysate was obtained from the Deuteration Lab of the ILL. Next to the samples listed above, vanadium was measured to determine the resolution function and D_2O for the background correction. Since the lysate is denaturated, its signal can be neglected for a first analysis. The scattering signal of the empty can is subtracted from the ones of the samples. The obtained signal was thus described by:

$$S(q,\omega)=R(q,\omega)\otimes[\beta(q)\cdot[A_0(q)L(\gamma,\omega)+(1-A_0(q))L(\Gamma+\gamma,\omega)]\beta_{D_2O}\cdot L(\gamma_{D_2O},\omega)]$$

An example spectra with fit is shown in figure 1.

Previous measurements (Experiment number 8-04-759) gave access to the specific volume of lysate of ν=1.11ml/g. With this information, the total volume fraction can be calculated

$$\varphi=\varphi_{Protein}+\varphi_{Lysate}$$

In Figure 2, the apparent diffusion coefficient is plotted as a function of this volume fraction. As expected, the diffusion coefficient decreases with increasing volume fraction and with decreasing temperature.

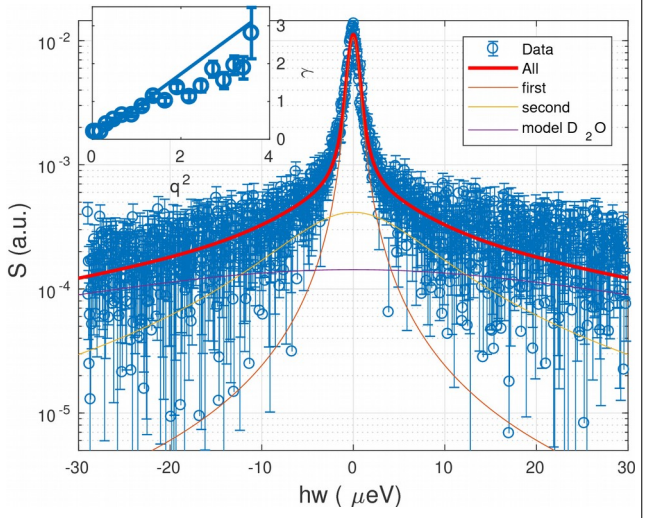

Figure 1: Example spectra of IgG: c_p=100ml/ml; c_{Lysate}=100mg/ml; T=295K; q=0.5678Å^{-1}
Inset: Width of the global diffusion as a function of q^2.

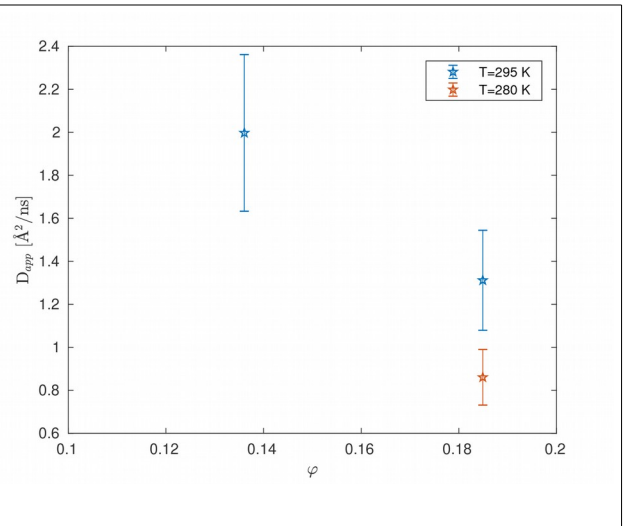

Figure 2: Apparent diffusion coefficient as a function of volume fraction.