# Experimental report

01/02/2016

**Proposal:** 8-04-754 **Council:** 4/2015

**Title:** Discerning static from transient clusters using neutron spin echo andbackscattering

**Research area:** Soft condensed matter

**This proposal is a continuation of 8-04-724**


**Main proposer:** Marco GRIMALDO

**Experimental team:** Marco GRIMALDO
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**Local contacts:** Ingo HOFFMANN


**Samples:** Bovine beta-lactoglobulin (BLG) proteins in aqueous (D2O) solution

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| IN15 | 2 | 2 | 17/11/2015 | 19/11/2015 |


## Abstract:

The presence of dynamic or transient clusters in protein solutions and its understanding in terms of colloid physics is of great current interest due its relevance for pathological pathways as well as for drug design [K.P.Johnston et al., ACS Nano 6, 1357 (2012)]. Earlier studies combining SAXS and neutron spin-echo have addressed this issue using the model protein lysozyme [L.Porcar et al., J.Phys.Chem.Lett. 1, 126 (2010)].

Here we propose a systematic study of the cluster formation in aqueous solutions of the new model protein beta-lactoglobulin (BLG). We propose to measure the diffusion function for several values of q below and above q_c using IN15.

We have previously obtained the short-time self-diffusion of BLG as a function of volume fraction phi in D2O indicating the presence of static clusters with a size that depends on phi. Moreover, we have obtained the diffusion functions for two samples on IN11, which, interestingly, disagree with the expectation of static clusters and do not converge to the short-time diffusion at high q. To resolve the arising questions, we clearly need IN15.

Due to the prevailent colloid physics aspects, we explicitly submit to college 9.

# Experimental Report

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|---|---|
| **Date of Report:** | January 30, 2016 |
| **Proposal:** | **8-04-754** **Scientific Council:** 4/2015 |
| **Title:** | Discerning static from transient clusters using neutron spin echo and backscattering |
| **This proposal is a continuation proposal** | |
| **Previous proposal number:** | **8-04-724** |
| **Research Area:** | Soft condensed matter |
| **Main Proposer:** | **GRIMALDO Marco** |
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| **Samples:** | Aqueous betalactoglobulin (BLG) solutions |

| **Instrument** | **Req. Days** | **All. Days** | **From** | **To** |
|---|---|---|---|---|
| IN15 | 2 | 2 | 17/11/2015 | 19/11/2015 |

**Abstract:**
In the last years in colloidal systems with a short range attraction and a longrange repulsion an equilibrium cluster phase which precedes gel formation has been discovered. In protein systems similar findings have been reported. However the existence and nature of these clusters is still debated. In contrast to colloids proteins are too small to be observed by microscopy. We have indications for cluster formation in yet unexplored betalactoglobulin (BLG) solutions. To decide whether the clusters are permanent or transient a high quality neutron scattering experiment was needed.

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| No. | Wavelength (Å) | Angle (°) | q (1/Å) | | |
|---|---|---|---|---|---|
| 1 | 12 | 3.5 | 0.0246 | 0.0335 | 0.0424 |
| 2 | 12 | 5.47 | 0.0420 | 0.0511 | 0.0601 |
| 3 | 12 | 8.76 | 0.0717 | 0.0808 | 0.0899 |
| 4 | 12 | 10.96 | 0.0916 | 0.1007 | 0.1099 |
| 5 | 8 | 12.5 | 0.1584 | 0.1720 | 0.1857 |
| 6 | 6 | 20.0 | 0.3467 | 0.3648 | 0.3829 |
| 7 | 6 | 30.0 | 0.5253 | 0.5430 | 0.5608 |

Table 1: List of measured configurations.

**Experimental:** Graphite, $D_2O$ and two samples, 144 mg/ml BLG and 72 mg/ml BLG were measured at the configurations listed in Table 1. Due to the size of the detector each configuration allows for simultaneously measuring at three q-values. BLG was dissolved in $D_2O$. The concentration was determined using UV-vis spectroscopy. For the NSE measurement the solutions were filled in quartz cuvettes.

**Results:** Figure 1 shows the previously obtained results from IN16B and IN11. On the left hand side the translational diffusion coefficients as extracted from the IN16B data are shown versus the protein volume fraction. They may only be fitted by a hard sphere model if clusters with an increasing number of monomers are assumed[1-3]. On the left hand side the IN16B data (high q) is shown together with data taken at IN11. The errorbars of the IN11 data are too large to allow for a conclusion.

Figure 2 shows the results from IN15. The left part shows the measured intermediate scatttering functions for the 72 mg/ml BLG sample. The signal of $D_2O$ is subtracted as background. Only the first out of the three measured q values is shown for each configuration (Table 1). The data at the two higher q values is comparable. The data obtained with the first five configurations is of high quality and in all cases may be fitted with a straight line at least up to ∼50 ns. The data obtained using configurations 6 and 7, i.e. the high q data, is not so good. From these data no diffusion coefficients can be calculated.

The right part of Figure 2 shows the diffusion coefficients that were calculated from the single exponential fits to the data. The diffusion coefficients nicely show a minimum at around 0.06 $Å^{-1}$. In 72 mg/ml BLG the diffusion coefficients are larger than in 144 mg/ml. In addition to the data obtained during this beamtime, apparent diffusion coefficients calculated from measurements at IN16B are shown. The order of magnitude is similar.

**References:**

[1] Roosen-Runge, F. *et al.*, Protein self-diffusion in crowded solutions, *Proc. Natl. Acad. Sci. U.S.A.*, **108**, 11815-20 (2011)

[2] Grimaldo, M. *et al.*, High-resolution neutron spectroscopy on protein solution samples, *EPJ Web of Conferences*, **83**, 02005 (2015)

[3] Grimaldo, M. *et al.*, Diffusion and Dynamics of $\gamma$-Globulin in Crowded Aqueous Solutions, *J. Phys. Chem. B*, **118**, 7203-09 (2014)

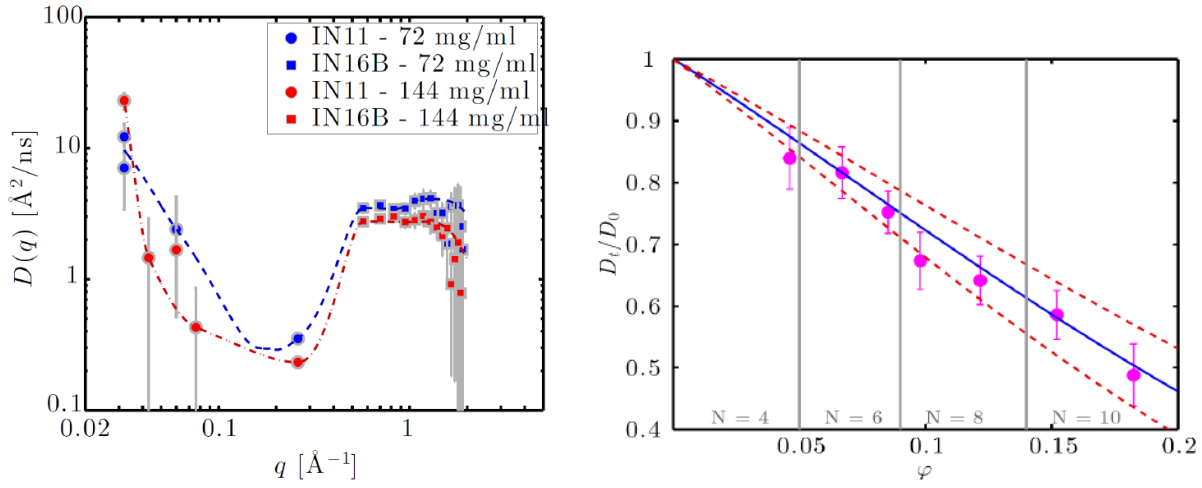


Figure 1: Left: Diffusion ceofficients from IN16B and IN11. Right: Translational diffusion coefficients from IN16B versus volume fraction of BLG. The data can only be described by the model for colloidal hard spheres (blue solid and red dashed lines) if clusters with an increasing number of monomers are assumed. The red dashed lines are for an error of 5% in the volume fraction [1-3].

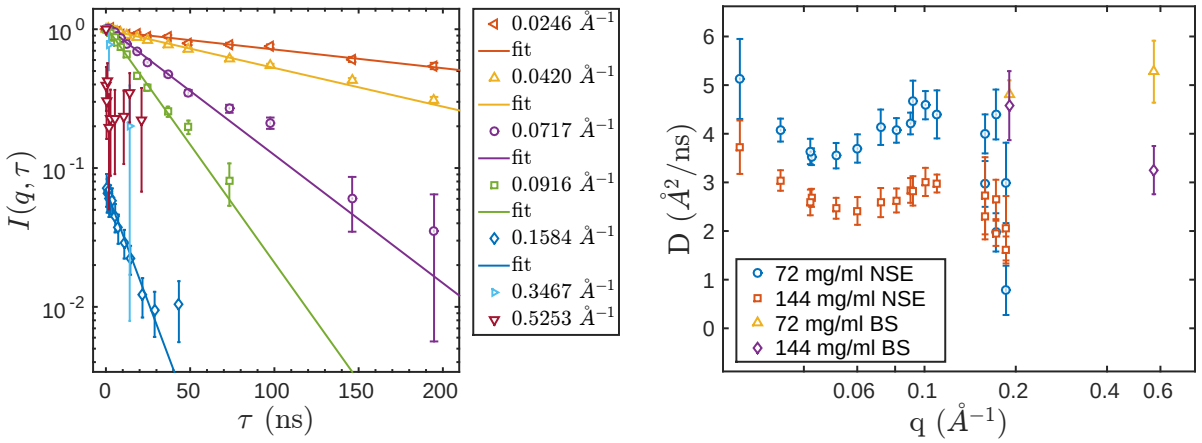


Figure 2: Left: Intermediate scattering functions for 72 mg/ml BLG. $D_2O$ was subtracted as background. Right: Calculated diffusion coefficients