Experimental report

07/02/2017

Proposal: 8-04-804 **Council:** 10/2016

Title: Characterizing the formation of liquid-liquid phase separation by tuning the system by using its lower critical solution temperature

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Christian BECK

Experimental team: Christian BECK
Lena BUHL
Michal BRAUN

Local contacts: Tilo SEYDEL

Samples: D2O
Bovine Serum Albumin
YCl3

Instrument	Requested days	Allocated days	From	To
IN16B	4	3	28/01/2017	31/01/2017

Abstract:

The presence of multivalent salt ions can induce complex phase diagrams in aqueous protein solutions including, with increasing salt concentration, a condensed and a re-entrant dissolution regime. Within the condensed regime, a liquid-liquid phase separation (LLPS) can occur. This LLPS regime may be of fundamental biological and medical interest, because it can play an important role in the self-organization of proteins, both in normal contexts of biological function as well as in pathological pathways. The LLPS regime manifests itself macroscopically by a separation of the originally homogeneous protein solution into microdroplets of a protein-rich phase suspended in the bulk liquid of a protein-poor phase. It is assumed that the LLPS is associated with the formation of protein clusters or aggregates, but the dynamics aspects of the transition are poorly understood. With the frameworks and methods established by our group, we propose a high-resolution backscattering study to access the slow self-diffusive dynamics of protein clusters systematically resolving their nature.

Experimental report: 8-04-804

Experimentalists: C. Beck, M.K. Braun, L. Bühl, T. Seydel
Local Contact: T. Seydel
Co-Proposer: M. Grimaldo, O. Matsarskaia, F. Roosen-Runge, F. Schreiber, F. Zhang
ILL & University of Tübingen

February 7, 2017

1 Sample preparation

Samples were prepared by weighing the desired mass of protein and calculating based on this the amount of total solution needed to obtain the desired protein concentration. The YCl_3 was added after the protein was dissolved in D_2O from a stock solution with $c_s^{stock} = 100\,\mathrm{mM}$. The samples were filled into Al sample holders which were sealed with indium to prevent evaporation in the cryofurnance.

2 Overview

We measured two samples with a protein concentration of 200 $\frac{\mathrm{mg}}{\mathrm{ml}}$ BSA with YCL_3 concentrations of $c_s/c_p = 7$ and $c_s/c_p = 8$ respectivlely. For both samples, the same measurements were performed: Starting from $T = 280\,\mathrm{K}$, the samples were heated up to the temperatures $T = 295$, 310, 320 K. After the desired temperature was reached, the samples were coold down back to $T = 280\,\mathrm{K}$. This procedure was repeated three times measuring with fixed window scans at the energies $\Delta E = 0\,\mu\mathrm{eV}$, $1,3\,\mu\mathrm{eV}$ and $3\,\mu\mathrm{eV}$ respectively. At all four temperatures, whole QENS spectra were recorded for 2,25 hours.

In addition to the samples vanadium was measured at room temperature to obtain the resolution function.

To be able to model the water contribution later, a full QENS spectrum was collected of D_2O at $T = 320\,\mathrm{K}$. During cooling down to $T = 280\,\mathrm{K}$ fixed window scans with the energys mentioned above were measured in an alternating way.

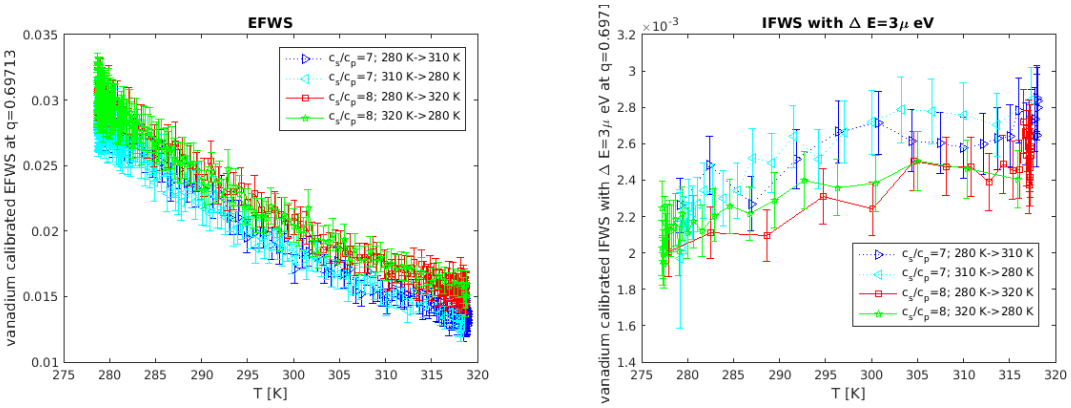
To have on the one hand a high point density with changing temperature to capture potential changes and to have on the other hand good statistics, the measuring times for one fixed window scan are adapted to the energy transfer:

Energy transfer ΔE	measuring time
$0\,\mu\mathrm{eV}$	30 s
$1.3\,\mu\mathrm{eV}$	90 s
$3\,\mu\mathrm{eV}$	3 min

For full QENS spectra we measured 2,25 hours.

3 Preliminary results

As shown in figure 1a the collected data indicate that the system returns to the initial state and that it is thus in this temperature range and within the given salt and protein concentrations reversible. A change to higher salt concentrations (from $c_s/c_p = 7$ to $c_s/c_p = 8$) does influence the system and lead to a higher elastic signal. at higher energy transfers ($\Delta E = 3\,\mu\mathrm{eV}$) a non reversibility can be observed (see figure 1b).



(a) Elastic fixed window scan

(b) inelastic fixed window scan at $\Delta E = 3\,\mu\text{eV}$

Figure 1: Fixed window scans during the temperature ramps. The shown data is for $q \simeq 0.7\text{Å}^{-1}$.

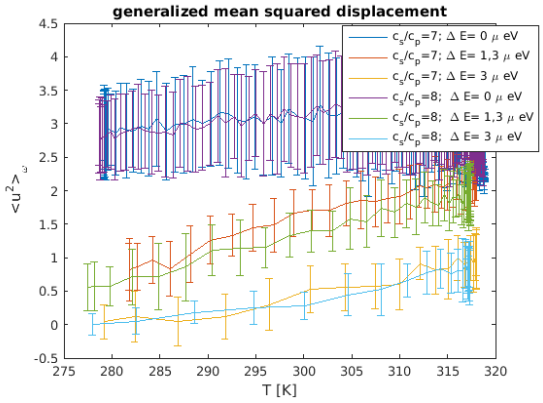


Figure 2: Generalized mean squared displacement as a function of temperature for the different offset energies and different salt concentrations. Data shown is based on a rough fitting without any corrections (see text).

Based on Ref [1], a very brief analysis was performed by determining the (generalized) mean squared $< u^2 >_\omega$ displacement:

$$< u^2 >_\omega = -\lim_{q\to 0} \frac{3}{q^2} \log(S(q,\omega)/R(\omega)) \tag{1}$$

Although the water contribution, empty cell corrections, Paalman-Pings corrections, etc were neglected up to this point, it can be seen in figure 2 that the increasing salt concentration seems not to influence the generalized mean squared displacements.

References

[1] EPJ Web of Conferences **83**, 02015(2015) Felix Roosen-Runge, Tilo Seydel: A generalized mean-squared displacement from inelastic fixed window scans of incoherent neutron scattering as a model-free indicator of anomalous diffusion confinement