Experimental Report

Proposal: **9-13-474** **Council:** 10/2012

Title: Looking at the diffusion in crowded eye lens protein mixtures using acolloid-based approach

This proposal is continuation of: 9-13-390

Researh Area: Soft condensed matter

Main proposer: **STRADNER Anna**

Experimental Team: STRADNER Anna
SCHURTENBERGER Peter
OBIOLS-RABASA Marc

Local Contact: FARAGO BELA

Samples: beta crystallin and gamma crystallin in D2O

Instrument	Req. Days	All. Days	From	To
IN15 Std+Small echo	8	8	26/06/2013	04/07/2013

Abstract:

Current approaches in proteomics mainly focus at the molecular level to understand the set of protein-protein interactions. However, there is an increasing awareness that a quantitative understanding also requires comprehension of crowded protein mixtures, as the concentrated protein background strongly influences the diffusion of individual proteins as well as their effective binding constants by non-specific interactions with the surrounding proteins. We have thus started to implement a colloid-physics based approach, where we use the eye lens cell as a model system and study the interactions between the crystallin proteins in concentrated solutions and mixtures using a combination of scattering techniques and computer simulations. Here we apply NSE to measure the diffusion of beta crystallin in the presence of a concentrated solution of a second lens protein, the gamma crystallin. We will use partially deuterated gamma crystallins and contrast match them using D2O buffer. We will look at different mixing ratios at total protein concentrations similar to those in the eye lens.

Looking at the diffusion in crowded eye lens protein mixtures using a colloid-based approach

The mammalian eye lens cells are made up of so-called crystallin proteins, which are divided into three principal classes, α-, β- and γ-crystallin. In the lens, these proteins are present in mixtures at total concentrations between approximately 200 and 400 mg/ml, the nucleus exhibiting a higher protein concentration than the cortex. In spite of this high protein concentration, the lens has to stay flexible in order to be able to properly adapt its focus. With age, this ability to change focus is gradually lost, resulting in what is known as presbyopia. Even though not completely understood yet, it is presumed that the origin of presbyopia is strongly connected to an age-related increase of the stiffness of the nucleus of the lens, where the protein concentration is the highest. We are speculating that the reason for this increase in stiffness is an age-related change in the protein-interactions, leading to an arrest transition and thus leaving the protein mixture in an arrested glassy state instead of a more flexible fluid-like state.

After having investigated the protein dynamics in α- and γ-crystallin solutions and their mixtures during earlier NSE measurements, we now turned to β/γ-mixtures, at volume fractions up to 0.24. Earlier studies of the individual proteins have suggested that β-crystallins can be described as polydisperse hard spheres, whereas γ-crystallins exhibit short-range attractive interactions [1 and references therein].

Our original plan for the NSE experiments on the β/γ-mixtures was to look at the short time dynamics of β-crystallin in the presence of partially deuterated γ-crystallin (D-γ-crystallin) whose contribution to the signal would have been matched out by an H_2O/D_2O solvent with the appropriate mixing ratio. To this end, our collaborators in Halle/Germany provided us with D-γ-crystallins of different deuteration degree. The exact match points of these proteins were determined by SANS (performed at FRM II in Munich, January 2013). However, while preparing and handling the protein samples for these experiments, it became apparent that the solubility of D-γ-crystallin is considerably lower than that of its hydrogenated equivalent, making it impossible to reach the concentrations that we were aiming at for the NSE experiments. We thus had to change our plan and use hydrogenated γ-crystallin instead.

Φ	% of β	% of γ
0.05	100	0
0.12		
0.17		
0.24		
0.29		
0.35		
0.45		
0.24	75	25
	50	50
	25	75
0.12	75	25
	50	50
	25	75

Table 1: Total protein volume fractions and β/γ mixing ratios in the samples used for NSE experiments.

First of all, we investigated the dynamic behaviour of pure β-crystallins. We measured samples with volume fractions between Φ = 0.05 and 0.45 at a temperature of T = 25°C. Each sample was measured at several q-values between 0.03 and 0.2 Å^{-1} in order to obtain the q-dependent diffusion coefficient (cf. figure 1a). The nearest neighbour peak in the structure factor of pure β-crystallin is located around q = 0.06 Å^{-1} while q = 0.2 Å^{-1} roughly corresponds to the nearest neighbour peak of pure γ-crystallin. We observe that increasing the volume fraction of β-crystallin leads to a slowing down of the dynamics. Since a small and unexpected temperature dependence was observed in the SAXS measurements carried out in parallel to the NSE experiments, the sample with Φ = 0.35 was also measured at a temperature of 20°C and 35°C (cf. figure 1b).

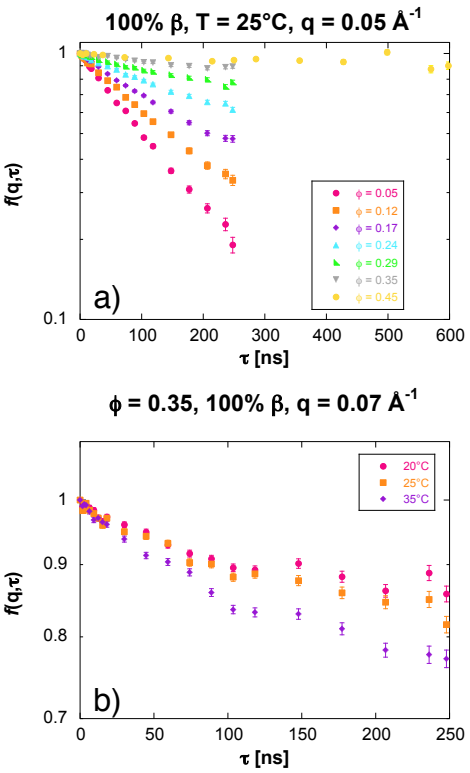

Figure 1: a) Intermediate scattering functions at T = 25°C and q = 0.07 Å^{-1} for Φ between 0.05 and 0.45 of pure β-crystallin samples. **b)** Intermediate scattering functions at q = 0.07 Å^{-1} (i.e. close to the structure factor peak) and different temperatures of a pure β-crystallin sample with Φ = 0.35.

Most of the temperature dependence observed in the NSE data is however due to the T-dependent viscosity of the solvent, but to draw a final conclusion, the data from NSE and complementary techniques needs to be analyzed in more detail.

We then focused on the mixtures, for which we measured protein samples with two different total volume fractions and three different mixing ratios (cf. table 1) at a temperature of T = 25°C. Each sample was again measured at several q-values between 0.03 and 0.2 Å^{-1}. Since lowering the temperature is expected to increase the short-range attractions between the γ-crystallins, the two samples with the highest γ-content were also measured at a temperature of T = 20°C and T = 18°C, respectively. Figure 2 shows a selection of the obtained intermediate scattering functions. Decreasing the relative amount of β-crystallin, as well as increasing the total protein volume fraction, leads to a slowing down of the dynamics. Since both proteins used for these experiments were hydrogenated, we find ourselves in need of detailed model calculations and theoretical input to be able to distinguish the contributions of the individual proteins to the total scattering signal. Static and dynamic light scattering as well as SAXS experiments and phase diagram studies have been performed in parallel to complement the NSE data. This extended set of experimental data together with the simulations that our collaborators at FZ Jülich are working on, will hopefully lead to a better understanding of the interactions in concentrated mixtures of eye lens proteins and their effect on the protein dynamics under crowded conditions.

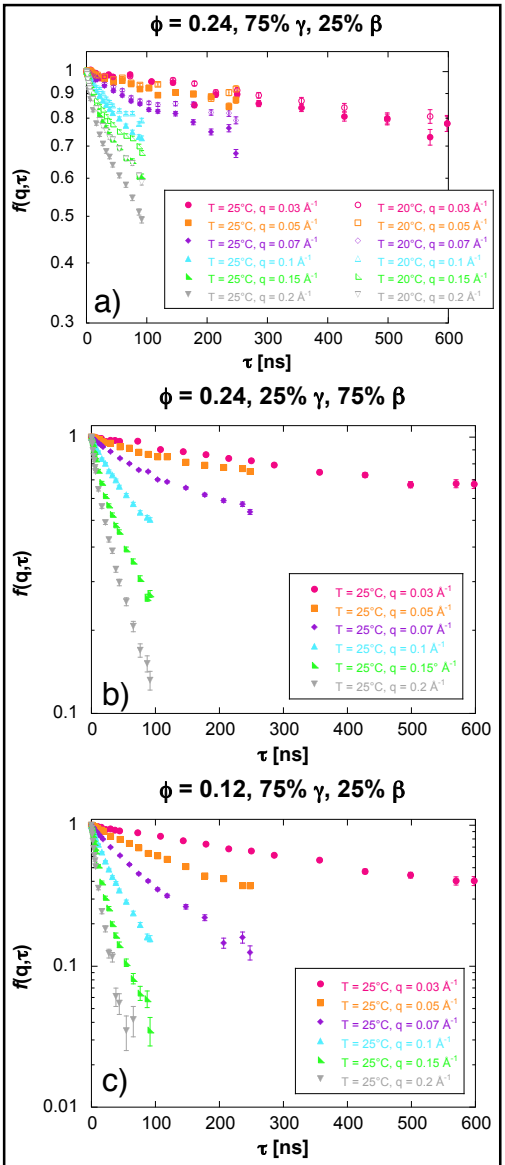

Figure 2: Intermediate scattering functions at different temperatures and q-values for
a) Φ = 0.24; β/γ mixing ratio = 25/75
b) Φ = 0.24; β/γ mixing ratio = 75/25
c) Φ = 0.12; β/γ mixing ratio = 25/75

References:

1) A. Stradner, G. Thurston and P. Schurtenberger, J. Phys. Condens. Matter. 17, S2805 (2005)