ESRF	**Experiment title:** Kinetics of liquid-liquid phase transitions in concentrated solutions of lens proteins/polymer mixtures : time-resolved SAXS studies	**Experiment number**: SC 727
Beamline: ID2	**Date of experiment**: from: 15/09/00 to: 17/09/00	**Date of report**: 21/02/01
Shifts: 6 (block allocation with SC732)	**Local contact(s)**: S. FINET	*Received at ESRF:*

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Report:

Six shifts were available for SC-727. The first positive outcome was that the high flux, the X-ray image intensifier - FReLoN CCD camera and the rapid stopped-flow device available at the ID2 beamline render feasible time-resolved studies of phase separations in binary mixtures of proteins and polyethylene glycol (PEG).

I- The first control experiemnts were done to determine the minimum exposure time necessary to record α-crystallin spectra with sufficient signal to noise ratio and statistics. This time was found to be between 50 and 100 ms.

II- The second trial was to check for how long the sample could be irradiated before being damaged. With a 1Å wavelength and a sample holder kept at about 12°C, about 20 to 40 shots could be made, with about 1s dead time in between. Then, the sample starts to aggregate. One has to be very careful to avoid such aggregates, since they stick on the capillary walls and are difficult to wash out. If possible, it is preferable to use the same capillary for the whole experiment to subtract the same background.

III- The kinetics of phase separation upon the addition of PEG 8K (or PEG + ammonium sulphate) to α-crystallin solutions were then studied as a function of time for different initial protein and PEG concentrations. The separation was found to proceed immediately after mixing (80 ms) and was essentially complete after a few minutes. An example of the intensity curves recorded for a final mixture of α-crystallins 40 mg/ml, 10% PEG 8K and ammonium sulphate 0.1 M, is given in figure 1 and the corresponding structure factors are shown in figure 2.

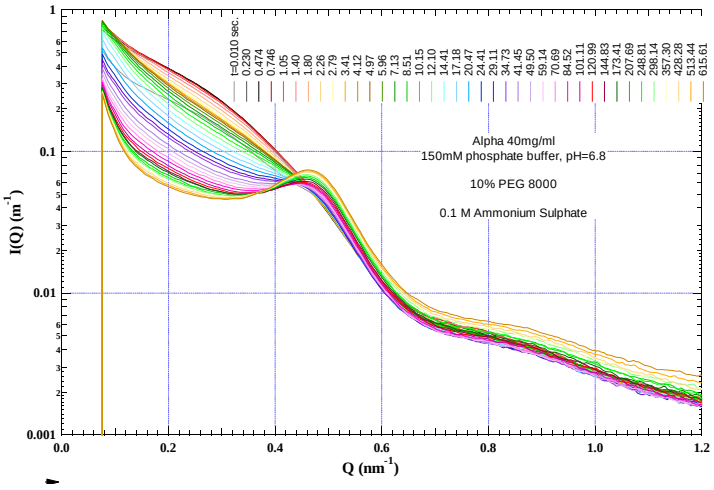

Figure 1

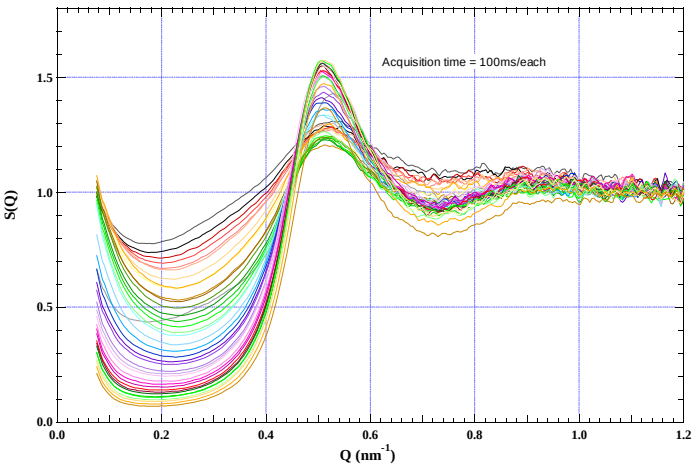

Figure 2

The structure factors are obtained by the division of the scattered intensity by the form factor, recorded at low protein concentration in a separate experiment. As can be seen in the figures, upon mixing, the scattered intensity at low angles increases, which indicates that a strong attraction is induced between proteins. The separation between the protein-rich and the polymer-rich phases then starts immediately. The maximum that appears around q=0.5 nm-1, is the signature of the liquid-like ordering in the concentrated protein droplets. The concentration within the droplets increases until the phase separation is complete. In some cases, the final droplets were large enough and concentrated enough to sediment at the bottom of the capillary tube. In fact, the main limitation comes from the reproducibility of the background level, especially at high angles because of the concentration fluctuations and gradients.

IV- In physiological buffer, the alpha-crystallins behave like charged spheres (there is no van der Waals component probably because of the open alpha-crystallin quaternary structure). The interaction parameters therefore depend upon only two parameters, hard core diameter and charge. The best fit parameters were found respectively equal to 170 Å and 50 charges. After the phase separation induced by PEG, or PEG plus salt, it is clear from the shape of the structure factors in figure 2 that to fit the structure factors an additional attraction is needed. The attraction required has the characteristics of a depletion attraction, with a potential range equal to the polymer radius of gyration. The maximum of the structure factor in the concentrated phase is displaced as compared to the position of the structure factor in concentrated alpha-crystallin solutions without PEG. The peak position in figure 2 can only be fitted with a much smaller hard core, of diameter 130 Å. Such a displacement has been observed in previous experiments of colloid-polymer mixtures (e.g. Ye et al. Phys. Rev. E **54**, 6, 1996), but for a fixed colloid concentration, the particle diameters do not change very much with the polymer concentration and are close to those from the corresponding pure colloidal suspensions. This effect could be linked to the quaternary structure of the biological macromolecules. It could indicate that in the presence of PEG or PEG and salt the proteins may behave as soft spheres. Since PEG is of general use in protein crystallisation, it could be important to clarify the point. It might be one of the reasons why PEG is so successful for crystal nucleation and growth.

From static studies, the range of the depletion-induced attraction was found to vary as a function of PEG size and concentration. We wish to perform time-resolved experiments as a function of PEG size, PEG concentration, temperature, and salt concentration to analyze the relationship between the kinetic parameters and the interaction parameters.