ESRF	**Experiment title:** **Influence of Phase Separations on the Crystallization of Urate Oxydase and BPTI**	**Experiment number:** **SC-1269**
Beamline: ID02	**Date of experiment:** from: 04/02/2004 to: 07/02/2004	**Date of report:** 25/02/2005
Shifts: 9	**Local contact(s):** Dr S. Finet	*Received at ESRF:*

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Report:

For several years, we have been using SAXS to understand the fundamental principles, which govern crystallization of biological macromolecules (1-4). It was thus shown that a better knowledge of interaction potentials between biological macromolecules in solution (coulombic and hard sphere repulsion, van der Waals and depletion attraction…) makes it possible the description of thermodynamic behaviors i.e. solubility, crystallization or liquid-liquid phase separation. Thanks to studies performed on the beam line D24 at LURE (ORSAY) with different proteins, we have shown that two types of additives (salts and neutral polymers) that both induce attractive interactions, were playing a major role in crystallization depending on the size of the protein (5) and that different phenomena could be observed (crystallization, gel-like structures, phases separations or precipitation) as a function of the range of the attractive potential. In the case of liquid-liquid phase separation (LLPS), SAXS patterns in static experiments on D24 at LURE were characterized for different macromolecules by the appearance of a correlation peak at a constant position $2R_g.s=1$ and by an increase of the intensity at very low angle.

Since very low angles ($q_{min}=0.018nm^{-1}$) were accessible with the larger distance (10m) on ID02, the questions that we wanted to answer with these kinetic experiments were: what was the origin of the increase at small angle (aggregation, size of droplets, number of droplets); did the correlation peak correspond to proteins in contact in droplets? Did the height of the peak and the size of droplets vary with time? Can crystals grow from droplets?…

Experimental methods and Results

The kinetic experiments were done with UoAf (urate oxidase from *Aspergillus flavus*) and BPTI (bovine pancreatic trypsin inhibitor) since their phase diagrams were perfectly known in the conditions studied (6-8).

With BPTI, the liquid-liquid phase separation was induced by decreasing temperature of the solution of protein with salt (350mM KSCN). Since no T-jump device was available on ID02, we used the stopped-flow device as temperature control. With UoAf, the LLPS was observed using time-resolved X-ray scattering and was induced by rapid mixing of the protein with crystallizing agent (PEG8000) with the ID02 stopped-flow device at constant temperature (20°C). In each case, two distances were studied: 1m50 to measure the form factor at large angles to systematically control the nature of scattering species in solution and 10m to measure the scattering signal of the presumed droplets in the very low angle range.

<u>BPTI results</u>

The thermo-induced LLPS experiments in acidic condition, in presence of 350mM KSCN allowed us to confirm (8) the decreasing of apparent concentration of the solutions submitted to a decrease of temperature (typically spinodal decantation phenomenon) (Fig.1), and therefore to measure the quantity of oligomeric species in poorly concentrated phases (monomer/decamer mixtures) (Fig.2). But due to a bad control of temperature in the cell, we could not observe the correlation peak characteristic of the LLPS, nor the droplet formation.

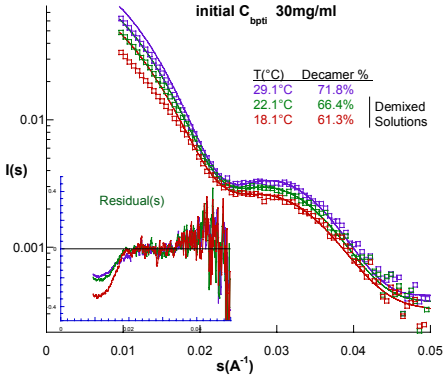

Fig.1: Evolution of the scattering intensity of BPTI at pH 4.5 in KSCN 350mM (open squares), during a thermo-induced LLPS. The lines represent the best fit. In the inset plot of the fitting residuals vs s.

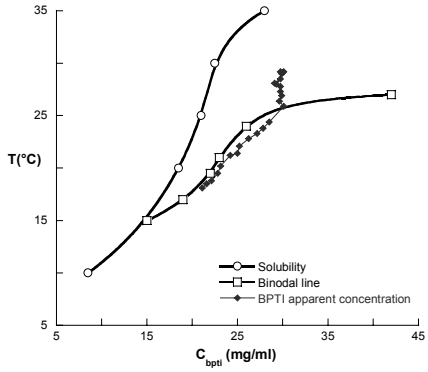

Fig.2: Evolution of the BPTI apparent concentration during the thermo-induced LLPS.

UoAf results

According to previous results (6), we chose 4 conditions in the UoAf/PEG8000 phase diagram corresponding to different states in the LLPS at constant temperature (20°C): UoAf 10.5mg/ml, PEG8000 8%, 10%, 11% and 12%. With the larger distance accessible on ID02 (10m), we could observe and measure different phenomena up to 250nm: formation of droplets or gels, decantation, as a function of the position in the phase diagram (Fig.3).

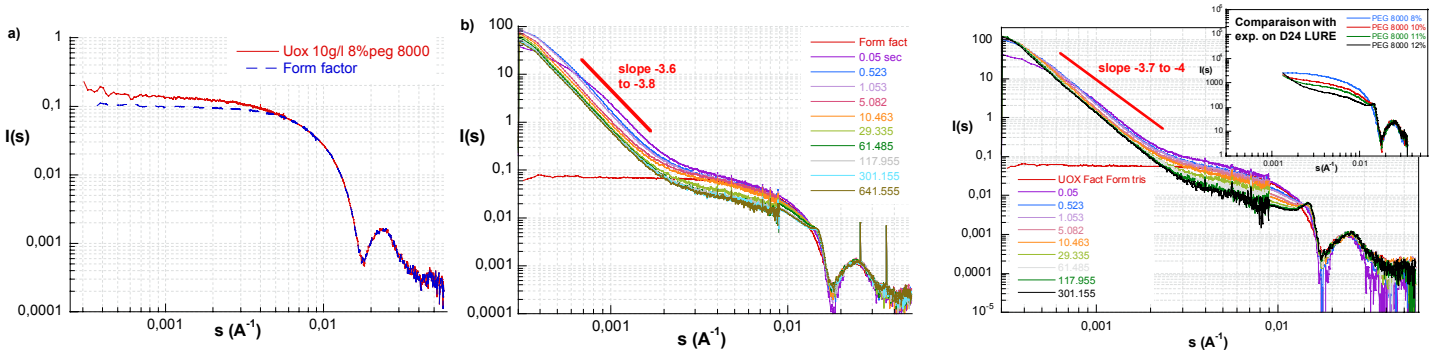

Fig.3: Scattering intensities of UoAf 10.5g/l as a function of % PEG from 1m50 to 10m a) 8%PEG8000, b)11%PEG8000and c) 12% PEG8000. In the inset comparaison with experiments performed at LURE on D24, which show the lower limits in low-q and large- q.

For example with 12%PEG (Fig.3c), the increase of the correlation peak as a function of time and the evolution of intensity with an exponent -4 in the very low-q domain seems indicating of globular domains (droplets which grow as a function of time).

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