ESRF	**Experiment title:** "Structure to function relationship of the chaperone alpha-crystallin"	**Experiment number**: Experiment SC-3247
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Shifts: 9 shift(s)	**Local contact(s)**: Louiza Zerrad, Petra Pernot, Adam Round	*Received at ESRF:*

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Report:

Cataract, eye lens clouding due to light scattering, is a leading cause of blindness and can result from protein condensation in hyperthermic and stressful conditions [i], when altered intermolecular interactions lead to dense phases that can compromise cell and organ function. Mammalian eye lens cells contain concentrated solutions of proteins called crystallins. Crystallins are divided in three main classes: α, β and γ crystallins. Among these, the most abundant are the α-crystallins, which are capable of chaperon-like activity, the ability to bind unfolded proteins to avoid their aggregation and precipitation in cell. When β and γ crystallin unfold during thermal, chemical or electromagnetic stress, they become substrates of alpha crystallin[i].

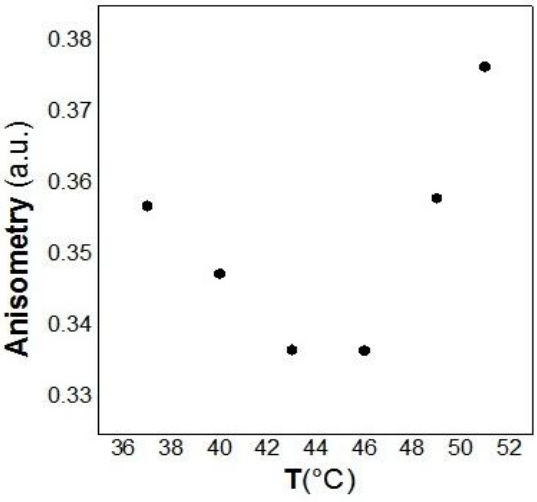

Fig. 1 Shape analysis of alpha crystallin *The anisometry of the particle, obtained from Dammin structures, decreases during the transition, this indicates a transition toward a more spherical form at 46°C, but since at 37°C and 51°C values are similar the protein maintains an elliptical symmetry before and after the transition.*

α−crystallin exhibits a phase transition at nearly T_C=45 °C, involving a quaternary structural modification and enhanced or reorganized hydrophobic surfaces[ii]. Beyond this temperature it has been demonstrated an enhanced chaperon activity of the protein[iii]. Furthermore it has been shown that also ATP activates the chaperon activity[iv]. We therefore studied temperature and ATP effects on alpha crystallin quaternary structure and on its interactions with gamma and beta crystallin, as their impact on lens transparency in both physiological and in hyperthermic conditions is an important step towards cataract prevention.

We have analyzed alpha crystallin behaviour at different Temperatures and we have seen a doubling in molecular weight of the protein beyond the Transition Temperature[v]. By means of the *ab-initio* software Dammin we have obtained tridimensional reconstructions of alpha crystallin and we have analyzed the anisometry of the quaternary structures (fig.1). Alpha crystallin has an ellipsoidal shape before and after transition, as can be deduced by tridimensional reconstructions[v] and by the higher values of anisometry (Fig.1). Interestingly, at 45°C the particle reaches the minimum value of anisometry which is a marker of a

more spherical simmetry. Hence we have hypotesized a rearrangment of subunits prior the acquisition of the activated form at high temperature[v].

We also studied the effect of ATP on quaternary structure of alpha crystallin and on interactions with γ–crystallins. We are currently analyzing interactions with β–crystallins. To obtain an overall shape analysis of alpha-gamma complexes (ratio 1:1 in concentration), we extrapolated P(R) distributions from SAXS data (Fig.2). General considerations about the shape of the protein can be deduced from the P(R): at 37°C both in presence and in absence of ATP, P(R) has a Gaussian shape indicating that the protein has a globular structure that is globally not influenced by the presence of the substrates. We are currently analyzing the structures at different temperatures, as we think that ATP could change the Tc value, stimulating an enhanced chaperone activity at lower temperatures.

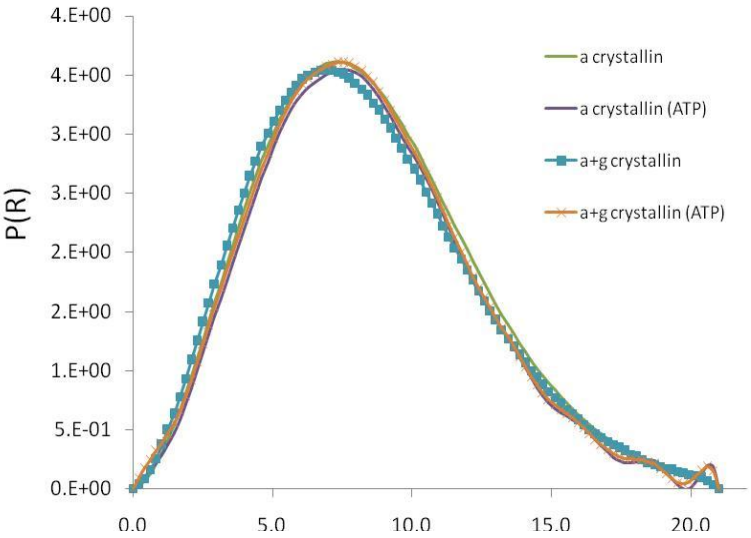

Fig. 2 P(R)distributions of alpha crystallin and mixtures of crystallins at 37°C. *The P(R) can give a overall shape information of the protein in solution. The form of the P(R) is similar for alpha crystallin alone and alpha and gamma crystallin mixtures, both in presence and absence of ATP in the buffer solution.*

If we consider alpha and gamma crystallin mixtures we see that there aren't evident structural changes. We obtained reconstructions of alpha crystallin and alpha and gamma crystallin complexes in presence and absence of ATP with the *ab initio* software Dammin (Fig.3). Alpha crystallin alone (Fig. 3a and 3e) and in presence of ATP at 37°C (Fig. 3b and 3f) shows almost an identic elliptical structure (NSD of aligned structures is 0.618). In presence of gamma crystallin (Fig. 3c and 3g) and in presence of both gamma crystallin and ATP (3d and 3h) the structures shows subtle structural modifications that we are currently analyzing. We think that changing the α/γ ratio would lead to deeper structural modifications of the protein complex, and to determine the interaction potential between α– and γ–crystallins. To achieve these necessary measurements, and to extend our results from suspensions to cytoplasmic extracts and lens, we submitted a proposal for SAXS beamline at ESRF on march 2012.

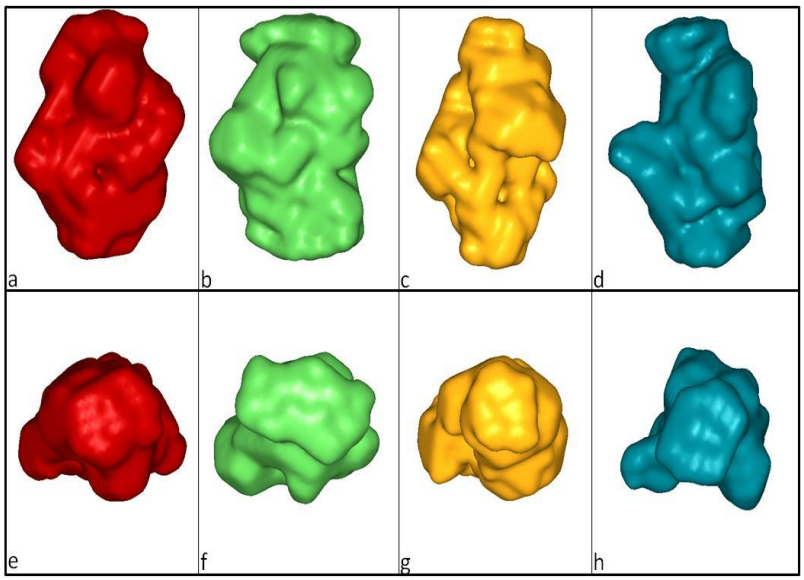

Fig.3 Dammin reconstructions of different complexes of alpha crystallin frontal (a,b,c,d) and lateral (e,f,g,h) view. *Alpha crystallin alone (a;e) and alpha crystallin in presence of ATP (b;f) show a similar elliptical structure. In presence of gamma crystallin (c;g) and in presence of both gamma crystallin and ATP(d;h) the structure shows subtle structural modifications that we are currently analyzing.*

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[v] Article submitted