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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

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All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** **Investigation of the temperature dependent interaction potential of cataractogenic protein α-crystallin**	**Experiment number**: MX-789
Beamline: ID 14-3	**Date of experiment**: from: 4/4/09 to: 7/04/09	**Date of report**: 26/02/2010
Shifts: 9	**Local contact(s)**: 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,ii], 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. Among those, the most abundant are the α-crystallins, which are globular, polydisperse, multisubunit, 800 kDa proteins with a diameter of about 18 nm, whose interactions are well described with a simple hardsphere colloid model[iii,iv].They exhibit a structural phase transition at nearly T_C=45 °C[v], involving a quaternary structural modification and enhanced or reorganized hydrophobic surfaces. α–crystallin aggregation is induced by heat and Calcium ions[3]. In particular, heat modifies the quaternary structure of α-crystallin[5], and Ca^{2+} decreases its thermal stability by promoting partial unfolding of the protein[vi]. Quantifying and understanding crystallin interactions and their impact on lens transparency, both in physiological and in hyperthermic conditions, is therefore an important step towards cataract prevention. Furthermore the characterization of the aggregation kinetics of the lens proteins is a fundamental tool in understanding the molecular origin of the disease, in which a subtle interplay between protein attractions, repulsions, and entropy governs condensation and the molecular mechanisms leading to protein aggregation.

We already followed by means of static and dynamic light scattering kinetics of aggregation in α-crystallin suspensions above and below *Tc* and we developed a kinetic model that describes the growth kinetics as a two step-process, a nucleation phase that leads to the formation of critical nuclei followed by an aggregation phase, in which the preformed critical nuclei are the basic aggregating units[vii]. The quantitative modeling of the kinetics by means of population balance equations (PBE) was combined with an extensive experimental investigation using light scattering techniques in order to determine the rate constants of both the phases of nucleation and aggregation (K_{nuc}, K_{agg}) and the size of the critical nuclei of the aggregation. Plotting the rate constants in an Arrhenius plot has shown that the aggregation kinetics are strongly influenced by the transition temperature *Tc* , as can be seen by the jump in the expected exponential trends at $1/T=1/Tc$ (Fig.1). From the same plot we obtained the free energies associated with the activation processes and we found that at *Tc* there is an increase in the free energies of activation with temperature, of 1.1 Kcal/mol for the nucleation process and 8.2 Kcal/mol for the aggregation process. The structural transition of α-crystallin is also accompanied by an increase in the dimensions of the critical nuclei from 23 nm to 27 nm, and by a contemporary decrease of their number. The overall result is the formation of a less number of larger and more

stable critical nuclei. In summary, α-crystallin above *Tc* exhibits a delay in the aggregation phase that preserves the lens from a premature opacification in hypertermic and stressful conditions, and can be related to the temperature dependent chaperone effect of the α-crystallin already observed in literature[viii].To study the effect of the transition on the Quaternary structure, X-Ray SAXS measurements were performed on α-crystallin suspensions at different temperatures (37-51°C).

The Intensity angular distributions are reported in figure 2. It is evident, increasing temperature, the increase of the intensity in the low s region ($s<0.28$ nm^{-1}) togheter with the decrease of the intensity in the high s region ($s>0.28$ nm^{-1}). The change in intensity distribution displays a clean isosbestic point at $s=0.28$ nm^{-1}.

In figure 3 the gyration radius (black circles), molecular weight (open squares) and anisometry (open triangles) of α-crystallin obtained with the software DAMMIN[ix], that recovers the shape of the particle from the scattering distribution profiles, are reported in function of temperature. Below *Tc* the α-crystallin oligomer shape has a bean-like structure, with an anisometry $(I_1-I_3)/(I_1+I_3)$ ~0.4 (where I_1 and I_3 are respectively the maximum and the minimum eigenvalue of the inertia tensor) . The radius is ~6.8 nm and the molecular weight is ~900 Kda. When T_C is reached, there is an abrupt increase of the radius (~8 nm) and of the molecular weight (~1600 Kda). The anisometry is 0.3, therefore the shape becomes more spherical. The DAMMIN 3D structures under and above T_C are reported in fig.2, respectively at the left and at the right of the red dotted line traced in corrispondence of T_C. It is possible to estimate from the ratio between molecular weight and the volume of the particle the density of the oligomer ρ, which is ~0.96 Kg/m^3 below T_C and ~0.80 Kg/m^3 over T_C.

X-ray SAXS measurements, performed above and below *Tc* at different concentrations (1-20mg/ml) don't reveal significative changes in the quaternary structure. The quaternary structure transition is reversible, and the transition kinetic is still under analysis.

These data are in correlation with our light scattering measurements that highlited a peculiar behaviour of the self-aggregation of α-crystallin. The structural quaternary modification, consisting in the formation of a bigger, but less-dense oligomer could in fact be linked to the protective mechanism that preserve crystalline and other unfolded proteins from aggregation. To gain further insights the first steps of heat and calcium induced aggregation below and above T_C need to be investigated by SAXS. We will expect a sharp reduction in the strength and the range of the attractive potential of both the protein-protein and nucleus-nucleus interaction potentials in correspondence of *Tc*. This would mean that the structural transition at *Tc* is linked to an allosteric chaperone function leading to the stabilization of the critical nuclei, with the overall effect of preserving the lens from the premature protein aggregation.

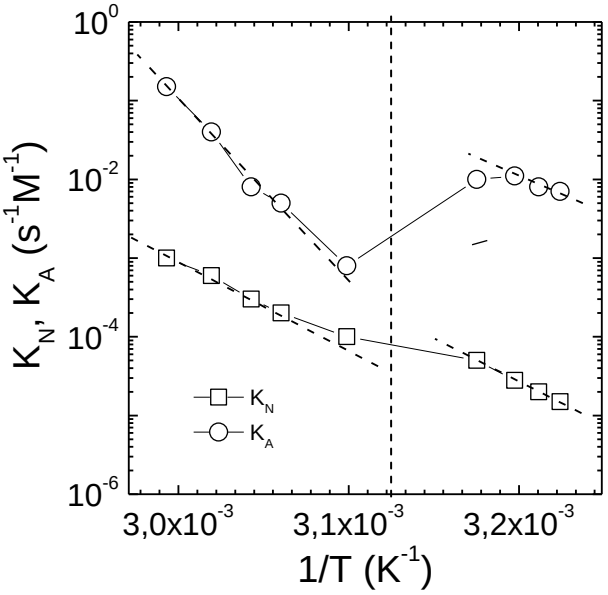

Figure 1. *Arrhenius Plot of the aggregation and nucleation rates K_{nuc} (squares), K_{agg} (circles) of α-crystallin suspensions ([α]=1.5 mg/ml in 10 mM Tris-HCl buffer, pH 7.4). The dotted line is traced at $1/T=1/T_C$*

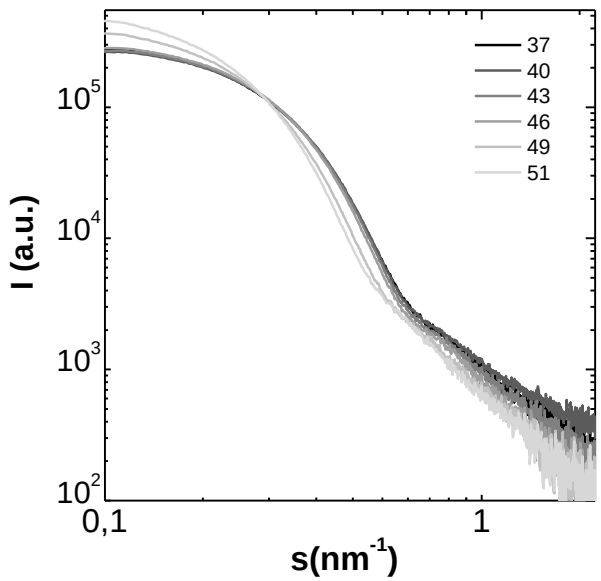

Figure 2. *X-ray solution scattering profiles of an α-crystallin solution (1.5 mg/ml) recorded at different temperatures (from 37° to 51°C).*

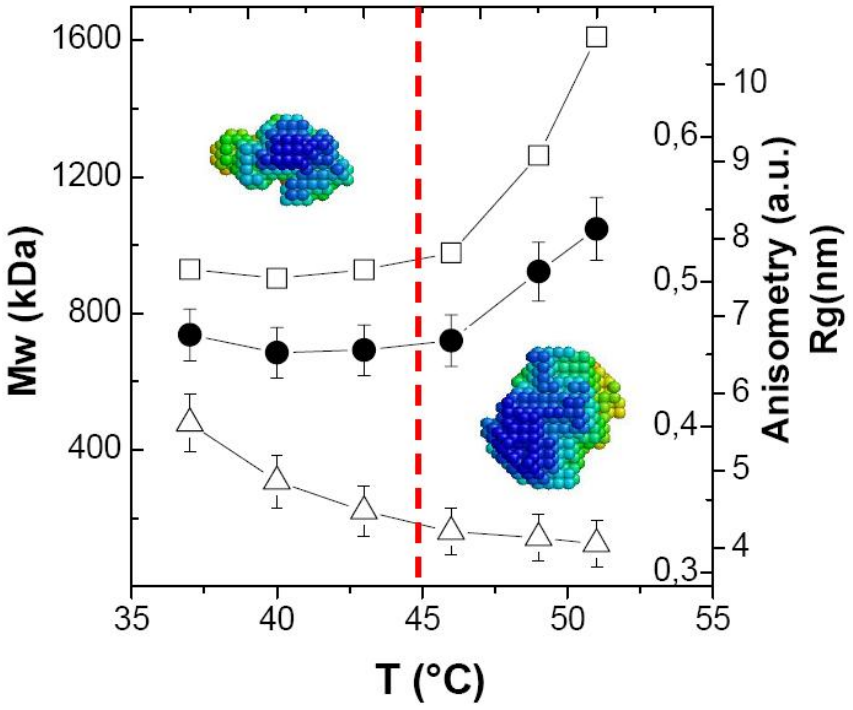

Figure 3. *Gyration radius (black circles), molecular weight (open squares) and anisometry (open triangles) of α-crystallin in function of temperature. Below Tc the α-crystallin oligomer shape has a bean-like structure, with an anisometry of about 0.4 . The radius is ~6.8 nm and the molecular weight is ~900 Kda. When T_C is reached, there is an abrupt increase of the radius (~8 nm) and of the molecular weight (~1600 Kda). The anisometry is 0.3, indicating that the shape becomes more spherical. The* DAMMIN *3D structures under and above T_C are also represented, respectively at the left and at the right of the red dotted line traced in corrispondence of T_C.*

REFERENCES

[i] H.Bloemendal et al., Prog.Biophys.Molec.Biol.86,407(2004)

[ii] F. Andreasi et al., Biophysical Journal, Vol. 69, 2720-2727 (1995).

[iii] A.Tardieu, International Journal of Biological Macromolecules 22, 211(1998)

[iv] S.Finet and A.Tardieu, J.Cryst.Growth 232, 40 (2001)

[v] M. T. Walsh et al., JBC Vol *266*, No. 30, Issue of October ' 25, PP, 20079-20084.(1991)

[vi] L.J. Del Valle et al, Biochimica et Biophysica Acta 1601 100– 109 (2002).

[vii] G.Maulucci, "Kinetics of nucleation and aggregation in α-crystallin suspensions" PhD thesis, Univ. "Roma 3"(2008)

[viii] B. Raman and C.R. Rao, PNAS Vol. 280, No. 23, Issue of June 10, pp. 21726–21730, (2005)

[ix] D.Svergun, *Biophys. J.*, **76**, 2879-2886 (1999)