ESRF	**Experiment title:** Structure and function of small Heat Shock Proteins under stress	**Experiment number**: SC2641
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Shifts: 9	**Local contact(s)**: M. Fernandez	*Received at ESRF:*

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Report:

The aim of the proposal SC2641 was to study the temperature and the pressure effect on the structural and functional properties of the small heat shock proteins (sHSP) which presents exceptional associative and chaperone-like properties, i.e. the ability to protect other proteins from various cellular stress.

The pressure was transmitted with a liquid medium (in our case, water). With this system, we were able to measure the scattered intensity from ambient temperature to 60-70°C and from ambient pressure to 300 MPa. The solutions were contained in a sample-container of about 100 µl.

SAXS was previously used to characterise the pressure and temperature transitions of native calf lens α-crystallins, recombinant human αB-crystallin and yeast HSP26 [1]. The recent collaboration with the group of P. Vicart allowed us to study the human αB-crystallin R120G mutant, which is responsible for a desmin-related myopathy and a cataract. Different αB-crystallins mutants have also been expressed and purified: R120D, R120C, and R120K. Mutation of the Arg120 residue in the human αB-crystallin sequence has been shown to be associated with a significant ability to aggregate in cultured cells and an increased oligomeric size coupled to a partial loss of the chaperone-like activity *in vitro* [2]. The ID2 setup allowed studying the temperature and pressure-induced structural transitions of the mutants through the exchange subunit process. The capacity to increase in size with temperature or pressure, while remaining soluble, had disappeared with the R120G mutant and was found reduced for the R120K and R120D mutants. The deficit of quaternary structure plasticity was well correlated with the decrease in chaperone-like activity previously observed, demonstrating the

importance of the R120 residue for structural integrity, both static and dynamic, in relation with function [3,4].

In the present study, small angle X-ray scattering was used to follow the temperature and pressure-induced structural transitions of human/bovine αA-crystallin and human Hsp27, 2 other members of the sHSP family. They are very important as cellular partner of αB-crystallin: αA-crystallin is eye lens specific whereas αB-crystallin is also found in other tissues (like muscle, heart, lung ...). These results allow us to compare their behaviour, reflecting their differences or complementarities in structure and function [5].

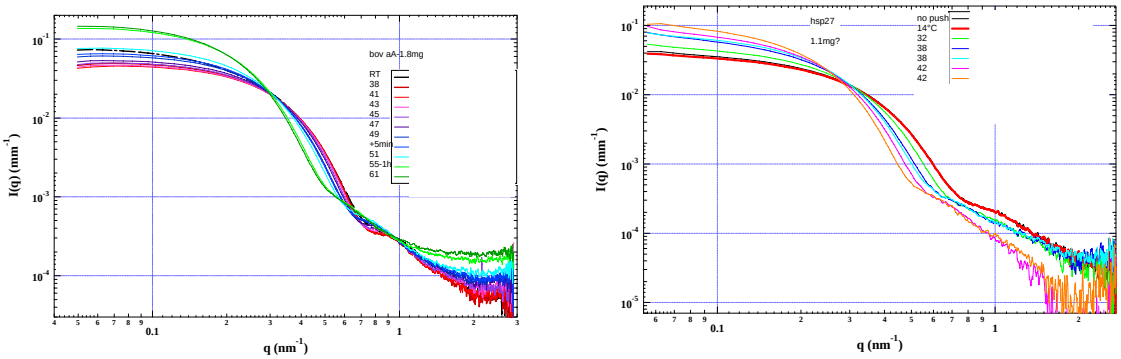

Figure 1. Temperature–induced structural transitions of bovine alphaA-crystallin (left) and human Hsp27 (right) as observed by SAXS.

Furthermore, in collaboration with the group of K. Lampi at the Oregon Health Science University, we have studied the temperature-induced aggregation of deamidated human βB2-crystallin and the incomplete rescue by α-crystallin chaperone [6]. These results allowed us to propose a potential mechanism for cataract formation in vivo which may involve the accumulation of deamidated β-crystallin aggregates.

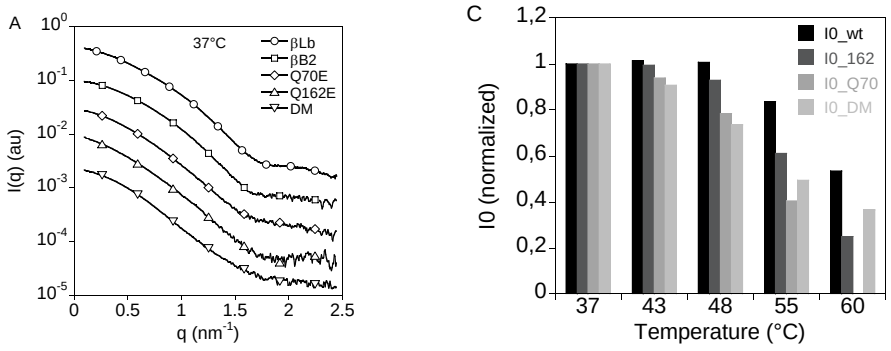

Figure 2. Normalised scattering intensity of betaB2-crystallins and deamidated mutants (left) and temperature–induced aggregation (right) as observed by the SAXS intensity extrapolated at zero angle.

[1] Skouri-Panet F, Quevillon-Cheruel S, Michiel M, Tardieu A and Finet S. (2006) sHSPs under temperature and pressure: the opposite behaviour of lens alpha-crystallins and HSP26. BBA 1764, 372-38.

[2] Simon S, Michiel M, Skouri-Panet F, Lechaire JP, Vicart P and Tardieu A. (2007) Residue R120 is essential for the quaternary structure and functional integrity of human αB-crystallin, Biochemistry 46, 9605-14.

[3] Michiel M, Skouri-Panet F, Duprat E, Simon S, Férard C, Tardieu A and Finet S. (2009) Abnormal behavior of αB-crystallin R120 mutants could originate from mutation induced modifications of the dimeric substructure. Biochemistry, 48(2):442-53

[4] Tardieu A, Michiel M, Skouri-Panet F, Duprat E, Simon S, Finet S, From sequence to pathology : the required integrity of the structure-dynamics-function relashionships at the molecular level, Small Stress Proteins in Human Diseases, Edited by S Simon and AP Arrigo, NovaSciences (NY) Ed. in press

[5] Finet S, Michiel M, Skouri-Panet F, Férard C, Tardieu A, Duprat E. in preparation

[6] Michiel M, Duprat E, Skouri-Panet F, Finet S., Tardieu A, Lampi KJ. Aggregation of deamidated human βB2-crystallin and incomplete rescue by α-crystallin chaperone, Accepted in Eye Experimental Research