ESRF	**Experiment title:** Small Heat Shock Proteins quaternary structure and gamma-crystallins denaturation in the pressure/temperature plane	**Experiment number**: SC2041
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Shifts: 9	**Local contact(s)**: S. Finet	*Received at ESRF:*

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Report:

The aim of the proposal SC2041 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 (among them, gamma-crystallins) from various cellular stress.

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 α-crystallins was known to increase in size from 60°C, the wild type αB-crystallin progressively increased in size with increasing temperature, from 43 to 60°C, before aggregating after 60°C, whereas the HSP26 dissociated into dimers. Similar but reversible transitions were observed as a function of pressure, from 250 MPa for the crystallins and 150 MPa for the 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 recently 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].

In the present study, small angle X-ray scattering was used to follow the temperature and pressure-induced structural transitions of human αB-crystallin and its R120G, R120D, R120K mutants. We have performed high temperature and high-pressure SAXS experiments of the

different protein solutions with the ID2 pressure setup. The solutions were contained in a sample-container of about 100 µl. 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 70°C and from ambient pressure to 300 MPa, as shown in the figure below.

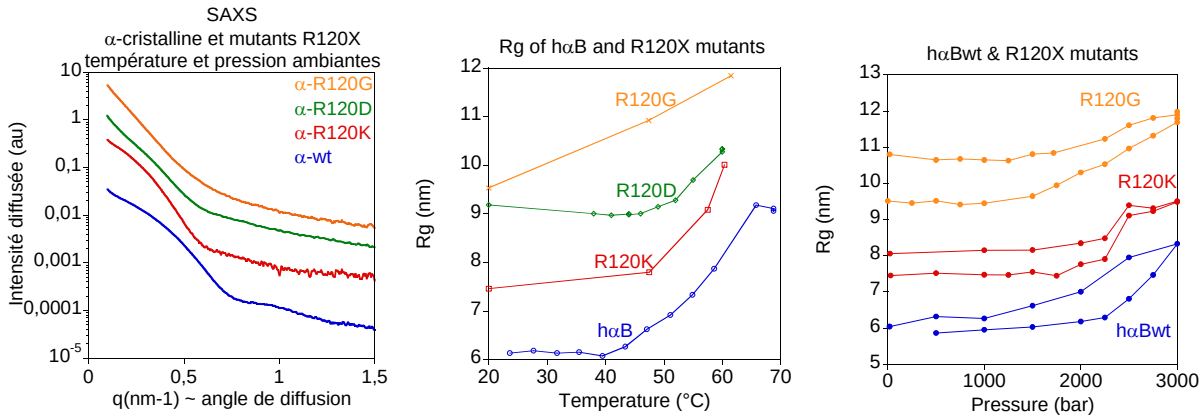

Figure. Temperature and pressure–induced structural transitions as observed by SAXS. (a) Normalized X-ray scattering intensity curves of hαB-WT, R120K, R120D and R120G mutants at ambient temperature and pressure. For the sake of clarity, the curves were vertically shifted. (b) Radius of gyration (R_g) corresponding to the temperature-induced transitions from ambient to 66°C. (c) Pressure–induced structural transitions of hαB-WT, R120K and R120G mutants as represented by the variation of R_g between 0.1 and 300 MPa. A complete hysteresis cycle is shown: the lower curves were recorded during the pressure increase; the upper curves correspond to the return to ambient pressure.

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 R120K mutant, which preserves the particle charge, was the less impaired. The deficit of quaternary structure plasticity was well correlated with the decrease in chaperone-like activity previously observed. Molecular dynamic simulations and *in silico* mutagenesis at the R120 position suggested a destabilization of the dimeric substructure by the R120 mutations. The whole of the results demonstrated the importance of the R120 residue for structural integrity, both static and dynamic, in relation with function [3].

[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. Abnormal behavior of αB-crystallin R120 mutants could originate from mutation induced modifications of the dimeric substructure. Submitted to *Biochemistry*.