ESRF	**Experiment title:** Protein stability and interactions in solution at high pressure studied by SAXS	**Experiment number**: SC 732
Beamline: ID2	**Date of experiment**: from: 15/11/00 to: 17/11/00	**Date of report**: 28/02/01
Shifts: 6 (block allocation with SC727)	**Local contact(s)**: S. FINET	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Jean-Louis HAZEMANN* & Denis RAOUX Laboratoire de Cristallographie BP 167 - 25 av. Des Martyrs – 38042 Grenoble CEDEX

Stéphanie FINET* ESRF BP 220 - 38043 Grenoble CEDEX

Françoise BONNETÉ, François GUYOT* & Annette TARDIEU Laboratoire de Minéralogie et Cristallographie - Case 115, 4 place Jussieu - 75252 Paris CEDEX 5

Michela PISANI* Istituto di Scienze Fisiche, Universita' degli Studi di Ancona, Via Brecce Bianche, I-60131 Ancona

Report:

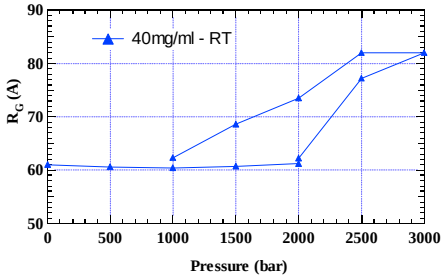
Two days were available for the phase separation project (SC 727) and two days for the high-pressure project (SC 732). Two series of experiments were done 15-16 September and 15-16 November 2000.

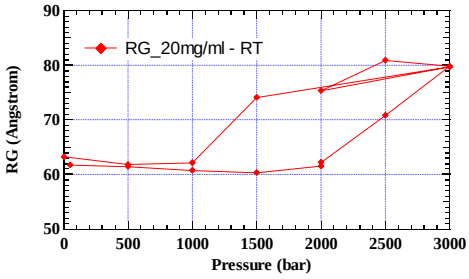
The aim of the proposal SC732 was to study the pressure effect on the interactions and the stability of the α-crystallins, one of the lens protein family, which presents exceptional associative and chaperone-like properties.

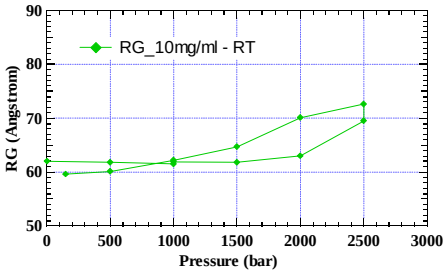
We have performed high-pressure experiments with solutions of α-crystallins at different protein concentrations. We used the available pressure cell on ID2, with diamond windows. The solutions were contained in a sample-container of about 100 µl. The pressure was transmitted with a liquid medium (in our case, water or alcohol). With this system, we were able to measure the scattered intensity from ambient pressure to 3000 bars.
These first results are particularly promising. We report below the variation of the radius of gyration (R_G) as a function of the pressure, for different protein concentrations. We performed the experiments from ambient

pressure, with a regular increase of pressure up to 3000 bars. The pressure was then decreased back to atmospheric pressure.

During the pressure increase, the radius of gyration remains constant up to 1000 bars, and then it significantly increases with pressure. The increase of the radius of gyration indicates an increase of the particle size. At high pressure, either the proteins experience pressure-induced aggregation or the stable state corresponds to a higher number of subunits, because of subunit exchange. Indeed, at room temperature and atmospheric pressure the alpha-crystallins are able to exchange subunits. In all the tested conditions, we then observed a decrease in radius of gyration as pressure decreases. However, the radius of gyration vs pressure diagrams clearly show some hysteretic behaviour above 1000 bar. In these experiments, we had only partial reversibility, since one shoulder in the intensity curves disappears at high pressure and is not recovered. These diagrams offer a first hint of pressure stability field of α-crystallins as a function of pressure







Before delivering a coherent model of the evolution of α-crystallins at high pressure, several parameters need to be analysed. First, with these preliminary experiments, we did not study the effect of the time, i.e. the rate of the aggregation phenomena. We recorded the scattering curves immediately after reaching the desired pressure. We also only began to study the effect of the temperature (10mg/ml, at 35, 45 and 60°C). A preliminary result showed that the combination of temperature and pressure favours the denaturation of the proteins. This point needs also to be further studied.

During these 2 days, we were only able to study one pressure scan at each protein concentration. Whenever we had to open the pressure cell (to change the gaskets or the sample solution), the diamond windows were displaced and had to be recenter to minimise the parasitic scattering (because of microdefects in the windows). Yet, because of the absorption of the windows, the radiation damage was reduced as compared with standard set-up (capillary for example), but the scattering intensity at low protein concentration was quite weak and the form factor was difficult to obtain.