ESRF	**Experiment title:** Dynamics structure of lens crystallins : pH study with time-resolved SAXS	**Experiment number**: SC 727
Beamline: ID2	**Date of experiment**: from: 02/09/00 to: 05/09/00	**Date of report**: 26/02/01
Shifts: 9	**Local contact(s)**: S. FINET	*Received at ESRF:*

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Report:

It is known that at acidic pH, the alpha-crystallins loose subunits and reorganize to form smaller size particles. We have used the stopped-flow equipment avialable on ID2 to follow the changes as a function of time after a pH jump from 6.8 to about 3.0.

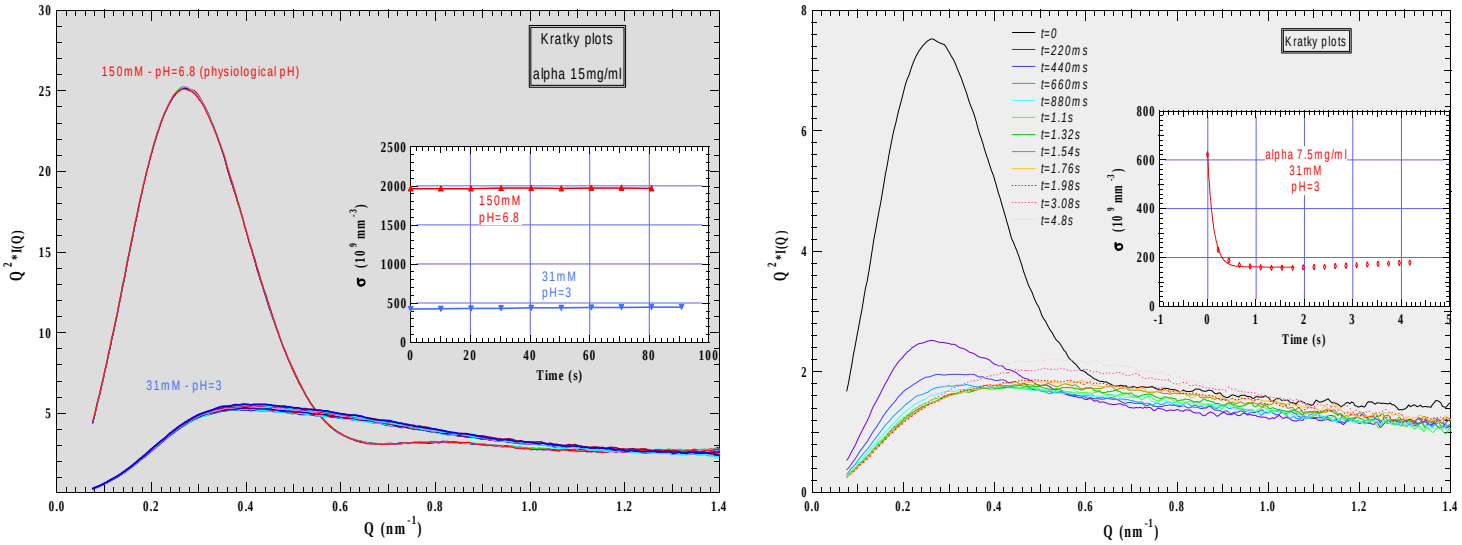
The idea was that by "peeling" the crystallins as a function of time and pH, we would be able to follow the reverse course of the building up of the alpha-crystallin oligomers and therefore to discriminate between the different quaternary structure models that have been proposed.

Kratky plots and the corresponding integrated intensities were used to follow the change in the quaternary structure of α-crystallins with pH jumps.

The figure 1 shows Kratky plots, $Q^2I(h)=f(Q)$, for static experiments at 15mg/ml. Each curve corresponds to 100ms exposure. In the Guinier region ($QR_G<1$), the radius of gyration R_G of globular particles is given by

$$I(Q) = I(Q=0) \exp(-R_G^2 Q^2/3)$$

The radius of gyration is stable with time. However, a small increase can be seen after a long exposure, which reveals the onset of protein aggregation due to the radiation damage. The inset depicts the corresponding integrated intensities $\sigma = \int_{Q1}^{Q2} I(Q)\, Q^2\, dQ$, with $Q_1=0.075$ and $Q_2=0.5$ nm^{-1}. For each static condition, σ remains constant.



The figure 2 shows Kratky plots of kinetic experiments for 7.5mg/ml, pH=3 & 31mM phosphate buffer. The time zero corresponds to the end of the mixing sequence. The corresponding σ is plotted as a function of time (diamond). We observed an important modification of the protein quaternary structure during the first second, and only a weak evolution after.

A single exponential fit to the time evolution of the integrated intensity (solid red line) gave the result:

$$\sigma(t) = k_0 + k_1 \exp(-k_2 \cdot t)$$

with the coefficients k_0=161.3, k_1=459.8 and k_2=8.3 s^{-1} (i.e. 0.33 s). The change in the Kratky plots (dashed lines) and the increase of σ in the last curves are attributed to radiation damage.

Yet, a precise model of the quaternary structure of the smaller particles was difficult to determine since the polydispersity was high. In addition, the smaller particles were more sensitive to radiation damage, since after about five seconds, a strong increase near the origin could be seen, indicating the onset of protein aggregation.

We therefore do not intend to pursue these experiments for the moment. We prefer focus on others proposals which have been proposed with crystallins.