ESRF	Experiment title: **Time-resolved diffuse x-ray scattering studies of retinal proteins**	**Experiment number**: CH2521
Beamline: ID09B	**Date of experiment**: from: 6th September 2007 to: 9th September 2007	**Date of report**: 25/08/2007
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Time resolved x-ray scattering has emerged as a powerful technique for studying the rapid structural dynamics of small molecules in solution [1-6]. The beamline ID09B at ESRF has seen most of this development and the ongoing technical developments at the beamline makes it possible to extend these methods to study protein dynamics. We aimed with this experiment (CH-2521) to take the step of extending our methodology developments within the area of small-molecule photo-chemistry [1-6] to study reaction dynamics of membrane proteins, which is our major focus for the laboratory [7-9]. Active membrane transport proteins are fundamental to the cell. They catalyze essential reactions such as the import of metabolites and export of waste, establish and harvest proton motive force in bioenergetics, and restore homeostasis following many cellular signalling and other essential processes. They are also major drug targets for improving human health.

The target proteins under study during this experiment were bacteriorhodopsin (bR) and proteorhodopsin (pR), both light-activated proton pumps residing in the membrane of certain bacteria. Several photo-intermediates in the photocycle of bR have been structurally determined, while the photocycle of pR (including its groundstate) remains unresolved. Hence, bR makes out a good validated system for a first attempt to directly visualising its reaction pathway, and in combining this within studies of pR breaks new ground.

To activate the proteins, a laser pulse (527 nm) of 125-250 μJ focussed on a capillary containing solubilised sample of either bR or pR, was used. The capillary was translated during data collection to avoid radiation damage.

For bacteriorhodopsin the following time points were collected: 2 μs, 20 μs, 65 μs, 200 μs, 650 μs, 2 ms, 20ms and 100 ms. In addition, a dark image (laser off, x-rays on) was collected before and after each set of time points. After subtracting the dark image the difference spectra were normalised, averaged and corrected for heating. This last correction is necessary because as the photo-excited protein molecules relax captured energy is dissipated into the surrounding solvent, which consequently expands [10]. Three difference spectra (after averaging) for bacteriorhodopsin are illustrated below:

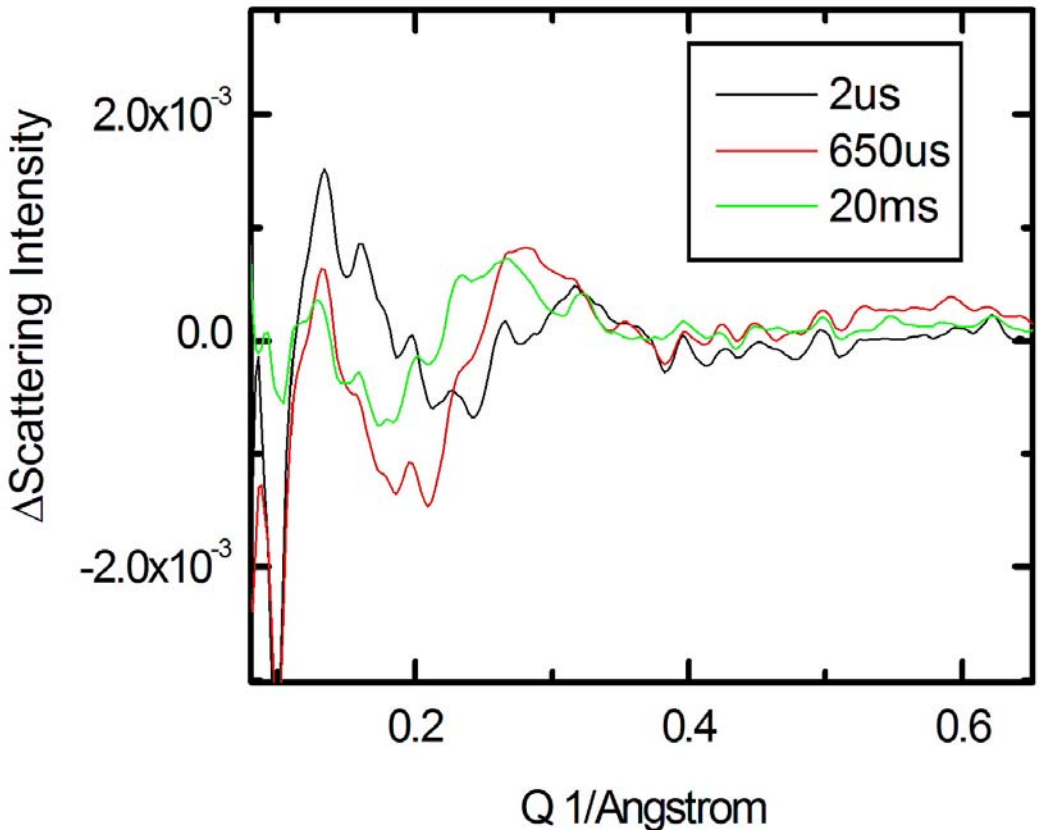

Fig2. The difference scattering for three time-delays (2 μs, 650 μs and 20 ms) of bR is depicted.

The resulting difference scattering curves for bR show that it is possible to follow the evolution of features associated with structural changes within retinal proteins. This time dependency agrees well with in-house spectroscopic data following the decay of spectral intermediates. We are currently working on assigning these wide-angle x-ray scattering different spectra with structural changes within the protein.

Similar experiments on proteorhodopsin gave the following signal:

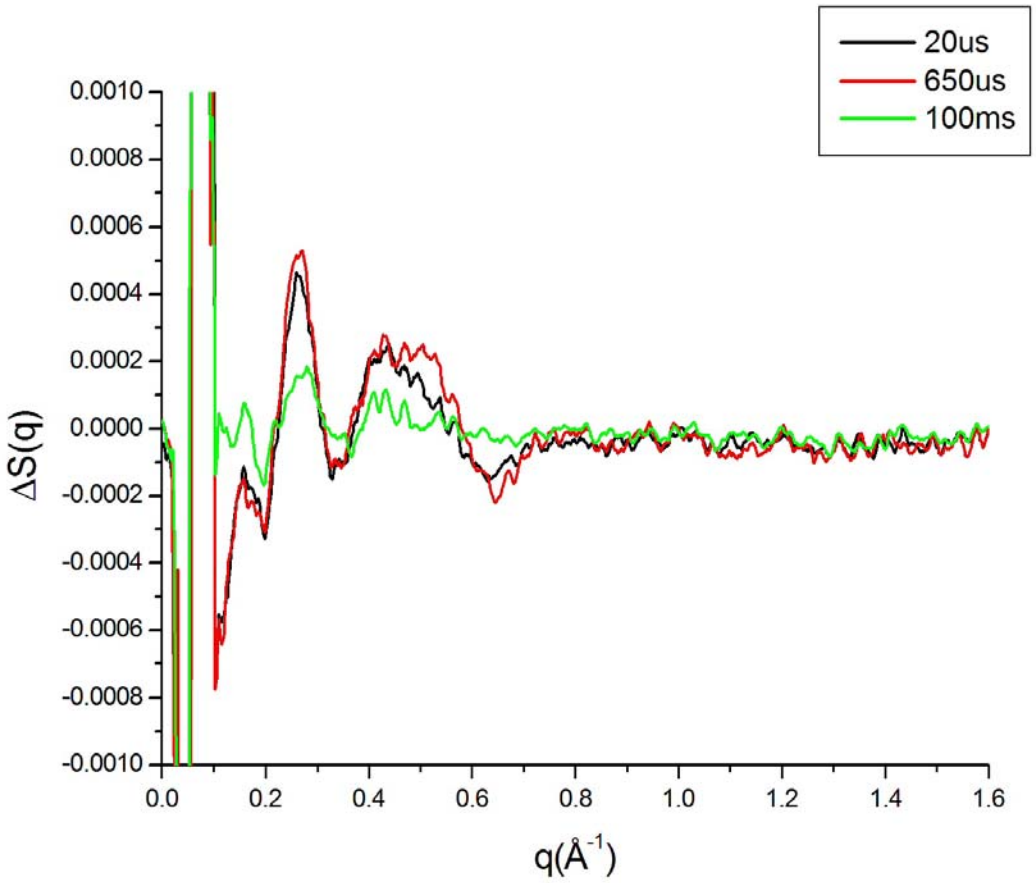

Fig3. The difference scattering for three time-delays (20 μs, 650 μs and 100 ms) of pR is depicted.

Again high-quality difference spectra could be resolved. Clearly, proteorhodopsin behaves somewhat differently when compared with bacteriorhodopsin. This was expected since the half-life of its photocycle is slower than that of bR as shown by in-house spectroscopic measurements. We believe that together the bR and pR experimental data along with its interpretation is sufficient to serve as a foundation for a publication.

During this experiment we also performed test-studies on the photosynthetic reaction centre, and this gave no structural changes, thus providing a good control. We also tried some preparative experiments on proteorhodopsin containing a heavy-atom substituted retinal. This latter experiment is preparative for future studies whereby heavy atoms introduced into the protein provide specific marker sites for highlighting structural changes.

We also made several technical tests, and realise that we have to develop a new sample environment if we are able to move to 1 kHz repetition rates for these experiments (and hence considerably better signal-to-noise) rather than the 10 Hz used in the current study. These innovations are now under development.

References.

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