ESRF	**Experiment title:** **Light driven structural changes in rhodopsins probed by time-resolved WAXS**	**Experiment number**: LS 2589
Beamline:	**Date of experiment**: from: 9 Nov 2016 to: 14 Nov 2016	**Date of report**: March 1st 2017
Shifts:	**Local contact(s)**: Martin Nors Pedersen	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Four protein samples were transported to the ESRF for this experiment. Visual rhodopsin was prepared in two detergents: CHAPS and DDM; and samples of a channelrhodopsin 2 chimer and sensory rhodopsin II were also prepared.

Many technical problems were encountered with the beamline. The first step was to black-out the hutch so as not to damage the visual rhodopsin samples (which are damaged upon exposure to light). This was successful. There were a lot of problems with aligning the back-stop for the X-ray beam due to the helium cone. It turned out that when the cone was filled with helium it expanded at the back and pushed the back-stop up against the detector, which tilted the back-stop and this caused very strong X-ray scattering onto the X-ray detector. This problem was eventually identified by the local contact (Martin Nors Pedersen) and the beamline scientist (Michael Wulff). However the level of X-ray scattering from the helium cone was still too strong for these studies, and eventually it was realized the thte problem was the choice of plastic material for the entry point of X-rays into the helium cone. This was replaced and from memory it was mylar which was acceptable. Finally we discovered that the choice of undulator spectrum allowed a lot of the second harmonic to be reflected from the mirror and contaminated our time-resolved difference spectrum. These problems took the first two full days to identify and solve.

Time resovled wide angle X-rays scattering data were recorded from all four samples, with priority initially being given to studies of visual rhodopsin since we had complementary data on an ultrafast time-scale collected at the LCLS. These studies went well, but we had the complication that samples could only be exposed onece to light otherwise they were damaged. Thus we could not reuse these samples. Good quality TR-WAXS data were recorded using rhodopsin isolated in two different detergents, and both gave good results in the X-ray scattering (**Figure 1A,B**). We are looking to merge these data with data recorded at the LCLS to make a paper during 2017.

We also recorded TR-WAXS data from both channelrhodopsin 2 and sensory rhodopsin II and it worked well. Both proteins were active and gave a structural signal. Scientifically we wish also to study the sensory rhodopsin II in complex with its transducer protein – and we could not do this since we did not succeed with the production of the transducer protein in advance of this experiment. For channel rhodopsin we had a construct which contained significant glycolyation and we have since established an expression strain without this problem. Non-glycolyated protein will be better for structural modeling whereas the glycolyated form may be difficult to recover a consistent structural interpretation of the difference WAXS data.

In other studies we have used an IR-illumination to heat the samples to get difference WAXS measurements of the effect of sample heating. In this study we did not bring the IR system but in the future it may be valuable to set this up. Overall a lot of time was lost due to technical difficulties and it would be good for ID09 to standardize these types of studies so as to use beamtime more effeciently. We would be willing to assist in this if possible.

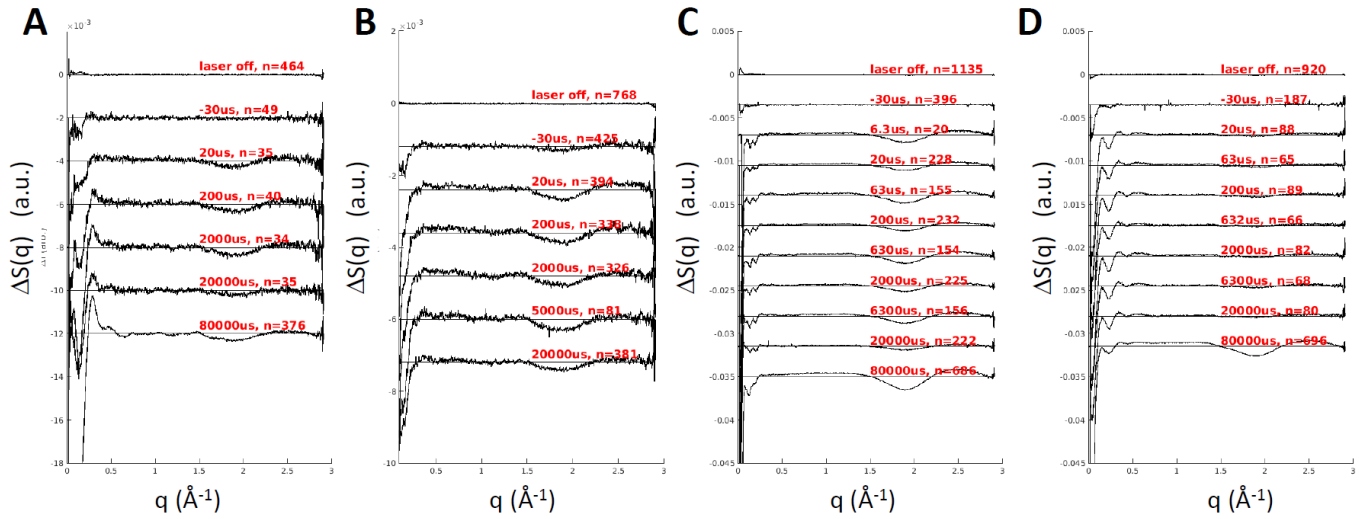

Figure 1: TR-WAXS data on visual rhodopsin in (**A**) DDM and in (**B**) Chaps; (**C**) TR-WAXS data recorded from channel rhodopsin II; (**D**) TR-WAXS data recordd from sensory rhodopsin II. The last points in all traces were steady state measurements to allow the influence of sample heating to be separated using linear algebra methods.