Interim report; Long term proposal LS-2051

Under our long term proposal 'Crystal structure of light activated states of rhodopsin', we have had three sessions with access to the microfocus technology. We had to test combined light-and-temperature protocols for activating rhodopsin molecules *in situ* in the frozen crystal lattice of the trigonal form, since the temperature threshold for various transitions in the photocycle are not known for the crystalline state. We were able to show on three separate occasions that, provided we can find a diffracting rhodopsin crystal 8-15 μm in width, the diffraction was preserved to 3.6Å resolution *post* treatment and a complete data set could be recorded from the treated crystal. One data set gave a sufficiently clear map, which however appeared indistinguishable from the ground state, indicating that the temperature during activation was sub-threshold for chromophore isomerization. The remaining data sets were rejected due to perfect merohedral twinning and crystal disorder, respectively. We aim to refine the light-and-temperature protocols for activation, by using microspectrophotometry to characterise the activation products in the frozen crystal lattice.

We need to find a way to have more reliable high resolution diffraction and therefore, in collaboration with Dan Oprian, we have started to work on recombinant rhodopsin. The material is only available in sub-milligram quantities, but we were still able to produce microcrystals and show that they diffract to high resolution. Our present assessment is that diffraction of these crystals is more reliable and these crystals will be a firm basis for further activation studies.

We have diverted some hours to explore 5-10 μm crystals of another GPCR, and obtained diffraction to 4Å resolution. Our preliminary data has helped that project to obtain separate ESRF support (MX-63).

Milestones achieved.

1. We have now completed the ground state structure of rhodopsin. A manuscript on the crystallisation and the microfocus strategy is ready for submission. A second paper on the detailed structure of rhodopsin in an untwinned $P3_1$ crystal form is being written. ID13 beamline staff member Manfred Burghammer will be a co-author.
2. Three data sets after illumination of rhodopsin at elevated temperatures were collected. Two were rejected due to merohedral twinning and crystal disorder; the third showed no difference to the ground state, indicating we must further explore the temperature effect on light activation.
3. Microcrystals of recombinant wild type rhodopsin and mutated rhodopsins showed diffraction to 3.5Å resolution. 10–20 degrees of data could be collected from a single crystal (smaller than 10μm).
4. A data set of rhodopsin in complex with a G protein peptide was collected.

Long-term access to the ID13 facilities was and is essential to the success of this project. We would like to thank Manfred Burghammer and Christian Riekel for their support and expertise.