ESRF	**Experiment title:** Progress in structural studies of LHCII and other micro-crystals of membrane proteins	**Experiment number**: MX-27 & MX-100
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Shifts: 2	**Local contact(s)**: Edward Mitchell , Stéphanie Monaco & Joanne McCarthy	*Received at ESRF:*

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Report:

Structural studies of LHCII and other micro-crystals of membrane proteins

The most abundant chlorophyll-containing protein in plants is the membrane protein light harvesting complex II (LHCII). This protein transfers absorbed light to the photosystem reaction centres where charge separation takes place. Moreover, LHCII has an important role in balancing the distribution of light to the photosystems, via a mechanism involving (de-)phosphorylation and (de-)oligomerization of LHCII.

To date the only known structure of LHCII from green plants is based on electron microscopy study of two dimensional crystals by Kühlbrandt and co-workers [1]. The model shows a trimer of LHCII molecules and has given insight into light harvesting by plants. However, the model does not include the N-terminal domain that contains the phosphorylation sites, is of modest resolution and lacks complete description of the various cofactors. We aim to obtain the high resolution structure of LHCII in various biological relevant states by using X-ray crystallography, in order to understand plant photosynthesis and the regulatory role of LHCII.

Bright green, small lozenge shaped crystals have been grown under a variety of conditions using vapour diffusion and micro-batch crystallization. These crystals, however, showed very poor diffraction characteristics. Under the present experiments, we have screened series of

additives and alternative crystallization conditions. These experiments have established a combination of detergents, additives and cryo-protectants that improved the diffraction limit from 20 Å to 6 Å. In the best case, diffraction spots to 3.7 Å were observed in a highly twinned crystal at the microfocus beamline ID13. Clearly further optimization is required. Currently, we are continuing to optimize the crystallization, paying attention to homogeneity and stability, crystallization kinetics and cryo-protection. Screening of additives is further extended. As the visual appearance of the LHCII crystals is not correlated with their diffraction properties, screening of crystals using synchrotron radiation remains required.

Structural characterization of a variety of other membrane proteins is undertaken, which include proteins from the (bacterio)rhodopsin, aquaporin, P-type ATPase and transhydrogenase family. Initial crystallization experiments giving promising leads have been optimized to micro-crystals. Under the present experiment, these micro-crystals have been screened to verify that the crystals are indeed proteins. Currently optimization of these crystals is on going, and a modest amount of access to the microfocus beamline ID13 will certainly be necessary as the programme develops.

References

[1] Kühlbrandt, W., Wang, D.N., and Fujiyoshi, Y.: Atomic model of plant light-harvesting complex by electron crystallography. *Nature* **367**, 614-621 (1994).