ESRF	**Experiment title:** Three dimensional crystals of the light-harvesting chlorophyll a/b protein complex from plants chloroplasts	**Experiment number**: LS 1613
Beamline: ID14 2	**Date of experiment**: from: 26-avr-00 8:00 to:27-avr-00 7:00	**Date of report**: 29/8/00
Shifts: 3	**Local contact(s)**: LESCAR Julien	*Received at ESRF:*

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Report:

Light-harvesting chlorophyll a/b protein complex (LHC-II) is the major collector of solar energy in all plants. It binds about half of the chlorophyll in green plants and it is probably the most abundant membrane protein on earth. LHC-II is a trimer in the photosynthetic membrane of nearly identical monomers, each consisting of 232 amino acids. Each monomer binds and orientates a minimum of 12 chlorophyll molecules, two luteins and a neoxanthin for light harvesting and energy transfer. Although the structure of LHC-II has been determined at 3.4 Å resolution by electron microscopy of two-dimensional crystals, this is not sufficient to allow a complete understanding of the mechanism of energy transfer from LHC-II to the reaction centre, since the effective resolution in the *z* direction is 4.9 Å. In fact the chemical difference between Chl *a* and Chl *b* (a formyl group instead of the methyl group at the 7-position in the chlorin ring of the Chl *a*) is too small to be detected at this level of resolution. In addition the orientations of the chlorophyll tetrapyrroles have not been determined unambiguously. This is important for understanding the photochemical processes of energy trapping and transmission. This project aims at solving the structure of LHC-II at high resolution so as to fully understand the mechanism of light-harvesting and energy transfer to the reaction centre in chloroplast membranes. LHC-II was purified from pea leaves by a standard procedure. Crystals grow by vapour diffusion in hanging drops. Hexagonal plates appear in a few days at 20 °C, measuring 0.2x0.2x0.01 mm. Crystals were frozen in liquid nitrogen after harvesting with a loop from the drops. However during the last

experiment performed at the ID 14 (endstation EH2, ESRF, France), it has not been possible to collect a complete data set. The crystals tested showed a too high degree of mosaicity to be useful for data analysis.

Recently we have been able to obtain small crystals from expressed LHC-II 25 Kda apoprotein in *E.coli*. After solubilisation of the inclusion body the apoprotein is bound to a Ni-column via a His-tag. Refolding of the protein, pigments binding and trimerisation occur during column elution with mixed lipid-detergent micelles (H. Rogl, K. Kosemund, W. Kühlbrandt, I. Collinson: *FEBS Letters* 432 (1998) 21-26). Eventhough these crystals are currently too small for data collection a further improvement of the size is expected.

On the basis of this work the energy transfer pathway from light-harvesting protein complex to the reaction centre can be solved and so make clear one of the most fascinating aspect which supports all life on earth.