ESRF	**Experiment title:Three dimensional crystals of the light-harvesting chlorophyll a/b protein complex from pea chloroplasts**	**Experiment number**: LS-1371
Beamline: ID-14 3	**Date of experiment**: from:8-Feb-99 8:00 to:9-Feb-99 8:00	**Date of report**: 16-Feb-99
Shifts:3	**Local contact(s)**: Dr. Laurence Dumon	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Matteo Lamborghini, Johan Ph. Zeelen and Werner Kühlbrandt

Max-Planck-Insitut für Biophysik,

Abt. Strukturbiologie

Heinrich-Hoffmann-Str.7,

D-60528 Frankfurt/M.,

Germany

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 and two carotenoids (lutein) 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 tetrapyroles have not been determined unambiguously. This is important for understanding the photochemical processes of energy trapping and transmission. This project aims to solve 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 standart 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. Recently, at the ID 14 (endstation E3, ESRF, France), one crystal only, over almost forty tested, was used for data collection under a stream of cold nitrogen. The complete data required a rotation range of 90° using 1° steps . The exposure time was of 240 seconds per image and reflections were recorded up to 3.4 Å. Evaluation of these data show a fairly large unit cell (a=b= 127.5 Å, c= 135 Å α=β=90° and γ= 120°, in the space group of P321or P622) and a quite high level of mosaicity (almost 2° as a preliminary estimation). Experimental conditions were optimised allowing us to find better concentration and application of the cryo protectant. Improvement on the data quality is expected to be collected using smaller oscillation range and increasing the exposure time per image. Other attempts to find a different protein source have been successful. Very recently LHC-II has been purified from spinach leaves and reproducibly crystallised under several different conditions. These crystals are larger in all three dimensions and preliminary cryo-experiments show diffraction better than 3.0 Å. On the basis of this work the energy transfer pathway from light-harvesting protein complex to the reaction centre, a question of outstanding interest, can be solved and so make clear one of the most fascinating aspect which supports all life on earth. Recently, there have been reports of light induced conformational changes in LHC-II and it may be possible to study these by X-ray diffraction of single crystals.