ESRF	**Experiment title: Determination of structures of Photosystem II proteins involved in oxygen evolution**	**Experiment number**: LS-2083
Beamline: ID141	**Date of experiment**: from: 10/2/02 to: 11/2/02	**Date of report**: 25/7/2002
Shifts: 3	**Local contact(s)**: Ed Mitchell	*Received at ESRF:*

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Report:

This project was designed to collect data from a number of proteins connected with the function of Photosystem II. The proteins that measurements were made on were:

1. The reaction center of Photosystem II (RCII) from spinach, dimeric form. RCII is a large membrane bound pigment protein complex (MW~300kDa). We have previously obtained crystals of a monomeric form of theis complex which diffracted to 6.5Å (at ESRF). In this experiment, isolated and purified a new dimeric form of RCII, which crystllized under different conditions. The crystals were small (>0.1 mm), and we were not able to obtain a diffraction pattern on our in-house diffractometer. We were succesful in our attempts at ESRF to mount the crystals after a breif treatment in silicon oil, the crystals appeared to freeze well, however the diffraction was poor, ~15Å at best. However, the experiment showed the feasability of using the dimeric form, which we are now working on to improve.

2. Phycocyanin and allophycocyanin from *Synechococcus vulcanus*. We have previously collected data to 1.5Å on phycocyanin (at CHESS). We attempted to obtain higher resolution diffraction, however none of the crystals diffracted better. We have also isolated allophycocyanin and obtained small crystals, which did not diffract.

3. The MntC solute binding protein, a manganese transport protein from *Synechocystis* PCC sp.6803. This protein was crystallized after overexpression. The crystals diffracted to 2.6Å, and we obtained a full data set to 2.8Å (Table 1). This has been used for structure determination using molecular replacement (Fig. 1), and a preliminary report has **been accepted for publication in Acta Crystallographica D**. The abstract of this paper is:

Preliminary X-ray crystallographic analysis of a soluble form of MntC, a periplasmic manganese binding component of an ABC-type Mn transporter from *Synechocystis* sp. PCC 6803

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Abstract

Manganese is recruited in microorganisms by way of ABC type transporter systems. We report here the expression, purification and preliminary crystallographic analysis of a soluble form of the MntC solute binding protein component of the MntABC manganese import system from the cyanobacterium *Synechococystis* sp. PCC 6803. The protein (321 amino acid residues) was expressed as a exclusively in inclusion bodies, which required unfolding and refolding in the presence of manganese, prior to purification. The purified protein was crystallized in the presence of PEG and zinc. The crystals belong to space group $P6_222$, with unit cell parameters of a=b=128.1Å, c=90.0Å with a single molecule in the asymmetric unit. The crystals diffract to 2.6Å under cryoconditions using synchrotron radiation.

We attempted to collect derivative data sets using lead and mercury derivatives, but appeared to have a problem with our back-soaks. Due to limited homology, the use of molecular replacement has led to only a partial solution (Fig.1). In order to proceed with structure determination we intend to collect multiwavelength data and determine the structure of the protein using MAD phasing

Table 1

Summary of crystal parameters and data collection statistics.

X-ray diffraction data were collected at ESRF using beamline ID-14, on an ADSC Quantum-4 CCD detector with X-ray radiation at λ = 0.933Å. Values in parenthesis are for the highest resolution shell (2.9-2.8Å)

Space Group	$P6_222$
Unit-cell dimensions (Å, ^{o})	a = b =128.1, c = 90.0, $\gamma = 120^o$
Resolution range (Å)	25 - 2.8
No. of reflections	204033
No. of unique reflections	11170
R_{merge} [a]	0.084
Completeness (%)	87.9 (93.6)
Multiplicity	4.7 (6.1)
I/σ(I)	9.7 (3.5)

[a] $R_{merge} = ((\Sigma_h\Sigma_i|I(h)_i - <I(h)>)/(\Sigma_h\Sigma_i|I(h)_i))$

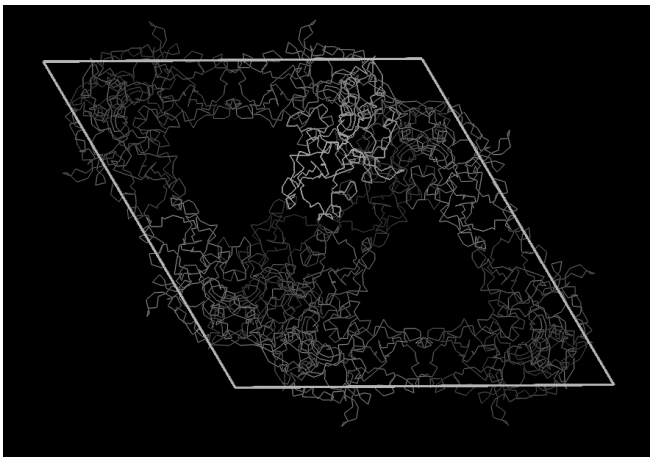

Figure 1. Crystal packing of the temporary MntC model (Cα trace) in the hexagonal unit cell, showing the crystal contacts. The figure was prepared with InsightII (MSI).

4. <u>The manganese stabilizing protein (MSP) from spinach RCII.</u> This protein is a putative "natively unfolde protein" or "molten globule" however we have obtained micro-crystals. No diffraction could be seen for a number of crystals we measured.

All together we performed measurements on more than 35 crystals during our 23 hour time slot, and collected 15 data sets.