



**Experiment title:**  
Crystallographic Investigations on Structure and Function  
of Photoactive Proteins

**Experiment  
number:**  
MX-134

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**Report:**

Photosystem II (PSII) is located in the thylakoid membrane of higher plants, algae and cyanobacteria that catalyzes the oxidation of water to atmospheric oxygen.

We are elucidating the three-dimensional structure of the PSII purified from the thermophilic cyanobacterium *Thermosynechococcus elongatus*. Up to now we obtained electron density maps at relatively low resolutions (Zouni *et al.* 2001), the most recent model determined at 3.2 Å resolution (Biesiadka *et al.*, 2004), collected during the same proposal (MX 134) period.

We analysed the cofactor composition of our protein samples and of re-dissolved crystals by reversed phase chromatography and other methods (Kern *et al.*, 2005). In particular we were interested to which extent the binding pocket of the plastoquinone-9 (PQ9) was occupied, as we could not detect PQ9 in our electron density maps. Our experiments suggest that only 50 % of the PSII reaction centres contain PQ9 bound into the Q<sub>B</sub> site (Kern *et al.*, 2005). Therefore we added different PQ9 homologues to our crystallization setups, to stabilize the binding region of latter cofactor.

Crystals grown in presence of PQ9 homologues, showed the same morphology as native ones. We collected 5 complete datasets with 5 different compounds. Inspection of electron density maps did not allow precise localization of the PQ9 homologues. One possibility might be that PQ9 homologues are rather unspecifically bound to the protein or integrated in the detergent belt surrounding the protein.

Furthermore we collected data of MHC class I molecules which can cause Ankylosing Spondylitis (AS) an autoimmune disease genetically linked to HLA-B27. The subtype HLA-B\*2705 of this MHC class I molecule is strongly associated with AS while HLA-B\*2709 is not. The only difference between the two alleles is the HLA heavy chain at position 116 (Asp in B\*2705 and His in B\*2709). We have collected diffraction data to high resolution and determined the structures of both subtypes in complex with a nonapeptide (mE) by molecular replacement.

**Table 1:** Crystallographic Data

	<b>HLA-B*2705 : mE</b>	<b>HLA-B*2709 : mE</b>
Space group	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
Unit cell ( <i>a,b,c</i> [Å])	51.0, 82.1, 65.3	51.0, 81.9, 65.4
Resolution [Å] <sup>a</sup>	30.0 – 2.0 (2.07 – 2.00)	30.0 – 1.4 (1.45 – 1.40)
Unique reflections	31317 (2869)	89668 (8610)
Completeness [%] <sup>a</sup>	94.8 (88.4)	95.1 (92.3)
I/σ <sup>a</sup>	10.0 (4.6)	15.5 (2.9)
R <sub>sym</sub> <sup>a, b</sup>	0.08 (0.251)	0.042 (0.47)

## Reference

Zouni, A., Witt, H.-T., Kern, J., Fromme, P., Krauß, N., Saenger, W., Orth, P. (2001) Crystal structure of photosystem II from *Synechococcus elongatus* at 3.8 Å resolution. *Nature* **409**, 739-743.

Biesiadka, J., Loll, B., Kern, J., Irrgang, K.-D. and Zouni, A. (2004) Crystal structure of cyanobacterial photosystem II at 3.2 Å resolution: a closer look at the Mn-cluster. *Phys Chem Chem Phys*, **6**, 4733-4736.

Kern, J., B. Loll, C. Lüneberg, D. DiFiore, J. Biesiadka, K.-D. Irrgang and A. Zouni (2005) Purification, characterisation and crystallisation of photosystem II from *Thermosynechococcus elongatus* cultivated in a new type of photobioreactor. *Biochim Biophys Acta* **1706**: 147-157.

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