# Application of Magnetic Circular Dichroism spectroscopy to the study of the OEC in Photosystem II from cyanobacteria and higher plants.


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## Photosystem II and MCD

Photosystem II (PSII) is the light driven water-splitting enzyme in all oxygenic photosynthesizing organisms. Each PSII core complex contains 35 chlorophylls and the Oxygen Evolving Complex (OEC), an $Mn_4O_5Ca$. PSII absorbs four photons for every molecule of oxygen released, transitioning to a distinct S-state with each photon absorbed. **Figure A**

It has previously been shown that the OEC absorbs in the near infrared [1]. Changes in the EPR spectrum in the S2 and S3 states are induced by illumination with light between 720 and 850nm at low temperature. The action spectra for both the S2 and S3 state (**Figure I**) has a maximum at ~760nm. A good analogue for the PSII OEC is oxidised manganese catalyse, which contains two μ-oxo-bridged manganese (III). The MCD shows a weak negative absorption between 670 and 800nm[3].

Absorption from the OEC was expected to be too weak to be measured through basic optical spectroscopy, with a molar extinction coefficent of >100, compared to $10^6$ for chlorophyll. We therefore used circular dichroism (CD) and magnetic circular dichroism (MCD) to search for this absorption.

Chlorophylls give a B-term MCD signal, which increases linearly with applied magnetic field, and follows the total optical absorption, which is largely temperature independent between 1.8K and 40K [4]. This allows us to distinguish between absorption from chlorophyll and from the cytochromes and the OEC, which are highly temperature dependent and not linear with increasing magnetic field.

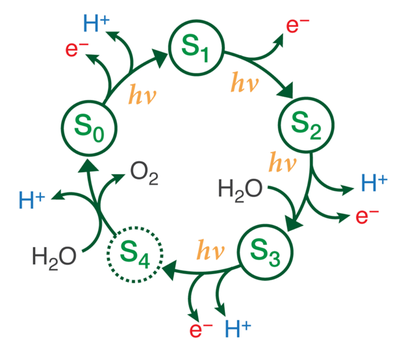


**Fig. A**
Basic overview of the Kok cycle

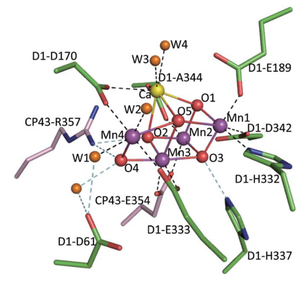


**Fig. B** Local structure of the $CaMn_4O_5$ cluster [2]

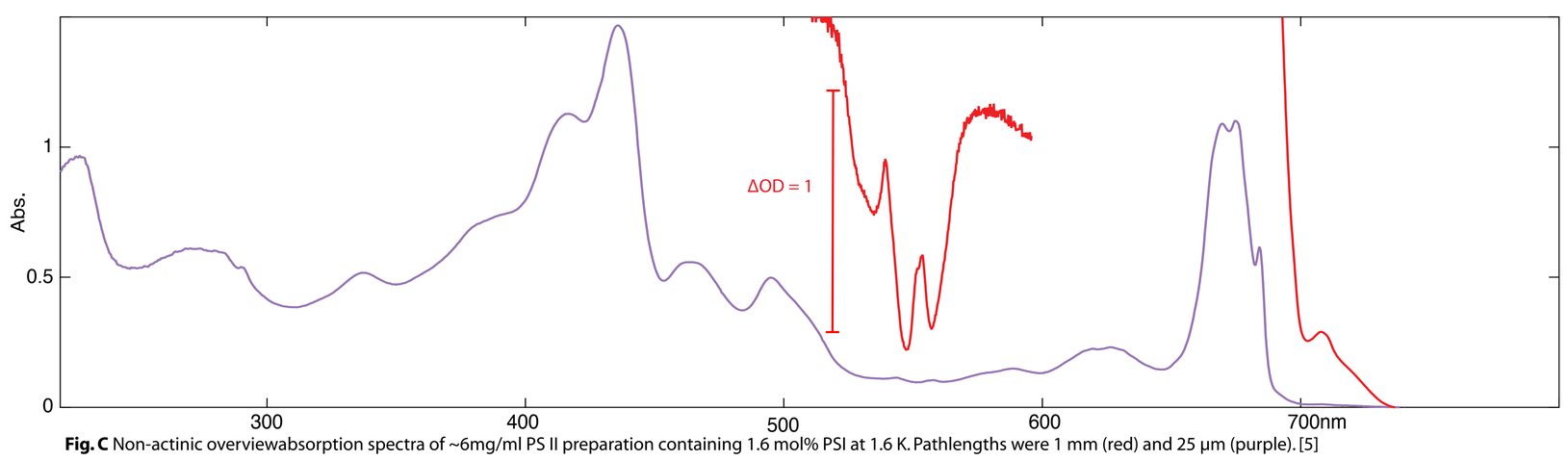


**Fig. C** Non-actinic overviewabsorption spectra of ~6mg/ml PS II preparation containing 1.6 mol% PSI at 1.6 K. Pathlengths were 1 mm (red) and 25 μm (purple). [5]

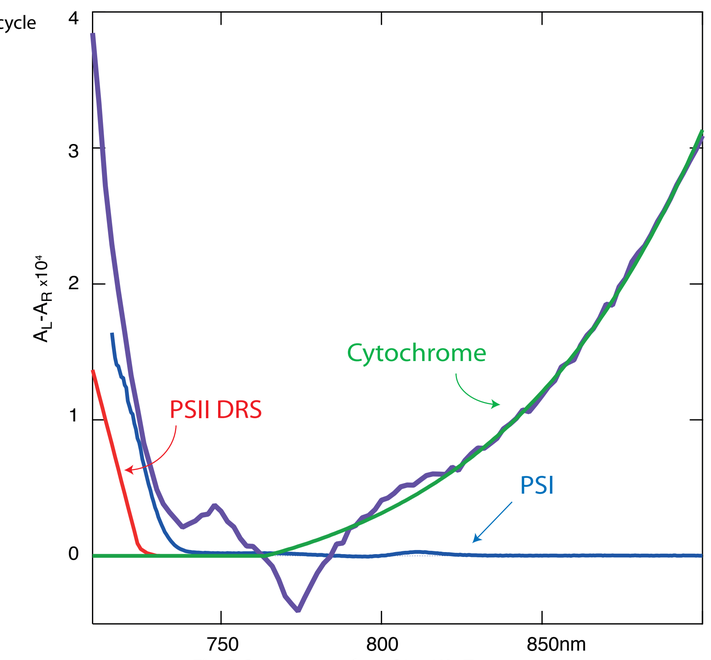


**Fig. D** Breakdown of the S2 1.8K 5T sprectrum

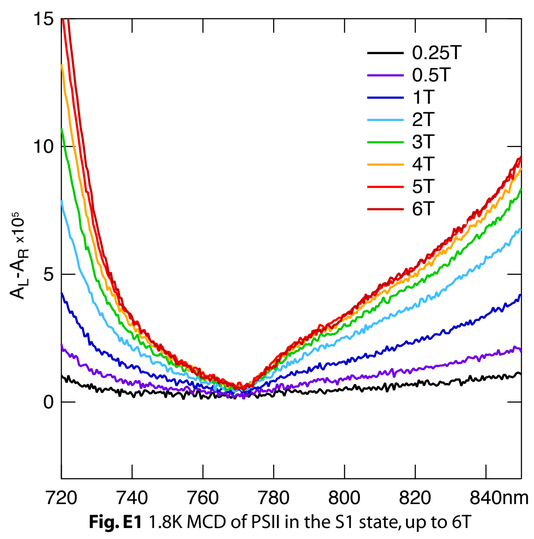


**Fig. E1** 1.8K MCD of PSII in the S1 state, up to 6T

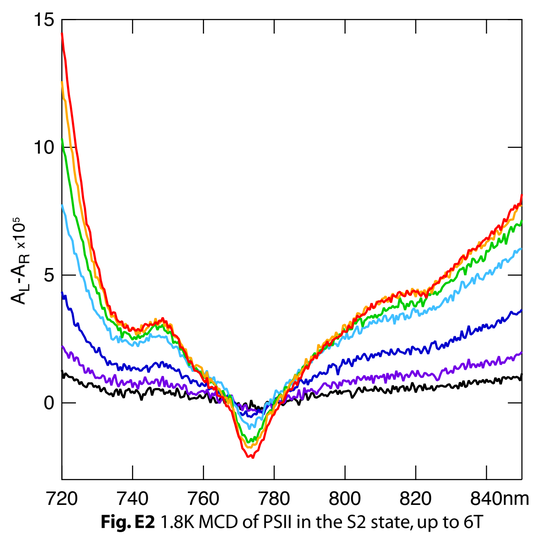


**Fig. E2** 1.8K MCD of PSII in the S2 state, up to 6T

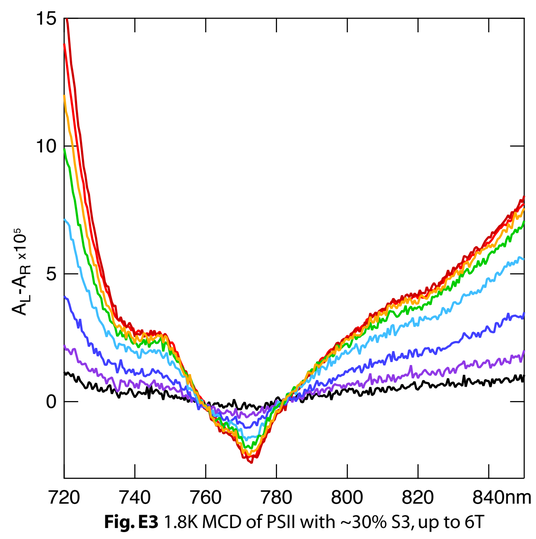


**Fig. E3** 1.8K MCD of PSII with ~30% S3, up to 6T

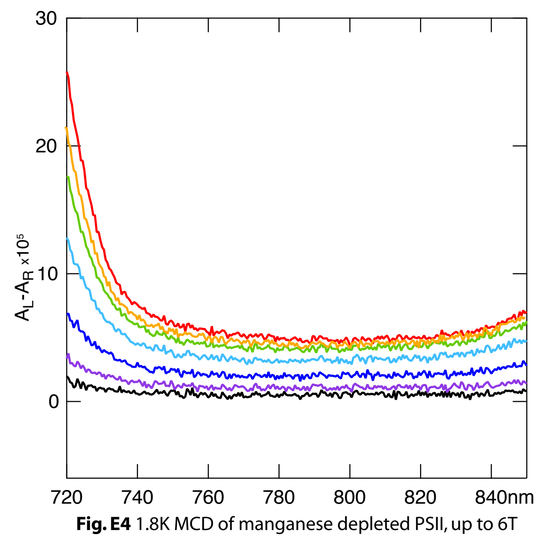


**Fig. E4** 1.8K MCD of manganese depleted PSII, up to 6T

## Results

At wavelengths shorter than 710nm, absorption from the chlorophylls of PSI and PSII dominates, obscuring absorption from the OEC [5]. PSI has a distinctive strong negative CD and positive MCD, peaking at 710nm and extending to 740nm. A weaker positive MCD band that extends to 900nm and a derivative feature at 800nm, assigned to P700+, can be seen in measurements of isolated PSI trimers. [5, 7]

A broad absorption from the PSII deep red state (DRS) extends out to 725nm [5]. As the MCD of PSI and the PSII DRS are B-terms, we can identify a temperature dependent absorption band underneath. A combined ligand to metal charge transfer band (LMCT) from cytochrome c550 and b559 give a strong positive MCD and extends from 800nm onwards, and peaks at 1540nm[5]. Both the cytochrome LMCT band and the OEC signals are highly temperature dependent and show a similar field dependence, making it difficult to uncouple the two overlapping absorptions. **Figure D** shows the best estimate of the LMCT absorption in this region, along with the absorption from PSI and the DRS.

Field dependent MCD spectra of Photosystem II from *T.vulcanus* the S1, S2 and S3 states are shown in **Figure E1**, **E2** and **E3**.[5] Washing the PSII core sample with 1M Tris removes the OEC, along with cytochrome c550. This results in the loss of the sharp 772nm feature as shown in **Figure E4**, as well as half of the cytochrome LMCT MCD. These results confirm that this feature has to be associated with the OEC.

The S2 spectra shows a sharp positive feature at 748nm and a negative at 772nm on transitioning from S1 to S2. The VTVH curve for these bands fit the analytical solution for an S=1/2 system.[6] Illumination with a Ti:S laser at 780nm results in a depletion of the 772nm band and the creation of another band at ~785nm, shown in **Figure F**, which saturates significantly faster with increasing field. This is consistent with EPR experiments showing a conversion from the g=2 split signal to a g>4.1 signal upon low temperature illumination [1].

The transition from S2 to S3 results in a broader negative band at 763nm shown in **Figure E3**. The conversion rate to S3 in this sample is ~30%. The S3 feature saturates faster with increasing field than the S2 g=2 signal.

Figure F is the 5T 1.8K spectra of PSII cores from spinach in the same region. A distinct positive band at 786nm in the S1 spectra is lost on conversion to S2. The negative band in the spinach S2 spectrum occurs at the same wavelength as in the T.vulcanus sample (772nm), but is approximately 5 times weaker.

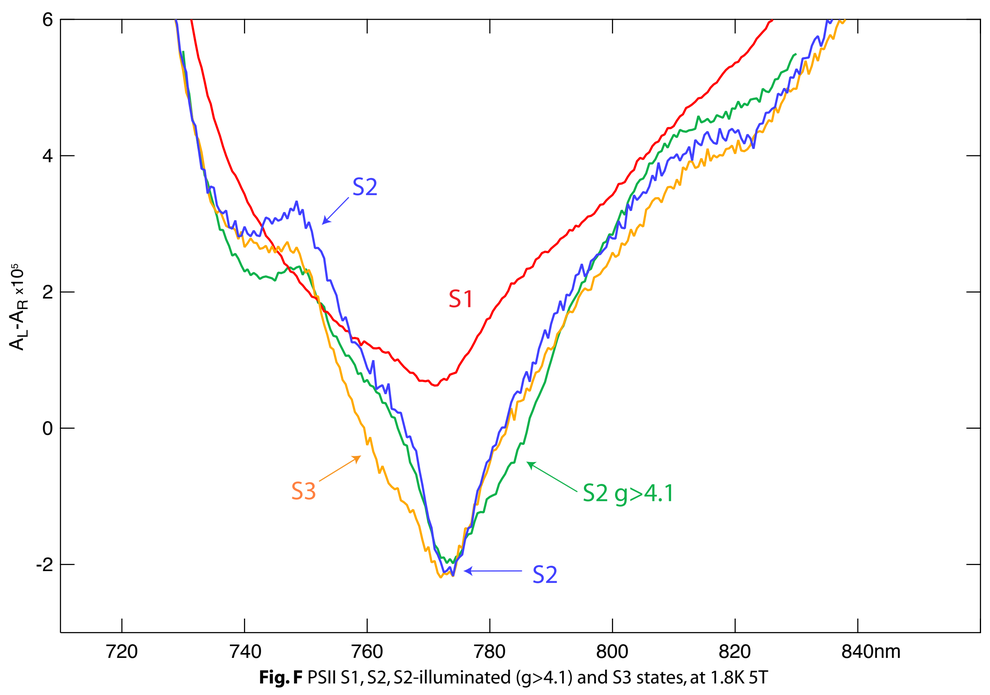


**Fig. F** PSII S1, S2, S2-illuminated (g>4.1) and S3 states, at 1.8K 5T

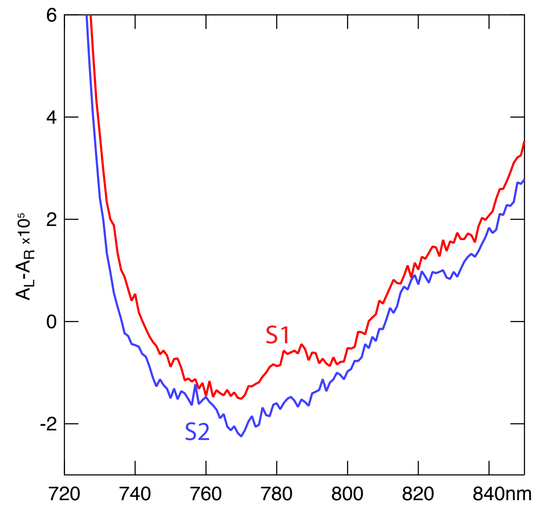


**Fig. G** 1.8K 5T MCD of spinach PSII cores in the S1 and S2 state

## Further work

Higher S-state measurements are pending, along with studies of PSII from other organisms and from modified PSII cores. Studying the magnetic properties of the OEC can give us insights into the mechanism of oxygen evolution, as the geometry and overall oxidation state of the OEC is still a matter of debate [9]. Our experimental results will act as constraint on the various models of water oxidation.

The procedures we have developed to study Photosystem II can also be applied to other metalloenzymes.


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