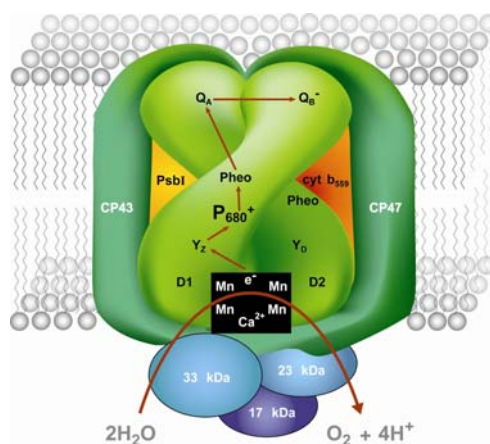


# 1. Photosynthetic Water Oxidation

Supervisor: [Warwick Hillier](#)

## Photosynthetic Water Splitting – How Atmospheric Oxygen is Made

Water is a ubiquitous solvent in biology and is the stuff of life. Biological proteins are surrounded, solubilised and indeed stabilised by this molecule. A few select enzymes utilise water as a substrate, and to study them requires methods that can discriminate the substrate water from the solvent water. We have developed a  $^{16}\text{O}/^{18}\text{O}$  stable isotope exchange assay which probes the binding of the substrate water to the oxygen evolving complex (OEC) in Photosystem II (PSII). We know from previous studies that there are two independent sites but do not know exactly where these sites are located in the OEC. Using the  $^{16}\text{O}/^{18}\text{O}$  isotope exchange assay we hope to gain further insight into the nature of substrate water binding and the mechanism of photosynthetic water splitting through studies of: (1) site-specific protein mutants of the OEC in cyanobacteria; (2) bioinorganic mutants of the catalytic core in the OEC; (3) the interaction of various inhibitor molecules that perturb the water splitting reaction; and (4) secondary isotope effects using deuterated water. This project involves mass spectrometry and aspects of various biochemical and biophysical techniques.

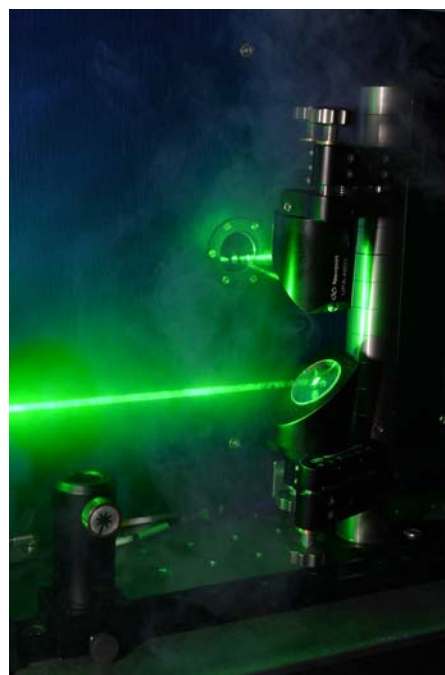


### Example

Hillier, W. and Wydrzynski, T. (2008)  $^{18}\text{O}$ -Water Exchange in Photosystem II: Substrate Binding and Intermediates of the Water Splitting Reaction. *Coordination Chemistry Reviews* 252, 306-317.  
<http://dx.doi.org/10.1016/j.ccr.2007.09.004>

## Protein Dynamics – Controlling Enzymatic Catalysis

Infrared spectroscopy gives information on the vibrational modes of chemical bonds. In biology it can be used to determine the secondary structure of a protein (i.e. the  $\alpha$ -helix,  $\beta$ -sheet and random coil content), as well as the structural dynamics of enzymatic proteins, where the movement of amino acid side chains is part of the catalytic mechanism. We use this technique to study metallo-proteins such as photosystem II, cytochrome *c* oxidase, superoxide dismutase, catalase, and bacterioferritin. The infrared snapshots of reaction intermediates give us a powerful insight into how protein chemistry works at the molecular level. This project involves FTIR (Fourier Transform Infra-Red) spectroscopy and biochemical preparation of samples.



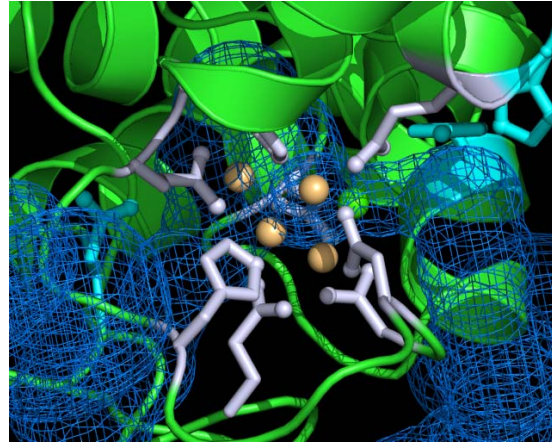
### Example

Strickler, M.A., Hillier, W. and Debus, R.J. (2006) No evidence from FTIR spectroscopy that Glutamate 189 of the D1 polypeptide ligates the Manganese cluster in Photosystem II. *Biochemistry* 45, 8801-8811.

<http://dx.doi.org/10.1021/bi060583a>

## Oxygen and Water Channels in Proteins – Maximizing Chemical Reactions

Photosystem II intakes  $\text{H}_2\text{O}$  and oxidises it to  $\text{O}_2$  while cytochrome *c* oxidase intakes  $\text{O}_2$  and reduces it to  $\text{H}_2\text{O}$ . Like many proteins the reaction needs to be controlled and regulated. Part of this regulation and control must occur through substrate entry and product exit into and out of a buried catalytic site. We are interested in developing a project to study the channels and substrate access in PSII and other proteins. This project will involve making mutants of amino acid residues that form the proposed channels and will involve computational analysis of substrate and product trajectories through the protein matrix.

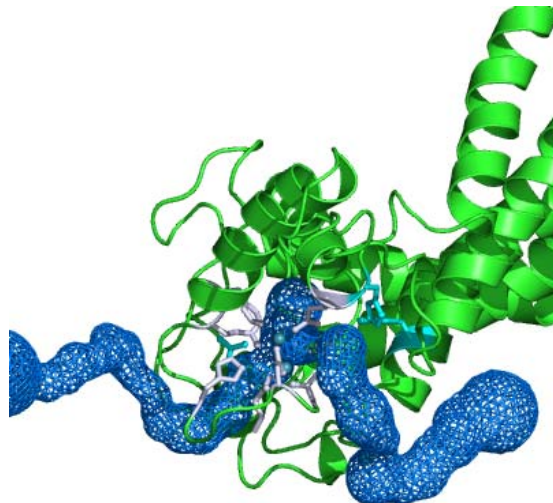


### Example

Murray, J.A and Barber, J (2007) Structural characteristics of channels and pathways in photosystem II including the identification of an oxygen channel. *Journal of Structural Biology* 159, 228–237  
<http://dx.doi.org/10.1016/j.jsb.2007.01.016>

Ho, F.M and Styring, S (2008) Access channels and methanol binding site to the  $\text{CaMn}_4$  cluster in Photosystem II based on solvent accessibility simulations, with implications for substrate water access. *Biochim Biophys Acta* 1777, 140–153  
<http://dx.doi.org/10.1016/j.bbabi.2007.08.009>

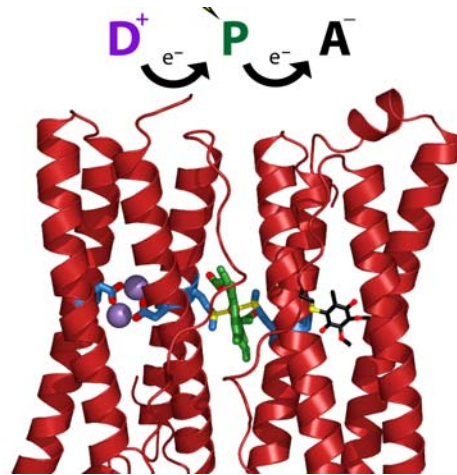
### Water channel on right



## 2. Protein Engineering

### Engineering Model Photoactive Proteins – Artificial Photosynthesis

With the recent advances in the understanding of the molecular structure and function of Photosystem II and hydrogenase enzymes, the time is now ripe to apply this knowledge in the development of photocatalysts for the production of carbon-neutral solar fuels such as hydrogen. By introducing light-activated cofactors based on Photosystem II into well-defined protein scaffolds, such as bacterioferritin, we can evaluate those protein interactions that are essential for the efficient splitting of water into H<sub>2</sub> and O<sub>2</sub>. If this can be achieved, then an ideal fuel cycle can be envisioned where H<sub>2</sub> with O<sub>2</sub> are generated from water in the light and energy is released when the H<sub>2</sub> and O<sub>2</sub> are combusted into water.



#### Reference

Hay S, Wallace BB, Smith TA, Ghiggino KP and Wydrzynski T (2004) Protein engineering of cytochrome b562 for quinone binding and light-induced electron transfer. *PNAS* *101*: 17675-17680.  
<http://dx.doi.org/10.1073/pnas.0406192101>

Wydrzynski T, Hillier, W. and Conlan B (2007) Engineering model proteins for Photosystem II function. *Photosynthesis Research* *94*, 225-233.  
<http://dx.doi.org/10.1007/s11120-007-9271-0>

## Re-Engineering Metal Binding Sites in Proteins – Why the Protein is Important

Biology has a raft of redox active metallo proteins critical for the bio-energetic reactions in cells. We are interested in identifying metal specificity in catalytic pockets and changes to the amino acid ligands upon metal oxidation (i.e. the movement of side chains and redistribution of charge) that are inherent to the mechanisms of catalysis. This study is approached by amino acid modification and spectroscopic analysis of the modified proteins.



### Example

Conlan, B., Hillier, W. and Wydrzynski (2007) Engineering model proteins for Photosystem II function. *Photosynthesis Research* 94, 225-233.

<http://dx.doi.org/10.1007/s11120-007-9271-0>

## **Engineering Cobalt proteins**

Recent discovery of a water oxidation catalyst using cobalt raises some intriguing questions about the catalytic activity of this element. Biology does not make extensive use of cobalt as is found in relatively low abundance. However, we would like to explore cobalt chemistry in protein as it may provide novel and interesting reactivity. This project will employ synthetic biology and molecular engineering of proteins to make a water oxidation catalyst.

### Example

Kanan, M.W. and Nocera, D.G. (2008) In Situ Formation of an Oxygen-Evolving Catalyst in Neutral Water Containing Phosphate and  $\text{Co}^{2+}$ . *Science* 321: 1072-1075

<http://dx.doi.org/10.1126/science.1162018>

## **Respiratory Oxygen Reduction – The Powerhouse of Aerobic Life**

Molecular oxygen ( $\text{O}_2$ ) is a thermodynamically reactive molecule once kinetic barriers are breached by supplying a sufficient activation energy. Over the course of billion years, biology has evolved several systems to utilise  $\text{O}_2$  from the atmosphere to drive chemical reactions. We are interested in studying and modifying enzymes that react with  $\text{O}_2$ . Various research projects may be built around the proton back-leak in proton pumping oxidase,  $\text{O}_2$  mismatch chemistry, or substrate ( $\text{O}_2$ ) affinity in oxidase enzymes. A number of experimental approaches will be applied including mass spectroscopy.

### Reference

Hillier W (2008) The Significance of  $\text{O}_2$  for Biology. *Encyclopedia of Ecology* (Ed) Sven Erik Jørgensen Elsevier pp 3543-3550.

<http://dx.doi.org/10.1016/B978-008045405-4.00283-4>