

PHD AND HONOURS PROJECTS

PHOTOBIOENERGETICS GROUP

[Group Web Link](#)

The research in the **Photobioenergetics Group** focuses on the light reactions of photosynthesis, whereby solar energy is captured by Photosystem II and Photosystem I and converted into chemical energy. In this research we use and develop a broad range of novel techniques in biophysical chemistry, biochemistry, molecular biology and plant physiology.

Our primary interest is in the first part of photosynthesis - the capture and conversion of light energy by Photosystem II that leads to the oxidation of water. In evolution, this process has been the main biological energy input to the living world and the only one responsible for generating all atmospheric oxygen (O₂).

We also study the light-induced inhibition of Photosystem II, how plants protect themselves in excess light, the dynamic structure/function relationships of the photosynthetic apparatus with particular reference to order/disorder, and the supramolecular organization of various protein complexes under dark and varying light conditions.

Our main goal is to contribute to the understanding of the basic principles in the photosynthetic process, so that they can be applied to optimize both natural photosynthesis and bio-inspired artificial systems. Specific goals include the use of available genetic information to engineer artificial proteins that are modelled on Photosystem II and designed to convert light energy into useful chemical or electrical energy, and biomimetic systems that produce hydrogen (H₂) through the photo-decomposition of water ([Molecular Biofuels](#)).

1. OXYGENIC REACTIONS

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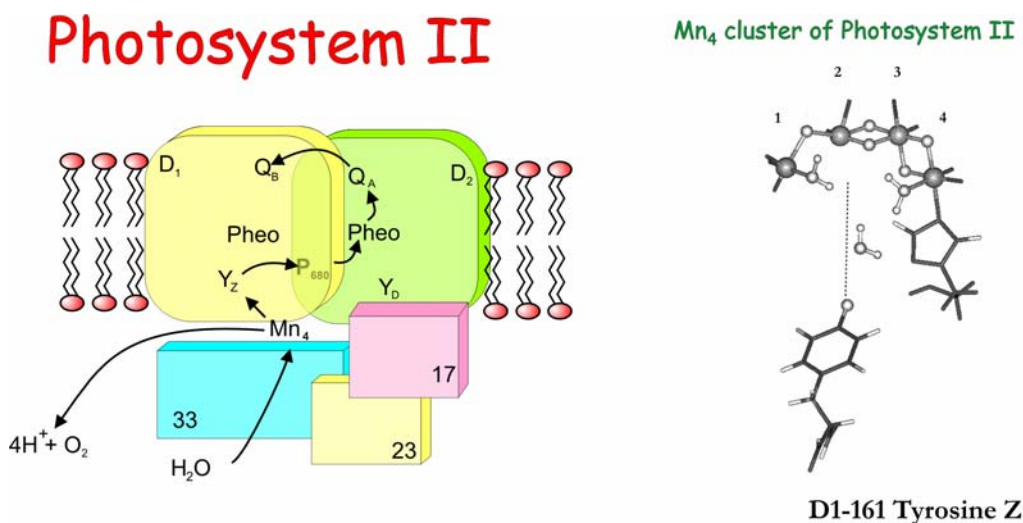
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1.1 Secondary Isotope Effects on the ^{18}O Exchange Reactions of the Substrate Water in Photosystem II

Water is a ubiquitous solvent in biology and proteins and membranes are surrounded and solvated by this molecule. A select few enzymes utilise water as a substrate, and to study them requires that methods be developed to discriminate *substrate* water from *solvent* water. We have recently developed isotope exchange techniques using stable isotopes for monitoring the binding of the substrate water to the O_2 -evolving complex (OEC) of photosystem II (1). The current data show that one substrate water molecule is bound to the OEC throughout the entire four-step catalytic cycle, but with varying affinities; whereas the second substrate water molecule is bound (at least) during the last step, just prior to O-O bond formation. To gain further insight into the nature of the substrate binding sites, a research project can be organised around ^{18}O exchange measurements of photosystem II samples in the presence of D_2O (deuterated water). The results from these measurements will provide information regarding predicted H-bonding interactions at the catalytic site and how H-bonding may facilitate O-O bond formation.



1.2 Substrate and Product Channels in Photosystem II

In many enzymatic mechanisms, such as carbonic anhydrase II, an important parameter in determining the reaction path is one of substrate accessibility to the catalytic site. The role of the channel is to orient the substrate with respect to the catalytic site to maximize chemical bond breakage or formation. This may also be the case for the oxygen-evolving complex (OEC) in Photosystem II, where the order binding of the two substrate water molecules to the catalytic site is required to insure the O-O bond formation leads to the kinetically inert triplet state di-oxygen (O_2) rather than to the formation of radical oxygen intermediates, such as hydrogen peroxide, that damage the protein matrix. The access of solvent water to the catalytic site in Photosystem II may be controlled by discreet channels through the protein matrix, connecting the catalytic site with the external aqueous phase. With the refined 3D crystal structure of the Photosystem II complex and the recent application of simulation programs to determine solvent contact surfaces within the protein interior, two publications appeared which predict the existence of several full length channels in Photosystem II that may transport substrate water, protons and O_2 . Based on these predictions site-specific amino acid residues lining the channels are identified. As a short or long term project, the channel hypothesis for Photosystem II can be tested by making site directed mutants at the entry, exit and constriction points of the proposed channel. Effects on these mutants would then be evaluated on O_2 yield, kinetics, substrate exchange and proton release.

Techniques: site directed mutagenesis; mass spectrometry; kinetic O_2 measurements; biochemical procedures for handling photosynthetic samples from higher plants

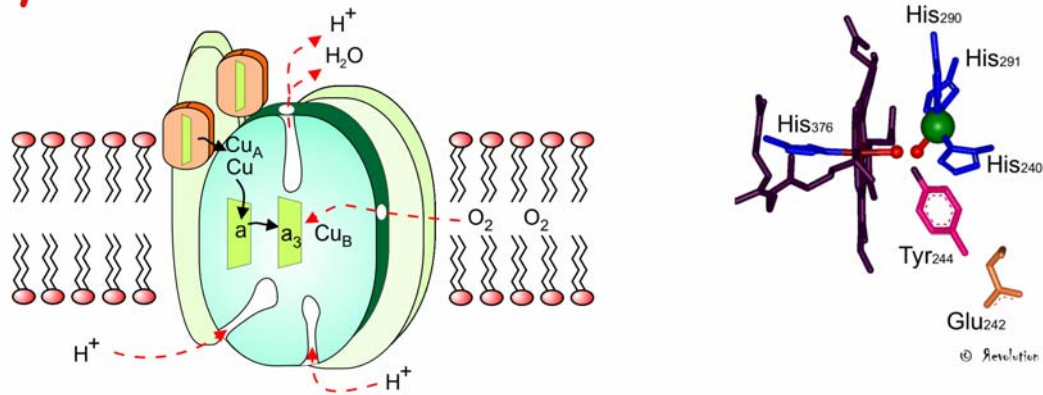
References:

- Wydrzynski T, Hillier W and Messinger J (1996) On the functional significance of substrate accessibility in the photosynthetic water oxidation mechanism. *Physiol Plant* 96, 342-350
- Loll B, Kern J, Saenger W, Zouni A and Biesiadka J (2005) Towards complete cofactor arrangement in the 3 Å resolution structure of Photosystem II. *Nature* 438, 1040-1044
- Murray J M and Barber J (2007) Structural characteristics of channels and pathways in photosystem II including the identification of an oxygen channel. *Journal of Structural Biology* (on-line)
- Ho F M and Styring S (2007) Access channels and methanol binding site to the $CaMn_4$ cluster in photosystem II based on solvent accessibility simulations, with implications for substrate water access. *Biochim Biophys Acta Bioenergetics* (in press)

1.3 Oxygen reduction by Cytochrome c Oxidase

Molecular oxygen (O_2) is a thermodynamically reactive molecule and, over the course of several billion years, biology has evolved several systems to utilise O_2 from the atmosphere to drive chemical reactions. Some of these processes are involved in preventing reactive oxygen chemistry destroying living cells. Other processes harness O_2 reduction in a fundamentally important proton pumping mechanism to pump protons across a membrane and dissipate this to generate ATP. The pumping element itself is a molecular machine called Cytochrome *c* Oxidase (CcO).

Cytochrome c Oxidase



The CcO molecular machine has been structurally characterised; yet, insight into the molecular pump is lacking detail. It is believed that specific channels for the protons exist in the enzyme and these pathways interact *via* specific amino acid residues to translocate protons across the membrane in a unidirectional manner. This pumping machinery is closely coupled to the O_2 reduction chemistry. It is proposed that students might investigate the mechanism of this molecular machine by investigating issues relating to proton back-leak across the membrane, O_2 miss match chemistry, or substrate (O_2) affinity. The goal of this research is to understand age-related processes in mitochondria.

Techniques: Optical / vibrational / mass spectrometry and biochemical procedures for sample isolation from recombinant photosynthetic bacteria.

Reference:

Michel H (1999) Proton pumping by cytochrome *c* oxidase. *Nature* 402: 602-603

2. PROTEIN ENGINEERING

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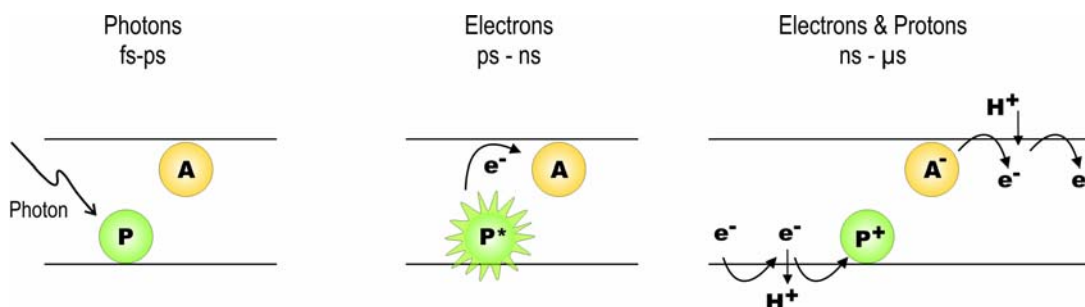
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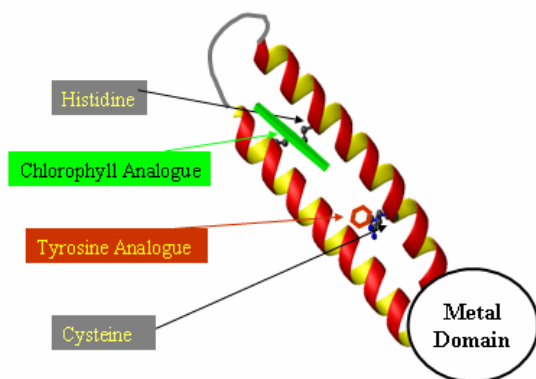
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The essence of a reaction centre:



2.1 Engineering Light-Activated Proteins



The natural photosystems of photosynthetic organisms are the most efficient photochemical conversion systems. Electron flow originates in the photosystems at a special reaction centre where light causes a rapid charge separation between a photoexcited chlorophyll donor and an acceptor molecule. To minimize back reaction, the protein matrix arranges a series of secondary redox cofactors with specific positions and tuned redox potentials along the desired transfer pathway.

The goal of this project is to construct efficient photoactive biocatalysts that are simplified versions of photosynthetic reaction centres, ultimately to split water into O_2 and H_2 in a non-polluting solar/hydrogen fuel cycle. Our approach is to engineer robust natural proteins to contain structural motifs for binding chlorophyll pigment analogues and redox-active cofactors such as quinones and metal atoms. We have created an artificial reaction centre by replacing the heme in the *E. coli* cytochrome b562 protein with a light-sensitive zinc-chlorin molecule and introduced a covalently linked quinone acceptor molecule within electron tunnelling distance of the pigment. Upon illumination a charge separation is achieved with 20% efficiency. Our next step is to introduce a metal centres that will donate electrons to the light activated zinc-chlorin. In this case we have identified bacterioferritin from *E. coli* as a robust protein scaffold to engineer a light-sensitive pigment, a quinone acceptor and

a multinuclear metal centre. By modifying the protein environment, we aim to establish a directional electron transfer using the energy of light. This project will involve the production of proteins in *E. coli* as the expression system and biophysical characterization of photoactive proteins.

Techniques: molecular biology; biochemical procedures for protein purification; optical and EPR spectroscopy

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. Proc Natl Acad Sci USA 101: 17675-17680

3. ASTROBIOLOGY

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3.1 Characterisation of oxygenic reactions in algae living in extreme environments

Liquid water is the absolute requirement for life as we know it. The search for extraterrestrial life thus begins with the search for water. This year a landing probe is being sent to search for water beneath the ground surface in the northern polar region. With a possible maximum temperature of 24°C at the equator during mid-summer, there is a good possibility that some form of life exists, or at least has existed in the past, on the Martian planet. More spectacularly, the Galileo space probe has obtained strong evidence for the presence of large oceans below the surface on three of Jupiter's moons; Europa, Ganymede, and Callisto. Indeed, the expectation for the possibility of life on Europa is considered to be so great, that the Galileo space probe was purposely diverted into the Jovian atmosphere on 21 September 2003, and incinerated in order to prevent any possible future contamination of Europa.



From a thermodynamically perspective water is a very stable molecule and oxidizing water requires a considerable energy input. The splitting of water molecules is readily achieved on Earth by the light-driven photochemical reactions performed by chlorophyll-*a* containing algae and plants, generating molecular oxygen as a by-product. In the reverse process, molecular oxygen is reduced back to water releasing energy. This reaction is catalysed by the ubiquitous respiratory enzyme cytochrome *c* oxidase and the released chemical energy is used to drive the metabolic pathways necessary for life. The water/oxygen cycle is the key biogeochemical cycle that sustains the existence of most life living on Earth. Extraterrestrial life based on a water/oxygen cycle may possibly contain similar metabolic pathways, which, in turn may be modified to adapt to the more extreme environments. In preparation for the exploration of extraterrestrial life, a new venture has been established within the PBE Group to probe the oxygenic reactions of photosynthetic algae living in extreme earth environments, including strains from African soda lakes, geothermal hot springs, intertidal pool stromatolites, low-temperature Antarctic deserts, and high-temperature Australian deserts. This project would use a combination of biochemistry and spectroscopy to investigate biodiversification of photosynthesis and respiration. An important goal from this work is measurement of isotopic fractionation ratios of oxygen. These ratios provide isotopic reference signatures for future extraterrestrial surveys within the solar system.

4. THYLAKOID STRUCTURE AND FUNCTION

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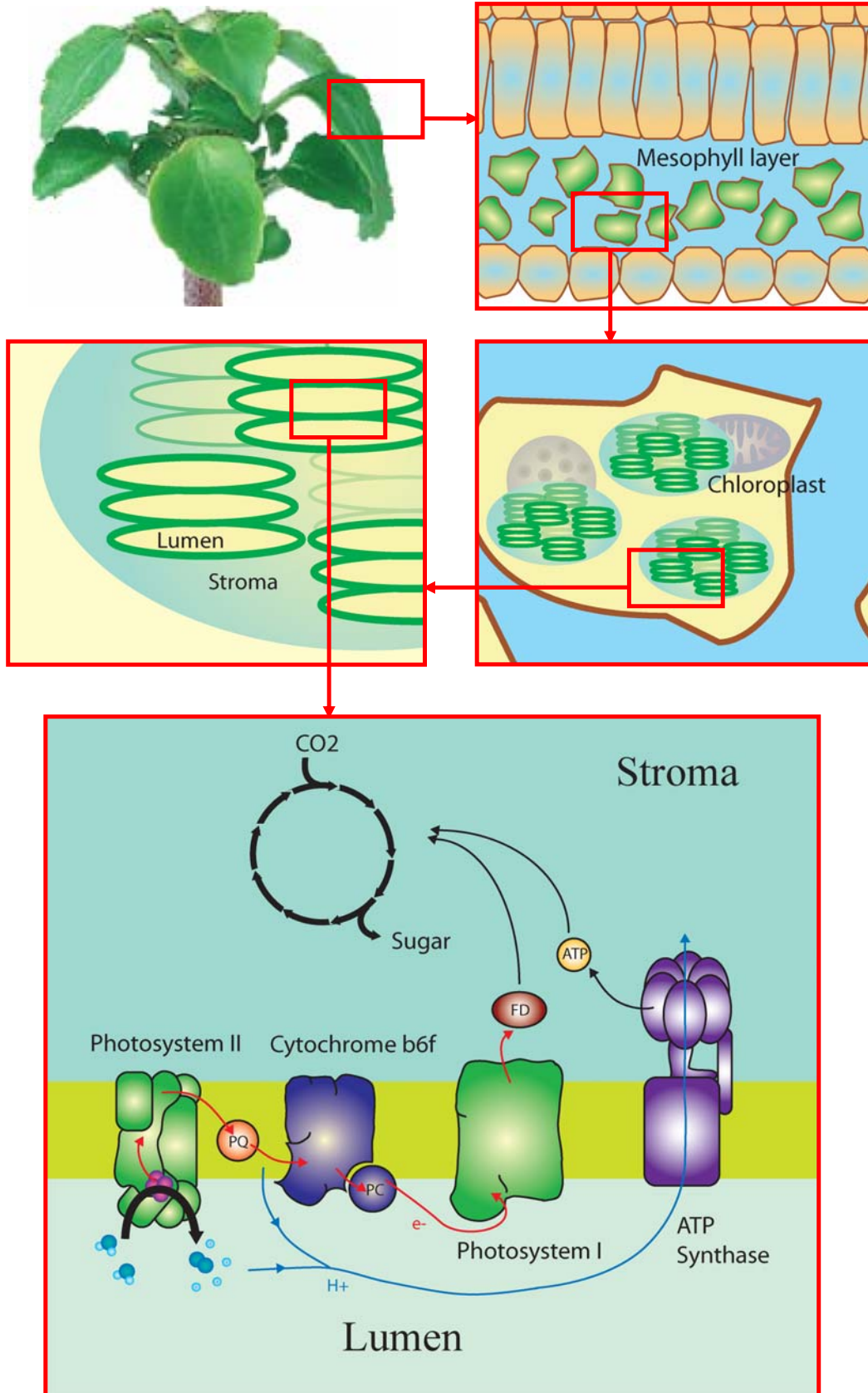
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4.1 Entropy as a novel determinant of photosynthetic structure and function

Chloroplasts, the powerhouse of higher plants and recently-evolved green algae, almost invariably have a granal structure: flattened thylakoid sacs stacked up to form orderly grana, interconnected by non-stacked thylakoids and bathed in an aqueous stroma phase, all enclosed in a double-membrane envelope. Obviously, chloroplasts are an open system through which both energy and mass flow.

But why are grana so ubiquitous in the plant world? What is Nature's driving force for selecting a granal structure? This project seeks to (1) demonstrate the involvement of disorder (*entropy*) in thylakoid stacking, (2) investigate the functional implications of entropy-assisted thylakoid stacking, (3) establish that the functional consequences in turn enable chloroplasts to increase entropy production, a thermodynamic imperative, and (4) relate the evolution of a granal structure to the evolutionary increase in *complexity*, defined as the energy flow through an open system per unit time per unit mass.



Drawn by Adele Williamson

Techniques: kinetic *in vivo* optical spectrophotometry; chlorophyll fluorescence techniques; electron microscopy; biochemical techniques; oxygen measurements.

References

Chow WS, Kim E-H, Horton P and Anderson JM (2005) Stacking of thylakoid membranes in chloroplasts: the physicochemical forces at work and the functional consequences that ensue. *Photochemical & Photobiological Sciences* 4: 1081-1090

Kim E-H, Chow WS, Horton P and Anderson JM (2005) Entropy-assisted stacking of thylakoid membranes. *Biochimica et Biophysica Acta* 1708: 187-195

Chow WS (1999) Grana formation: entropy-assisted local order in chloroplasts? *Australian Journal of Plant Physiology* 26: 641-647

4.2 Dynamic architecture of plant thylakoid membranes between light and dark

Recent intermediate resolution of the atomic protein complexes of plant thylakoid membranes reveals an intriguing aspect of their organization into highly organized oligomeric assemblies; these supercomplexes enhance structural and functional stability compared to monomers. In contrast, little is known about the dynamic interactions of thylakoid supercomplexes *in vivo* in the dark, in limiting light, in saturating light or in excess light where photosystem II becomes progressively photoinactivated. As well as long-term adjustments of thylakoid composition by gene expression in response to environmental cues (photosynthetic acclimation), existing supercomplexes are rapidly reorganized by dynamic changes in protein structure and/or supramolecular organization within the membrane in response to light. The spectral quality of light induces limited state transitions, while light quantity induces flexible partition of absorbed energy between use in photosynthesis and dissipation as heat over widely fluctuating light irradiance that involves non-photochemical quenching, photosystem II photoinactivation and D1 protein repair. Our goal is to study the structural dynamics of the supramolecular organization of the thylakoid protein complexes, particularly the dynamic structure of photosystem II in the light and in the dark, and how dynamic structural changes in grana stacking in higher plants assist function.

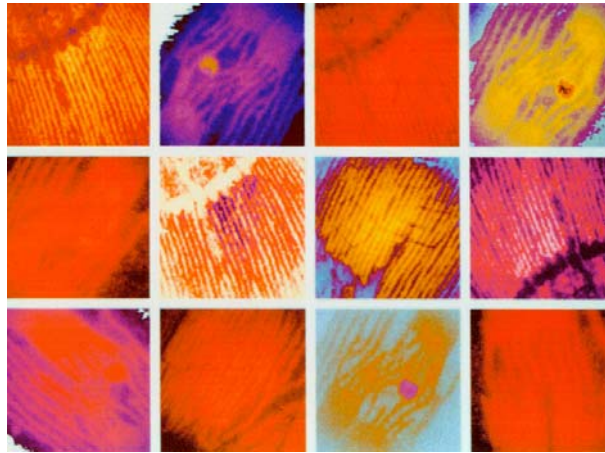
Techniques: kinetic optical spectrophotometry; chlorophyll fluorescence techniques; biochemical techniques; oxygen measurements, and sophisticated electron microscopy including Single Particle analysis coupled with electron cryo-microscopy (A/Prof. Ben Hankamer, Institute of Molecular Biosciences, The University of Queensland)

References

Chow WS, Kim E-H, Horton P and Anderson JM (2005) Grana stacking of thylakoid membranes in higher plant chloroplasts: the physicochemical forces at work and the functional consequences that ensue. *Photochemical and Photobiological Sciences* 4: 1080-1090

Anderson JM (2002) Changing concepts about the distribution of photosystem II and photosystem I between grana-appressed and stroma-exposed thylakoid membranes. *Photosynthesis Research* 73:157-164

Anderson JM (2000) Strategies of photosynthetic adaptations and acclimation. In: "Probing Photosynthesis; Mechanisms, Regulation and Adaptation" in Mohanty, P. and Pathre, Y. Eds.) pp. 283-292, Taylor and Frances, London



False-colour visualization of photosynthetic membranes

5. TRACKING ELECTRON TRANSFERS IN LEAVES

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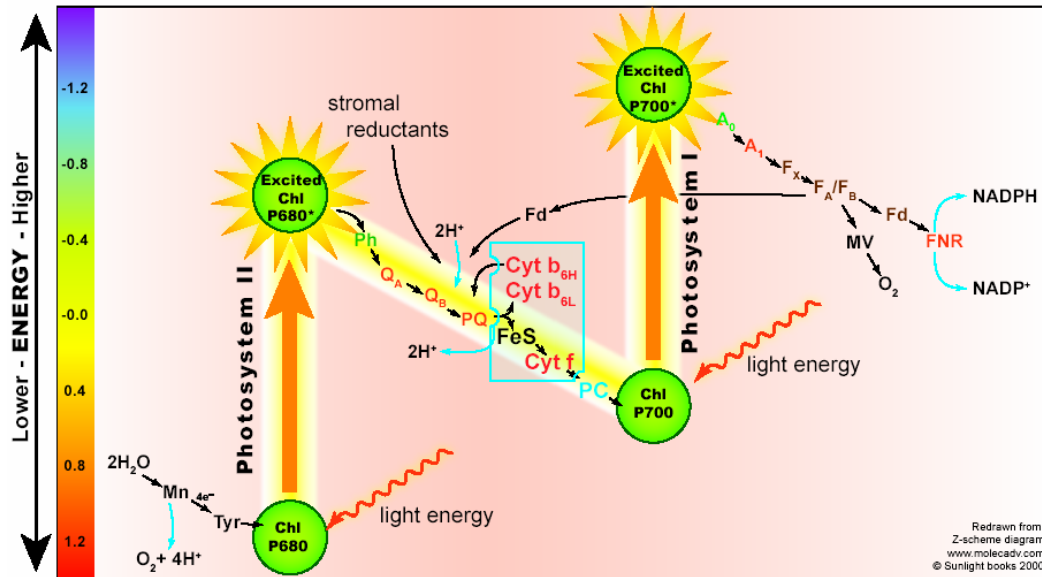
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5.1 Pathways of photosynthetic electron flow under environmental stress

The two photosystems in the chloroplast work in series to deliver electrons from the substrate water to NADP^+ in two light-driven uphill steps, conserving the light energy as chemical energy in NADPH and ATP.



In addition to this linear electron flow, there is a cyclic electron pathway mediated by ferredoxin around Photosystem I, another Photosystem I cyclic pathway involving NADPH, a Q cycle around the cytochrome *bf* complex, as well as electron donation from stromal reductants. A challenge is to quantify these separate electron fluxes, all except one of which pass through P700, the primary electron donor in Photosystem I, and to do so in leaves without having to isolate the chloroplasts. How do these fluxes vary under different environmental conditions? What roles do the electron fluxes play in the protection of the photosynthetic apparatus under environmental stress, such as high light and/or drought? The overall goal is to decipher the various electron fluxes in situ, with leaves functioning in defined environmental conditions.

Techniques: kinetic *in vivo* optical spectrophotometry; chlorophyll fluorescence techniques; biochemical techniques; oxygen measurements.

References:

Chow WS and Hope AB (2004) Electron fluxes through Photosystem I in cucumber leaf discs probed by far-red light. *Photosynthesis Research* 81: 77-89

Chow WS and Hope AB (2004) Kinetics of reactions around the cytochrome *bf* complex studies in intact leaf disks. *Photosynthesis Research* 81: 153-163

Fan, D-Y, Nie Q, Hope AB, Hillier W, Pogson BJ and Chow WS (2007) Quantification of cyclic electron flow around Photosystem I in spinach leaves during photosynthetic induction. *Photosynthesis Research*. In press. Doi 10.1007/s11120-006-9127-z