AuPS/ASB Meeting - Newcastle 2007

Symposium: Artificial photosynthesis

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Chair: Tony Collings

Redesigning photosynthetic charge separation

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The x-ray structure of bacterial photosynthetic reaction centers transformed our view of the relationship between structure, spectroscopy and function and has been the basis of nearly every physical and biological measurement on photosynthetic assemblies for the last 20 years. The most striking finding was the presence of two reasonable electron transfer pathways, the L and M (or A and B) branches, yet under ordinary conditions, electron transfer occurs only along the L branch of chromophores. Many labs have engineered local changes to probe the origin(s) of this unidirectional electron transfer. Two groups pursued larger-scale symmetrization in *Rb. capsulatus:* the D_{LL} mutant (Robles *et al.*, 1990), in which D helix residues M192-M217 that make contact with most of the reactive components were replaced with those at L165-L190; and the *sym1* mutant (Taguchi *et al.*, 1992) in which residues M187-M203 were replaced with L160-L176. Neither led to electron transfer to the M-branch; however, both produced novel phenotypes. Remarkably, D_{LL} RCs assembled, but with the primary electron acceptor H_L missing, so L-side electron transfer is blocked. The i>sym1 mutant led to the surprising discovery that P is much more difficult to oxidize to P^+ than in wild type, and this was traced largely to the replacement of Phe at position M195 with His, which can hydrogen bond to the ring I acetyl group of the M macrocycle of P, P_M (Stocker *et al.*, 1992). Subsequently, the role of residue M195, the symmetry related residue at position L168, and those at positions L131 and M160 in *Rb. sphaeroides*, were all shown to systematically affect the oxidation potential of P, primarily by adding or removing hydrogen bonds to the acetyl or keto carbonyl groups of the two halves of P (Lin *et al.*, 1994). This strategy has been extensively developed by many groups, but the D_{LL} mutation has been much less studied because, as originally described, pure reaction centers could not be isolated.

We have returned to the D_{LL} system in the context of contemporary knowledge about mutations that affect co-factor redox properties and mild methods for isolation of reaction centers. Since electron transfer to H_L cannot occur, this system is ideal for probing the role of B_L, since B_L^- should still be formed, and for observing M-side electron transfer. We have recently reported that systematic mutations in the vicinity of P, BL, BM and HM can produce a very large yield of M-side electron transfer (Chuang *et al.*, 2006). To date, no evidence has been found for the presence of appreciable yields of $P^+B_L^-$ at room temperature. The results suggest that reaction energetics dominate native unidirectional electron transfer.

While significant progress has been made re-engineering the reaction center, nearly every step was based on empirical findings, rather than predictions. Electrostatic calculations can provide a basis for the analysis of reaction energetics, however, the level of structural information that is needed is rarely available. We have developed vibrational Stark shifts in proteins as a precise local and directional measure of electrostatic energy changes upon mutations.

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Artificial photosynthesis and the bio-mimetic production of hydrogen

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Natural photosynthesis results in the photo-chemical conversion of visible light quanta into high energy chemical species. Many reactions in the overall process occur with efficiencies unmatched by man-made catalytic systems and operate close to the thermodynamic limit. Such key steps include:

- the electro-chemical splitting of water into H^+ and molecular Oxygen
- the capture and conversion of Carbon Dioxide into energy rich substances (*i.e.*, foods)
- the primary conversion of sunlight into electro-chemical energy.

Although whole photosynthetic organisms can be genetically manipulated to directly produce molecular hydrogen, the process at present has very low quantum efficiency, in part because of fundamental incompatibilities between oxygenase and hydrogenase enzymes operating in close proximity. However oxygenic photosynthesis has 'solved' the most chemically demanding reaction in the electrolytic decomposition of water into H_2 and O_2, *vs* the anodic oxidation of water (or OH^-) to molecular oxygen and protons. The Mn containing water splitting catalytic site in photosystem II (PSII) performs this reaction at close to thermodynamically limiting efficiency (< 0.2 V over-voltage), at a high turnover rate (~103 s^{-1}), under mild external pH and in the presence of significant concentrations of environmentally common anions, such as Cl^-. A bio-mimetic electrolysis system based on the natural PSII catalytic site would have substantial thermodynamic and kinetic advantage.

The PS II oxygen evolving complex (OEC) contains 4 Mn and 1 Ca in a compact, exchange coupled cluster. While full structural detail of the site is yet to be resolved, most of the protein ligands, which define the cluster geometry, are located in a very small region near the C terminus of the D1 polypeptide of the PSII reaction centre. This suggests that functioning catalytic site analogs may be assembled from small model peptides or other synthetic constructs. Proposals for such a structures have been made and some existing Mn complex OEC models show promise of useful catalytic function. (for details, see Pace, 2005).

Recently computational chemistry has been employed to explore possible intermediate structures and catalytic pathways to water oxidation which the Mn cluster might utilise (Petre *et al.*, 2007). This approach shows great promise and reveals aspects of the system essentially inaccessible by other techniques. These and other results allow the possibilities of a practical, fully bio-mimetic cathodic water oxidiser to be assessed.

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Water-soluble chlorophyll-binding proteins (WSCPs) as well-defined systems by which to probe and modify chlorophyll-chlorophyll and chlorophyll-protein interactions

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Natural photo-systems are the most efficient, flexible and adaptable photovoltaics known. Chlorophyll(Chl) pigments are utilised as stable light harvesting components, and yet the same Chls, when bound at specific protein sites, spontaneously charge separate upon optical excitation to perform the primary photochemistry of photosynthesis. The properties of Chls that are important for light absorption, energy transfer and charge separation are strongly influenced and controlled byChl-Chl and Chl-protein interactions. Understanding these processes is important for artificial photosynthesis.

We report laser-based spectroscopic studies of a natively reconstituted cauliflower WSCP (see Schmidt *et al.*, 2003 and Hughes *et al.*, 2006). In the presence of Chl, this protein forms tetrameric units. During this process there are just *two* Chls bound per protein tetramer. The natural function of WSCPs is not fully understood, but may act as Chl transport proteins during Chl biosynthesis and/or catabolic pathways, and possibly as aChl scavenger when the plant is under stress. Chl bound to WSCP is protected against photo-induced singlet-oxygen formation (Schmidt *et al.*, 2003) and, with the absence of any carotenes in WSCP, the protection mechanism is not known.

Our previous circular dichroism (CD) and magnetic circular dichroism (MCD) studies (Hughes *et al.*, 2006) have established that in natively reconstituted Chl-WSCP, the two Chls are in a single and remarkably well defined protein environment. Furthermore, the two Chls are in a 'sandwich' configuration (see Figure), leading to a relatively strong exciton coupling between the pigments. One consequence of this arrangement is a *weak* lowest energy optical excitation of the system.

We report laser-induced spectral hole-burning measurements of this system. Hole-burning measurements allow further characterisation of the exciton-coupling in Chl-WSCP as well providing excited state lifetimes. The width of spectral holes provide lifetimes, without the need for time-domain experiments. These measurements also potentially identify the mechanism for the protection of Chl against photo-degradation due to singlet oxygen. We also use hole-burning to study the electron-phonon coupling of Chl-WSCP and Chl excited-state vibrational frequencies. We investigate ourprevious suggestion (Hughes *et al.*, 2006) of the presence of a unique high-frequency phonon mode (~90 cm^{-1}) for Chl-WSCP. Excited-state vibrational frequencies can be used as indicators of Chl-protein interactions such as the Mg^{2+} ligation state and hydrogen bonding of the protein to the Chl peripheral groups. Such interactions may play critical roles in the Chl-binding and WSCP tetramerisation processes as well as in the photo-protection mechanism.

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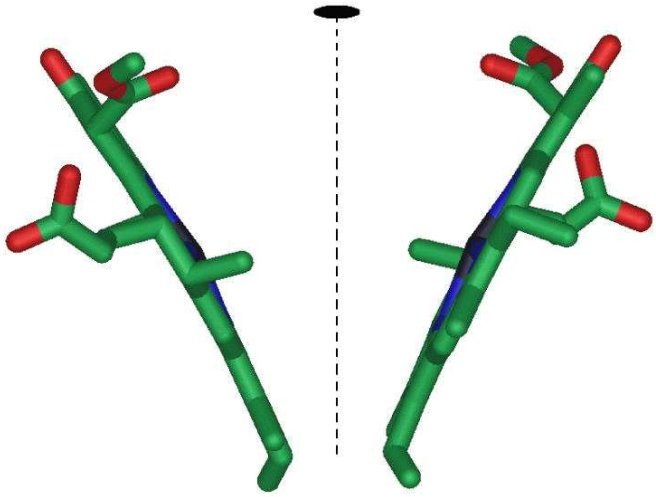
Hughes JL, Razeghifard R, Logue M, Oakley A, Wydrzynski T & Krausz E. (2006) *Journal of the American Chemical Society*, **128:** 3649-58.



'Sandwich' geometry of Chls in WSCP