$Pheo_{D1}$ photo-reduction. The area of the narrow (2nm FWHM) bleach at 683.8nm is fully commensurate with that of an isolated $pheophytin_a$, indicating a weak coupling to its neighbouring pigment, the accessory chlorophyll Chl_{D1}. Also, a highly structured second derivative pattern is seen in the change in the CD at 683.8 nm upon photoreduction. This is also interpreted as indicative of a weak $Pheo_{D1}$-Chl_{D1}interaction.

Corresponding EPR measurements of the trapped $Pheo_{D1}^-$ - which give rise to a "split signal" centered about g~2.0 - agree quantitatively with optical results. A theoretical model of the split signal interaction was developed using spin Hamiltonian formalism, in which the reduced pheophytin interacts with the pseudo spin ½ quinone-iron coupled complex. It establishes that the magnitude of this coupling is minimal with J~|50G|. The EPR analysis requires the presence of the two lowest doublets of the quinone-iron system to simulate the observed spectrum, implying the signal is a composite of two distinct spectral patterns. Consequently, the $Pheo_{D1}$ can be considered electronically isolated from Q_A and only weakly coupled to Chl_{D1}.

PS2.21
Purification and biochemical characterization of PSI-LHCI supercomplex in *Chlamydomonas reinhardtii*

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The unicellular green alga Chlamydomonas reinhardtii contains nine distinctive light-harvesting complexes I (LHCI complexes) designated Lhca1-9 (1) as well as fourteen photosystem I (PSI) polypeptides (PsaA-L, N, and O) (2). It appears that the PSI-LHCI supercomplex in C. reinhardtii is larger than that in higher plants that contain only four distinctive Lhca polypeptides (3). Since it is of interest to characterize the structure of the PSI-LHCI supercomplex in C. reinhardtii, we have improved a method to purify the PSI-LHCI supercomplex on a larger scale in order to carry out structural analysis. Thylakoid membranes were purified, washed with NaBr, and subsequently solubilized with n-dodecyl-b-D-maltoside. The resulting extracts were fractionated on sucrose density gradient. We optimized sucrose density gradient for ultracentrifugation for a larger sample scale and for a better separation of PSI and PSII complexes. The resulting PSI-LHCI enriched fractions were subsequently applied onto DEAE column. The conditions for elution buffer were optimized to eliminate minor contamination of other polypeptides. A highly purified PSI-LHCI supercomplex retaining PSI activity was obtained. The preparation contained most constituent PSI polypeptides except two peripheral subunits such as PsaN and PsaO. We found that PsaG is rather unstable in PSI complex and is easily dissociated from PSI complex. We also found that PsaG is highly susceptive to protease digestion. 1. Takahashi, Y. et al. Biochemistry 43 (2004) 7816-7823 2. Hippler, M. et al. Protist 153 (2002) 197-220 3. Ben-Shem, A. et al. Nature 426 (2003) 603-635

PS2.22
Spectroscopic investigation of electron and proton transfer in the photosynthetic reaction center of *Rhodobacter sphaeroides*

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The reaction center (RC) from the purple nonsulfur bacterium *Rhodobacter sphaeroides* uses light energy to reduce Q_A, the primary electron acceptor, and then, Q_B, the secondary electron acceptor. Previous Fourier-transform infrared (FTIR) measurements led to the suggestion of an electron transfer mechanism involving an intermediary electron donor [1]. Fe^{2+} has been excluded as an intermediary electron donor by time-resolved X-ray absorption on the Fe-K-edge [2]. We study RC mutants by FTIR spectroscopy to search for the intermediary electron donor and to better understand molecular mechanisms of the coupled electron and proton transfer reactions. We showed that after reduction of Q_A, protons are taken up via Asp210 and transported in a Grotthuss mechanism via a protonated water chain to Q_B.

Mutation of Asp210 to Asn leads to a deceleration of oxidation of Q_A^-. Using time-resolved FTIR spectroscopy we characterized molecular reaction mechanisms of this mutant and found that Q_B^- formation precedes Q_A^- oxidation even more pronounced than in the wild type RC [3]. We proposed that specific bands belong to the intermediary electron donor. Time-resolved continuum bands were recorded and indicate protonation state changes of a protonated water cluster [4].

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PS2.23
What is the origin of the highly dispersive quantum efficiencies for secondary donor oxidation at low temperature in Photosystem II?

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$_A$ reduction following illumination at low temperature using green light is highly dispersive [1]. The data can be described [1] by three nearly equal populations that each differs in QE by approximately an order of magnitude from $\sim 10^0$ to $\sim 10^{-3}$. With direct excitation of the weakly absorbing lowest energy optically accessible absorption band of PSII (700-730 nm), the QE *decreases* by up to $\sim 10^4$ [1].
We have extended our studies to *Thermosynechococcus elongatus* PSII core complexes. Our aim is to determine the origin of the dramatic 10^0-10^4 decrease in QE of Q_A reduction that follows primary charge separation induced by direct excitation of the weak long wavelength (700-730 nm) absorption band of PSII.
We have used optical and EPR spectroscopy at low temperatures (<20 K) of both spinach and *Thermosynechococcus elongatus* PSII core complexes to quantify the secondary electron donors that are oxidised following either green or deep red (700-730 nm) illumination. Distinctly different electrochromic patterns were obtained for deep red vs. green illumination.
We address the question of whether a particular secondary donor can be associated with a subset of PSII exhibiting a particular range of QEs, and address the origin of the dramatic decrease in QEs that we observe. The mechanism of primary charge separation is discussed in light of these results.

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PS2.24
Triplet States in Photosystem II Reaction Centers Studied

with EPR and ENDOR Spectroscopy

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The chlorophyll triplet state ^{3}P680 in photosystem II (PS II) is formed by recombination of the primary radical pair. In D1/D2 complexes of PS II from spinach this state was investigated using time-resolved EPR and ENDOR spectroscopy.
The zero field splitting (zfs) parameters were determined over a large temperature range (10 □250 K) with time-resolved EPR (pulse and transient) spectroscopy. The results showed that the triplet exciton is localized on a single chlorophyll at low temperature and delocalized over several (≥ 2) pigments at higher temperature. The spin density distribution has been determined for the triplet chlorophyll from a comprehensive set of the ^{1}H hyperfine coupling constants (hfc) which could be measured by orientation selection pulse ENDOR spectroscopy at Q□band. These results agree well with previous observations in frozen solution at X□band[1]. Due to improved sensitivity and resolution more hyperfine couplings are resolved. The analysis supports the localization of the triplet on a single chlorophyll. DFT calculations using the ORCA program[2] are presented for the triplet states for various chlorophylls in the PS II reaction center yielding both the zfs and hf parameters. The agreement between experimental and theoretical values allows for the determination of the details of the electronic structure of pigment triplet states in photosynthesis.
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PS2.25
A model for temperature-dependent peak shift of the bacterial reaction-center absorption

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Absorption spectra of the purple-bacterial reaction center have three bands in the near-IR region. They are ascribed to the Q_y electronic transition of bacteriochlorophyll-*a* (Bchl) and bacteriopheophytine-*a* molecules.
The lowest one of them, assigned to the lower excited state of the special pair of Bchls, exhibits a striking temperature dependence, decreasing markedly its energy with lowering temperature. The other two bands do not show such a temperature dependence.
A model for this temperature dependence shift of the special-pair absorption is given, by constructing potential energy surfaces for the electronic excited and the ground state of the special pair.
These surfaces are composed of the interaction with the protein vibration and the electronic energy. The excited state of the special pair is the exciton state, whose energy is sensitive to the intra-pair distance and the mutual angle. Their variations make the excited-state energy surface markedly anharmonic.
Employing these energy surfaces, the absorption band was calculated as a function of temperature. The large peak shift with temperature could successfully be reproduced. This mechanism does not rely on the thermal expansion which has been assumed so far to be effective in the peak shift without detailed calculation. The peak shift is due to the density-of-states difference between the energy surfaces.

PS2.26
Effect of capsaicin on the photosynthetic performance of *Scenedesmus obliquus* cultures *in vivo*

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Capsaicinoids are the principle pungent of capsicum fruits with capsaicin and dihydrocapsaicin to be responsible for more than 90% of the pungency. Capsaicin has been reported to act as a competitive inhibitor in vitro in both, plant PSII and bacterial reaction center. Due to this inhibitory action on the photosynthetic apparatus and the structural similarity with known herbicides, capsaicinoids could be exploited as herbicides or as leads for synthetic herbicide designing. The purpose of this work is to further investigate the action of capsaicin in the photosynthetic apparatus and to test its in vivo activity in cultures of the green alga *Scenedesmus obliquus*. In this direction, chlorophyll fluorescence was used to screen the photosynthetic performance of the cell cultures of *S. obliquus*, following capsaicin supplementation. Increasing concentrations of capsaicin showed a reduction of the photosynthetic efficiency (Fv/Fm) of the cultures, indicating that capsaicin exhibits in vivo activity. Further processing of the fluorescence induction kinetics by JIP-test analysis provided significant information on the alterations induced in the structure and function of the photosynthetic apparatus. These results not only confirm that the capsaicin inhibitory action is a common trait among photosynthetic species, but also prove that Q_B is the binding site for capsaicin in vivo, inducing the closure of the PSII reaction centers. Further work is in progress in order to investigate the possible phytotoxical action of capsaicinoids on intact higher plants.

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PS2.27
***In vitro* reconstitution of the cytochrome b_{559} from higher plants**

R Picorel (EEAD-CSIC)

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The cytochrome (Cyt) b_{559} is a membrane-protein component of the photosystem II reaction center but its function still remains unclear. This Cyt consists of two polypeptide subunits, a and ß. The a-subunit (9 kDa) is encoded by the *psbE* gene and the ß-subunit (4.5 kDa) by the *psbF* gene. Both genes are of chloroplastic origin and each polypeptide spans the thylakoid membrane with a single a-helix. The two polypeptides are bound by a heme group coordinated by two histidines, one from each subunit. Our work is aiming to obtain *in vitro* reconstitution of the plant Cyt b_{559} for future structural and functional studies. To that end we have cloned and overexpressed each subunit from sugar beet in *E. coli* as a fusion protein using the expression vector pMALc2x. When the fusion protein expression was induced at standard conditions, most of that protein appeared as inclusion bodies. This fraction was purified by washing with Triton X-100 and denatured with 7 M urea. For reconstitution, we mixed the two fusion proteins with equimolar amounts of heme. The reconstitution was carried out by dyalisis against

gradually decreasing urea concentrations, and, finally the free heme was removed by chromatography to avoid spectral interferences. The reconstitution was analyzed by UV/Vis spectroscopy; *i.e.*, the reconstituted Cyt b_{559} redox difference spectrum was very similar to that of the native Cyt b_{559} from higher plants, with a typical maximum of the a-band and b-band at 559.5 nm and 530 nm, respectively.

PS2.28
The Electron Acceptor Quinone A_1 of Photosystem I Investigated by Pulse EPR and ENDOR Spectroscopy

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The quinone vitamin K_1 (VK_1) is part of the electron transfer chain in photosystem I (PS I). After light-induced charge separation the spin polarized radical pair $P_{700}^{+}A_1^{-}$ (protein bound VK_1 is denoted A_1) is formed. The protein bound quinone exhibits physical properties different from those of VK_1 *in vitro*. The X-ray structure of PS I indicates that VK_1 in the A_1 binding site is involved in asymmetric hydrogen bonding with the protein.
EPR and ENDOR spectroscopy were used to investigate the light-induced radical pair $P_{700}^{+}A_1^{-}$ and the stationary radical anion A_1^{-} and were compared with VK_1^{-} *in vitro*.[1] One- and two-dimensional TRIPLE spectroscopy was used to determine relative orientations and signs of the hyperfine (hf) coupling tensors. Variable mixing time (VMT) ENDOR[2] was employed to determine the absolute signs of the hf coupling constants in the radical pair $P_{700}^{+}A_1^{-}$. We exchanged the quinone in the A_1 binding site of *menB* mutant PS I with fully deuterated vitamin K_3. This allowed us to differentiate between proton hyperfine couplings from the quinone and from the protein environment. Furthermore, DFT calculations were performed on a model of the A_1 binding site, which yielded hf tensors in very good agreement with the experiment. The experimental and theoretical results clearly show that the single-sided H-bond to A_1^{-} is indeed the crucial factor that determines the particular electronic structure of this radical.

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[2] Epel *et al*., *Appl. Magn. Reson.*, 2006 , 30, 311-327

PS2.29
Redox Reactions of the Non-Heme Iron of Photosystem II: An EPR Spectroscopic Study

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We have used EPR spectroscopy to investigate the redox chemistry of the non-heme iron of photosystem II, focusing on the cryogenic electron-transfer reactions of iron, Q_A and a variety of secondary electron donors, namely chlorophylls, carotenoids and tyrosine D. Glycolate coordination to the iron was used in combination with potassium ferricyanide to preoxidize the non-heme iron completely and prime it for photoreduction. The oxidized Fe^{3+} and Q_A centers operated as a single, one-electron acceptor site: EPR quantitation of both the oxidized donor yield and the Fe^{2+} Q_A^{-} yield found no evidence for the formation of more than one oxidized secondary electron donor per PSII. The photooxidized chlorophyll and carotenoid secondary electron donors were shown, for the first time, to be capable of charge recombination with photoreduced Fe^{2+}, oxidizing the iron in a temperature-dependent fashion below 300 K. Two redox populations of Fe^{3+} were revealed at low temperatures. One population was photoreduced at the lowest attainable temperatures, while the other was fully photoreduced only at temperatures above *ca.* 140 K, having an apparent reduction potential below that of the Q_A / Q_A^{-} couple (–80 mV) at lower temperatures. It is hypothesized that the redox activity of the non-heme iron depends upon the existence of a facile proton-transfer pathway linking the site to the stromal surface of the protein, and that the redox activity of the non-heme iron may probe redox-coupled proton-transfer reactions around the Q_B site. Supported by the DOE, Office of Basic Energy Sciences, Division of Chemical Sciences DE-FG02-05ER15646.

PS2.30
An investigation into thermodynamics of the thermal denaturation of the reaction centre of *Rhodobacter sphaeroides in vitro* and reconstituted into liposomes.

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The thermodynamics of thermal stability of the reaction centre (RC) of *Rhodobacter sphaeroides* isolated in detergent micelles and reconstituted into liposomes has been investigated. The temperature dependence of the absorbance spectra and circular dichroism spectra were investigated in the ultra-violet region and the visible region to investigate the thermal stability of the protein component of the RC, and the co-factors in their native binding pockets, respectively. The isolated RC in detergent micelles shows three melting transitions at 8 °C, 45 °C and 113 °C. For these three transitions the melting entropies (ΔH_m) are 210 kJ mol^{-1} 320 kJ mol^{-1} and 412 kJ mol^{-1} and specific heat capacities (ΔC_p) are 0.68 kJ K^{-1} mol^{-1}, 0.5 kJ K^{-1} mol^{-1} and 0.2 kJ K^{-1} mol^{-1}, respectively The transition at 8 °C is attributed to a conformational change affecting the carotenoid binding site. The 45°C melting point was observed when monitoring both protein and co-factor absorption, while the transition at 113 °C was monitored in the UV region only. In reconstituted RCs only one melting transition was observed at 83 °C in the visible region of the absorption spectrum. Moreover, we observed a lower ΔH_m (140 kJ mol^{-1}) and a substantial increase of ΔC_p (2.2 kJ K^{-1} mol^{-1}) in the liposome-reconstituted RC. The balance between entropic and enthalpic contribution to the temperature dependent free energy of melting provide a microscopic description of the temperature stabilisation of the RC conferred by a membrane-mimicking environment.

PS2.31
Electronic structure of axial histidines in photosynthetic reaction centres of *Rhodobacter sphaeroides*

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Histidine residues are the axial ligands to primary electron donor chlorophylls in all known photosynthetic reaction centres (RC). In bacterial reaction centres, mutation of the axial histidines has been demonstrated to affect kinetics of electron transfer and symmetry of the

electron spin distribution. In the present study, different Magic angle spinning (MAS) NMR techniques are applied in order to explore the electronic structure of the histidines co-ordinating to Mg^{2+} in bacterial reaction centres. ^{15}N MAS NMR spectra of [$^{13}C_6$,$^{15}N_3$]-histidine labeled bacterial reaction centers clearly show two different classes of axial histidines in RC. Out of four magnesium bound histidines, one show very different electronic structure in which magnesium bound nitrogen resonates 6 ppm downfield compared to rest of the three histidines. This data was further confirmed by 2D heteronuclear (^1H-^{13}C) dipolar correlation spectrum that shows clear separation of axial histidines into two catogories based on the chemical shifts of d carbon and d proton of axial histidines. The DFT calculations corroborate that one of the axial histidine in reaction centre may be carrying partial negative charge in ground state due to partial displacement of the proton of pros nitrogen of the imidazole ring. We propose that this could be the axial histidine ligated to one of the special pair bacteriochlorophyll in the active branch of bacterial reaction centres and might be involved in tuning the properties of the active branch.

PS2.32
Proton uptake in the reaction center mutant L210DN from *Rhodobacter sphaeroides* via protonated water molecules

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The reaction center (RC) of the purple nonsulfur bacterium *Rhodobacter sphaeroides* uses light energy to reduce and protonate a quinone molecule, Q_B (the secondary electron acceptor), to form quinol, Q_BH_2, which is released from the RC. Asp210 in the L-subunit was shown to be a catalytic residue in this process. In the L210DN mutant the proton uptake pathway is disturbed, which slows down the proton transfer to Q_B and leads to a deceleration of the re-oxidation of Q_A^- in the $Q_A^-Q_B$ à $Q_AQ_B^-$ transition. Previous Fourier-transform infrared (FTIR) measurements led to the suggestion of a mechanism of electron transfer involving a intermediary electron donor X [1]. Q_B^- formation in the L210DN mutant precedes Q_A^- oxidation even more pronounced than in the wild type RC [2]. The structure of the L210DN mutant was solved to 2.5 Å. There are no major structural differences as compared to the wild type protein. We found Q_B in the distal position and a chain of water molecules between Asn210 and Q_B, which is highly conserved in wild type RC structures. Continuum absorbance changes in FTIR-spectra indicate deprotonation of a protonated water cluster, most likely of the water chain between Asn210 and Q_B. In addition an H/D exchange spectrum of the L210DN mutant in the ground state evidences a water molecule with a dangling hydroxyl group.

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PS2.33
Secondary Donors in Low-Temperature Optical Spectroscopy of Photosystem II

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At low temperature (5K) light-induced charge separation in photosystem II is followed by charge stabilisation due to reduction of $P680^+$ by secondary donors such as carotenoid (Car) or chlorophyll (Chl) which compete with $P680^+$ Q_A^- charge recombination.

In order to study charge stabilisation in photosystem II at low temperature we utilised a newly developed CCD-based absorption spectrometer providing minimal actinic fluence and reasonable time (100 ms) and spectral (0.5 nm) resolution while covering a large spectral window from 470 – 1100 nm. This enabled us to follow the electron transfer reactions in photosystem II by monitoring the specific signals for Q_A^-, Chl^+ and Car^+ simultaneously in the wavelength and time domain.

We show that, as a result of inducing multiple turnovers in photosystem II during the course of illumination with either continuous green light or saturating laser flashes, different donors are generated. These include two different carotenoids, chlorophyll and a third as not yet identified donor. The relative amounts of these donors change with the number of photons absorbed per reaction centre. In a prolonged illumination Car^+ is largely replaced by the other more stable donors, resulting in slower recombination kinetics in the dark after illumination. In addition the kinetic separation of different donor species enables us to identify spectral features to either Car or Chl oxidation processes.

PS2.34
The lipid environment influence on spectral and kinetic properties of semiquinones in bacterial photosynthetic reaction centres.

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Quinones perform a key role in the energy conversion of biological systems, coupling oxydation/reduction reactions to proton uptake or release across membranes. In bacterial photosyntesis a cyclic electron flow occurs between the Reaction Centrer (RC), the bc_1 complex and the cytochrome c_2; this movement of electrons is accompanied by a net flow of protons from the periplasmic to the cytoplasmic space. Electrons are shuttled beteween the membrane-spanning RC and bc1 complex by the hydrophobic ubiquinone-10 (UQ_{10}). In the RC crystal structure two molecules of UQ_{10} are found in distinct binding sites, termed Q_A and Q_B. The quinone in the Q_A pocket acts as a single electron carrier and is firmly associated to the protein, while the one in the Q_B pocket can undergo to double reduction and protonation and is loosely bound. When isolated RC are hit by a light flash, the charge separated state $D^+Q_B^-$ (or $D^+Q_A^-$ in the presence of Q_B inhibitors) is produced. Here D is the primary donor, a bacteriochlorophyll dimer. In the presence of a suitable electron donor to D, a relatively stable semiquinone is formed. The properties of the two semiquinones are mainly modulated by the protein environment, but fine adjustments are achieved by the interaction with the protein surrounding, namely the lipidic bilayer. We have reconstituted the RC in liposomes made by different and physiologically important phospholipids and recorded the spectra of the semiquinones with the aim of study protein-lipid interactions that are involved in the fine tuning of these key intermediates.

PS2.35
Influence of bilayer thickness on photosynthetic Reaction Centres function.

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The photosynthetic Reaction Centre (RC) is a transmembrane pigment-protein complex where the energy associated to the electromagnetic solar radiation is converted into chemical energy in the form of a charge separated state. Following absorption of a photon the bacteriochlorophyll dimer (D) reaches its singlet excited state (D*) and then transfers an electron through a chain of cofactors to the final electronic acceptor Q_B. In absence of electron donors to D, a charge recombination reaction occurs with a lifetime in the seconds timescale, strongly influenced by the protein environment. The membrane-spanning portion of the RC is about 35 Å and is normally embedded in the intracitoplasmic membrane where the most representative phospholipid is palmitoyl-oleoyl- phosphatidylcholine (POPC) with side chains of 16 (saturated) and 18 (monounsaturated) carbon atoms (n_c) forming a 44 Å thick bilayer. To assess the effect of a complete or partial covering of the transmembrane portion, RC have been reconstituted in liposomes made by 1,2 diacyl – phosphatidylcholine with n_c spanning from 9 to 14, ensuring that the bilayers formed are in the liquid-crystalline phase. RC embedded in liposomes made by POPC, showing a charge recombination lifetime of 2.2 s, was taken as a reference system. A discontinuity in the charge recombination reaction lifetime was found between n_c = 12 and n_c = 14 corresponding to a bilayer thickness of 32 and 37 Å respectively.

PS2.36
The Distant Protein Environment influences the Re-dox Potentials of the Quinone Acceptor A_1 and the F_X Iron-Sulphur Cluster in Photosystem I: TR EPR Study of Mutants of the D575<

I Karyagina (FU Berlin)

Multifrequency TR EPR is applied to measure the kinetics of electron transfer (ET) from A_1 to F_X in site-directed variants of the amino acids $D575_{PsaB}$ and $D566_{PsaB}$ in Photosystem I. Electrostatic calculations predict that the partial negative charges associated with these Asp residues play a significant role in modulating the Red-Ox potentials of A_1 and F_X. To test this prediction, the side chains of residues 575_{PsaB} and 566_{PsaB} were changed from negatively charged (Asp) to neutral (Ala) and to positively charged (Lys). The rate of ET from A_{1A} to F_X was found to decrease slightly in the $D575_{PsaB}$ but increase in the $D566_{PsaB}$ variants from wild type in the sequence D/A/K. These results are consistent with the expectation that changing the partly negatively charged Asp residue will shift the Red-Ox potential of nearest cofactors to more positive values and this shift will depend on the distance between the cofactor and the Asp residue position. According to the X-ray structure model (1JB0), the Red-Ox potentials of A_{1A} should experience a larger shift than A_{1B} and even larger than F_X in the $D575_{PsaB}$ variants. But F_X should experience a larger shift than either A_{1A} and A_{1B} in the $D566_{PsaB}$ variants. As a consequence, the driving energy (the Red-Ox potential difference) and the ET rate from A_{1A} to F_X will decrease in the former and increase in the latter case. The experimental findings agree to a large extent with the predictions from electrostatic calculations as will be discussed in detail.

PS2.37
Bacterial reaction centers with bound Mn are capable of light-controlled enzymatic activity

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The reaction center from Rhodobacter sphaeroides has long been used as a model for photosystem II, and our group has incorporated features of photosystem II such as a highly oxidizing primary donor that is capable of oxidizing tyrosine residues. We have also introduced a Mn cofactor bound at a location analogous to that of the Mn cluster of photosystem II. In addition to characterizing the new light-driven electron transfer reactions involving Mn as a secondary donor to the oxidized bacteriochlorophyll dimer, we are examining the modified reaction centers for the types of functional properties found in other Mn-binding enzymes. For example, superoxide dismutase catalyzes the conversion of superoxide anions in a critical antioxidative reaction. An optical assay used to quantify the amount of superoxide dismutase activity by measuring the concentration of superoxide anion was employed to test for such activity in reaction centers. The modified reaction centers with bound Mn were found to be capable of reacting with superoxide. This enzymatic activity was present only when the reaction centers were exposed to light and only when the bacteriochlorophyll dimer was highly oxidizing. These results indicate that the superoxide conversion proceeds from the oxidized state of the Mn cofactor. The modifications of the reaction center provide a pathway by which a phototroph capable of enzymatic activity could have been an intermediate in the evolutionary development of organisms containing oxygen-evolving complexes. The ability to regulate the enzymatic activity using light provides the possibility of developing new light-controlled enzymes.

PS2.38
Mutational analysis of the EC2-EC3 environment in PS1

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Photosystem I (PS1) has two branches of cofactors (A and B), which are potential electron transfer pathways to the FeS cluster F_X. Each branch consists of two chlorophylls (ec2 and ec3) and a phylloquinone (PhQ). P_{700} has been thought to be the site of primary charge separation, but mutations to the H-bond donor near P_{700} seem to have no effect on directionality (Li et al., 2004, *Biochemistry* 43:12634-47) or on the rate of primary charge separation (Holzwarth et al., 2006, *Biophys. J.* 90:552-65). Mutation of the H-bond donor to $ec3_A$ (PsaA-Tyr696) or $ec3_B$ (PsaB-Tyr676) had a marked effect on directionality. The PsaA-Y696F mutation increased the amplitude of the faster kinetic component (assigned to electron transfer from PhQ_B) and decreased the amplitude of the slower kinetic component (assigned to electron transfer from PhQ_A), while the PsaB-Y676F mutation had the opposite effect. Recent ultrafast data shows that these mutations slow primary charge separation, implicating ec3 as one of the species involved in this step. We have also been able to obtain high-field EPR (413 GHz) spectra of photo-accumulated "A_0-" with clear resolution of the g-tensor for WT and both mutants. We are examining other mutations to the environment of $ec2_A$ and $ec2_B$, specifically an alanine close to the pair (PsaA-Ala684 and PsaB-Ala664) and the H-bond partner to the axial ligand H_2O (PsaB-Asn591 and PsaA-Asn604). Like PsaA-Y696F, the PsaA-A680D mutant shows a similar increase in the faster component, indicating redirection of electrons down the B branch.

PS2.39
Triplet photoprotection by carotenoid in intact photosystem II cores

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Two ß-carotenes are located in the D1-D2 reaction center (RC) of photosystem II (PSII) in the X-ray structure.[1] Despite the presence of two carotenes and a high yield of triplet chlorophyll production, no triplet carotene quenching has been observed so far in the RC of PS II. We have used nanosecond transient spectroscopy to study the triplet kinetics and photoprotection in intact PS II cores from *T. elongatus*. We report for the first time efficient photoprotection by ^{3}Chl-to-^{3}Car transfer. We assign the ^{3}Car to the D_2 carotene, assigning a photoprotective role to the otherwise inactive branch of the RC.

[1] Loll, B., Kern, J., Saenger, W., Zouni, A., Biesiadka, J. *Nature* 438, 1040-1044 (2005).
[2] Telfer, A. *Photochem. Photobiol. Sci.* 4, 950-956 (2005).

PS2.40
The dual-branched electron transfer in photosystem I

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There exists clear evidence - based on the bi-phasic reduction of the iron-sulphur cluster F_x - that in PS I electron transfer (ET) occurs in both branches. However, the relative efficiencies and in particular the mechanism and the rates of the early ET steps have not been determined. We present here ultrafast transient absorption measurements on two mutants (A-Y696F, B-Y676F) from *C. reinhardtii* We show that primary CS slows down in both mutants relative to the w.t. We were able to separate the kinetics in both branches and determined all the rates and yields of the early ET steps. The data provide clear evidence for efficient ET along both branches.

PS2.41
Charge separation and energy transfer in the Photosystem II core complex studied by femtosecond mid-infrared spectroscopy.

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The core of photosystem II (PSII) of green plants contains the reaction center (RC) proteins D1D2-cytb559 and two core antennas CP43 and CP47. We have used time-resolved visible pump/mid-infrared probe spectroscopy in the region between 1600 and 1800 cm^{-1} to study the energy transfer and charge separation events within PSII cores. The absorption difference spectra in the region of the keto and ester chlorophyll modes show spectral evolution with time constants of 3 ps, 27 ps, 200 ps and 2 ns. Comparison of IR difference spectra obtained for the isolated antennas CP43 [1], CP47 [2] and the D1D2-RC [3] with those measured for the PSII core allowed us to identify the features specific for each of the PSII core components. From the presence of the CP43 and CP47 specific features in the spectra up to time delays of 20-30 ps, we conclude that the main part of the energy transfer from the antenna's to the RC occurs on this time scale. Direct excitation of the pigments in the RC leads to radical pair formation of $P_{D1}^+Pheo_{D1}^-$ on the same time scale as multi-excitation annihilation and excited state equilibration within the antennas CP43 and CP47, which occur within ~1-3 ps. The formation of the earlier radical pair $Chl_{D1}^+Pheo_{D1}^-$, as identified in isolated D1D2 complexes with time-resolved mid-IR spectroscopy [3] is not observed in the current data, probably because of its relatively low concentration. Relaxation of the state $P_{D1}^+Pheo_{D1}^-$, caused by a drop in free energy, occurs in 200 ps in closed cores. We conclude that the kinetic model proposed earlier for the energy and electron transfer dynamics within the D1D2-RC [3], plus two slowly energy transferring antennas C43 and CP47 explains the complex excited state and charge separation dynamics in the PSII core very well. We further show that the time resolved IR-difference spectrum of $P_{D1}^+Pheo_{D1}^-$ as observed in PSII cores is virtually identical to that observed in the isolated D1D2-RC complex of PSII, demonstrating that the local structure of the primary reactants has remained intact in the isolated D1D2 complex.

[1] Di Donato, M., van Grondelle, R., Groot, M. L., submitted to *J. Phys. Chem.*
[2] Groot, M. L., Breton, J., van Wilderen, L. J. G. W., Dekker, J. P., van Grondelle, R. (2004) *J. Phys. Chem. B.* 108, 8001-8006.
[3] Groot, M. L., Pawlowicz, N. P., van Wilderen, L. J. G. W., Breton, J., van Stokkum, I. H. M., van Grondelle, R. (2005) *Proc. Natl. Acad. Sci. U.S.A.* 102, 13087-13092.

PS2.42
(A_1^--A_1) FTIR Difference Spectra Obtained using Photosystem I Particles With ^{18}O and Specifically ^{13}C Labeled Quinones Occupying the A_1

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Time-resolved step-scan Fourier transform infrared difference spectroscopy (TRSS FTIR DS) has been used to produce (A_1^--A_1) FTIR difference spectra for PS I particles from *S. 6803* that have a 2-methyl naphthaquinone (2MNQ) occupying the A_1 binding site. 2MNQ is the same as phylloquinone except that the phytyl chain is replaced with a hydrogen atom. We have also obtained spectra for PS I particles containing 2MNQ that is ^{18}O labeled, and specifically $^{13}C_1$ or $^{13}C_4$ labeled. These spectra allow a clear demarcation between bands associated with the quinone and bands associated with other molecular groups in the binding site.
To aid in the interpretation of the (A_1^--A_1) FTIR difference spectra we have obtained FTIR absorption spectra for all labeled and unlabeled quinones studied, and have used density functional methods to calculate the FTIR absorbance and "anion minus neutral" absorbance difference spectra for all of the quinones studied. Finally, we have used QM/MM methods (ONIOM method) to calculate the vibrational properties of quinones *in* the A_1 binding site.

PS2.43
Calculated Electronic Spectra of Chlorophylls in Solution

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Chlorophylls and bacteriochlorophylls play a fundamental role in photosynthesis. They are used to capture and funnel solar energy to the reaction center. They are also the primary units used to convert excitation energy into chemical products. Knowledge of their function in various environments, from solution, to photosynthetic light harvesting

complexes, to reaction center protein complexes, is therefore of considerable importance. As a first step in developing a quantitative model of chlorophylls in various environments we have been using time dependent density functional theory to calculate the electronic properties of several chlorophyll structures in various solvents. Up until now most studies have been limited to isolated pigments in the gase phase. In particular, we have used time dependant density functional methods [B3LYP/6-31G(d)] in conjunction with the polarizable continuum model, to obtain fully optimized structures of chlorophylls *a, b,* c_1, c_2, d and bacteriochlorophylls *a, b, c, d, e* and *g*, in both polar and non-polar solvents. The calculated wavelengths for the Q_y, Q_x and soret bands of the different bacteriochlorophylls and chlorophylls were found to agree well with experimental spectra.
We have also used QM/MM methods to calculate the electronic properties of chlorophyll-*a* in the presence of 40 explicitly added solvent molecules. Solvent induced electronic band shifts calculated using this explicit solvent model are compared to the results obtained using the polarizable continuum model. In this way the applicability of the polarizable continuum model is assessed.

PS2.44
Calculation of the Vibrational Properties of Chlorophyll-*a*

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Chlorophylls are important pigments in photosynthetic processes, and they have been widely studied using vibrational spectroscopies. In spite of this, little work has been undertaken to quantitatively assess the vibrational structure of chlorophyll. With this in mind we have used density functional theory (DFT) to calculate the vibrational properties of chlorophyll-*a* (Chl-*a*) in the gas phase and in the presence of solvents. Gas phase calculations do not accurately simulate the vibrational properties of the carbonyl groups of either neutral or cationic Chl-*a*. That is, the calculated results do not agree with experimental observations.
Here we undertake calculations to investigate how different factors impact the carbonyl vibrational modes of Chl-*a*. Firstly, we use DFT in combination with the polarizable continuum model to investigate how different solvents impact the vibrational properties of Chl-*a*. Secondly, how additional peripheral groups modify the vibrational properties of Chl-*a* and Chl-*a*' are investigated. Thirdly, the effects of hydrogen-bonding to the carbonyl groups are investigated. Our calculations show that solvent effects, as described by the polarizable continuum model, cannot explain the differences between theory and experiment. Additional peripheral groups cannot explain the differences either. Hydrogen bonding to the carbonyl groups of Chl-*a* appear to be the only way to reconcile calculations with experimental spectra. We therefore propose that in all previously determined experimental IR spectra of Chl-*a* in solvent, hydrogen bonding effects predominate, possibly because water has never been completely removed in any of these previous experiments.

PS2.45
Photo-CIDNP MAS NMR on photosynthetic reaction centres

J Matysik (Leiden University)

The first step of photosynthesis, the charge separation upon light-induced electron transfer, is of unsurpassed efficiency having quantum yields close to unity. In all six RCs which have been tried, originating of various branches of the evolutionary tree, photo-CIDNP has been observed, despite the window of the conditions for the occurrence of this phenomenon is rather narrow. Hence, we suppose that the conditions allowing for the production of photo-CIDNP are conserved in evolution. On the other hand, despite of lots of efforts until now no photo-CIDNP has been reported from any artificial RC system, having also low quantum yield. Therefore, there may be a link between the fundamental conditions, allowing for the production of photo-CIDNP, and the efficiency of light induced electron transfer. In that case, understanding of these fundamental conditions could have a large impact on the synthesis of artificial RC systems.
Three mechanisms producing photo-CIDNP in RCs are proposed and in good agreement with experiments. In these experiments, NMR enhancement factors of above 10000 have been observed, making photo-CIDNP to a hot candidate to overcome the intrinsically low sensitivity and selectivity in solid-state NMR. Currently, the method is used to study the photochemical machinery of various RC systems at the atomic resolution. To obtain detailed insight into the mechanisms, the build-up of photo-CIDNP in RCs is studied with nanosecond-flash experiments, allowing for observation of the evolution of electron spin density during the birth of the radical pair.

PS2.46
The origin of the high redox force of photosystem II: A photo-CIDNP MAS NMR analysis

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Photochemically induced dynamic nuclear polarisation (photo-CIDNP) enhances the intensity of a NMR line by inducing a non-Boltzmann distribution of the nuclear spin states. The observation of photo-CIDNP by magic angle spinning (MAS) NMR in photosynthetic reaction centers (RCs) allows for a spectacular increase of NMR signals up to factor 10000, providing significant increase of sensitivity and selectivity of signals originating from cofactors involved into electron transport. Hence, photo-CIDNP MAS NMR allows analysing the photochemical machinery of RCs at the atomic and molecular level. Of particular interest is electron donor of photosystem II of plants, having the highest redox potential in living nature. Here, we present both ^{13}C and ^{15}N photo-CIDNP MAS NMR spectra of photosystem II. Combined with existing data from photosystem I, a direct comparison of the donors of both plant photosystems can be made. The comparison clearly demonstrates an inversion of the electron spin density distribution in the donor of photosystem II compared to photosystem I; latter resembles the spin density of an undisturbed chlorophyll molecule. Furthermore, at the donor of photosystem II, electron spin density is observed on a deprotonated histidine. A model, in which a tilted axial histidine affects the electron density distribution in the chlorophyll macrocycle, is proposed.

PS2.47
Time-Resolved High-Field EPR Spectroscopy of Natural Photosynthesis: Photoinduced Electron Transfer Pathways in Photosystem I.

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0, a chlorophyll molecule, and A_1, a phylloquinone. From A_1^- the electron is transferred to the [4Fe–4S] cluster F_X, and further to F_A and F_B, two iron-sulfur clusters held within an extrinsic protein subunit Thus, unlike Type II reaction center (RC) proteins, ET in PSI does not

terminate at two functionally distinct quinines. Is ET in Type I RCs likewise functionally asymmetric? Resolution of this basic, yet important, issue of ET directionality (uni *vs*. bi) in PSI has remained an experimental challenge.
Using high-field (HF) pulsed EPR technique we have resolved two distinct transient spectra of the $P^+A_1^-$ radical pair from PSI RC proteins of the cyanobacterium *Synechococcus lividus* and correlate their structures with kinetic data and the X-ray crystal structures of PSI. We demonstrate that the geometries of the two distinct donor/acceptor pairs correspond to the charge separated states along the A and B branches, and that our assignments of radical pair geometries are in excellent agreement with the X-ray crystal structure of PSI. Together with previously reported data, the concomitant structural and kinetic information obtained with HF EPR provide unambiguous evidence of bidirectional ET in PSI.

PS2.48
Photochemically induced dynamic nuclear polarization studies on photosynthetic reaction centers from diverse organisms

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Photochemically induced dynamic nuclear polarisation (photo-CIDNP) MAS NMR is a technique in studying the electronic structures of the cofactors involved in the electronic transfer in the RCs. Previously photo-CIDNP was observed in RCs from *Rhodobacter sphaeroides* (WT, R26) and PSII (spinach) which are type II RCs having quinone as the terminal electron acceptor. Here we present RCs from diverse photosynthetic organisms containing type I RCs that have iron sulphur clusters as terminal electron acceptors, in which photo-CIDNP has been observed by ^{13}C MAS NMR. These RCs range from photosystem I (spinach) [1], green sulphur bacteria (*Chlorobium tepidum)* [2] and isolated membrane fragments from heliobacteria (*Heliobacillus mobilis)* [3]. The ^{13}C photo-CIDNP spectral pattern in *Rhodobacter sphaeroides* WT, PSI and *Chlorobium tepidum* comprises of emissive (negative) signals, while both emissive and absorptive (positive) signals are observed in *Rhodobacter shaeroides* R26, PSII and *Heliobacillus mobilis*. Photo-CIDNP thus appears to be an inherent property of natural RCs. Hence, there may be a link between the fundamental conditions which result in photo-CIDNP and the efficiency of RCs in light induced electron transfer.

[1] Alia, E. Roy, P. Gast, H. J. van Gorkom, H. J. M. de Groot, G. Jeschke, J. Matysik (2004) J. Am. Chem. Soc. 126, 12819-26.
[2] E. Roy, Alia, P. Gast, H. van Gorkom, H.J.M. de Groot, G. Jeschke, J. Matysik (2007) *Biochem. Biophys. Acta*, in press.
[3] E. Roy et al., to be published.

PS2.49
The structure of plant photosystem I at 3.4 Å resolution

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A plant Photosystem I (PSI) is exquisitely organised, intricate, multi-subunit membrane supercomplex of protein and non-protein components that drive the photosynthesis process. Previously we determined the structure of a plant PSI, which provided the first a-carbon structural model of the supercomplex, containing the reaction center complex (RC) and the peripheral antenna (LHCI). Continuous and progressive improvement of purification procedures and optimization of crystal quality altered significantly the diffracting properties of crystals, enabling the recent determination of the X-ray crystal structure at 3.4 Å resolution (Amunts, A., Drory, O. and Nelson, N. The structure of a plant Photosystem I supercomplex at 3.4 Å resolution. *Nature,* 2007). The current crystal structure provides a picture at near atomic detail of 16 out of 17 protein subunits with an additional subunit (PsaN) being identified for the first time on the luminal side of the supercomplex. Positions of 3038 out of 3443 predicted amino acids were assigned as were those of 168 chlorophylls (65 revelaing the orientation of the Qx/Qy transition dipolar moments), 2 phyloquinones, 3 Fe4S4 clusters and 5 carotenoids. In addition the unique interactions between the LHCI and the RC were revealed. The structural information on proteins, co-factors and interactions between them provides a first glimpse at the fine architecture of nature's most efficient photochemical nano-machine.

PS2.50
Origin and function of the long-wavelength chlorophylls of PSI in the cyanobacterium *A. platensis*.

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PSI core antenna of cyanobacteria is highly enriched with the long-wavelength (or red) chlorophylls (Chl); the relative content of the longwave Chls in the cyanobacterium *A. platensis* is of about 10%. The red-most Chls in PSI complex of that cyanobacteria absorb at 740 nm and emit at 760 nm. The longwave bands in 77 K CD spectra of PSI complexes of *A. platensis* at 711 and 736 nm are indicative on the excitonic origin of red Chls. According to 77 K LD spectra, the transition dipole moments of the red-most states are oriented parallel to the membrane plane. Light-induced difference CD spectra of PSI complexes *A. platensis* give strong evidence for the delocalization of the excited singlet states in the reaction centre. Therefore P700 cannot be considered as a dimer but should be regarded as a multimer of the six nearly equally coupled reaction centre Chls. Energy absorbed by red Chls migrates uphill with high efficiency to P700 causing its oxidation. Fluorescence at 760 nm of the red-most Chl in PSI of *A. platensis* is quenched not only by P700 cation radical but also by P700 in triplet state thus indicating the protective role of red Chls.

PS2.51
Low quantum yield electron transfer pathways in PS II

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At low temperatures (<180 K) the electron transfer in photosystem II (PS II) is inhibited from Q_A^- to Q_B and from Y_Z to P_{680}^+. Depending on the initial redox state of cytochrome b_{559} different long-lived states are formed upon illumination (Q_A-P_{680}Cyt b_{559}, Q_A-$P_{680}Car^+$, Q_A-$P_{680}Chl_Z^+$). Their formation is interpreted as an electron transfer from alternate electron donors (Cyt b_{559}, Car, Chl_Z) to P_{680}^+, which occurs with low quantum yield besides charge recombination of Q_A-P_{680}^+. To determine their function in photosynthesis, the formation and decay of these states are investigated by means of Raman, EPR, absorption difference spectroscopy and LD experiments. DFT-calculations on β-carotene and its radical cation have been performed to assist with the assignment of Raman-signals arising in PS II samples after illumination at 77 K. Oxidation of $Chlorophyll_Z$ has been analyzed with the focus on

absorbance changes in the Q_Y and Soret region. All measurements were done using PS II from *Thermosynechococcus elongatus* as well as PS II from *Spinacia Oleracea*. Interestingly there are some quite remarkably differences in the formation and decay of the different closed states in these two organisms.

PS2.52
Temperature Dependence of the Reduction Kinetics of P680+ in Oxygen-Evolving PS II Complexes throughout the range from 320 K to 80 K

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The absorption of light by Photosystem II (PS II) induces a transmembrane charge separation between the primary electron donor P680, which is composed of P_{D1} and P_{D2}, and the plastoquinone acceptor Q_A. The photooxidized P680 is re-reduced by the redox active tyrosine Tyr_Z (D1-Tyr-161) which in turn accepts an electron from the oxygen-evolving complex.
Transient absorbance difference spectroscopy has been used to study the reduction kinetics of P680+ after the first flash given to dark-adapted oxygen-evolving PS II complexes from Thermos*ynechococcus elongatus* as a function of temperature between 80 K and 320 K.
The half-life of P680+ reduction by Tyr_Z increases from 20 ns at 300 K to about 4.2 µs at 150 K corresponding to an activation energy of 122 ± 3 meV. Analysis by nonadiabatic electron transfer theory yields edge-to-edge distance of about 9 Å in excellent agreement with the distance between P_{D1} and Tyr_Z in the recent structural model of PS II at 3.0 Å resolution [1]. In the range from 260 K to 150 K, the re-reduction of P680+ by Tyr_Z is increasingly replaced by the charge recombination of P680+ with QA-. It is proposed that reorganization processes which are required for the stabilization of the state P680TyrZox become blocked around 200 K.
[1] B. Loll, J. Kern, W. Saenger, A. Zouni, J. Biesiadka (2005) Nature, 438, 1040-104

PS2.53
Electron Transfer between Q_A^- and Q_B in *Rb. sphaeroides* Reaction Centers:Recent Advances from FTIR Difference Spectroscopy

J Breton (Service de Bioénergétique, CEA-Saclay)

Rhodobacter sphaeroides two ubiquinone molecules, Q_A and Q_B, play a pivotal role in the coupling of electron transfer to proton uptake. Three distinct observations derived from recent FTIR studies related to the $Q_A^-Q_B$?$Q_AQ_B^-$ reaction will be presented:
-- In contrast to native RCs where Q_B reduction elicits protonation of the single residue Glu L212 in the pH range 4-10, a large pH dependence of the protonation pattern of at least three distinct carboxylic acid residues has been observed in the AspL212/GluL213 swap mutant (Nabedryk et al., 2007, *Biochemistry 46*, 1176).
-- Comparison of the Q_A^-/Q_A FTIR difference spectra of native and CysM260 mutant RCs demonstrates that the unusually strong hydrogen bond between the carbonyl of Q_A and His M219 is not essential for efficient electron transfer from Q_A^- to Q_B. (Breton, J. et al; 2007, *Biochemistry 46*, in press).
-- On the basis of time-resolved FTIR measurements in native RCs, a new and unconventional mechanism has been proposed in which Q_B^- formation precedes Q_A^- oxidation (Remy, A. and Gerwert, K., 2003, *Nat. Struct. Biol. 10*, 637). The FTIR spectrum of the proposed transient acceptor (X^+/X) has been recently reported in the GlnL210 mutant (Hermes, S. et al., 2006, *Biochemistry 45*, 13741). The analysis of normalized steady-state Q_A^-/Q_A and Q_B^-/Q_B FTIR spectra provides compelling evidence that in both GlnL210 and native RCs the species X^+ and X are spectrally indistinguishable from Q_B and Q_B^-, respectively (Breton, J., 2007, *Biochemistry 46*, 4459).

PS2.54
A_0^-?A_1 electron transfer in *Chlamydomonas reinhardtii* Photosystem I with replaced A_0 axial ligand

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Replacement of methionine, the natural axial ligand to the primary electron acceptor (A_0) in Photosystem I, with a series of different amino acids results in dramatic increase of the A_0^- lifetime from ~20 ps in wild type to a few nanoseconds in the mutants in the case of *Chlamydomonas reinhardtii* [Ramesh et al. (2004) Biochemistry 43:1369–1375; Ramesh et al. (2007) BBA 1767:151-160]. This effect is similar independently if the mutation affects A-side or B-side A_0. This observation further confirms an existence of two equivalent primary electron acceptors in both symmetric branches of Photosystem I in *Chlamydomonas reinhardtii*, which makes this photosystem unusual among other photosystems (from purple bacteria, PS II) which are essentially unidirectional. However, it is still not clear if the bidirectionality of electron transfer in Photosystem I is complete, i.e. if the electron from A_0^- reaches A_1 in both branches or takes another route in the "non-active" branch. In order to solve this issue, in this contribution we will compare kinetics of A_0^- reoxidation to the kinetics of A_1^- formation in the case of both A-side and B-side A_0 mutants.

PS2.55
Triplet states in photosynthetic reaction centers of *Rb. Sphaeroides*

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The photoexcited triplet state of the primary donor P_{865} and of the carotenoid in the reaction center of *Rb. sphaeroides* wild type and mutants were investigated by pulsed EPR spectroscopy. Temperature dependence and time evolution of the triplet states were measured. Different mechanisms of triplet formation were observed: At T=10K, *Rb. sphaeroides* strain R.26-1 and 2.4.1 (wild type) and the double mutant Gly?Asp(M203)/Ala?Trp(M260)[1] form a triplet via the radical pair (RP) mechanism. The electron transport in this species proceeds exclusively via the A-branch of the RC. In the double mutant Leu?His(M214)/Ala?Trp(M260)[1] the intersystem crossing (ISC) mechanism is dominant. At T>30K, the radical pair mechanism also contributes to triplet formation. Electron transfer in this species proceeds via the B-branch but with very low triplet quantum yield. This process is temperature dependent. The zero-field splitting parameters of $^3P_{865}$ are the same for the RP and ISC triplet. The carotenoid takes over the triplet state from $^3P_{865}$. The amount of carotenoid triplet signal and the transfer rate are temperature dependent. Moreover, the rate of triplet transfer

differs in *Rb. sphaeroides* 2.4.1 and mutants. The data confirm that triplet exciton transfer from $^3P_{865}$ to Car proceeds via the accessory BChl, whereby the $^3P_{865}$?BChla transfer step is rate limiting.

[1]Paddock et al. (2005) Biochemistry 44, 6920-6928

PS2.56

Quantifying the excitation migration time in Photosystem II. Consequences for primary and secondary charge separation rates and the corresponding drop in free energy.

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The fluorescence decay kinetics of Photosystem II (PSII) membranes with open reaction centers (RCs), was compared after excitation at 420 and 484 nm. These excitation wavelengths lead to preferential excitation of Chl *a* and Chl *b*, respectively, which causes different initial excitation populations in the inner and outer antenna system. The non-exponential fluorescence decay appears to be 6.6 ± 0.8 ps slower upon 484 nm excitation for preparations that contain on average 2.3 LHCII (light-harvesting complex II) trimers per RC. Using a recently introduced method (*Biophysical J* 91:3776-3786, 2006) it is concluded that the average migration time of an excitation towards the RC contributes 34 ± 7% to the overall trapping time. This demonstrates that the exciton-radical pair equilibrium (ERPE) model that describes the kinetics of samples without outer antenna [*Proc. Natl. Acad. Sci .USA* 84, 1987], is not applicable for systems with outer antenna. This prompts us to introduce the MiCS model, which includes appreciable contributions from both the Mi(gration) time and the trapping or C(harge) S(eparation) time to the overall decay. It is conluded that the effective rate of primary charge separation of the entire RC (i.e. not only the primary donor) is $(3.7 \pm 0.5 \text{ ps})^{-1}$, the rate of secondary charge separation is $(155 \pm 42 \text{ ps})^{-1}$ and the drop in free energy upon primary charge separation is (825±106) cm^{-1}. This large drop in energy occurs faster than generally found for systems without outer antenna.

PS2.57

Transient EPR Studies of *In Vivo* Uptake of Substituted Anthraquinones by Photosystem I in Phylloquinone Biosynthetic Pathway Mutants of *Synechocystis sp.* PCC *6803*

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Deletion of the *menA* or *menB* gene in *Synechocystis sp.* PCC 6803 results in mutant strains of the cyanobacterium that are unable to synthesize phylloquinone. However, in the absence of phylloquinone, Photosystem I (PS I) incorporates plastoquinone allowing both strains to grow photoautotropically at low light. It has also been shown that the plastoquinone in PS I particles isolated from the mutants can be displaced by incubating the particles with naphthoquinones. Here we show that supplementing the growth medium with various substituted anthraquinones allows the mutants to grow under high light conditions. Chromatographic analysis of PS I particles isolated from cells grown in this way show varying levels of incorporation of AQ. The spin polarized transient EPR (TREPR) signals the samples are remarkably different from both the wild type and the mutants grown without anthraquinone. At room temperature, the spectra reveal that the AQ incorporated into PSI is active in electron transport and the kinetic traces show that electron transfer to the iron sulfur clusters occurs with lifetimes that depend on which anthraquinone has been incorporated. The spin polarization patterns also provide evidence that the rate of electron transfer from A_0 to AQ is slow and that significant singlet-triplet mixing occurs in the primary radical pair. Interestingly, the spectra at low temperature from all samples reveal only the fraction of the PSI complexes that still contain plastoquinone.

PS2.58

Identification of special pair and Chl_Z of Photosystem II in *Acaryochloris marina*

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The special pair and Chlorophyll$_Z$ (Chl_Z) of the photosystem II (PSII) in the Chl *d*-dominated cyanobacterium, *Acaryochlroris marina* MBIC 11017, were studied using FT-IR and electronic absorption difference spectroscopy. We purified photochemically active complexes consisting of a CP47, CP43', D1, D2, cytochrome *b*559, PsbI, and a small polypeptide. The pigment composition per two pheophytin (Phe) *a* molecules was 55 ± 7 Chl *d*, 3.0 ± 0.4 Chl *a*, 17 ± 3 a-carotene, and 1.4 ± 0.2 plastoquinone-9. The special pair was detected by a reversible absorption change at 713 nm (P713) together with a cation radical band at 842 nm. FT-IR difference spectra of the specific bands of a 3-formyl group allowed assignment of the special pair. The combined results indicate that the special pair comprises a Chl *d* homodimer. Two molecules of Chl_Z were also identified as Chl *d* using FT-IR difference spectra and UV/Vis absorption difference spectra. The potential of primary electron acceptor (Phe *a*) was shifted to a higher value than that in the Chl *a*/Phe *a* system. The overall energetics of PSII in the Chl *d* system are adjusted to changes in the redox potentials, with P713 as the special pair using a lower light energy at 713 nm. Our findings support the idea that changes in photosynthetic pigments combine with a modification of the redox potentials of electron transfer components to give rise to an energetic adjustment of the total reaction system.

PS2.59

Unexpected Difference in the P700 Redox Potential among Oxygenic Photosynthetic Organisms Revealed by Spectroelectrochemistry

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The redox potential of the primary donor of photosystem (PS) I, P700, determined over decades mainly by chemical redox titration, exhibits a heavy scatter from +375 to +525 mV vs. SHE (for review, see B. Ke, in Photosynthesis: "Photobiochemistry and Biophysics", Kluwer Academic Pubsihsers, Chap. 28, 2001). Though a part of this scattering would be experimental artifacts, the scattering of the P700 redox potentials values might reflect inherent differences in the properties of P700 among organisms which cannot be expected at a glance from highly conserved amino acid sequences of the PS I reaction center proteins. Thus, we have tried to measure the redox potential of P700 of various oxygenic photosynthetic organisms, such as cyanobacteria, red algae, green algae, and higher plants, precisely by spectroelectrochemical mean using an optically transparent thin-layer cell. Experimental conditions developed by us, which can determine the P700 redox potential with an error range of a few millivolts, revealed significant species-dependence of the P700 redox potential. The results showed that the P700 redox potentials are

spanning from +398 to +470 mV vs. SHE among different species. Furthermore, the P700 redox potential shifts to positive direction on the order of cyanobacteria, red algae, green algae and higher plants. Possible causes for the species-dependence of the P700 redox potential will be discussed by taking into account the spectroscopic properties of P700 and the natures of the external electron donor proteins, cytochrome c_6 and/or plastocyanin.

PS2.60
Fluorescence Lifetime Imaging of crystals of Photosystem I

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Photosystem I (PSI) is a multisubunit protein-pigment complex. It possesses Light-Harvesting Complexes (LHCs) that transfer absorbed energy to the core where the excitation energy induces charge separation in the reaction centre (RC). PSI is unique in binding red chlorophylls (Chls), with energy levels lower than that of the RC. These Chls may compete with the RC as an energy sink. The function of these red Chls is not fully known. Many time-resolved optical spectroscopy studies on PSI have revealed very heterogeneous excited state kinetics. Although this at least partly results from the intrinsic properties of PSI , including the presence of the red pigments , it might in principle also be caused by some sample heterogeneity. Here we show that the fluorescence kinetics of (highly homogeneous) crystals of PSI from pea are identical to those of PSI in solution. This justifies the structure-based modelling of spectral properties of PSI . Our new data indicate that the energy flow from LHC to the core proceeds mainly via the red Chls.

PS2.61
Site-directed mutagenesis of cytochrome *b*559 in the cyanobacterium *Thermosynechococcus elongatus*

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Cytochrome *b*559 (Cyt *b*559) is an intrinsic and essential component of the photosystem II (PSII) reaction centre in all photosynthetic oxygen-evolving organisms, but its function, although widely investigated, still remains unresolved. Most of the functional hypotheses propose that Cyt *b*559 may participate in secondary electron transfer pathways protecting PSII against oxidative damage. Mutational studies have not succeeded in demonstrating this redox function of Cyt *b*559 in PSII because most of the mutants obtained are impaired in the functional assembly of PSII holocomplex. We have constructed a series of site-directed mutants, each carrying a single amino acid substitution, in the thermophilic cyanobacterium *Thermosynechococcus elongatus*, in order to modify the redox potential of the heme without altering the assembly properties of PSII. We have obtained 19 mutant strains of Cyt *b*559: 10 in a-subunit (R8I, R8L, I14A, I14S, R18S, W20L, W20Y, I27A, I27T and F31Y) and 9 in ß-subunit (W20I, W20F, W20T, V21T, V28S, V28T, I31A, I31G and F32Y). Efficient liquid cultures of all mutant strains have been obtained. The midpoint redox potential of Cyt *b*559 in PSII-enriched membranes from 8 of the strains (WT, a-I14A, a-I14S, a-I27A, a-I27T, a-R18S, ß-V28T, ß-F32Y) have been measured. Some of these mutants showed relevant differences in redox properties of Cyt *b*559 compared with WT. We have also analyzed the PSII functionality of these 8 strains by polarographic and thermoluminescence techniques. The a-R18S mutant strain showed the most important effects.

PS2.62
Primary events in cyanobacterial photosystem I complexes studied using femtosecond selective excitation of antenna and reaction center chlorophylls

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The photosystem I (PS I) complex from cyanobacteria contains ~90 light-harvesting antenna chlorophyll (Chl) molecules and the reaction center (RC) electron-transport chain, which includes P700 (Chl dimer), A_0 (one or the two Chl monomer molecules), A_1 (one or the two molecules of phylloquinone), and the iron–sulfur clusters. The flash-induced kinetics of primary events in PS I complexes from *Synechocystis sp.* PCC 6803 was studied using femtosecond transient absorption spectroscopy within 400 – 800 nm spectral range at different excitation wavelengths. This approach provided an opportunity to observe the excitation energy transfer between antenna Chl molecules, the formation of excited state P700* and the appearance of ion-radical pair (IP) states in RC. It was shown that 20 fs laser pulses centered at 680 and 700 nm predominantly excite antenna Chl molecules, while the pulse at 720 nm mostly results in selective excitation of the RC primary electron donor P700.

The comparison between the flash-induced kinetic spectra showed that:

1) excitation of antenna leads to energy transfer and formation of P700* within ~3-5 ps, while selective excitation of P700 leads to very fast (~50 fs) formation of P700*;

2) generation of the primary IP (most probably $P700^+A_0^-$) state accomplishes in ~5-8 ps irrespective of selective excitation of either antenna Chls or P700;

3) disappearance of A_0^- and formation of secondary IP state ($P700^+A_1^-$) is completed within ~30 ps.

PS2.63
The chloroplast encoded PSI-J subunit is required for formation of the plastocyanin binding domain of photosystem I

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Photosystem I (PSI) is located in the thylakoid membrane of chloroplasts where it is involved in light-mediated electron transport form plastocyanin on the luminal side to ferredoxin on the stromal side. The plastid encoded *psaJ* gene encodes a small hydrophobic subunit containing one transmembrane helix. The function of PSI-J in higher plant PSI is so far unknown. Tobacco plants with an inactivated *psaJ* gene were constructed by chloroplast transformation. The mutant plants were devoid of PSI-J protein and grew photoautotrophically but were slightly smaller and paler than wild-type caused by a 15-20% reduction in the content of PSI indicating that PSI-J is important for assembly or stability of PSI. The functional size of the PSI antenna was not affected suggesting that PSI-J is not involved in binding of LHCI. The specific PSI activity measured as $NADP^+$ photoreduction revealed a 55% reduction in electron transport in the absence of PSI-J. No significant difference in the second order rate constant for electron transfer from

reduced plastocyanin to oxidized P700 was observed in the absence of PSI-J. Instead, a large fraction of PSI was found to be inactive. Immunoblotting analysis of PSI complexes revealed a secondary loss of the luminal PSI-N subunit. Presumably the absence of PSI-J affects the conformation of PSI-F which in turn affects the binding of PSI-N. This together renders a fraction of the PSI particles inactive. Thus, PSI-J is an important subunit of PSI that together with PSI-F and PSI-N is required for formation of the plastocyanin binding domain of PSI.

PS2.64

Structure of Radical Pairs $D^{\cdot+}Q_A^{\cdot-}$ in Photosynthetic Reaction Centers Cooled to Cryogenic Temperatures in Neutral and Charge Separated States: A High-Field EPR/PELDO

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Photosynthetic reaction centers (RCs) from *Rb. sphaeroides*, upon excitation by light, undergo electron transfer from a dimeric bacteriochlorophyll donor (D) to a primary ubiquinone acceptor (Q_A). Structural changes associated with the charge separated state, $D^{\cdot+}Q_A^{\cdot-}$, have been suggested from the difference in electron transfer kinetics in RCs cooled in the dark and under illumination.[1] The goal of the present work is to determine the nature of these suggested structural changes. RCs containing only Q_A, were frozen in the dark and under continuous illumination to trap the DQ_A and $D^{\cdot+}Q_A^{\cdot-}$ states, respectively. The $D^{\cdot+}Q_A^{\cdot-} \rightarrow DQ_A$ recombination kinetics was monitored using time-resolved W-band EPR at 90 K. In RCs cooled prior to illumination, the charge recombination time was 25 ms (fast), in agreement with previous reports.[1,2] When the same RCs were warmed to room temperature and recooled under continuous illumination, a stable radical-pair ($D^{\cdot+}Q_A^{\cdot-}$) EPR signal was observed in ~70% of the sample. The charge-recombination time corresponding to the ~30% fraction of the sample was 120 ms (slow).

PELDOR experiments on spin-correlated $D^{\cdot+}Q_A^{\cdot-}$ radical pairs were performed on the states with "fast" and "slow" kinetics. The results showed similar relative orientations of the pair partners in both states. Since it is not likely that both cofactors move similarly during illumination, it is concluded that the same orientation for $Q_A^{\cdot-}$ is observed in RCs cooled in the dark and in cyclic RCs cooled under illumination. This suggests that the difference in their kinetics is due to a different structural change of the redox cofactors, presumably involving the binding site of the intermediate pheophytin.

[1] Kleinfeld et al., *Biochemistry* 23, 5780 (1984).

[2] Zech et al., *Appl. Magn. Reson.* 13, 51 (1997).

PS2.65

Study of intersystem electron transfer in the chlorophyll *d* containing cyanobacterium *Acaryochloris marina* and a reappraisal of the redox properties of P740

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A far red light driven oxygenic photosynthesis takes place in *Acaryochloris marina*, a marine prokaryote whose major pigment is chlorophyll *d*. The PS1 primary donor, named P_{740}, was identified as being composed of Chl *d* instead of Chl- *a* (Hu et al. PNAS, 1998). Its midpoint potential was lower than in Chl *a* organisms (335mV instead of 420-470mV), apparently compensating for the low quantum energy absorbed (1.68eV instead of 1.77eV).

This would leave unmodified the reducing power resulting from charge separation, as argued by Hu et al., but would be expected to affect intersystem electron transfer. Indeed, if the midpoint potential of cytochrome *f* is similar to that in Chl *a* organisms (350-370mV), electron transfer would be uphill and might reduce considerably the efficiency of the photosynthetic chain. Hence, we investigated P_{740} and Cyt *f* re-reduction kinetics to assess the equilibrium constant between the two complexes. We found a value of ~15 which is consistent with the Cyt *f* midpoint being ~100mV lower than that of P_{740}. We then directly measured, by equilibrium redox titration, the midpoint potentials of both P_{740} and Cyt *f* and found respectively 430mV and 330mV. Both values are similar to those found in Chl *a* organisms.

This indicates that the change in Chl type is not associated with a modification of the midpoint potential of P_{740}, as previously reported. Rather, we propose that the lower reducing power may affect the energetics of electron flow on the acceptor-side of PS1. Kinetic consequences will be discussed.

PS2.66

Primary radical pair P^+Bphe^- lifetime in *Rhodobacter sphaeroides* with blocked electron tranfer to Q_A. Effect of o-phenantrol

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Transient absorption spectroscopy with a time resolution of ~1 ns was applied to study the decay of the primary radical pair (PRP) P^+Bphe^- in *Rhodobacter sphaeroides* R-26 reaction centers with blocked electron transfer from $Bphe^-$ to Q_A. The block in electron transfer was realized in two ways: either by reducing or by removing Q_A. We have found very different kinetics for the PRP decay in these two cases. When Q_A was reduced to Q_A^-, the decay was clearly biphasic with exponential lifetimes of t_1 = 3 ns (63%) and t_2 = 11 ns (29%). Fit with a single exponential gave a value of 5 ns. Reaction centers with Q_A removed were characterized by slower and monophasic decay with t = 12 ns. Addition of 10 mM o-phenantroline slowed down the PRP decay in reaction centers with Q_A^- nearly twice, to t = 9 ns, and had almost no effect in Q_A-depleted reaction centers. O-phenantroline had also no effect on the PRP lifetime in chromatophores with Q_A reduced where t = 9 ns. We propose that the negative charge on Q_A accelerates the PRP decay by repulsive interaction with the negative charge on Bphe. This effect is partly removed by o-phenantroline-mediated protonation of the sites in the vicinity of Q_A.

PS2.67

The physiological relevance of electron transfer involving redox-active centres bound to the PsaA and PsaB subunits of Photosystem I.

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Photosystem I (PS I) is a large macromolecular complex located in the thylakoid membranes of oxygenic phototrophs, and is an essential component of oxygenic photosynthesis. Recent literature is pointing towards the involvement of both the redox chains bound to the reaction centre subunits, PsaA and PsaB, in electron transfer in PS I. The effect of point mutations (histidine to methionine) of the axial ligand for the primary electron acceptor A_0 on photosynthetic electron transfer in PS I is investigated in two mutants of the green alga *C. reinhardtii* (PsaA:M684H, PsaB:M664H). Both mutations affect the stability of the PS I reaction centre, the accumulation of which is approximately halved compared to the wild-type. PS II accumulation is not altered the mutants, compared to the wild type. In whole cells of the PsaA:M684H mutant the rate of linear electron transfer (ET) under saturating light conditions is decreased by only 5-10% compared to the wild-type, whereas in the PsaB:M664H mutant the rate is decreased by 90%. Under limiting light conditions these rates are decreased by 50% (PsaA:M684H) and 90% (PsaB:M664H) relative to WT. The maximal rate of PS I mediated ET is decreased by 30-35% in the PsaA:M684H mutant and by ~75% in the PsaB:M684H. These functional differences resulting from mutations affecting the PsaA-bound or the PsaB-bound cofactors are not fully explained by previous spectroscopic investigations. We interpret the results in terms of an asymmetric effect of the axial donor substitution to A_0 on the maximal photochemical efficiency of PS I.

PS2.68
Redox potential of chlorophyll *d*

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a plays a key role. In 1996, a Chl *d*-dominated cyanobacterium *Acaryochloris marina* was discovered. In the PS I RC of *A. marina*, Chl *d′* functions as the primary electron donor P740: a heterodimer of Chl *d/d′*, like Chl *a/a′* for P700. The primary electron acceptor of PS II in *A. marina* has been defined as Phe *a*, however, whether Chl *d* acts as the primary electron donor in PS II is a matter of controversy. One of the reasons for this uncertainty is due to the absence of data about the redox potential of Chl *d* that is needed to be compared with that of Chl *a*. The oxidation potential of Chl *d* was found to be +0.88 V vs. SHE in acetonitrile, which was higher than that of Chl *a* (+0.81 V), and lower than that of Chl *b* (+0.94 V). The oxidation potential order, Chl *b* > Chl *d* > Chl *a*, can be explained by inductive effect of substituent groups on the conjugated p-electron system on the macrocycle. Corresponding pheophytins showed significantly high values around +1.2 V, which are rationalized in terms of an electron density decrease in the p-system by the replacement of magnesium with more electronegative hydrogen. Consequently, oxidation potential of Chl *a* was the lowest of all Chls. The results will help us to broaden our views on questions about photosystems in *A. marina*.

PS2.69
Oxidation potential of Chl *a* is the lowest of all Chls

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In 1996, a Chl *d*-dominated cyanobacterium *Acaryochloris marina* was discovered. In the PS I RC of *A. marina*, Chl *d′* functions as the primary electron donor P740: a heterodimer of Chl *d/d′*, like Chl *a/a′* for P700, and Chl *a* as the primary electron acceptor. In PS II, however, whether Chl *d* acts as the primary electron donor in PS II is a matter of controversy, although the primary electron acceptor has been defined as not Phe *d* but Phe *a*. Quite recently, we first found that Chl *d* has higher oxidation potential than Chl *a*, and lower potential than Chl *b*. Phes *a*, *b*, and *d* showed much higher potentials than corresponding Chls. For water oxidation, very high oxidation power is believed to be needed, but oxidation power of Chl *a* is found to be the lowest of all Chls. To explain the enigma, we want to propose a unique model for O_2 evolution. In our model, oxidation potential of Chl *a*(or *d*) is not high enough to oxidize water, but the stepwise positive shifts of oxidation potentials of the Mn-complex take place during the S-cycle to create the great high oxidation power to oxidize water. Lower oxidation states of the Mn-complex may accept holes from $P680^+$, but higher oxidation state(s) cannot do this and should utilize photon energy to attain the final state to oxidize water.

PS2.70
Electron and nuclear dynamics in many-electron atoms and molecules in bacterial

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L of each electron in many-electron atom is L = mvr = nh and similar to N. Bohr's expression for one-electron atom. According to that the energy expression for many-electron atoms was found and used for the energy calculations in molecules, particularly with conjugated bonds, including chlorophyll like molecules and reaction centers (RCs).
The nuclear wave packet motion was observed by fs-oscillations in stimulated emission from the primary electron donor P* at 900 nm and 940 nm which was accompanied by a coherent formation of the charge separated states $P^+B_A^-$, $P^+H_A^-$ and $P^+H_B^-$ (where B_A, H_B, and H_A are the primary and secondary electron acceptors, respectively) in native, pheophytin-modified and mutant RCs. Wave packet motion on the 130-150 cm^{-1} potential surface of P* was found to be due to the internal shift of the pyrrol ring I of P_B with respect to P_A molecule in the dimer P. This leads to the formation of an exciplex with an electron density shift from P_A to P_B accompanied by the stimulated emission spectrum shift from 900 nm to 940 nm and the electron density transfer to the primary electron acceptor B_A with partial formation of the $P^+B_A^-$ state.

PS4 - The Water Oxidising Enzyme

PS4.1
Structure of the Photosynthetic Mn_4Ca Cluster Using X-ray Spectroscopy

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Single crystals of Photosystem II (PS II) isolated from thermophilic cyanobacteria have been studied by X-ray diffraction (XRD) with resolutions between 3.0 and 3.8 Å. These studies have localized electron density associated with the water-oxidizing Mn_4Ca cluster within the large complex of PS II peptides, but the limited resolution is short of what is needed to place individual metal atoms precisely in the cluster. Examination of the orientation dependence of the EXAFS of single crystals of PS II can provide structural information about the Mn sites at a resolution higher than that is presently available from single-crystal X-ray diffraction.
We have successfully collected single crystal XANES and EXAFS data from the native S_1 state with the X-ray e-vector parallel to the a, b, and c axes of the crystal, under non-damaging conditions by monitoring the Mn K-edge for any X-ray induced Mn reduction. The EXAFS spectra show that the Fourier peaks are clearly dichroic, demonstrating an asymmetric Mn cluster. We have used the EXAFS dichroism to evaluate the Mn cluster geometry. Three Mn_4Ca models which satisfy the trend of EXAFS dichroism were further fit into the ligand environment obtained from XRD, in order to discriminate between the several symmetry-related orientations which arise from the crystal symmetry. Furthermore, single crystals in the S_1 state were illuminated either by continuous illumination or by laser flashes to create intermediate S-states (S_2 and S_3). Polarized XANES and EXAFS spectra from these crystals show unique orientational dependence.

PS4.2
DFT-QM/MM Structural Models of the Oxygen-Evolving Complex of Photosystem II

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The mechanism of water splitting in photosystem II is described in terms of complete structural models of the oxygen evolving complex (OEC) in the S0-S4 states. The models include the influence of the surrounding protein environment according to state-of-the-art quantum mechanics/molecular mechanics (QM/MM) hybrid methods applied in conjunction with the X-ray crystal structure of PSII from the cyanobacterium Thermosynechococcus elongatus. The resulting structural models are validated through direct comparisons of simulated extendend-X-ray absorption fine structure (EXAFS) spectra with the experimental spectra of S-state intermediates. The models provide a detailed tentative description of the water splitting mechanism as determined by structural rearrangements in the oxomanganese cluster, rearrangements of water molecules around the metal cluster and changes in the oxidation states of metal centers. The formation of an additional mu-oxo bridge between Mn(3) and Mn(4) not present in the X-ray crystal structure of the S1 state is proposed for the S3, S4 and S0 states of the OEC. The models are consistent with the hypothesis that dioxygen is produced by the reaction of water with an oxyl radical in the S4 state of the catalytic cycle.

PS4.3
Oxygen, water, proton and quinine channels in PSII

J Murray (Imperial College London), J Barber (Imperial College London)

The photosystem II (PSII) reaction centre must allow the entry and egress of substrates (water, plastoquinone), and products (protons, oxygen and plastoquinol), with the intermediate transfer of electrons within the enzyme. Using recent structural information on PSII a putative oxygen channel has been identified which is about 21Å in length, leading from the water splitting site to the lumen. This channel follows a path along the lumenal surface of CP43, passing across the interface of the large extrinsic loop which joins the fifth and sixth transmembrane helices of this chlorophyll binding protein. In so doing it seems to minimise interactions with the excited states of chlorophylls bound within the PSII complex, especially those that constitute the primary electron donor, P680. Two additional channels leading from the water splitting site, and also exiting at the lumen, were also identified. Their hydrophilic nature suggests that they probably facilitate the delivery of water to, and protons from, the water oxidising site. The acceptor side of PSII is associated with a cavity that is accessible to solvent on the stromal side and to the membrane. We use single particle electron microscopy to propose a possible role for this cavity.

PS4.4
Electronic and Geometric Structures of the Mn_4O_xCa Cluster in the S_0 and S_2 States of the Oxygen-Evolving Complex of Photosystem II Based on Pulse

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The heart of the oxygen-evolving complex (OEC) of photosystem II is a Mn_4O_xCa cluster that attains five different oxidation states (S_0 to S_4) during catalysis. Four very similar high resolution models of this cluster were recently obtained employing polarized EXAFS spectroscopy.[1] Using DFT calculations we confirm that these models are chemically reasonable, and interpret on that basis our previously reported ^{55}Mn-hyperfine coupling constants of the S_0 and S_2 states[2] using Y-shaped spin-coupling schemes with up to four non-zero exchange coupling constants. This analysis rules out the presence of one or more Mn(II) ions in S_0, and thereby establishes that the oxidation states of the manganese ions in S_0 and S_2 are Mn_4(III, III, III, IV) and Mn_4(III, IV, IV, IV), respectively. By applying a 'structure filter' we (i) show that the above models are fully consistent with EPR and ^{55}Mn-ENDOR data, (ii) assign the Mn oxidation states to individual Mn ions and (iii) propose that the known shortening[3] of one 2.85 Å Mn-Mn distance in S_0 to 2.75 Å in S_1 corresponds to a deprotonation of a m-hydroxo bridge between Mn_A and Mn_B, i.e. between the outer Mn and its neighboring Mn, which belongs to the m_3-oxo bridged moiety of the cluster. The results are

summarized in a molecular model for the $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ transitions.

[1] Yano et al. (2006) Science 314, 821-825
[2] Kulik et al. (2005) J. Am. Chem. Soc. 127, 2392-2393
[3] Robblee et al. (2002) JACS 124, 7459-7471

PS4.5
pH dependence of the S_0 Split EPR signal in photosystem II, induced by 5K illumination

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Upon illumination of PSII an electron is extracted from the $CaMn_4$ cluster via tyrosine-161 on the D1 polypeptide, to form a neutral tyrosine radical $Y_Z^{\bullet}$. When illuminating at cryogenic temperatures, water oxidation is blocked but the primary charge separation can still occur. At 5K, illumination results in EPR signals originating from $Y_Z^{\bullet}$ in magnetic interaction with the $CaMn_4$ cluster, offering a way to probe for oxidation of Y_Z in active PSII. We have studied how pH affects the formation of the Split EPR signal from Y_Z in the S_0 state. S_0 was induced by 3 flashes. Then pH was adjusted between pH 4.0-9.0. The S_0 split EPR signal was induced by illumination in the cavity at 5 K. Maximum signal intensity was observed around pH 6.3. On the acidic and alkaline sides the signal intensity decreased with apparent p*Ks* of ~4.7 and ~7.9, respectively. The signal decayed in the dark on a similar time scale over the whole pH range. The EPR studies showed that cryogenic oxidation of Cyt b_{559}, Car, and/or Chl_Z can compensate for the loss of the split radical donor (Y_Z) at both high and low pH. As described earlier, the presence of methanol induces a change in the spectral shape of the Split S_0 signal. Recent results from these methanol induced changes at different pH will be presented and discussed.

PS4.6
Water binding to the Mn_4O_xCa cluster in Photosystem II studied by advanced pulse EPR spectroscopy

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Mn_4O_XCa cluster acting together with the nearby D1-Tyr161. During the stepwise proton-coupled electron transfer, the Mn_4O_XCa cluster undergoes a cycle composed of five distinct intermediates termed S_i states (i = 0-4). Molecular oxygen is released during the $S_3?[S_4]?S_0$ transition.
The 'when' and 'where' of substrate water binding belong to the important open questions about photosynthetic water splitting. Mass spectrometry data show that at least one water molecule is bound up to the S_2 state, and that both substrate waters are bound in the S_3 state. However, due to kinetic limitations, they are unable to determine if the fast exchanging substrate water molecule is also bound in the S_0 to S_2 states, and no direct information is obtained about the modes and sites of substrate binding in the OEC. In the paramagnetic S_0 and S_2 states binding of water to the Mn_4O_XCa cluster can be also studied by advanced EPR spectroscopy via couplings of the unpaired spin on the cluster with directly bound or nearby isotopically labeled water.
In the present study we extend previous EPR studies by employing advanced X- and Q-band pulse EPR spectroscopy, two-pulse ESEEM, two-dimensional three-pulse ESEEM, HYSCORE and Mims ENDOR and variously isotopically labeled water, $H_2{}^{16}O$, $D_2{}^{16}O$ and $H_2{}^{17}O$ to investigate water binding to the S_2-state.

PS4.7
A detailed structural model for the eukaryotic LHCII-PSII supercomplex

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Photosystem II (PSII) core complex X-ray structures have so far been derived from preparations isolated from prokaryotic cyanobacteria. These have provided significant information for understanding PSII functionality, including water splitting, but as yet no high-resolution structure has become available for eukaryotic PSII. Such knowledge will be required to reveal spatial excitonic linkages between the pigments of the intramembrane light-harvesting components (LHCII) and the chlorophylls of the reaction centre core. Furthermore, differences in the eukaryotic water splitting environment also need to be investigated. To this end we have utilised the published X-ray structures of a cyanobacterial PSII core (Ferreira et al., 2004), LHCII (Standfuss et al., 2005), PsbP (Ifuku et al., 2004) and PsbQ (Calderone et al., 2004) proteins to construct a model of eukaryotic LHCII-PSII supercomplex using 16 to 17 Å resolution 3D density maps from spinach and *Chlamydomonas*, as determined by electron cryo-microscopy and single particle analysis. We tentatively identify the positioning of chlorophylls, in order to consider energy transfer pathways between the different subunits, and discuss the positioning of the extrinsic protein subunits of the water-splitting site.
Calderone V, et al., (2004) EMBO Rep. 4, 900-905
Ifuku K, et al., (2004) EMBO Rep. 5, 362-367
Ferreira K, et al., (2004) Science 303, 1831-1838
Standfuss J, et al., (2005) EMBO J. 24, 919-928

PS4.8
Structure of the Photosynthetic Mn_4Ca Cluster Using X-ray Spectroscopy

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Single crystals of Photosystem II (PS II) isolated from thermophilic cyanobacteria have been studied by X-ray diffraction (XRD) with resolutions between 3.0 and 3.8 Å. These studies have localized electron density associated with the water-oxidizing Mn_4Ca cluster within the large complex of PS II peptides, but the limited resolution is short of what is needed to place individual metal atoms precisely in the cluster. Examination of the orientation dependence of the EXAFS of single crystals of PS II can provide structural information about the Mn sites at a resolution higher than that is presently available from single-crystal X-ray diffraction.
We have successfully collected single crystal XANES and EXAFS data from the native S_1 state with the X-ray e-vector parallel to the *a*, *b*, and *c* axes of the crystal, under non-damaging conditions by monitoring the Mn K-edge for any X-ray induced Mn reduction.[3] The EXAFS spectra

show that the Fourier peaks are clearly dichroic, demonstrating an asymmetric Mn cluster. We have used the EXAFS dichroism to evaluate the Mn cluster geometry. Three Mn_4Ca models which satisfy the trend of EXAFS dichroism were further fit into the ligand environment obtained from XRD, in order to discriminate between the several symmetry-related orientations which arise from the crystal symmetry. Furthermore, single crystals in the S_1 state were illuminated either by continuous illumination or by laser flashes to create intermediate S-states (S_2 and S_3). Polarized XANES and EXAFS spectra from these crystals show unique orientational dependence.

PS4.9
Electronic Structure and Oxidation State Changes in the Mn_4Ca Cluster of Photosystem II

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A detailed electronic structure of the Mn_4Ca cluster is required before two key questions for understanding the mechanism of photosynthetic water oxidation can be addressed. They are whether all four oxidizing equivalents necessary to oxidize water to O2 accumulate on the four Mn ions of the OEC, or do some ligand-centered oxidations take place before the formation and release of O2 during the S3 ® [S4] ® S0 transition, and what are the oxidation state assignments for the Mn during S-state advancement. X-ray absorption and emission spectroscopy of Mn, including the newly introduced resonant inelastic X-ray scattering spectroscopy have been used to address these questions. XAS and EPR of Mn model complexes and single crystals have also been used to understand the details of the electronic structure of the Mn_4Ca cluster in PS II. The relevance of these data from Mn complexes to the Mn_4Ca cluster in the OEC will be discussed. The present state of understanding of the electronic structure and oxidation state changes of the Mn_4Ca cluster in all the S-states, derived from all these techniques will be presented.

PS4.10
Role of phosphatidylglycerol in oxygen-evolving complex of photosystem II

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Our previous studies with *pgsA* mutant of *Synechocystis* sp. PCC6803 which is defective for the biosynthesis of phosphatidylglycerol revealed that PG is essential for electron transport from Q_A to Q_B on the acceptor side of photosystem II (PSII). In this study, we analyzed the properties of PSII with *pgsA* mutant both *in vitro* and *in vivo* in details to clarify the role of PG in the donor side of PSII, namely, oxygen-evolving complex (OEC). Analyses of purified PSII complexes indicated that PSII from PG-depleted *pgsA* mutant sustained only 50% of the oxygen-evolving activity compared to wild-type cells and dissociated the extrinsic proteins of PsbO, PsbV and PsbU, and manganese ions. The released PsbO re-bound to PSII when PG was added back to the PG-depleted mutant cells, even when de novo protein synthesis was inhibited. The extent of inactivation of oxygen evolution in PG-depleted mutant cells by heat treatment or dark incubation resembled those of *?psbO* and *?psbV* mutant cells. These results suggest that PG plays an important role in the binding of extrinsic proteins required for sustaining a functional Mn cluster.

PS4.11
Calcium requirement for S-state transitions

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The functional role of the Ca^{2+} ion in the oxygen-evolving complex of photosystem II is still under debate. The S_3 state is not formed in the absence of Ca^{2+}. Re-interpretation of the literature on methods of Ca^{2+} depletion (Miqyass et al., 2007) led us to conclude that also the oxidation of S_1 to S_2 requires Ca^{2+}. Here we confirm this interpretation. Ca^{2+} depletion of BBY membranes by the salt-wash procedure was studied by measuring flash-induced S-state transitions in UV absorbance. Using 1 M KCl rather than NaCl, we found that Ca^{2+} is replaced within 30 minutes in the dark, leaving PSII in an inactive S_1 state. A saturating flash caused Y_Z oxidation only. Nevertheless, 30 minutes exposure to room light in the presence of 1 mM EGTA produced the inactive stable S_2 state, in which also Y_Z oxidation was largely inhibited and flashes induced mainly $P_{680}{}^{+}Q_A{}^{-}$ recombination. Apparently, Y_Z• can still oxidize S_1 to S_2 but the flash yield is negligible, suggesting that the reaction takes much longer than the few seconds lifetime of Y_Z• in these conditions and is at least 5 orders of magnitude slower than in the presence of Ca^{2+}. In spite of a good S_2 yield after minutes of illumination, this indicates an essential functional role of Ca^{2+} in the S_1 to S_2 transition.

Key words: Calcium, S-state, Oxygen evolution, Photosystem II.

PS4.12
Mono-manganese mechanism of dioxygen evolution catalyzed by unique Mn4Ca cluster in PSII

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The molecular mechanism of the water oxidation reaction in photosystem II (PSII) remains a great mystery in life science. This reaction, which takes place in the oxygen evolving complex (OEC) incorporating four manganese, one calcium and one chloride cofactors, is light-driven to cycle five intermediates, S0 through S4, to produce four protons, four electrons and lastly one dioxygen, as indispensable resources in biosphere. Despite of recent advancements of X-ray crystallography models, which established the existence of a catalytic Mn4Ca cluster ligated by seven protein amino acids, its functional structure has been a subject of intense debate. In this paper, we present a chemically complete model for the Mn4Ca cluster and its surrounding enzyme field and its predominant reaction intermediates, together with our theoretical interpretations of the S-state dependence of ^(18)O exchange rates of two substrate water molecules, by using the hybrid DFT/B3LYP geometry optimization method to confirm good agreements with the 3.0 Angstrom resolution PSII model. Remarkably, we could verified that two substrates are bound to asymmetric cis-positions on the terminal Mn ion being triply bridged (μ-oxo, μ-carboxylato, and μ-hydroxo) to the Mn3CaO3(OH) core, by developing a generalized theory of ^(18)O exchange kinetics in OEC to obtain an experimental evidence of the cross exchange pathway. Furthermore, it will be shown that the O-O bond formation can readily take place along the cross exchange pathway between neighboring oxo radical and hydroxo anion, which are both bound to the terminal Mn(IV) ion, but only the latter is H-bonding with the trimeric part.