During photoinhibition of PSII membranes by excess light (4000 lux), a protective role of an exogenous acceptor PpBQ has been observed. In view of the fast and steady-state phases were less pronounced and, in addition, process of photoreactivation was monitored. We have observed strong photoinhibition effect on phases of Mn4O5Ca-cluster. Under simultaneously action of Cd2⁺ [(P680QA+

To investigate the photoinhibition sites in PSII at different pH (pH 5.5, 7.0, 8.5) and at the action of heavy metals (Cd2⁺ and Co2⁺ ions).

While it can generally be said that cellular function is critically dependent on the fidelity of cargo transport, processive transport is even more important in the axons and dendrites of neurons, where a cell must regulate populations of molecules on length scales that can range up to meters. Consequently, much effort has been made to investigate the translocation of cargos in neurons and the properties of the motors responsible therein. Though biocompatible fluorophores have become increasingly powerful tools for study of motor-driven transport, they suffer from photobleaching and require bright illumination which can be toxic to live cells. Most conventional fluorescent approaches are further limited by the lack of orientation information they provide. On the other hand, with the small diameter of neurites and the high levels of traffic they support through a crowded environment, orientation of the cargoes relative to the cytoskeletal tracks they are moving on can be vital. We present an experimental approach making use of dark field optical microscopy and gold nanorods as reporters of both lateral translocation as well as orientation of cargo in neurons. Using relatively low illumination intensity, we can measure dynamics of single cargoes moving in the image plane and resolve changes in the azimuth and polar angles all at millisecond time scales. Furthermore, the gold nanorods can be specifically delivered to the cell body or axon terminal by culturing the neurons in microfluidic devices with separate chambers, enabling the investigator to resolve differences between retrograde and anterograde transport. The ability to track axonal transport with a high temporal and spatial dynamic range reveals several kinds of orientational changes of moving cargos that correlate with transport dynamics, allowing more detailed inferences into changes in the activity of molecular motors.

Photosynthesis

Donor Side-Induced Photoinhibition in Photosystem II

To investigate the photoinhibition sites in PSII at different pH (pH 5.5, 7.0 and 8.5) and at the action of heavy metals (Cd2⁺ and Co2⁺ ions) were monitored by delayed fluorescence of chlorophyll a in the millisecond range (ms-DF). During photoinhibition of PSII membranes by excess light (4000 μmol photons m⁻² s⁻¹) the fast and steady-state phases of ms-DF induction curve were measured. We have observed strong photoinduction effect on phases of ms-DF at the elevated pH values. At acidic pH photoinduction of the fast and steady-state phases were less pronounced and, in addition, protective role of an exogenous acceptor PpBQ has been observed. In view of our previous results we suggest that change in equilibrium between Y2° [P680QA] → (Y2° *) and Mn4O5Ca-cluster [P680QA] → (S1 ¹ ¹) taking place when pH of medium is changed from low to high values. At high pH this leads to the donor side induced photoinhibition and the destruction of the Mn4O5Ca-cluster. Under simultaneously action of Cd2⁺ and Co2⁺ ions and photoinduction PSII become very sensitive to action of inhibitory illumination against the background of heavy metals. This inhibitory effect appears very strongly in the case of inhibition of donor side of PSI by Cd2⁺. The main target of this action may be for partner for recombination with PbPsbA, depend on medium pH, either Yz or Mn4O5Ca-cluster on the donor side for Cd2⁺ and Co2⁺ ions and between QA and Qb on the acceptor side for Co2⁺ ions.

Two Dimensional Broadband Electronic Spectroscopy of Photosystem II Core Complexes

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Studying the energy transfer and conversion processes in natural photosystems has both fundamental importance and relevance to the development of artificial light harvesting devices. Of particular interest is the D1/D2-cyt b559 re- action center of photosystem II (D1D2 RC), where the primary charge separation events associated with the evolution of molecular oxygen in photosynthesis occur. Using transient 2D electronic spectroscopy (2DES), the energy transfer and charge separation processes in this system can be studied with femtosecond temporal resolution. Previously we have reported 2DES studies of the D1D2 RC in the Qy region where the electronic transitions associated with charge separation are found. This region contains overlapping transitions from all of the constituent chlorophyll and phophytin pigments in the D1D2 RC, complicating the interpretation of the spectroscopic dynamics. Here we present broadband 2DES data spanning 460 - 700 nm along the detection axis, enabling the use of other electronic transitions to facilitate understanding of the energy transfer and charge separation processes. We discuss preliminary 2DES results on highly functional core complexes from BBD particles, which include the D1D2 RC along with the CP43, CP47 and psbO subunits. By studying the more intact and functional core complex, we aim to determine whether more reduced systems such as D1D2 RC experience changes in excitonic couplings or fundamental charge separation as a result of purification.

Selective Abolishment of Electron Transfer at A1 Site in Cyanobacterial Photosystem I with Minimal Structural Disturbance

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Photosystem I/PSI is an integral membrane protein complex that harvests solar energy into reducing power. PSI electron transfer cofactors consist of 6 chlorophylls, 2 phylloquinones (PhQs) and 3 Fe₃S₄ clusters. The electron transfer in PSI of the cyanobacterium Synechocystis sp. PCC 6803 has been studied in two site-directed variants, M688HpsaA and M668HpsbB variants. In those two variants, the Met residue that acts as axial ligand to the primary electron acceptor A₀ has been substituted with a His either on the PsA(M688HpsaA) or the PsbB(M668HpsbB) subunits. Room temperature transient EPR study indicated that the Met to His mutation on the A-sided (M688HpsaA) variant slowed down the forward electron transfer from A₁₁₁ to Fx. This might resulted from the formation of a second hydrogen bond to the C1 carbonyl oxygen of PhQ in the A₁₁₁ site. Flash-induced absorbance change at 480nm confirmed the slower forward electron transfer from A₁₁₁ to Fx in the M688HpsaA variant. Likewise, forward electron transfer from A₁₁₁ to Fx was also slowed down in the B-sided variant. This result supported the formation of a second hydrogen bond to the PhQ in the A₁₁₂ site as well. Furthermore, we detected new components of charge recombination kinetics in these two variants. Each of the components corresponded to charge recombination between A₁₁₁ to PsA⁺⁺ in M688HpsaA PSI and A₁₁₂ to PsbB⁺⁺ in M668HpsbB PSI, respectively. We propose that the Met to His mutation abolished the forward electron transfer from A₁₁₁ to Fx in the M688HpsaA variant and A₁₁₂ to Fx in the M668HpsbB variant. This is consistent with the establishment of a second hydrogen bond to the PhQ. Two models of the primary charge separation in PSI are discussed in the context of this finding.

Theoretical Study of Electron Transfer Rate from Phylloquinones to Iron-Sulfur Cluster (F₅) in Photosystem I

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Photosystem I (PSI) is one of the two multi-subunit, pigment-protein complexes that drive the initial processes of light utilization in oxygenic photosynthesis. The PSI complex has approximately 100 packed chlorophyll (Chl) molecules that form the antenna and functional redox cofactors comprising the reaction center (RC). The latter cofactors are arranged in two pseudo-symmetric branches, starting from P700, and then split into two branches with the ec2 and ec3 chlorophylls and phylloquinone (A₁) on each branch, and then rejoin at the iron-sulfur cluster F₅. All of these are bound by the heterodimer of the PsAα and PsbAα subunits, while the terminal F₅ and F₆ clusters are bound by PsAc. Electron transport (ET) along the two branches has been studied by various investigators, and it was established that both branches are active in electron transfer, but that electron transfer to F₅ was ~10-fold faster from PhQA than from PhQ₇.
New results from electron paramagnetic resonance showed that the orientations of the PhQ radicals are different which affect the strength of hydrogen bonding between PQ and the protein backbone. We are investigating these different rates of electron transfer and orientation of PhQ radicals through molecular dynamics simulation and time dependent density functional theory (TD-DFT) methods.

3358-Pos Board B513
A Novel Spectral Marker for Fe-Starvation in Cyanobacteria and its Association with IsiA in the Process of Light-Harvesting
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Iron-limiting growth conditions were established for Synechococcus sp. PCC 7002. Time-resolved optical spectroscopy of Fe-starved cells revealed a light-induced 520 nm spectral signal that may represent the electrochromic bandshift of carotenoids. The isiA and isiB were inactivated, respectively. Only the isiA mutant showed differences in growth rate and pigment content relative to the wild type grown in Fe-depleted medium. Further, the signal did not appear in Fe-starved isiA mutant. We conclude the signal serves as a marker for Fe-starvation and is associated with the IsiA protein. Under iron-limitation, IsiA associates with PS I, forms the PS I-IsiA supercomplex and increases the overall light-harvesting efficiency. We propose the spectral signal observed actually arises from the β-carotene near the quinones in PS I when the electron acceptors are transiently reduced by high light intensity. Under the intensity regime employed, the PS I-IsiA supercomplex in Fe-starved cells processes more energy per unit time than the PS I in Fe-replete cells, and thus back ups electrons to quinones more prominently. Time-resolved optical and EPR spectroscopies further demonstrated the carotenoid electrochromic bandshift signal and the semi-quinone signal.

3359-Pos Board B514
Characterization of 2D PSII Crystalline Arrays in Thylakoid Membranes Highly Efficient in Photo-Protective Energy Dissipation by Atomic Force Microscopy
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Non-photochemical quenching (NPQ) is an important mechanism by which photosynthetic organisms balance the light energy necessary for carbon fixation while preventing photo-damage. NPQ requires elaborate dynamic reorganization of the photosystem II (PSII) supercomplex and its closely associated light-harvesting complex II (LHCII). Currently, structural details directly related to NPQ and PSII-LHCII arrangements are scarce and in debate. The suppressor-of-quenching protein SOQ1 has recently been shown to prevent the formation of a slowly relaxing quenching pathway when chloroplasts are exposed to high light. We have used atomic force microscopy (AFM) to characterize the PSII organization in grana thylakoid membranes isolated from Arabidopsis thaliana wild type (wt) and soq1 mutant plants acclimated to different illumination conditions. To analyze the AFM data, we developed sensitive algorithms that automatically detect and discriminate between different types of crystalline packing observed in the membranes. We find that PSII density, extent of PSII array formation, and crystalline packing type depend strongly on illumination and mutant status. Surprisingly, pair correlation function and nearest-neighbor distribution analyses indicate that high-light-acclimated crystalline arrays from wt membranes are similar to those found in low-light-acclimated membranes from soq1 membranes. These different PSII arrangements can be correlated with the high levels of NPQ characteristic of the mutation. A potentially new type of 2D crystalline packing present in the low-light-acclimated soq1 membranes could be associated with slow relaxation of NPQ.

3360-Pos Board B515
Protection of Chloroplast In Vivo against Oxidative Stress
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For increasing of condition due to that chloroplast in native tissue will be riched by highly reactive oxygen species (ROS) leaves of cucumber and pumpkin plants were spraying with paraquart (methyl-viologen: 1.1‘-dimethyl 4,4‘-bipyridinium) (MV, 100µM). The states of donor and acceptor sides of Photosystem II (PS II) on the base of different characteristics of the millisecond delayed fluorescence of chlorophyll a (ms-DF) in leaves were analyzed. At the treatment by MV the sharp decay of characteristic value of ms-DF of reaction center of PS II by 80-90%, value of ms-DF, characterizing of state donor side of PS II and oxygen-evolving MnO4Ca-cluster and Yh, by 65-70% was revealed. The changes of different phases of kinetic induction curvature of ms-DF indicate that products, possibly ROS, formed in the photosynthetic units surrounding of PS I might bring to damage of PS II. Well known an antioxidant-sodium ascorbate has restored damages appearing under action of MV, possibly by quenching of O2* and *OH. Spraying of leaves by paraquat together with Plumbagin - (50mg/l 2 methyl-5-hydroxy-1,4-naftoquinone) from (Ceratostigma plumagoides) plant roots has restored fast phase of ms-DF induction curve almost to the control value and slow phase representing donor side of PS II more than in three times. At the same time steady state level of fluorescence has increased by 50-75% too. The suggestion is made that plumbagin plays a decisive role in the protection of chloroplasts oxygen-evolving complex possibly on the stage of water oxidation against photooxidative stress by quenching of O2 and *OH, being generated under action of paraquat.

3361-Pos Board B516
C-Terminal M4 Peptide Fragment of Albino 3 Interaction with cpSRP-43
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The protein complexes responsible for insertion of light harvesting chlorophyll binding protein into the thylakoid membrane of the chloroplast are the concern of this investigation. Critical for this insertion, the membrane bound protein Albino 3 has been shown to bind a cpSRP-FTSY-LHCP complex. The following study implicates the strongly conserved C-terminus of Albino 3 as an imperative binding interaction in this process. Isothermal titration calorimetry and 1H-15N HSQC were used to understand the binding of a 16 residue segment from this region of Albino 3 known as M4. The data show that this segment binds with 14μM affinity and that electrostatic interactions with E327 and E356 on the 43kDa subunit of cpSRP are essential for this event. Importantly, the interaction of M4 with Albino 3 is not observed when two domains of cpSRP-43, CD2 and CD3, are separated. Far UV and intrinsic fluorescence experiments also show that, upon M4 binding, moderate conformational changes in CD2-CD3 occur.

3362-Pos Board B517
Theoretical Studies on Excited States of Bacteriochlorophyll A in Solutions
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Bacteriochlorophylls and bacteriochlorophylls play important roles in photosynthesis, and thus the excited-state properties of chlorophylls and bacteriochlorophylls in solutions and proteins have been studied for a long time both experimentally and theoretically. It is experimentally known that the S6-S5 absorption energy of bacteriochlorophyll a (BChl a) in solution is quite insensitive to solvent, though the BChl a has polar functional groups. We investigate the excited-state properties of BChl a in several solutions by using QM/MM-RWFE-SCF method (J. Chem. Theory Comput. 8 (2012), pp 322-334), in which the geometry of a quantum mechanically treated molecule (BChl a, in this case) can be optimized effectively on a free energy surface defined with thermal distribution of the surrounding molecular environment (solvent) obtained by molecular dynamics simulation with a molecular mechanics force field. We employ TDDFT method for the excited electronic structure calculation, and find that excited-state properties of BChl a strongly depend on the density functional. We can reproduce the solvent insensitivity by adjusting the parameter of the density functional, and find that the solvent insensitivity results from the counterbalance between the redshift due to hydrogen bond interaction and the blueshift due to increase in dipole moment. It is expected that the density functional with the adjusted parameter can be applied to BChl a in proteins.