present the results of a Molecular Dynamics (MD) simulation study suggesting that the surrounding membrane composition contributes to the propensity for asymmetric changes in GPCR dimers. This inference is reached from a comparison of the prototypic rhodopsin dimer simulated in explicit POPC lipid-containing membrane [Filizola et al., 2006, JCAMD *20*:405–16] with new MD simulations of the same dimeric system in 2:1 POPC/Cholesterol (CHOL) mixed membranes. Specifically, the dynamic behavior of the two interacting protomers of the rhodopsin dimer is found to be different in the two simulations and leads to structural differences resulting from the asymmetric distribution of CHOL around the protomers. The protomer surrounded by the larger number of CHOL molecules is more rigid compared to the other subunit, which may affect the ability to undergo the specific conformational changes required for activation. These results are in line with recent experimental findings [Niu et al., 2002, J. Biol. Chem. *277*(23):20139–20145] showing that high cholesterol content in cell membrane has a suppressing effect on the activation of rhodopsin.

2382-Pos Imaging Heptahelical Receptors in Nanoscale Apolipoprotein Bound Bilayers

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Board B497

Apolipoprotein A-I and phospholipids self-assemble into discoidal complexes ideally suited for studies of transmembrane proteins including heptahelical G protein-coupled receptors (GPCRs). We have used zebrafish apo A-I to form such discoidal bilayers, called Nanoscale Apolipoprotein Bound Bilayers (NABBs), containing GPCRs in a controlled, soluble, native-like membrane-mimetic environment. We demonstrate a rapid and quantitative method of incorporation of GPCRs into NABBs using bovine rhodopsin as a model system. We developed methods to control the ratio of receptor to NABB and imaged the rhodopsin-NABBs using electron microscopy with two independent labeling techniques to measure stoichiometry and receptor orientation. Rhodopsin reconstituted into NABBs is dramatically more stable than rhodopsin solubilized in commonly used detergents. We conclude from fluorescence G protein activation assays of rhodopsin-containing NABBs that a single rhodopsin is necessary and sufficient for coupling to the G protein transducin. We are currently investigating the feasibility of rapid incorporation of heterologously-expressed receptors into zebrafish apo A-I NABBs in order to avoid long-term exposure to detergents and maximize functional receptor reconstitution.

2383-Pos Are PUFAs G-protein Signal transducers on a Microsecond Timescale?

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Board B498

Polyunsaturated fatty acids (PUFAs), ubiquitous in eukaryotes, are absent in prokaryotes as are G-proteins and G-protein coupled receptors (GPCRs). I propose that GPCRs use PUFAs to signal G-proteins. Rhodopsin is an example. Over 80% of the lipids chains in its disc membrane are 22-carbons long with six double bonds (DHA). The dominant membrane-spanning protein in the rod outer segment (ROS) membrane is rhodopsin. It signals to transducin, a G-protein. One photon activates 300 to 500 transducins. GPCRs and G-proteins always have lipid anchors. The low energy state of PUFAs is helical. In the ROS the main PUFA is DHA (C22:6) It may be stretched to full length (22 carbons) expanding the bilayer locally. Let's say rhodopsin expands perpendicular to the bilayer as a consequence of absorbing a proton. Aided by Helix VIII and its 2 adjacent lipid anchors Helix VII may expand and drag lipids out of the bilayer. This unstable state would then collapse and sends a soliton across the membrane. As the soliton (tsunami) peak hits transducins it separates their α- from their $\beta\gamma$-subunits. The erect PUFAs would amplify such a signal. In this model, GTP's cleavage destabilized the G-protein making it inactive and sensitive to cleavage by the wave. The wave crosses the entire ROS disc in 2 microseconds. Transducin site-specific mutations near the lipid anchor on the α- subunit interfere with G-protein cleavage. The model would presumably also apply to hormone GPCRs. The hormones bind to the interior of the GPCR even the large peptide hormones. The PUFAs, primarily on the inner monolayer, need only activate a few G-proteins to activate an enzyme. The wave need not travel as far.

Photosynthesis

2384-Pos Charge And Protein Dynamics In The Reaction Center Of Purple Bacteria *Rhodobacter Sphaeroides* Studied By Ultrafast Mid-infrared Spectroscopy

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Board B499

Time-resolved visible pump/mid-infrared probe spectroscopy was applied to investigate electron transfer and radical pair/protein relaxation at room temperature in the *Rb. sphaeroides* RC. Wild-type RCs both with and without the quinone electron acceptor Q_A, the Alanine M260W and Tyrosine M210W mutants, were excited at 600 nm, 800 nm, 794 nm and 860 nm. The region between 1600 cm^{-1} and 1800 cm^{-1} encompasses absorption changes associated with carbonyl stretch vibrational modes of the cofactors and protein. A sequential analysis and simultaneous target analysis of the data showed a free energy drop in P_LP_M bands due to charge-transfer

within the P. The electron localization on P_L or P_M changed in time and was finally established as $P_L^- P_M^+$. After photoexcitation of the RC the P excited singlet state (P*) decayed on a time scale of 3.6 ps to state $P^+B_L^-$ in WT and AM260W mutant, while in case of the Tyrosine mutant the P^* state decayed multi-exponentialy on a time scale of 12.5 ps and 39 ps. This is the first report of the mid-IR absorption spectrum of B_L; the 9-keto C=O stretch of B_L was located around 1678 cm^{-1}, indicating a relatively strong hydrogen-bond with the protein. The $P^+B_L^-$ state decayed in ~0.8 ps in WT and AM260W RCs, while in YM210W it was about one order of magnitude slower. In case of selectve excitation of B_L the ~10% of direct charge separation, leading to $P^+B_L^-$ formation, was observed. Relaxation of the $P^+H_L^-$ radical pair on the 20–30 ps time scale was accompanied by a minor change in a band at 1640 cm^{-1}, which we tentatively ascribe to an amide C=O. The $P^+Q_A^-$ state is formed in ~250 ps in WT and mutants RCs.

2385-Pos Binding of Strontium and Calcium Ions to Reaction Centers from Rb. sphaeroides Increases the Rate of Charge Recombination

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Board B500

The rates of electron transfer in photosynthetic reaction centers (RC) are affected by binding of transition metals[1]. After screening different metal ions we found that electron transfer rates were also affected by addition of $SrCl_2$ and $CaCl_2$. To determine the binding site, 2.4 Å resolution x-ray diffraction data were collected on tetragonal crystals of *Rb. sphaeroides* RCs incubated with 0.3M $SrCl_2$. Electron density maps revealed a 17 sigma difference density peak at the glutamic acid cluster of H43, H79, and H81 and the carbonyl backbone oxygen of Ser L4. Anomalous difference maps from data collected near the strontium absorption edge showed this peak was due to strontium. X-ray data collected to 2.4 Å on RC crystals incubated with 0.3M $CaCl_2$, resulted in an 8 sigma difference density peak at the same site, about 17 Å from Q_A on the cytoplasmic surface of the RC. Due to the proximity of the site to Q_A, metal binding is expected to modulate the redox potential of Q_A and affect its electron transfer reactions. Addition of Sr^{2+} and Ca^{2+} increased the recombination rates for the reaction $D^+Q_A^- \rightarrow DQ_A$ by 20% and 10%, with K_D of 2 mM and 10 mM respectively. The increase in rate is opposite to the effect expected from an electrostatic stabilization of Q_A^- by the bound metal. We suggest that the specific binding of Ca^{2+} and Sr^{2+} to this site produces conformational changes that destabilize the charge separated state.

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References

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2386-Pos Snapshots of Biological Proton-Coupled Electron Transfer: Electron-Nuclear Double Resonance Spectroscopy of Tyrosine Intermediates in Photosystem II

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Board B501

The solar water-splitting protein complex, photosystem II (PSII), catalyzes one of the most energetically demanding reactions in Nature by using light energy to drive water oxidation. Proton-coupled electron transfer (PCET) reactions, which are exquisitely tuned by smart protein matrix effects, are central to this water-splitting chemistry. PSII contains two symmetrically placed tyrosines, Y_D and Y_Z. The functions of these tyrosines are quite distinct, a versatility provided by their local environments in PSII. Y_Z is kinetically competent and is proposed to be directly involved in the PCET reactions of water oxidation. In contrast, the Y_D PCET redox poises the catalytic Mn_4 cluster and may electrostatically tune the adjacent redox-active chlorophyll and beta-carotene in the photoprotection pathway of PSII. This study focuses on disentangling individual steps of the photo-induced PCET events that lead to the formation of the redox-active tyrosyl radical, $Y_D^\bullet$, in PSII. Owing to the cryogenic turnover properties of PSII, it is possible to photooxidize Y_D such that both proton and protein motions are limited. Controlled annealing this state allows proton movement and relaxation of the protein environment. It is our hypothesis that the electron transfer, proton movement and associated conformational changes of the Y_D-binding pocket are the individual steps of the PCET reaction. Using pulsed high-frequency EPR and ENDOR spectroscopy, we elucidate the sequence of events that lead to PCET at the Y_D site of PSII. These studies provide direct '*snapshots*' of functional PCET intermediates and, for the first time, make it possible to detail the mechanism of PCET in biological energy transduction.

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2387-Pos Theoretical Prediction of the Absorption and CD Spectra of B800-B850 Bacteriochlorophylls in LH2 Antenna Complexes

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Board B502

A general method, that combines molecular dynamics (MD), quantum chemistry (QC) and many-body theory, is used to calculate and compare the linear absorption (OD) and circular dichroism (CD) spectra of B800-B850 bacteriochlorophyll (BChl) aggregates of the LH2 light harvesting complexes from Rps. acidophila (ACI) and Rs. molischianum (MOLI) purple bacteria. The individual BChls are treated as two-level quantum systems coupled to the classical environment composed of the protein matrix and the fully solvated lipid membrane. The dynamics of the nuclear degrees of freedom of the environment is described by MD simulations, while the time evolution of the energy gap fluctuations of the BChls is calculated using semi-empirical QC calculations. Finally, by employing a polaron model, the sought OD and CD spectra of the B800–B850 system are obtained from the calculated time autocorrelation function of the energy gap fluctuations of the individual BChls. The obtained results are found to be in good agreement with the available experimental data. A detailed analysis of the differences and similarities between the results corresponding to the LH2 antenna complexes from ACI and MOLI are also provided.

2388-Pos Oxygen Concentration in Photosynthetic Membranes

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Board B503

Production of oxygen by oxygenic photosynthetic organisms is expected to rise oxygen concentration within their photosynthetic membranes above normal aerobic values. These raised levels of oxygen may affect function of many proteins within photosynthetic cells. However, experiments on proteins *in vitro* are usually performed in aerobic (or anaerobic) conditions and the oxygen contents of membrane is not known. Using theory of diffusion and measured oxygen production rates we estimated the excess levels of oxygen in photosynthetic cells. We show that for an individual photosynthetic cell suspended in water oxygen level is essentially the same as that for a non-photosynthetic sell. These data suggest that oxygen protection mechanisms may have evolved after the development of oxygenic photosynthesis in primitive bacteria and was driven by the overall rise of oxygen concentration in the atmosphere. Substantially higher levels of oxygen are estimated to occur in closely packed colonies of photosynthetic bacteria and in green leafs.

2389-Pos Structure-Function Studies on the Iron-Sulfur Clusters in the Photosynthetic Reaction Center of Chlorobium vibrioforme

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Board B504

Chlorobium vibrioforme is a green-sulfur bacterium that performs anaerobic photosynthesis using iron-sulfur clusters as the terminal electron acceptors. The reaction-center (RC) contains three iron-sulfur clusters denoted F_X, F_A and F_B. F_X is an inter-polypeptide cluster held by the homodimeric core of the RC. By homology with other Type-I RC's, F_X is expected to be a [4Fe-4S]-cluster. However, it has not been well characterized by either EPR or Mössbauer spectroscopy. Based on analogy with other homodimeric inter-polypeptide [4Fe-4S]-clusters, it is likely that F_X has a mixed S=1/2 and S=3/2 ground state. Photoaccumulated samples of Chlorobium-membranes exhibit EPR-resonances around g=6, typical of a S=3/2 system. The magnetic properties of F_X in solubilized membranes and RC's are under investigation. The terminal iron-sulfur clusters, F_A and F_B are harbored in the PscB subunit of the RC. The presence of two [4Fe-4S]-cluster binding motifs suggests that PscB is likely to be derived from a dicluster ferredoxin. However, its EPR spectrum is axial in nature, characteristic of a single [4Fe-4S]-cluster. This is in contrast to other dicluster ferredoxins wherein a complex EPR spectrum is obtained due to dipolar interaction of the two clusters. The identity of the clusters in PscB is being probed by Mössbauer spectroscopy.

The protein environment around the clusters in PscB is highly sensitive to ionic strength as evidenced by a change in the EPR spectrum after adding 500mM NaCl. The PscB subunit dissociates from the Chlorobium-RC in the presence of high ionic strength. The EPR resonances from F_A and F_B in photoaccumulated RC samples disappear when the ionic strength is raised, and the RC loses its ability to photoreduce NADP+. We propose that NaCl disrupts the tertiary structure of PscB resulting in its dissociation from the RC.

2390-Pos Glutamate-354 of the CP43 Polypeptide Interacts with the Oxygen-Evolving Mn_4Ca Cluster of Photosystem II: A Preliminary Characterization of the Glu354Gln Mutant

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Board B505

In the recent X-ray crystallographic structural models of photosystem II, Glu354 of the CP43 polypeptide is assigned as a ligand of the oxygen-evolving Mn_4Ca cluster. A preliminary characterization of the CP43-Glu354Gln mutant of the cyanobacterium *Synechocystis* sp. PCC 6803 will be presented. The steady-state rate of O_2 evolution in the mutant cells is only about 20% compared to wild-type, but the kinetics of O_2 release are essentially unchanged and the O_2 flash yields show normal period-four oscillations,

although with lower intensities. Purified PSII particles exhibit an essentially normal S_2 state multiline EPR signal, but exhibit a substantially altered S_2-*minus*-S_1 FTIR difference spectrum. The intensities of the mutant EPR and FTIR difference spectra ($> 75\%$ compared to wild-type) are much greater than the O_2 signals and suggest that CP43-Glu354Gln PSII reaction centers are heterogeneous, with a minority fraction able to evolve O_2 with normal O_2 release kinetics and a majority fraction unable to advance beyond the S_2 or S_3 states. The S_2-*minus*-S_1 FTIR difference spectrum of CP43-Glu354Gln PSII particles is altered in both the symmetric and asymmetric carboxylate stretching regions, implying either that CP43-Glu354 is exquisitely sensitive to the increased charge that develops on the Mn_4Ca cluster during the S_1 to S_2 transition or that the CP43-Glu354Gln mutation changes the distribution of Mn(III) and Mn(IV) oxidation states within the Mn_4Ca cluster in the S_1 and/or S_2 states.

2391-Pos Fluorescence Lifetime Imaging of Chlamydomonas reinhardtii Following High Pressure Application

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Spinning-disk confocal fluorescence lifetime-resolved microscopy SPD-FLIM is used to measure changes in the spatial distribution of chlorophyll fluorescence within cells of the alga Chlamydomonas reinhardtii following the application of high hydrostatic pressure ($<$1100 bar). Spatial inhomogeneities in the fluorescence intensities and lifetimes are compared to that of cells at atmospheric condition. Upon pressure application, the fluorescence intensity initially increases. The maximum value of intensity, and the time that this maximum is reached (500–1000 seconds), depends on the pressure level. The fluorescence eventually decreases to a plateau value, which is the same for all pressures $>$500 bar. By measuring the spatial distribution of the fluorescence lifetime changes, SPD-FLIM provides information about the integrity of the photosynthesis system and structural changes in the chloroplasts.

2392-Pos Protein-Cofactor Interactions: Effects of a Single Amino Acid Change on the Redox Properties of Protein-Bound Cofactors

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Board B507

Many critical biological processes depend on electron transfer (ET) between protein-bound cofactors that are held at optimal distances by the protein matrix that modulates their redox properties. The approach described here assesses the effect of a single amino acid substitution on Photosystem I (PSI). PSI is a multisubunit pigment-protein complex that converts photons to chemical bond energy. It consists of the PsaA/PsaB heterodimer that harbors six chlorophylls, two phylloquinones, and one [4Fe-4S]-cluster, F_X. The phylloquinone in the ET chain oxidizes A_0^-, the primary electron acceptor and reduces F_X. The crystal structure indicates that the phylloquinone is H-bonded to $Leu722_{PsaA}$/$Leu706_{PsaB}$ and is pi-stacked with $Trp697_{PsaA}$/$Trp677_{PsaB}$. Apart from electrostatic considerations, these two features are deemed important in modulating its low redox potential (-770mV). Here, the H-bonded $L722_{PsaA}$ residue is replaced with a bulky Trp residue. Low temperature transient electron paramagnetic resonance experiments indicate that the orientation of phylloquinone is unaltered in the variant. However, the methyl hyperfine coupling (hfc) structure (quartet with relative intensities 1:3:3:1 centered near g_{yy} component of the g-tensor) is less pronounced, indicating a weakening or possible loss of the H-bond. Further, the hfc lines are temperature dependent, signifying an increase in the flexibility of the quinone in its binding pocket. High temperature studies show that the ET from the quinone to F_X is an order of magnitude faster in comparison to wild-type. Further, the preceding ET step from A_0^- to quinone remains unchanged. Thus, the quinone in the variant has a more negative midpoint potential than in native PSI. Temperature dependence studies indicate that the ET from the quinone to F_X has lower activation energy than in native PSI. Further experimentation will characterize the H-bond and map the distribution of quinone orientations.

Computational Methods - I

2393-Pos CHARMM-GUI: Graphical User Interface for CHARMM Users

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Board B508

Molecular dynamics simulations of proteins have provided deeper insights into their functions and interactions with surrounding environments at the atomic level. However, writing input files for molecular dynamics simulation software can be challenging for some time, especially if the input files are involved with sophisticated tasks like solving PB (Poisson-Boltzman) Equation, solvating a protein in an implicit solvent, and building a realistic protein/membrane complex. The CHARMM-GUI (http://www.charmm-gui.org) website is a graphical user interface for molecular dynamics for biology scientists. Using this interface, a scientists can read and modify PDB from various sources and generates versatile input files from solvating a protein in an explicit or implicit solvent and solving PB (Poisson-Boltzman) Equation to compute solvent accessible surface to building a fully explicit protein-membrane complex. The interface also provides an easy access to set up the periodic boundary condition and environment for molecular dy-