wild-type RBS COX; however, the α-region of the reduced spectrum for E90H(SII) displayed a slight red shift of the absorbance maximum by 1 nm, resembling that seen for the SIII-depleted oxidase. E90H(HII) and E90H(HII) mutant proteins were determined to contain stoichiometric amount of each subunit by SDS-PAGE gel, similar to wild-type COX. Electron transfer rates observed at pH 6.5, for E90H(HII)/H212(EII) and E90H(HII)/COX, were decreased by 17 and 39% respectively, as compared to WT COX. The mutants exhibit little or no suicide inactivation when compared to the SIII-depleted RBS COX. pH dependence of electron transfer activity for E90H(HII)/ H212(EII) and E90H(HII) were also not altered when compared to WT enzyme. Proton pumping experiments on reconstituted E90H(HII) and E90H(HII)/ H212(EII) in liposomes will be discussed.

4291-Pos Board B510
An Anhydrous Proton Transfer Pathway in the Cytochrome B6F Complex
Syed Saif Hasan1, Eiki Yamashita2, Danas Banulis3, William A. Cramer1,
1Purdue University, West Lafayette, IN, USA, 2Osaka University, Osaka, Japan, 3Lithuanian Research Centre for Agriculture and Forestry, Babtai, Lithuania.

Cytochrome b6f, a hetero-oligomeric energy transducing membrane protein complex, catalyzes proton-coupled electron transfer reactions of the substrate quinone to generate as much as two-thirds of the total proton gradient used for ATP synthesis in oxygenic photosynthesis. Proton transfer pathways within b6f have remained unidentified due to limitations of crystallographic resolution 1,2,3,4,5. using new crystallographic information based on a 2.70 Å crystal structure of the native complex, and structures obtained in the presence of quinone analogue inhibitors, tridecyl-stigmatellin (TDS) and 2-nonyl-4-hydroxyquinoline-N-oxide (NQNO) (resolutions 3.07 Å and 1.25 Å respectively), two structures have been seen in close proximity to heme cn on the electrochemically negative (n) side of the b6f complex, an anhydrous proton uptake pathway is defined that delivers protons from the n-side aqueous phase to plastocyanin bound at heme cn. A hydrated channel is identified on the electrochemically positive (p) side of the complex, that may provide a route for proton exit to the p-side aqueous phase. These n- and p-side pathways contribute to the first complete description of quinone-mediated transmembrane proton transfer.


4292-Pos Board B511
Electrostatically Constrained Pathway of Intra-Monomer Electron Transfer in the Cytochrome B6F Complex of Oxygenic Photosynthesis
Purdue University, West Lafayette, IN, USA.

The preferred pathway of trans-membrane electron transfer in the symmetric dimeric cytochrome b6f complex, involving four b-hemes organized as two pairs in symmetric monomeric units, was studied by simultaneous measurement of heme b redox state by absorbance measurement and heme-heme excitation interaction by excitonically split circular dichroism spectra in the Soret band. The rate of heme reduction by dithionite, in the dimeric or monomeric complex, coincides with an increase in amplitude of the split CD spectrum. The similarity of the time course of absorbance and CD changes, together with crystal structure information on inter-heme orientation and separation, imply that chemical reduction of the intra-monomer heme pair bn and bp occurs preferentially in the monomeric unit of the dimer. This preference is attributed to the negative free energy of the heme excitation interaction. The relatively small contribution of inter-monomer excitation interaction is attributed to the distance and angular dependence of the interactions. A slow rate of heme reduction is a consequence of an electrostatic energy barrier caused by injection of electrons into the apolar protein interior without compensating charge, inferred from an increased rate of heme reduction and generation of the split CD signal at acidic pH, a kinetic deuterium isotope effect, a red shift in the Q-band absorbance maximum of the integral chlorophyll a attributed to a Stark Effect associated with the internal electrical field, and a similar time course of heme reduction and generation of the Stark Effect. Thus, the Stark Effect arises from injection of electrons into the hydrophobic protein core without charge-compensation that would occur physiologically through H⁺ uptake and translocation by the complex. Funding-NIH-GM038323. (SDZ and SSHA share first authorship).

4293-Pos Board B512
Increased Superoxide Production in the Cytochrome B6F Complex: A Function for the Enigmatic Chlorophyll-A
Danas Banulis1, Jason T. Stofleth2, Syed Saif Hasan1, William A. Cramer1,
1Lithuanian Research Centre for Agriculture and Forestry, Babtai, Lithuania, 2University of California, San Diego, CA, USA, 3Purdue University, West Lafayette, IN, USA.

The structural basis for superoxide production in cytochrome bc complexes is currently unknown. Understanding the mechanism of generation of deleterious reactive oxygen species and partition of electron transfer in the branched quinol oxidation pathway in cytochrome bc complexes. The specific rate of superoxide production, normalized to the electron transfer rate, was determined for the yeast cytochrome bc1 complex (provided by B. L. Trumpower) and the cytochrome b6f complex from spinach thylakoid membranes and cyanobacteria. Although electron transfer rates were comparable in bc1 and b6f complexes, the specific rate of superoxide production was 10-20 fold greater in the b6f complex. Whereas antimycin A, a specific n-side quinone analogue inhibitor of the cytochrome bc1 complex, caused a large increase in the superoxide production rate of the bc1 complex, no comparable effect was found for NQNO, an n-side quinone analogue inhibitor in b6f, as defined by spectrophotometry and a crystal structure. These differences between bc1 and b6f complexes imply an increase in branching ratio for reduction by plasto-semiquinone of O2 to O2-, relative to reduction of heme bp for trans-membrane electron transfer. The change in branching ratio is ascribed to a longer semiquinone residence time in the p-side binding niche, due to steric restriction of the quinone binding site by the chlorophyll phytyl chain, as seen in a crystal structure. The presence of this phytyl chain can be seen to result in a smaller accessible volume for binding sites of a p-side quinone analogue inhibitor. The longer residence time of quinone/semiquinone would facilitate trans-membrane signaling, e.g., activation of n-side LHC kinase (NIH GM-38323).

4294-Pos Board B513
Investigation of Excited State Charge Redistribution of the Reduced Anionic Flavin in DNA Photolyase and Simple Solvents by Stark Spectroscopy
Temple University, Philadelphia, PA, USA.

The two-electron reduced anionic form of flavin adenine dinucleotide (FADH⁻) catalytically active redox cofactor in DNA photolyase. This enzyme repairs UV-induced cyclobutane pyrimidine dimers (CPD) via a photo-induced electron transfer (PET) reaction, in which the FADH⁻ is excited with blue light. The UV-visible absorption spectra of reduced anionic flavins, characterized decades ago, show a peak at ~350 nm with a broad shoulder at ~420 nm. We have shown, through earlier linear dichroism experiments, that these absorption bands arise from two distinct electronic transitions, a controversy that remains up to the time. Here we have used Stark spectroscopy as a complementary approach to substantiate this result. The sensitivity of the amplitude and band shape of the Stark spectra unambiguously resolves the broad absorption band structure into two separate transitions; in fact, an analysis of the Stark data is not possible under the assumption of a single electronic transition. The magnitude of the difference dipole moment of the low energy S0 → S1 transition (~420 nm) is about three times smaller than the S0 → S2 transition (~350 nm). These experimental assignments were supported with TD-DFT calculations which help to assign the direction of charge redistribution. A picture emerges in which the xylene moiety of the isoalloxazine ring becomes electron-rich upon excitation into either absorption band. Interestingly, this part of the isoalloxazine ring is in close proximity to the bound CPD, suggesting that this is the site for PET to the CPD.

4295-Pos Board B514
Engineering and Tuning of Oxygen Reactivity in Heme Protein Maquettes
Molly M. Sheehan, Lee A. Solomon, Goutham Kodali, Christopher C. Moser, P. Leslie Dutton.
University of Pennsylvania, Philadelphia, PA, USA.

There is a dearth of quantitative data related to reactive oxygen species (ROS) production, particularly superoxide, from protein systems. Understanding of ROS chemistry in proteins is necessary for avoiding damaging oxidative reactions involved in biological processes including aging and ischemic injury. These reactions are also necessary to control in order to develop robust artificial enzymes and photosystems for alternative energy production. Here we show multi-faceted control over the oxygen reactivity of ferrous heme containing designed protein maquettes. We demonstrate control over superoxide production through both inner and outer sphere electron transfer (ET) mechanisms. Inner sphere ET occurs through oxygen binding and both oxygen
on and off rates effect detectible superoxide production rates and total yields. On rates are controlled via helical strain and core packing while off rates are controlled via water accessibility to the heme. The faster the on rate, the less superoxide produced due to more complete formation of the oxyferen steady-state. Stable oxyferen states with half times on the order of seconds produce superoxide below the level of detection and biological relevance. When the oxyferen state cannot be formed due to lack of helical strain, outer sphere ET occurs at rapid rates on the order of NADPH oxidases. Faster rates of ET are observed for more solvent-exposed hemes. In all cases when the superoxide production rate is faster, we observe lower total yields. This effect is due to increased dismutation or peroxide when there are higher local concentrations of superoxide. The engineering principles we learned for superoxide control could be used to understand natural ROS processes as well as develop durable artificial ET protein systems.

2496-Pos Board B515
Physical Chemical and Engineering Principles underlying the Construction and Electrochemical Properties of Man-Made Protein Maquettes
Many key biological functions are accomplished through complicated system of oxidoreductases. Even though a significant number of these enzymes have been structurally characterized, attempts to reproduce their functions have not been successful. This work examines engineering of a simplified 4-alpha-helix-bundle protein, called a maquette, which lacks complexity. This feature allows us to reconcile the function of each amino acid such that mutations have minimal effects. Using simple maquettes with a variety of structural topologies - ranging from molten globular tetramers to natively structured monomers - we determine what aspects of the protein structure control the rate of protein-cofactor assembly. This minimalism also allows for a more controlled study of how the electrostatic environment of the protein affects the midpoint potential of heme b. By altering charges on the surface, we were able to raise the Em of heme in maquettes by approximately 240 mV, whereas single point mutations in the heme-binding site only raised the midpoint by 10 mV. Another benefit of maquette simplicity is that they are able to bind a variety of heme b analogues. This feature allowed us to probe the effect of ring substituents on both the assembly rate and the porphyrin’s midpoint potential. We show that porphyrin solubility is a rate-limiting step of the assembly process and that the different ring substituents can alter the midpoint by over 400 mV. This culminates with the design of a simple two-component system wherein two proteins designed with different midpoints split by approximately 200 mV and can transfer a single electron. This work is the first step toward designing systems in which electrons can be shuttled between chains of completely artificial proteins.

2497-Pos Board B516
Designing Protein Maquettes for Interprotein and Transmembrane Electron Transfer
Bryan A. Fry1, Gregory R. Weinman2, Geetha Goparaju1, Christopher C. Moser3, P. Leslie Dutton3, Bohdana M. Discher1.1University of Pennsylvania, Philadelphia, PA, USA; 2Johns Hopkins University, Baltimore, MD, USA.
Protein-driven electron transfers are essential steps in a wide range of biochemical processes. Electron transfer rates are governed primarily by the distance between redox centers and by the driving force originating from midpoint potentials and coupled catalytic reactions. The structural complexity of natural proteins contrasts with the relatively simple rules of cofactor selection and placement that, in principle, govern the electron transfer behavior. Here we present two examples of minimal 4-alpha-helical bundle proteins (“maquettes”) that leverage these basic design principles to illustrate functional electron transfer. Both proteins support bis-histidine ligation of natural heme cofactors within a tetrahedral bundle. The first of these maquettes is BT6, a water-soluble monomeric protein with a net charge of −14. Reduced heme-bound BT6 transfers electrons to cytochrome c at 2×10⁻⁷ M. A similar electron transfer between the natural heme-protein cytochrome and cytochrome c during apoptosis. As in the neuroglobin-cyt c system, we believe that the rapid electron transfer from glutamate-rich BT6 to lysine-rich cytochrome c is enhanced through electrostatic interaction between the two proteins. The second maquette discussed here is AP6, a amphiphilic helix that assembles into a homotetrameric bundle in a phospholipid bilayer. AP6 incorporates six bis-histidine binding sites to ligate six heme cofactors spanning a flow through experiments to probe transmembrane electron transfer, mixing the soluble redox dye indigotrisulfonate with AP6 liposomes encapsulating oxidizing K₃Fe(CN)₆. In the presence of protein and heme, transmembrane electron transfer rates are significantly faster than in the absence of either.

2498-Pos Board B517
The De Novo Engineering of Artificial Flavoproteins
Flavins are ubiquitous redox active cofactors that participate in a host of biological processes such as O₂ activation, DNA repair, aromatic hydroxylation, phototaxis and magnetoreception. The diverse utility of the flavin cofactor stems from its ability to accept one or two electrons and/or facilitate hydride transfer. These processes can occur over a wide range of midpoint potentials (~360 to +160 mV) by specific interactions with the protein matrix into which the flavin is inserted. In this work, we aim to understand the biophysical basis of this control by designing and synthesizing tetra-helical protein maquettes with flavins as redox cofactors. We covalently couple differentially substituted novel flavin analogues via a cysteine linkage at a designated location. Our results demonstrate successful coupling of two flavin analogues that produced systems with very different midpoint potentials. We show a light activated electron transfer between the flavin and a bis-his ligated heme under continuous illumination conditions to our maquette as well as light activated electron transfer between a tropotphan and the flavin. We also show light independent oxidation of nicotinamide analogues by the flavomaukette. These results not only help us understand the natural oxidoreductases but also bring us closer to realizing fully synthetic flavin enzymes for novel catalysis.

2499-Pos Board B518
Photosynthetic Reaction Center Performance under Physiologically Relevant Energetic Changes
Zhenyu Zhao1, A. William Rutherford2, Chris C. Moser3, P. Leslie Dutton1.1University of Pennsylvania, Philadelphia, PA, USA; 2Imperial College, London, United Kingdom; 3Pennsylvania State University, State College, PA, USA.
Keywords: Photosynthesis, Reaction Center, Photosystems.
Natural photosystems undergo significant changes in driving forces for single or even multiple steps of electron transfers during actions under transmembrane electric field (Rh. Sphaeroides) or during assembly (Photosystem II). These changes could have significant influences on the performance parameters of these systems or serve a protective function against oxidative damages. The poster discusses the effects of the transmembrane electric field across Rh. Sphaeroides reaction center on its photochemical efficiencies, defined as the yield of a photon-generated charge-separated state in redox potentials between associated electron donors and acceptors, and explore whether the otherwise suboptimal machinery is optimized for functions under relevant membrane potentials. Surprisingly, efficiencies of the photosystems can increase significantly under transmembrane loads. We further examine whether PSII make use of its free energy to select redox potentials of cofactors that dodge and minimize the destructive effects of singlet oxygen or partly reduced oxygen. The results of the analysis indicate that significant protection against singlet oxygen damage in PSII is difficult to achieve.

2500-Pos Board B519
Electrochemical Investigation of the Radical SAM Enzyme, BtrN from Bacillus Circulans
Stephanie J. Maiocco1, Tyler Grove2, Lauren Sites2, Squire J. Booker2, Sean J. Elliott1.1Boston University, Boston, MA, USA; 2Pennsylvania State University, State College, PA, USA.
Radical SAM enzymes catalyze a variety of reactions involved in biological pathways including the biosynthesis of antibiotics, cofactors, and biosynthesis and repair of DNA. Measurement of the electrochemical characteristics of radical SAM enzymes has been limited due to the typically buried location of the radical SAM cluster within the enzyme. The midpoint potential of a radical SAM cluster has only been determined for lysine aminomutase using spectrroelectrochemical titrations with the use of mediators. This study presents the first direct electrochemical measurement of a radical SAM enzyme using the technique of protein film voltammetry (PFV). BtrN from Bacillus circulans is an emerging class of radical SAM dehydrogenases, which catalyze the third step in the biosynthetic pathway of the antibiotic butirosin. BtrN has been shown to contain a second [4Fe-4S] cluster in addition to the canonical radical SAM [4Fe-4S] cluster. Nonturnover PFV has characterized the electrochemical properties of these clusters and provided insight into the mechanism of electron transfer. Additionally, PFV has been used to characterize the substrate binding on the clusters. These results provide insight into the catalytic mechanism of BtrN and the electrochemical characteristics of radical SAM enzymes in general.