on and off rates effect detectable 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 oxyferrous state. Stable oxyferrous states with half times on the order of seconds produce superoxide below the level of detection and biological relevancy. When the oxyferrous 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
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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 measurable effects. Using single monomers, we performed stopped flow 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.

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Designing Protein Maquettes for Inteiprotein and Transmembrane Electron Transfer
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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-α-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 × 10−9 M s−1 versus −100 mV in the case of the native b-heme protein cytochrome and cytochrome c apo- tosisc. 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, an amphiphilic helix that assembles into a homotetrameric bundle in a phospholipid bilayer. AP6 incorporates six bis-histidine binding sites to ligate 6 of the 8 heme cofactors spanning stopped flow 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 Flavorproteins
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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 designed 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-histidine ligated heme under continuous illumination conditions to our maquette as well as light activated electron transfer between a tryptophan and the flavin. We also show light independent oxidation of nicotinamide analogues by the flavomauquette. These results not only help us understand the natural oxidoreductases but also bring us closer to realizing fully synthetic flavin enzymes for novel catalysis.

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Photosynthetic Reaction Center Performance under Physiologically Relevant Energetic Changes
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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
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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 spectrotelectrochemical 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] 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 effect of 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.