method of freezing, glycerol concentration, and pH. Small Angle X-Ray Scattering experiments are planned to determine if significant structural changes are apparent on forming Prz-cyt *c*.

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Comparative Studies of Human Indoleamine 2,3-Dioxygenase (IDO) and Tryptophan Dioxygenase (TDO)
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The majority of our dietary Tryptophan (L-Trp) is oxidized to NAD via the the kynurenine pathway. The first and rate-limiting step of the pathway, the conversion of L-Try to N-formyl kynurenine, is catalyzed by two heme-based dioxygenases, tryptophan dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO). Although the two enzymes catalyze the same chemical reaction and exhibit high structural similarity, they perform distinct physiological functions: TDO is a hepatic enzyme that is linked to Trp homeostasis, while IDO is an ubiquitous enzyme that is involved in a variety of important immune related conditions, such as fetal tolerance and cancer immune escape. Recently, IDO has attracted a great deal of attention owing to its potential as a therapeutic target for cancer. Despite of intense reserach, the dioxygenase reaction mechanisms of IDO and TDO are poorly understood. Our recent spectroscopic and kinetic studies indicate that the two reactions follow the same ferryl-based two-step mechanism, challenging the general conception that the two atoms of dioxygen are inserted into the substrate simultaneoiusly. In this work, the structural differences between IDO and TDO are systematically evaluated. The implication of the data on the functional differences between the two enzymes will be discussed.

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Time-Resolved Enthalpy and Volume Changes for Co-Photorelease from Indoleamine 2,3-Dioxygenase
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Indoleamine 2,3-dioxygenase (IDO) is a heme protein catalyzing the first and rate limiting step in the metabolism of L-tryptophan via the kynurenine pathway. The enzyme is responsible for the insertion of dioxygen into the indole ring generating l-formyl-kynurenine. However, the mechanisms underlying ligand binding and the resulting protein conformational changes leading to the reaction products are not well understood. Photoacoustic calorimetry was utilized to probe conformational dynamics associated with CO photo-release from the ferrous form of IDO in the presence and absence of L-tryptophan. At pH 7.7 three kinetic phases are resolved in the absence of L-tryptophan: a fast phase occurring on the timescale of the instrument response (<50ns) corresponding to an endothermic process (30 kcal mol^{-1}) with a volume expansion of 11mL mol^{-1}, an approximately 500ns exothermic volume expansion (−43 kcal mol^{-1}) of approximately 7mL mol^{-1} and a 1.4μs endothermic (96 kcal mol^{-1}) volume expansion of ~6mL mol^{-1}. Ionic strength data indicate large contributions to the observed enthalpy and volume changes arising from electrostriction. The observed rates differ in the presence of L-tryptophan; the lifetime of the 500ns phase decreases to ~200ns and the lifetime of the 1.4μs phase increases 2.7μs whose thermodynamic and volumetric parameters are also sensitive to ionic strength. The results are compared to CO-photolysis from myoglobin and other heme proteins. On the basis of the data, a number of models for ligand migration are proposed.

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Designing Transmembrane Electron Transport in Amphiphilic Protein Maquettes
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Abstract: Electron transfers between protein-bound redox cofactors 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 that originates from the redox mid-point potentials or coupled catalytic reactions. The structural complexity of natural redox proteins contrasts with the relatively simple rules of cofactor placement that, in principle, govern the electron transfer behavior. Rather than focusing on the structural details of a specific natural protein, we have designed general protein structural scaffolds (“maquettes”) to accommodate a variety of functions. In this work we demonstrate transmembrane redox reactions via AP6, an amphiphilic tetrahelical maquette, and via APC, a disulfide-linked dimer comprising two dihelical subunits. In both proteins, histidine residues facing the interior of the helices coordinate several redox-active heme cofactors. We performed stopped flow experiments to probe transmembrane electron transfer, mixing soluble electron-donoating species with protein liposomes encapsulating oxidixing $K_3Fe(CN)_6$. In the presence of protein and heme, transmembrane electron transfer rates are significantly faster than in the absence of either. We also employed Langmuir-Blodgett deposition to produce oriented protein samples in lipid bilayers. The orientation of the maquettes in the membrane is investigated through UV-Vis linear dichroism and circular dichroism spectroscopy.

Membrane Protein Structure II

2380-Pos Board B150
Prediction of Thermostable Mutants of G-Protein Coupled Receptor Proteins using Knowledge-Based Energy Functions
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G-protein coupled receptor (GPCR) proteins form the largest family of membrane proteins and are important drug targets. The three-dimensional crystal structure of a protein is critical for drug design. However, membrane proteins are exceedingly difficult to crystallize. One successful strategy recently used to crystallize GPCR proteins is to derive thermostable mutants that crystallize more readily than the wild type protein. One bottleneck in the identification of thermostable mutants is the laborious number of experiments that need to be done to determine the stability of a mutant. Another major bottleneck is the large number of possible mutants that need to be screened. For a GPCR protein of typically 300 residues length there are approximately 6’000 possible single-point mutants; the ways to combine two mutations are more than 16 million.
Our aim is to cut the experimental costs by predicting which mutations may increase the thermostability of a GPCR protein. One of our computational models for the structural stability as a function of single-point mutation to alanine is based on experimental data for two different specific GPCR proteins with 34 % sequence identity. Our model increased the recall rate and the specificity of the predictions by 30 % and shrank the number of mutants that need to be tested in experiment by 40 %. Application of the same model to another GPCR protein with 25 % sequence identity showed the same enrichment of correct predictions, with a decrease of 25 % in the number of mutants to be tested. The predictions were calculated from a knowledge-based energy function for the structural stability of two GPCR proteins. We develop such functions from theoretical considerations and from experimental data such as the thermostability and the existing crystal structures of GPCR proteins.

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Quantitative Comparison of GPCR Interactions with the Lipid Bilayer of the Cubic and Lamellar Mesophases
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Recent successes in the crystallographic determination of structures of transmembrane (TM) proteins in the G protein-coupled receptor (GPCR) family have established the lipidic cubic phase (LCP or “in meso”) environment as useful for growing diffraction quality crystals. The mechanism underlying in meso crystallogenesis is currently at a descriptive level. To begin developing a quantitative, energy-based nucleation and crystallization mechanism we are conducting molecular dynamics studies of the GPCR, rhodopsin, reconstituted into the LCP using the coarse-grained representations of the Martini force-field. The first aim is to quantify differences in the hydrophobic/hydrophilic exposure of the GPCR to lipids in the cubic and lamellar phases. Simulations of a single rhodopsin molecule in these monoolein-based mesophases showed more energetically unfavorable hydrophobic-hydrophilic interactions between the protein and lipid in planar bilayers of the lamellar phase. The reduced level of hydrophobic mismatch in the LCP, by contrast, is attributable to the highly curved geometry of the cubic phase that provides for more efficient shielding of the protein from unfavorable hydrophobic exposure. Since hydrophobic mismatch can drive oligomerization (Mondal et al., BJ 2011 - in press), these differences suggest that compared to the