excitation laser beam and monitored by the oxidation state marker bands. We investigate the pressure dependence of the iron-histidine stretching mode and compare with results on isolated hemoglobin and myoglobin. A pressure dependent shift suggests a conformational change of the heme environment with pressure.

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Intradimeric but not Interdimeric Interface Control the Function of Hemoglobin

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Chemical modifications of the sulhydryl groups located at the $\alpha 1\beta 1$ interface in tetrameric human hemoglobin – a region usually considered inert– altered significantly its oxygenation properties: both the affinity for oxygen and cooperative ligation were substantially reduced. Still, these derivatives responded to allosteric effectors, such as pH and organic phosphates. Electron paramagnetic resonance of the nitrosyl derivatives showed a hyperfine splitting around g ~ 2 indicating the cleavage of the bond between the α-heme iron and the proximal histidine, which indicates the presence of a low affinity conformation of hemoglobin. Compared to native hemoglobin, a reduced dimerization was also confirmed by isothermal titration calorimetry.

^{1}H-NMR measurements, on the other hand, showed unexpected results. Both the O_2-ligated and CO-ligated forms of the derivatives exhibited spectra typical of the R-conformation, i.e., the absence of the typical T-makers around 11 and 14 ppm even in the presence of strong allosteric effectors, whereas the unligated form of this derivative showed the typical T-makers (these signals originate from specific interdimeric interactions.)

These ^{1}H-NMR results stand diametrically opposed to the measured oxygenation characteristics and are entirely inconsistent with the classical T - R dichotomic view that seemed to describe satisfactorily the correlation structure-function of hemoglobin for decades. The studied derivatives represent a case in which there is a complete divorce between the ^{1}H-NMR-determined ''structure'' and oxygenation characterized ''function''.

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Two Tyrosyl Radicals Stabilize High Oxidation States in Cytochrome *C* Oxidase for Efficient Energy Conservation and Proton Translocation

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The reaction of oxidized bovine cytochrome *c* oxidase (bC*c*O) with hydrogen peroxide (H_2O_2) was studied by electron paramagnetic resonance (EPR) to determine the properties of radical intermediates. Two distinct radicals with widths of 12 and 46 G are directly observed by X-band EPR in the reaction of bC*c*O with H_2O_2 at pH 6 and pH 8. High-frequency EPR (D-band) provides assignments to tyrosine for both radicals based on well-resolved *g*-tensors. The wide radical (46 G) exhibits *g*-values similar to a radical generated on L-Tyr by UV-irradiation and to tyrosyl radicals identified in many other enzyme systems. In contrast, the *g*-values of the narrow radical (12 G) deviate from L-Tyr in a trend akin to the radicals on tyrosines with substitutions at the ortho position. X-band EPR demonstrates that the two tyrosyls differ in the orientation of their β-methylene protons. The 12 G wide radical has minimal hyperfine structure and can be fit using parameters unique to the post-translationally modified Y244 in bC*c*O. The 46 G wide radical has extensive hyperfine structure and can be fit with parameters consistent with Y129. The results are supported by mixed quantum mechanics and molecular mechanics calculations. In addition to providing spectroscopic evidence of a radical formed on the post-translationally modified tyrosine in C*c*O, this study resolves the much debated controversy of whether the wide radical seen at low pH in the bovine enzyme is a tyrosine or tryptophan. The possible role of radical formation and migration in proton translocation is discussed.

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Capturing Insertion and Dynamics of Membrane-Bound Cytochrome P450 3A4 using a Novel Membrane Mimetic Model

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Cytochrome P450 (CYP) enzymes constitute a large family of enzymes present in a wide variety of organisms that are involved in the metabolism of xenobiotics. In humans, CYP3A4 is the most abundant isoform in the liver and is responsible for the metabolism of a large variety of drugs. CYP enzymes are anchored in the cellular membrane by a transmembrane alpha helix and by the insertion of an unknown hydrophobic region from their globular domain into the lipid bilayer. Despite its high relevance to drug entry and binding, an experimental membrane-bound structure of CYP3A4 has not been reported to this date, and only soluble structures are currently available. Molecular dynamics (MD) simulations of other soluble CYP structures have suggested that the presence of the lipid bilayer might initiate important conformational changes in CYPs, but due to the limited lipid motion in such studies, the nature of these changes are still largely uncharacterized. In order to study the interaction of CYP3A4 with a membrane, we performed MD simulations employing a highly mobile membrane mimetic (HMMM) model developed by our group. The HMMM model allows the unbiased association of CYP3A4 with a phosphatidylcholine (PC) bilayer, providing an all-atom description of this process for the first time. The enhanced lipid mobility achieved by the HMMM model allows for a detailed description of the dynamics of CYP3A4, reveling the mechanism of opening and closing of the tunnels from the active site upon membrane binding. In particular, it is observed that the presence of the PC bilayer induces the closing of the access tunnel going through the BC loop of the globular domain. The resulting membrane-bound model exhibits an orientation that is in close agreement with experimental data.

2375-Pos Board B145

Characterization of Binding and Electron Transfer Between Shewanella Cytochrome C Peroxidase and its Monoheme Electron Donor C5

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Bacterial diheme cytochrome c peroxidases are a critical part of the detoxification pathways in the periplasmic space that convert hydrogen peroxide to water. The turnover of peroxide requires a two-electron reduction, and these reducing equivalents must be delivered to the peroxidase by soluble electron transfer proteins. This study focuses on the diheme cytochrome c peroxidase from Shewanella oneidensis (So CcP). We have shown previously that a monoheme electron transfer protein from Shewanella oneidensis, So c5, is capable of both activating and turning over the So CcP protein during in vitro peroxidase assays. The nature of the protein-protein interface is not known, especially since two different molecules of So c5 must bind So CcP, either sequentially or simultaneously, during the catalytic cycle. We postulate that for efficient in vivo activity, oxidized So CcP should preferentially bind to the reduced So c5, and the interaction should weaken substantially once the electron has been transferred. In our current work, we show that charge-reversal amino acid mutations on the putative protein-protein interface failed to substantially change the Km for So c5 in peroxidase activity assays. However, surface charge-reversal mutations did have subtle effects consistent with changes in the electron transfer pathways as measured by protein film voltammetry. We further investigated the interaction between So c5 and So CcP using a surface acoustic wave binding method. Binding between So CcP and So c5 is very weak, with observed dissociation constants around 20 μM, and the interaction can be modulated by surface charge-reversal mutations. However, the rate of dissociation of oxidized So CcP from oxidized So c5 is surprisingly slow, suggesting our current models of the in vivo catalytic cycle of So CcP may need to be revisited.

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Pyrazole Cytochrome C Complexes

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Cytochrome (cyt) *c* is well known to perform cellular functions unrelated to its role in respiratory electron flow, including signaling in the apoptosis pathway and peroxidation of lipids. These appear to involve a significant conformational change that allows access to the heme cleft. We have investigated the possible facilitation of such changes by small polar molecules that bind to the heme with displacement of the methionine. Among these, we found pyrazole (Prz) to bind weakly to ferricytochrome *c* with a strongly sigmoidal concentration dependence, with a midpoint ≈750mM and a Hill coefficient of 2.5, as measured in UV-Vis Spectroscopy at pH 6.5. Stopped-flow experiments show biphasic kinetics for binding of Prz and cyt *c*. Tentatively we model this behavior with a two-step binding reaction where Prz initially binds rapidly but weakly to displace the methionine ligand, with $k_{fast} \approx 30 s^{-1}$. The equilibrium is pulled over by a slower process, with $k_{slow} \approx 0.05 s^{-1}$, that is poorly understood. The slow phase contribution is small at pH 6.5, but increases at more alkaline pH. CW X-band EPR studies suggest the existence of two low-spin Prz-cyt *c* complexes, with g-values at $g_x = 1.55$, $g_y = 2.28$, $g_z = 2.83$ and $g_x = 1.60$, $g_y = 2.28$, $g_z = 2.73$, respectively. The ratio of one complex to the other as seen in EPR is highly dependent upon environment conditions including, but not limited to,

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*.

2377-Pos Board B147

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.

2378-Pos Board B148

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.

2379-Pos Board B149

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.

2381-Pos Board B151

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