data for small peptide systems to calibrate torsional parameters in the OPLS-AA/L forcefield. Our parameterization successfully reproduces observed puckered state ratios for prolyl rings and the coupling between ring puckering and the cis versus trans state of prolyl peptide bonds. Improvements to forcefield parameters and the improved Monte Carlo moves were tested for their ability to reproduce a range of experimental data for different PRRs including polyproline. Analysis of sampled ensembles suggests that prolyl peptide bonds have a higher than previously appreciated likelihood of sampling cis isomers in PRRs.

2043-Pos Board B29
Conformational Selectivity:Targeting the "death" Conformation of Mutsalpha
Freddie R. Salsbury Jr., Karin Scarpinato, Aksana Vasilyeva.

Enzymes/Heme Proteins II

2044-Pos Board B30
His93Gly Myoglobin Cavity Mutant: A Versatile Scaffold for Modeling Heme Protein Active Sites. Applications to Novel Heme Transport and Redox Proteins
John H. Dawson.
The proximal ligand His93Gly myoglobin (H93G Mb) cavity mutant is a versatile scaffold for the preparation of model heme complexes of defined ligand system (1). In particular, the difference in accessibility of the two sides of the heme iron center offers the advantage of forming ambient-temperature mixed-ligand heme model complexes, which are very difficult to prepare with model systems in organic solvents. Moreover, in the H93G Mb system, the protective environment provided by the protein allows for the formation of relatively stable oxyferrous and ferryl [Fe(IV)=O] complexes with variable ligands *trans* to the normally reactive dioxygen and oxo substituents. His93Gly myoglobin cavity mutant complexes with various exogenous ligands have been prepared as models for native heme iron active sites ligated by proximal Lys (amines), Asp or Glu (carboxylates), Tyr (phenols), seleno-Cys (selenols), Cys (thiols) and Met (thioethers). The ferrous, ferric and ferryl H93G Mb complexes described have been characterized with magnetic circular dichroism (MCD) and UV-visible absorption spectroscopy. MCD spectroscopy provides diagnostic spectral data sensitive to the nature of the axial ligands and to the spin state and oxidation state of the active site heme iron. Building upon this foundation, we have focused our attention on the use of the H93G Mb cavity mutant system to aid our investigation of the coordination structure of novel heme binding and transport proteins and heme-containing oxidative enzymes.
1) J. Qin, R. Perera, L.L. Lovelace, J.H. Dawson and L. Lebioda, *Biochemistry*, **45**, 3170-3177 (2006).

2045-Pos Board B31
Time Resolved Crystallographic Analysis of Cooperative Ligand Binding and Ligand Migration in a Dimeric Hemoglobin
William E. Royer, James Knapp, Zhong Ren, Hyun Sun Cho,
Philip Anfinrud, Vukica Srajer.
Invertebrate hemoglobins, which range in size from dimers to assemblies of hundreds of subunits, offer excellent models for the investigation of allosteric protein function. A recurring theme among cooperative invertebrate hemoglobins is a subunit pairing involving the heme-embedding E and F helices, despite a lack of sequence conservation in these helices. This is quite different from the assembly of vertebrate hemoglobins and suggests that such a pairing is amenable for regulating oxygen delivery.
We have carried out extensive time-resolved crystallographic analysis on the simplest of these hemoglobins, the cooperative homodimeric hemoglobin (HbI) from the blood clam Scapharca inaequivalvis. Limited ligand-linked subunit rotation permits the full allosteric transition to be followed in crystals of HbI. These studies have been combined with mutant and functional studies along with conventional crystallographic analysis to provide a comprehensive understanding of the communication between subunits and the movement of ligands through the protein. Investigation of ligand migration through this protein suggests, surprisingly, that ligands primarily escape through a distal histidine gate, despite its involvement in the subunit interface. As a result, ligand escape may require transient subunit movement, which is inhibited in the protein crystal lattice. These findings emphasize the central role of dynamics in protein function.
Our studies are also directed towards understanding cooperative protein function in larger invertebrate hemoglobin assemblies. Preliminary time-resolved crystallogaphic analysis on the tetrameric Scapharca HbII suggests similar structural transitions as HbI and offers the possibility of investigating how changes in one subunit directly impact a neighboring subunit in the heterodimeric halves of HbII. In addition, we will discuss new insights that may provide unifying themes for the basis of cooperativity among diverse invertebrate hemoglobins that form EF dimeric pairing.

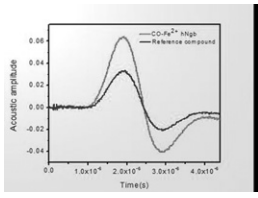
2046-Pos Board B32
Site(s) of Tyrosyl Radical Formation in Dehaloperoxidase A and B
Reza A. Ghiladi, Rania Dumarieh, Jennifer D'Antonio.
Dehaloperoxidase (DHP) is a dual-function hemoprotein that, in addition to being the putative coelomic hemoglobin of *Amphitrite ornata*, exhibits a broad substrate specificity for the catalytic oxidation of mono-, di-, and trisubstituted halophenols, thus distinguishing DHP as the first globin shown to possess a biologically relevant peroxidase activity. Both isoenzymes of DHP, termed A and B, have been shown to oxidize trihalophenols to dihaloquinones in a dehalogenation reaction that utilizes hydrogen peroxide as the oxidant. We have shown that the initially formed heme intermediate in wild-type DHP is not Compound I as is often the case in peroxidases, but rather is a combination of an iron(IV)-oxo and a tyrosyl radical that together have similarity to the Compound ES intermediate of cytochrome c peroxidase. In order to possibly identify the site(s) of this radical species in DHP, we have studied the tyrosine mutants DHP A (Y34F), DHP A (Y38F), DHP A (Y34F/Y38F), DHP B (Y28F), DHP B (Y38F), and DHP B (Y28F/Y38F), and studied their reaction with hydrogen peroxide using a combination of stopped-flow UV-visible and rapid-freeze quench electron paramagnetic resonance spectroscopies. Spectroscopic evidence for the formation of both Compound I and Compound ES will be presented. We have further characterized these mutants using biochemical assays to determine their effect on the catalytic activity of the enzyme, and relate these results to the structure of the heme active site and the formation of the catalytically-attenuated species Compound RH. Such mutagenesis studies of DHP provide critical insight into the mechanistic details of the H_2O_2-dependent oxidative dehalogenation reaction catalyzed by dehaloperoxidase, present a clearer description of the function of DHP at the molecular level, and lead to a better understanding of the paradigms of globin structure-function relationships.

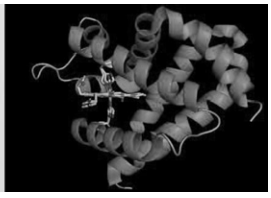
2047-Pos Board B33
Computer Simulation of Chemical Reactivity of Heme Proteins
Dario A. Estrin, **Luciana Capece**.
We present an investigation of the molecular basis of chemical reactivity modulation in selected heme proteins using a combination of classical molecular dynamics and hybrid quantum-classical (QM-MM) simulations. Results will be presented for:
i) Tryptophane and indoleamine dioxygenases, two related heme proteins of physiological relevance that catalyze the oxidative ring cleavage reaction of L-tryptophan to N-formyl kynurenine. We will show the molecular basis of the different selectivity of these proteins, and a detailed analysis of the reaction mechanism, which shows that the 2 atoms of dioxygen are inserted into the substrate via a consecutive 2 step reaction.
ii) Analysis of the molecular basis of hexacoordination in human neuroglobin. Our results suggest that protein oxidation through the formation of a disulfide bridge promotes the stabilization of the pentacoordinated species, thus favoring the reactive state and suggesting a O2 storage function for neuroglobin. Results obtained using high pressure simulations of neuroglobin and myoglobin suggest that the equilibrium between the 5c and the 6c states in globins is largely controlled by the structure and dynamics of the CD region.

2048-Pos Board B34
Time-Resolved and Steady-State Spectroscopy of Native and Mutated Thermobifida Fusca Hemoglobins
Stefania Abbruzzetti, Alberto Boffi, Alessandra Bonamore, Enrica Droghetti,
Alessandro Feis, Paolo Foggi, Cristina Gellini, Agnese Marcelli,
Francesco P. Nicoletti, Piero R. Salvi, Giulietta Smulevich, Cristiano Viappiani.
Thermobifida fusca hemoglobin (TFH) is a prototypical bacterial (class 2) hemoglobin. It has been identified in a thermophilic actinobacterium and overexpressed. The heme cavity properties are mainly related to the polarity and H-bonding capability of the ''distal'' amino acids: tyrosine (B10 in the myoglobin helix notation), tyrosine (CD1), tryptophan (G8).
Single, double and triple phenyalalanin (Phe) mutants of these key residues have recently become available. We have applied several techniques of time-resolved and steady-state optical spectroscopy to the study of THF carbon monoxide complexes, in an effort to correlate structural and dynamic properties of the protein active site.

i) Femtosecond transient absorption has shown that CO can rebind from TFH distal cavity in few ns. TrpG8 substitution with the apolar Phe accelerates the recombination, which takes place in hundreds of ps.
ii) Laser flash photolysis has allowed to extend the study of the dynamics to longer times. The yield of the geminate rebinding and the kinetics of recombination from the solvent are both influenced by the single TrpG8 → Phe mutation. Further changes are observed when the three polar amino acids are replaced by Phe. Rate constant distributions have been obtained by the maximum entropy method.
iii) Photoacoustic measurements have yielded both volume and enthalpy changes following CO photodissociation in the ns range. The thermodynamic parameters can be related to those measured by laser flash photolysis.
iv) Resonance Raman spectroscopy of seven THF mutated variants has given evidence for a H-bonding network in the distal cavity, which involves the bound CO.
All the results point to an overwhelming role for TrpG8. This is in agreement with a recent study on a related bacterial hemoglobin from *B. subtilis*.

2049-Pos Board B35
Conformational Dynamics in Neuroglobin
Luisana Astudillo, Sophie Bernard, Valerie Derrien, Pierre Sebban, Jaroslava Miksovska.
Neuroglobin (Ngb) is a heme protein that belongs to the family of hexacoordinated hemoglobins. The physiological role of this protein is not well understood, but several plausible functions have been proposed such as oxygen carrier, oxygen sensor, NO scavenger and protectant against oxidative damage. To understand the mechanism of Ngb interactions with diatomic ligands we have determined kinetics and thermodynamics of conformational changes associated with CO dissociation and rebinding to human neuroglobin (hNgb) and rat neuroglobin (rNgb) using photoacoustic calorimetry, photothermal beam deflection, and transient absorption spectroscopy. The impact of the internal disulfide bond found in hNgb on the ligand migration was investigated by characterizing the time profile of structural changes associated with CO photo-dissociation from the following mutants: rNgbGly46Cys, hNgbCys46Gly, hNgbCys55Ser, hNgbCys120Ser, hNgbCys55SerCys120Ser and hNgb reduced with DTT. Moreover, to determine the role of amino acid residues located in the heme binding pocket on the ligand - protein interactions in Ngb, the kinetics and energetics of the ligand dissociation from His64Gln, His64GlnVal68Phe, Val68Phe, Phe49Ala, Phe49Leu and Phe49Asp mutants were determined.





2050-Pos Board B36
Allosteric Mechanism of Oxygen-Binding in Hemoglobin
K. Kanaori, Y. Tajiri, A. Tsuneshige, **T. Yonetani**.
The widely held structure-based mechanism of cooperative oxygen-binding in hemoglobin (Hb), in which the oxygen-affinity of Hb is regulated by the T to R-quaternary and associated tertiary structural transition, is based upon the assumption of the structure-function correlation: **([deoxy-state] = [T-quaternary structure]=[low-affinity state])** and **([oxy-state]=[R-quaternary structure]= [high-affinity state]**. Simultaneous measurements of the quaternary structure by proton NMR and the oxygen-affinity by oxygen-binding equilibrium have yielded the following structural/functional states of Hb: **(IV)** T(deoxy)Hb with extreme low-affinity, **(V)** R(oxy)Hb with extreme low-affinity, and **(VI)** T(oxy)Hb with extreme low-affinity in the presence of potent heterotropic effectors as well as more conventional **(II)** T(deoxy)Hb with low-affinity and **(III)** R(oxy)Hb with high-affinity in the absence of heterotropic effectors. These results indicate the above-mentioned assumed structure-function correlation is no longer valid. The structure-based allosteric mechanism of Hb describes merely ligation-linked structural allostery rather than the cooperative mechanism of Hb. The structure and the function (the oxygen-affinity, the cooperativty, and the Bohr effect) of Hb are regulated independently by a tug of war between the allosteric effects of oxygen (T–>R and increasing affinity) and the opposing allosteric effects of heterotropic effectors (T<–R and decreasing affinity) rather than the T to R-quaternary and associated tertiary structural transition. The oxygen-affinity is regulated by effector-linked dynamic structural changes.

2051-Pos Board B37
The Stretching Frequencies of Bound Alkyl Isocyanides Indicate Two Distinct Ligand Orientations Within the Distal Pocket of Myoglobin
George C. Blouin, John S. Olson, Edwin J. Heilweil, Angela R. Hight Walker.
Alkyl isocyanides (CNRs) have a long history of use as probes of steric constraints in the binding pockets of myoglobins (Mb) and hemoglobins (Hb). However, little is known about their conformations within those environments. The FTIR spectra of CNRs bound to sperm whale Mb have ν_{CN} bands at ~2075 and ~2125 cm^{-1} that have been assigned to *in* and *out* conformations, respectively. In the *in* conformation, the ligand points toward the protein interior, and the lower ν_{CN} results from donation of a hydrogen bond from the distal His64 (E7) to the bound isocyano group. In the *out* conformation, the ligand displaces the His64 side chain into solvent and away from the binding site. Support for this interpretation includes: (1) the similar dependence of ν_{CO} on the His64 conformation in MbCO; (2) the absence of the low-frequency ν_{CN} peak for CNRs bound in the apolar binding sites of H64A Mb, H64L Mb, and micelles containing model heme; and (3) a correlation between the fraction of photodissociated CNRs that are held in the binding pocket and rebind geminately over 100s of ns and the fraction of CNRs in the *in* conformation. CNRs that point out through an open His64 ''gate'' rapidly escape following dissociation from the heme iron.
The situation is more complex for CNRs bound to isolated subunits of human Hb. The FTIR spectra contain three ν_{CN} bands, and geminate rebinding followed at visible wavelengths occurs in multiple phases. To interpret these data, we are currently studying CNRs bound to Mb, Hb, and a heme/micelle model system by Raman spectroscopy to determine the dependence of ν_{CN} on Fe-CNR back bonding, and by time-resolved IR to assign the rebinding phases to their respective ν_{CN} bands.

2052-Pos Board B38
Single Molecule and Hole-Burning Spectroscopies for the Determination of Molecular Electric Fields in Proteins
Yi Hu, Bradley Moran, Hannah Wagie, Jorg Woehl, **Peter Geissinger**.
Research in recent years has been pointing to molecular electric fields, generated by the charge distribution of nuclei and electrons inside molecules, as significant contributor to protein function in a variety of phenomena ranging from enzymatic activity [1,2] to photosynthesis [3]. Gaining detailed, quantitative information about such fields from experiments, however, has remained a difficult task. In this work, we will outline our experimental approach to quantitatively determine the molecular electric fields at the oxygen binding site in the heme proteins myoglobin and hemoglobin using single molecule and hole-burning spectroscopies in combination with quantum-mechanical models for data analysis. While hole-burning measurements average over a subset of proteins in a sample, single protein studies will reveal the distribution of internal molecular electric fields. We will discuss in the framework of our models hole-burning measurements in myoglobin and the dependence of the resulting molecular electric fields in the protein on the input parameters of these models. Moreover, we will present measurements of absorption cross-sections and fluorescence quantum yields of various fluorescent heme derivatives, which will serve as molecular probes of the internal field at the protein active sites. The results on room temperature imaging of single molecules of protoporphyrin IX embedded in thin polymer films demonstrate that such experiments are indeed feasible on the level of single molecules.
[1] E.D. Getzoff, D.E. Cabelli, C.L. Fisher, H.E. Parge, M.S. Viezzoli, L. Banci, and R.A. Hallewell, Nature 358, (1992) 347.
[2] J.P. Hosler, J.P. Shapleigh, D.M. Mitchell, Y. Kim, M.A. Pressler, C. Georgiou, G.T. Babcock, J.O. Alben, S. Ferguson-Miller, and R.B. Gennis, Biochemistry 35, (1996) 10776.
[3] A.P. de Silva and T E. Rice, Chem. Commun., (1999) 163.

2053-Pos Board B39
6-Phosphofructo-2-Kinase/Fructose-2,6-Bisphosphatase (PFKFB) Modulates Slow Oscillations in Pancreatic Islets
Matthew J. Merrins, Richard Bertram, Arthur Sherman, Leslie S. Satin.
Glucose-dependent insulin secretion from beta-cells of pancreatic islets is pulsatile, but the source of the underlying oscillations remains unclear. We have developed a computational model of the beta-cell, termed the 'Dual Oscillator Model' (DOM), based on the hypothesis that slow oscillations in insulin secretion reflect slow oscillations in glycolysis, which then interact with fast oscillations arising from membrane electrical activity and Ca^{2+}. One version of the DOM predicts that glycolytic oscillations are generated by phosphofructokinase-1 (PFK1) via allosteric activation by its product fructose-1,6-bisphosphate (FBP), and terminated by depletion of the substrate