structural features of the tyrosines (phenoxyl ring rotation angle) or with the values of the parameters obtained from the DFT calculation. This allows identification of the tyrosine in the protein responsible for the observed radical.

2870-Plat
Archaeal Protoglobin Structures: Novel Ligand Diffusion Paths And Heme Reactivity Modulation
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Despite its strict anaerobic nature, M. acetivorans genome hosts genes related to O2 metabolism; among these, an open reading frame encodes for a ''protoglobin'' (NP_617780; Pgb). Pgbs are single domain heme proteins of ~195 amino acids, related to the N-terminal domain of archaeal and bacterial globin coupled sensor proteins (GCS; Freitas et al. (2004) Proc. Natl. Acad. Sci. USA 101, 6675-6680). Sequence comparisons indicate that Pgbs, despite their 30-35% larger size, are structurally related to single chain hemoglobins (about 150 amino acids, 3-on-3 α-helical sandwich,12-16% residue identity to Pgbs), and to the heme-based aerotaxis transducer sensor domain of Bacillus subtilis GCS. Pgbs bind O2, CO, and NO reversibly in vitro. Functional and evolutionary issues are openly debated: Pgb may facilitate O2 detoxification in vivo promoting electron transfer to O2, or may act as CO sensor/supplier in methanogenesis.
Our previous studies unravelled the 3D structure of M. acetivorans Pgb, and of its ligand binding properties (Nardini et al. (2008) EMBO Reports 9, 157-163). We showed that Ma-Pgb heme- domain is strongly related in tertiary and quaternary structure to the N-terminal domain of archaeal and bacterial GCSs. Contrary to known globins, however, Pgb-specific loops and a N-terminal extension completely bury the heme within the protein matrix. A new access routes to the heme, built by two Pgb-specific apolar tunnels reaching the heme distal site from locations at the B/G and B/E helix interfaces was highlighted. We present here structural and ligand binding properties of four Ma-Pgb mutants (at sites B10, B12, G8, G11) that were designed in order to probe the role of the heme access tunnels previously described. The atomic resolution structures will be discussed at the light of the kinetic parameters measured for the mutant Ma-Pgb.

2871-Plat
Structural Probes Of Reactive Intermediates Of Dehaloperoxidase From Amphitrite ornata
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The enzyme dehaloperoxidase (DHP) from the marine worm Amphitrite ornata is a unique hemoglobin that functions as a peroxidase, capable of converting 2,4,6-trihalo- phenols (TBP, TCP, and TFP) into the corresponding 2,6-dihalogenated quinones as well as other products. In this overview talk we discuss the structure and function of DHP using X-ray crystallography and nuclear magnetic resonance (NMR) to discuss the large differences between DHP function and hemoglobin function despite the strong structural similarities. The position of halogenated phenols inside the distal pocket is one anomalous feature of DHP that is not observed any other hemoglobin or myoglobin. The X-ray crystal structure of DHP reveals that the distal histidine is flexible and has two major conformations. The closed conformation (named by analogy with Sperm Whale myoglobin) is enforced by the binding of a sixth ligand to the heme iron. In the open conformation, observed in the deoxy DHP X-ray crystal structure, the distal histidine is in a solvent exposed conformation. The role of the histidine in coupling the binding of substrate and inhibitors will be discussed in the context of the mechanism for formation of compound I, compound II and a novel intermediate called compound RH that appears to be crucial to the cycling of DHP between hemoglobin and peroxidase function. The NMR data reveal that there are both interior and exterior binding sites for the substrate. This aspect will be discussed along with evidence from optical and EPR spectroscopy to understand the electron transfer kinetics of DHP.

2872-Plat
Structural Analysis of Hemoglobins and Myoglobins Using MD Simulations
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In the family of respiratory proteins, hemoglobins and myoglobins have been the first to be crystallized in '50. Despite their precise 3D structures are available at high resolution, some questions regarding the microscopic functioning remain yet open. The R to T switching mechanism in hemoglobins and the ligand escape process in myoglobins remain still under debate.
Thanks to the small size, myoglobin is the preferred candidate also for the more general structure-funcion paradigm. In the interior of myoglobin five main docking sites have been identified, especially with Xe NMR, and for long time these Xenon cavities have been classified as packing defects. Recently, it was shown that they might be involved in ligands migration path, even if mechanisms used by myoglobin to connect these cavities is still unknown as well as processes regulating its biologic functions. In this work we made use of standard MD simulations of solvated myoglobin to characterize internal cavities. Our principal results is that we have found several secondary cavities showing volume and occurrence at least comparable to that of Xenon cavities. In order to rationalize and in-depth analyze such a huge amount of data (ca. 30000 cavities/10 ns MD), special cluster-analysis was applied: we classified all cavities with respect to the position, size and occurrence as function of simulation time ascribing them to different clusters. This analysis implicitly highlights possible ligand migration paths for small ligands within the protein matrix allowing to quantitatively compare dynamical behaviour of different myoglobins towards different ligands. Our suggestion that the secondary cavities constitute the preferred path for ligand escape is also supported by explicit metadynamics simulations of ligand escape.

2873-Plat
Auto-Oxidation of Human Hemoglobin and the Roles of Distal Heme Pocket Substitutions
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This study investigates the auto-oxidation reaction of human normal adult hemoglobin (Hb A) and the effects of distal heme pocket substitutions, P_{50}, and tetramer-dimer dissociation on the rate and mechanism of auto-oxidation. Recombinant hemoglobins (rHbs) with single amino acid substitutions at helical positions E11 and B10 have been expressed in *Escherichia coli* and purified, as well as di-α linked and octameric rHbs. These rHbs include: rHb (αV62L), rHb (αV62I), rHb (βV67L), rHb (βV67I), rHb(αL29W), rHb(αL29F), rHb(α-Gly-α/β gene di-α/β), rHb(diαL29F), rHb(diαL29W), and rHb(βG83C). Auto-oxidation measurements were conducted with 32μM heme in MES buffer (pH 6.5) for 60 hours at 35 °C. A monophasic nature of auto-oxidation has been observed for Hb A and a biphasic nature for all other rHbs. In comparison to the other mutants, including di-α linked and octameric rHbs, rHb (αL29F) is most resistant to oxidation and rHb (αL29W) is the least resistant to oxidation. Characterization of three novel rHbs; (βL28F, βV67I), (αL29F, βV67I), (αL29F,αV62I) will test whether the fast and slow phases of the observed biphasic nature of auto-oxidation can be attributed to the mutated and wild-type subunits, respectively. Our studies will also provide new insights into the roles of amino acid residues in the distal heme pockets on the structure-function relationship in hemoglobin(Supported by NIH grants HL-024525 and GM-084614, HHMI, and The Arnold and Mabelle Beckman Undergraduate Research Scholars Program).

2874-Plat
The Use Of Glassy Matrices To Identify Intermediates In The Nitric Oxide Dioxygenase Reaction Of Hemoglobins And Myoglobins
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The nitric oxide dioxygenase (NOD) reaction of nitric oxide with ferrous oxy derivatives of Hb and Mbs is important from many physiological standpoints. Several mechanisms have been proposed; however, the experimental difficulties in probing this reaction in solution at physiological pH and ambient temperatures have precluded an unambiguous determination of the sequence and nature of intermediates. We have developed a method of following the progression of this reaction in glassy matrices that allows for the trapping and probing of key intermediates. The technique is based on incorporation of O_2 derivatives of Hb and Mb in a thin glassy matrix (derived from trehalose) that lines the inner wall of an optical quality tube. After purging the sample with dry nitrogen to remove the unbound excess oxygen, the tube is filled with NO. Absorption spectroscopy is used to follow the spectral progression initiated as the NO slowly accesses the heme bound oxygen. The spectra reveal an intermediate that resembles the spectrum attributed to the bound peroxynitrite intermediate. The final product under these condition is a species with a spectrum that is identical to that which is generated when met Hb(Mb) is incorporated into a glass in the presence of an excess of nitrate. The spectrum attributed to the nitrate