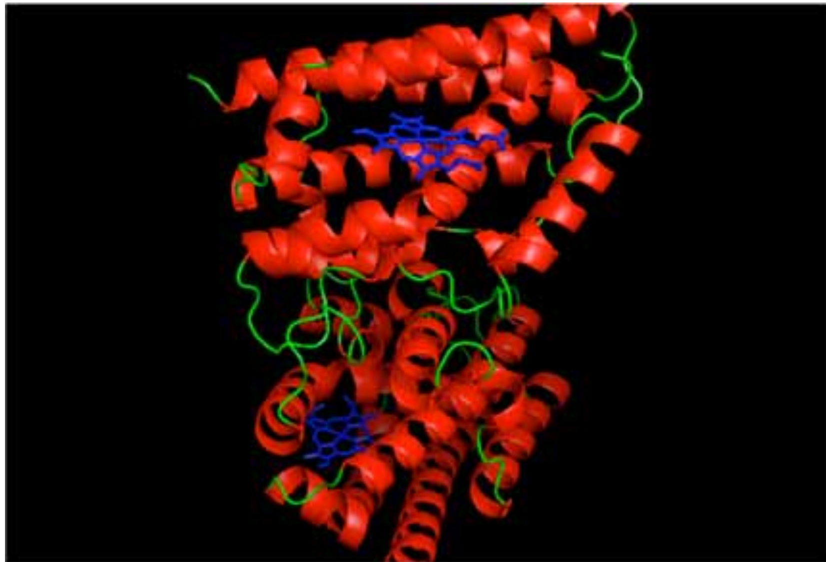
HUMAN HEME OXYGENASE-1

PDB ENTRIES:
1N45 (HO1 with HEME)
1NI6 (HO1 without HEME)
1S8C (HO1 with biliverdin)



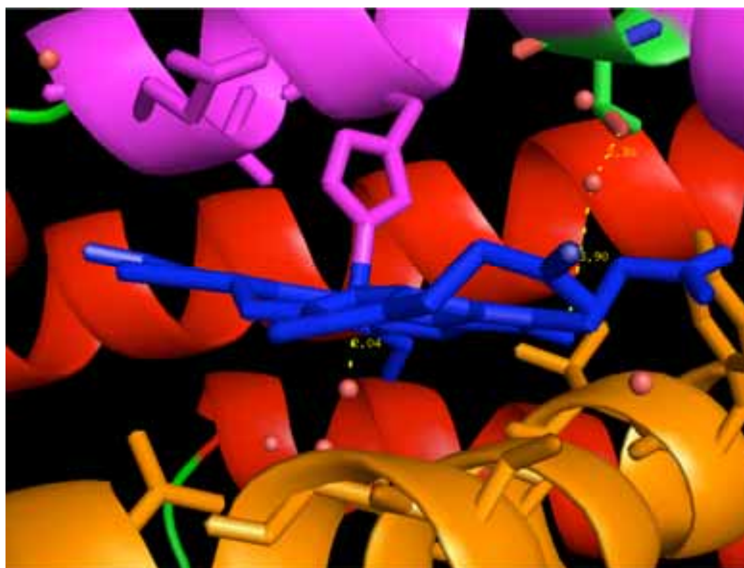
EVREN SABAN

Heme oxygenase (HO), originally discovered in 1968 by Tenhunen R, Marver HS and Schmid R., catalyzes the initial and rate limiting step in the oxidative degradation of heme. Together with the heme synthetic enzyme δ-aminolevulinate synthase it regulates the cellular levels of the prooxidant heme, yielding equimolar amounts of biologically active catabolites. Heme released from oxidized free hemoglobin constitutes a potentially harmful molecule due to its ability to intercalate into cell membranes where it promotes deleterious iron-dependent reactions leading to ROS generation and membrane lipid peroxidation. Acting in concert with NADPH-cytochrome P450 reductase as reducing agent, HO catabolizes the breakdown of heme, released mainly from hemoglobin of senescent erythrocytes, from myoglobin or cytochromes. HO-1 cleaves the α-methene carbon bridge and yields equimolar amounts of biliverdin IXα, carbon monoxide (CO), and iron. In mammalian cells, biliverdin is subsequently converted to bilirubin by bilverdin reductase, and released iron is used in intracellular metabolism or sequestered into ferritin.

Accumulating evidence suggests that HO-1 induction in addition to its role in heme degradation might confer cellular protection against oxidant insults and serve a vital function in maintaining cellular homeostasis. HO-1 has a diverse spectrum of cytoprotective effects that, apart from its ability to remove the pro-oxidant heme molecule, are mostly associated with the different end products of heme catabolism. The HO product biliverdin is rapidly converted into the bile pigment bilirubin which possesses potent free radical scavenging and antioxidant properties. Bilirubin has been demonstrated to provide cellular protection to neuronal cells exposed to oxidative injury by hydrogen peroxide as well as in a model of ischemic heart injury. Iron, released during the enzymatic degradation of heme, is cytotoxic due to its ability to generate ROS by Fenton chemistry, but is promptly sequestered into the iron storage protein ferritin, the synthesis of which is upregulated by iron. Ferritin exerts antioxidative effects that can be ascribed to its capacity to sequester iron, which lowers the prooxidant state of the cell by removing free iron, and to its ferroxidase activity, catalyzing the oxidation of ferrous iron to ferric iron to enable intracellular storage of iron in biological systems. HO-1 also upregulates an iron ATPase located in the endoplasmatic reticulum resulting in augmented cellular iron efflux. CO, the third catalytic product of HO-1 activity, can also mediate important cellular functions and has been shown to exhibit potent cytoprotective effects. This gaseous messenger can promote vasodilatation *via* activation of soluble guanylate cyclase and subsequent formation of cGMP. Therefore CO can maintain blood circulation at sites of inflammation, counteracting the deleterious effects of coagulation and thrombosis via the same mechanism, CO is able to suppress platelet activation or aggregation. Most interestingly, CO has also been reported to protect endothelial cells from undergoing apoptosis and to provide protection in models of lung injury, effects that are reported to be mediated *via* cGMPindependent pathways, such as activation of p38 MAPK. HO-1 induction is observed in a number of disease states, such as ischemic stroke or Alzheimer's disease. Evidence that the induction of HO-1 has therapeutic implications in the treatment of diseases associated with oxidative stress has been provided by various *in vitro* studies demonstrating a cytoprotective potential of this heat shock protein in models of lung injury, TNF induced apoptosis or ischemia/reperfusion injury. Recent studies in HO-deficient mice have further strengthened the important cytoprotective role of this protein. The mice lacked the ability to reuse iron and were

characterized by progressive anemia, tissue iron deposition, chronic inflammation, and delayed growth, as well as increased susceptibility to oxidative stress.

The structure of HO-1 with bound substrate, heme, has an R factor of 0.213 and free R factor of 0.265 at 2.08 Å resolution. There are two molecules of HO in the asymmetric unit, each with residues 10–223 resolved, along with a substrate heme in each enzyme molecule, and solvent. The first 9 and the last 10 residues of the 233-residue protein are not ordered in the electron density maps. The final model contains a total of 3,492 protein atoms, 86 heme atoms, 349 water atoms, and 1 non-water solvent, assigned as Cl^-. The fold of the conserved core of HO is novel when compared to known structures in the Protein Data Bank and is mostly a-helical. The heme is sandwiched between two helices termed the proximal and distal helices, with the heme d-*meso* edge and the



propionates exposed at the molecular surface (file:HO1.pse, F3, F4, F5). The electrostatic potential on the surface surrounding the exposed edge of the heme is positive. This provides a likely region for docking of the HO-1 electron donor, P450 reductase. The structure of P450 reductase29 shows a predominantly negatively charged surface. A recent crystal structure of P450BM-3 in a complex with the FMN domain of P450BM-3 reductase30 shows that binding of the FMN domain occurs on the electropositive surface surrounding the proximal face of the heme. The electropositive surface of HO-1 surrounding the exposed heme edge would be an ideal surface for the P450 FMN reductase domain to bind and also would provide the shortest route of electron transfer from FMN to the HO-1 heme.

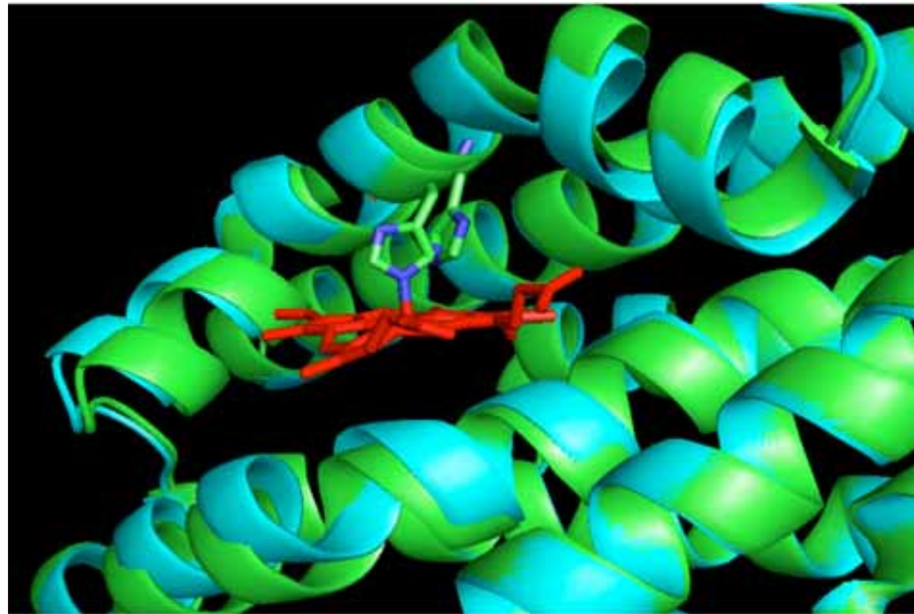
The heme pocket is formed mostly by two helices termed the proximal and distal helices. The proximal helix provides the His 25 heme ligand, along with heme contact residues Ala 28 and Glu 29, while Thr 21 contacts the heme through a water molecule. Phe 207 is also on the proximal side of the heme but is provided by a separate helix. Glu 29, underneath pyrrole A, is close enough to form an H-bond with His 25 (2.67 Å).

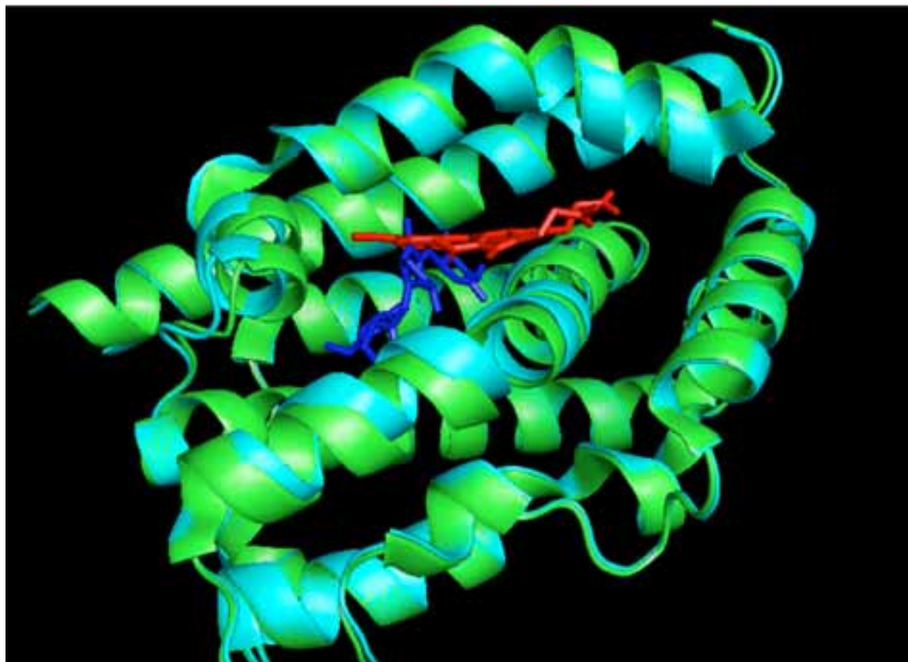
The distal helix sits over the opposite face of the heme where ligands bind and is kinked by about 50° directly over the heme Glycine residues in the highly conserved sequence Gly 139 Asp-Leu-Ser-Gly-Gly provide the required flexibility and also allow

the helix to approach closer to the heme than in true hemoproteins such as the globins and peroxidases. As a result, backbone atoms of Gly 139 and Gly 143 directly contact the heme. Other distal residues in contact with the heme include Tyr 134, Thr 135, Arg 136, Ser 142, and Leu 147, all supplied by the kinked distal helix.

In HO1.pse file F3, F4, and F5 shows the HEME contacts with HO1 protein.
In 1NI6.pse file F3 and F6 shows the alignment of 1N45 (with HEME) and 1NI6 (without HEME)
In 1S8C.pse file F2 shows the alignment of 1N45 (with HEME) and 1S8C (with biliverdin)

Here is the comparison of HO with substrate HEME and without substrate HEME (file 1NI6.pse F3 and F6). The distal and proximal alpha helices get closer when substrate HEME binds to HO1 (cyan: 1N45, green: 1NI6).





Comparison of HO1 with substrate HEME and with substrate biliverdin shows that upon iron release again proximal and distal helices get further away from each other (green: 1S8C, cyan: 1N45).

REFERENCES:

- David J. Schuller, Angela Wilks, Paul R. Ortiz de Montellano & Thomas L. Poulos *Crystal structure of human heme oxygenase-1. Nature Struct. Biol.* 6, 9, 1999
- Latesh Lad, David J. Schuller, Hideaki Shimizu, Jonathan Friedman, Huiying Li, Paul R. Ortiz de Montellano, and Thomas L. Poulos. Comparison of the Heme-free and -bound Crystal Structures of Human Heme Oxygenase-1. J. Biol. Chem. 278: 7834-7843.
- Characterization of heme oxygenase-1 induction in human endothelial cells, N. Bildner, Berlin, 2002
- D.J. Slebos, S.W. Ryter, A.MK. Choi. Heme oxygenase-1 and carbon monoxide in pulmonary medicine, Respiratory Research, 4, 7, 2003
- Crystal Structure of Human Heme Oxygenase-1 in a Complex with Biliverdin, Lad, L.; Friedman, J.; Li, H.; Bhaskar, B.; Ortiz de Montellano, P. R.; Poulos, T. L.; Biochemistry; 2004; 43(13); 3793-3801