Oxygen-Atom-Transfer Reactions: Fe

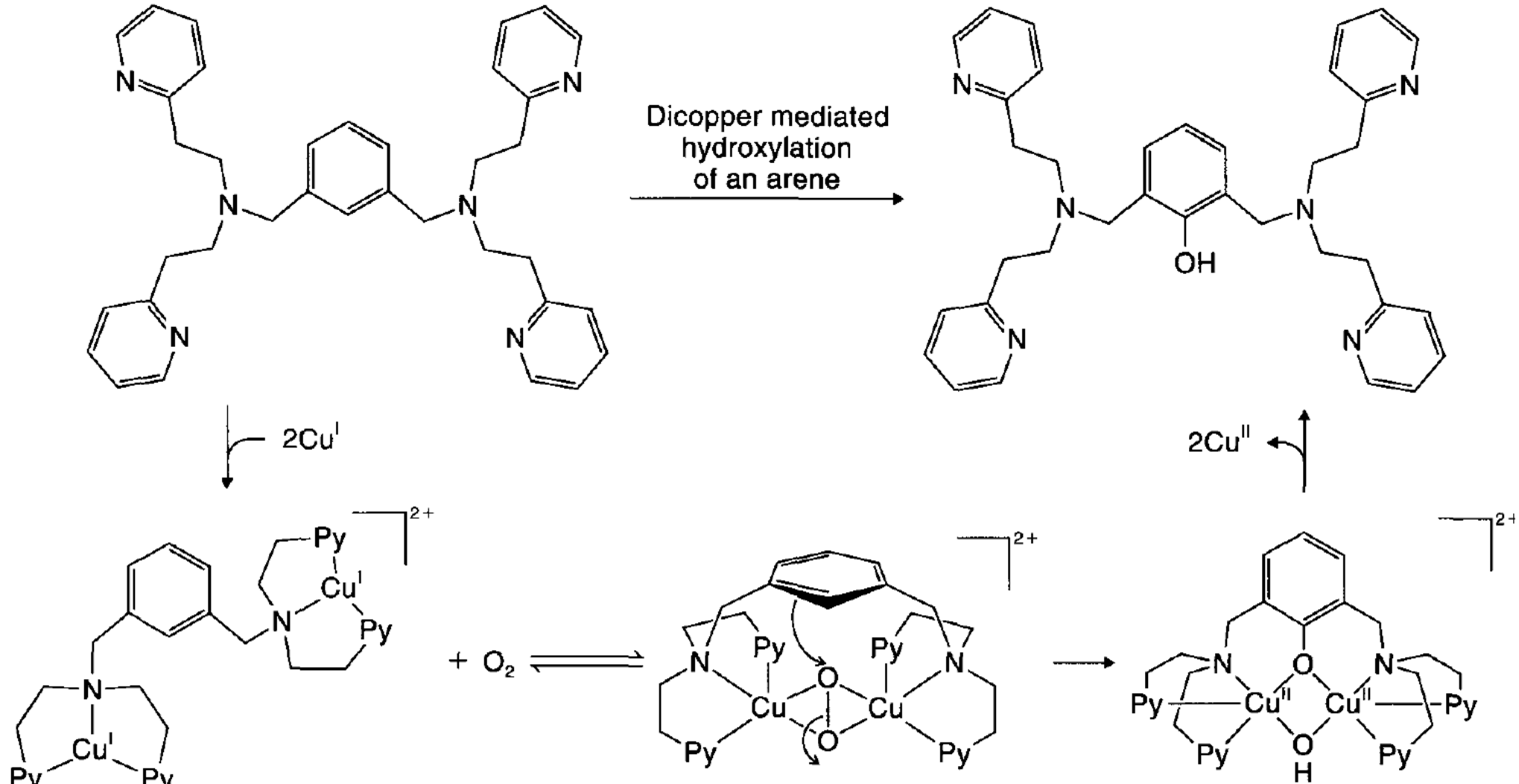
Chemical reactions in which an **oxygen atom** is transferred to a **substrate molecule**

Most common reagent for achieving this chemistry => O_2

Those reactions in which oxygen atoms are inserted into C-H or C-C bonds fall into two categories:

- **Two atoms** of the dioxigen molecule is transferred (**dioxygenases**)
- **Only one atom** is transferred (**monooxygenases**)

Metal center serves as an activator, rather than reversible carrier, of dioxygen.



Monooxygenase activity observed in a synthetic model system.

Cytochrome P-450.

Enzyme firstly isolated from rat-liver microsomes in 1958.

From its **optical spectroscopic properties**, the protein was known to be a cytochrome, literally, a "cellular pigment,"
-now synonymous with heme proteins-

The Soret band of the ferrous-CO cytochrome derivative did not have its usual absorption maximum at 420 nm; instead, the band appeared at 450 nm

→ “cytochrome P-450 (CYP450)“

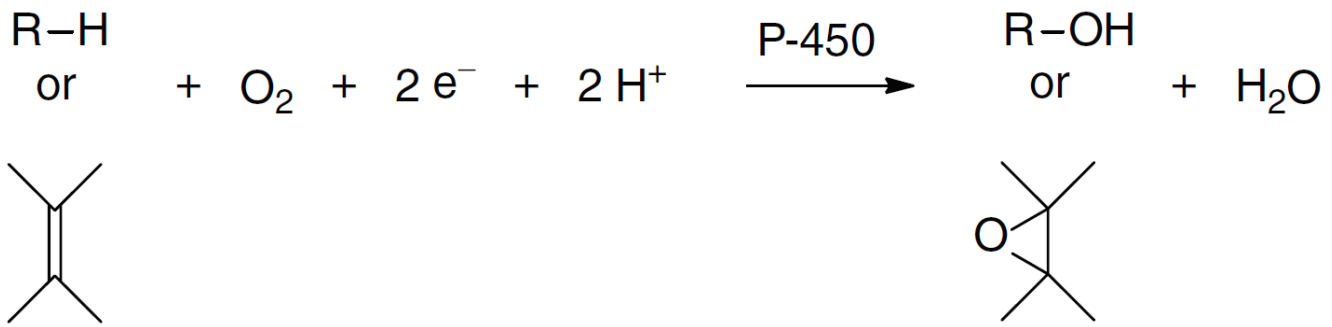
Enzymes of the CYP450 family catalyze the oxidation of several organic substrates;

Processes involved in **biosynthesis**, **metabolism**, the **detoxification** of harmful substances, and, in some cases, the **inadvertent generation** of highly active **carcinogens**.

Similarity with other metal-catalyzed processes:
catalysis involves the **controlled reaction of two ligands in the coordination sphere of the metal.**

The enzyme thus functions both as

- a substrate-selective "template" /spatially defined orientation: stereospecificity
- an electronic activator, providing a new reaction pathway with lower activation energy



Also:

$R_3N \longrightarrow R_3NO$

$R_2S \longrightarrow R_2SO$

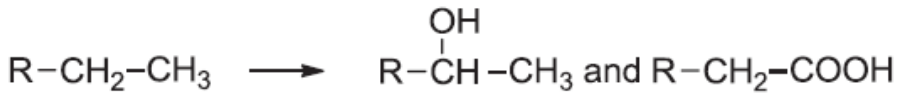
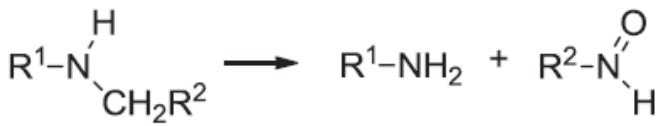
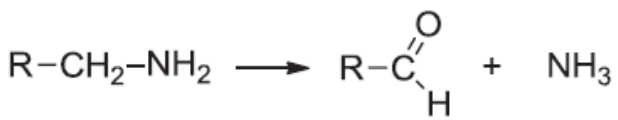
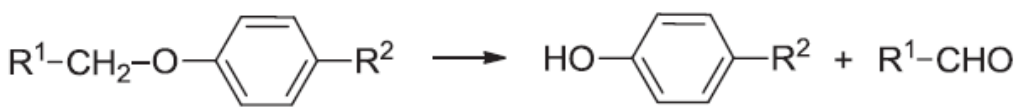
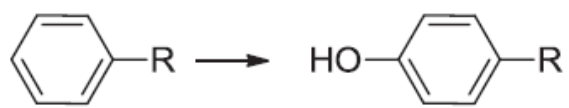
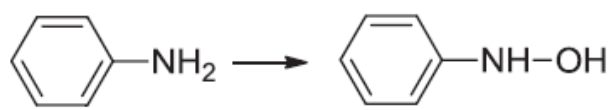
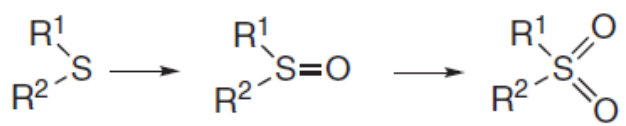
P-450-dependent monooxygenases are typical detoxification enzymes of the human liver

⬇

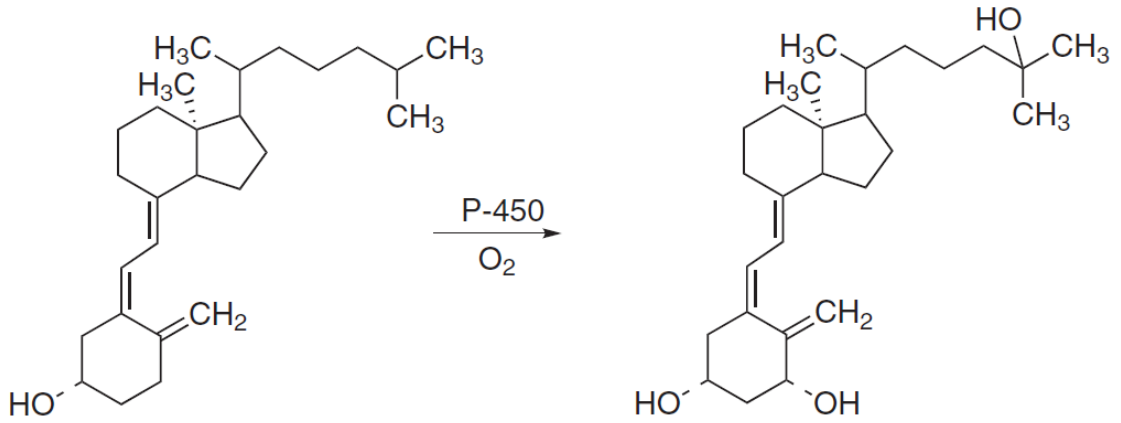
little substrate selectivity

Fatty acids, amino acids and hormones (steroids such as the estrogenic and androgenic sex hormones or prostaglandines) are metabolized by more **specific P-450 systems** in a stereospecific fashion.

Examples of the oxidative metabolism of pharmaceuticals by P-450 monooxygenation

Reaction type	Equation	Examples
oxidation of aliphatic chains		barbiturates
oxidative N-dealkylation		ephedrine
oxidative deamination		histamine norepinephrine mescaline
oxidative O-de-alkylation		phenacetin codein mescaline
para-hydroxylation of aromatic compounds		phenobarbital chlorpromazine
oxidation of aromatic amines		aniline derivatives
S-oxidation		phenothiazine

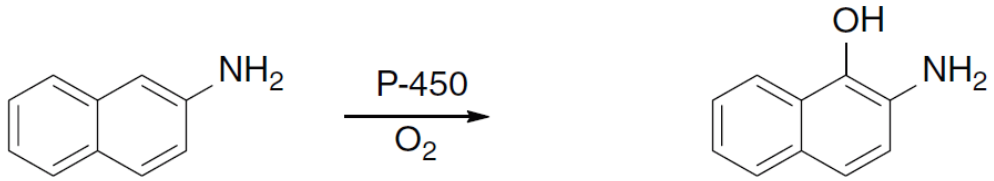
monooxygenation reactions of calciferols (vitamin D group) to active 1,25-dihydroxycalciferols



colecalciferol (vitamin D_3)

1,25-dihydroxy-colecalciferol

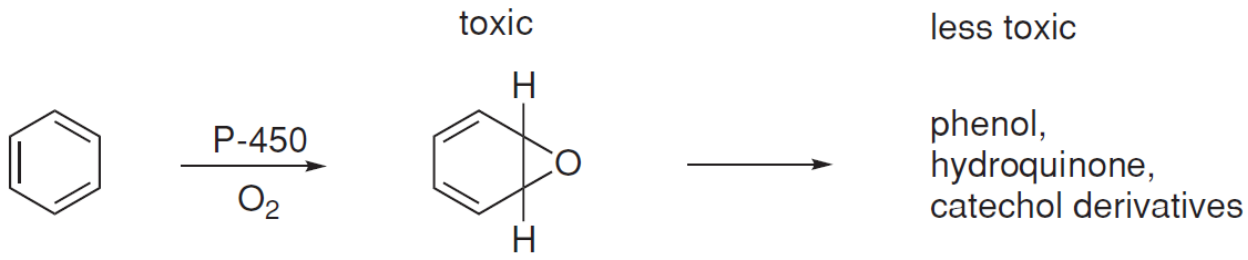
naphtylamine to the carcinogenic α-hydroxy-β-aminonaphthalene

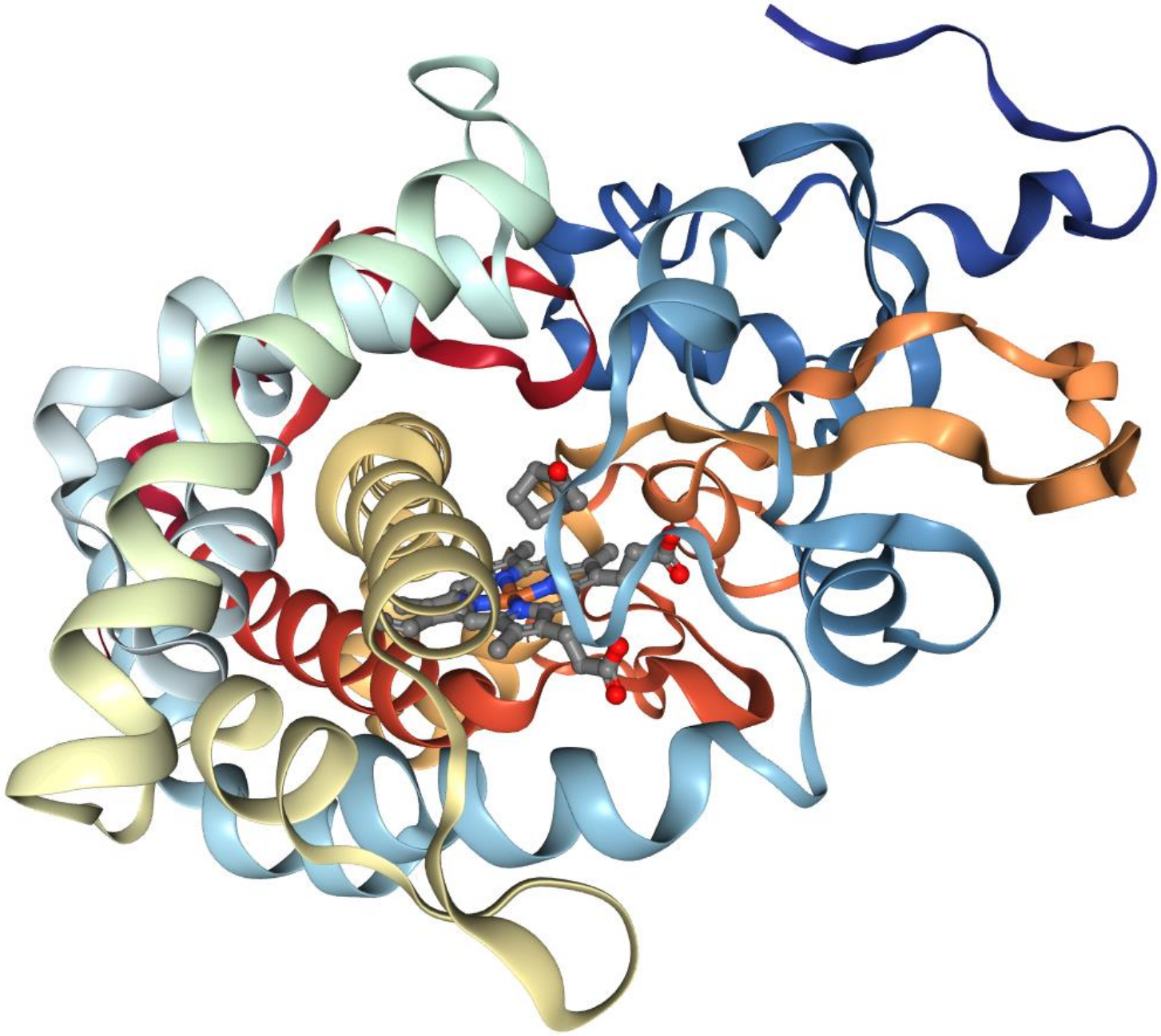


β-naphthylamine

α-hydroxy-β-aminonaphthalene (carcinogenic)

In the absence of oxidizable aliphatic side chains, the cytochrome P-450 enzymes catalyze the epoxidation of benzene or benzo[*a*]pyrene to yield mutagenic derivatives



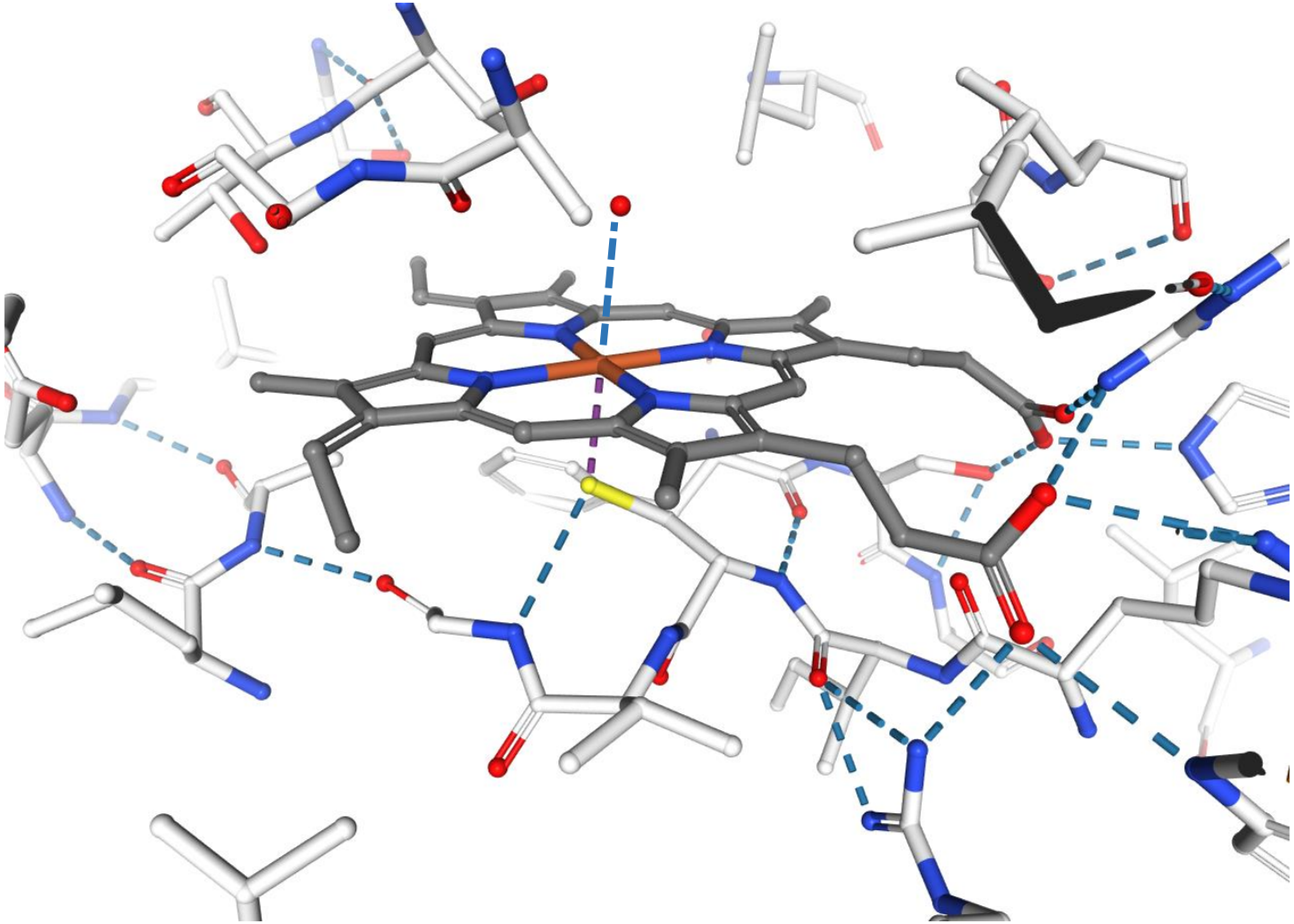


CYTOCHROME $P450_{CAM}$
PDB = 7CPP

Enzyme from ***Pseudomonas*** **putida.**

This soluble bacterial enzyme that hydroxylates camphor was the first P-450 to be purified and to have its crystal structure determined.

PDB = 1OG2
Structure of human cytochrome P450 CYP2C9



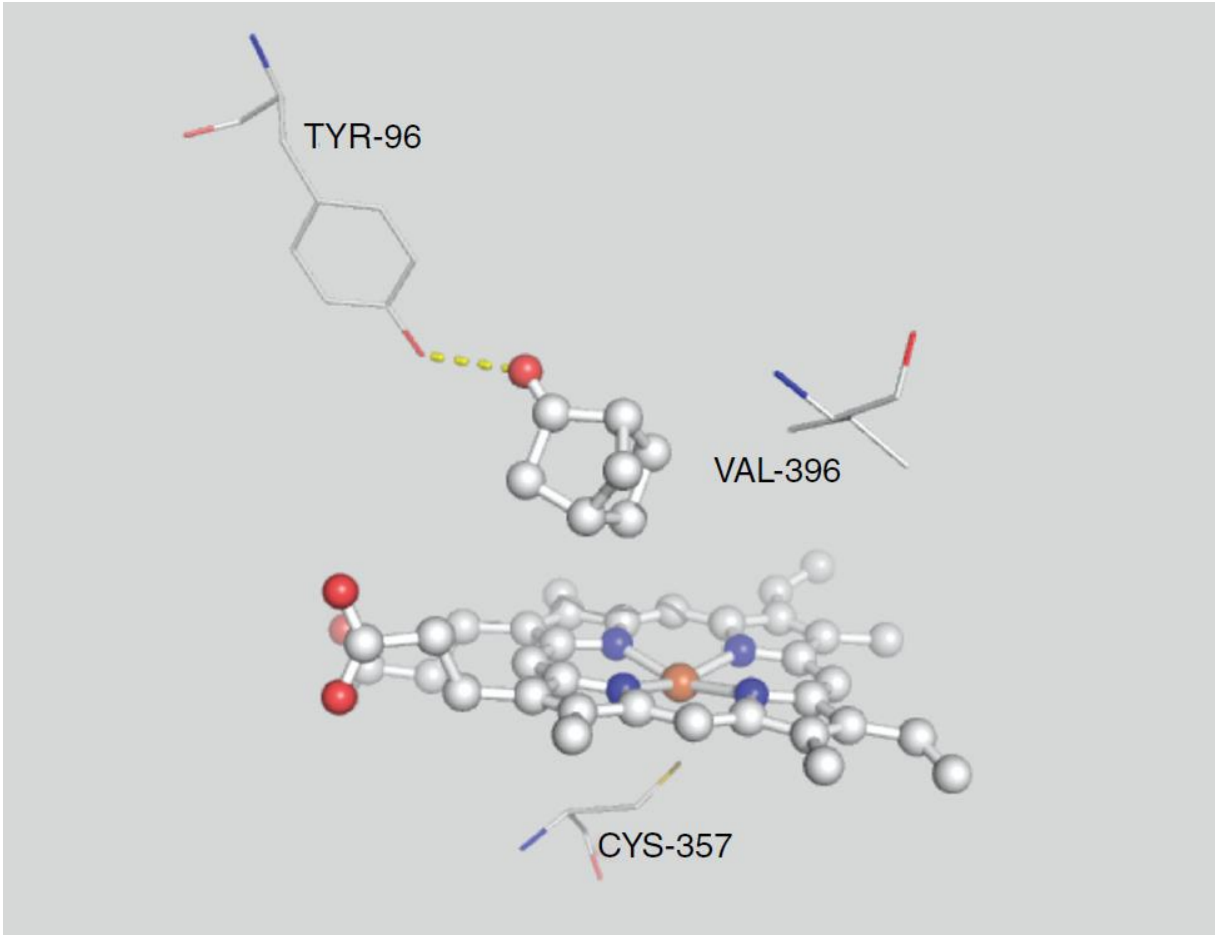
The activation of bound O_2 is influenced by the axial ligand: a cysteinate anion.

In contrast to π-accepting thioethers such as methionine, the **thiolates** are strong σ and π electron donors and **can stabilize high oxidation states** of metal centers.

Mechanism for dioxygen activation and monooxygen transfer

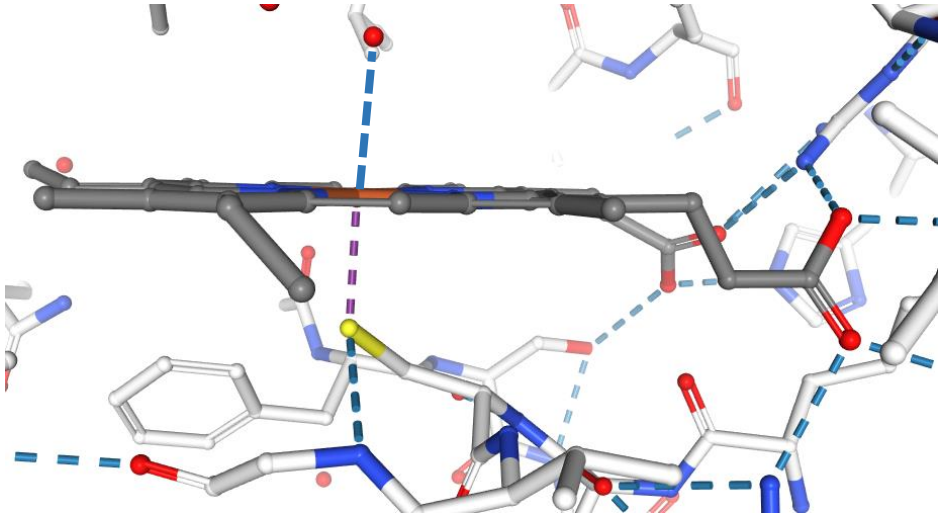
Everything starts from the predominantly low-spin iron(III) state **1** with six-coordinate metal (porphyrin, cysteinate, water)

Then the binding of the organic substrate (through hydrophobic and other interactions with the protein inside a cavity) closes to the axial coordination site of the heme system, thereby causing a transition to the high-spin iron(III) form **2**.



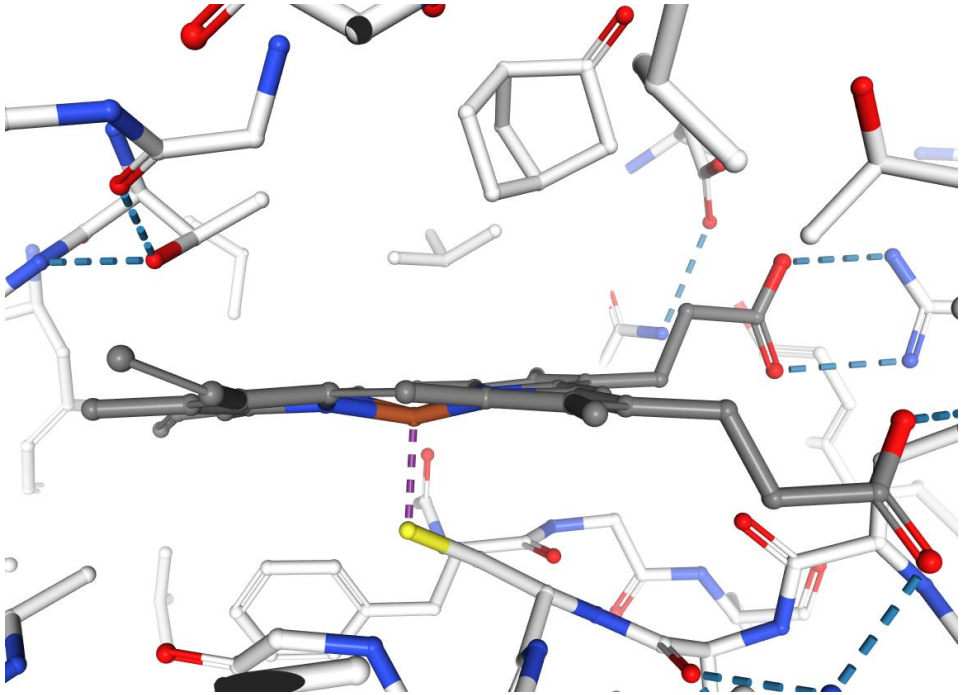
The loss of water generates an open coordination site

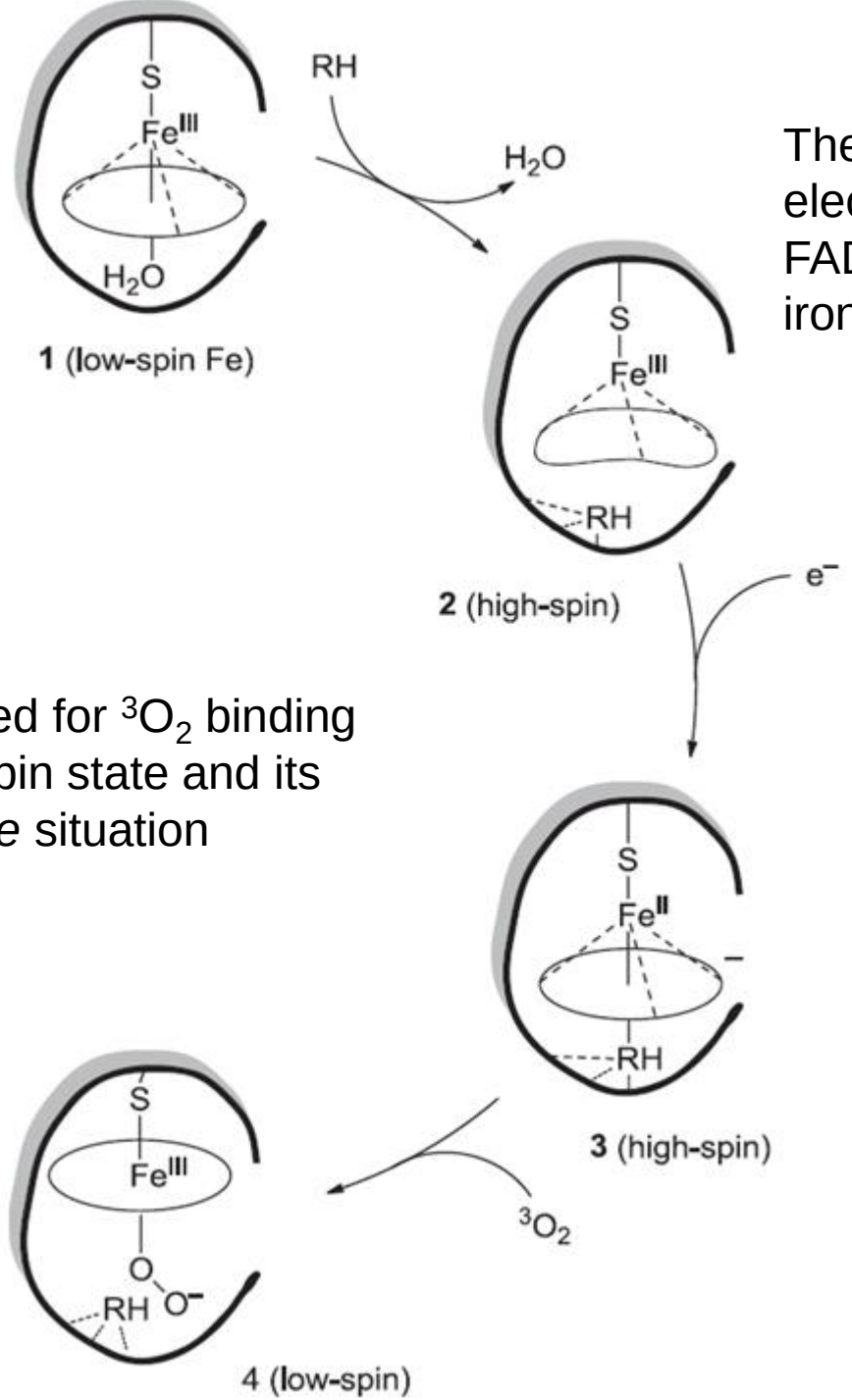
out-of-plane structure with a domed porphyrin ring



+ RH

- H_2O



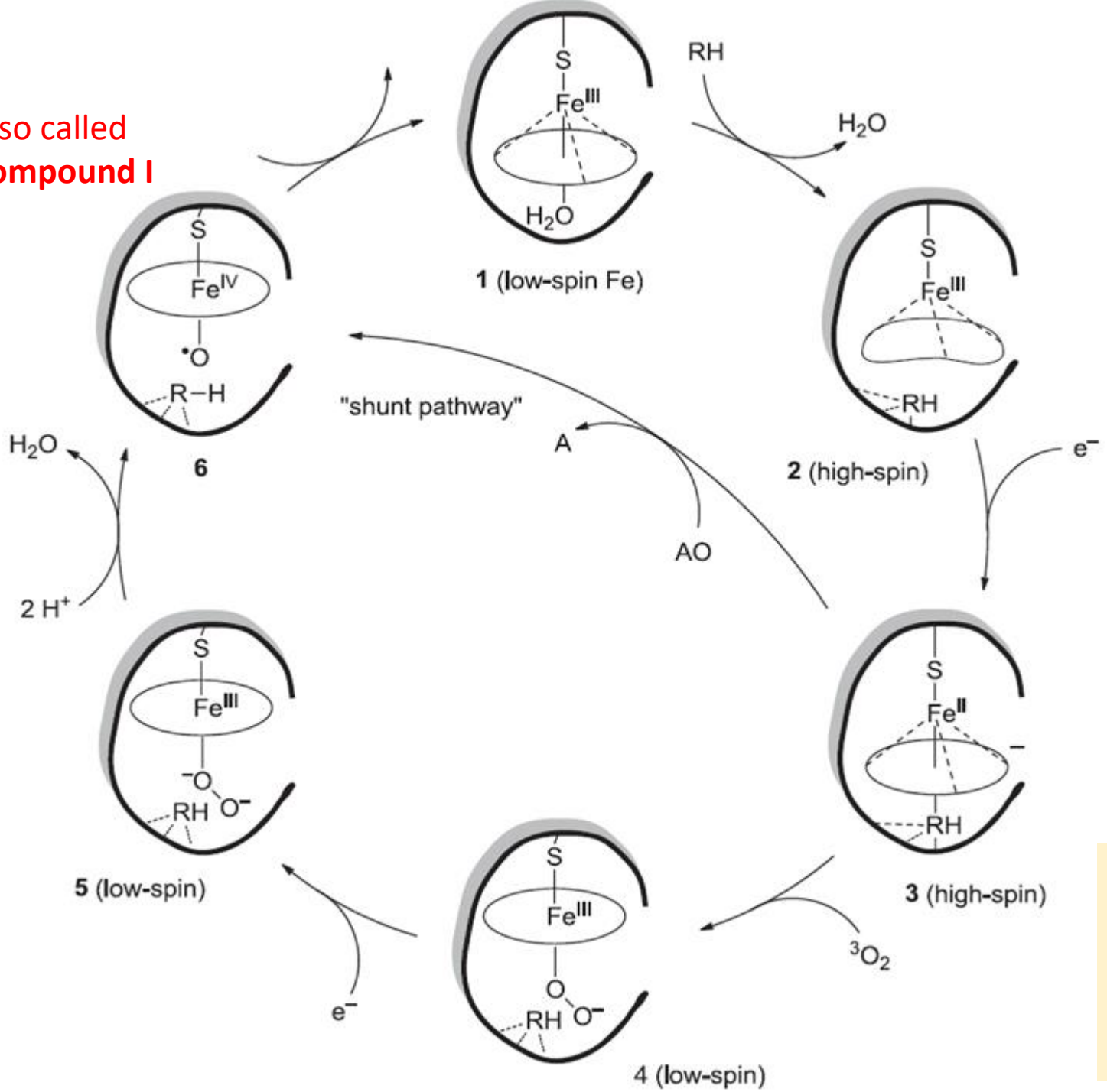


The next step is a one-electron reduction (via $FADH_2$) to give a high-spin iron(II) complex **3**

Complex **3** is predestined for $^3O_2$ binding because of its (S = 2) spin state and its pronounced *out-of-plane* situation

coordinatively saturated low-spin oxy form **4** with a hydrogen bond to a threonine side chain and a possible Fe(III)/$O_2^{\bullet-}$ oxidation state formulation

Also called
Compound I

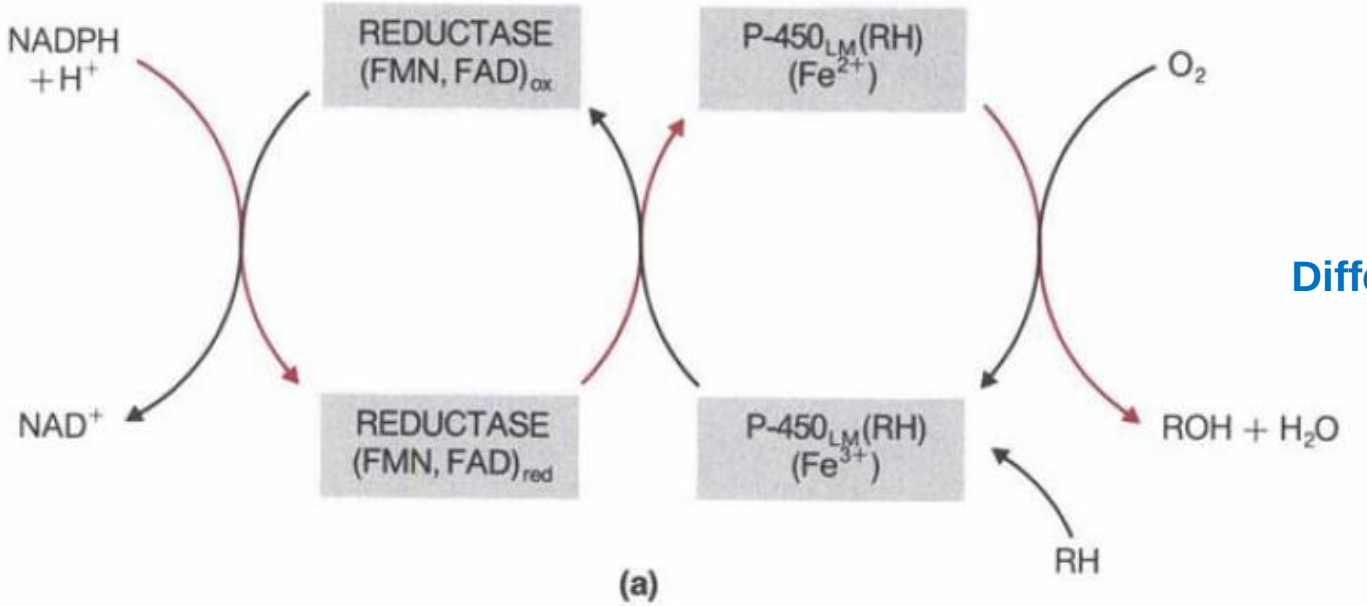


A second one-electron reduction forms a very labile low-spin peroxo iron(III) complex
5.

This species **adds two protons and releases one water molecule**, thereby cleaving the O–O bond.

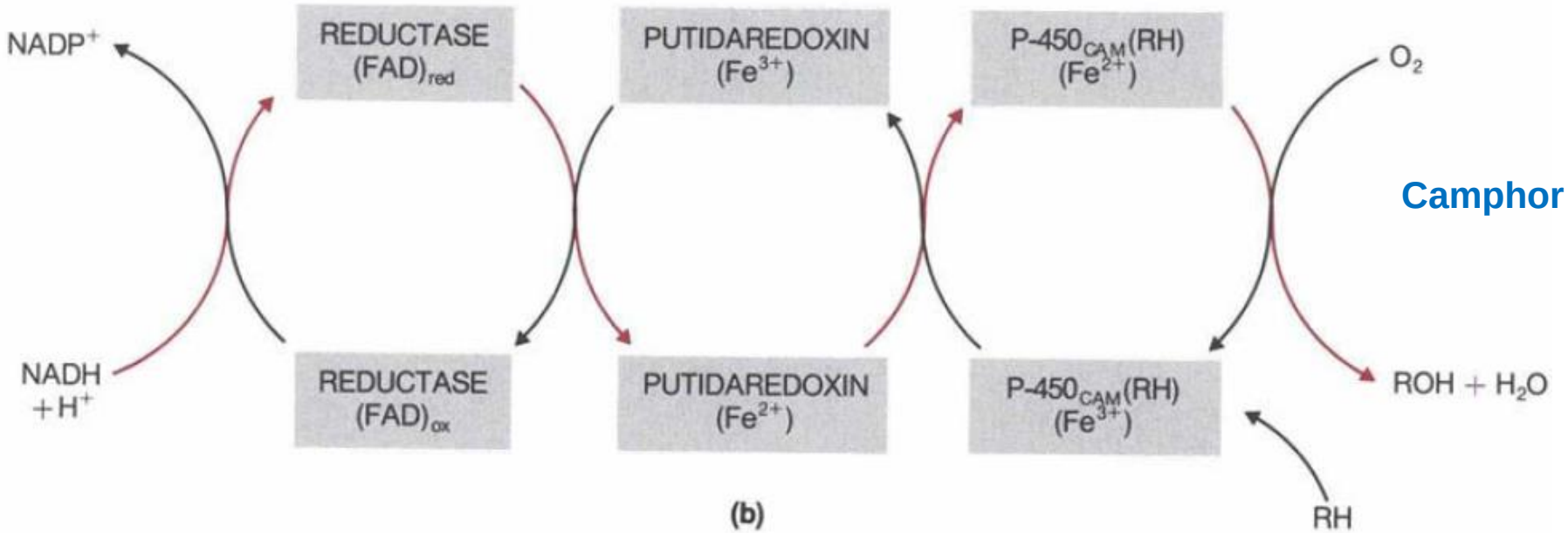
This cleavage requires two oxidation equivalents, which have to be made available intramolecularly; the result is a **reactive complex 6**, which can be formulated with $Fe(V)=O^{-II}$ or with $Fe(IV)\text{-}O^{\bullet -I}$

In the presence of external oxygenation agents, AO, such as peracids, iron(II) state **3** may directly yield compound **6**, the highly oxidized productive complex, via a “shunt” pathway.



(a)

Different substrates in liver microsomes

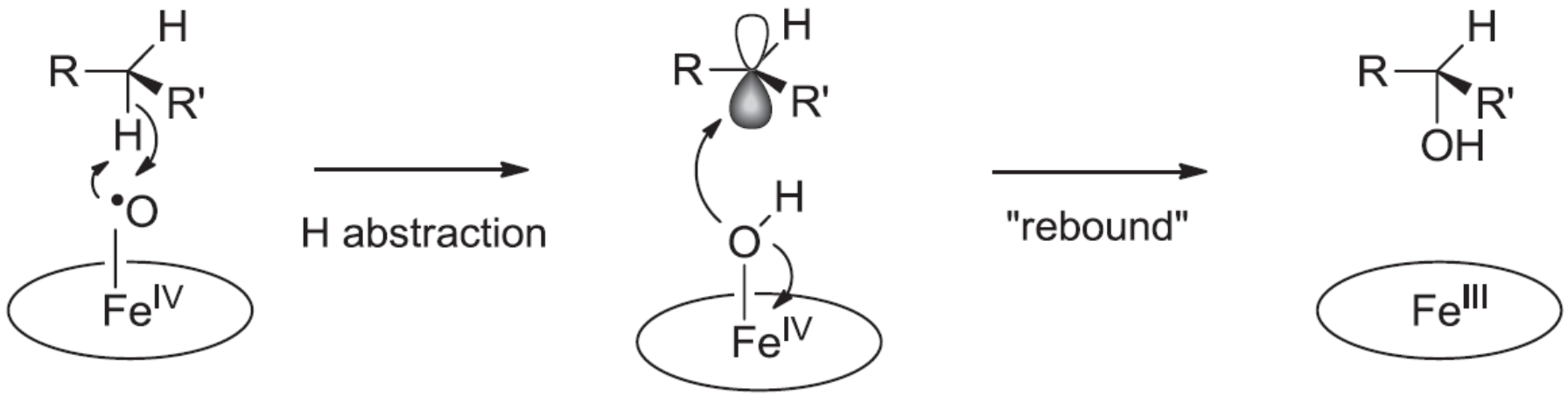


(b)

Camphor in a bacterial system

Reactive complex 6 collapses to yield the product and the initial state of the catalytic heme.

Reaction possibly involves **the transfer of a monooxygen ("oxyl") radical** to the substrate



Together these postulated steps have been termed a rebound mechanism. A value of 10^9 s^{-1} has been estimated for the radical recombination step, **fast enough that some radical rearrangements cannot compete with it**, for example, ring opening of cyclopropylmethyl radical (k ca 10^8 s^{-1}).

The high-valent oxo-iron center of the reactive heme group is stabilized by the electron-donating thiolate group, but it must also be regarded **in context with the surrounding porphyrin system**.

The porphyrin system may add or release single electrons to form radical ions.

For the highest oxidized states of the oxo-heme systems in peroxidases and in the P-450 enzymes a porphyrin radical anion, **$Por^{\bullet -}$ (S = 1/2) is coordinated to an oxoferryl(IV) fragment, $[Fe(IV)=O]^{2+}$, with tetravalent iron**.

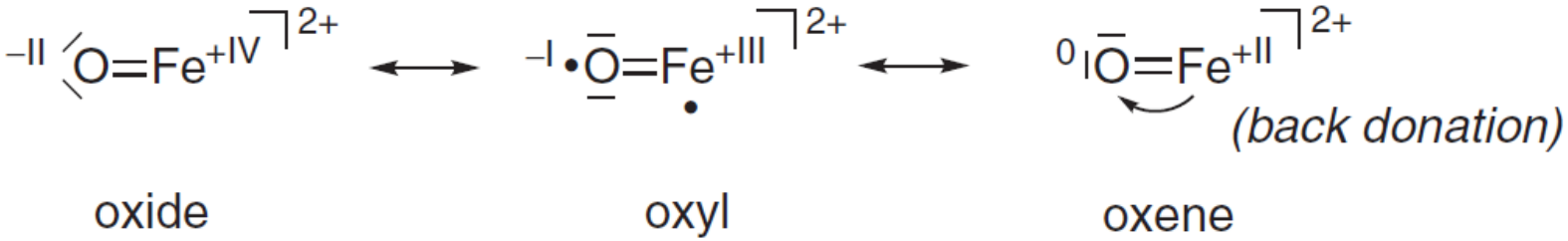
$$[Fe^{II}(Por^{2-})]^{0} \xrightarrow[-\ H_2O]{+\ O_2,\ 2\ H^+,\ e^-} [O^{-II}=Fe^{IV}(Por^{\bullet -})]^{\bullet +}$$

Even considering the highly activated nature of compound **6**, it remains surprising that
It is able to transfer the iron-bound oxygen atom to a substrate that is little or not at all activated

$[O^{-II}=Fe^{IV}(Por^{\bullet -})]^{\bullet +}$ implies that the coordinated monooxygen exists as oxide ligand O^{2-}.

However

a terminal monooxygen ligand can also be present as an oxyl radical anion ($O^{\bullet -}$), as the conjugated base of •OH or, in analogy with carbenes and nitrenes, even as neutral “oxene” ligand.



A **multitude** of conceivable **resonance structures**

x-ray spectroscopic and mechanistic results suggest that in many “oxo” complexes of transition metals, and particularly in the reactive state **6** of the P-450 system, the formulation with a **weakly bound radical oxygen atom** as reactive, **hydrogen-abstracting ligand** contributes significantly to the actual electronic structure.

“Genuine” oxide (O^{2-}) ligands, which might tolerate even organic ligands in the metal coordination sphere, are found mainly in metal complexes with extremely stabilized high-oxidation.

In the P-450 system, the **interactions of stabilizing cysteinate and reactivity-enhancing distal ligands** of the Fe–O(–O) moiety aid in controlling the reaction, particularly in **preventing an autoxygenation** alternative, which prevails in the case of the heme-degrading enzyme heme dioxygenase.

Methane Monooxygenase

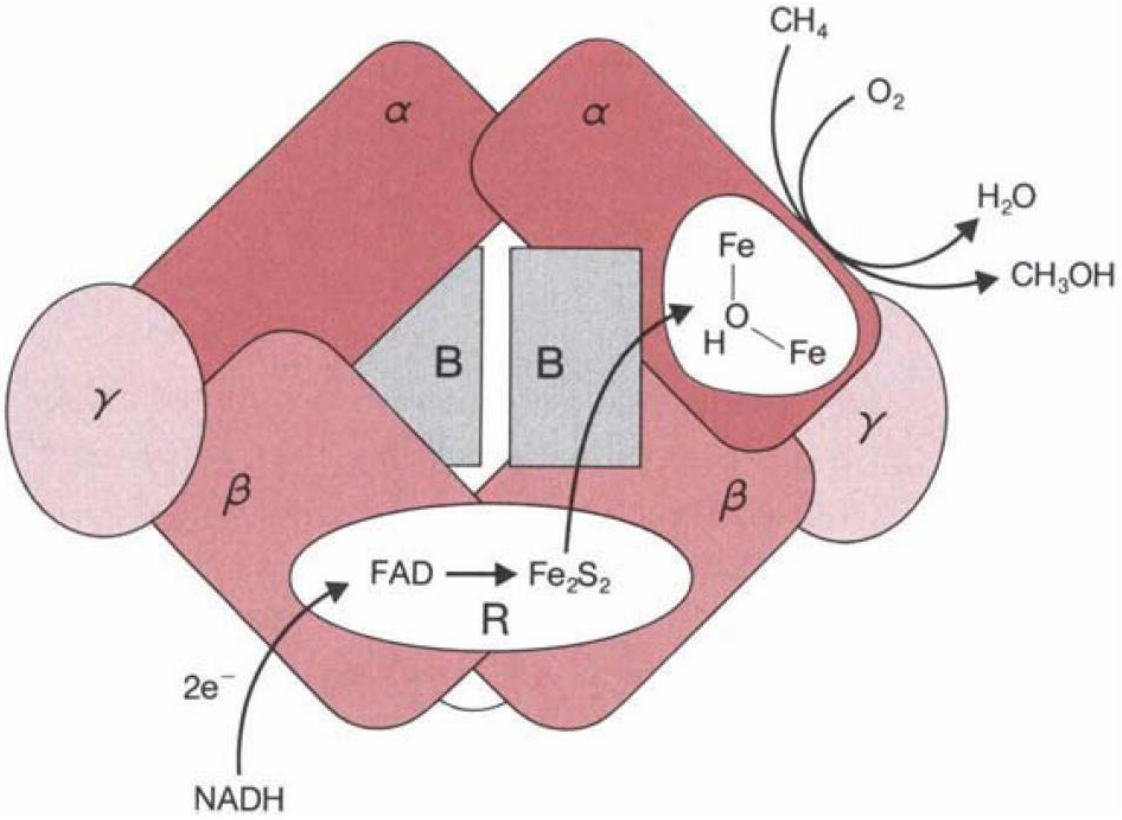
CH_4 ⟶ The most difficult hydrocarbon substrate
has a high C-H bond energy (104 kcal/ mol), no dipole moment, and no functionality to assist in binding to or reacting with a protein-active site.

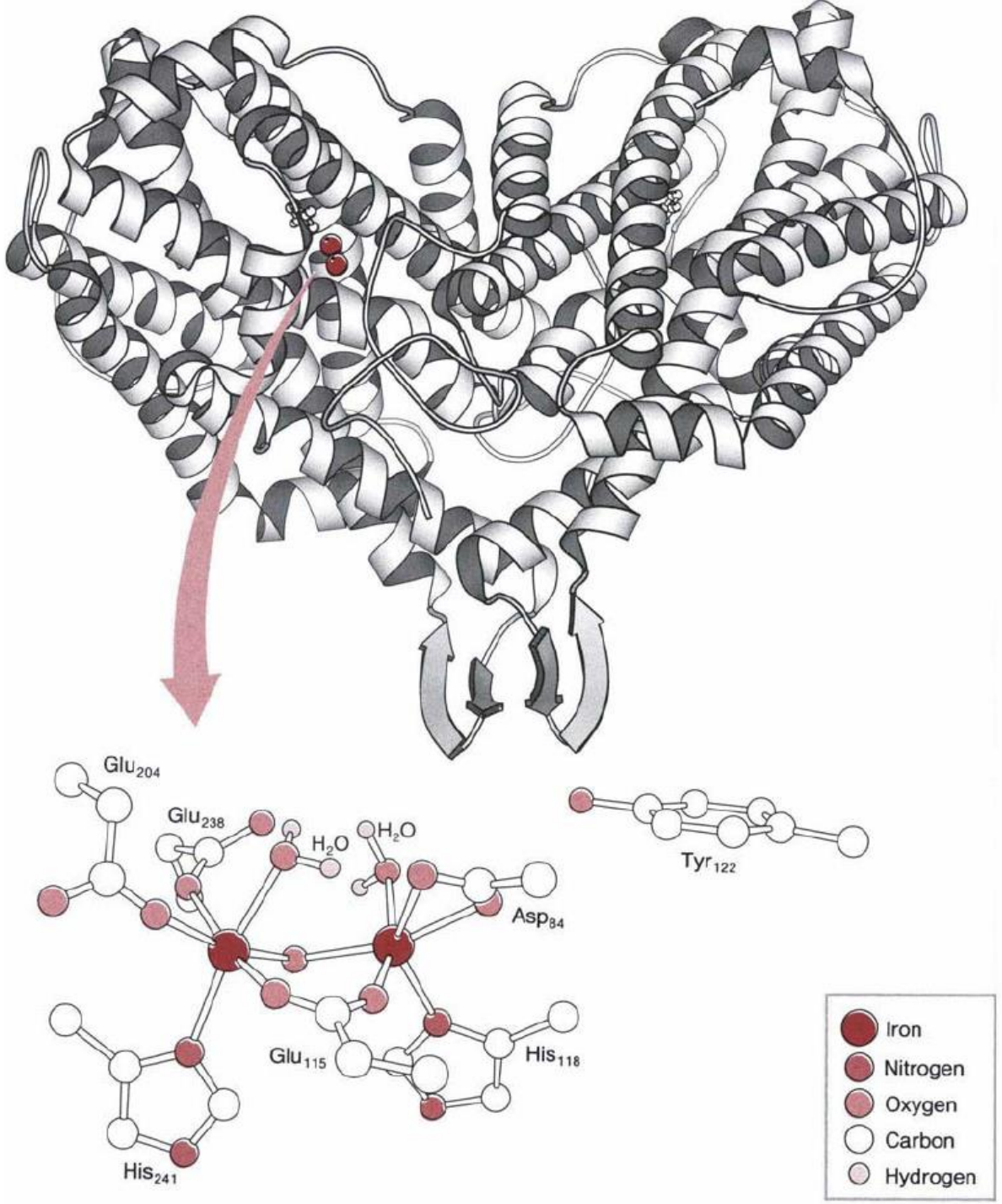
Cytochrome P-450 does not hydroxylate methane.

Who does?

Methane monooxygenase a multiprotein complex with a molecular mass of about 300 kDa, employed by methano*trophic* microorganisms which use CH_4 as source of carbon and energy

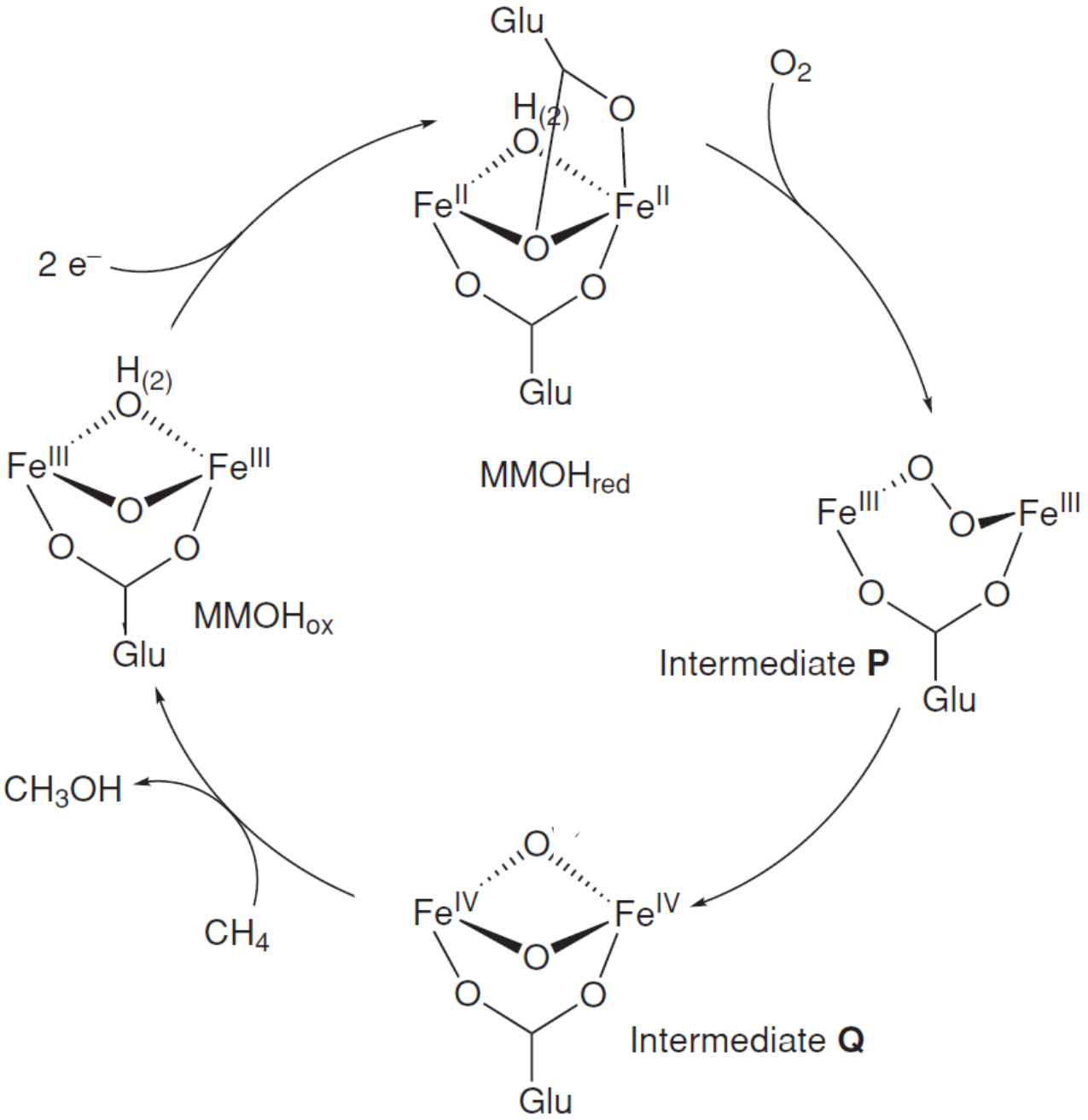
It **contains two diiron centers**, which have been characterized in several oxidation state combinations. Typical for the Fe(III)/Fe(III) form are the antiparallel spin–spin coupling of two high-spin Fe(III)





In accordance with spectroscopic data, a μ-hydroxo bridge has been found between the two coordinatively not fully saturated metal centers, in addition to a bidentate glutamate bridge (μ-η^1:η^1) and monodentate nonbridging glutamate and histidine ligands.

High-spin Fe(II)/Fe(II) form is active with respect to dioxygen activation.



Mechanism of reaction

It involves the formation of an **oxygenated dimetal center** with oxoferryl(IV) groups

it effects monooxygen insertion into the C–H bond of CH_4

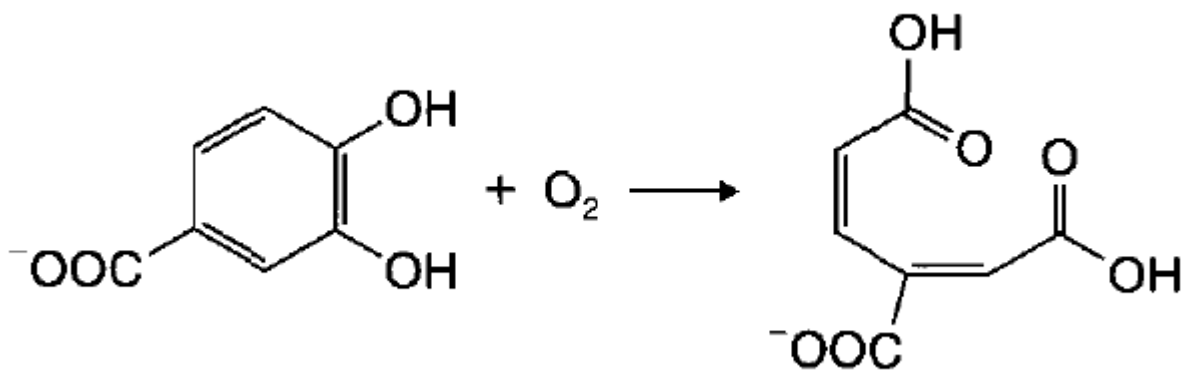
other hydrocarbons are also oxygenated with a certain stereospecificity.

Catechol and Other Dioxygenases.

Both cytochrome P-450 and MMO transfer only one atom of dioxygen to the alkane, the other being converted into water. This process requires energy in the form of reducing equivalents.

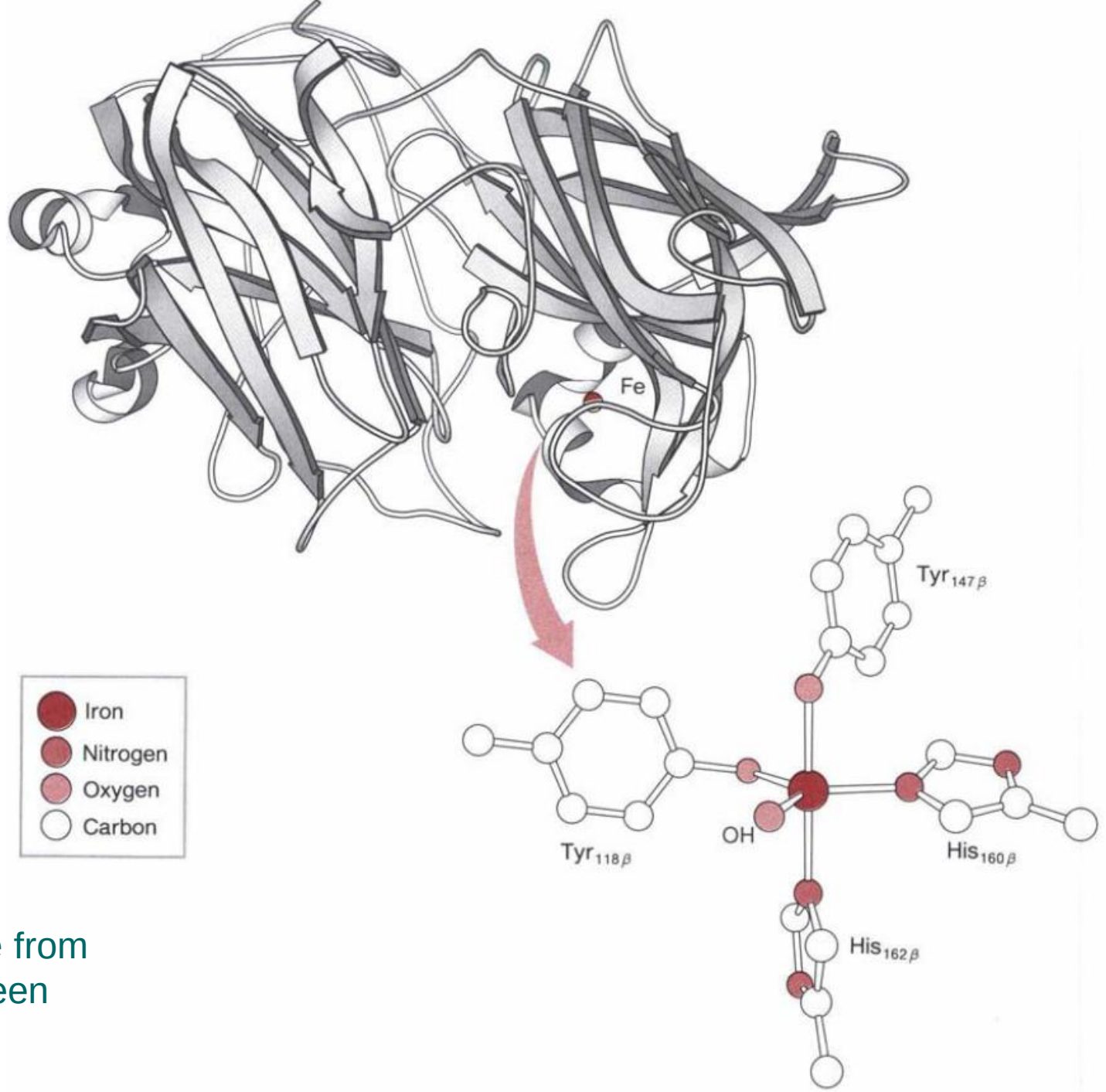
Dioxygenase enzymes, on the other hand, **incorporate both atoms of the O_2 molecule** into substrate.

The best-characterized enzyme of this class is protocatechuate 3,4- dioxygenase, which catalyzes the reaction:



In their oxidized state,
catechol 1,2-dioxygenases and related enzymes contain **high-spin iron(III) with tyrosinate ligands**

gives rise to a Ligand to Metal Charge transition in the visible spectrum.

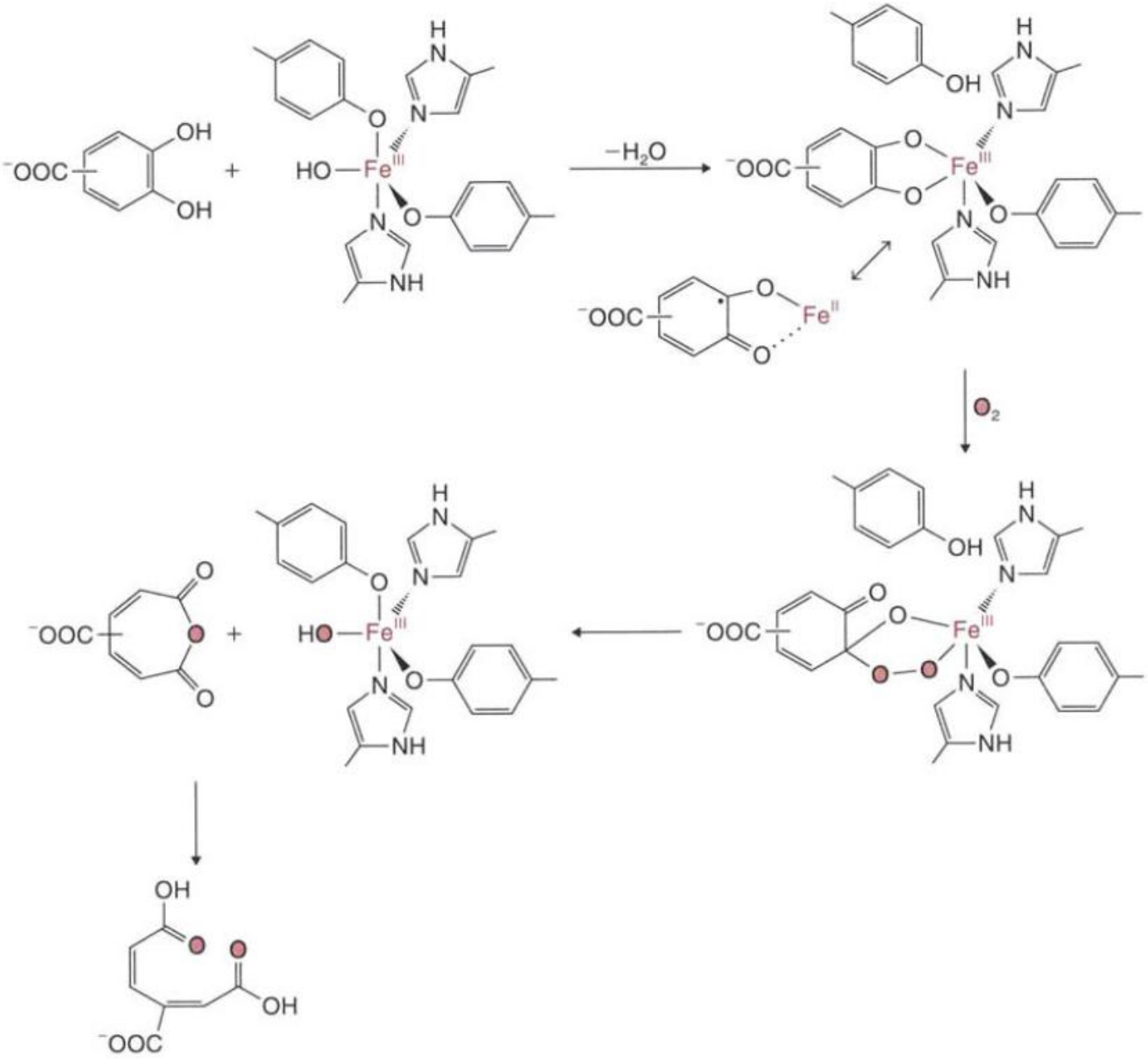


The crystal structure of protocatechuate 3,4-dioxygenase from *Pseudomonas aeroginosa* has been determined to 2.8 A resolution

Mechanistic hypothesis:

the binding of electron-rich catecholate to the electrondeficient and Lewis acidic high-spin Fe(III) center results in a **ligand-to-metal electron transfer** and corresponding weakening of the intradiol (O)CC(O)-bond of the new o-semiquinone radical.

$^3O_2$ activation may then proceed in a spin-allowed fashion via high-spin FeII before **the final product is formed through peroxidic intermediates**



Peroxidases: Detoxification and Utilization of Doubly Reduced Dioxygen

Heme-containing peroxidases and catalases are enzymes that are related to cytochrome P-450

also manganese-, vanadium- and selenium-containing peroxidases, as well as manganese-dependent catalases.

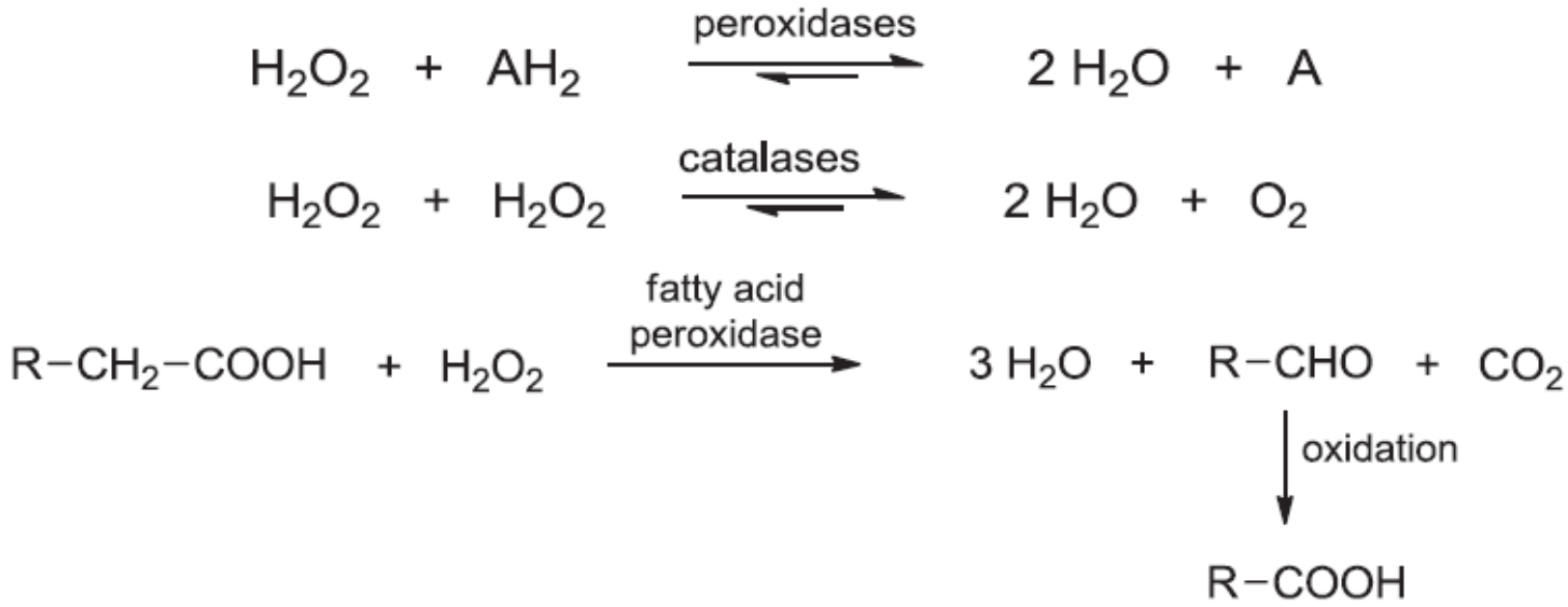
Peroxidases use the doubly reduced peroxidic form of O_2 to oxidize substrates of the type AH_2 to radical cations and their reaction products

Formation of peroxides is not a trivial problem

The peroxide oxidation state of dioxygen can be produced as an undesired intermediate in the course of photosynthetic water oxidation or via incomplete oxygen reduction during respiration (only about 80% of the dioxygen taken up by breathing is *completely* reduced).

Peroxidases are partly detoxification enzymes.

This is especially true for the catalases, since their second substrate is also hydrogen peroxide; overall, the resulting reaction is the enzymatically catalyzed disproportionation of metastable H_2O_2, the equilibrium constant being about 10^{36}.



There are numerous not easily oxidized compounds, such as fatty acids, amines, phenols, chloride and xenobiotic substances (toxins), that can serve as substrates for peroxidases.

Examples:

controlled oxidations of fatty acids during plant growth yield an α carbonyl carboxylic acid intermediate, which loses CO_2 (decarboxylation) to form an aldehyde with one less CH2 group; its oxidation product is the correspondingly shorter fatty acid

the coupling of tyrosines and their iodination to the thyroid hormones by thyreoperoxidases

the oxidation of cytochrome *c* by cytochrome *c* peroxidase (CCP)

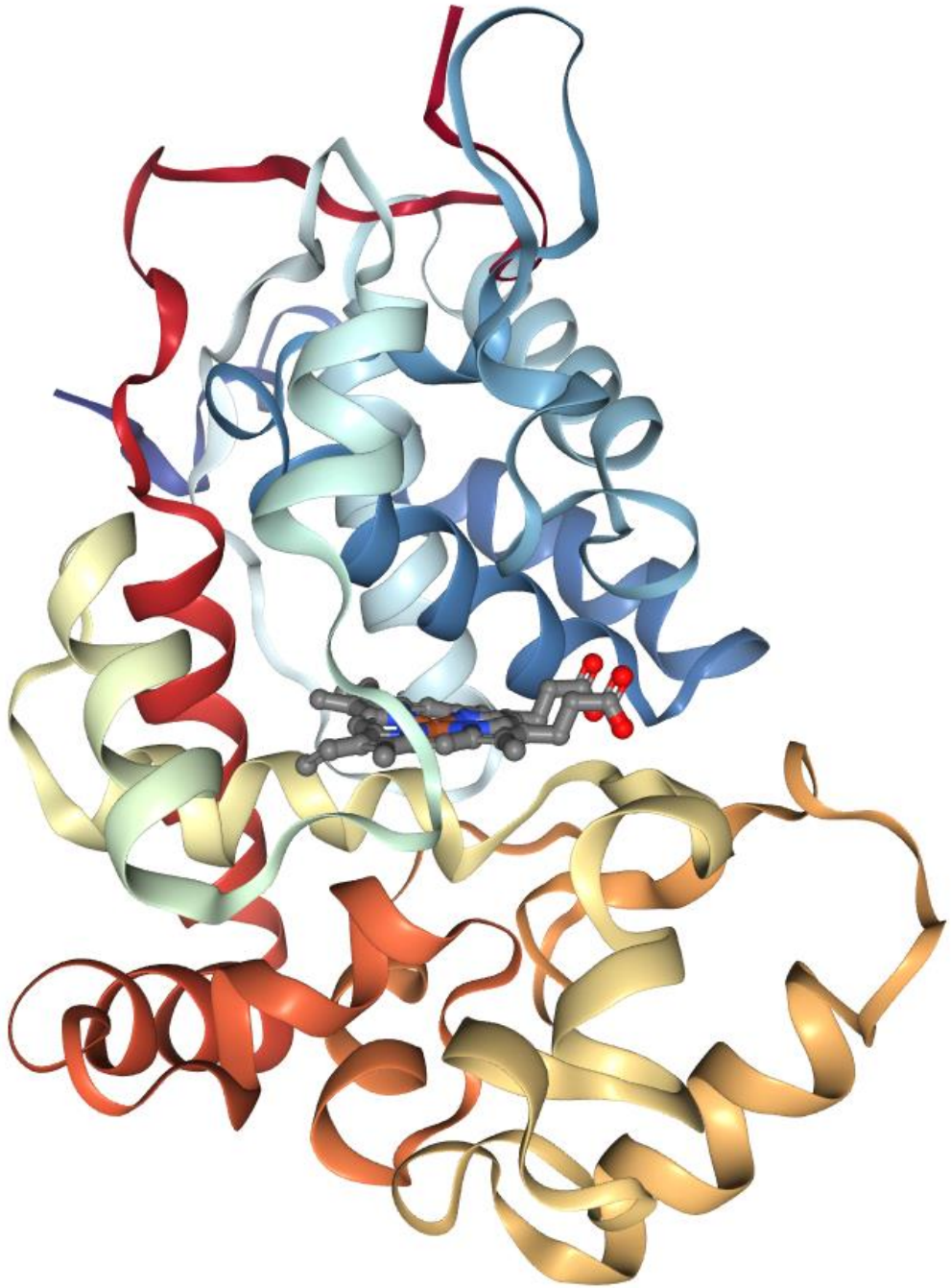
the oxidation of chloride to bactericidal hypochlorite by myeloperoxidase with cysteinate-coordinated iron

the oxidative degradation of lignin from wood by lignin peroxidase

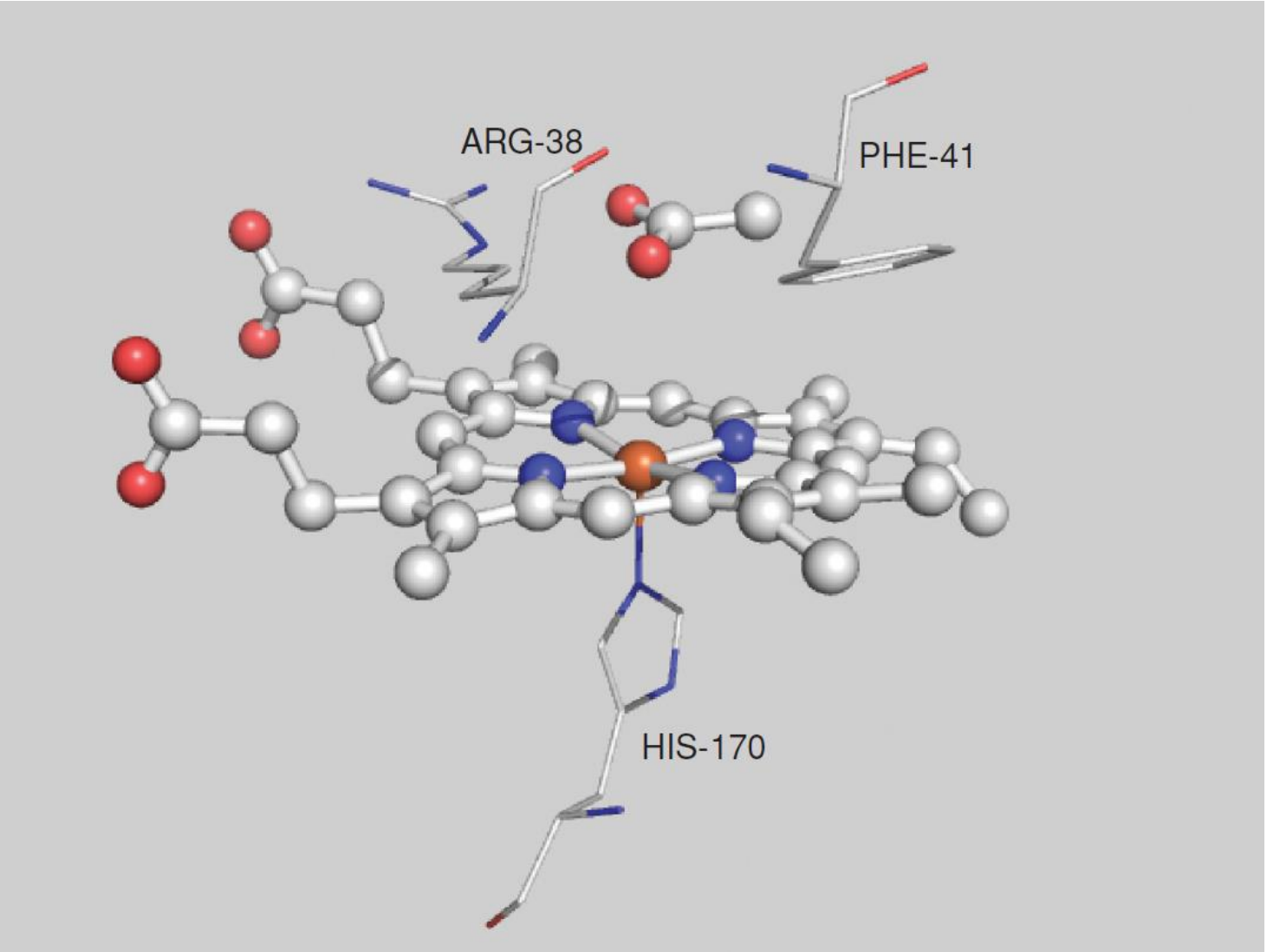
The most thoroughly studied enzyme in the peroxidase group is horseradish peroxidase (HRP), which has been investigated since the end of the 19th century

low molecular mass of ~40 kDa

Distinguished from the classical heme-containing catalases, which are associated proteins (tetramers) with total masses of 260 kDa and partially tyrosine-coordinated heme iron.

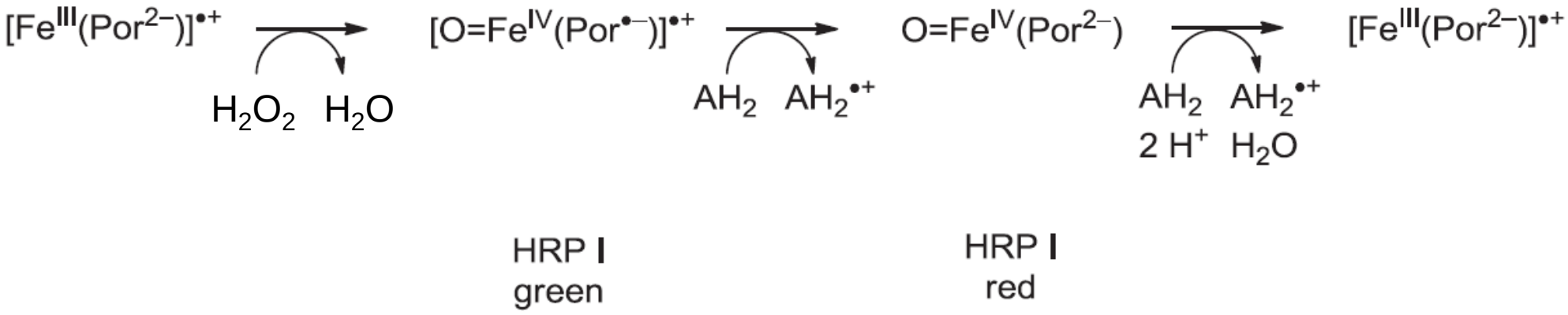


Under physiological conditions, the **resting state** of most heme peroxidases contains **high-spin iron(III)** (*S* = 5/2, half-filled d shell, *out-ofplane* structure) and, in contrast to the P-450 system, **an imidazole base from histidine as axial ligand**

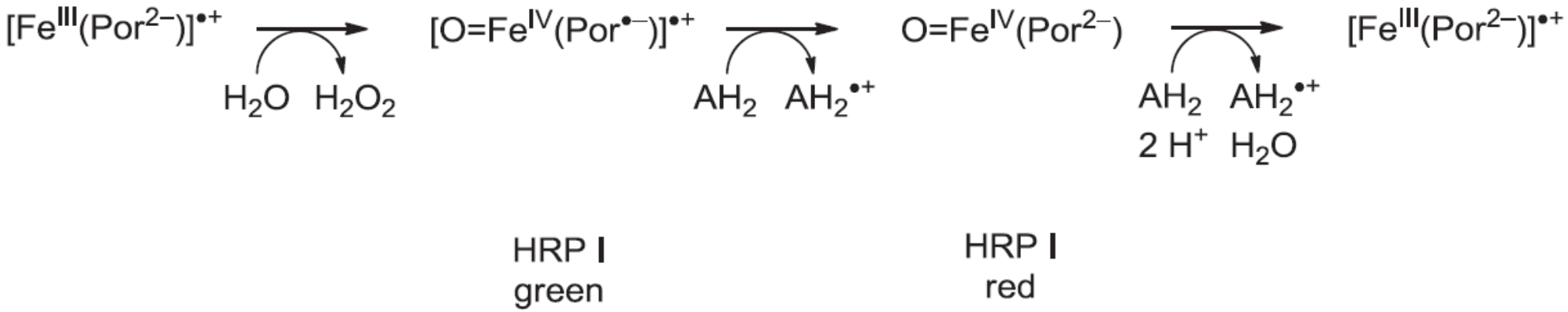


Protein-induced internally **monooxygen transfer from H_2O_2 to the iron center** under **release of water** adds two oxidation equivalents and leads formally to an "**iron(V)**" species
-most probably involving a dicationic oxoferryl(IV) center with a coordinated porphyrin radical -

This **very electron-deficient cationic intermediate** ("HRP I", E_0 >1 V) can react with the substrate in a **one-electron oxidation step**



The resulting second enzymatic intermediate shows only ***one*** **more oxidation equivalent** than the resting state but can still **undergo a second one-electron oxidation reaction**; according to physical measurements, this “HRP II” state contains an oxoferryl(IV) center (*S*=1) coordinated to a normal (i.e. dianionic) porphyrin ligand



Catalase enzymes

Catalase enzymes have evolved to effect the disproportionation of hydrogen peroxide

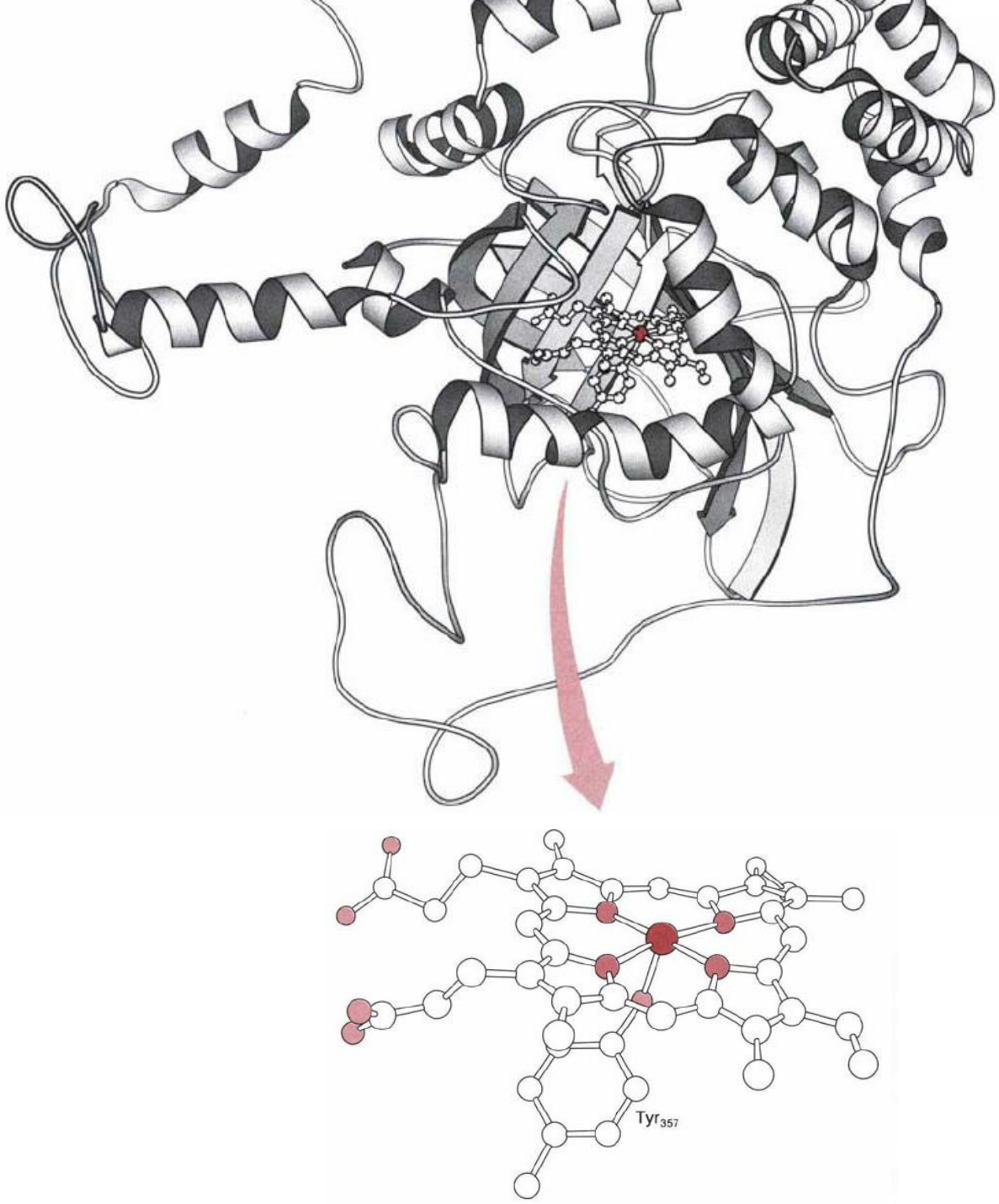
Catalases are multisubunit enzymes of M, 250,000 daltons that contain a heme group at their active site.

The structure of catalase from bovine liver has been determined crystallographically.

Coordinated to one of the axial positions is the deprotonated **phenolic oxygen atom of Tyr 357, a unique feature among heme proteins**.

The other **axial position is free to bind substrate**.

Situated in this distal site is the phenyl ring of Phe 160, which is parallel to and stacked on the plane of one of the porphyrin pyrrole rings, and hydrogen-bonding components His 74 and Asn 174, which are essential residues for the catalytic function of the enzyme



In the first step, the **substrate is reduced to water with concomitant oxidation of the enzyme**. The product of this oxidation, **Compound I**, has a heme redox level that is the same as that in the oxidized state of cytochrome P-450.

Compound I from catalase can oxidize formate, nitrite, and ethanol as well as hydrogen peroxide.

Oxidation of H_2O_2, to form dioxygen and water completes the catalytic cycle.

$$EFe^{III} + H_2O_2 \rightleftharpoons \text{Compound I} + H_2O$$

$$\text{Compound I} + H_2O_2 \rightleftharpoons EFe^{III} + H_2O + O_2$$

Controlling the Reaction Mechanism of the Oxyheme Group

Cytochrome P-450 and the heme peroxidases go through **reactive intermediate states with unusually high oxidation levels** of the iron centers.

BUT

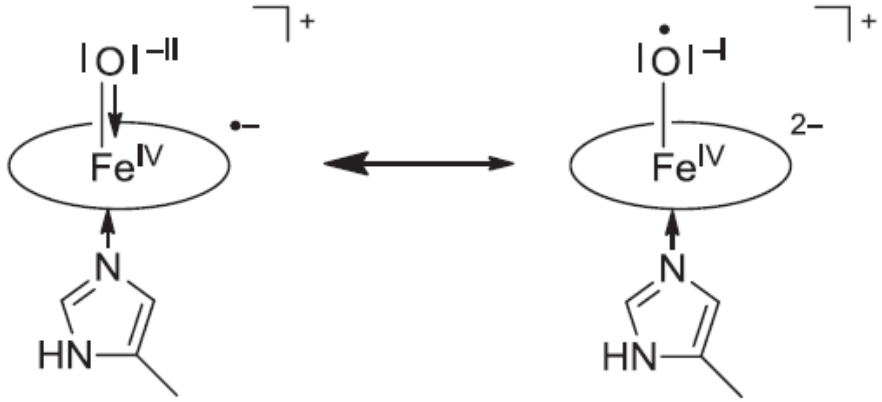
different reactivities:

- monooxygenase activity (monooxygen transfer, one O from O_2)
- direct electron withdrawal (formation of a substrate radical cation)

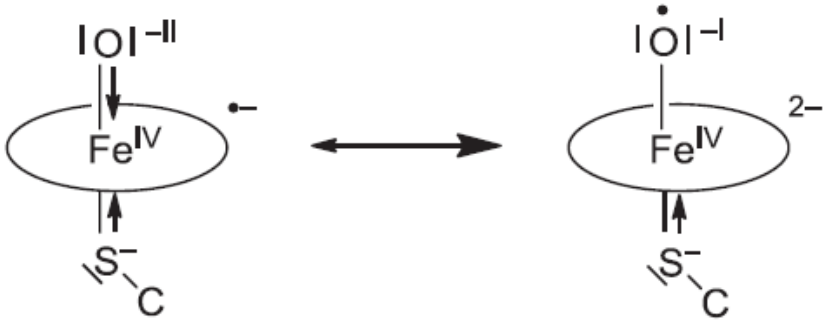
Unlike P-450, most peroxidase iron centers feature a neutral histidine as axial ligand, which can get deprotonated

Compared to the anionic thiolate ligand of the P-450 systems, neutral histidine is a weaker electron donor, which may possibly effect a **shift of the radical activity (spin density) from the iron-bound oxygen to the porphyrin π system**

peroxidases

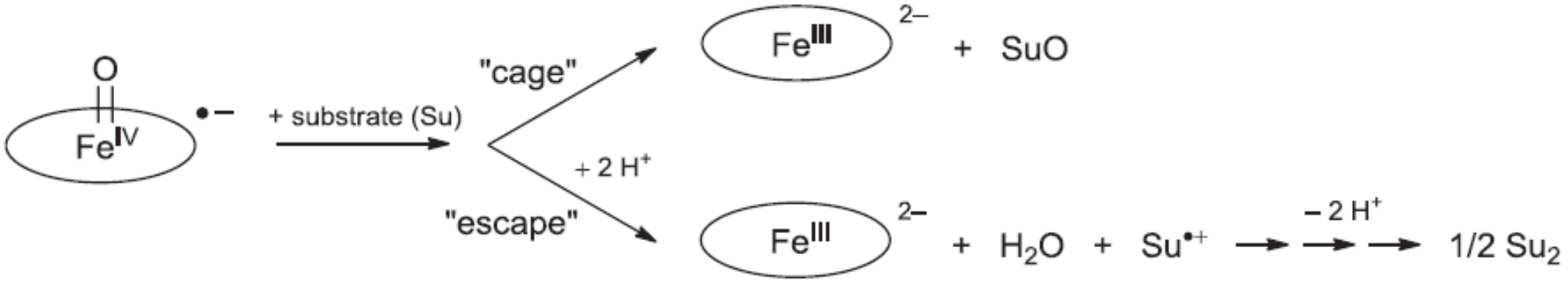


cytochrome P-450

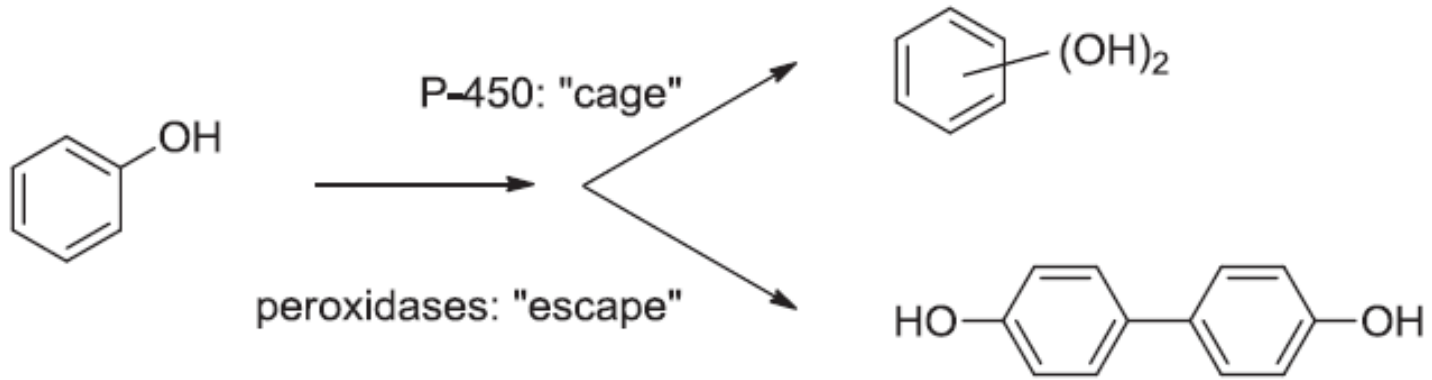


Electrophilic attack is then no longer connected to the oxygen transfer but consists of the **extraction of one electron from the substrate** and the conversion of the peroxidic oxygen to water.

Also the different protein environments is responsible for such different reactivities: in cytochrome P-450, the intermediate radicals that may be generated rapidly combine to yield the oxygenated products ("cage reaction"), while in peroxidases, a dissociation of the reactants can lead to the typical "escape" products of free radicals



example:



What about O_2 transport proteins?

The potentially high reactivity of oxyheme iron centers with regard to substrates has to be avoided at all cost in myoglobin and hemoglobin; otherwise, an autoxidation of these exclusively O_2-transporting and -storing systems would result

A condition that only occurs in a pathological context.