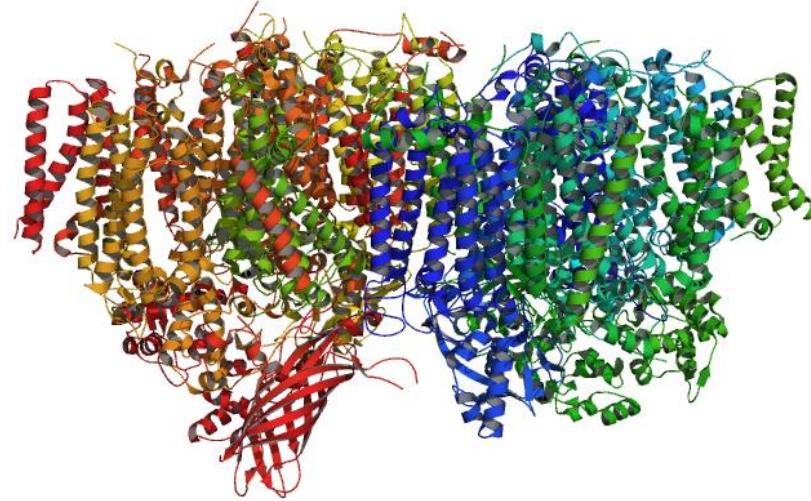
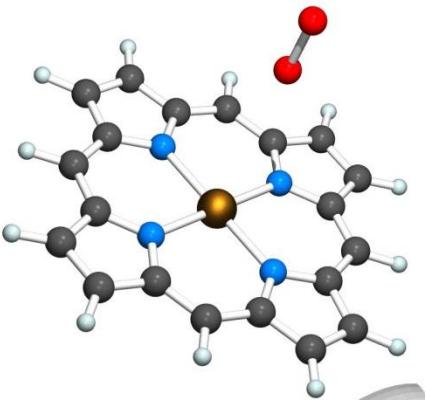
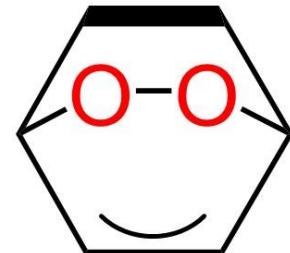


Bioinorganic Chemistry (BIC)

VI. Oxygen Metabolism



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Department of Chemistry



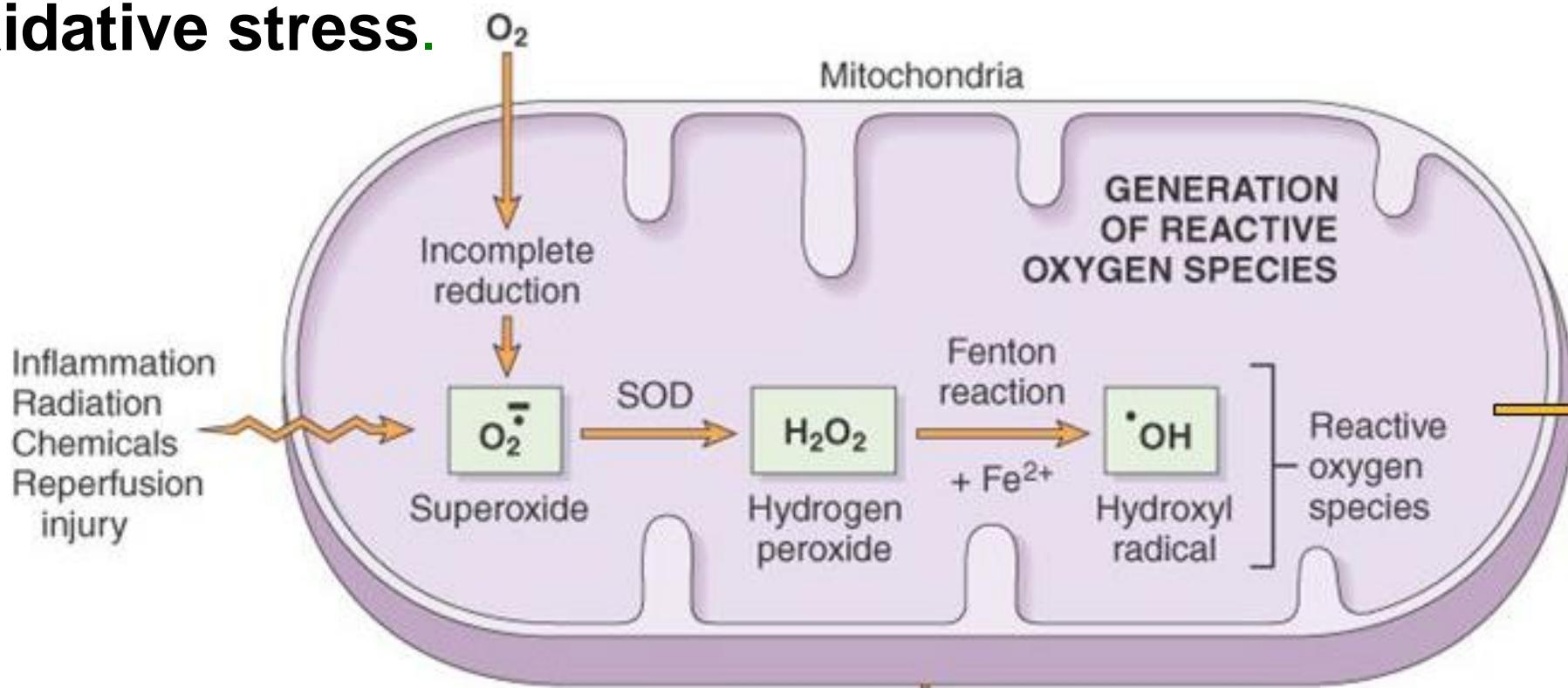
1. O₂ Toxicity & Reactivity

Key Features of O₂

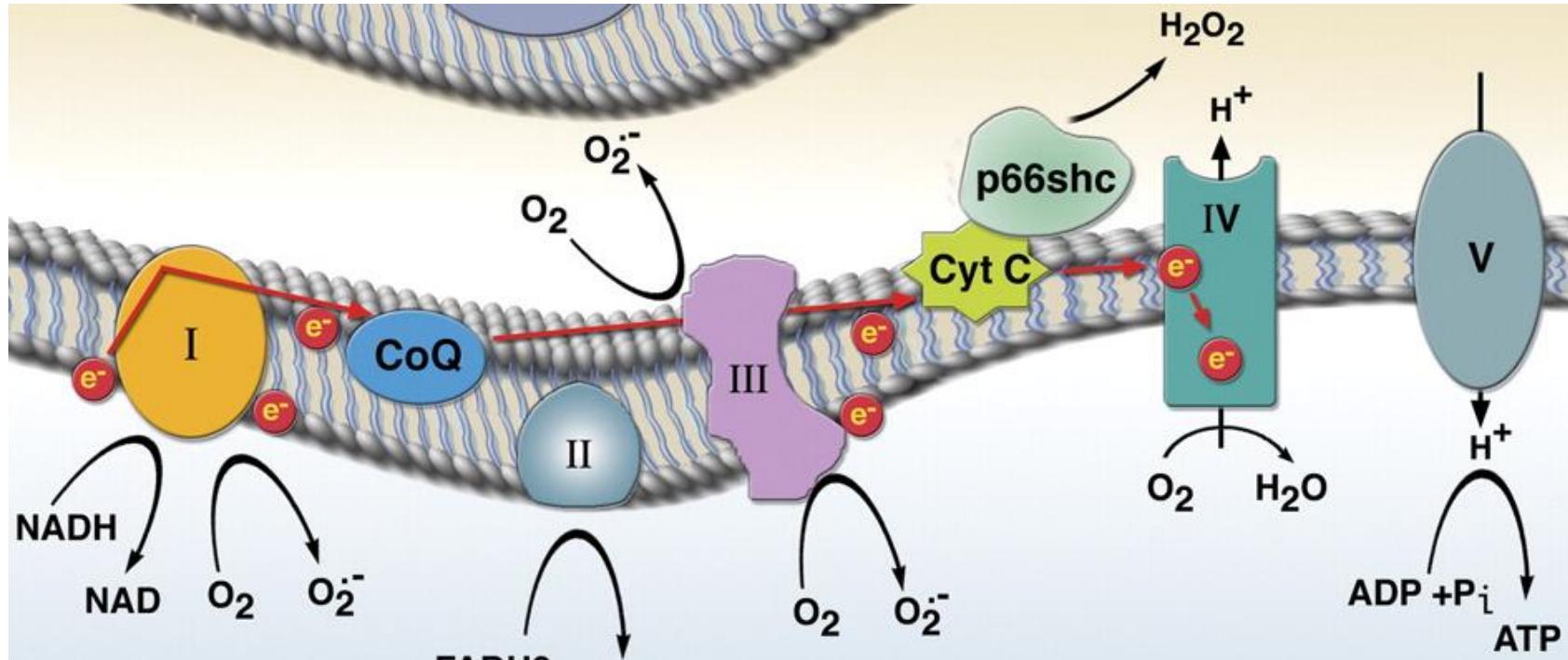
- Most non-photosynthetic biologic systems gain **energy** from **reduction** of an **O₂** oxidant to **H₂O** in **respiration**.
- Hemoglobin/myoglobin: transport, store & release O₂.
- Many enzymes can also use O₂ as an **O atom source** for many **enzymatic reactions/biosynthesis** of organic substrates.
- Reactions of O₂ with many molecules are generally **thermodynamically favorable**, but require **high barriers** without the catalyst or radical initiators. (If no or low barrier, our cells & bodies can be burned up!)

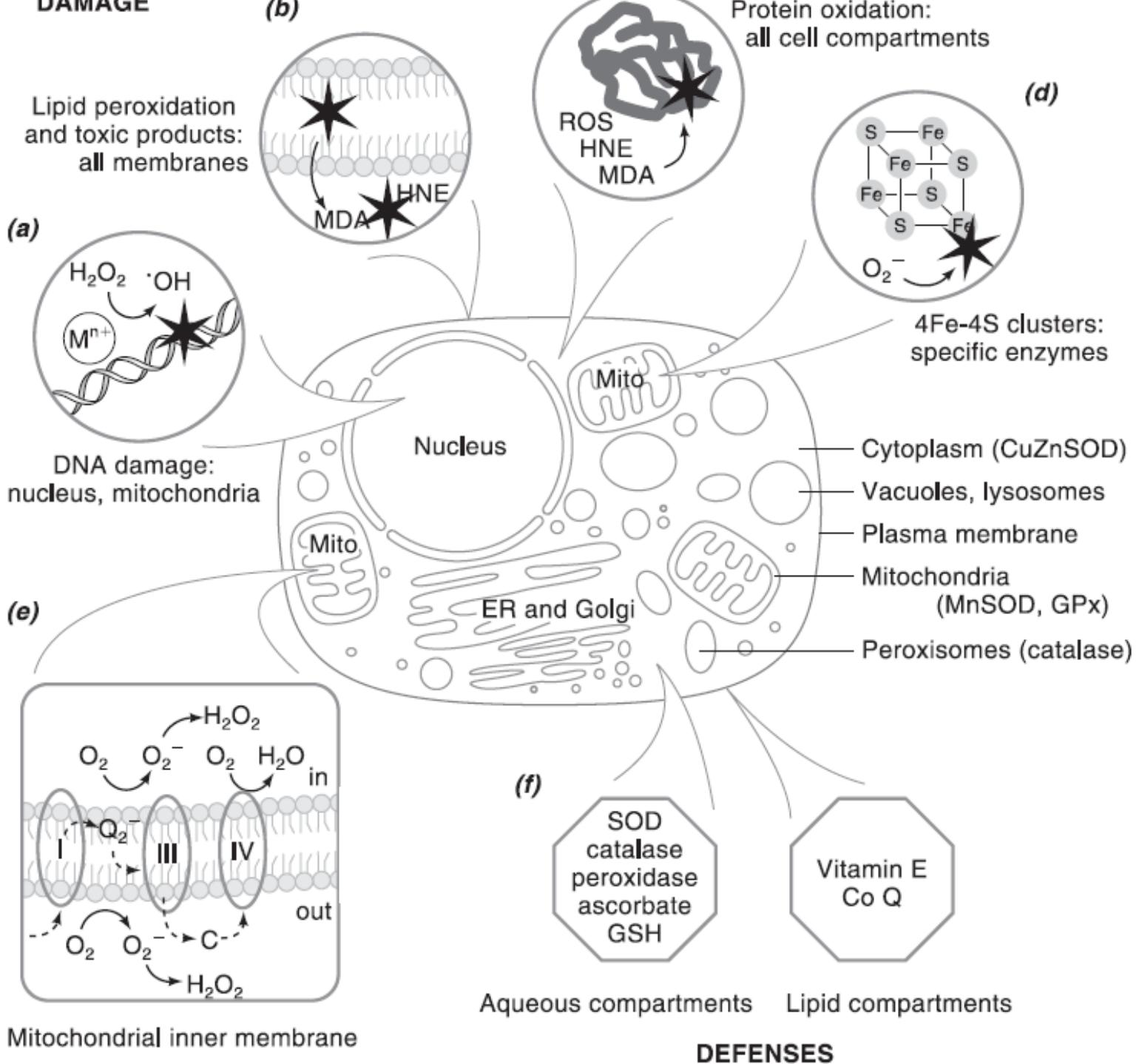
O₂ Toxicity

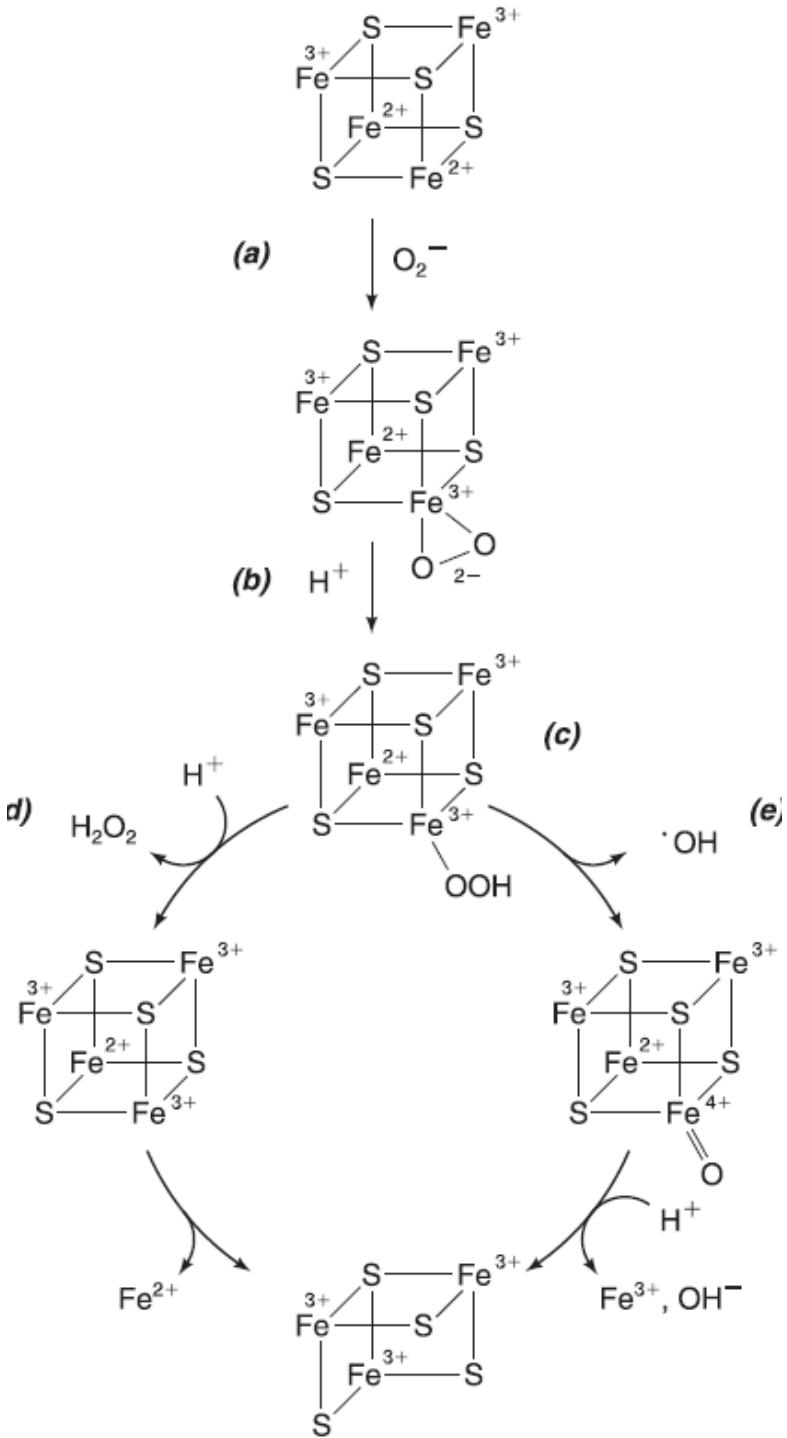
- Some non-enzymatic & enzymatic reactions of O₂ can generate partially-reduced compounds, e.g. O₂⁻ **superoxide** & H₂O₂ **hydrogen peroxide**, HO• **hydroxyl radical**, peroxy nitrite (ONOO) in aerobic cells. They can be called '**reactive oxygen species**' (**ROS**), & cause **oxidative damage** in our bodies & are agents of **oxidative stress**.



- HO \bullet & high-valent M=O & M-OH species can act as initiators of free radical autoxidation of lipids & proteins, nucleic acids, carbohydrates, & other organic molecules.
- O $_2^-$ is much less reactive than HO \bullet .
- Most H $_2$ O $_2$ & other ROS are derived from O $_2^-$ which is formed from reduction of O $_2$ by mitochondrial ET chain, primarily semiquinone (QH) in Complex III & secondarily NADH dehydrogenase (Complex I).



DAMAGE



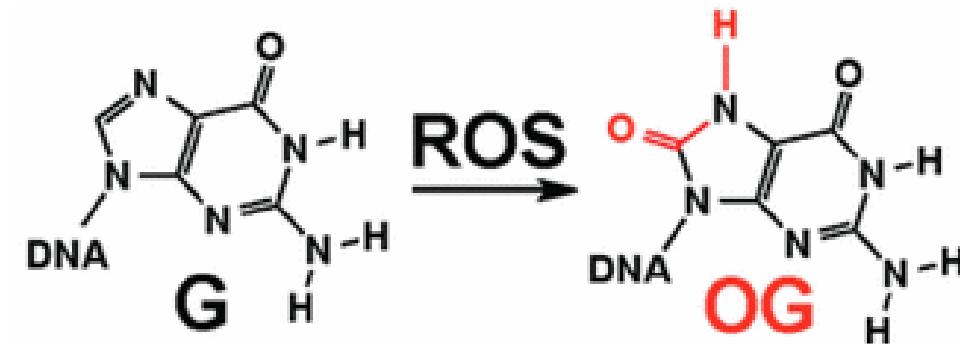
- Specialized systems (e.g. NADPH oxidase in leukocytes catalyzes the $1e^-$ reduction) form O_2^- & ROS to defend against pathogens (病原体).

- E.g. a Fe-S cluster-containing enzymes with single labile Fe atoms in the clusters.

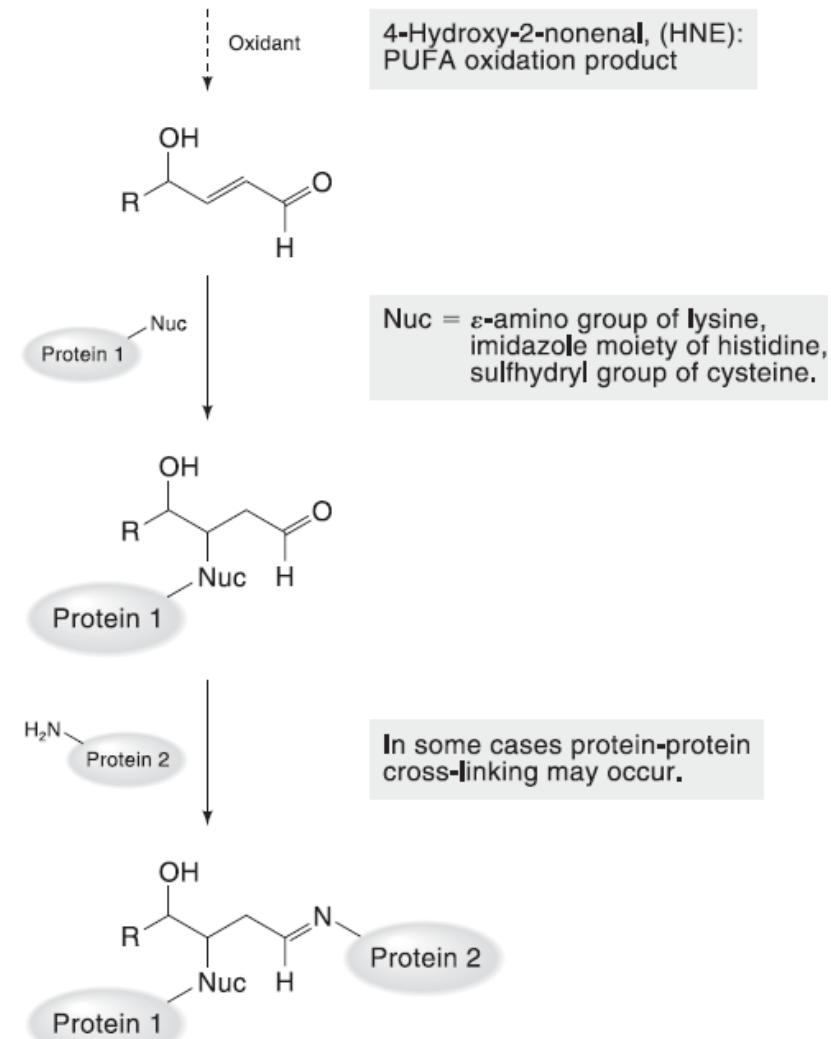
- Increase levels of free intracellular Fe \rightarrow more oxidative damage.

- **Proteins:** oxidative modification of their side chains (e.g. S-), backbone cleavage, & protein-protein dimerization.

- **Nucleic Acids:** oxidative damage of guanine (G) bases to form 8-oxo-7,8-dihydroguanine (OG) & subsequent oxidations products.



- **Lipids:** affects function of membranes & produces toxic aldehydes & ketones (e.g HNE).



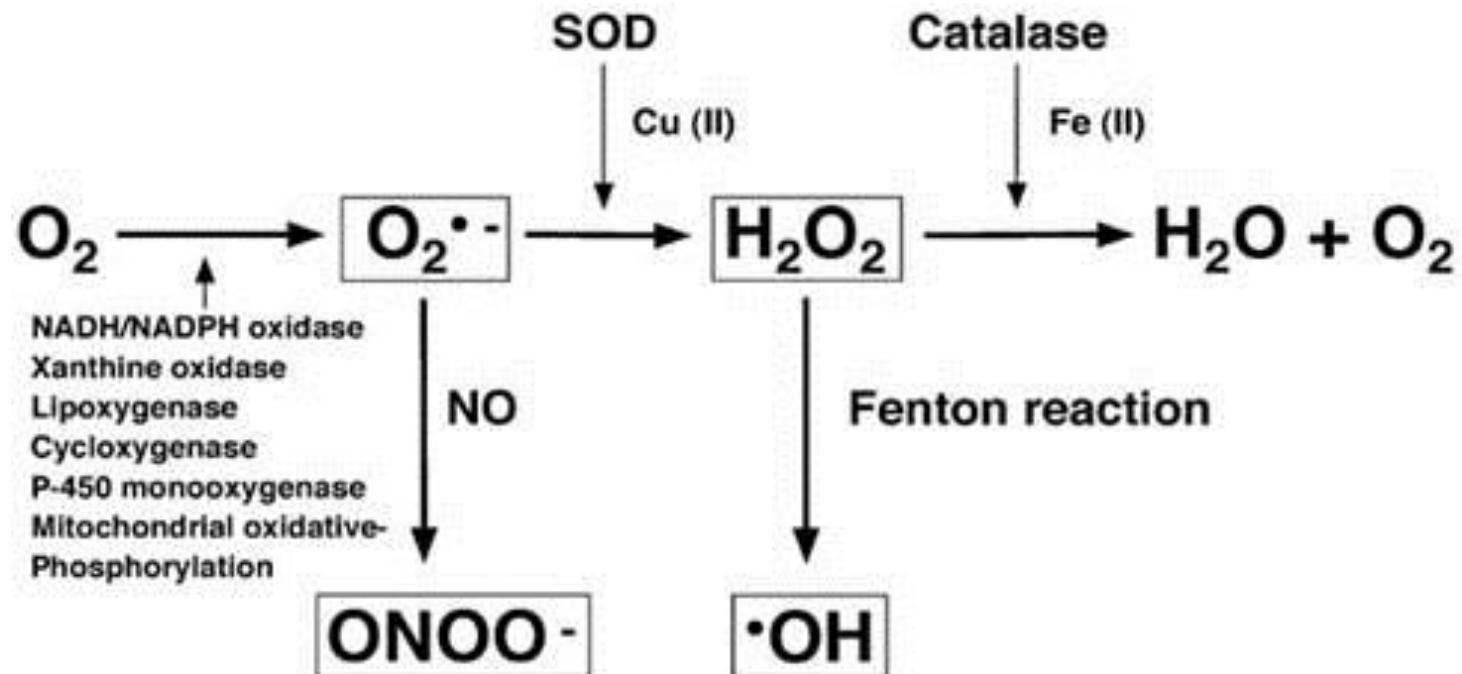
- Organisms use both **anti-oxidant molecules & enzymes** to defend against such oxidative stress.

- Major anti-oxidant **enzymes**:

(A) **Superoxide dismutase (SOD)** & **superoxide reductase (SOR)** enzymes for O_2^-

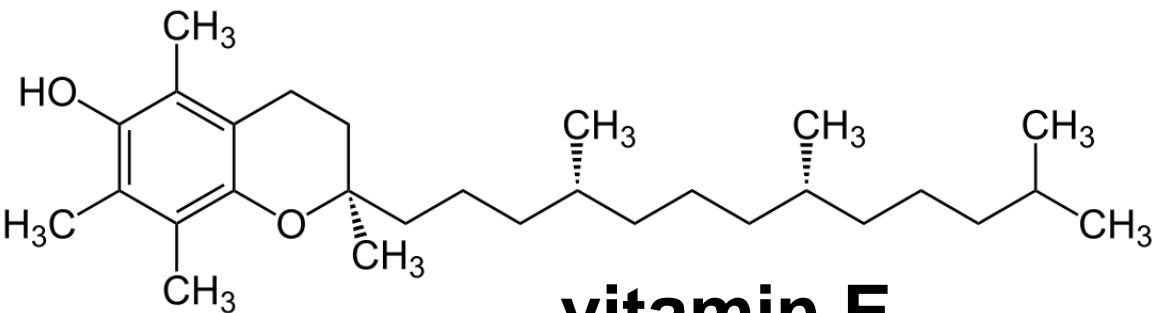
(B) **Catalase** & **peroxidase** enzymes for H_2O_2

- Anti-oxidant **molecules**: e.g. Vitamins C & E.



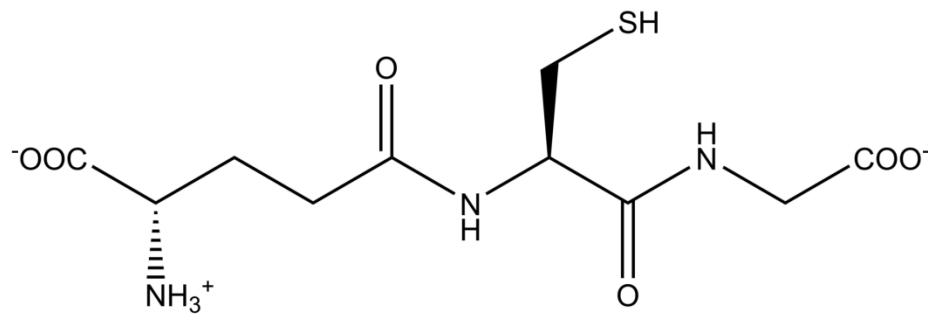
Low Molecular-Weight Anti-oxidants

- Many membrane-soluble antioxidants e.g. vitamin E, reduced ubiquinone (coenzyme Q) & NADPH.

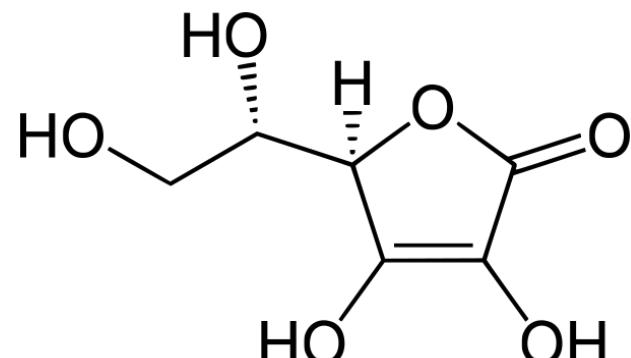


vitamin E

- Many cytosol (a lipid), glutathione, & ascorbate (vitamin C) are present in aqueous environment.



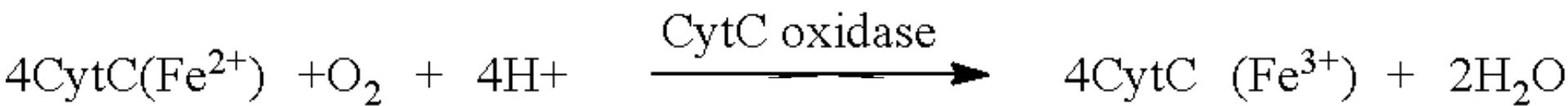
glutathione



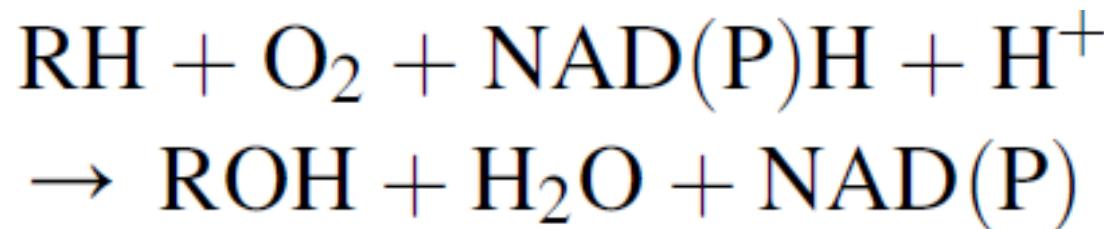
vitamin C

Two Common Types of Reactions with O₂

- **Electron-transfer** (ET) reactions **form** O₂⁻, H₂O₂ & H₂O: **oxidases** (e.g. Cyt c oxidase).

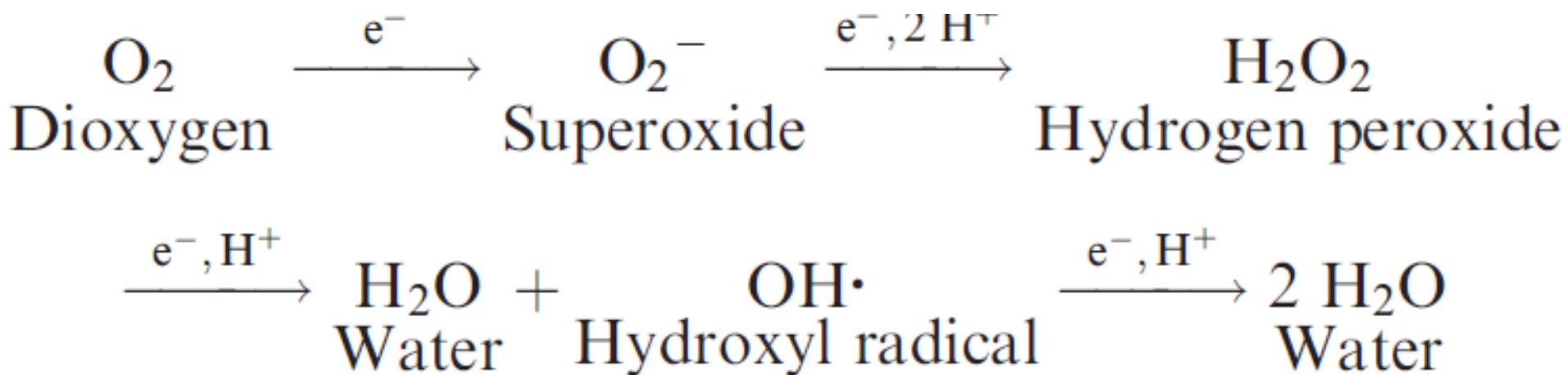


- **Oxygen-atom transfer** reactions involve **insertion** of at least one **O atom** of an O₂ molecule into the **substrate**: **oxygenases** (**monooxygenases (1O)** & **dioxygenases (2O)**).



ET Reactions

- For the $4e^-$ reduction of O_2 , most reducing agents can transfer at most $1e^-$ or $2e^-$ at a time.



- The **common product** without any catalyst is O_2^- , but this 1e^- reduction step is **thermodynamically unfavorable** → a **barrier**.

| <u>Reaction</u> | $E^\circ \text{ (V) vs. NHE}^a$ |
|---|---------------------------------|
| $\text{O}_2 + \text{e}^- \rightarrow \text{O}_2^-$ | −0.33 ^b |
| $\text{O}_2^- + \text{e}^- + 2 \text{ H}^+ \rightarrow \text{H}_2\text{O}_2$ | +0.89 |
| $\text{H}_2\text{O}_2 + \text{e}^- + \text{H}^+ \rightarrow \text{H}_2\text{O} + \text{OH}$ | +0.38 |
| $\text{OH} + \text{e}^- + \text{H}^+ \rightarrow \text{H}_2\text{O}$ | +2.31 |
| $\text{O}_2 + 2 \text{ e}^- + 2 \text{ H}^+ \rightarrow \text{H}_2\text{O}_2$ | +0.281 ^b |
| $\text{H}_2\text{O}_2 + 2 \text{ e}^- + 2 \text{ H}^+ \rightarrow 2 \text{ H}_2\text{O}$ | +1.349 |
| $\text{O}_2 + 4 \text{ H}^+ + 4 \text{ e}^- \rightarrow 2 \text{ H}_2\text{O}$ | +0.815 ^b |

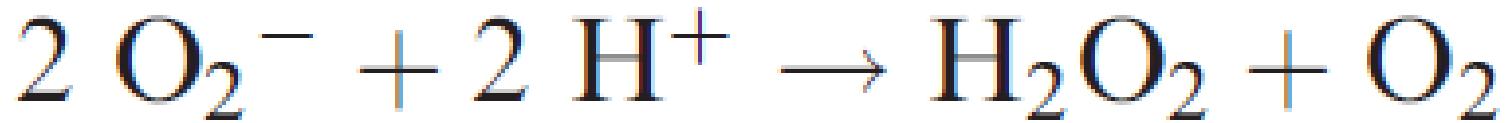
- A relatively **strong reductant** is required.

- When O_2^- is produced, it can quickly undergo disproportionation to give H_2O_2 & O_2 in aqueous solution (except at very high pH).

- 1e^- reduction of O_2 :



- Disproportionation of O_2^- :



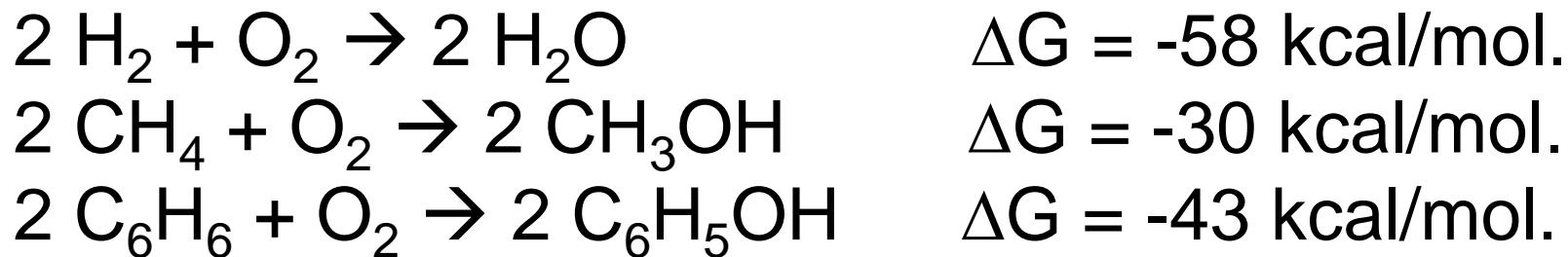
- 2e^- reduction of O_2 :



Oxygen-Atom Transfer Reactions

- The most important reactions to form C-O bonds in biology.

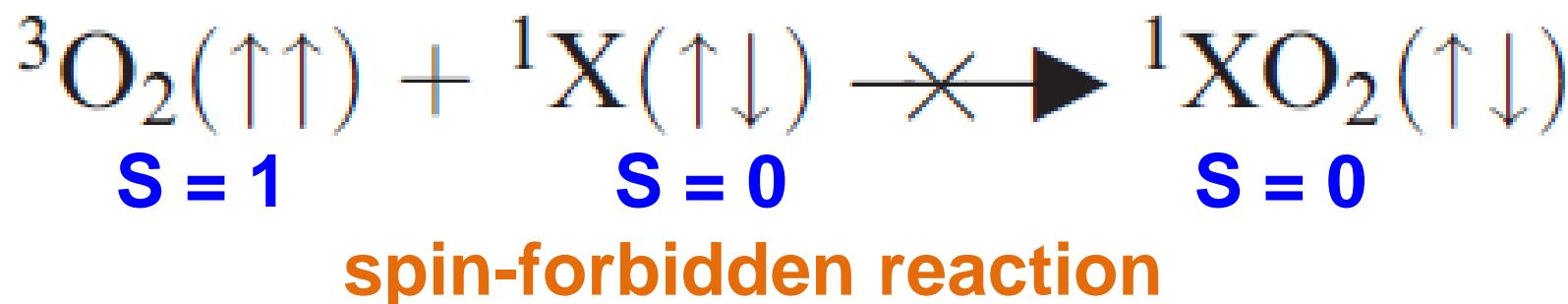
- **Favorable thermodynamics:**



- Although they are **highly exergonic**, they cannot occur spontaneously & rapidly at room temperature in the absence of a catalyst or free radical initiator, due to their barriers.

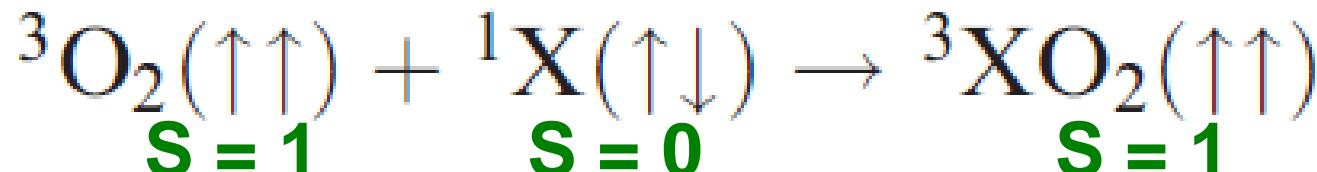
Kinetics for ET & Oxygen Transfer Reactions

- Origin of the main barrier: the (electronically) ground-state **O₂** with **2 unpaired electrons (triplet)** reacts with ground-state common **organic substrates** with **no unpaired electrons (close-shell singlet)**.
- (Uncatalyzed) Triplet-to-singlet spin conversions are **forbidden** by quantum mechanics & then are slow. A **spin-forbidden reaction** cannot occur in one concerted step:

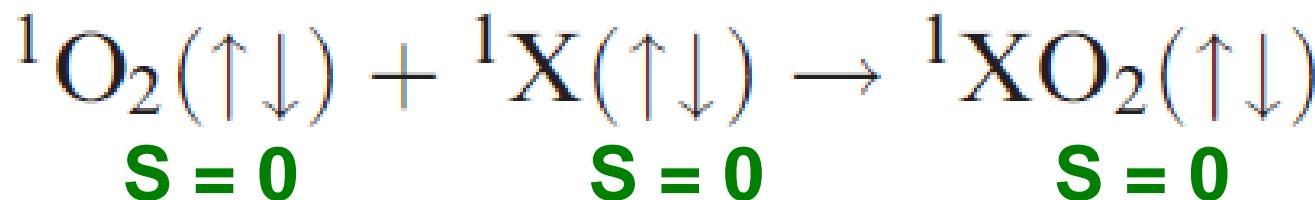


Alternative pathways:

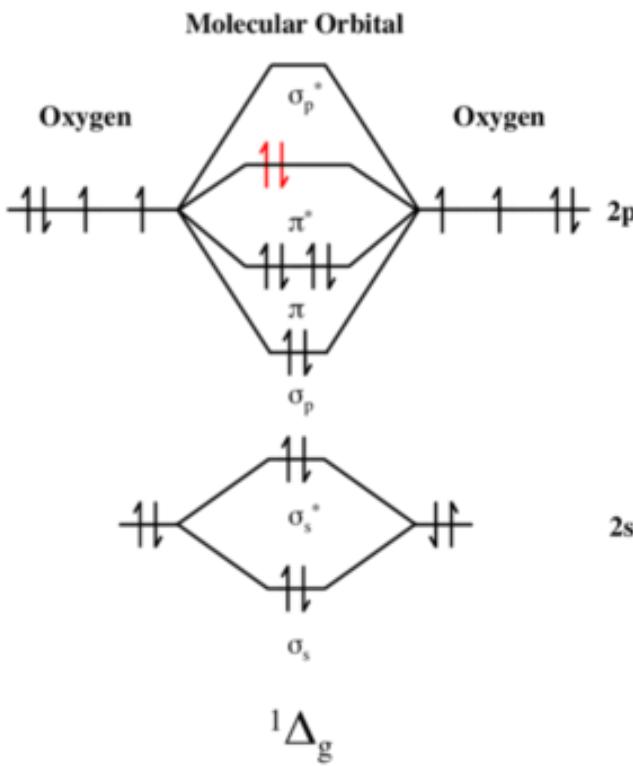
- The **spin-allowed** formation of the excited-state product:



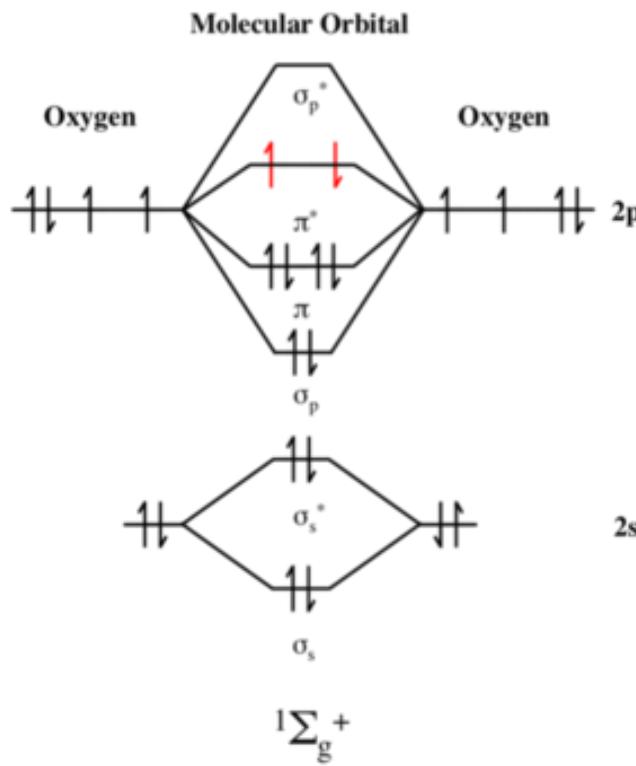
- The formation of the excited-state (reactive) singlet O₂ before the **spin-allowed reaction**:



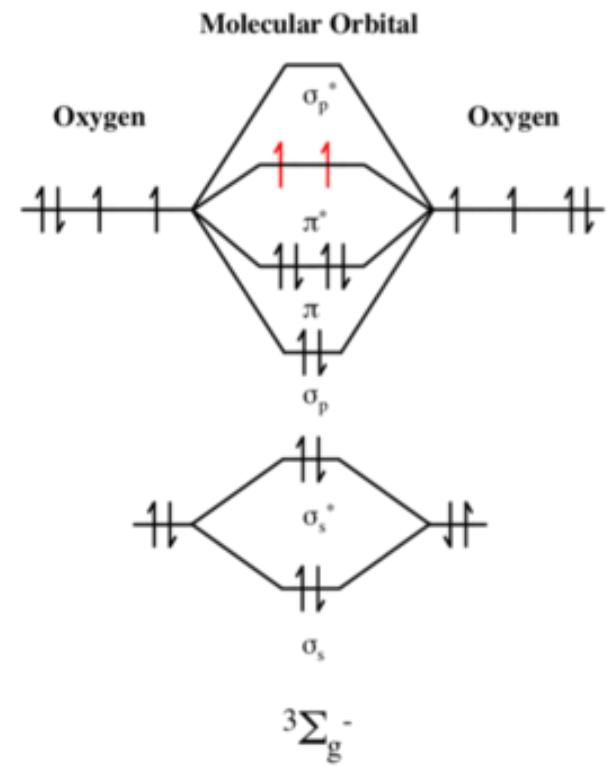
Molecular Orbital Diagrams & Energies



close-shell singlet
22.5 (S_1)

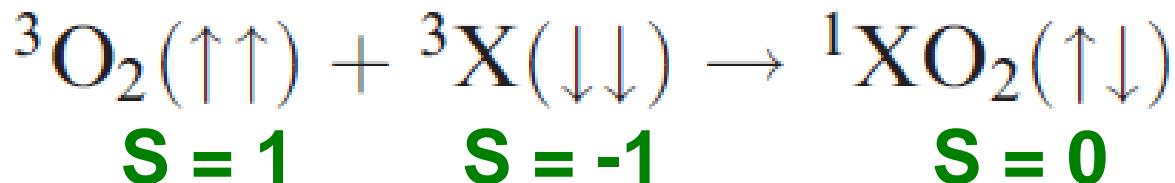


open-shell singlet
37.3
kcal/mol

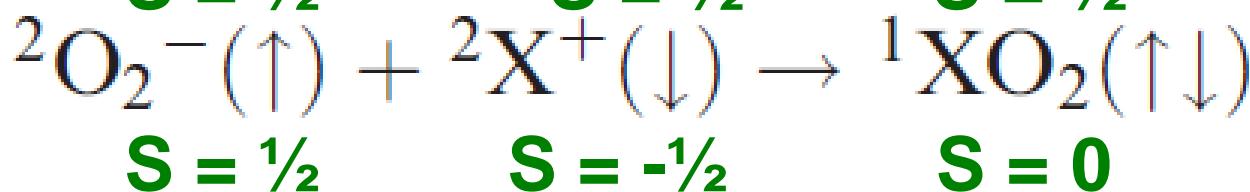
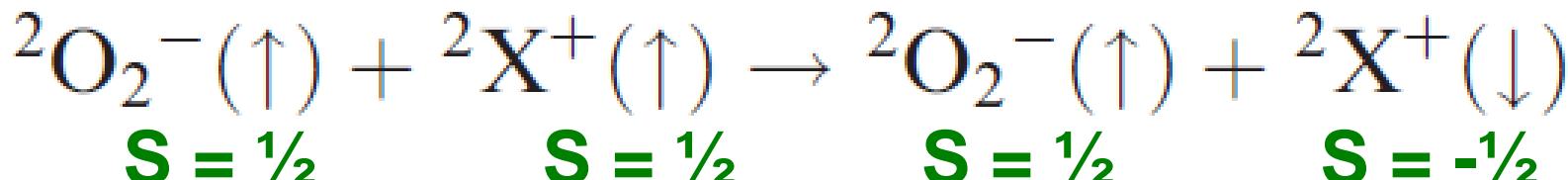
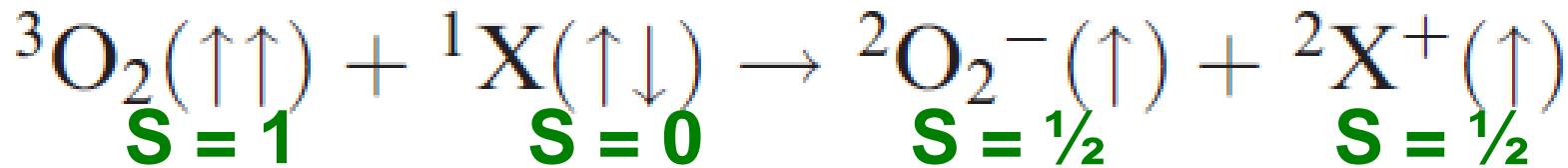


open-shell triplet
0.0 (GS, T_0)

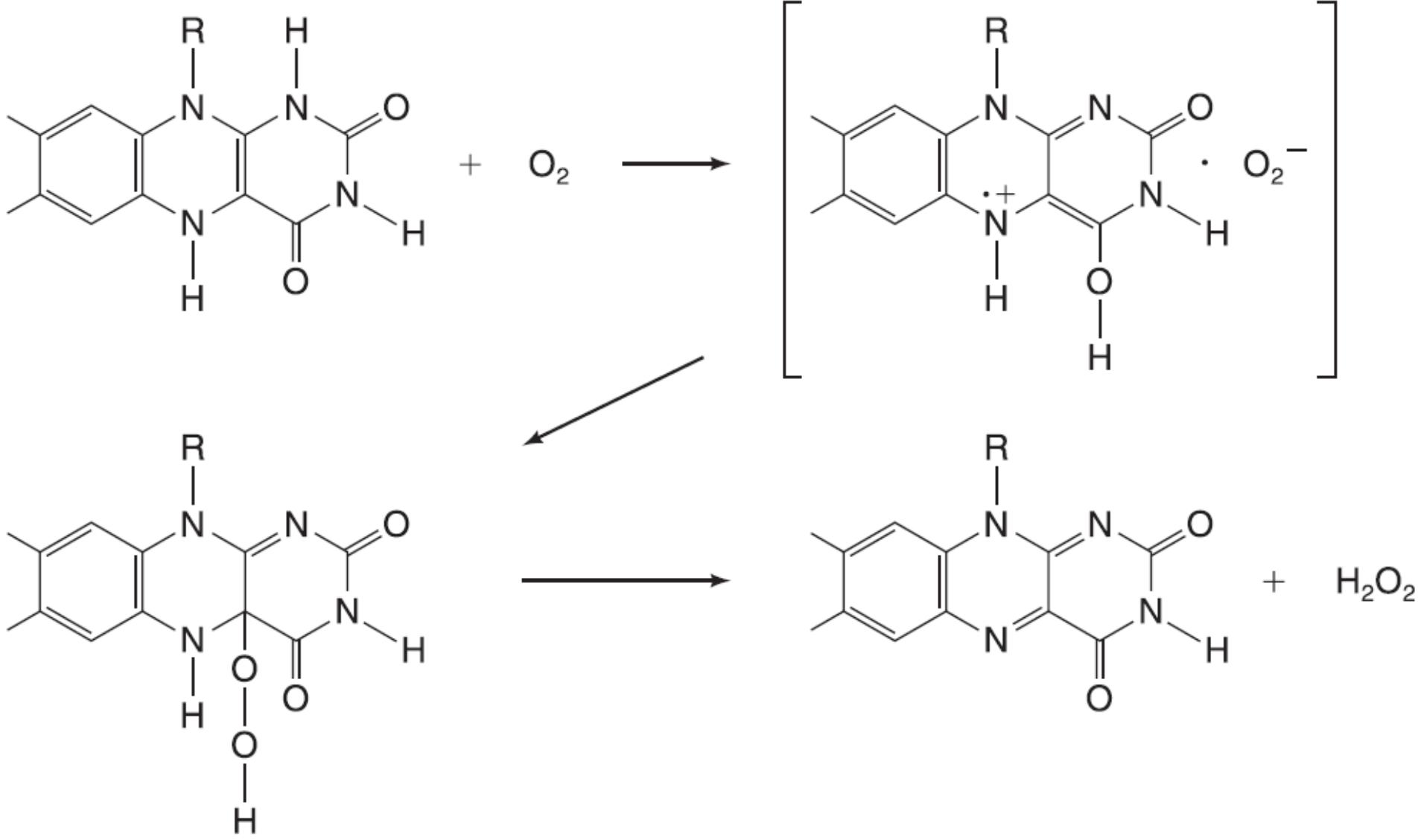
- Reaction with the excited-state triplet substrate:



- The **two sequential 1e redox** processes with a very good reductant to form an O_2^- intermediate (**common**):

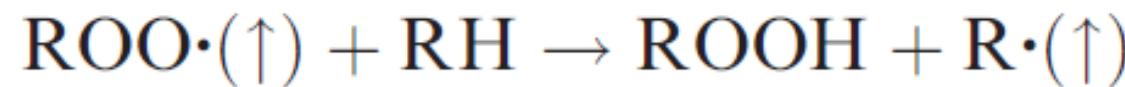
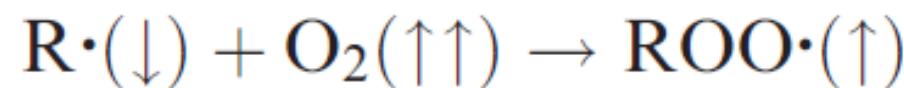
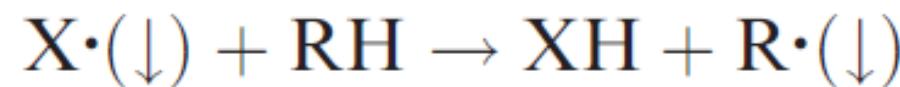
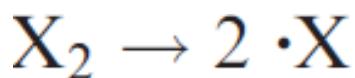


E.g. the spin-allowed reaction of O_2 with reduced flavins:



Free Radical Autoxidation Reactions

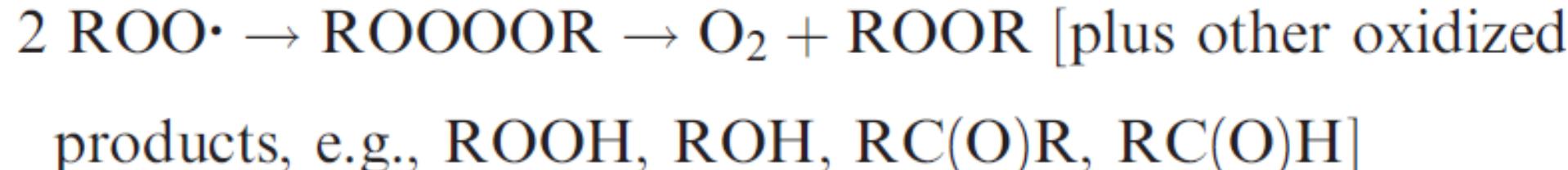
- Triplet O₂ can react with the organic substrate to give the oxygenated product in a **radical chain process** (a **spin-allowed** process) by adding a free-radical initiator.



Initiation:

Propagation:

Termination



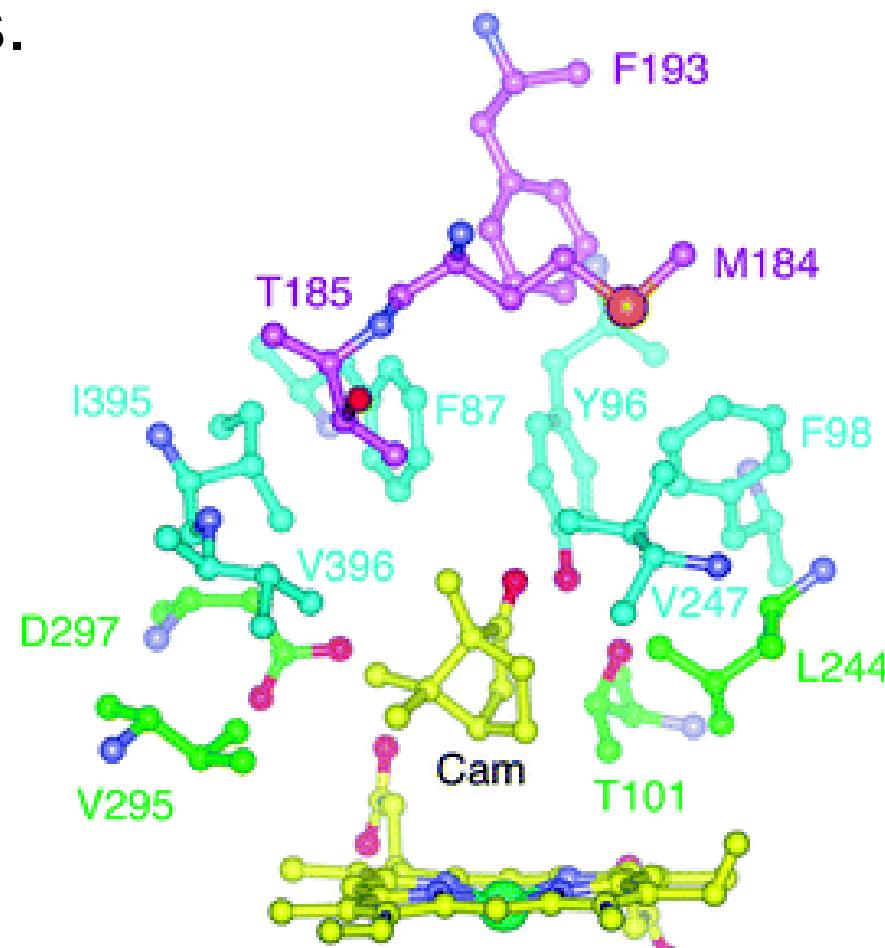
- A free radical initiator (e.g. peroxides with **a weak X-X bond**): a compound forms free radicals readily by a thermal or photo-chemical reaction.
- Free radical autoxidation is an **unselective oxidation** & believed to be **harmful** to biological systems, e.g. peroxidation of lipids in membranes.

Enzymatic Reactions

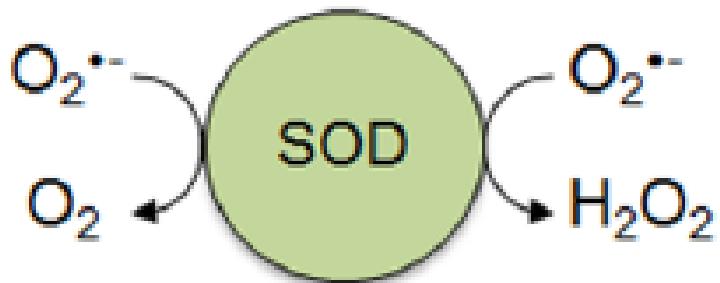
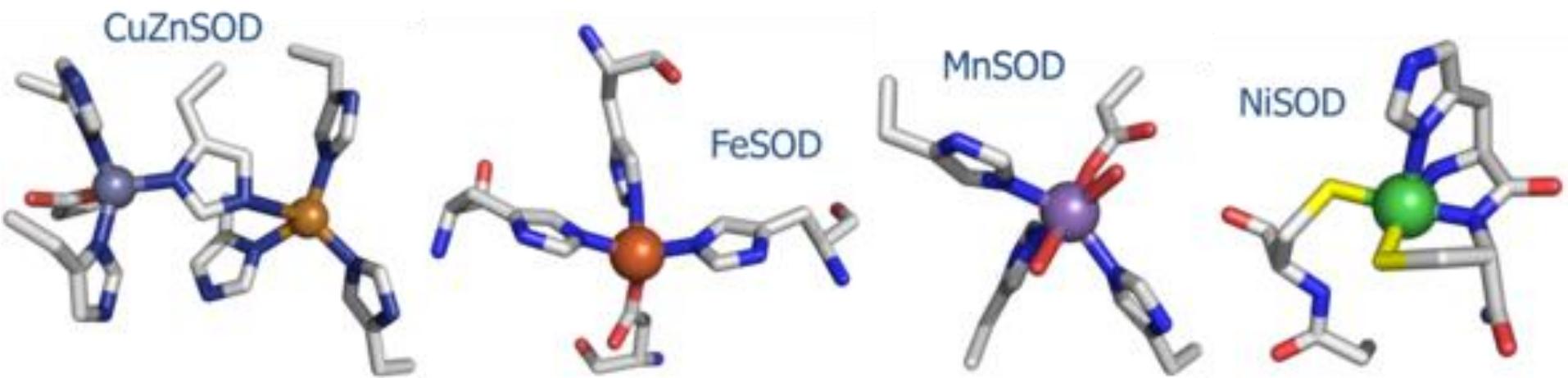
- ROS or other radical intermediates generated are generally **well controlled** & **not allowed to diffuse** from their catalytic site: **selective oxidation**.
- Compared to the slow or *unselective uncatalyzed* reactions of O_2 , **oxidase** & **oxygenase metallo-enzymes** catalyze the reactions in an **effective** & (chemo-, regio- & stereo-) **selective** manner to overcome the spin restriction & unfavorable 1st reduction (O_2) problems.
- Roles of these metalloenzymes: (1) **lower the barriers**, & (2) for oxygenases, **redirect the reactions** along different pathways to form different products.

E.g. monooxygenase cytochrome P450 catalyzes the reaction of O₂ with various organic substrates:

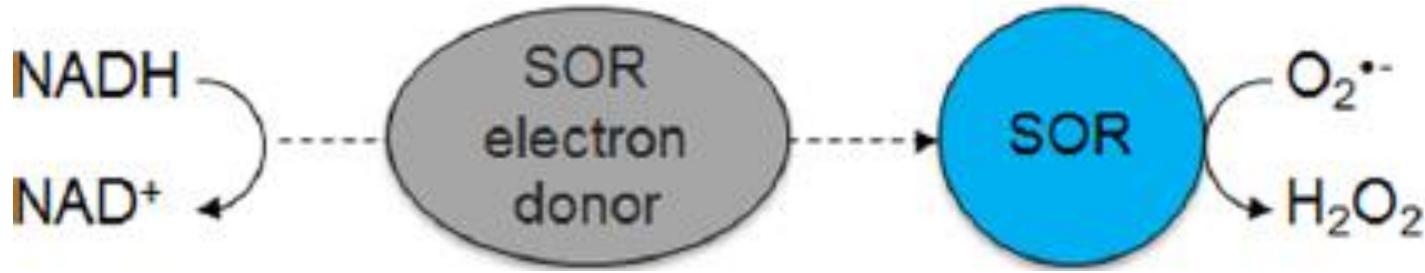
- Binding of **triplet O₂** to the **paramagnetic Fe(II)** ion in the active site (→ overcome the spin restriction).
- Multi-electron reduction of O₂ to form a very reactive high-valent **Fe(IV)-oxo** species.
- **Highly selective & stereo specific** oxygenation, as the substrate is directed to close to the oxo ligand.



2. Superoxide Dismutases (SOD) & Superoxide Reductases (SOR)



Metal = (Mn, Fe, Cu-Zn, Ni)

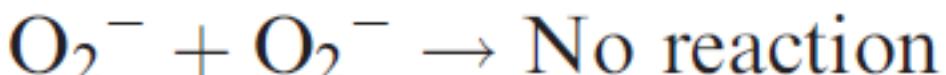
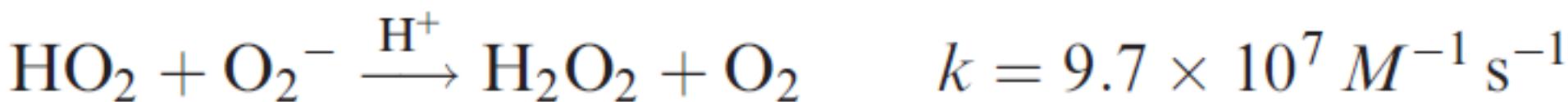


Metal = Fe

- In eukaryotes, the mitochondrial inner membrane releases toxic & reactive O_2^- as a byproduct of aerobic metabolism.



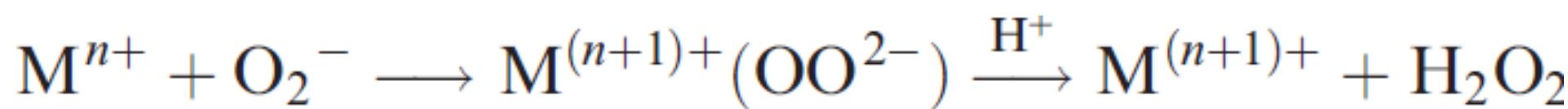
- Disproportionation** of O_2^- spontaneously occurs to form H_2O_2 & O_2 via pH-dependent pathways:



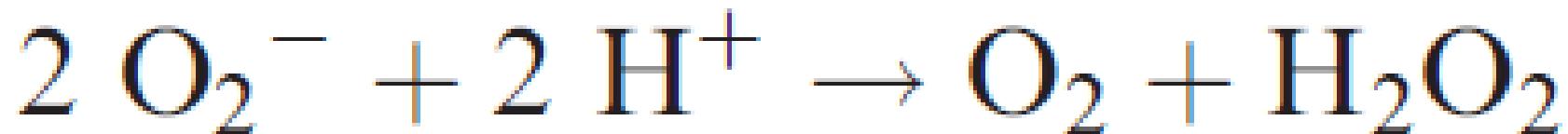


$$k = 1.7 \times 10^7 \text{ } M^{-1} \text{ s}^{-1}$$

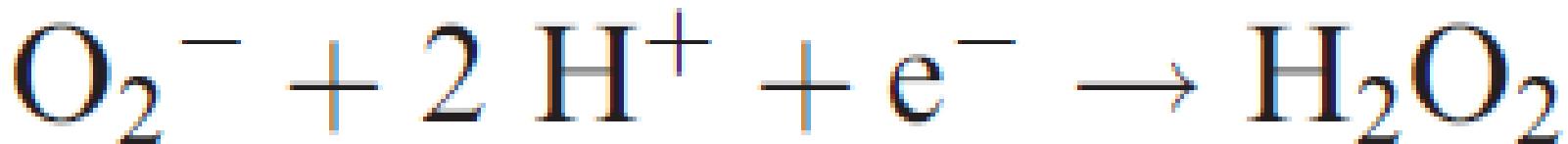
- A metal ion may reduce O_2^- to form a metal-peroxo complex before protonation.



- 2 classes of O_2^- detoxification enzymes: **superoxide dismutase (SOD)** & **superoxide reductase (SOR)**.
- **SODs** catalyze the **disproportionation** of O_2^- to give O_2 & H_2O_2 (O_2^- as the **oxidant & reductant**):



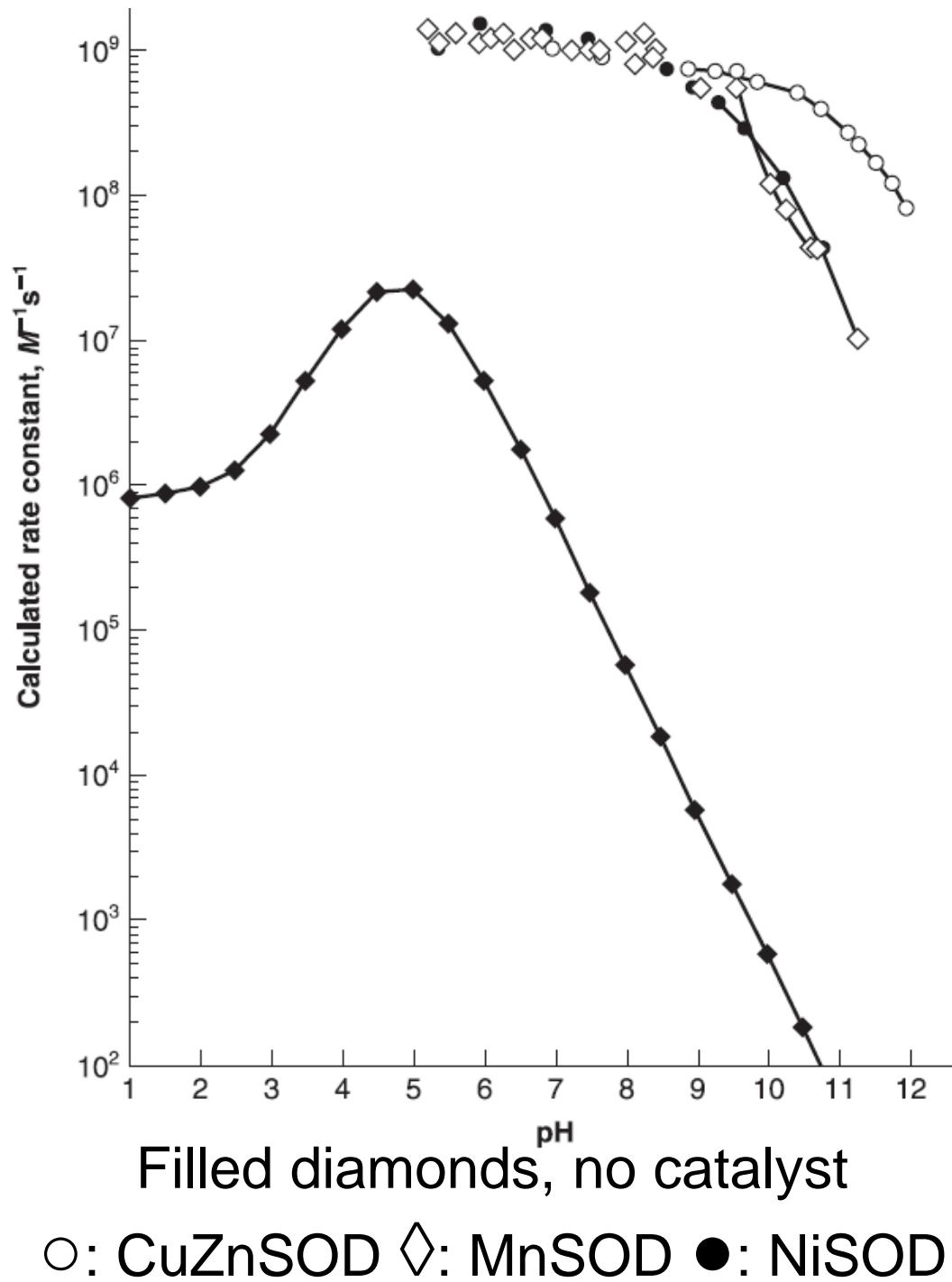
- **SORs** catalyze the **1e^- reduction** of O_2^- to give H_2O_2 by using NADH or NADPH as the ultimate e^- source:



- 3 classes of **SODs**:

 - (1) Copper-zinc SOD (**CuZnSOD**);
 - (2) Structurally-related iron & manganese SOD (**FeSOD** & **MnSOD**);
 - (3) Nickel SOD (**NiSOD**).

- Only 1 known class of **SOR** contains a **mono-nuclear non-heme** iron center in the active site.



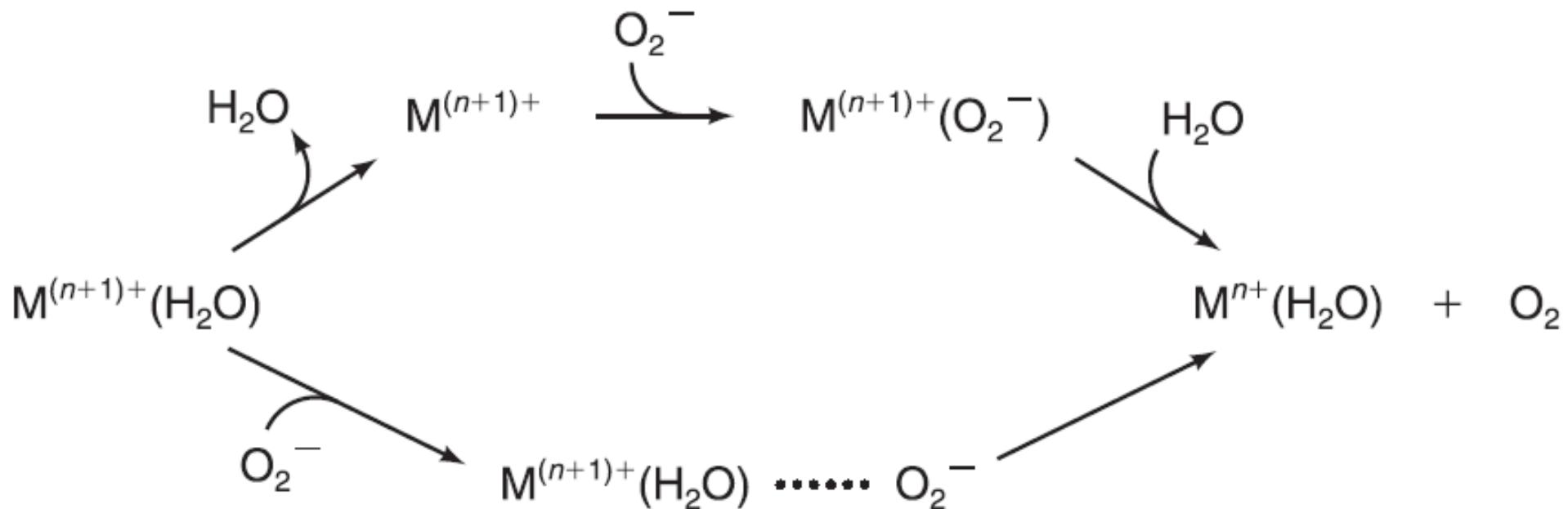
- **SOD**: Its mechanisms could be regarded as 2 individual steps: (1) oxidation of O_2^- by the **oxidized SOD** enzyme to form O_2 , & (2) reduction of O_2^- by the **reduced SOD** enzyme to form H_2O_2 .



- **SOR**: Its mechanisms involve only the 2nd reaction (reduction of O_2^- by the **reduced SOR** enzyme) followed by reduction of the oxidized SOR enzyme to regenerate the reduced state by reducing agents.

Oxidation of O_2^-

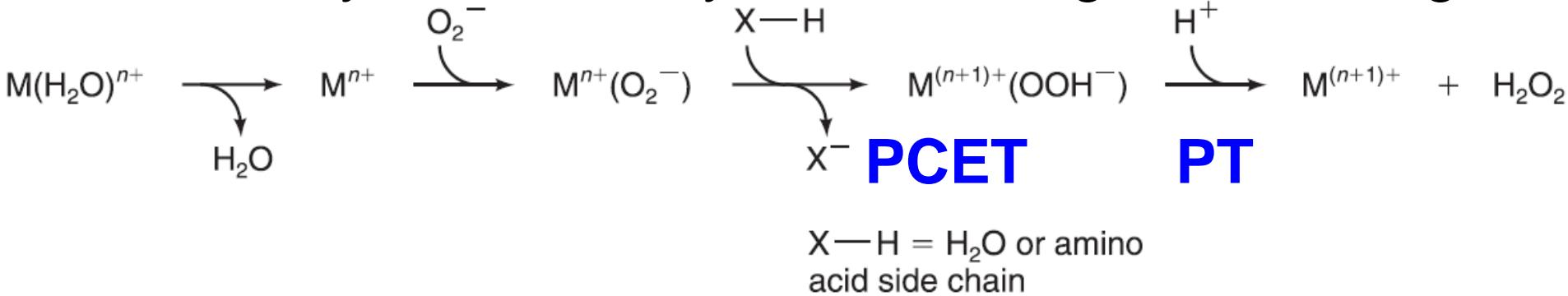
- 2 possible pathways: **inner- & outer-sphere pathways.**



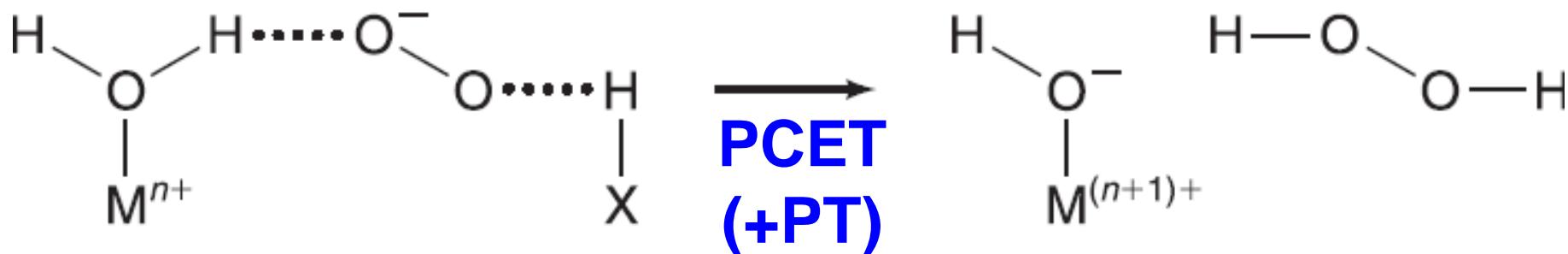
- The **inner-sphere** one (top): O_2^- coordination to the metal before ET.
- The **outer-sphere** one (bottom): O_2^- may be held close to the metal center by H-bonding with a metal-bound ligand (e.g. H_2O).

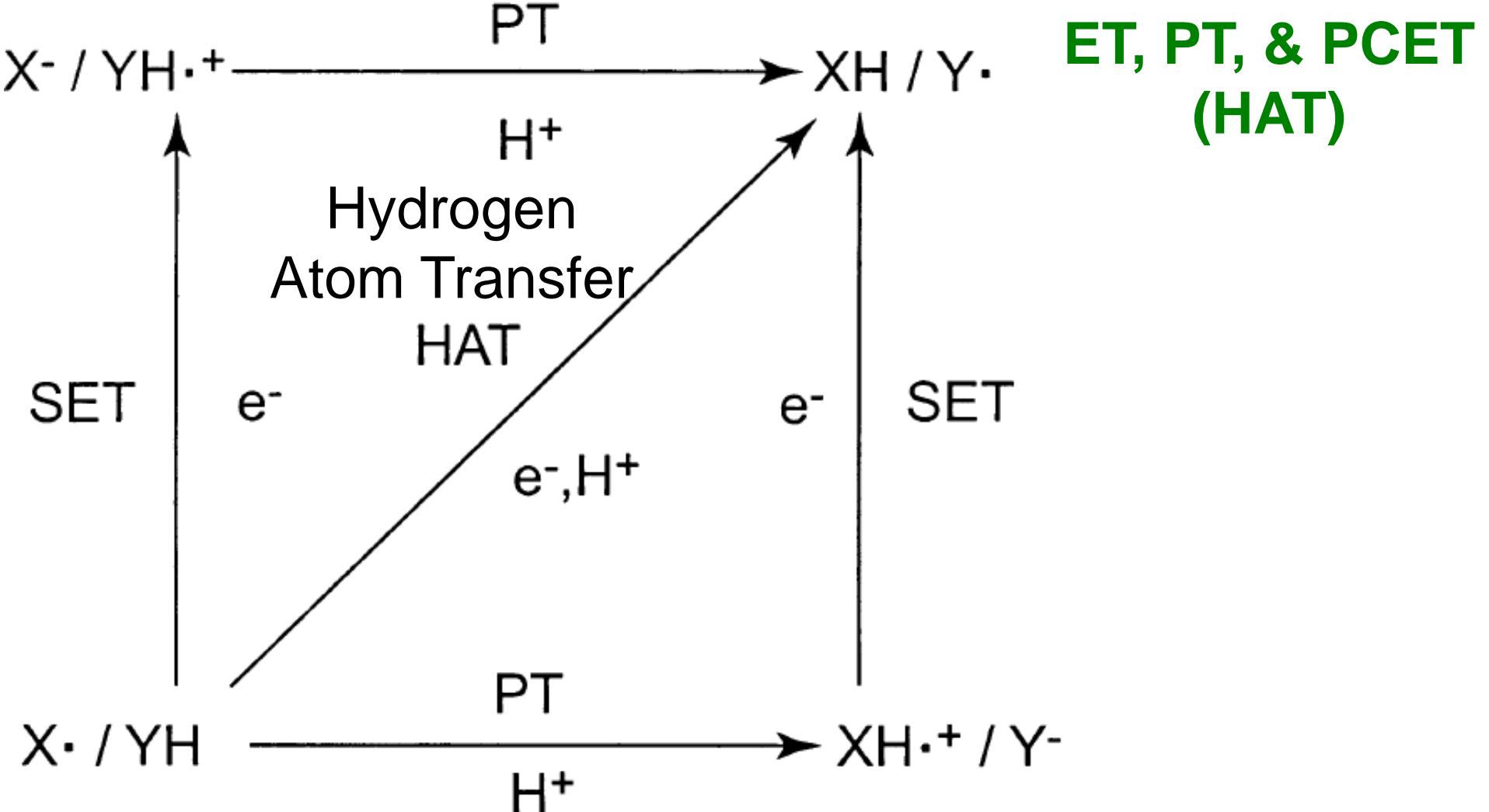
Reduction of O_2^-

- The reduction of O_2^- takes place via **proton-coupled electron transfer** (PCET), due to instability of O_2^{2-} .
- An **inner-sphere** pathway requires a vacant site on the metal, & may be limited by the rate of ligand exchange:

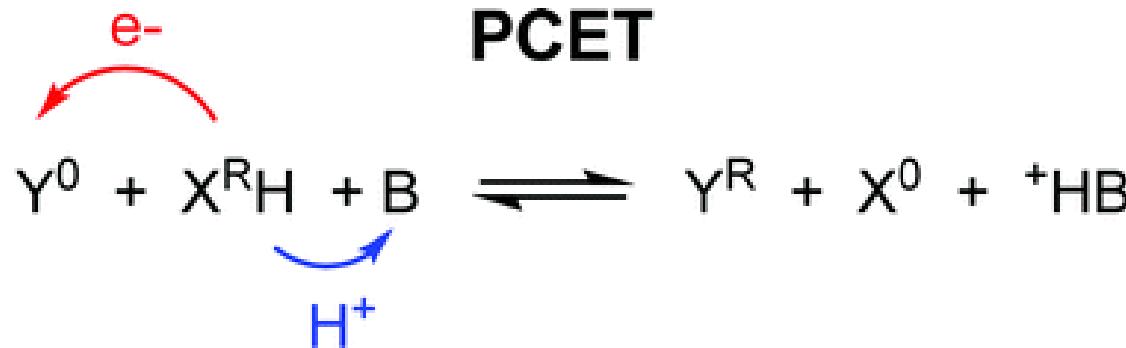


- An **outer-sphere** pathway does not form stable $M-OOH$ complexes:



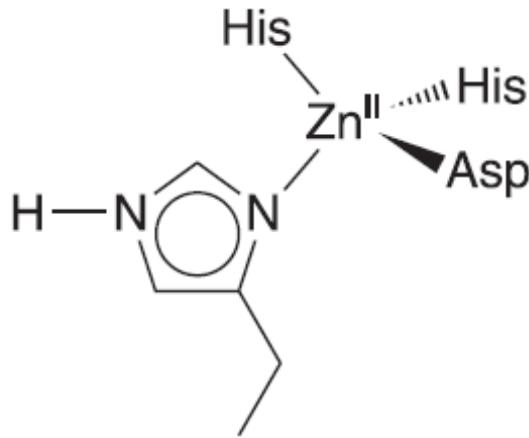
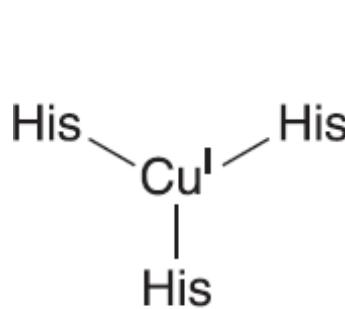


PCET:
Proton-coupled
Electron Transfer

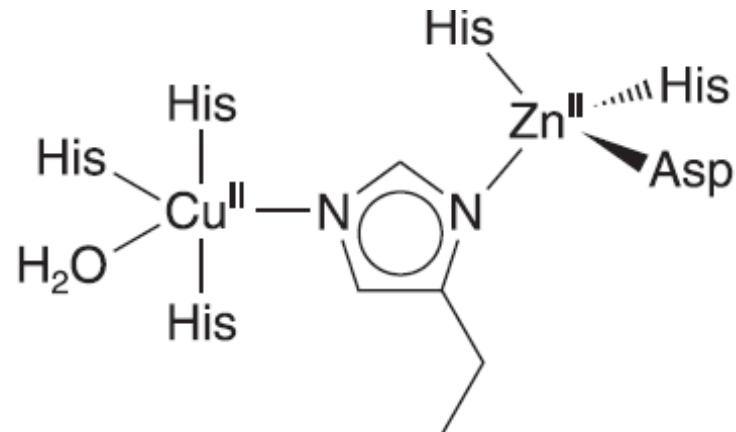


CuZnSOD

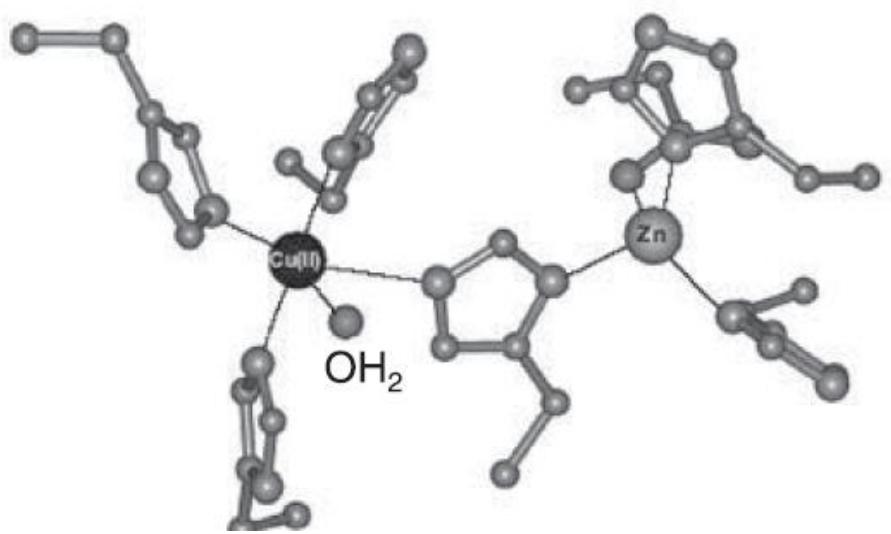
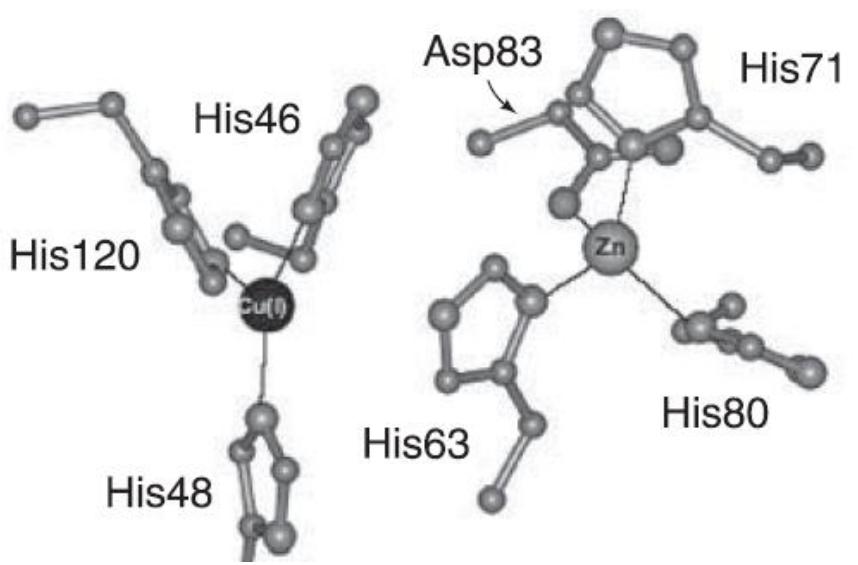
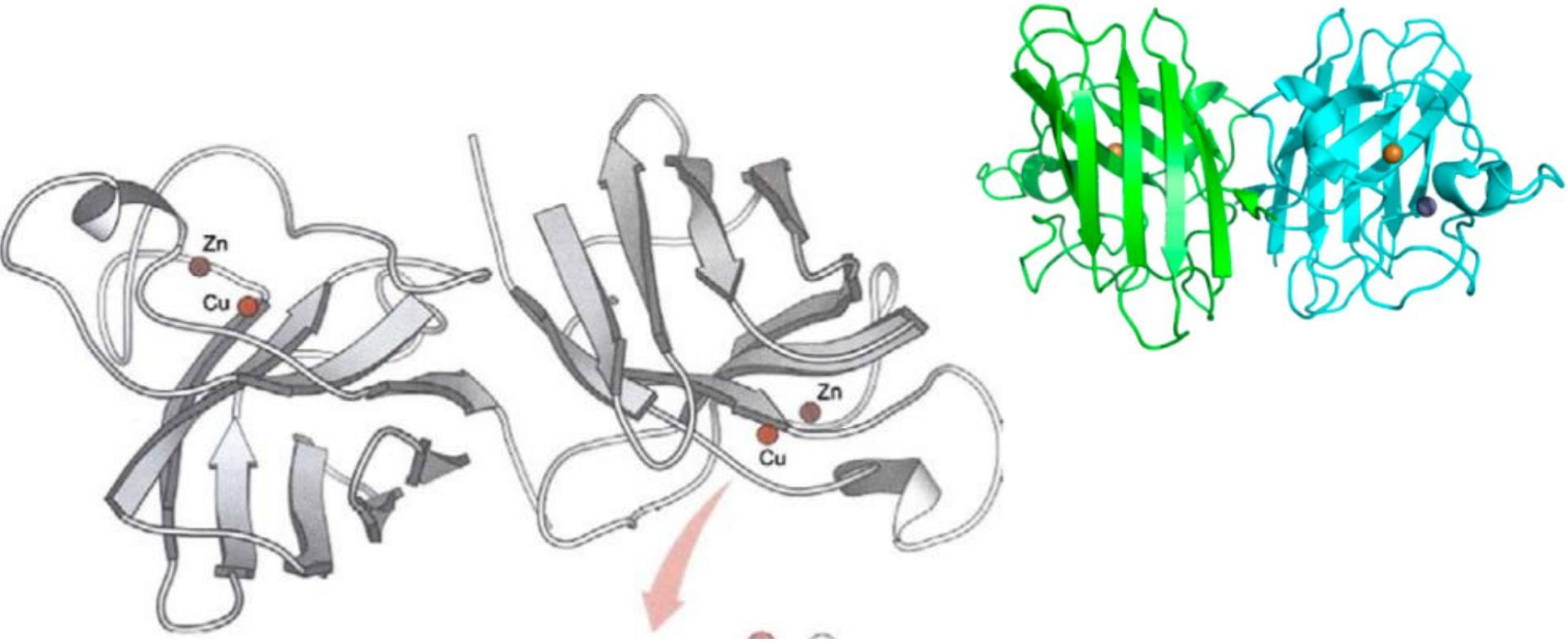
- Found in almost all eukaryotic cells & many prokaryotes.
- The overall protein structures of the reduced & oxidized forms are very similar, but not **the active site**.
- The active site has 6 His & 1 Asp.



Reduced
3-coordinate Cu(I)



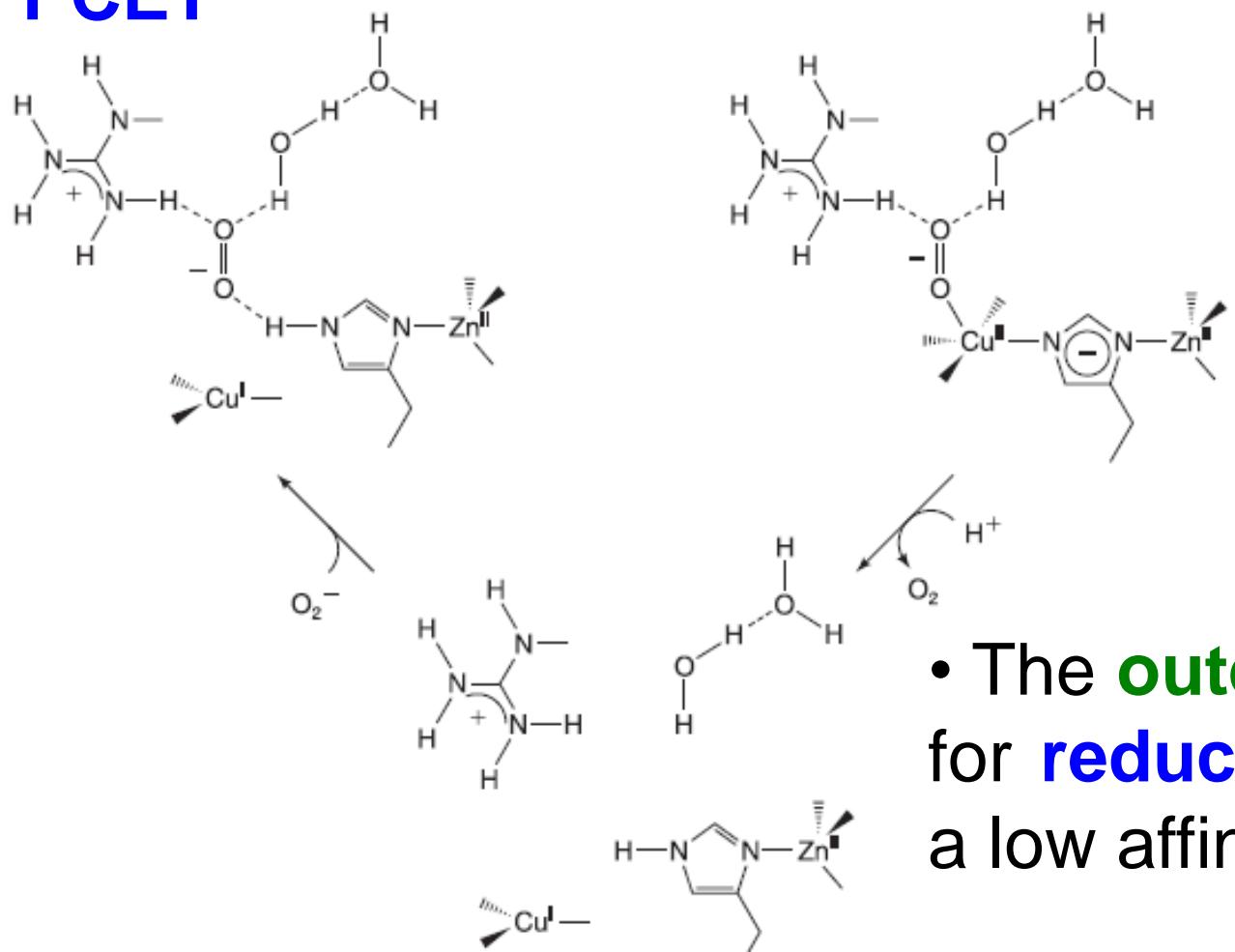
Oxidized
5-coordinate Cu(II)



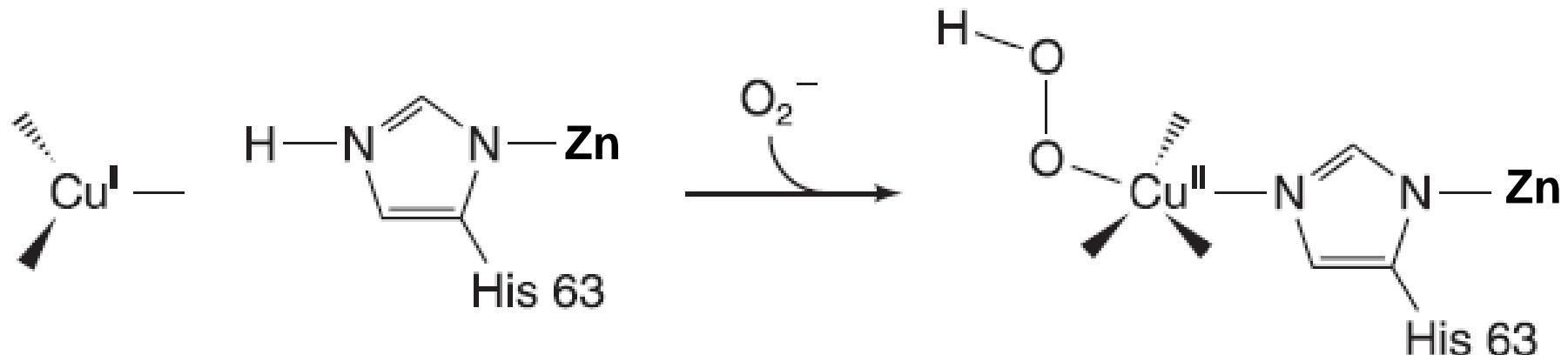
Proposed Mechanism

- The **inner-sphere** pathway for **oxidation of O_2^-** .
(Cu(II) → Cu(I))

PCET



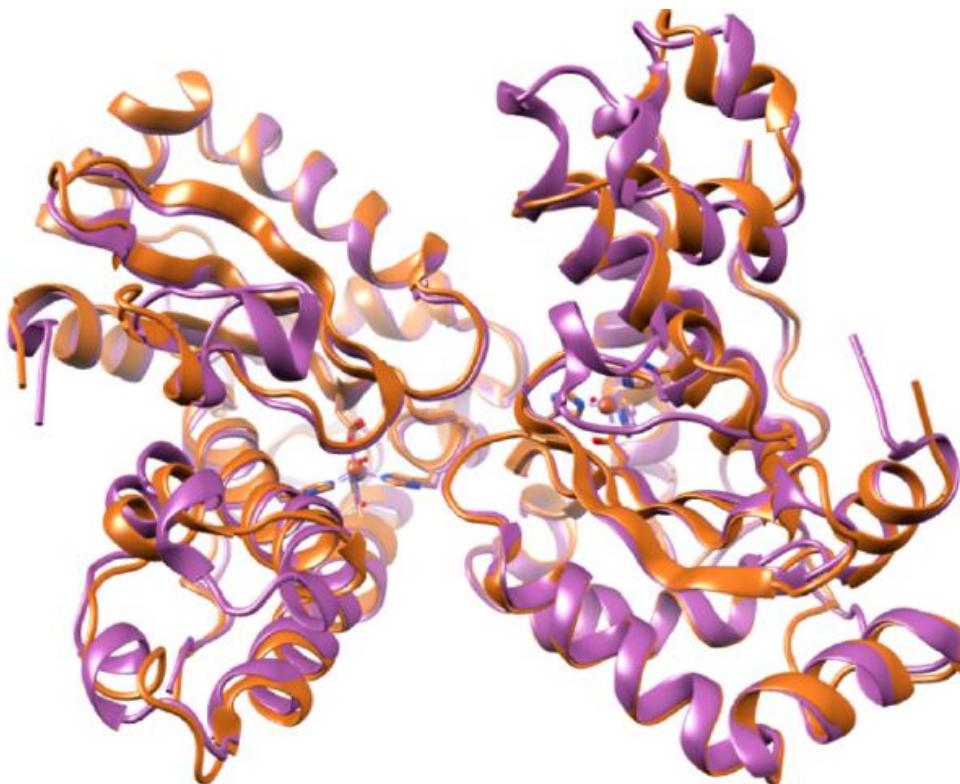
- The **outer-sphere** pathway for **reduction of O_2^-** , due to a low affinity of the **Cu(I)** ion.



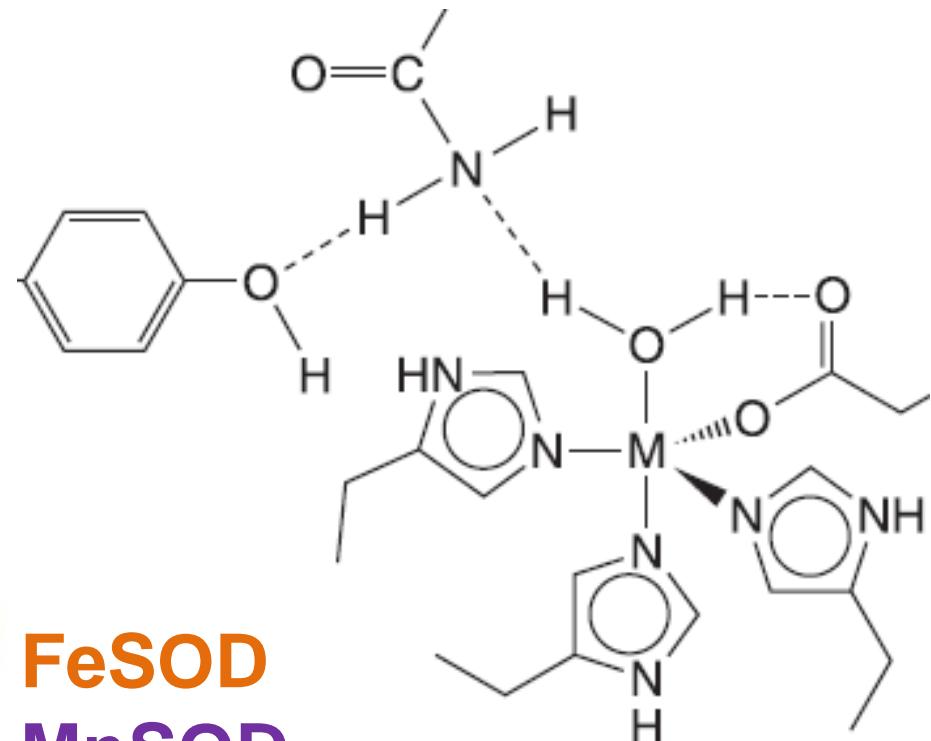
- The proton on **His63** possibly acts as a **proton donor** to promote the formation of the **Cu(II)-OOH** product.
- Removal of the **Zn** part makes the SOD activity pH dependent: affect the oxidation of the Cu(I) form (rate determining & pH dependent).
- The **Zn** part was proposed to induce a **weaker binding** of the **HOO⁻** part to the **Cu(II)** in an axial position: **rapid dissociation** of the **H₂O₂** product.

MnSOD & FeSOD

- MnSODs were found in prokaryotic & eukaryotic cells, while FeSODs were mainly found in prokaryotes.
- Very **similar protein structures & active-sites**, but most of them have high metal ion specificity with only one of the metal ions.



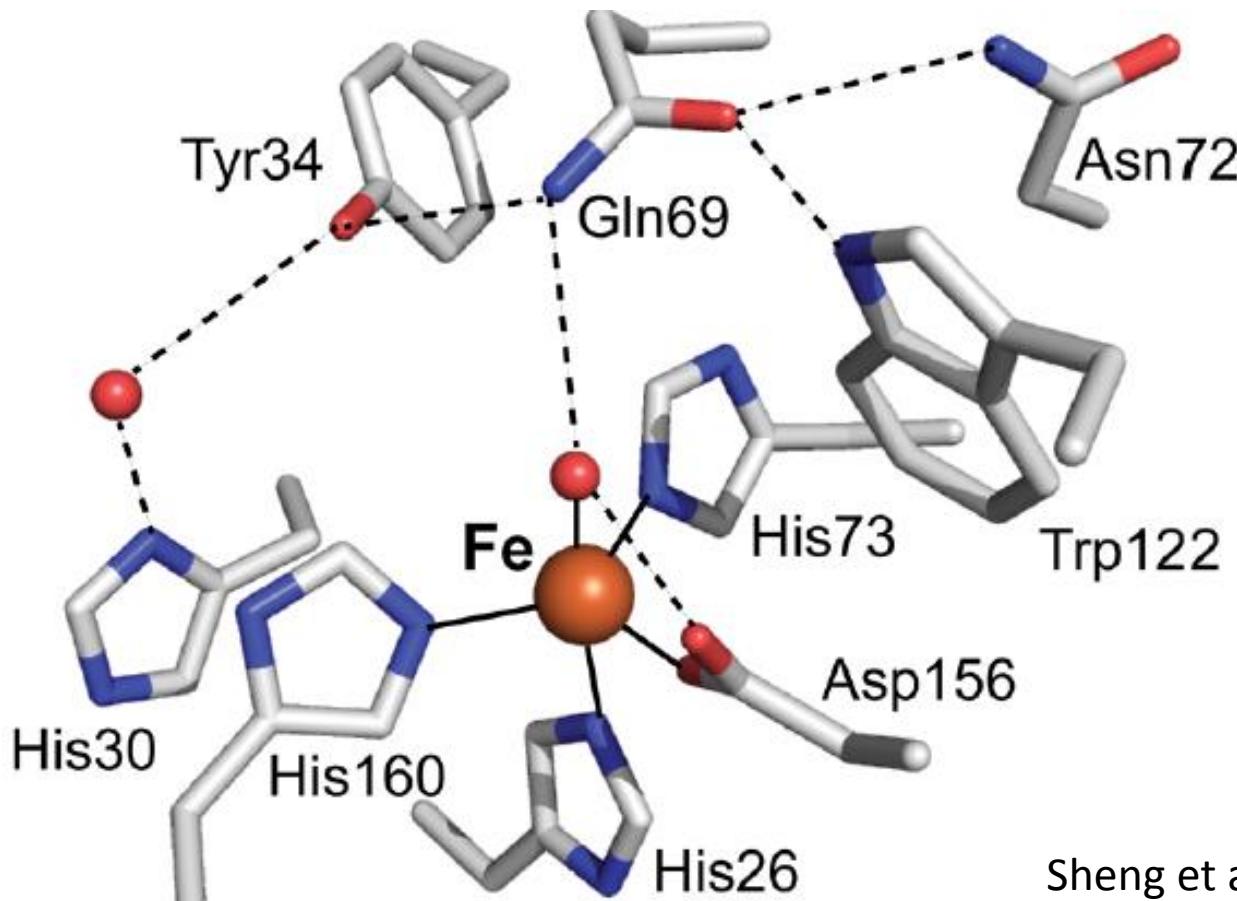
FeSOD
MnSOD



The 5-coordinate active site:

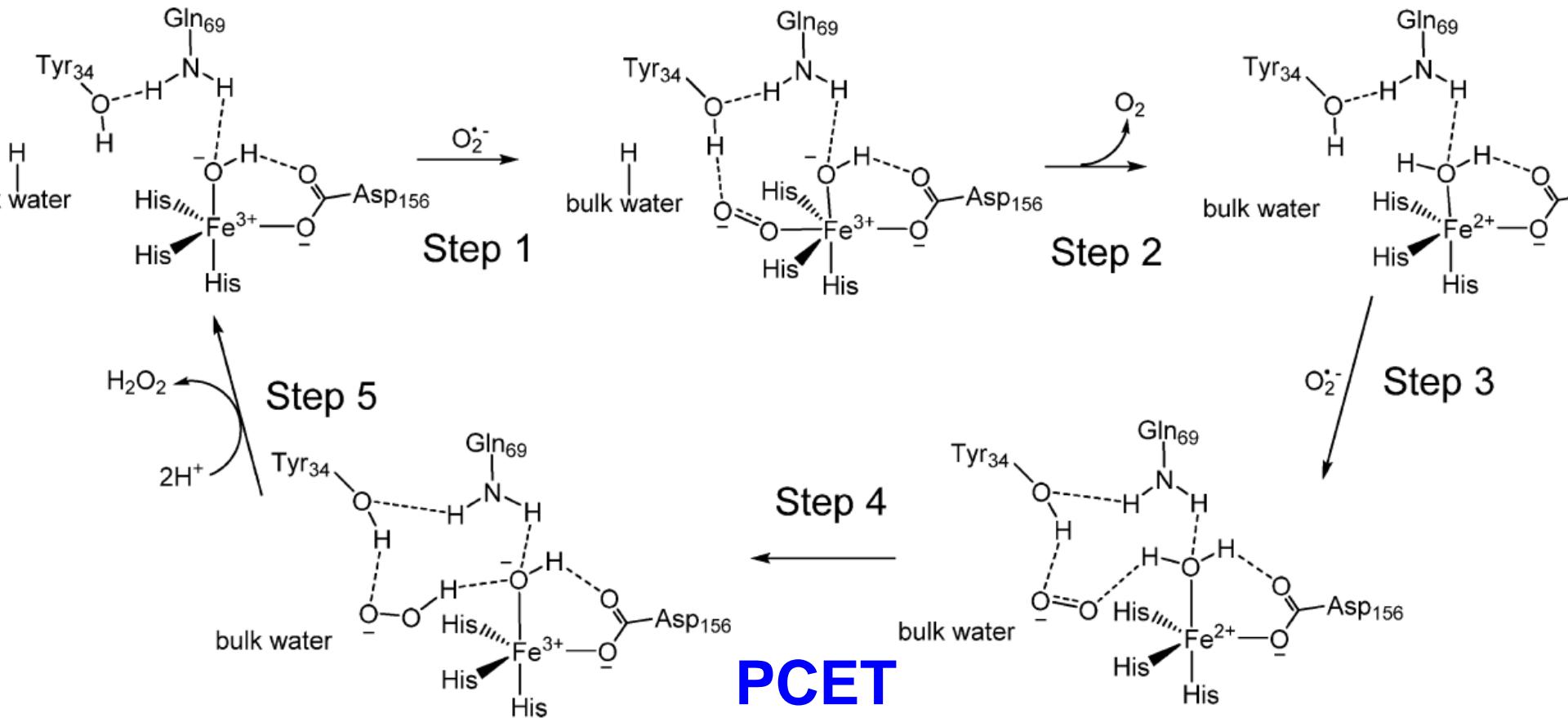
The reduced form with **M(II)**: 3 His, 1 Asp & 1 H₂O.

The oxidized form with **M(III)**: 3 His, 1 Asp & 1 OH.



Proposed Mechanism

- The **inner-sphere** pathway for the very **fast oxidation of O_2^-** ($\sim 10^9 \text{ M}^{-1}\text{s}^{-1}$): (**Fe(III) → Fe(II)**)

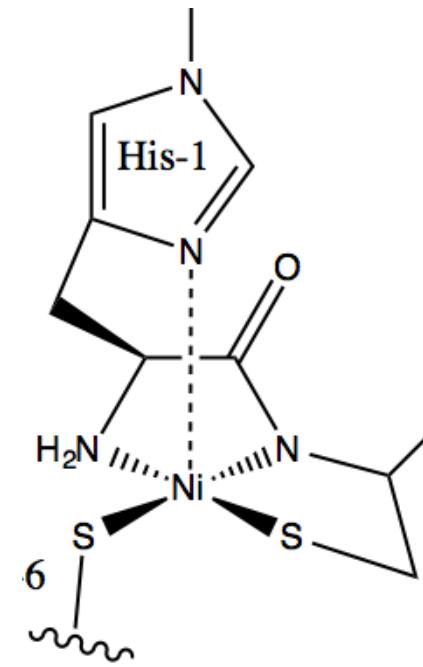
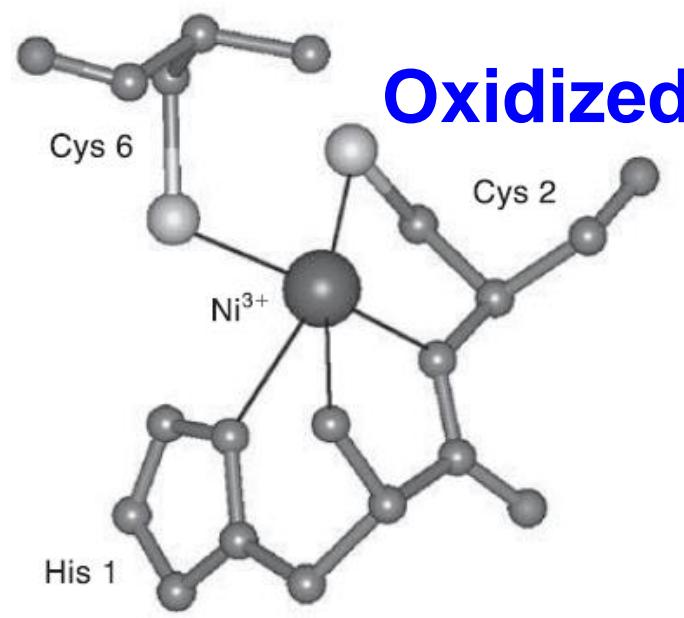
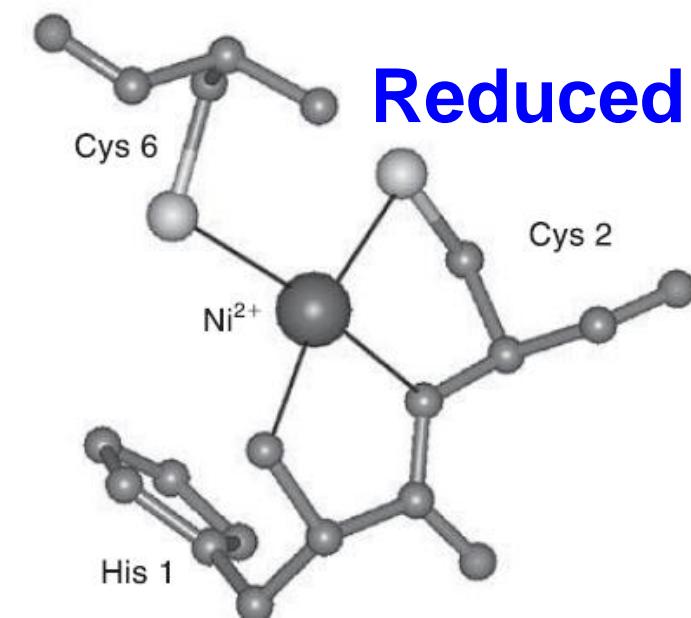
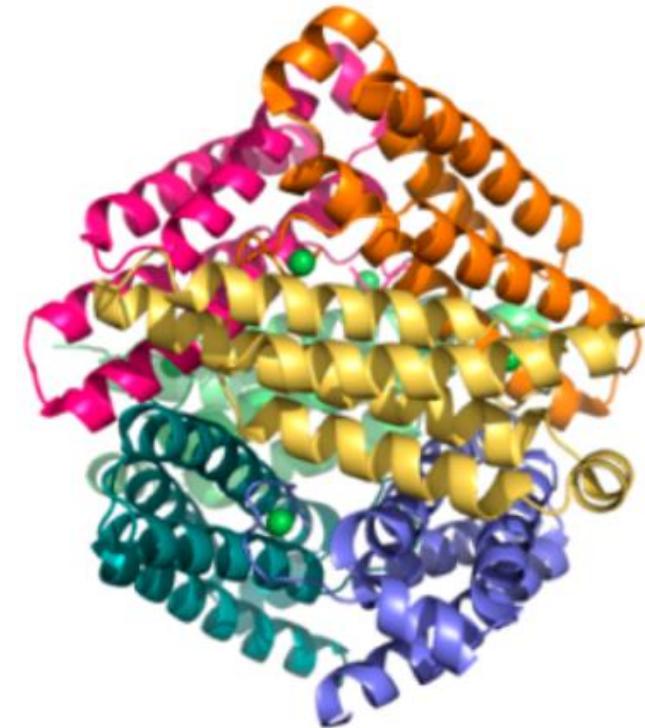


Sheng et al. Chem. Rev. 2014, 114, 3854

- The **outer-sphere** pathway for the **reduction of O₂⁻**.

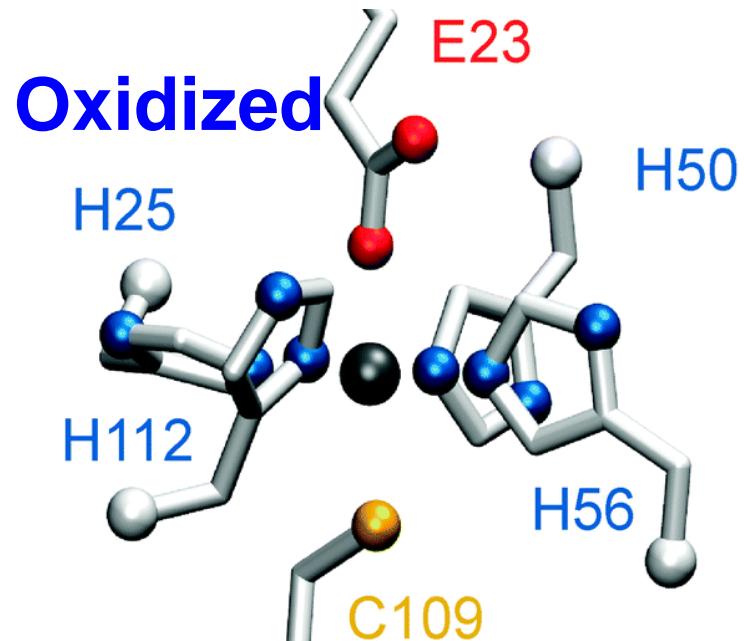
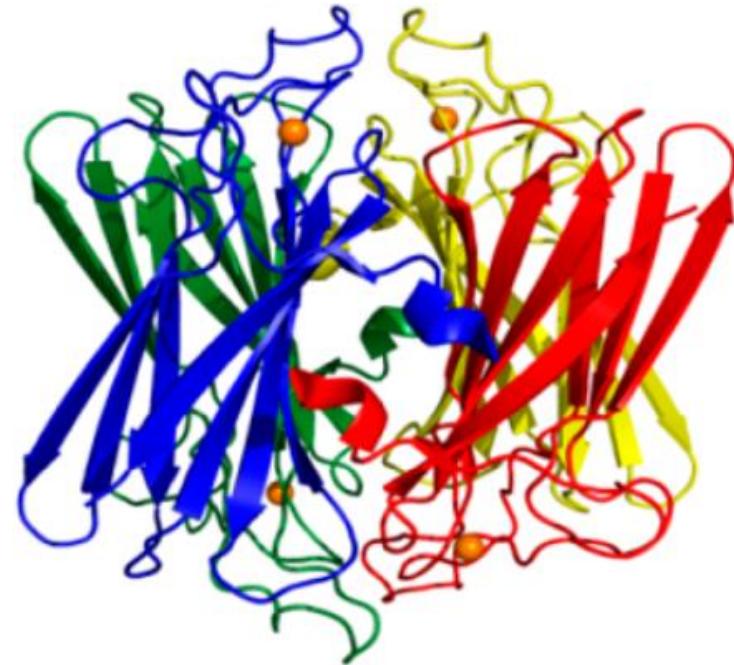
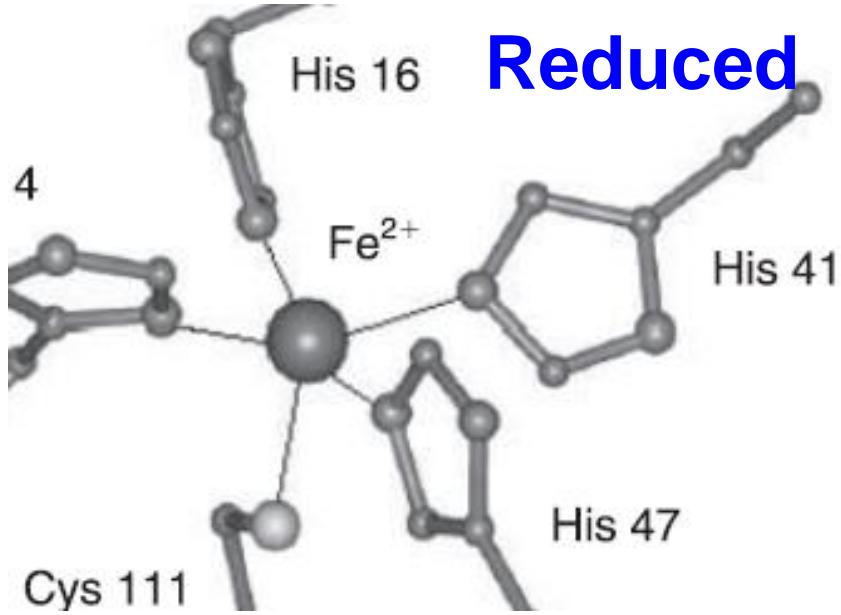
NiSOD

- Found in some *Streptomyces* species & cyanobacteria.
- **5-coordinate Ni(III) (oxidized)** with 2 Cys, 1 His + its amino group & 1 backbone amide N.
- **4-coordinate Ni(II) (no His).**
- Proposed **outer-sphere** pathway to protect oxidation of Cys.

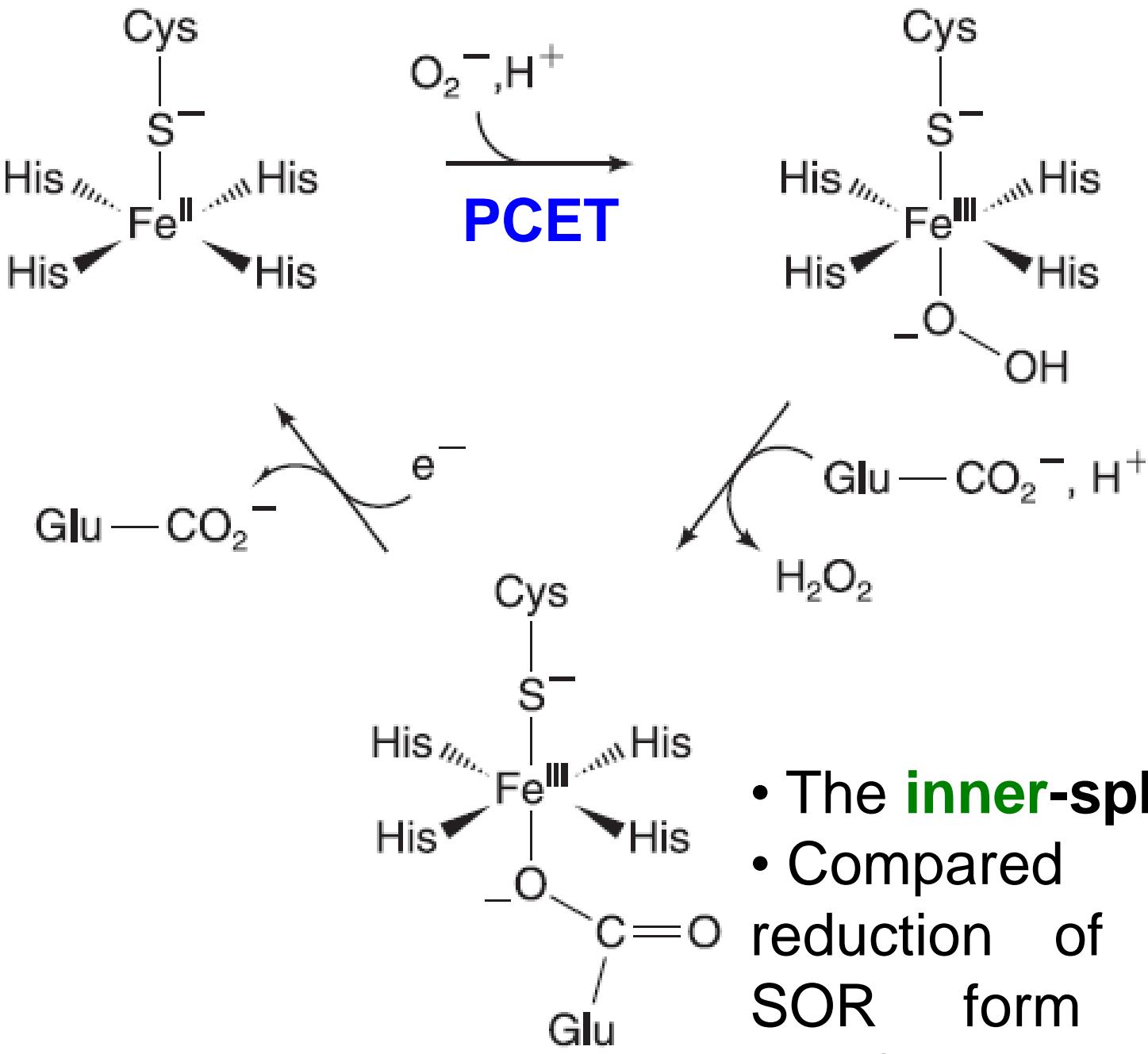


SOR (Fe)

- Found in some anaerobic & microaerophilic bacteria.
- Catalyze the **1e⁻ reduction of O₂^{·-}** to give H₂O₂.
- 5-coordinate reduced **Fe(II)** form with 4 His & 1 Cys.
- Glu can coordinate to the oxidized **Fe(III)** form.



Proposed Mechanism



- The **inner-sphere** pathway.
- Compared to SODs, reduction of the oxidized SOR form by O_2^- is slow/ineffective (Glu?).

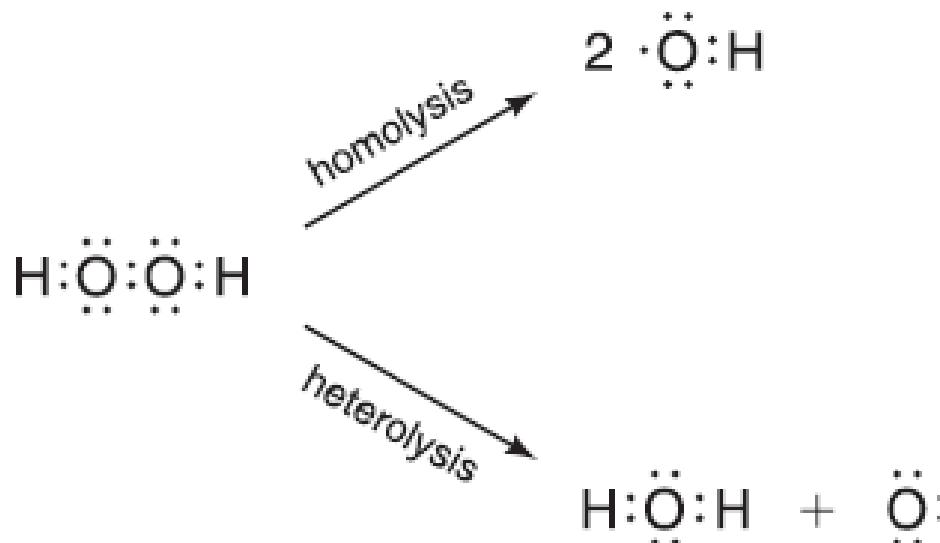
3. Peroxidases & Catalases

Peroxidase

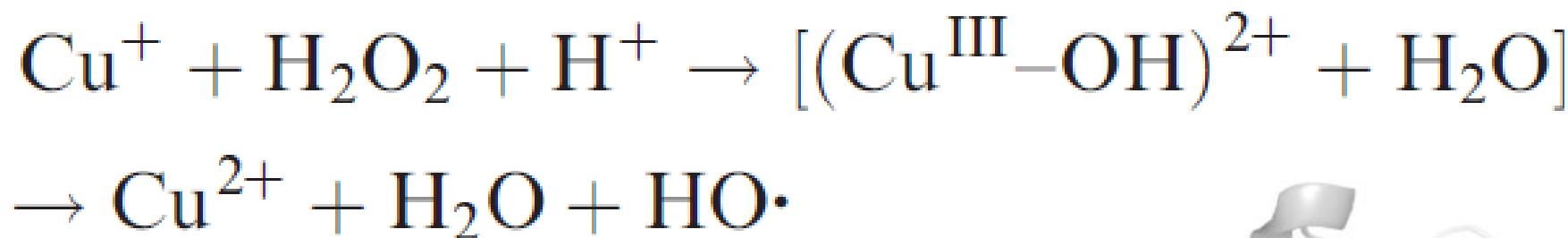
- H_2O_2 is used as the **oxidant** to oxidize different biologically important **molecules (S)**.



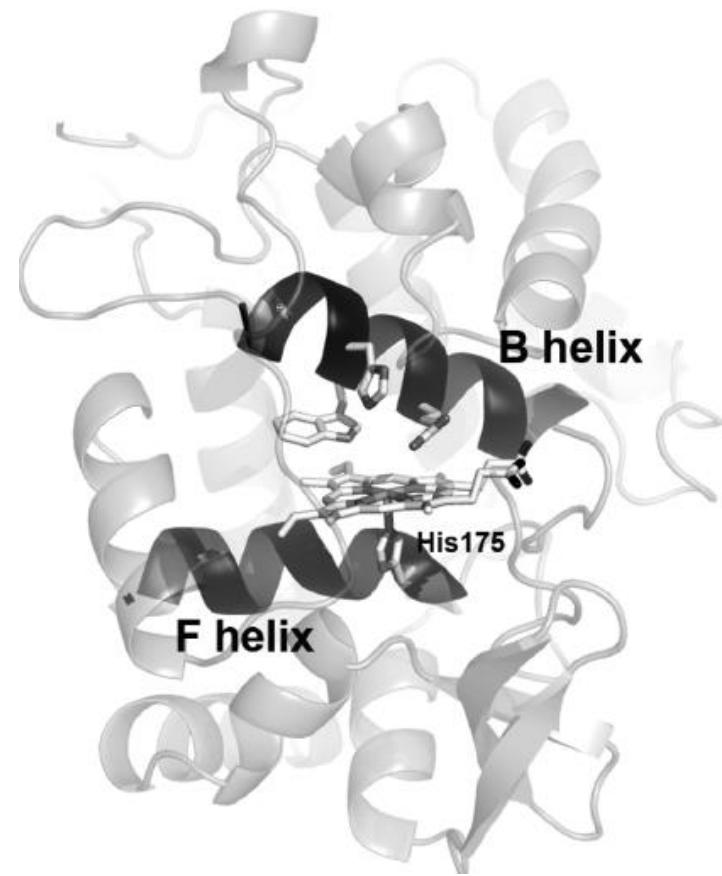
- **2 ways of the O-O cleavage:**
(homo- & heterolytic cleavage).



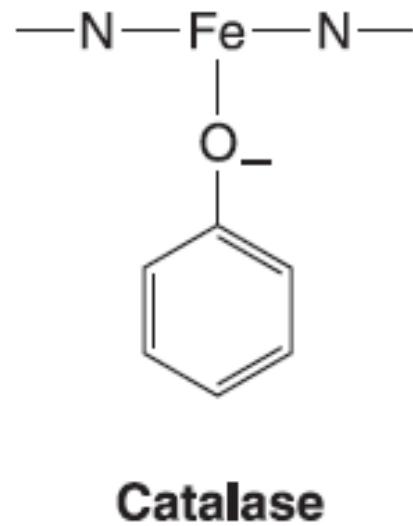
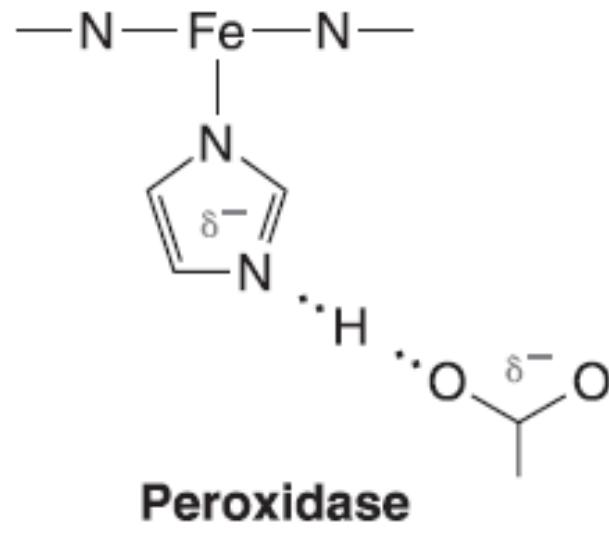
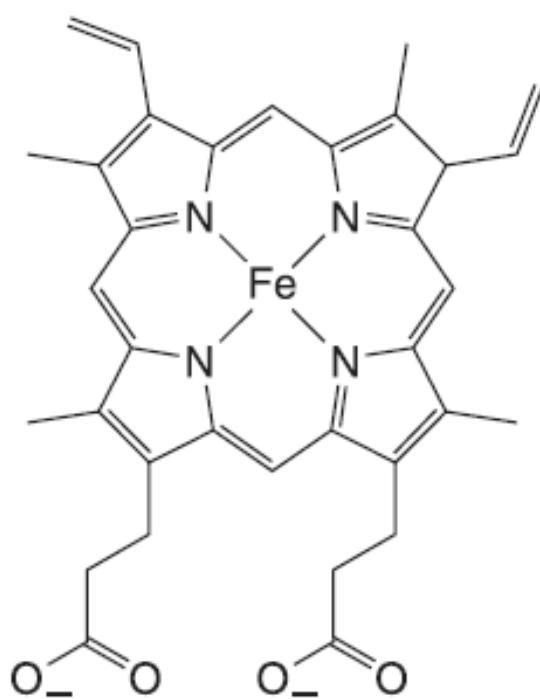
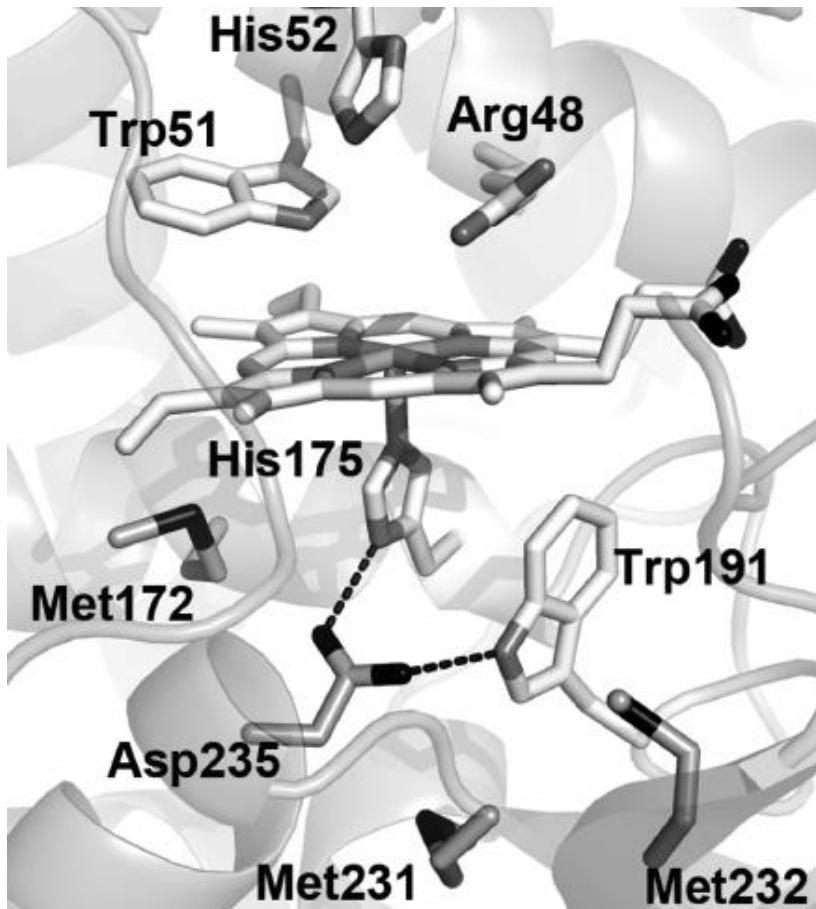
- Fe^{2+} or Cu^+ can catalyze the Fenton reaction to give reactive & toxic $\text{HO}\cdot$ radical (chemical reactions).



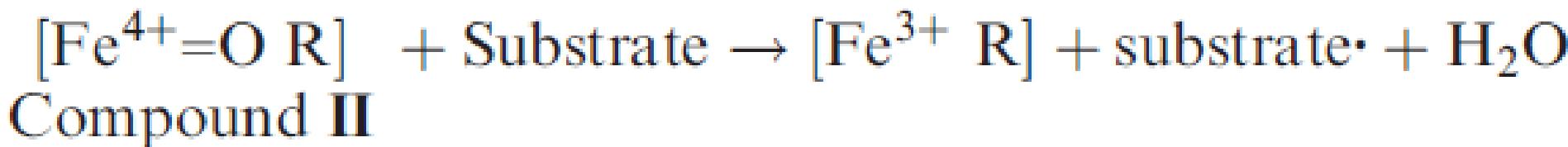
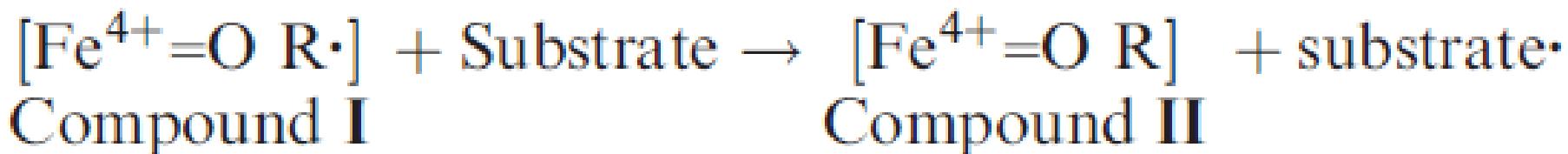
- **Peroxidases** undergo a **heterolytic cleavage** not to form toxic $\text{HO}\cdot$, but form H_2O & high-valent $\text{Fe}=\text{O}$.



- The proximal His forms a H-bond with the conserved Asp → more **anionic character** of that His to stabilize the Fe(III) center (not in the globins).

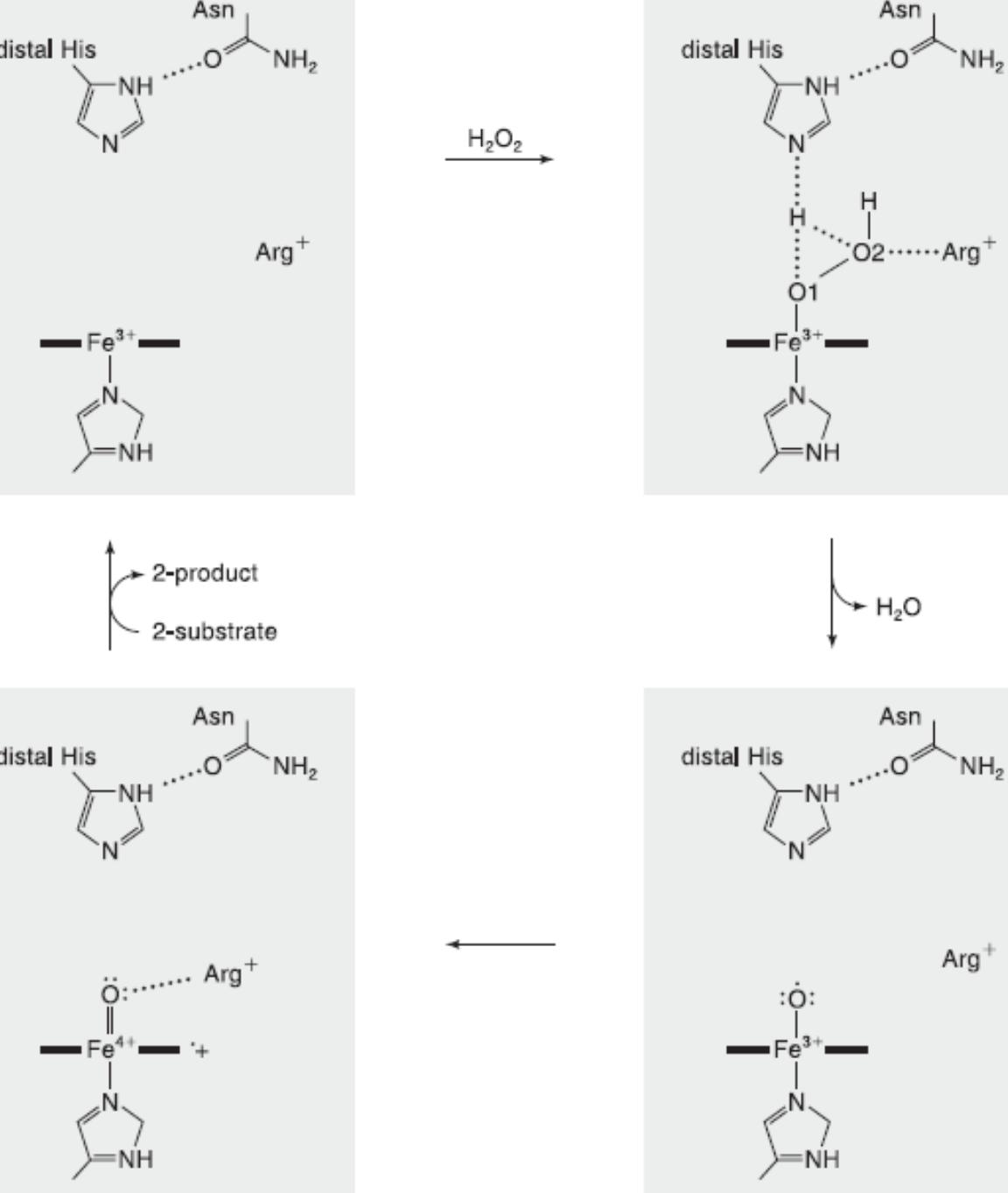


Proposed Mechanism



(R: porphyrin or amino acid side chain)

- (1) Heterolytic cleavage to form H_2O & high-valent **Compound I** (the porphyrin radical in most peroxidases, while Trp radical in Cyt c peroxidase).
- (2) **Reduction of Compound I** to **Compound II** by the 1st small aromatic substrate (e.g. phenol).
- (3) **Compound II** is further **reduced** to **Fe(III)** by the 2nd substrate.

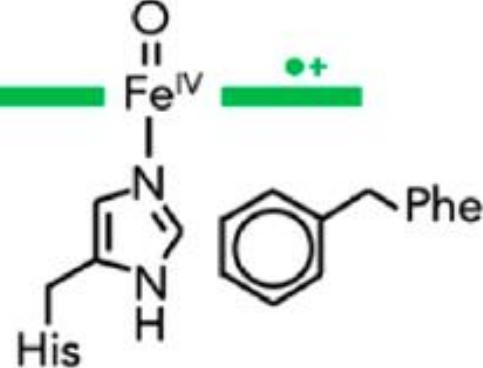


- The **distal His** acts as the general acid-base catalyst to promote the heterolytic cleavage.

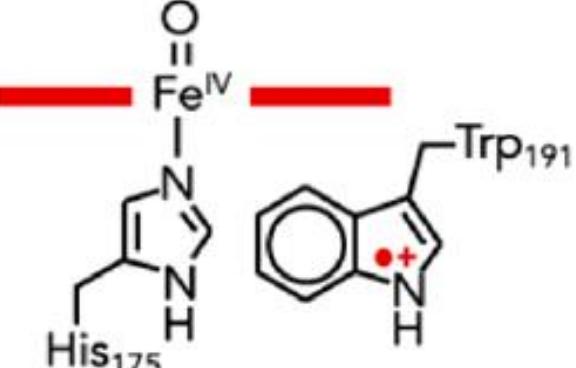
- Arg stabilizes the reactive oxo ligand.

Compound I

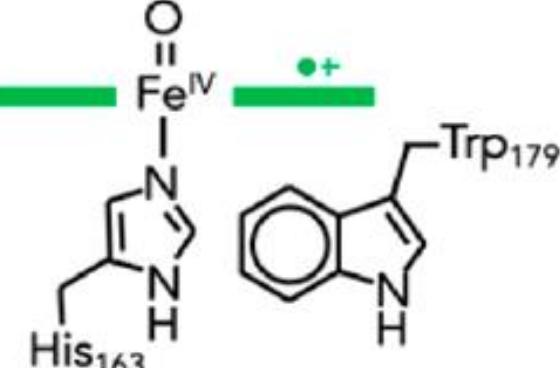
Hypothetical oxene intermediate



HRP

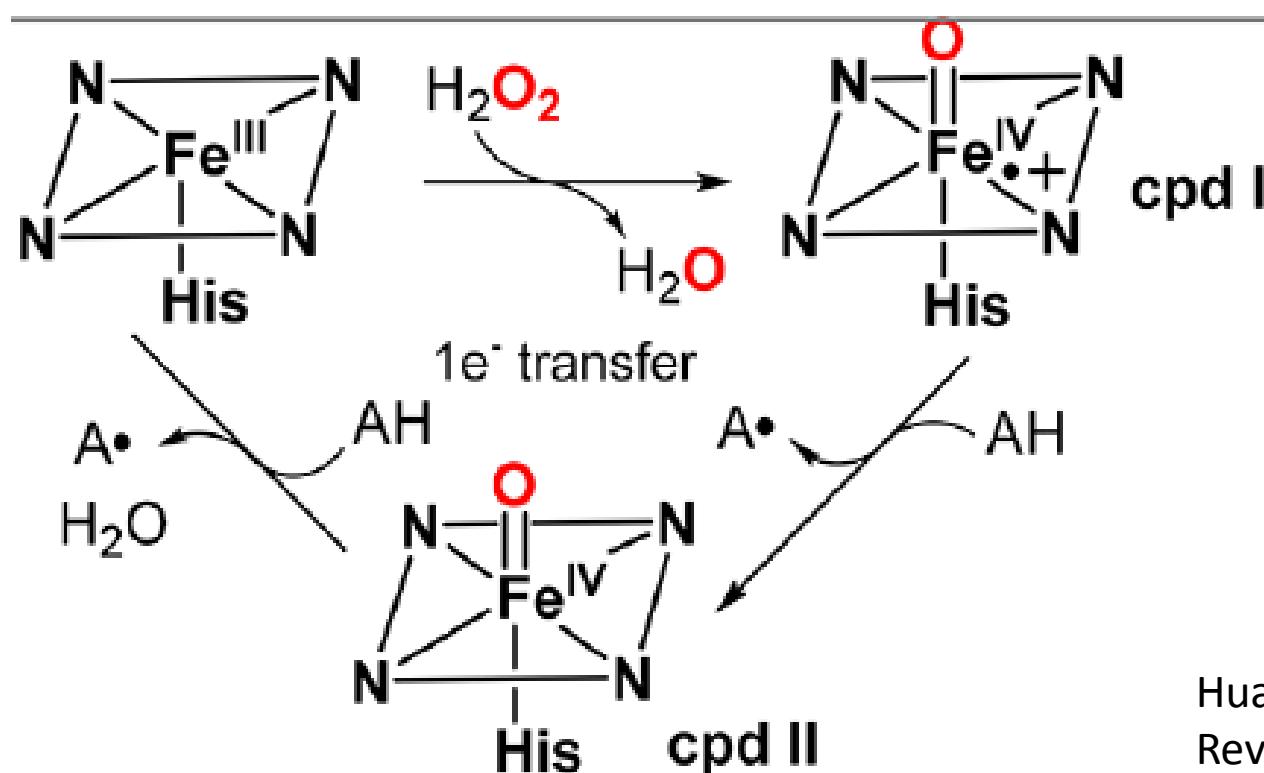


CcP

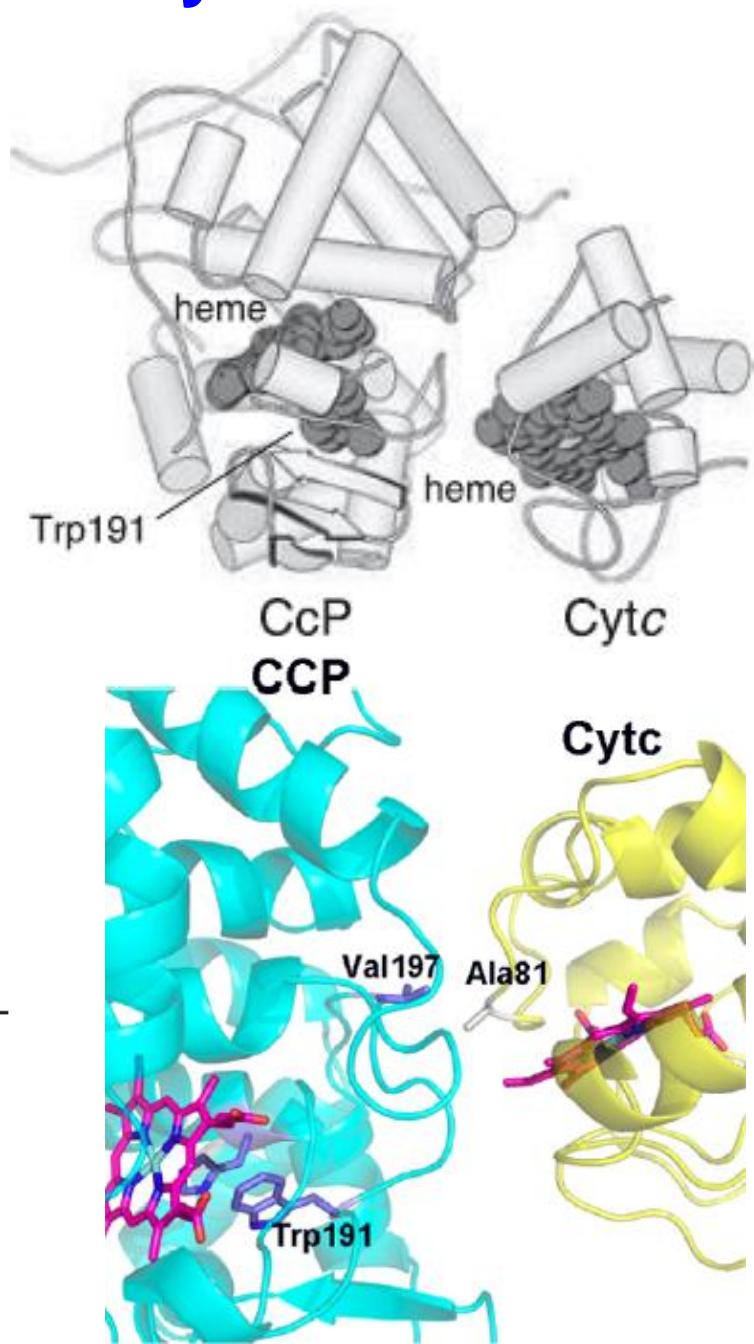
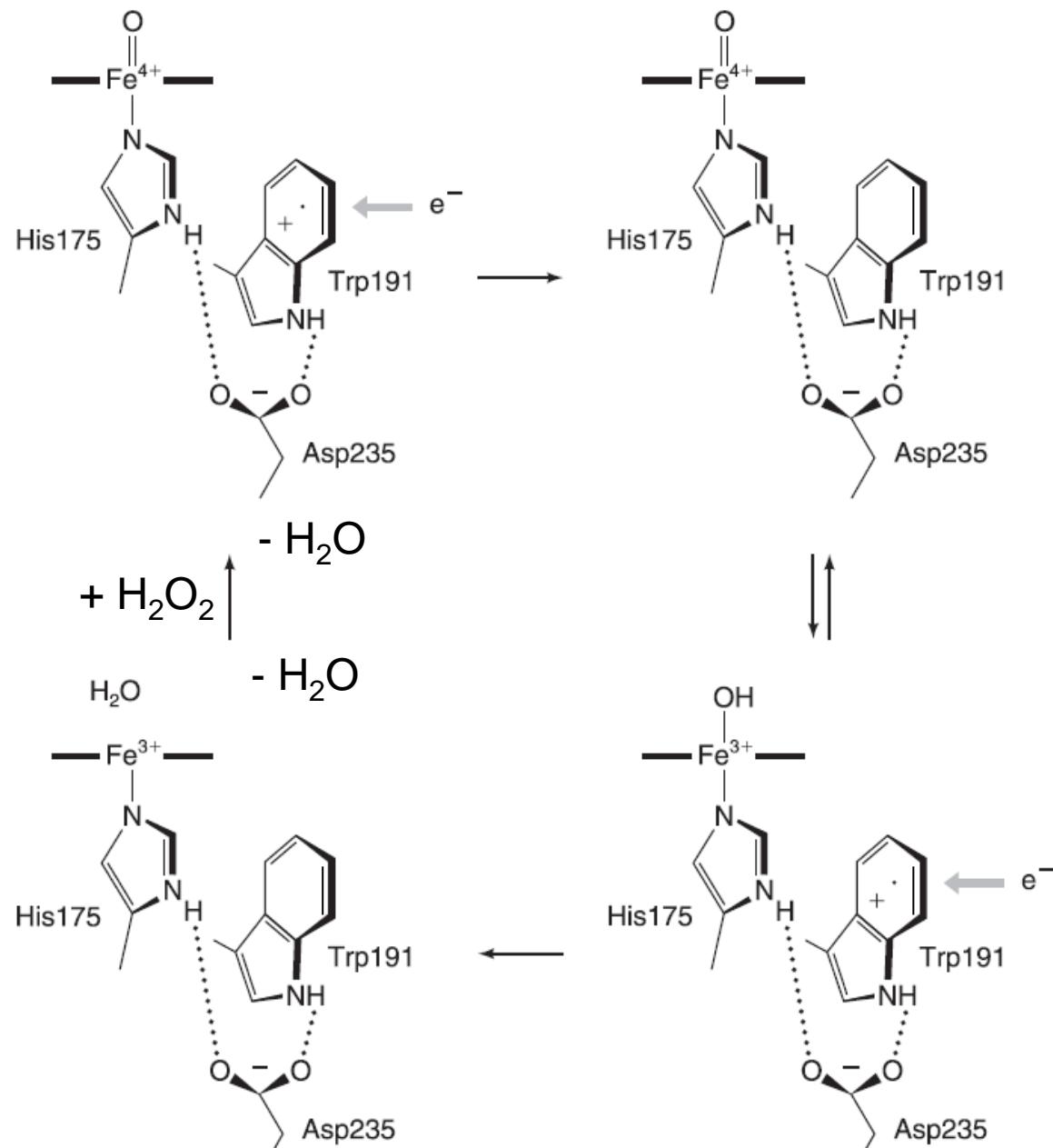


APX

Compound I (Cpd I)

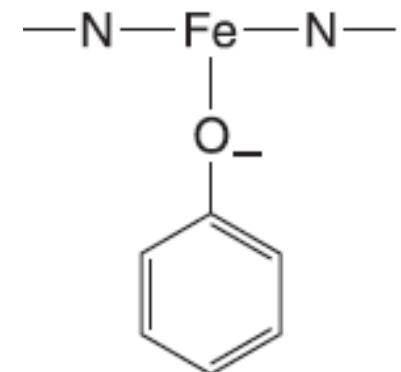
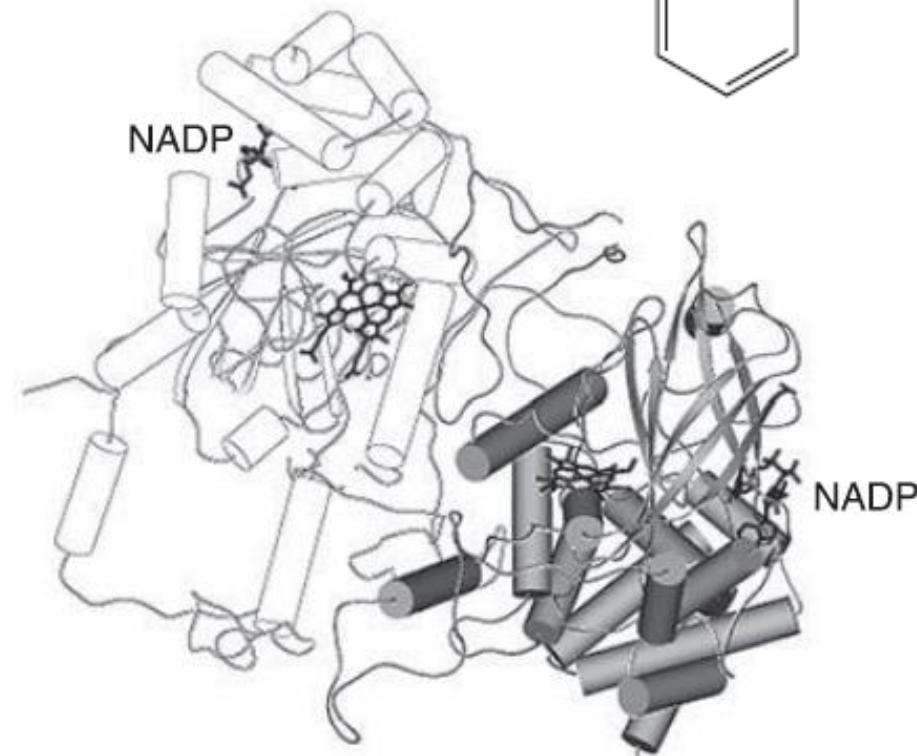
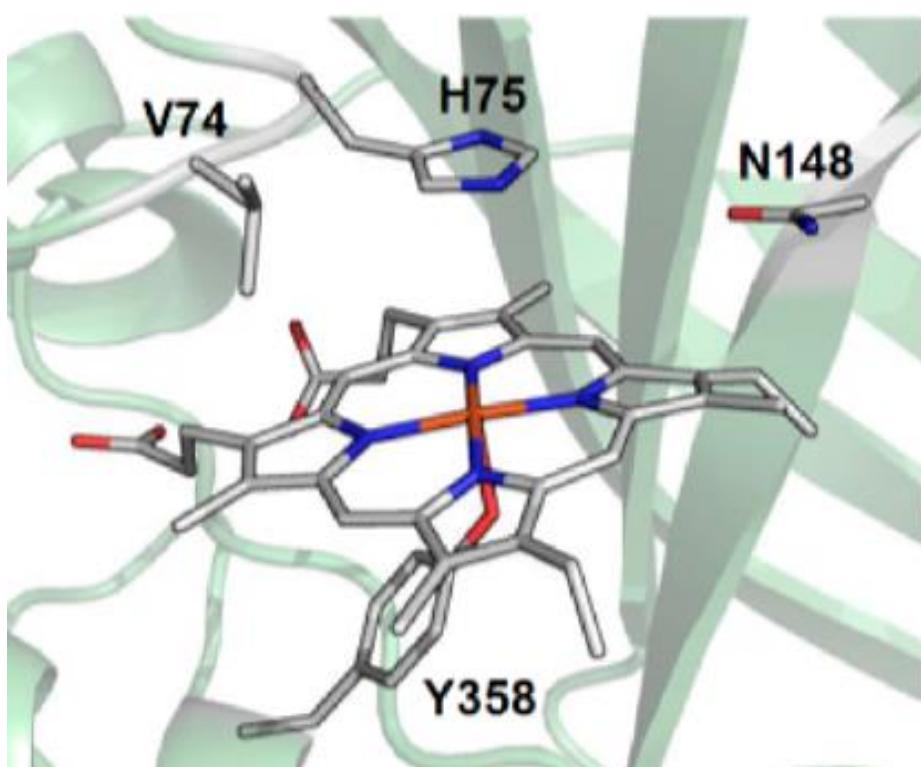


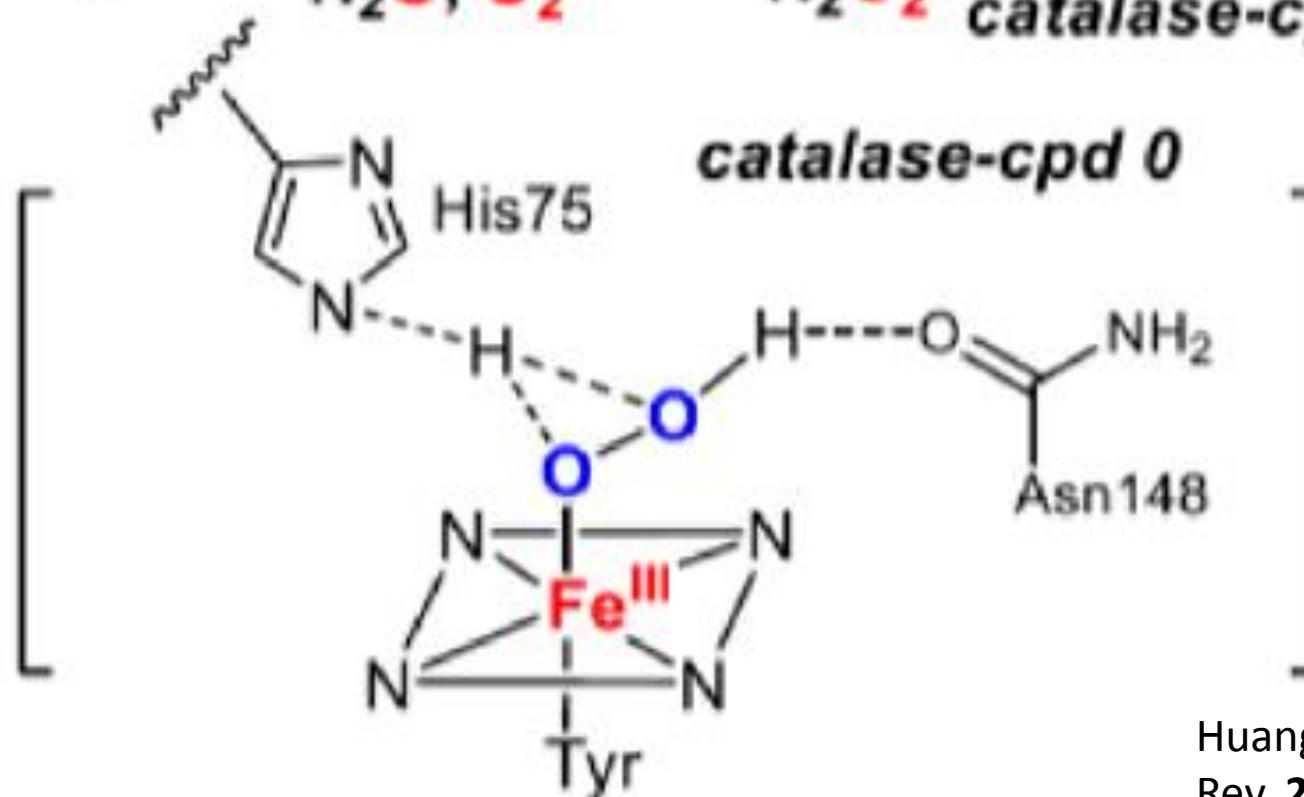
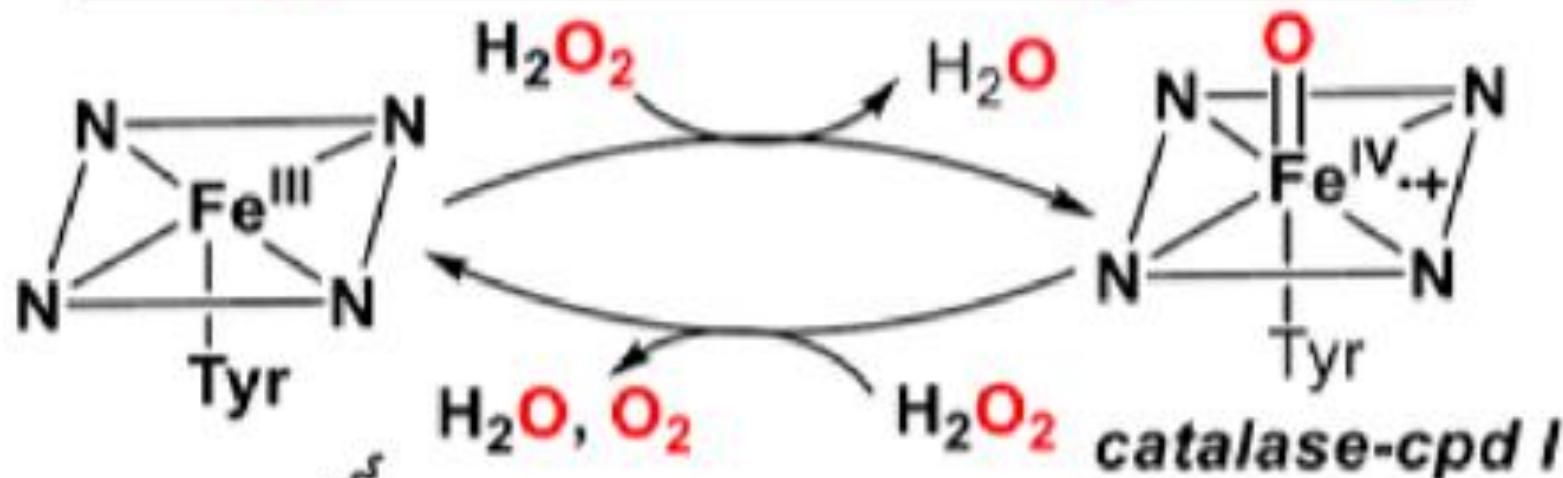
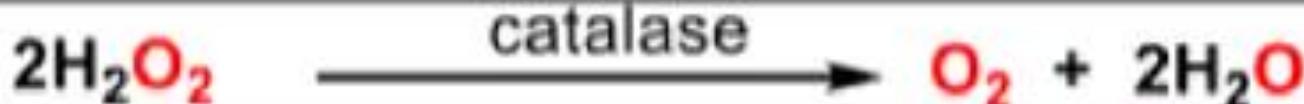
Proposed Reduction/ET step in Cyt c Peroxidase



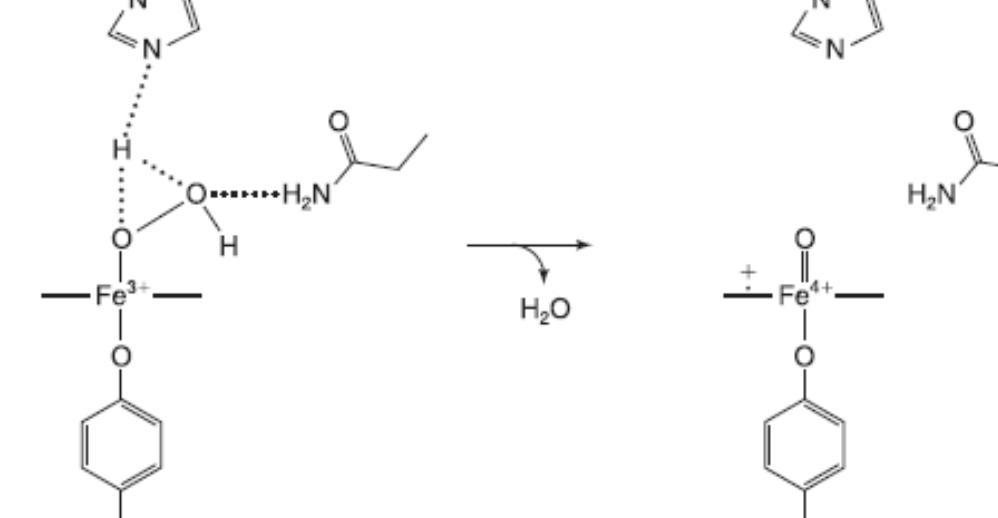
Catalases

- The distal His is parallel to the heme plane.
- The 1st example of hemes uses **Tyr** as the **proximal ligand**.
- **No oxidation of organic substrate** as in peroxidases.

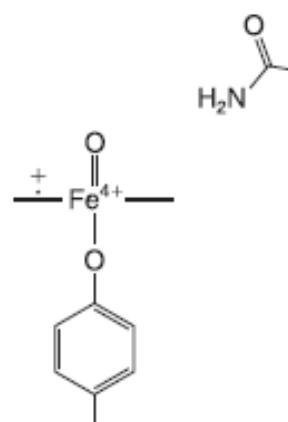




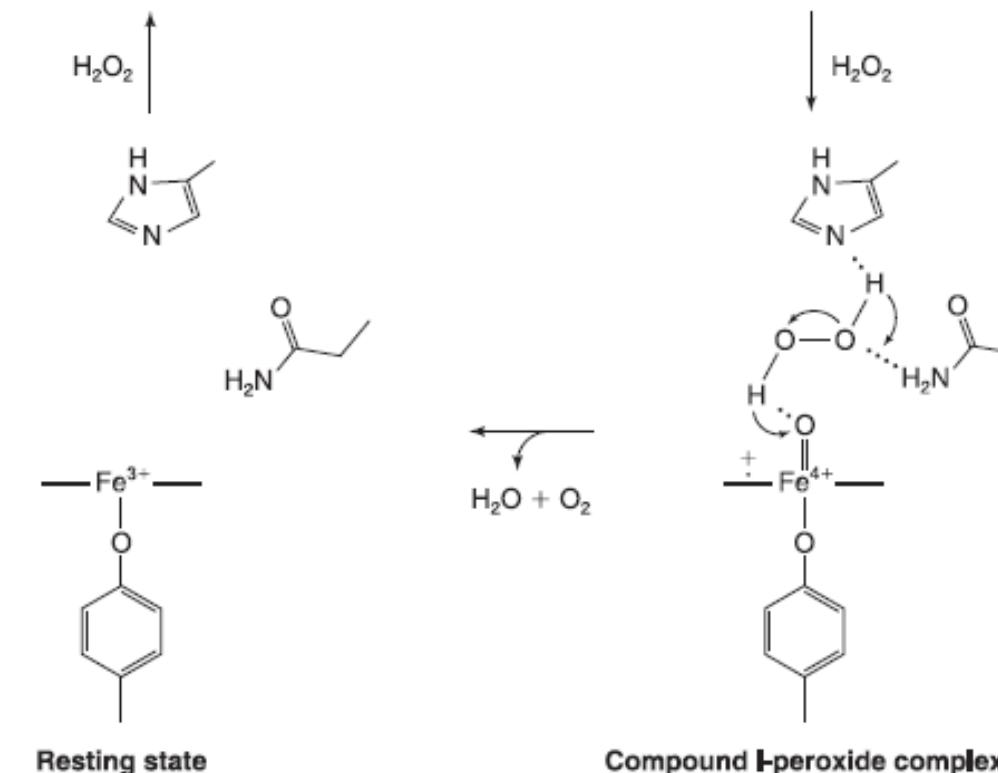
Proposed Mechanism



Peroxide complex



Compound I



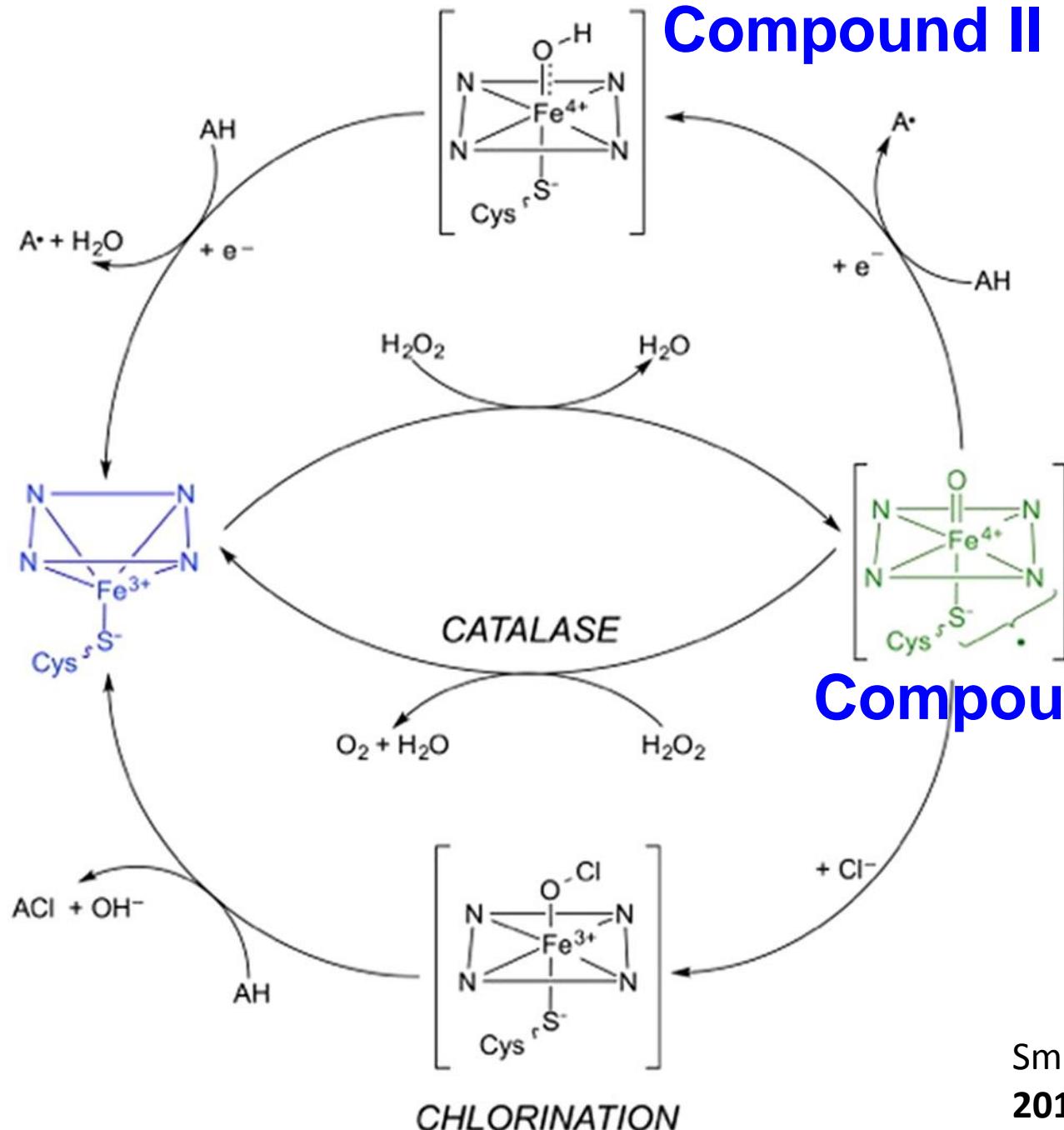
Resting state

- The distal His acts as the general acid-base catalyst to promote the **heterolytic cleavage**.

- A **2nd H_2O_2** molecule acts as the **reductant** to reduce Compound I in a 2e^- reduction process.

PEROXIDASE

Compound II



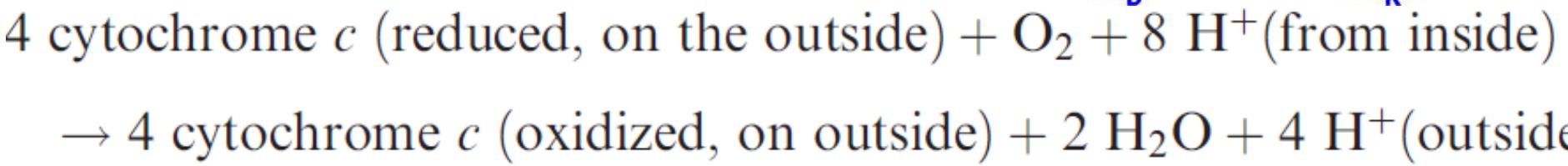
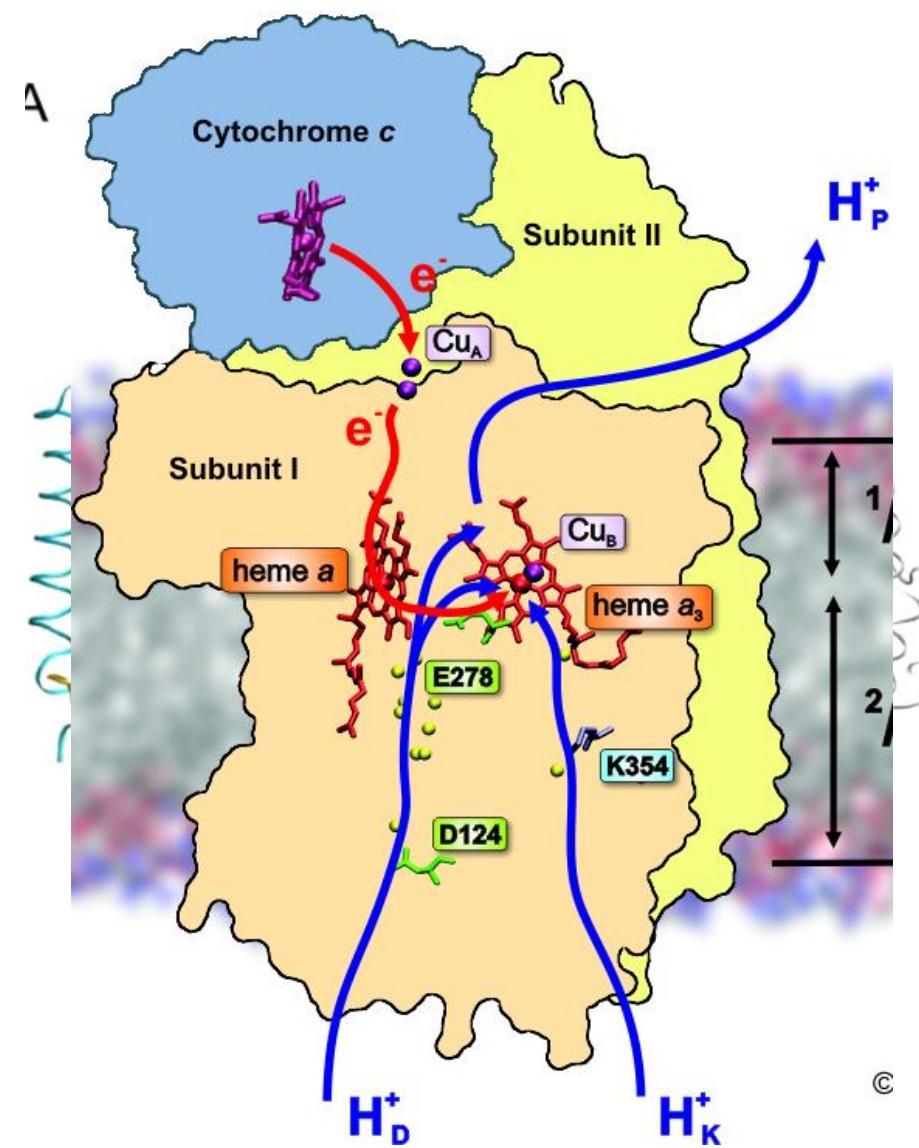
4. Cytochrome c Oxidase

Cytochrome c Oxidase

- **Complex IV** in the respiration chain.

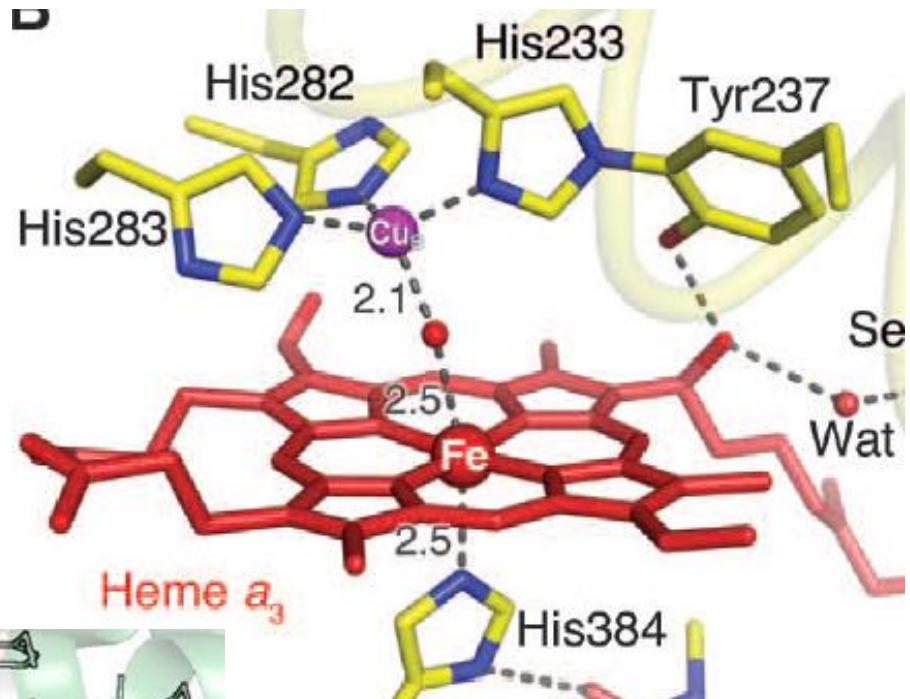
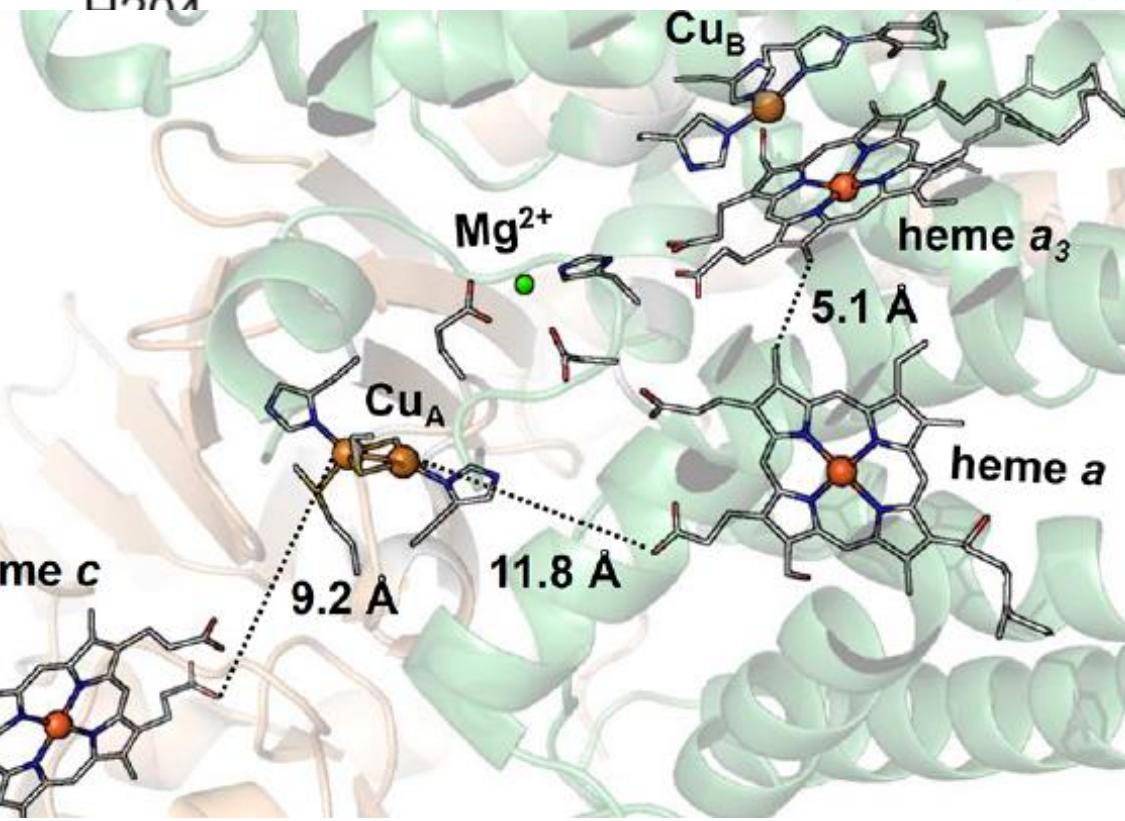
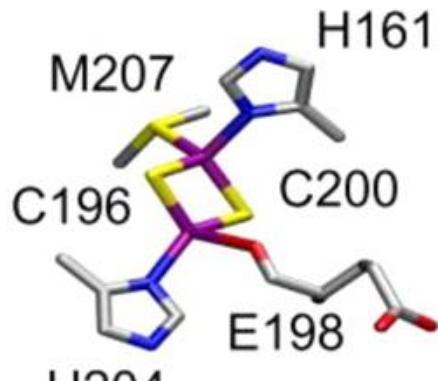
- Cyt c (e^- donor) & its electron acceptor Cu_A , heme a, heme a_3 , & Cu_B .

- With $4 H^+$, reduction of O_2 to form H_2O occurs at the bi-metallic heme a_3 - Cu_B center.

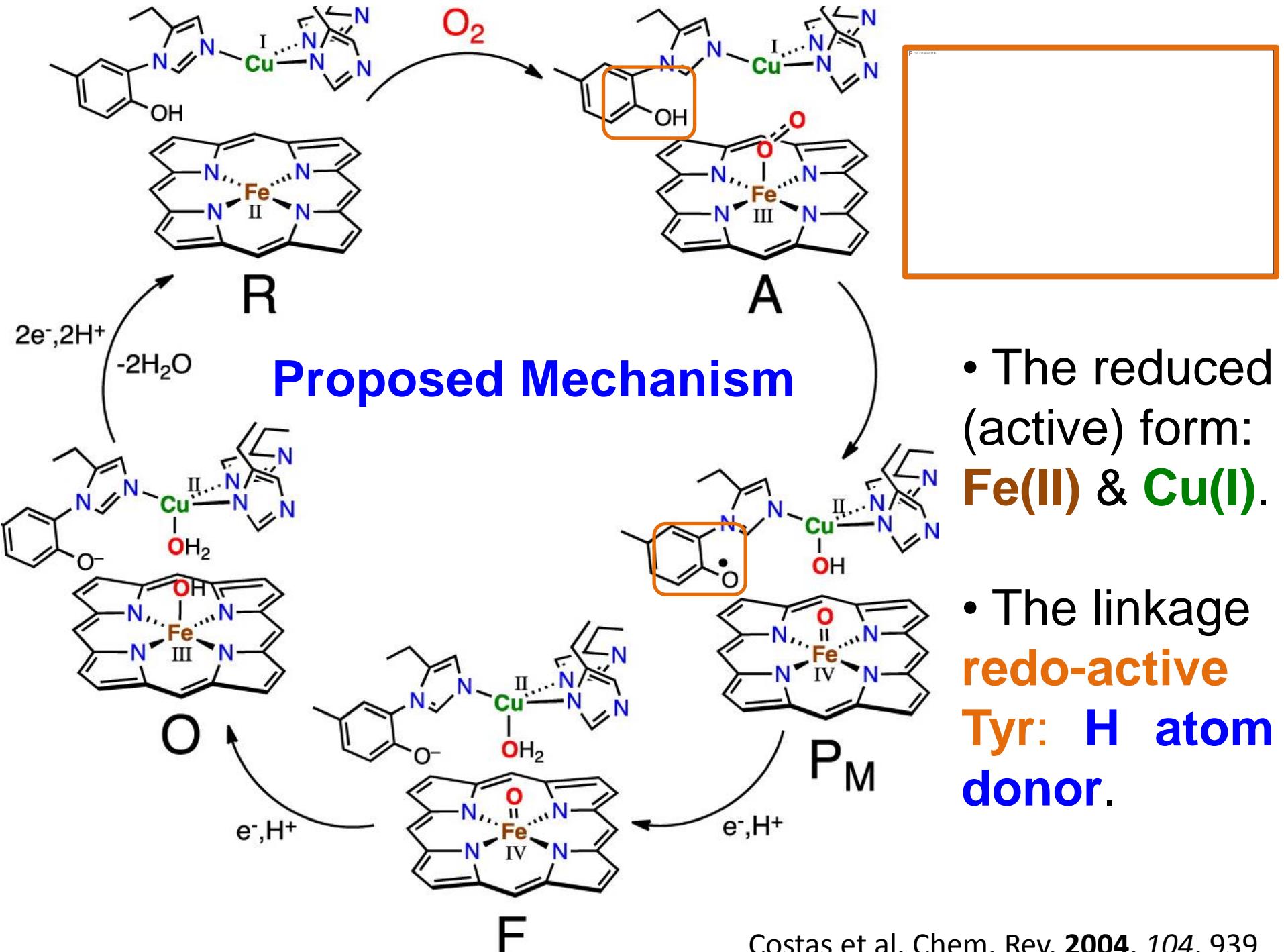


The Cofactors

CuA



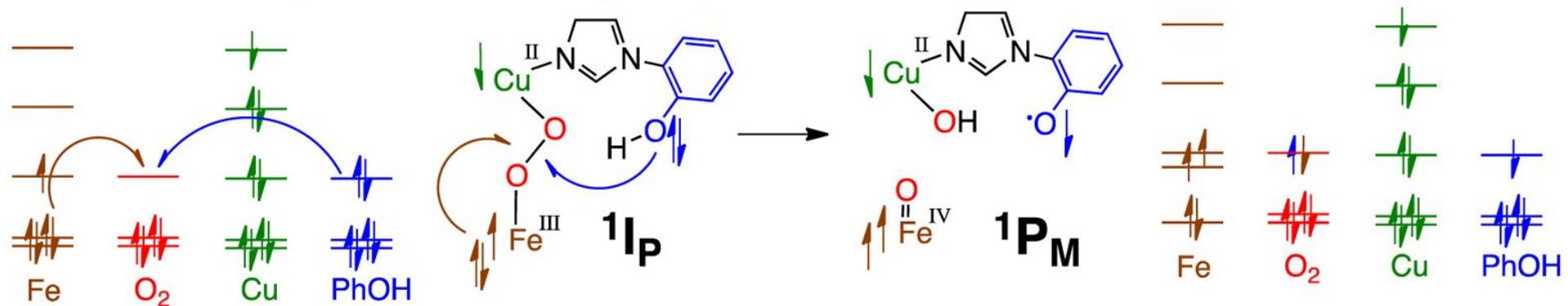
The bi-metallic
heme a_3 & Cu_B
center



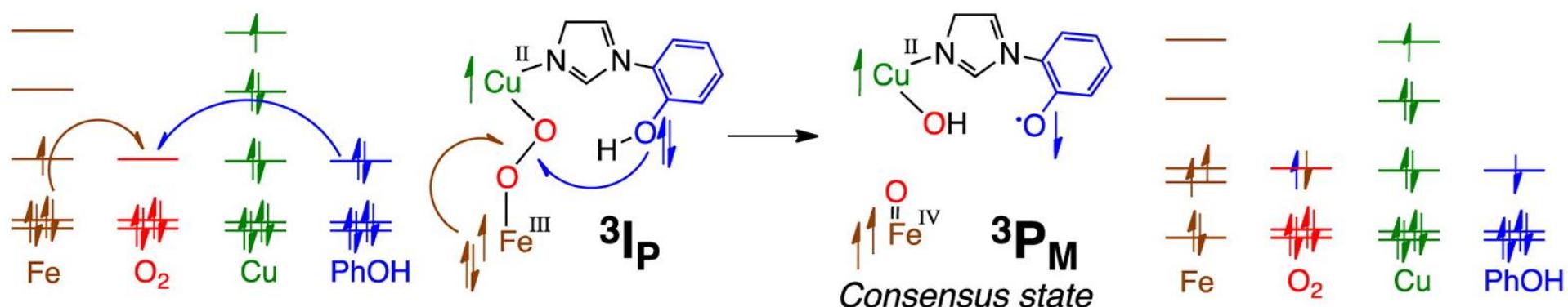
- The reduced (active) form: **Fe(II)** & **Cu(I)**.
- The linkage **redo-active** Tyr: **H** atom donor.

Proposed Electronic Structures

Anti-ferromagnetic coupling, $S_T = 0$

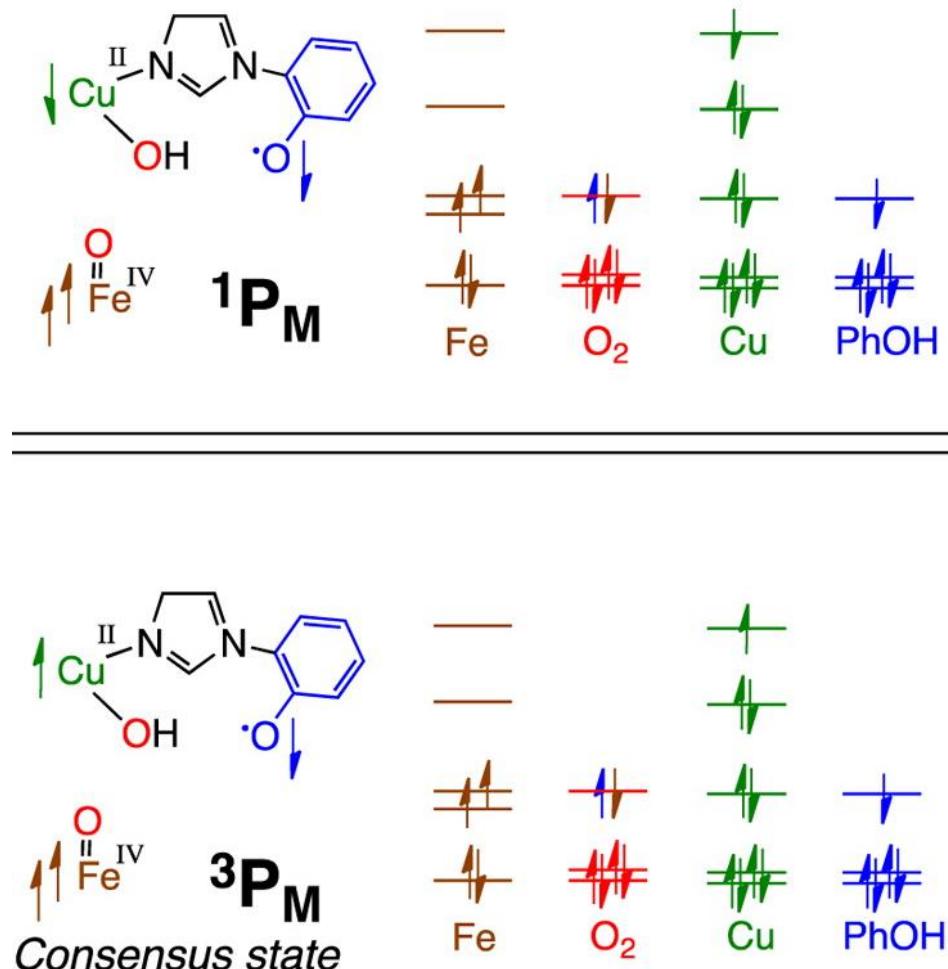
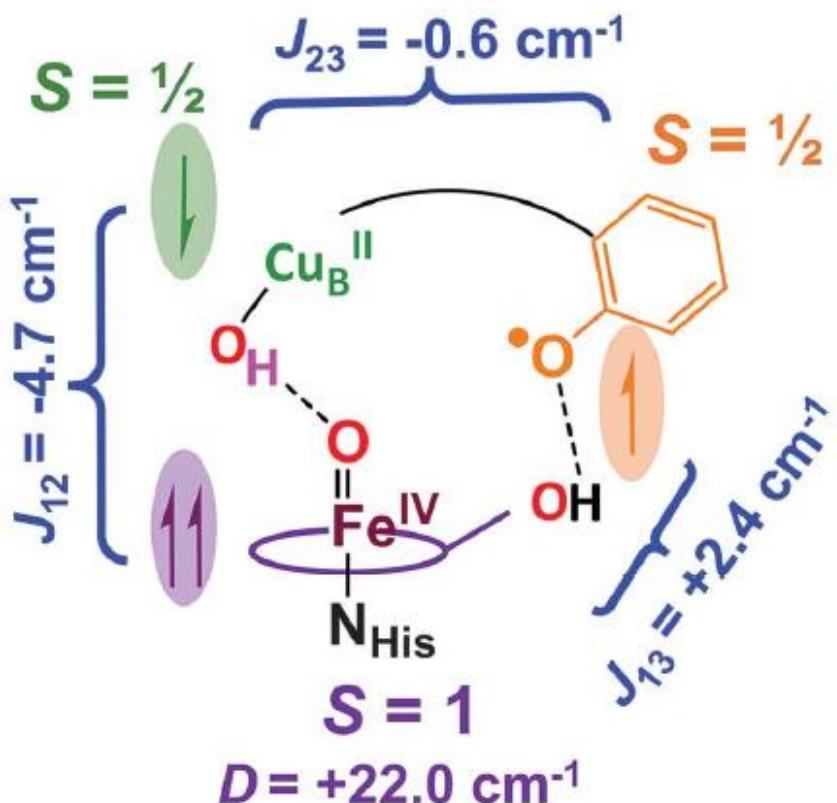


Ferromagnetic coupling, $S_T = 1$



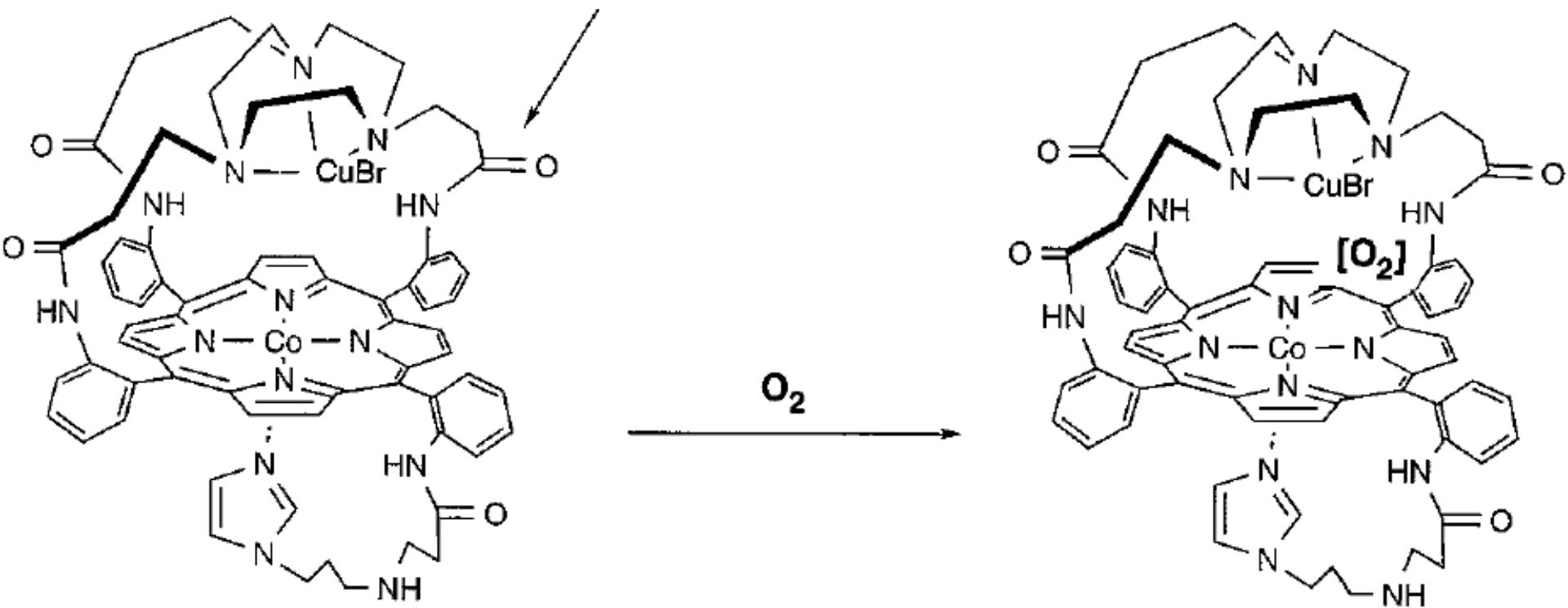
Electronic Structure of P_M (variable-temperature, variable-field magnetic circular dichroism spectroscopy)

D P_M Magnetic coupling scheme



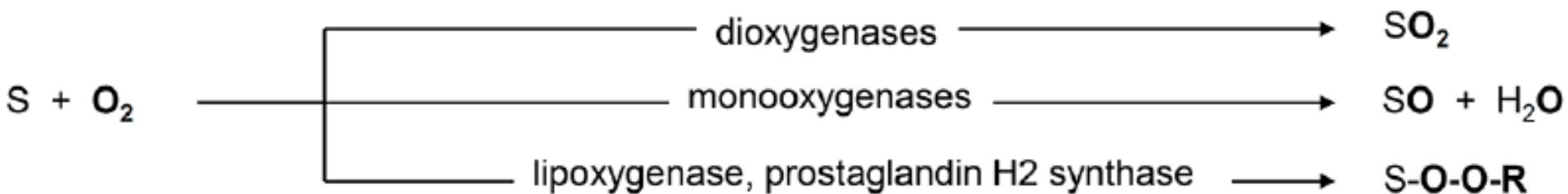
A Functional Model Related to Cytochrome c Oxidase and Its Electrocatalytic Four-Electron Reduction of O₂

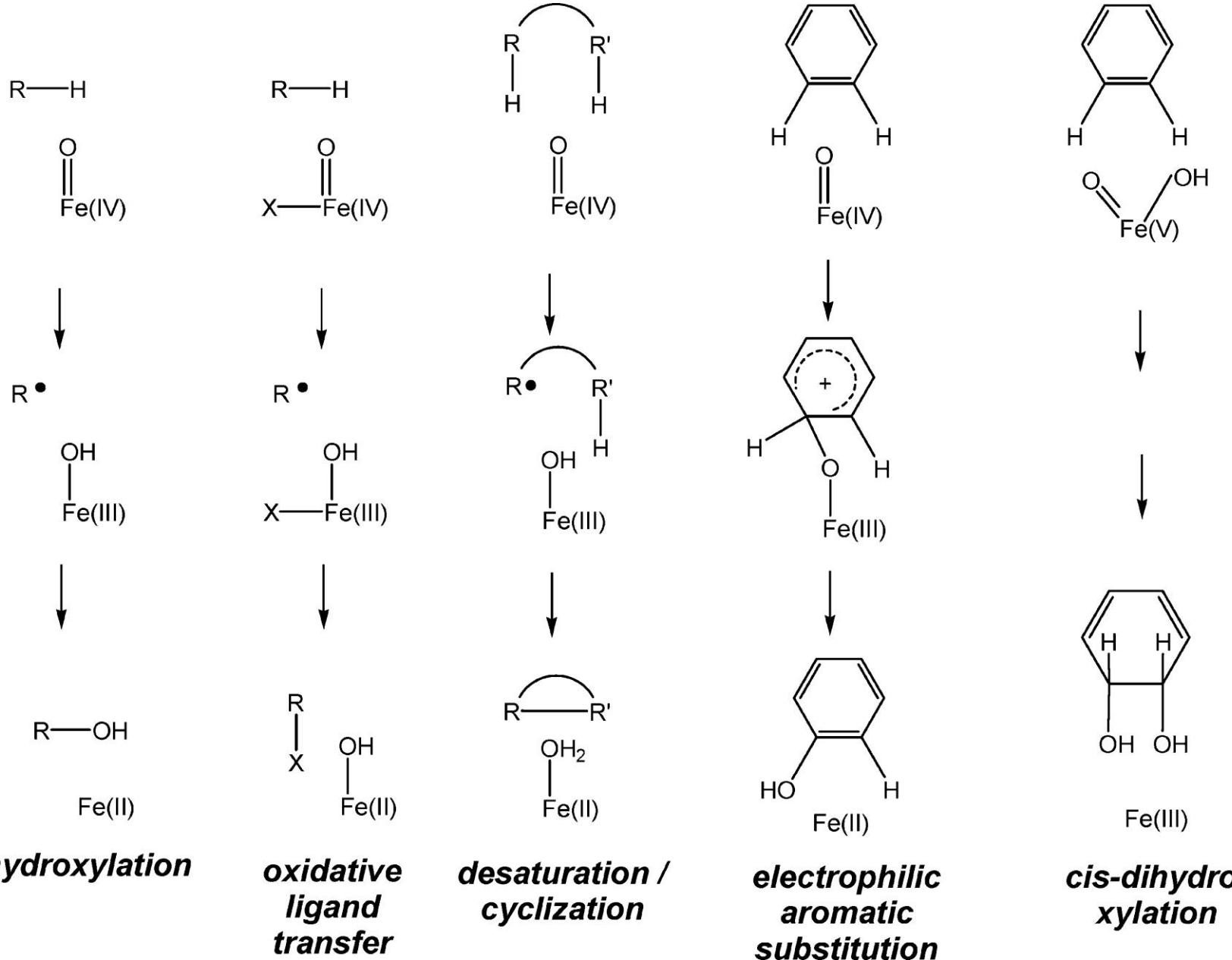
James P. Collman,* Lei Fu, Paul C. Herrmann, Xumu Zhang



5. Oxygenases (Heme, non-Heme & Cu)

Substrate oxidative transformations



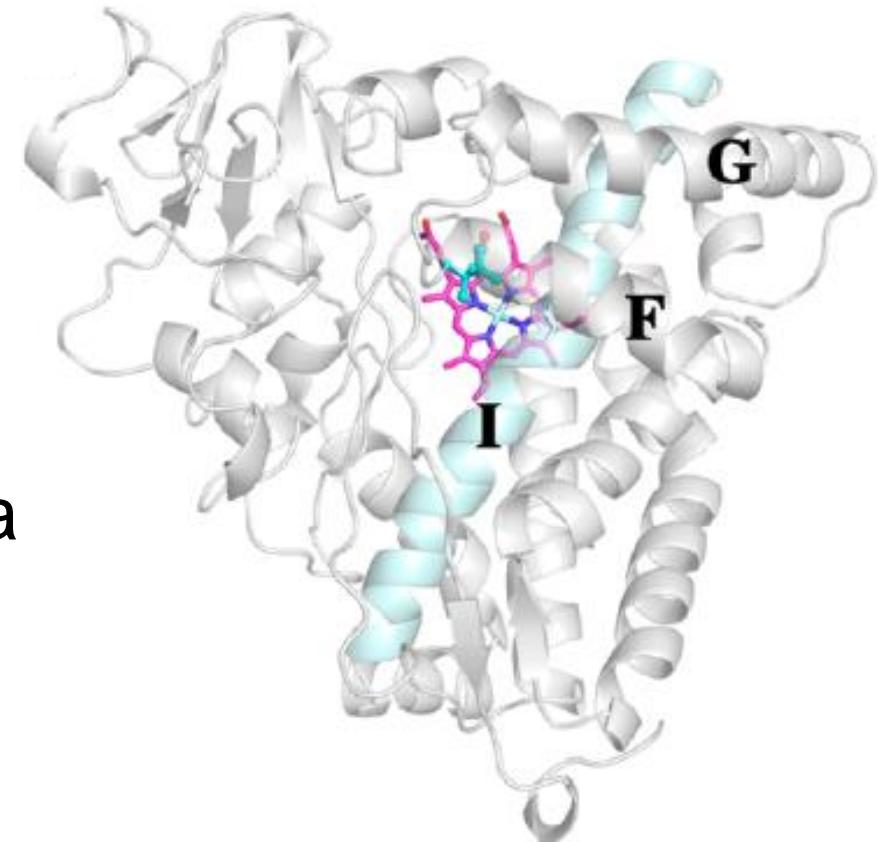


- **Reactive Fe-Oxo (Fe=O):** key intermediates in many Fe-containing oxygenases for many different reactions.

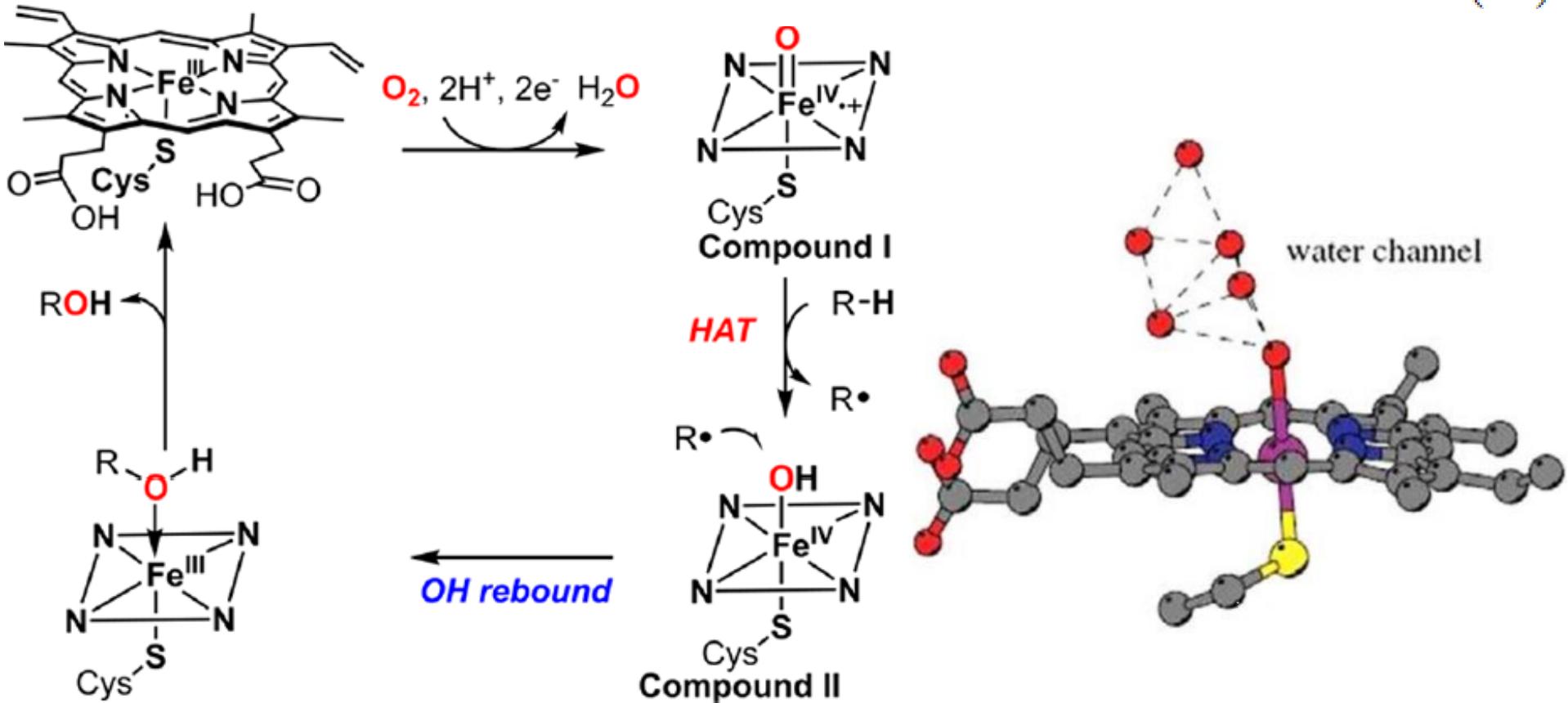
5a. Heme-containing Oxygenases

Cytochrome P450

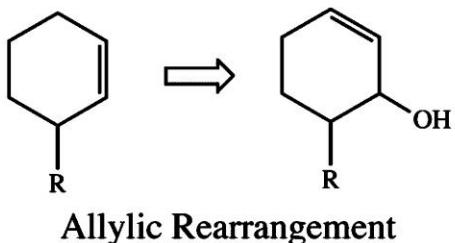
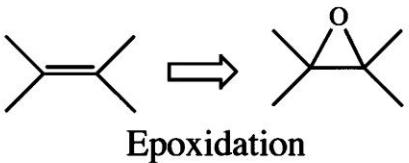
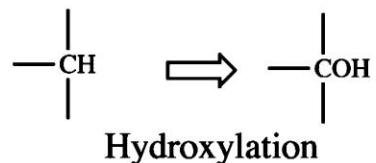
- This key type of heme-containing **monooxygenases** plays essential role in **hydroxylation** of endogenous physiological **substrates**, many drugs and other compounds foreign to the organism (**xenobiotic detoxification** in liver).
- Also involve biosynthesis of some compounds.
- Found in almost all mammalia tissues & organs, as well as in plants, bacteria, yeast, insects.



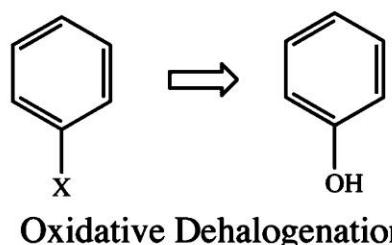
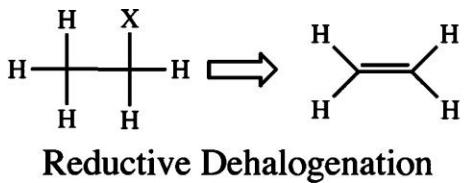
- A **proximal Cys** ligand for the heme.
- 2 e⁻ from NADH or NADPH (or FAD/FMN).



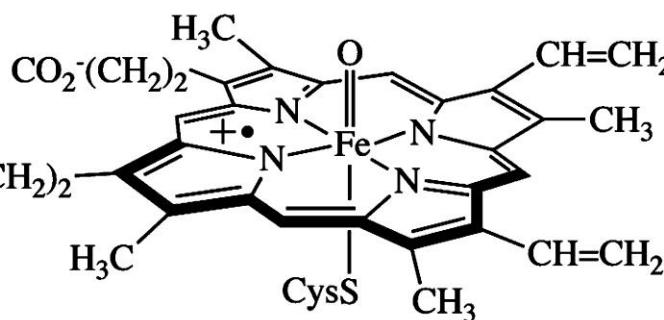
Hydrocarbons



Haloalkanes

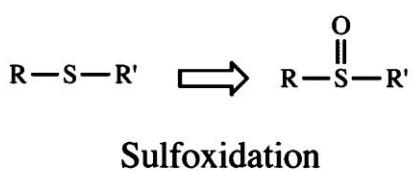


Broad Reaction Types

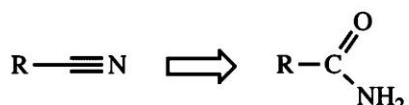


Cpd I

Thioethers



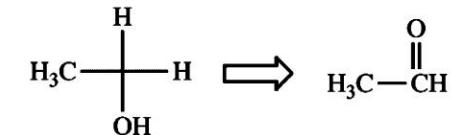
Nitriles



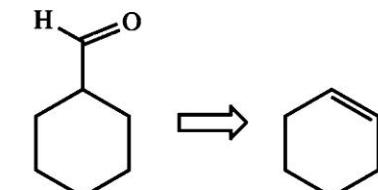
N-Oxides



Alcohols

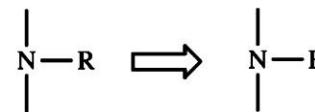


Aldehydes



Deformylation

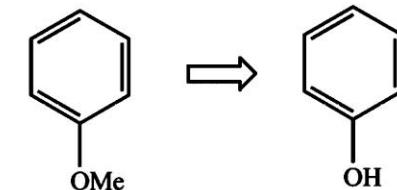
Amines



N-Dealkylation

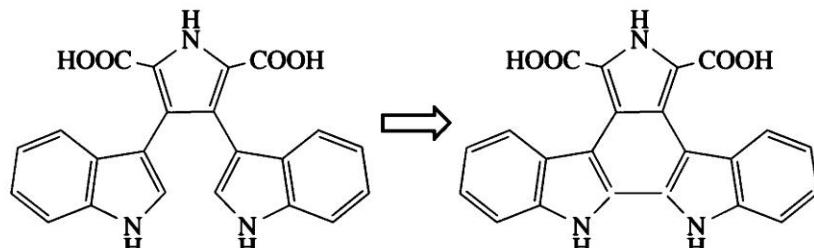


N-Oxygenation



O-Dealkylation

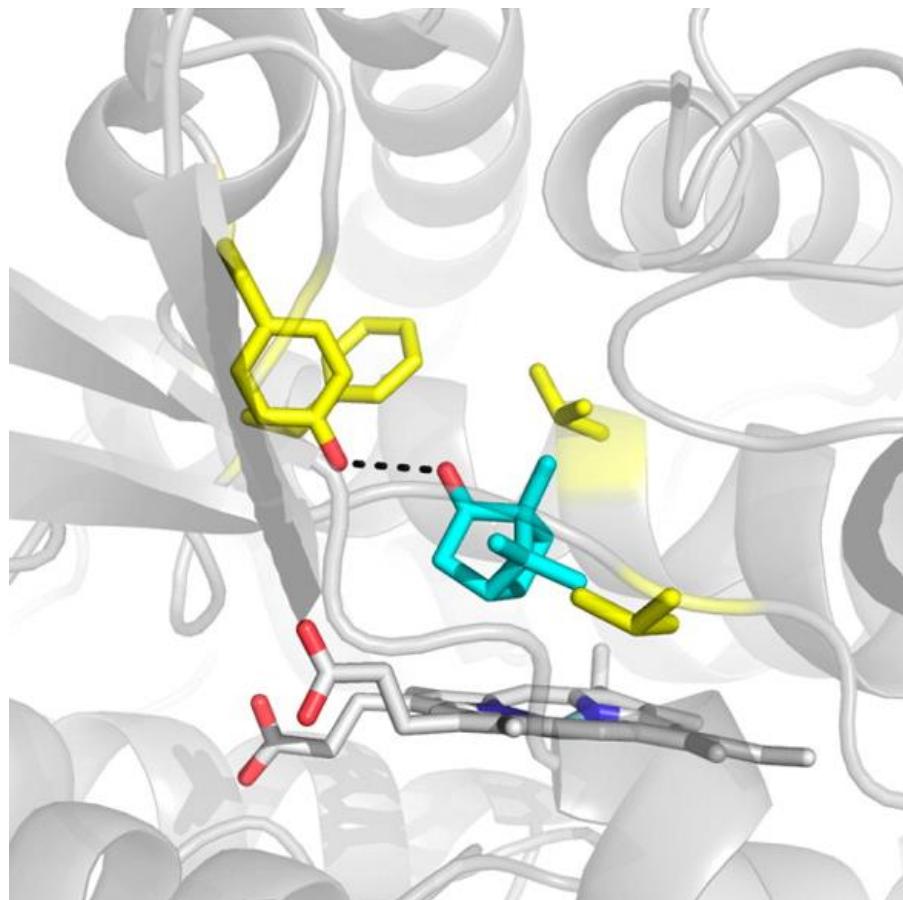
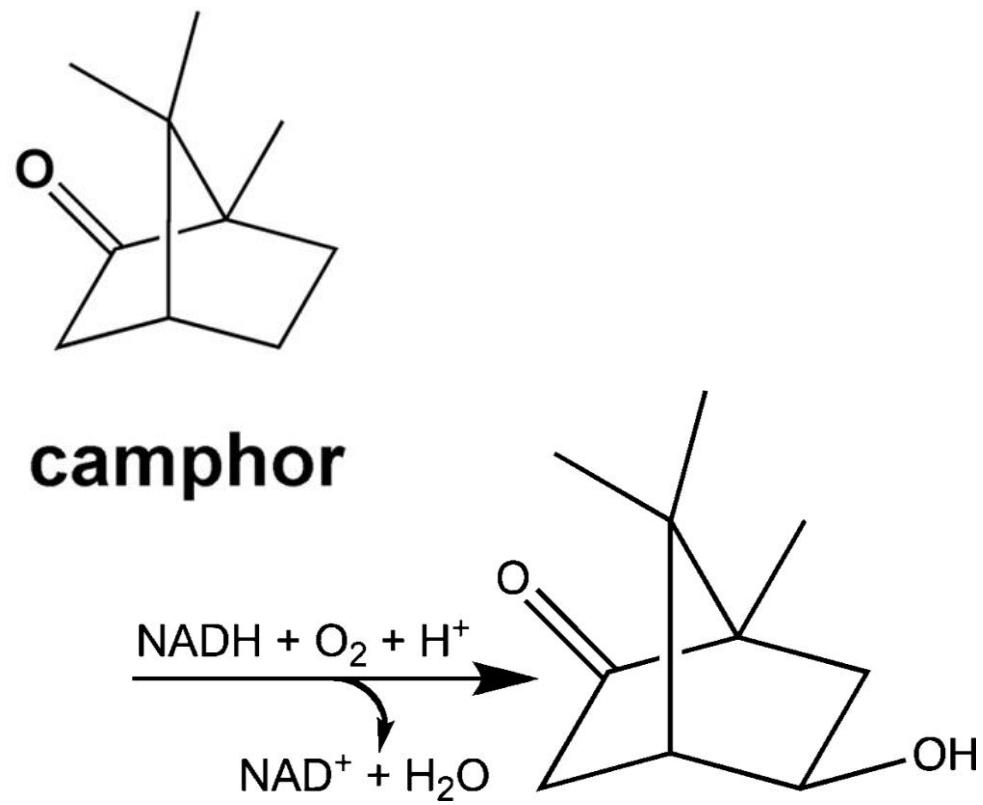
chromopyrrolic acid (CPA)

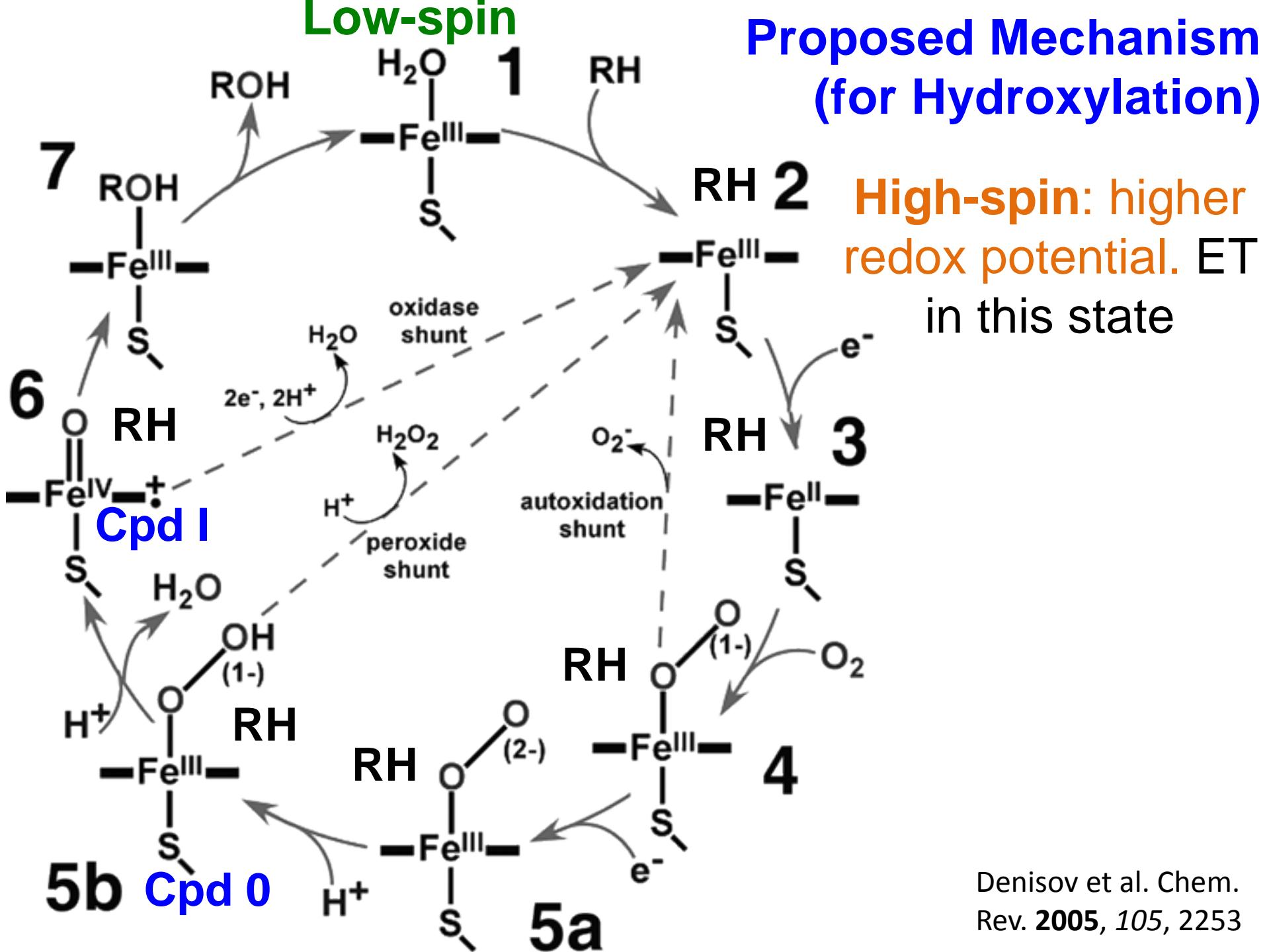


C-C coupling

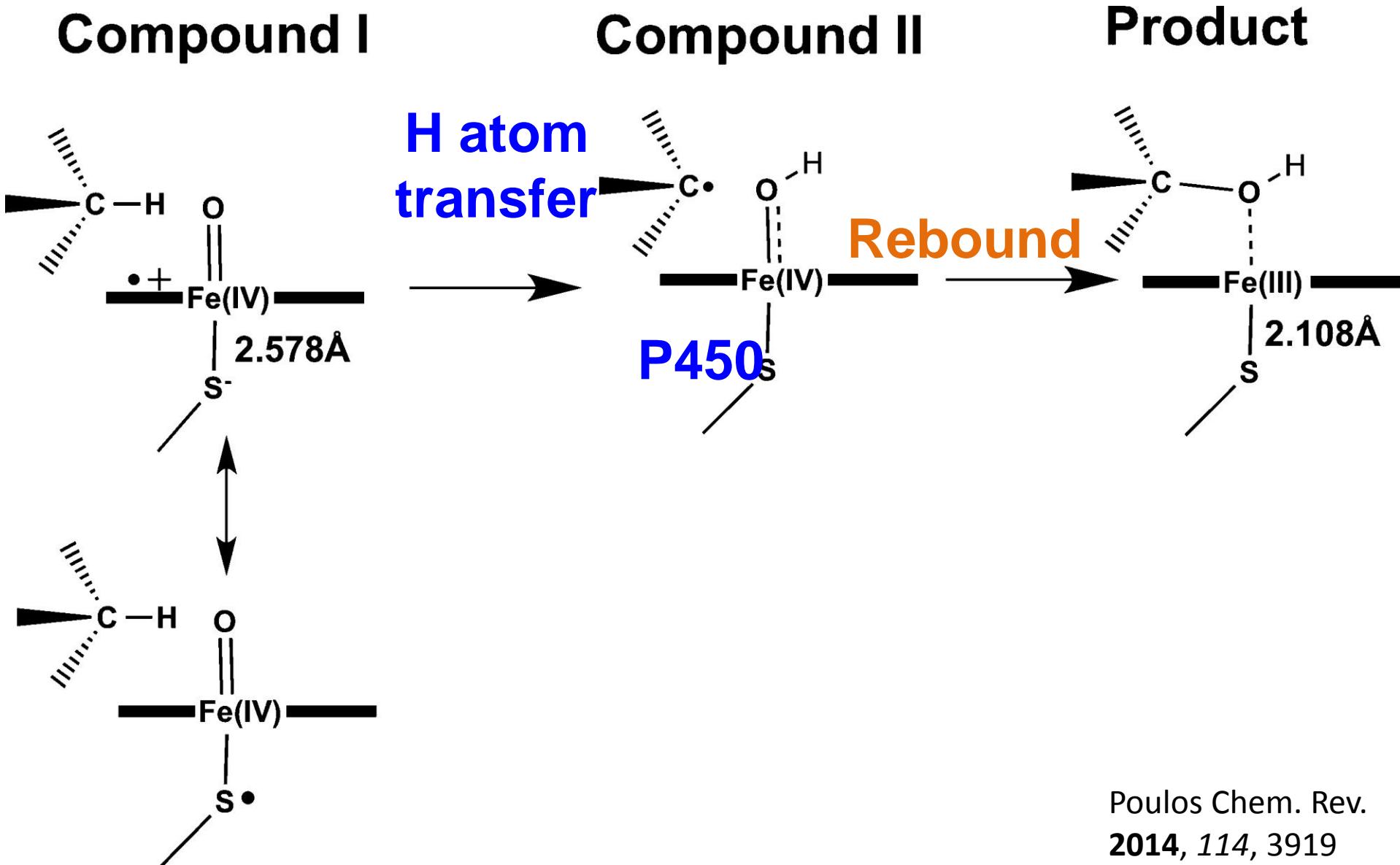
P450_{cam}

- P450_{cam} catalyzes the *regio-* & *stereo-selective* hydroxylation of camphor to give 5-exo hydroxycamphor.
- Selectively breaks the strong C-H bond.

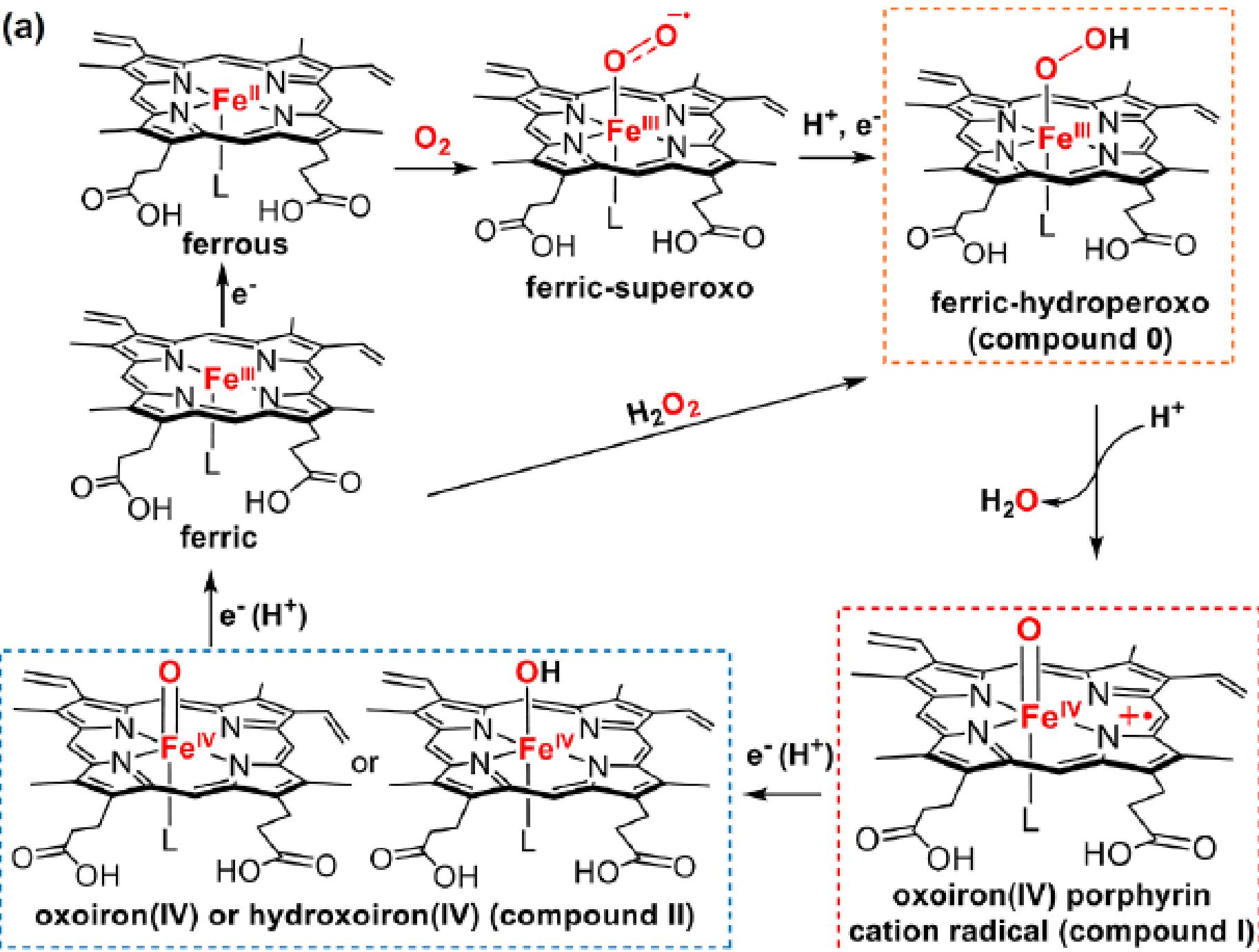




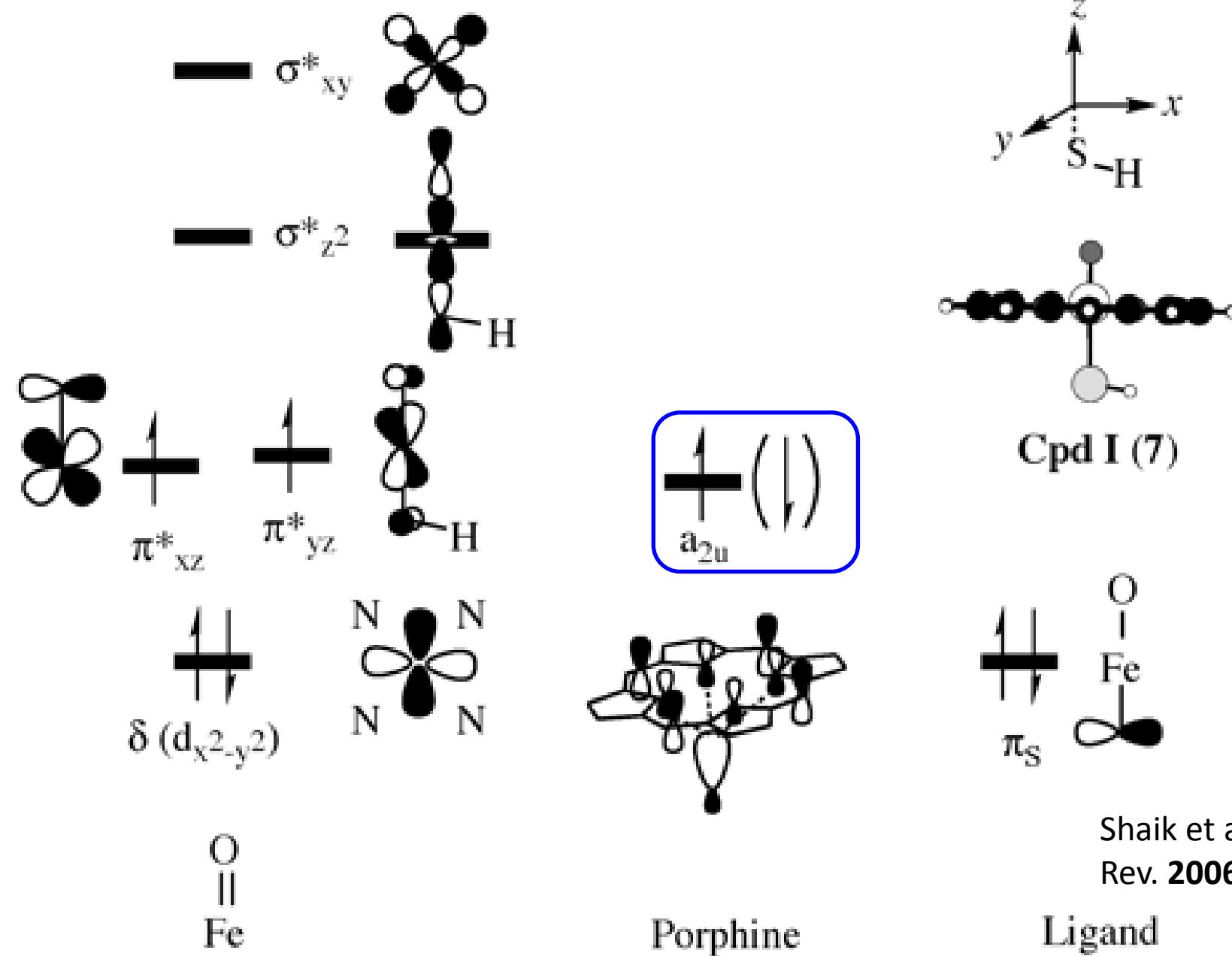
Proposed Detailed Mechanism for Hydroxylation (6→7)



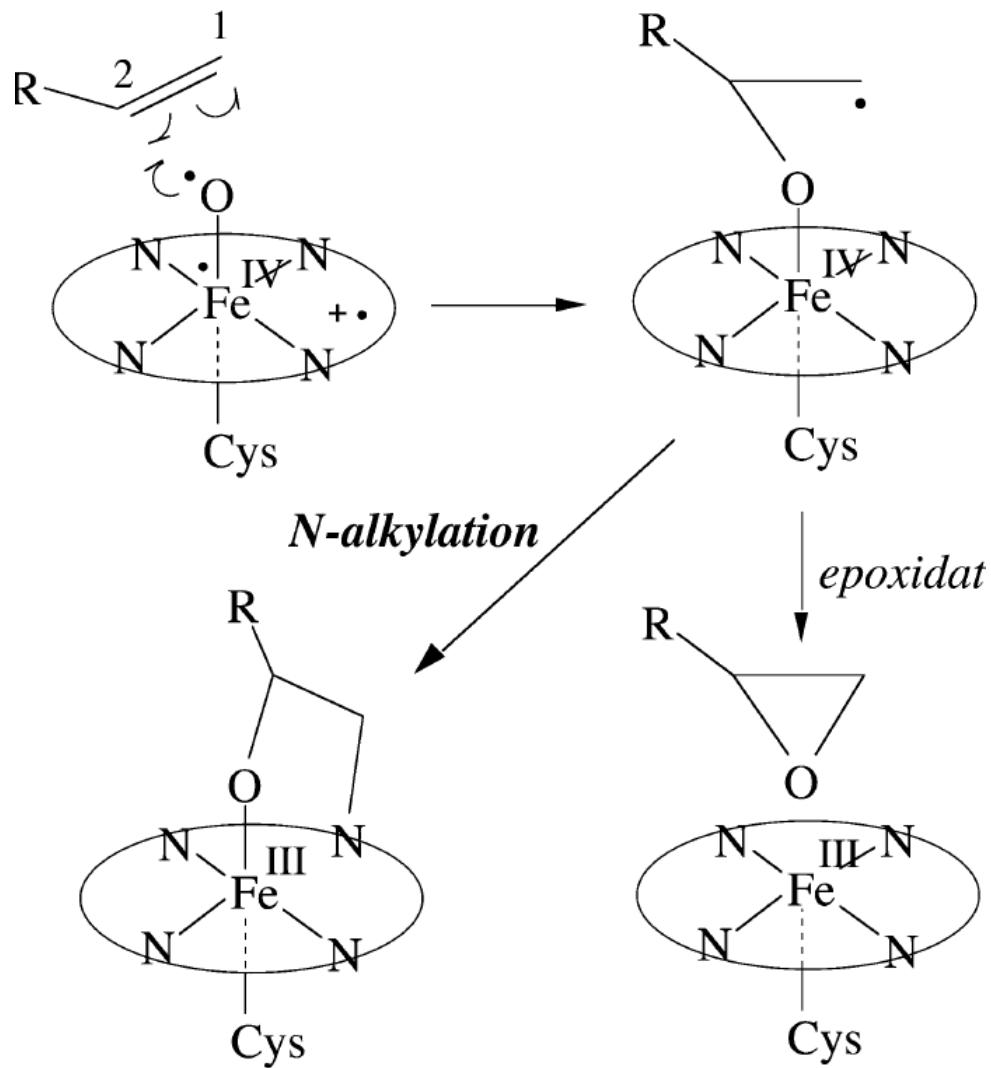
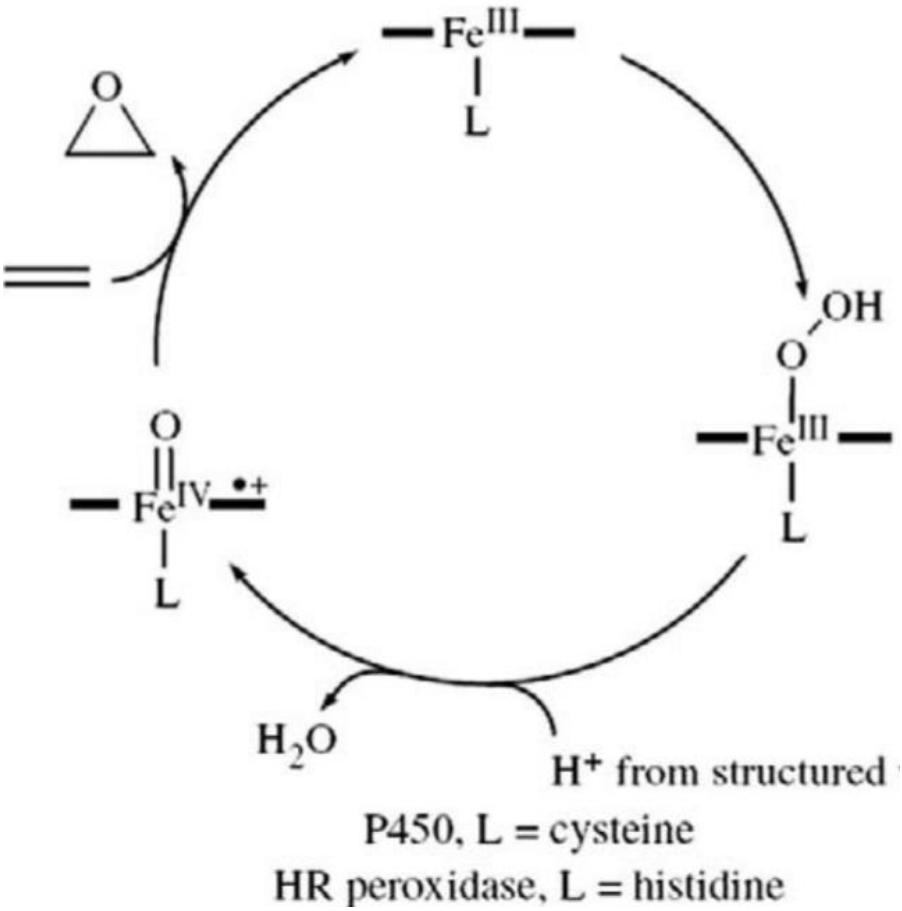
(a)



Proposed Electronic Structures in Compound I

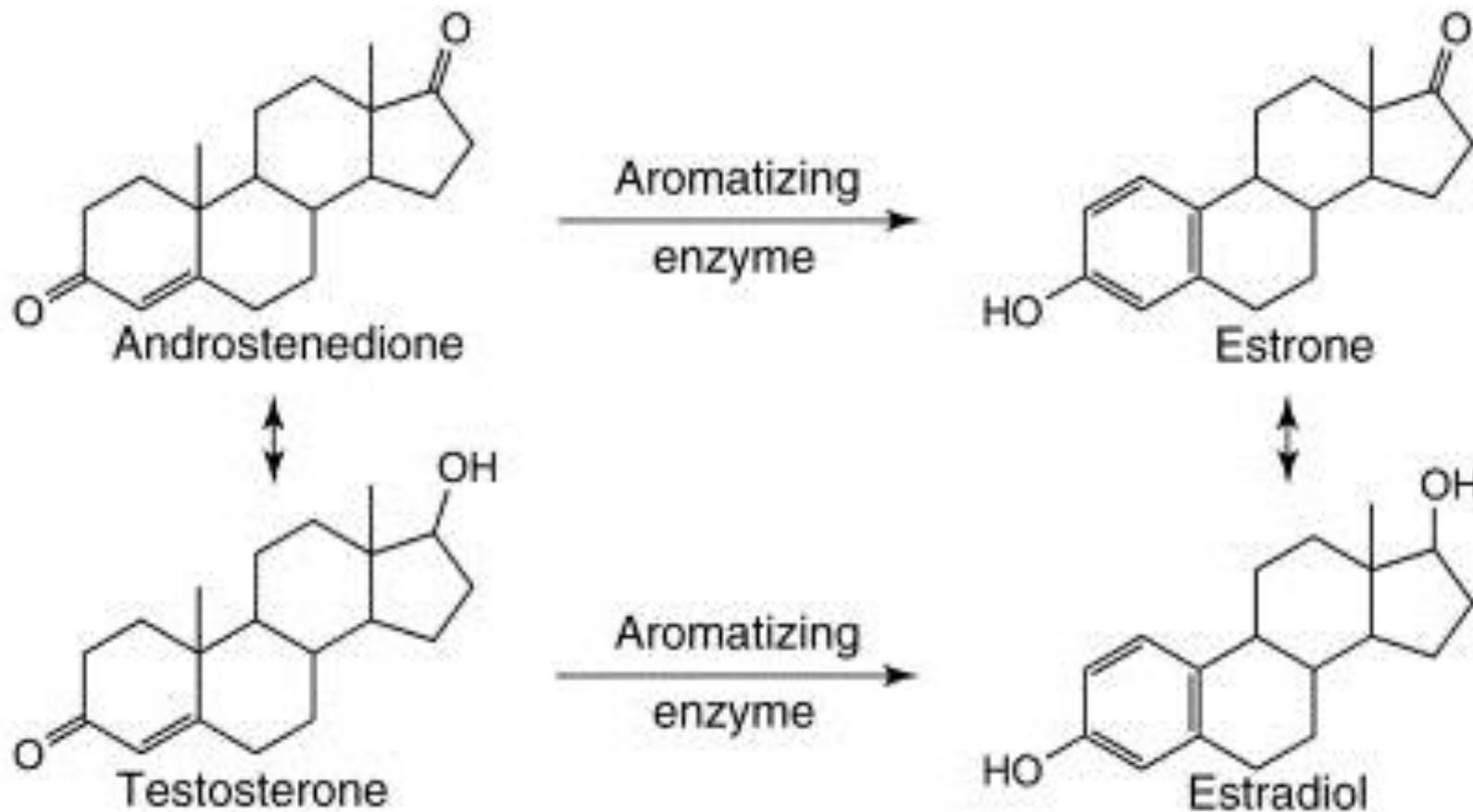


Proposed Mechanism (for Epoxidation)

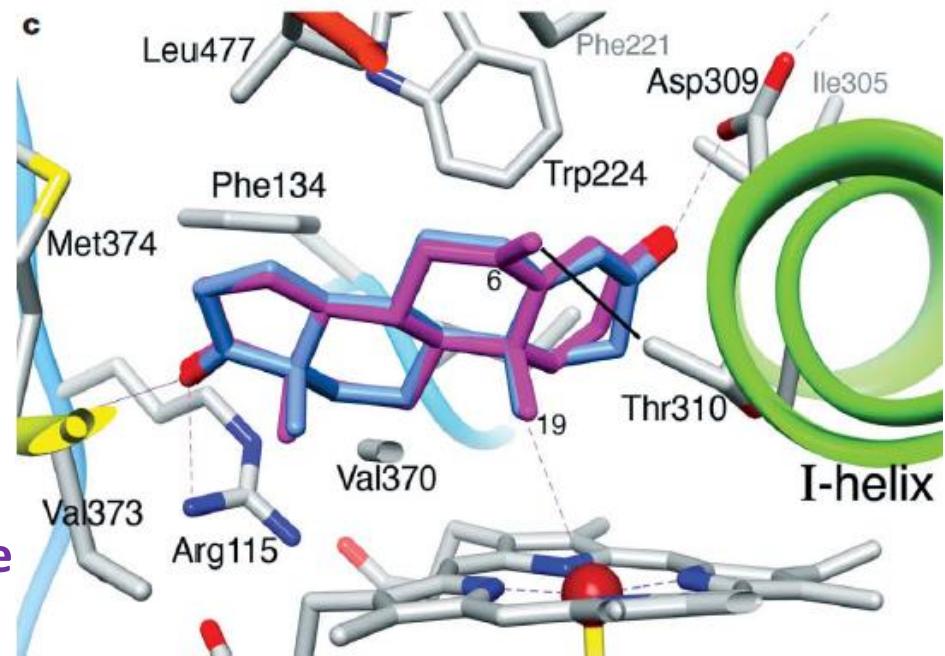
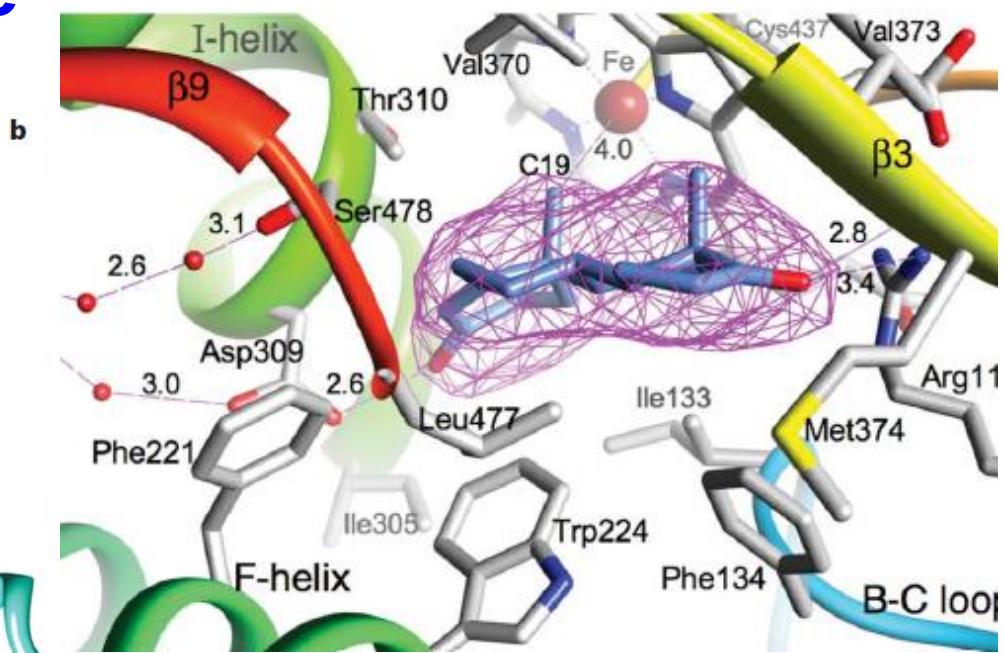
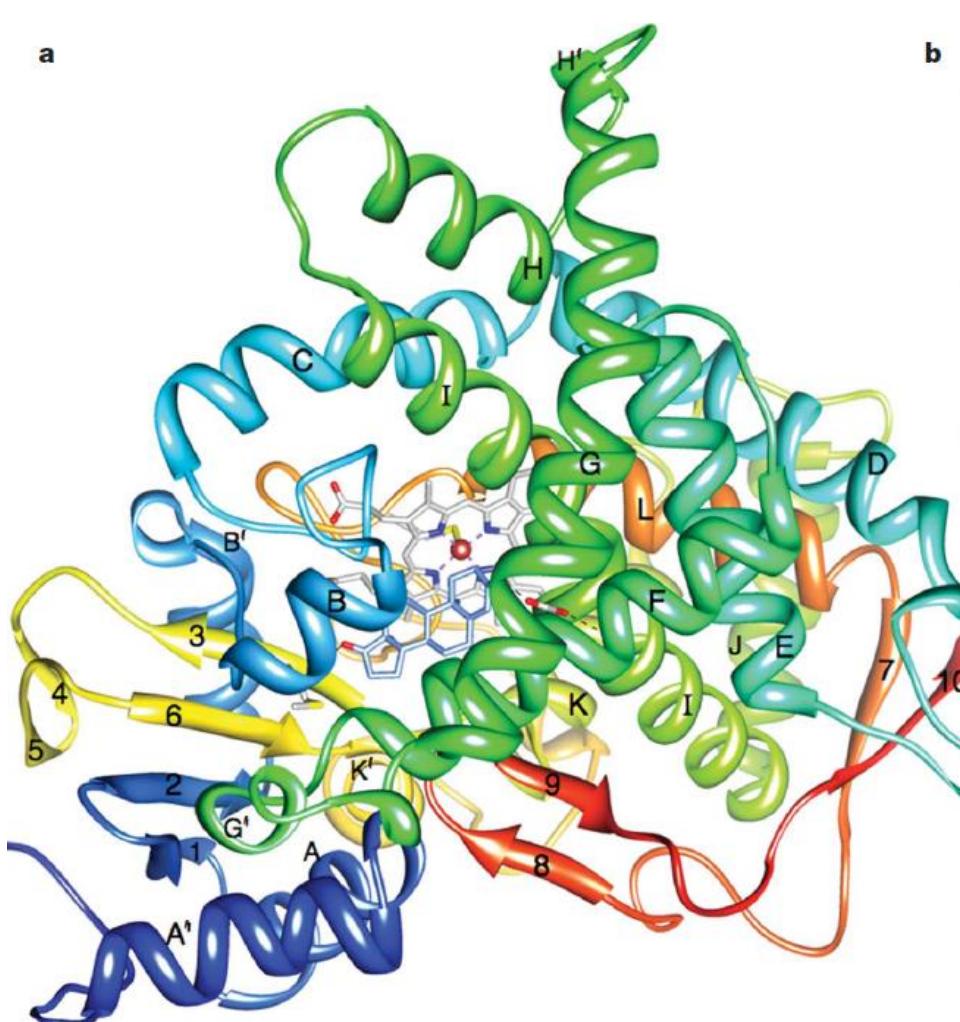


Aromatase

- A member of the cytochrome P450 family.
- Catalyzes the biosynthesis of all oestrogens (雌激素) from androgens.
- Aromatase inhibitors can be used to treat oestrogen-dependent breast cancer.

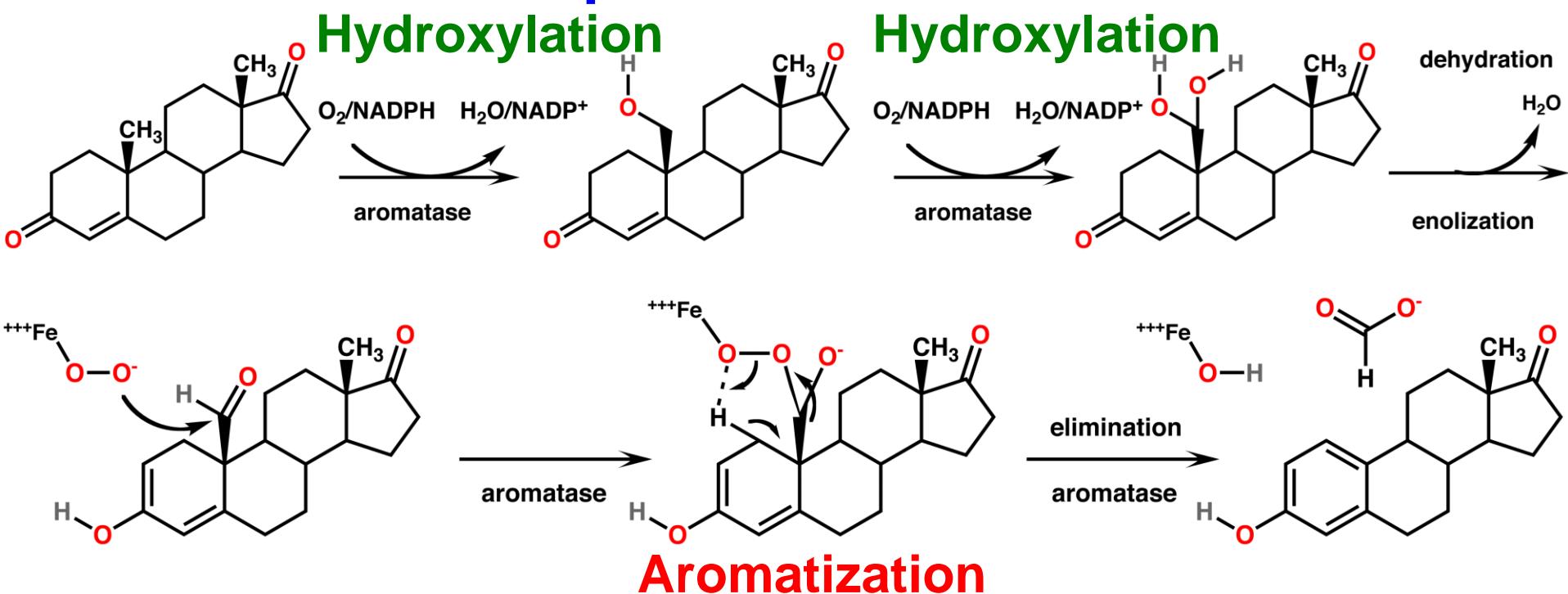


Crystal Structure



Modelling
of exemestane

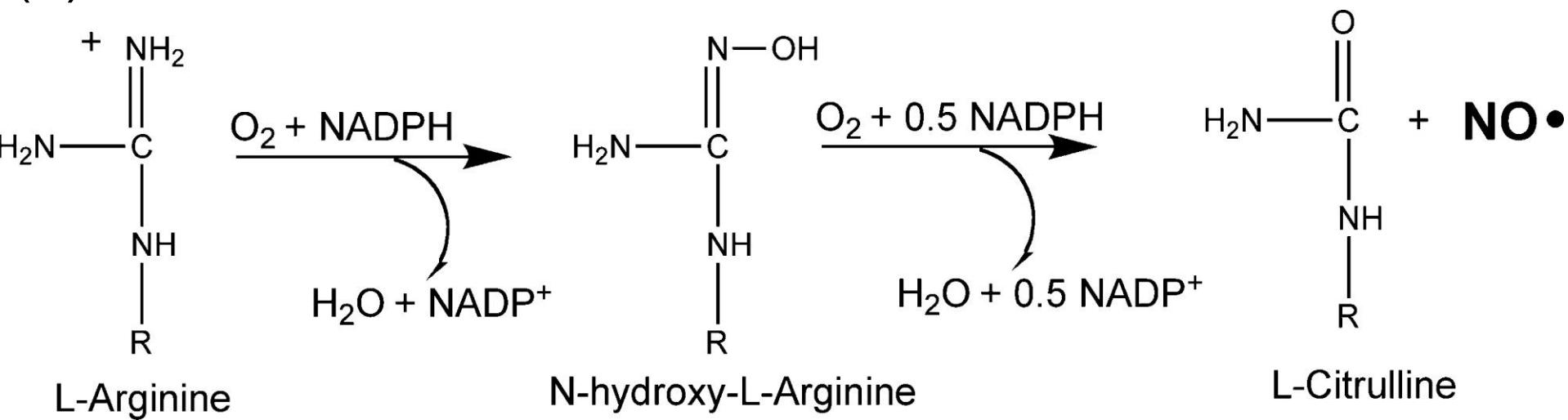
Proposed Mechanism



- **3-step process:** each step requires 1 O_2 , 1 NADPH, & its redox reductase.
- The first 2 steps: **Hydroxylation** at the C19-Me group;
- The last step: **Aromatization** of the steroid A-ring (using an unique **Fe(III)-peroxy** species).

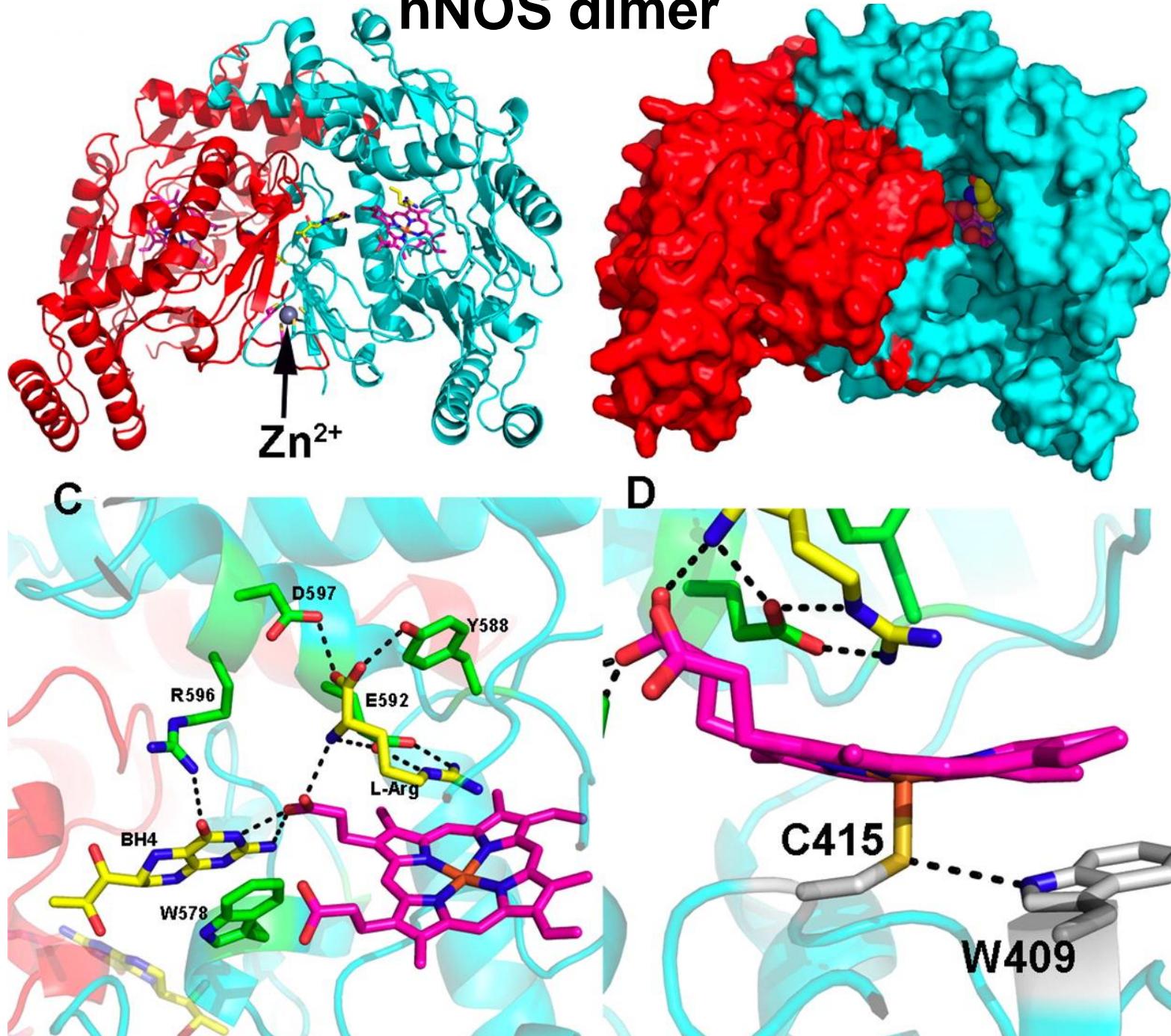
Nitric Oxide Synthase (NOS)

- Catalyzes **oxidation** of L-Arg to L-citrulline & NO (a key signaling molecule in the cardiovascular, immune & nervous systems).
- Similar to P450, a proximal Cys ligand is used.
- 2-step process:
 - (1) **Hydroxylation** of L-Arg to N^ω-L-hydroxyarginine (L-NHA) (similar to P450);
 - (2) Oxidation of L-NHA to form L-citrulline and NO.



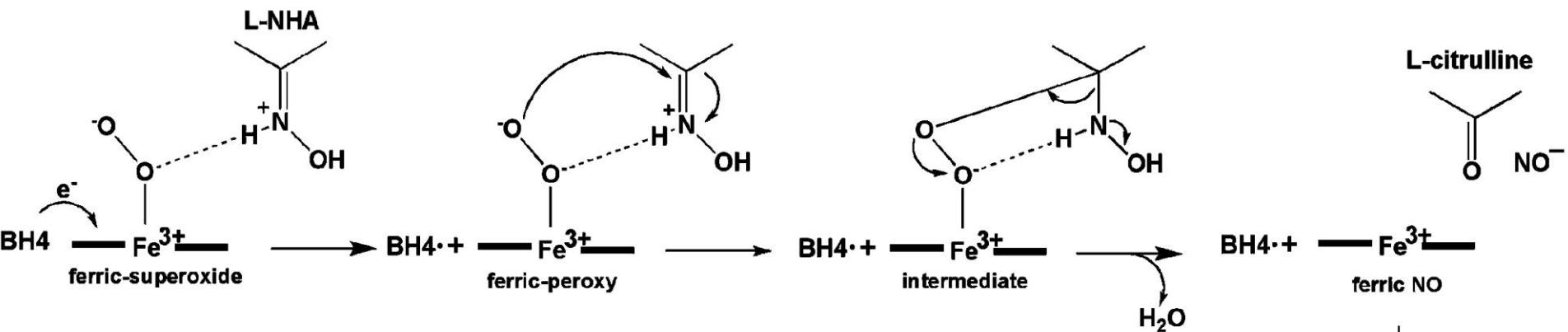
nNOS dimer

BH4
(tetrahyd-
robiopterin)
cofactor
as the e⁻
source.

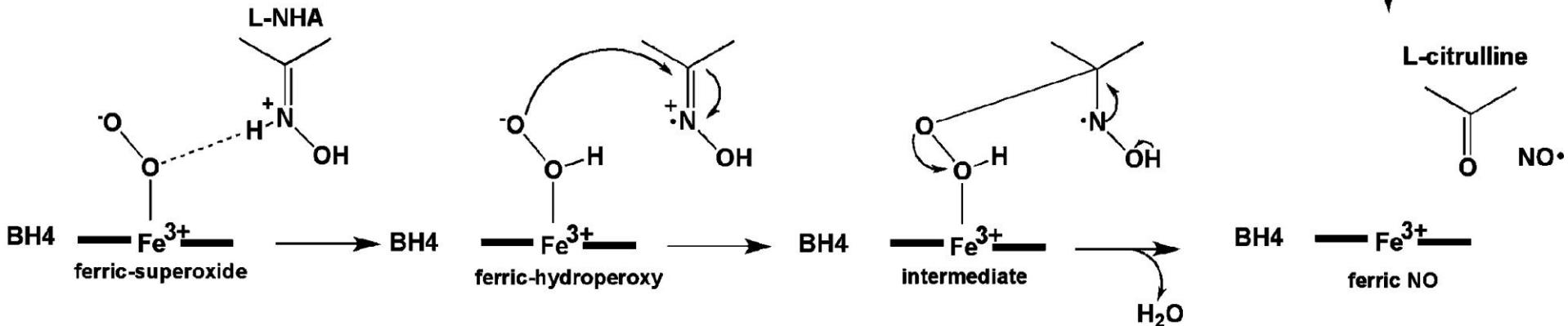


Proposed Mechanism for the Last Step

A. BH4 radical

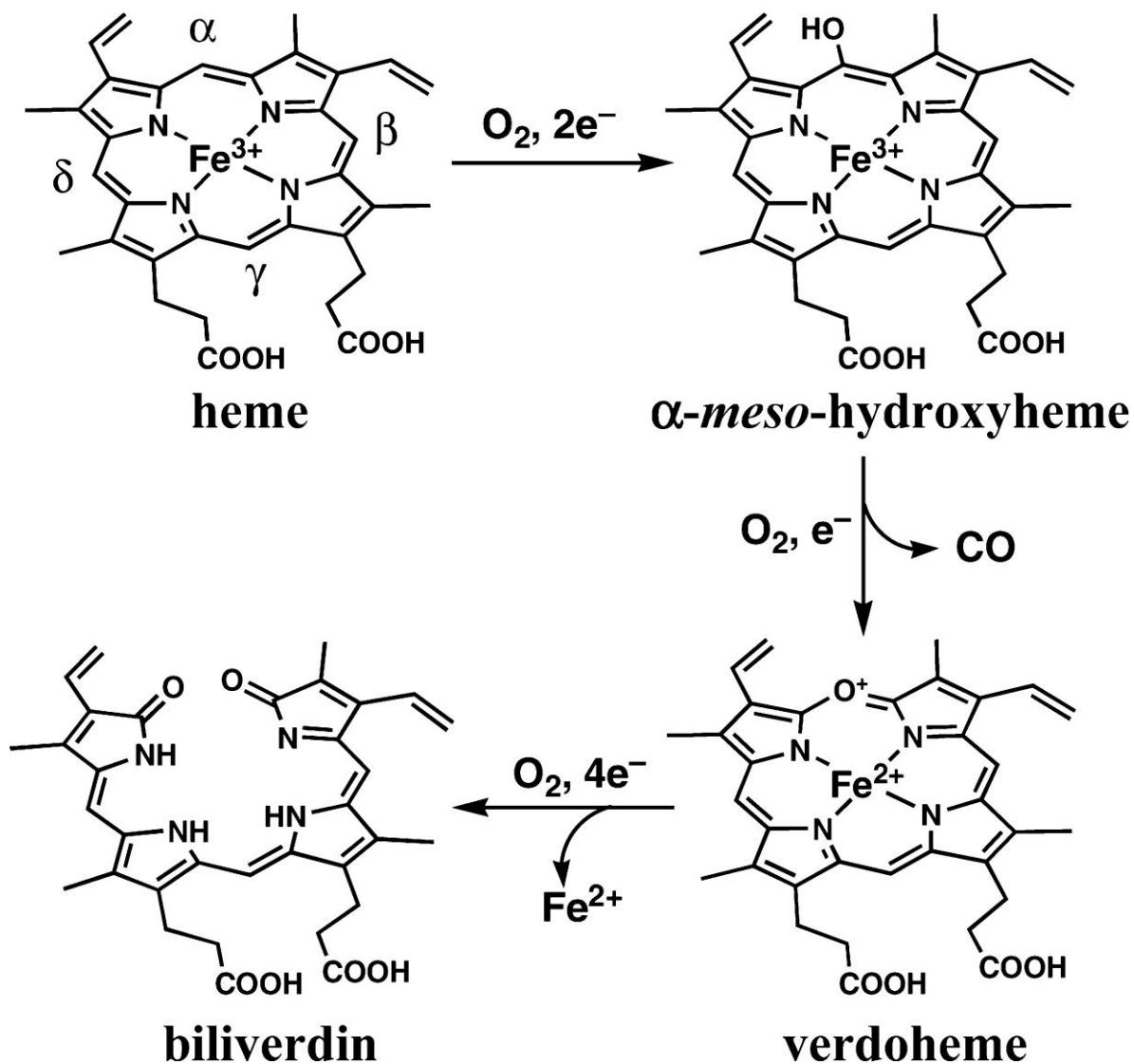


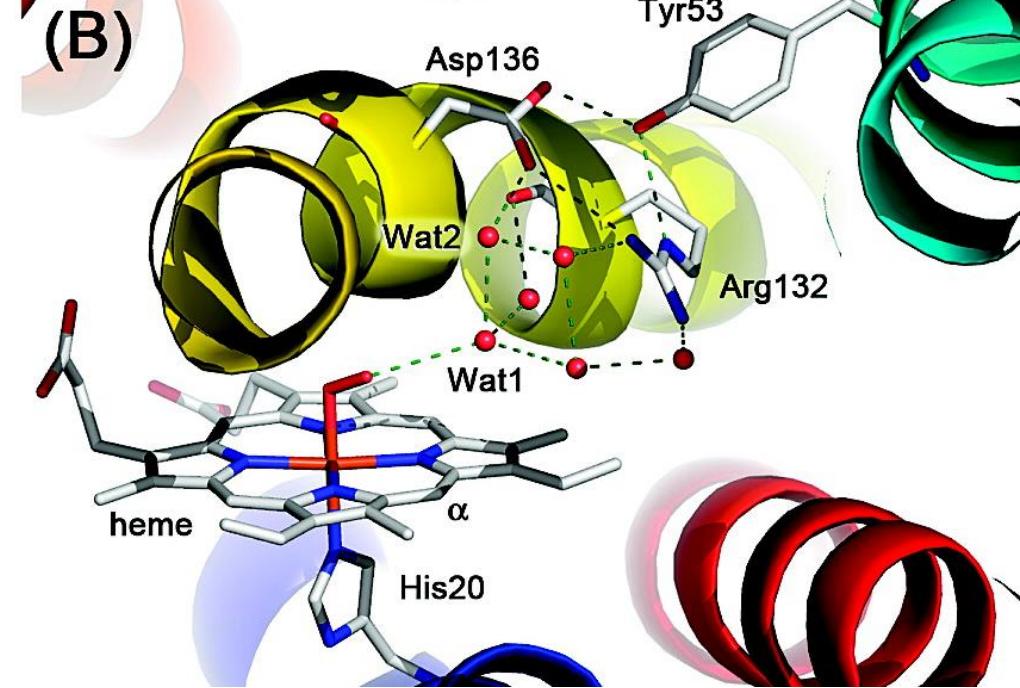
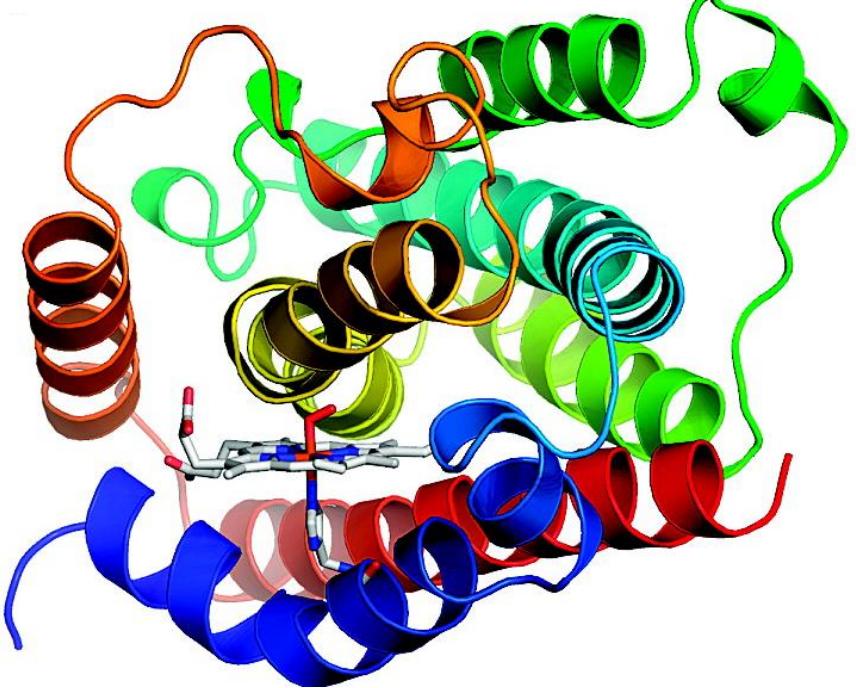
B. No BH4 radical



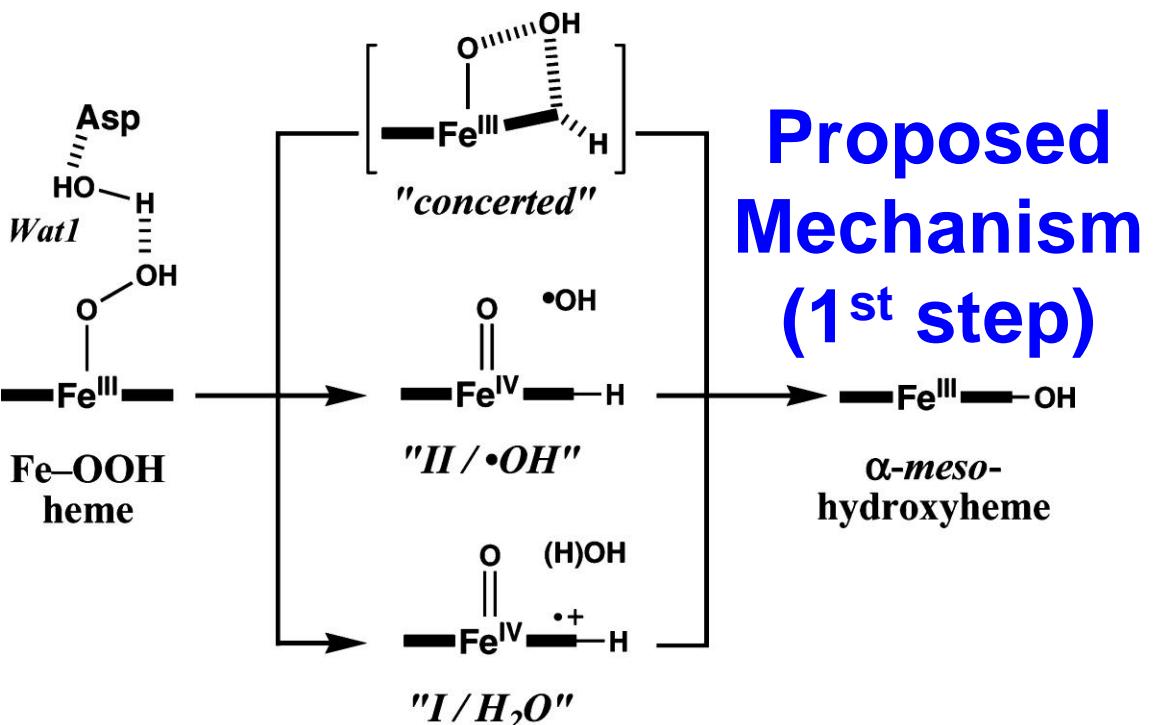
Heme Oxygenase (HO)

- Catalyzes degradation of heme proteins to release iron for further use.
- Different from the other heme enzymes, **heme** itself is the **substrate & cofactor for HO**.
- Selective oxidation of the α -meso heme C for most HOs.

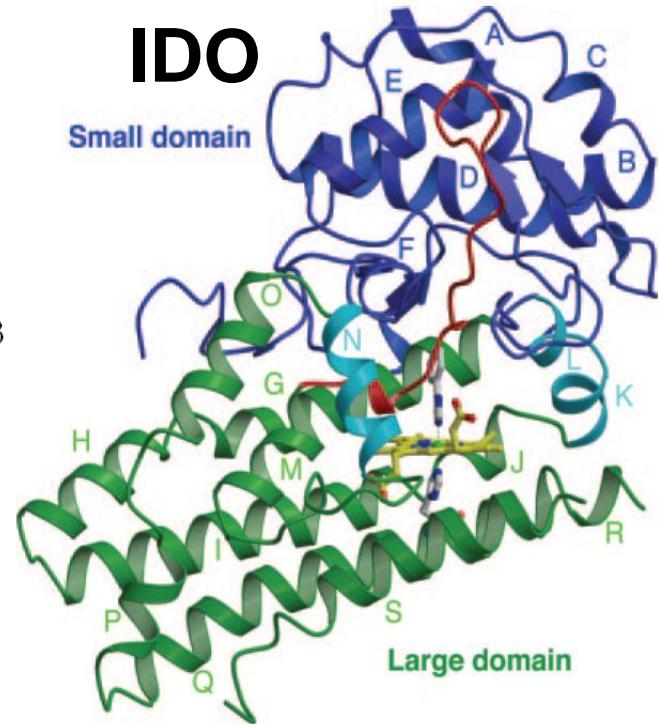
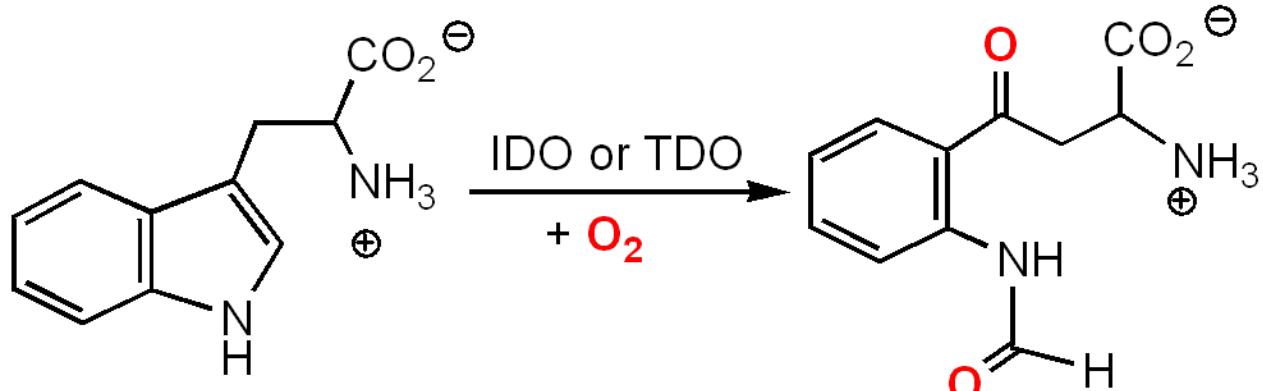




- A proximal His ligand.
- Flexible active site for substrate binding & product release.



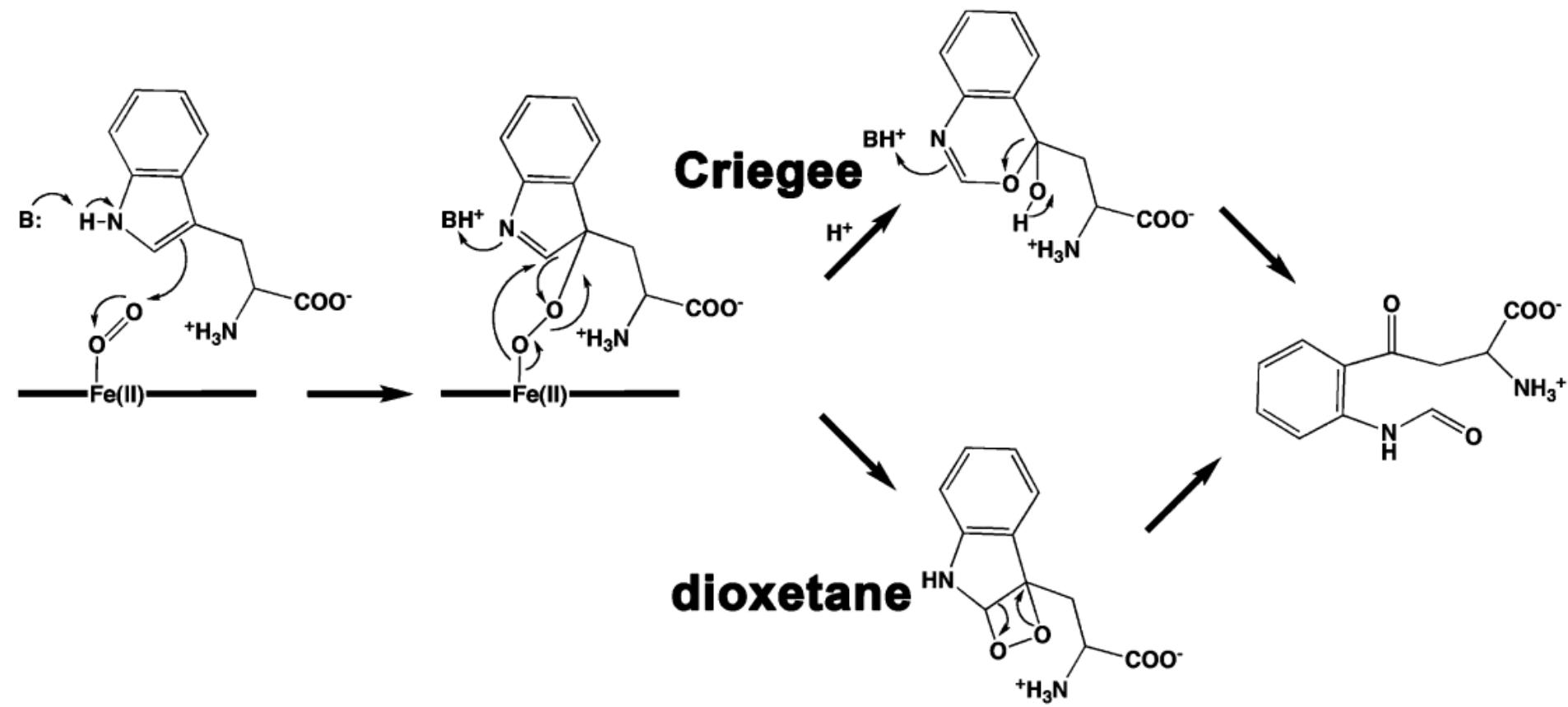
Indoleamine 2,3-dioxygenase (IDO) & Tryptophan 2,3-dioxygenase (TDO)



- Different from the other heme monooxygenases, IDO & TDO are heme-containing **dioxygenases**.
- Catalyzes conversion of L-Trp to N-formylkynurenone.
- A few key physiological roles (e.g. suppress T cell proliferation).

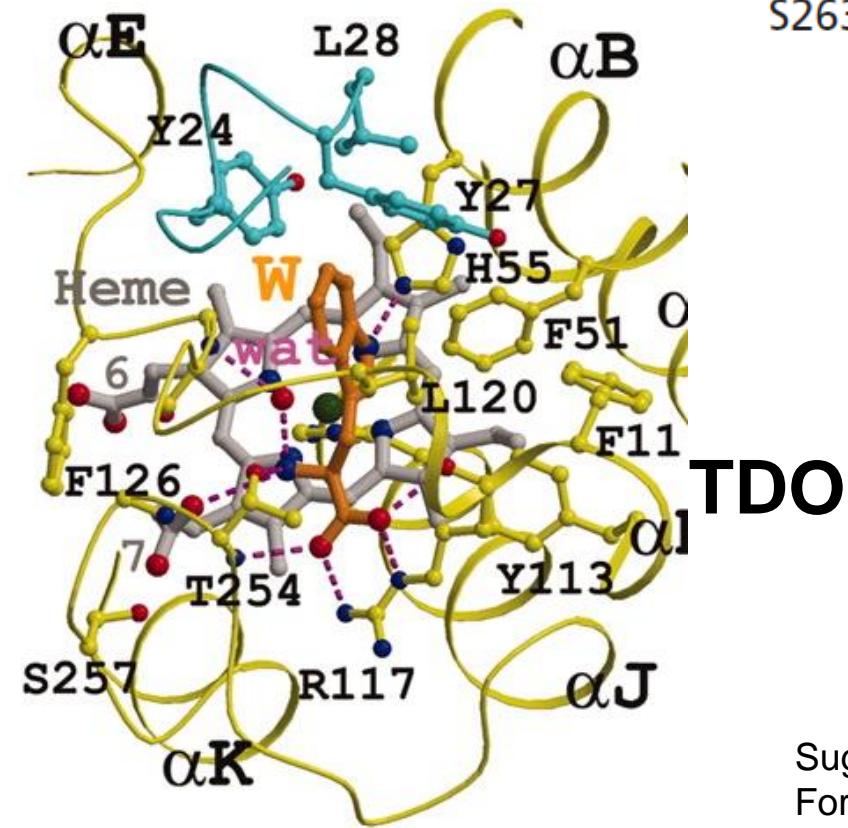
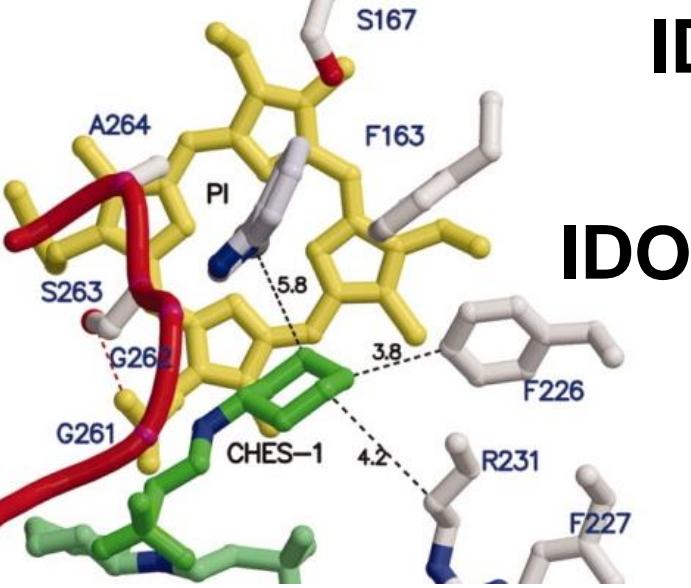
Kotake, Y.; Masayama, I. *Z. Physiol. Chem.* **1936**, 243, 237.
Yamamoto, S.; Hayaishi, O. *J. Biol. Chem.* **1967**, 242, 5260.

Proposed Generally-Accepted Mechanism



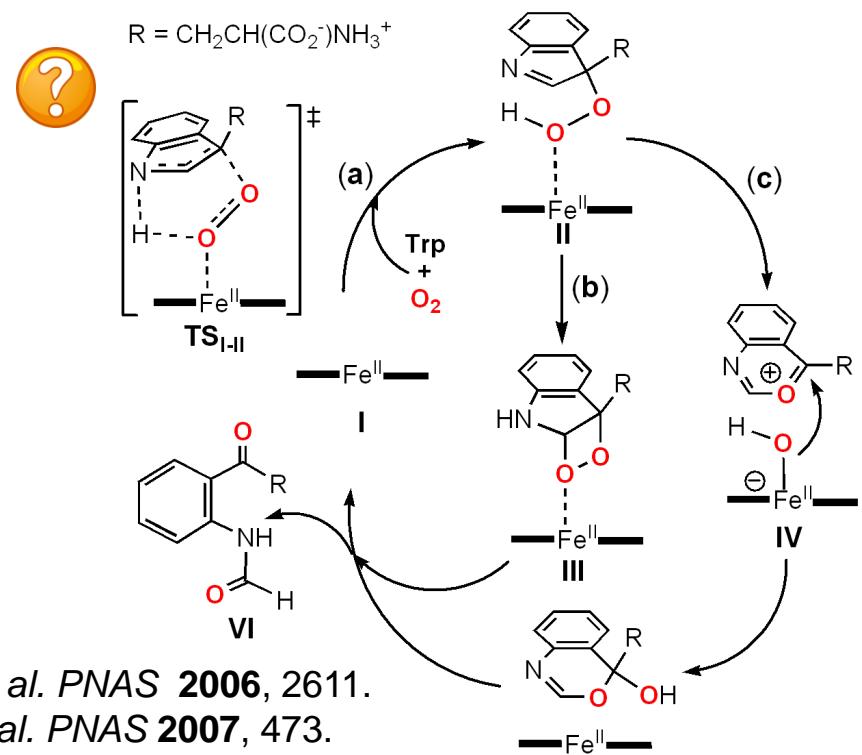
- Deprotonation by a base, electrophilic addition at C3 with $\text{Fe}^{(\text{II})}\text{O}_2$.
- No change of the Fe oxidation state.

IDO & TDO Active Sites

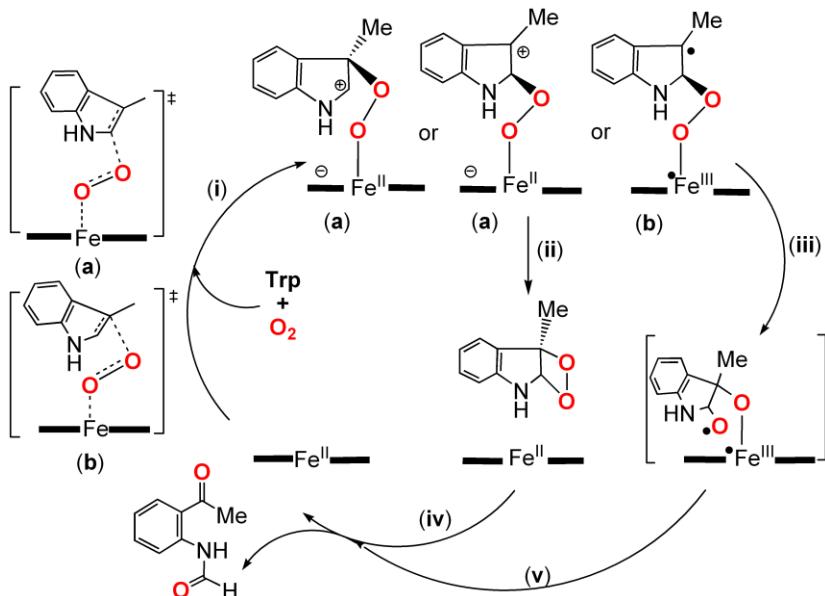


| Mutation | Activity* |
|-----------|-----------|
| Wild type | 126 ± 12 |
| C129A | 134 ± 6 |
| F163A | 148 ± 9 |
| S167A | 117 ± 5 |
| F226A | 1.3 ± 0.3 |
| F227A | 1.2 ± 0.5 |
| R231A | 2.3 ± 1.0 |
| S263A | 19 ± 7 |

Mutation: No critical polar residues in the active site.



Summary of the most favorable pathways (active-site model)



Chung, L. W.; Li, X.; Sugimoto, H.; Shiro, Y.; Morokuma, K. JACS **2008**, 130, 12298.

- ✗ Concerted addition and proton-transfer
- ✓ Direct (a) electrophilic and (b) radical addition from $\text{Fe}^{\text{II}}\text{-O}_2$ and $\text{Fe}^{\text{III}}\text{-O}_2^-$ species at either C2 or C3
- ✗ Widely-believed Criegee rearrangement
- ✓ Formation of dioxetane intermediate pathway (ii) or O-O cleavage pathway to give ferryl-oxo followed by oxo-attack (iii & iv)

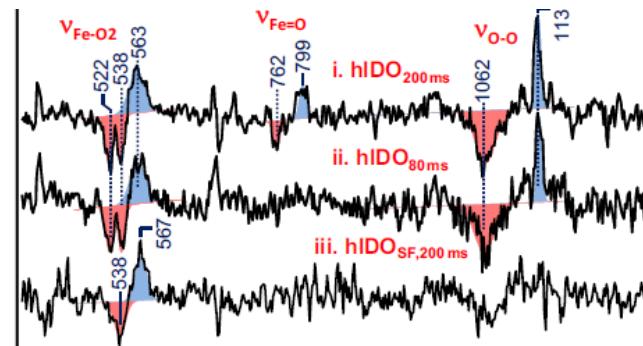
Subsequent Experimental Observations

| | L-Trp | | 1-Me-L-Trp | |
|--------------|--------------------------------------|-------------------------|--------------------------------------|-------------------------|
| | K_{cat} (s^{-1}) | K_M (μM) | K_{cat} (s^{-1}) | K_M (μM) |
| hIDO (wt) | 1.7 | 21 | 0.027 | 150 |
| hIDO (S167A) | 1.95 | - | 0.032 | 31 |
| xcTDO (wt) | 19.5 | 114 | - | - |
| xcTDO (H55A) | 2.86 | 133 | 0.048 | 59 |

Deprotonation is not necessary

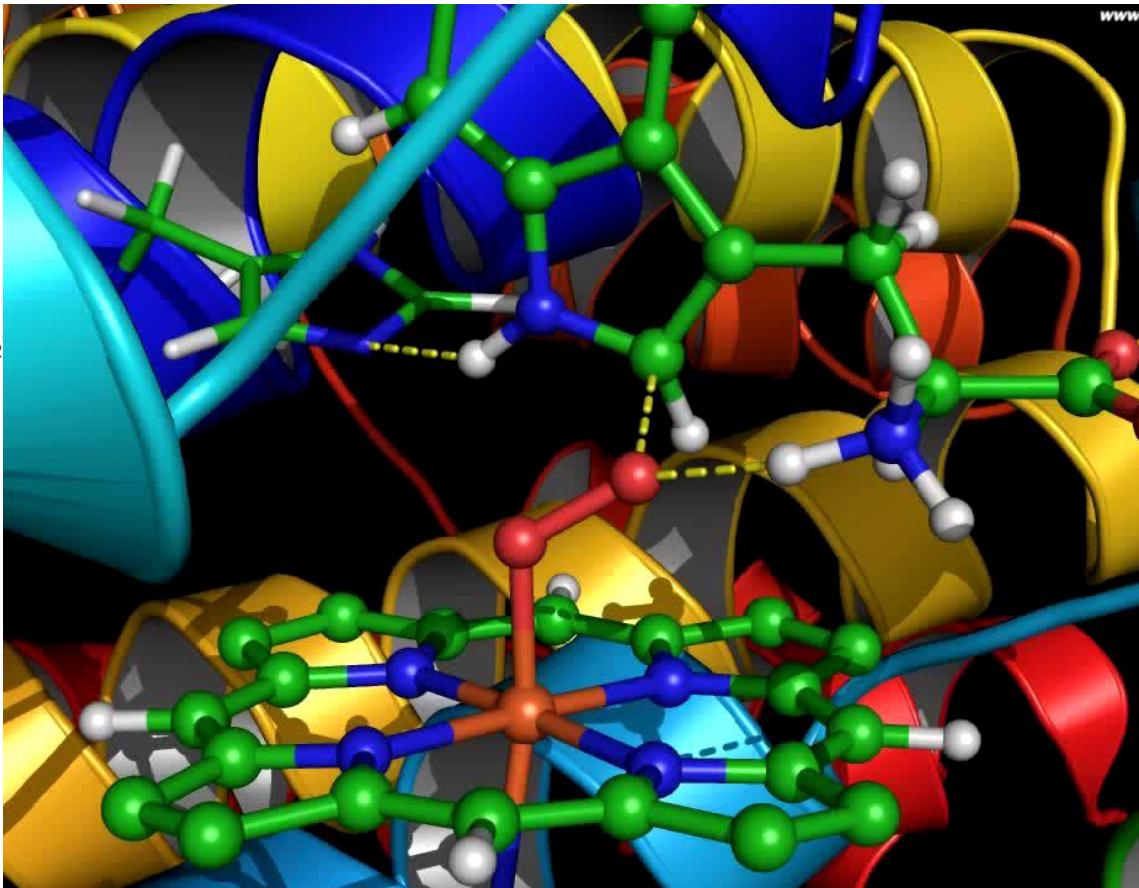
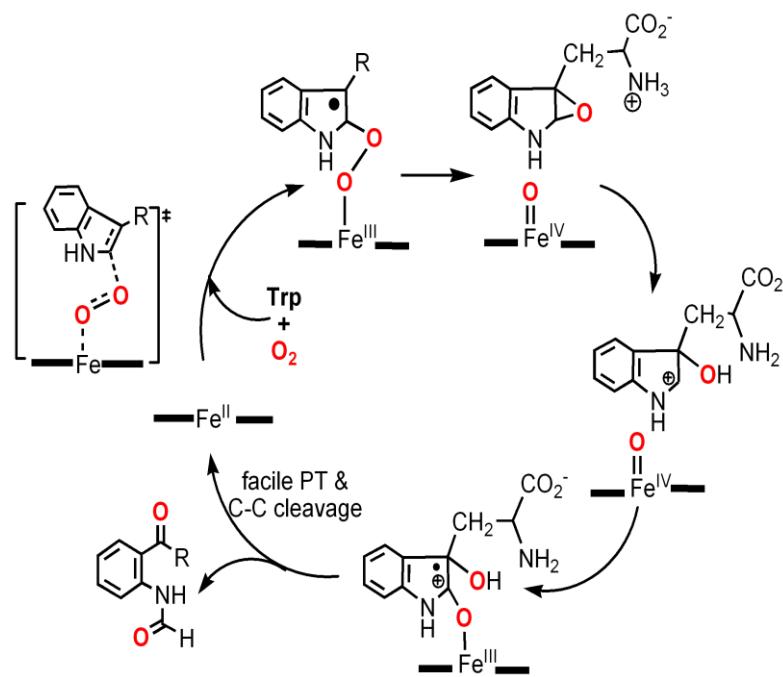
Chauhan, N. et al. JACS **2009**, 131, 134186.

Observed $\text{Fe}^{\text{III}}\text{-O}_2^-$ & $\text{Fe}^{\text{IV}}\text{-oxo}$ in IDO by resonance Raman



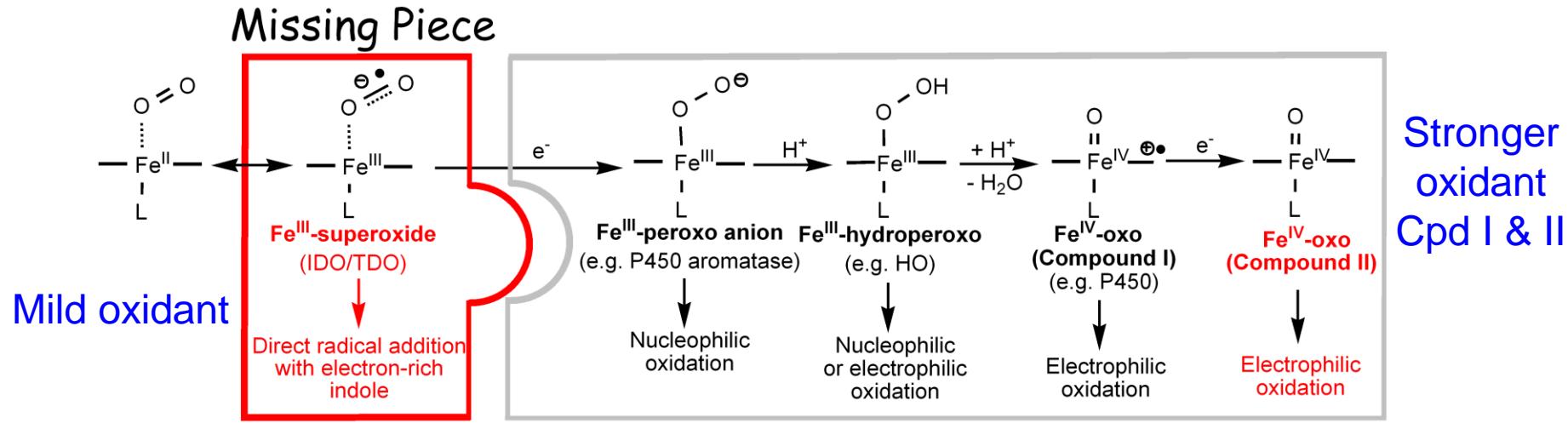
Lewis-Ballester, et al. PNAS **2009**, 106, 17371.
Yanagisawa, et al. Chem. Lett. **2010**, 36.

My QM/MM Study for a new Mechanism of TDO



Old Heme, New Heme Chem

O₂ activation and oxidation process in heme systems: Dual oxidants in TDO

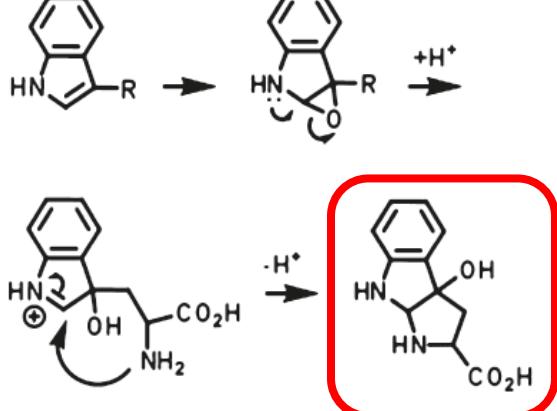


Trapping monoxygenated INT

- of special interest
- of outstanding interest

46. Chung LW, Li X, Sugimoto H, Shiro Y, Morokuma K: Density functional theory study on a missing piece in understanding of heme chemistry: the reaction mechanism for indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase. *J Am Chem Soc* 2008, **130**:12299-12309.

One of two very prescient and insightful computational papers from Chung et al. (see also [52]), which together present likely (as well as unlikely) mechanisms to guide experimental work. The computational data overall do not support base-catalyzed abstraction (subsequently confirmed experimentally [45••]), or the Criegee/dioxetane mechanisms previously proposed [8]; and they implicated both ferryl heme (subsequently identified experimentally [48••]), epoxide formation (suggested later [48••]), and ring opening of the epoxide (subsequently inferred from experimental work [53]).



Raven *JACS* **2011**, *133*, 16251.

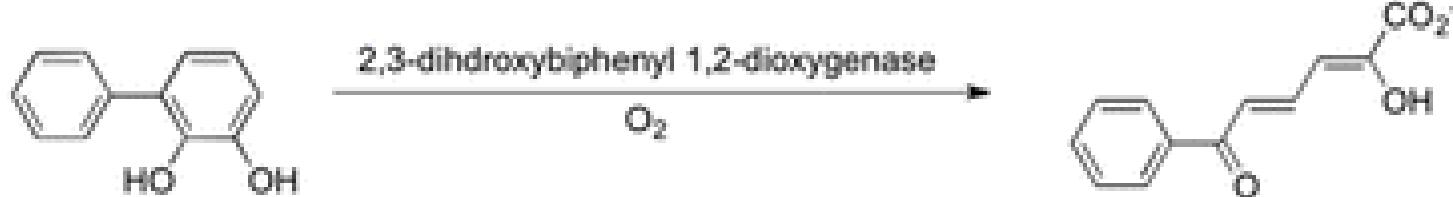
Raven *Curr. Opin. Chem. Biol.* **2012**, 60.

5b. Non-heme Oxygenases

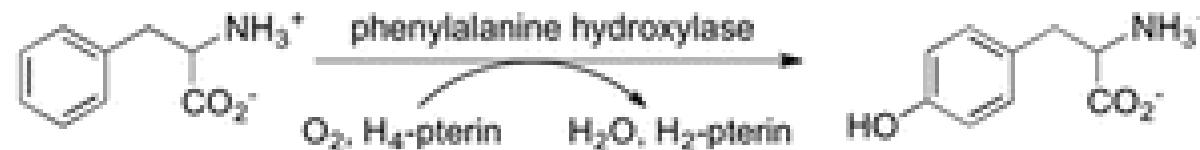
Examples of Non-Heme Oxygenases

Oxygen-Activating (Fe^{II})

Extradiol Dioxygenases



Pterin-Dependent Hydroxylases



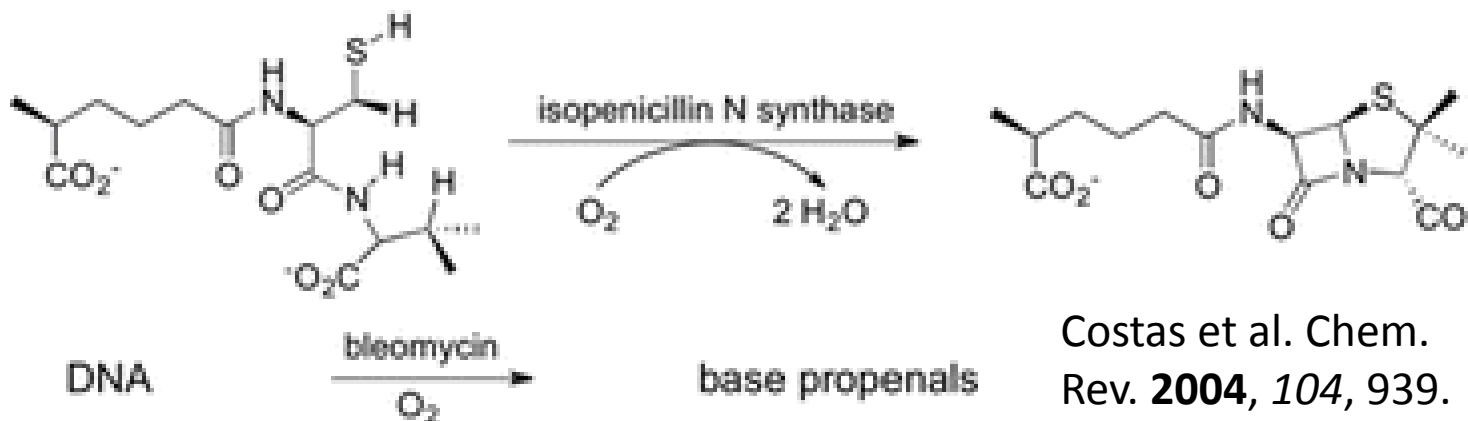
$\alpha\text{-KG}$ -Dependent Dioxygenases



Rieske Dioxygenases



Non-Redox Substrate Enzymes



Bleomycin

DNA

$\xrightarrow[\text{O}_2]{\text{bleomycin}}$

base propenals

Costas et al. Chem.
Rev. 2004, 104, 939.

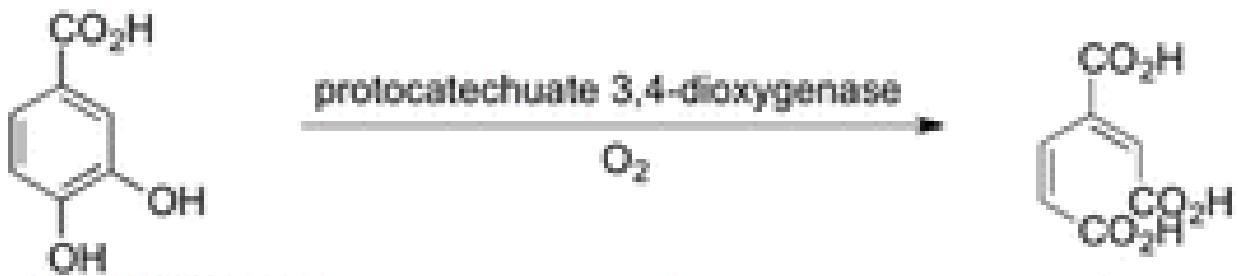
Examples of Non-Heme Oxygenases

Substrate-Activating (Fe^{III})

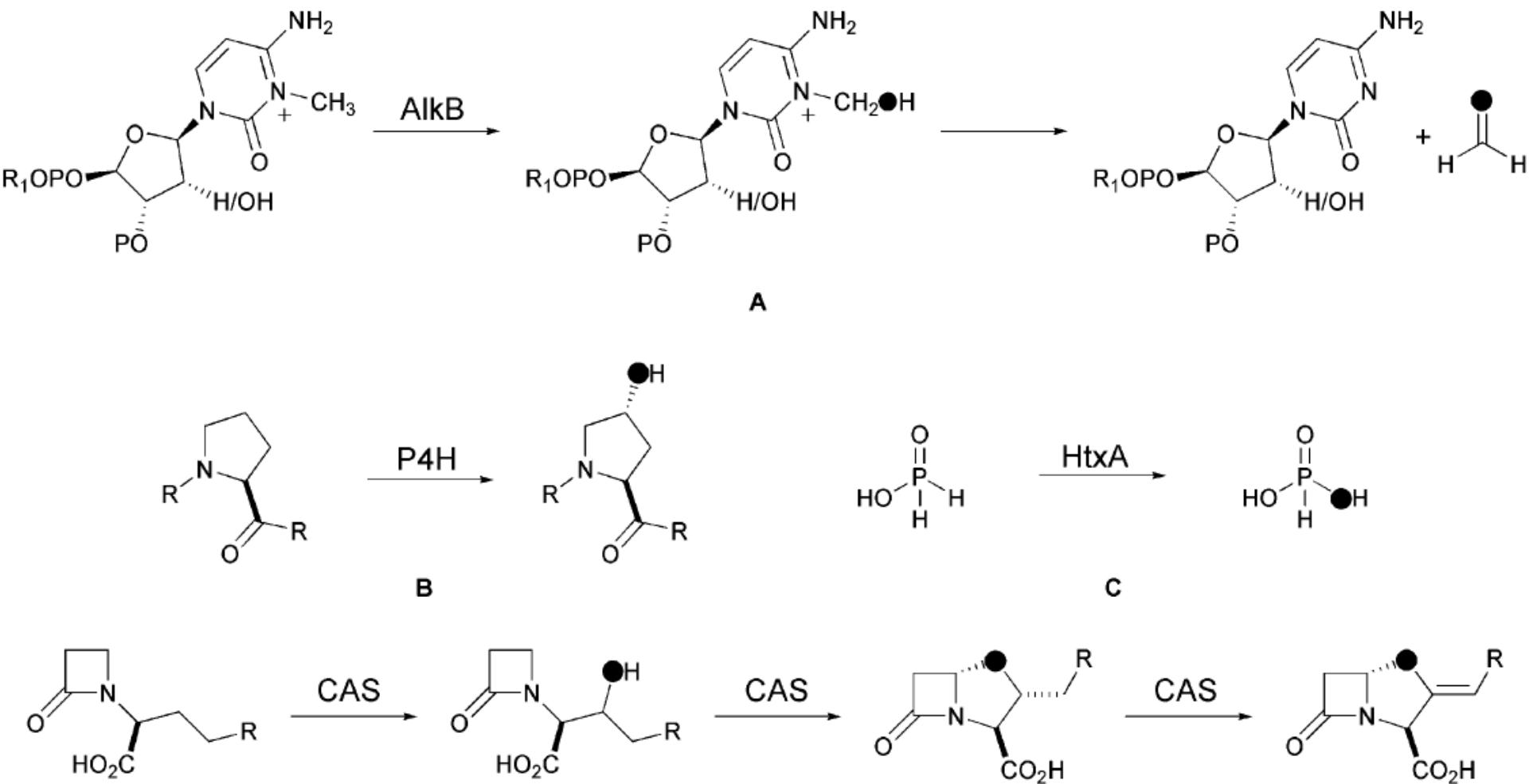
Lipoxygenases



Intradiol
Dioxygenases



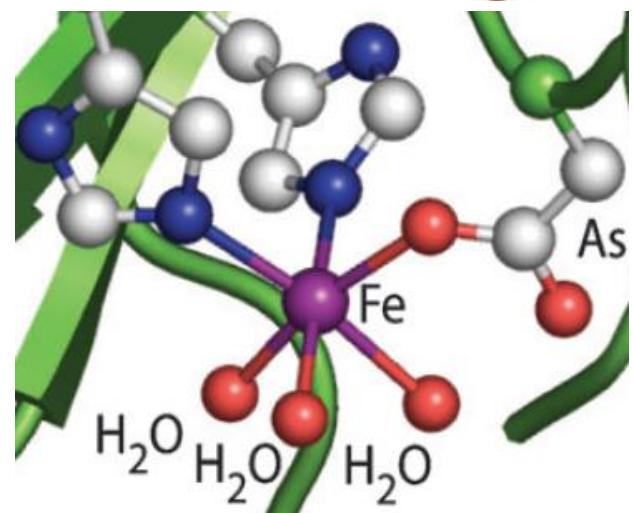
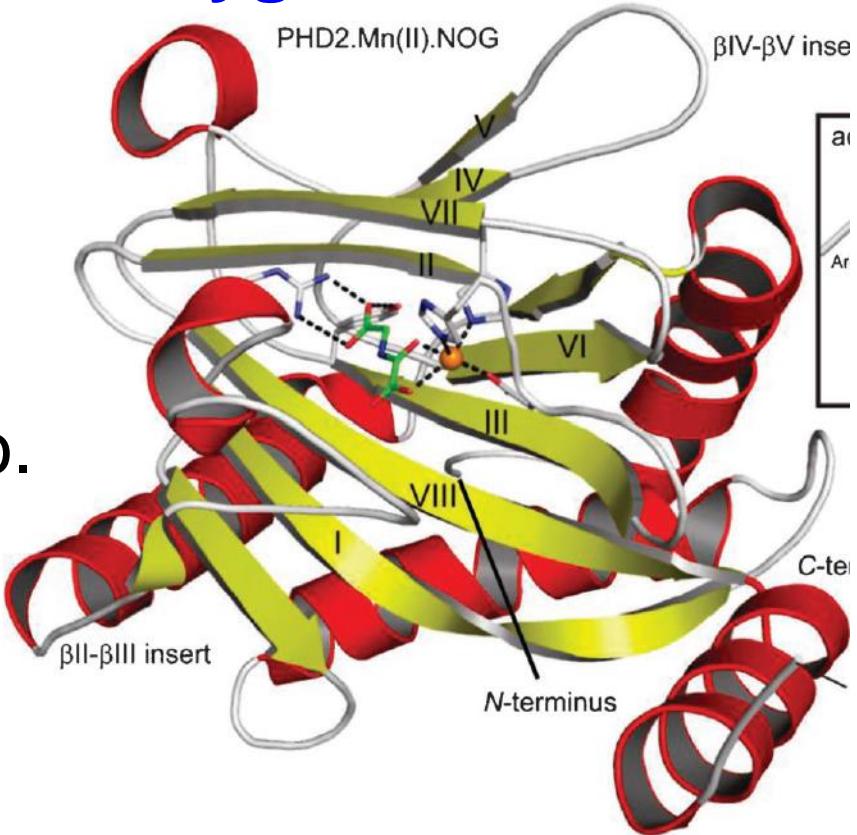
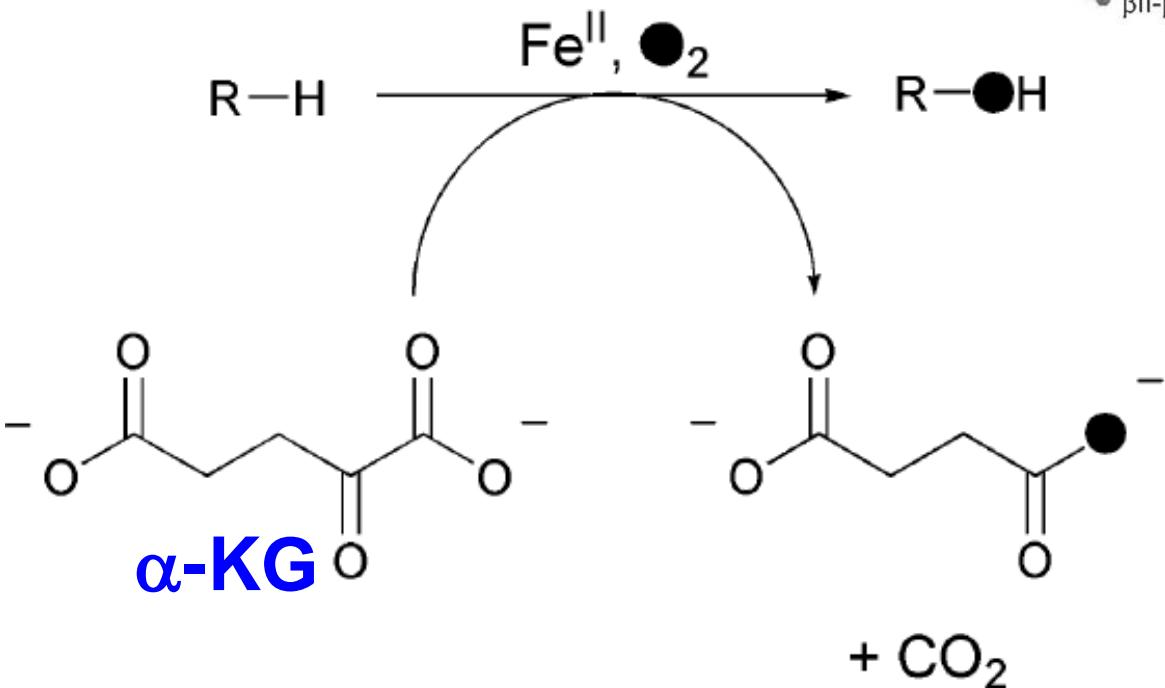
Examples of Non-Heme Oxygenases



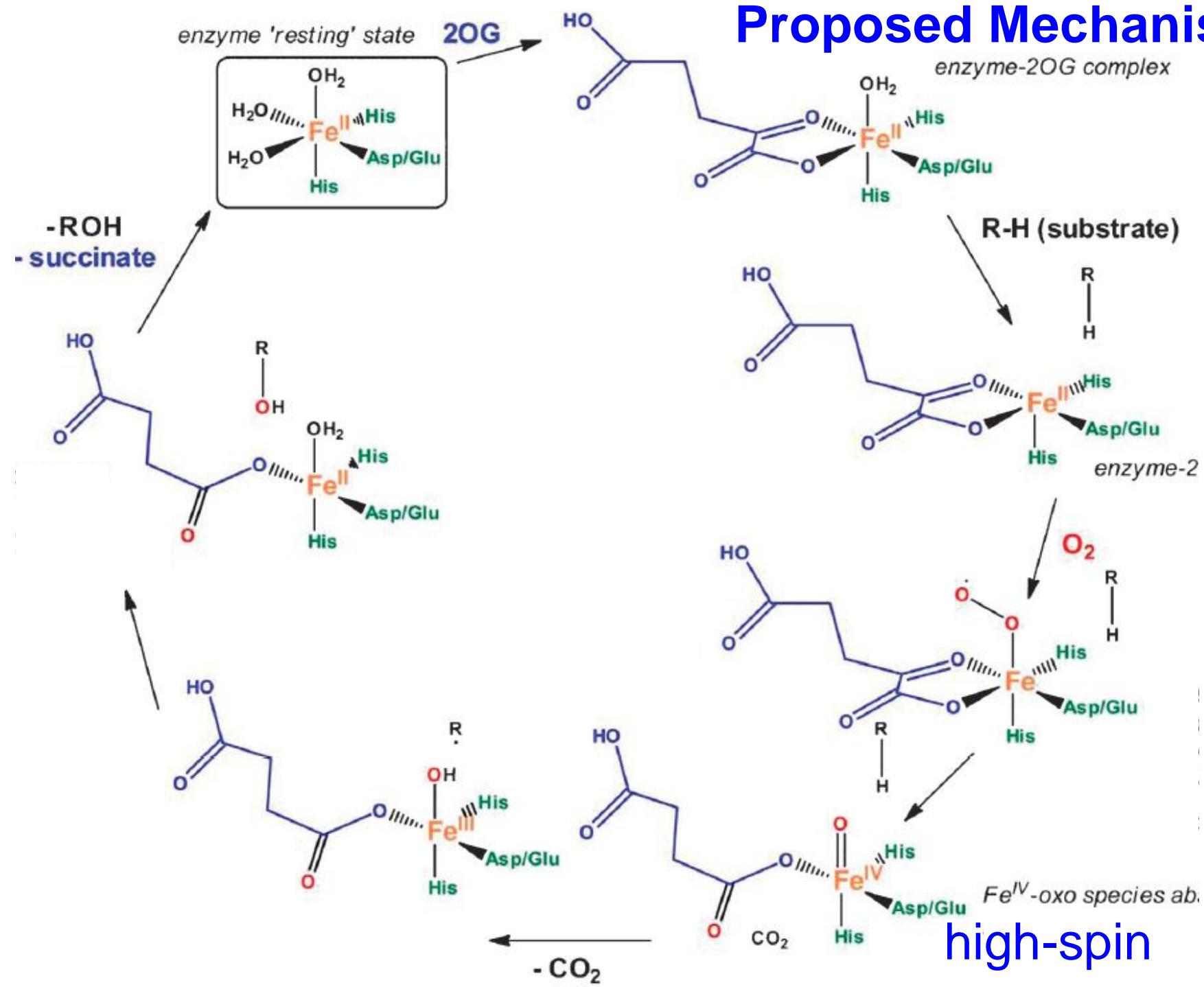
α -Ketoglutarate (KG) dependent Oxygenases

- A key class of oxygenases for wide-range oxidations.

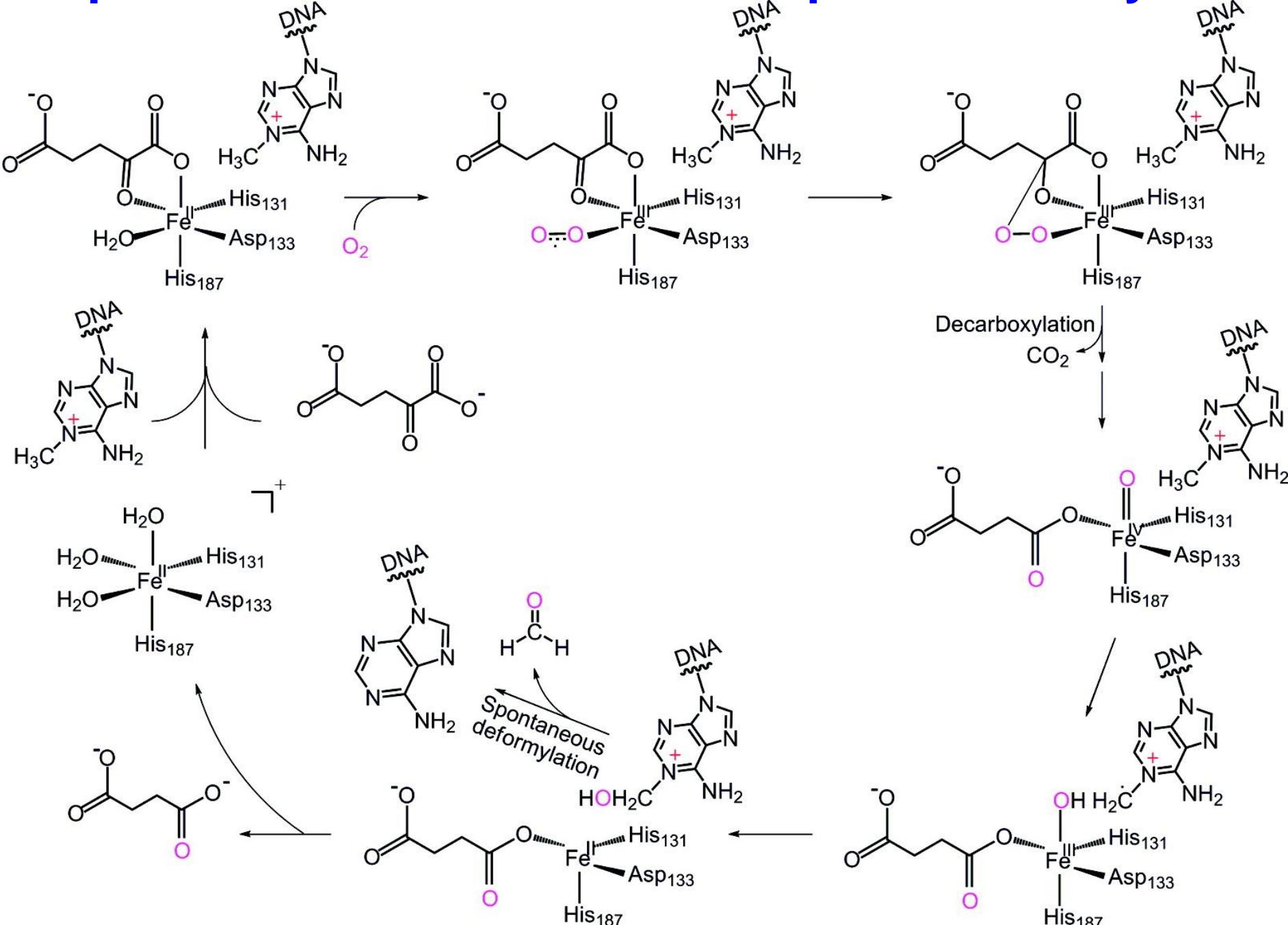
- **α -KG as the co-substrate** to activate O_2 & form Fe(IV)-oxo.
- **2-His+Asp/Glu** coordination.



Proposed Mechanism

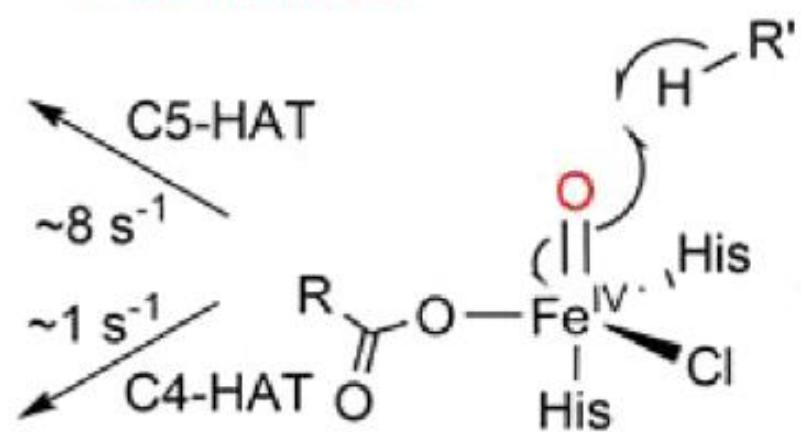
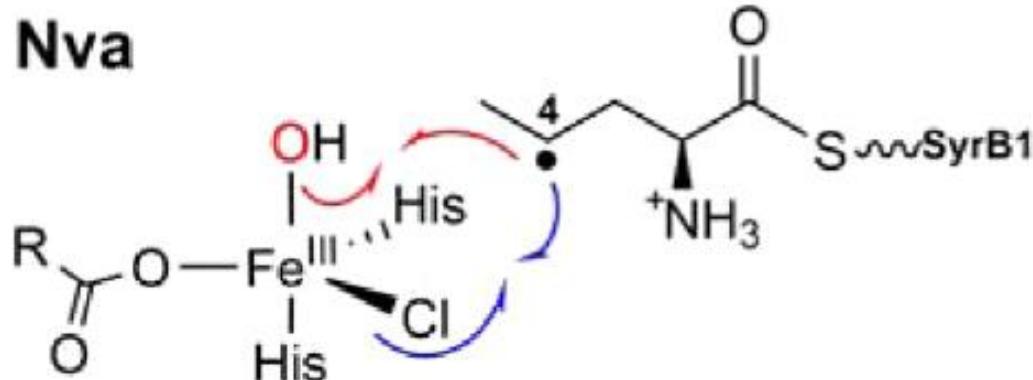
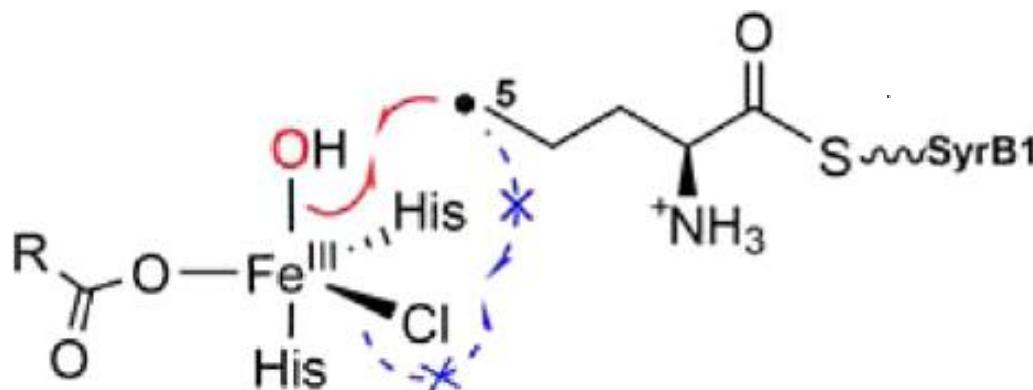
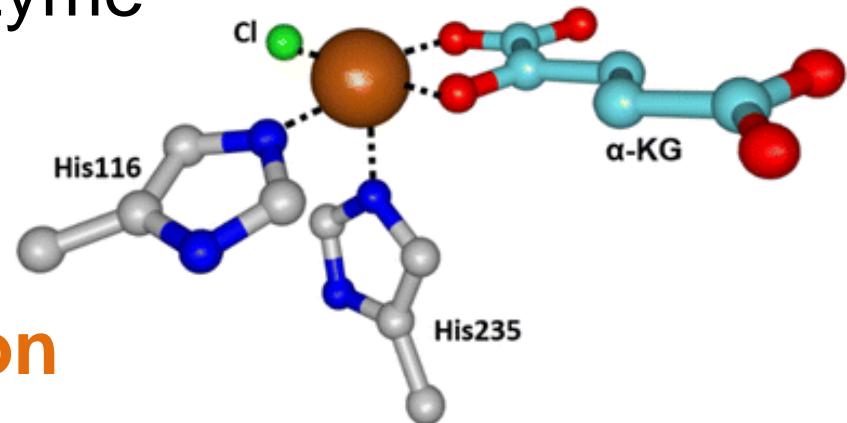


Proposed Mechanism for DNA repair: Demethylation



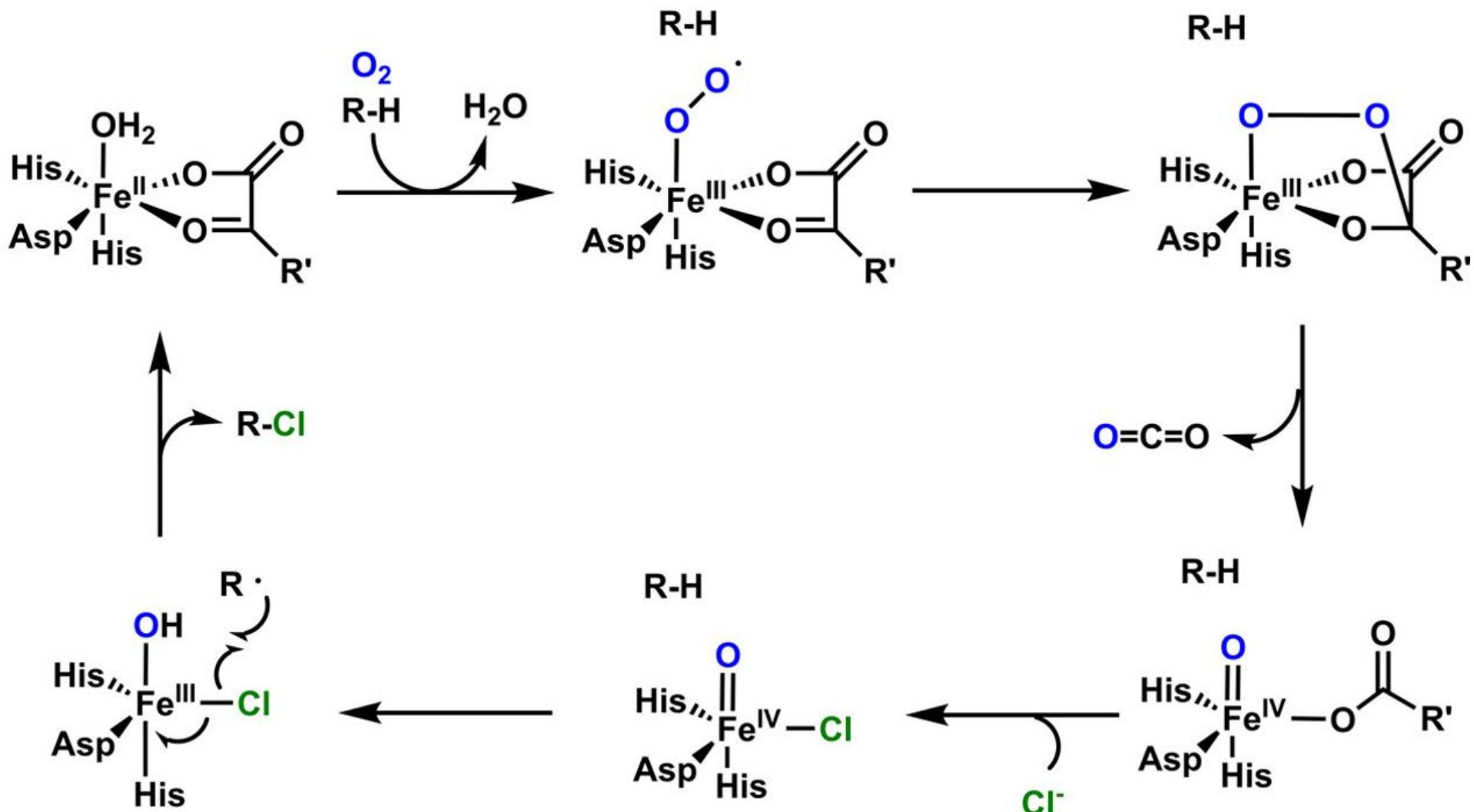
Halogenase SyrB2

- Syringomycin biosynthesis enzyme 2 (SyrB2)
 - 2 His + 1 Cl + 2-KG ligands.
 - Catalyze **halogenation** of the native substrate or **hydroxylation** of non-native substrates.



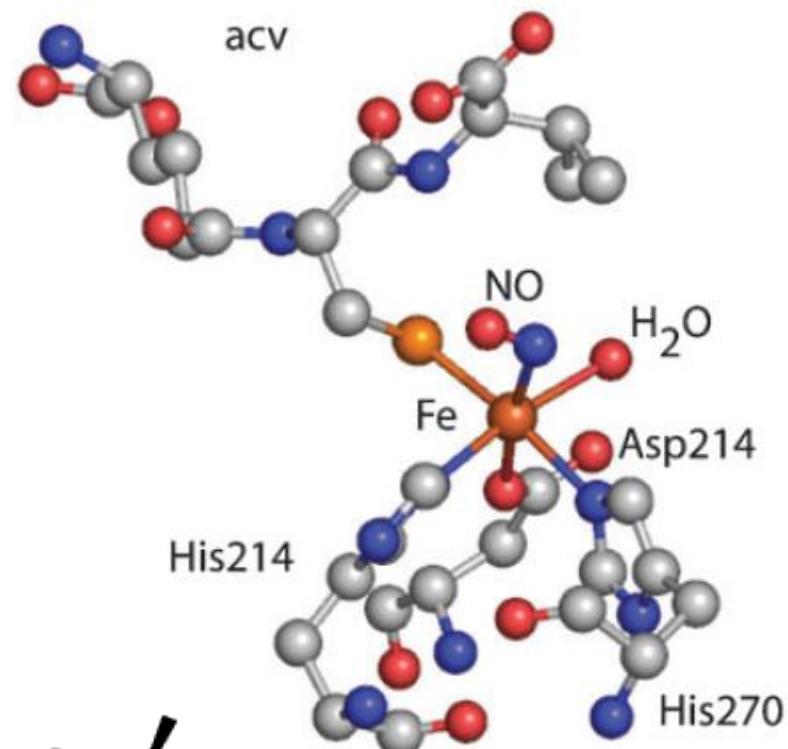
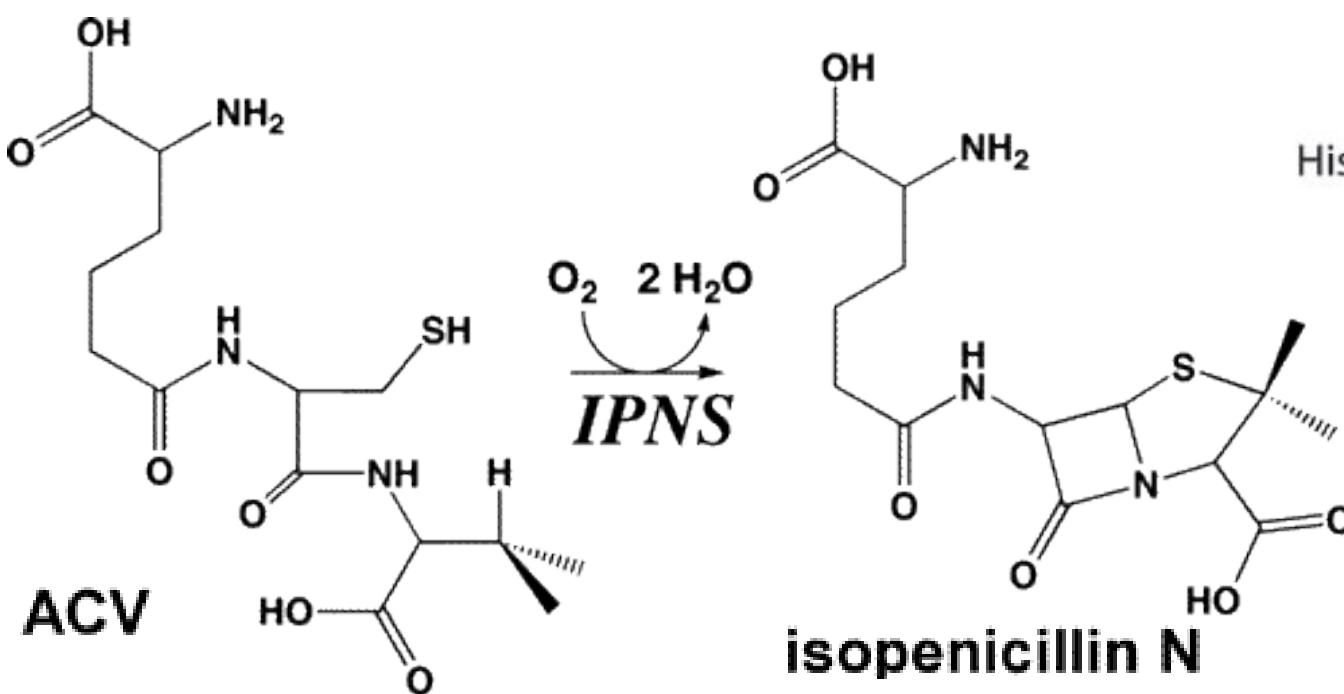
Proposed Mechanism

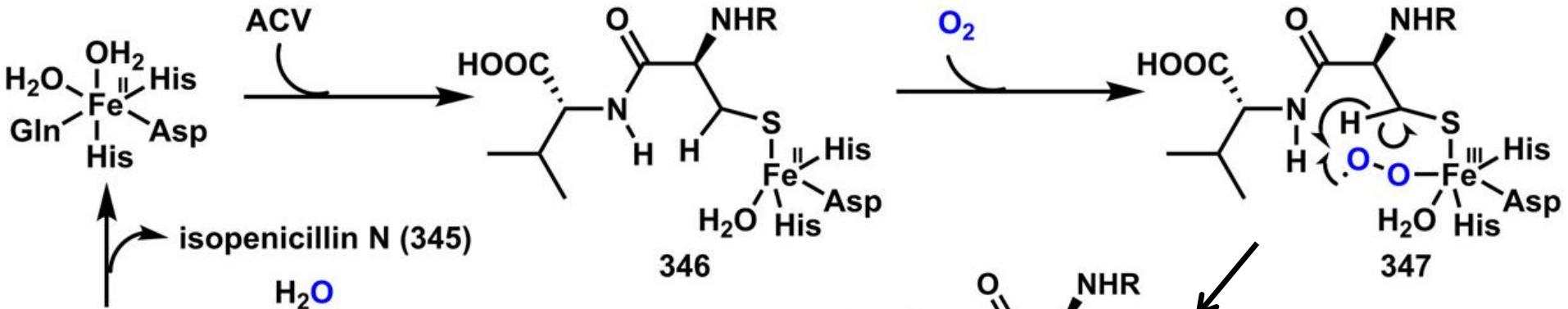
- The formation of the reactive $\text{Fe}=\text{O}$ for H atom abstraction.



Isopenicillin N-synthase (IPNS)

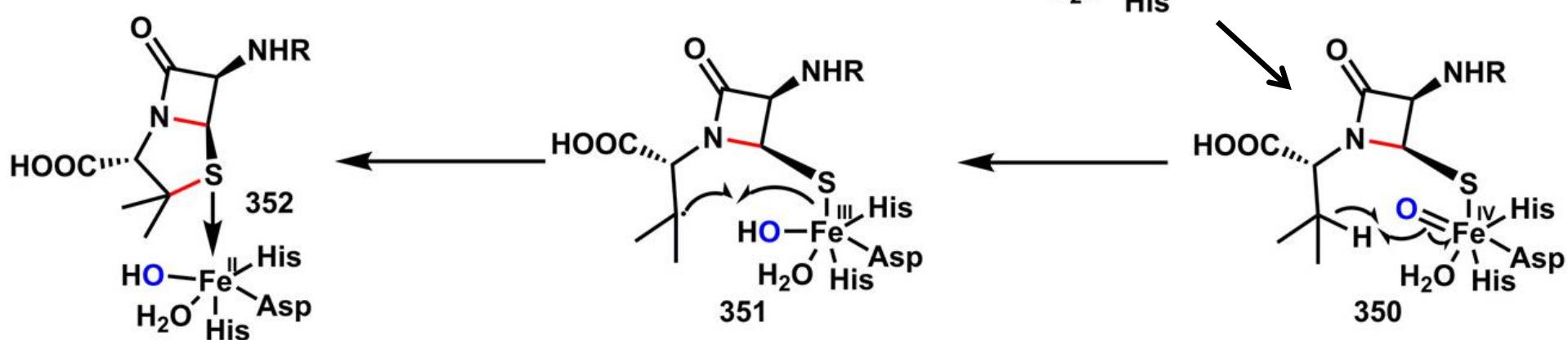
- Catalyzes the formation of isopenicillin N from δ -(L- α -amino adipoyl)-L-cysteinyl-D-valine (ACV): the key step in the biosynthesis of penicillin.
- 2 His + 1 Asp ligands.
- 2-KG is not required.





Proposed Mechanism

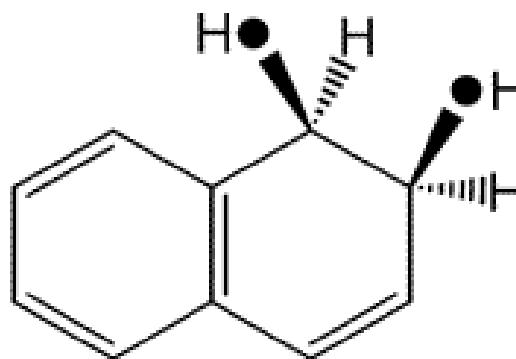
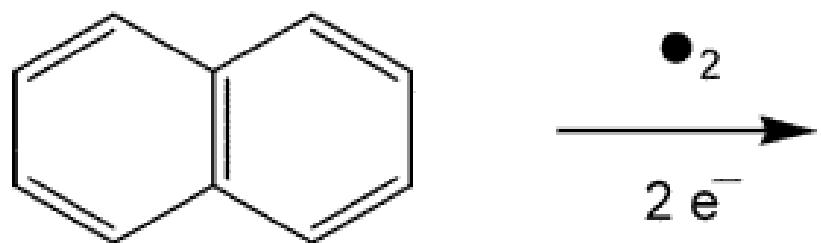
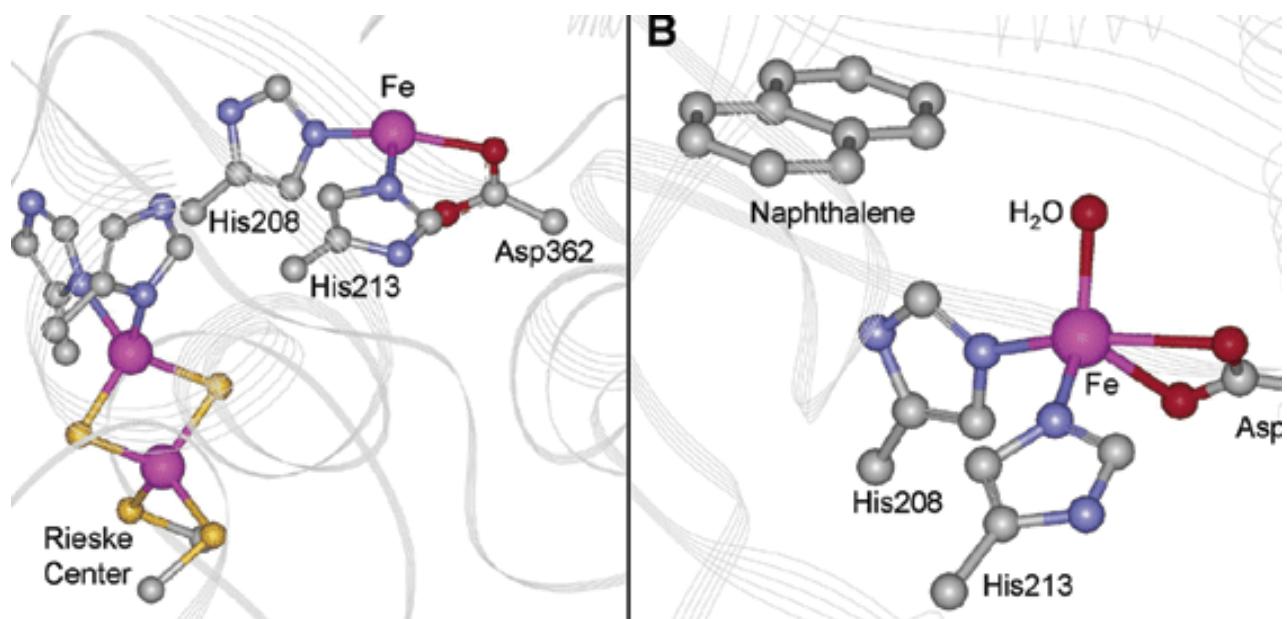
Tang et al. Chem. Rev.
2017, 117, 5226



Rieske cis-diol dioxygenases

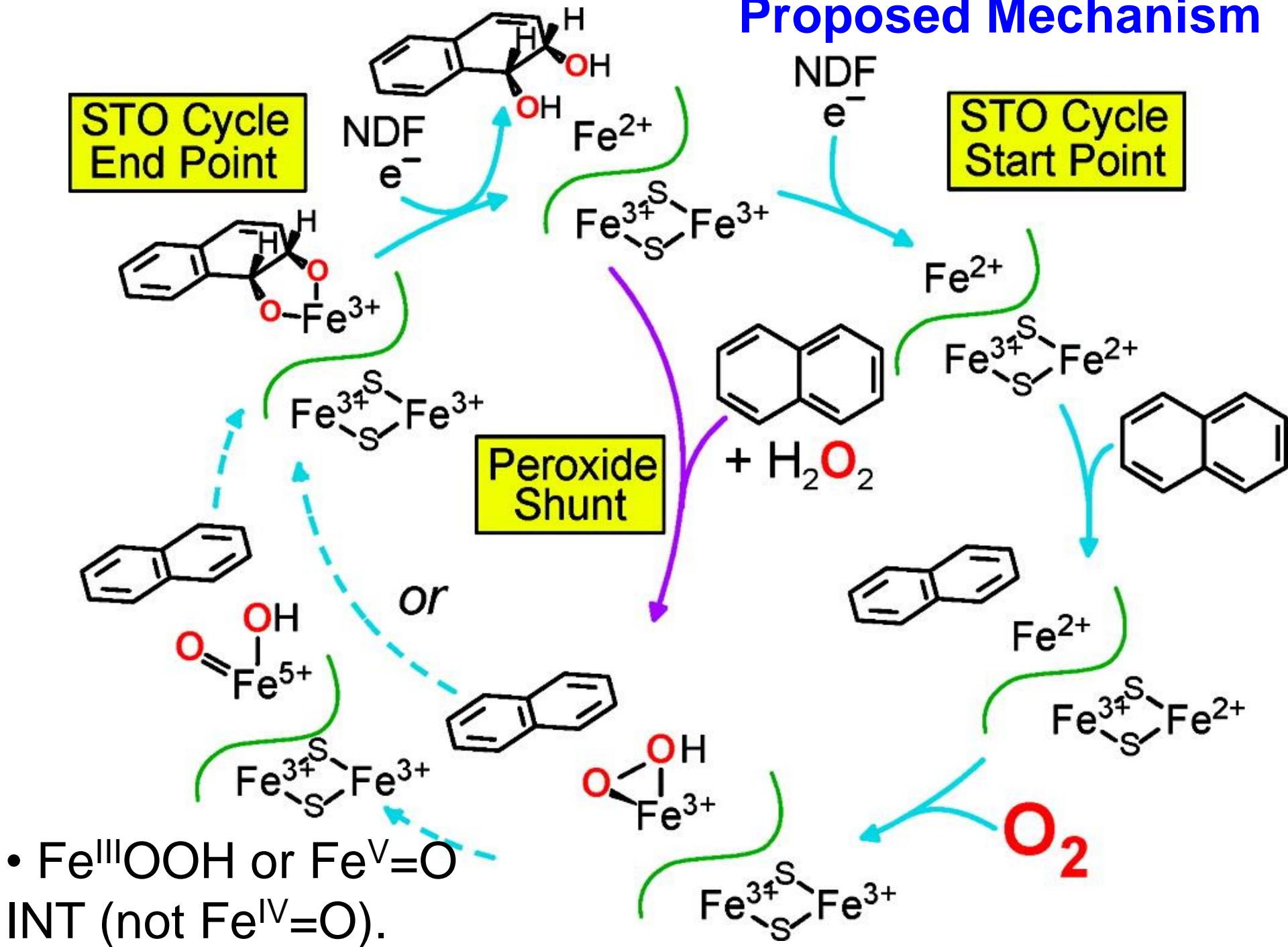
- Catalyzes cis-dihydroxylation of arenes to form cis-dihydro-diol products (the 1st step in the biodegradation of aromatic molecules by soil bacteria).

- 2 His + 1 Asp
& Rieske-type
[2Fe-2S] cluster
(as the e⁻ donor)
in the active site.



Costas et al. Chem.
Rev. 2004, 104, 939

Proposed Mechanism

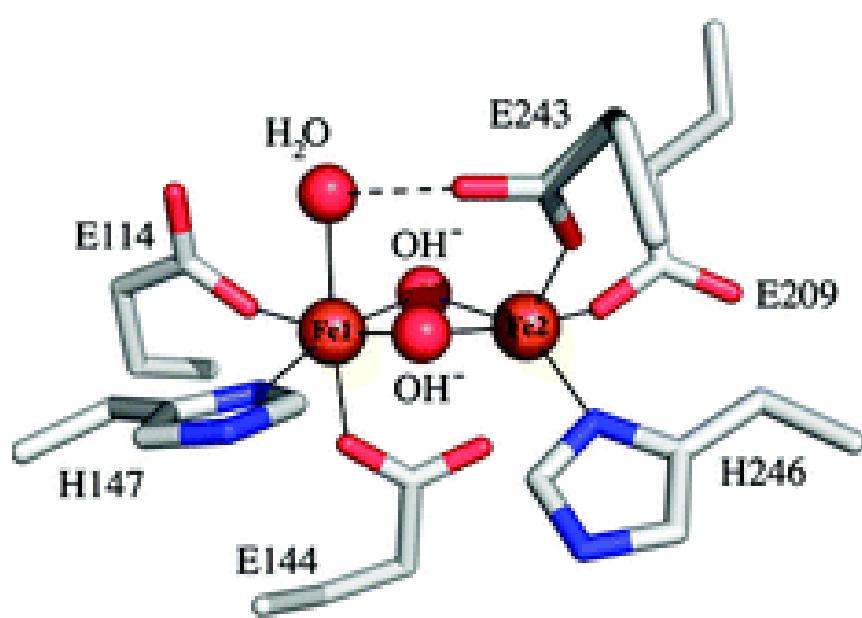
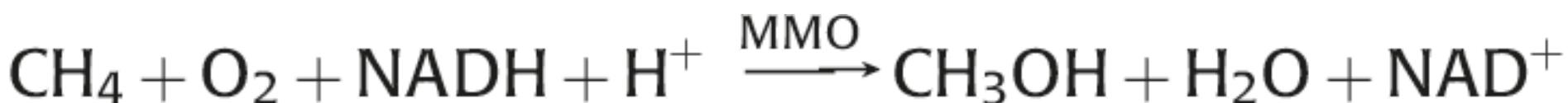


- $\text{Fe}^{\text{III}}\text{OOH}$ or $\text{Fe}^{\text{V}}=\text{O}$

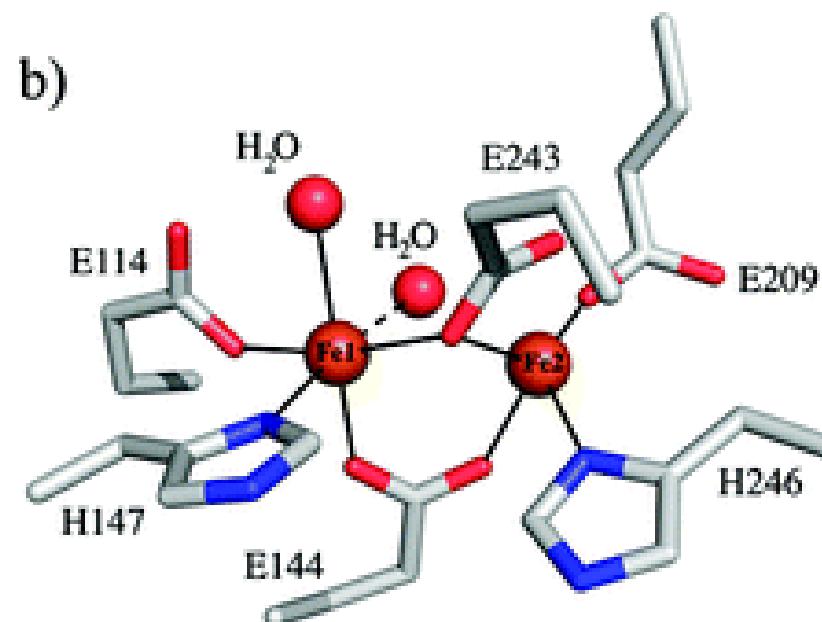
INT (not $\text{Fe}^{\text{IV}}=\text{O}$).

Soluble Methane Monooxygenase (sMMO)

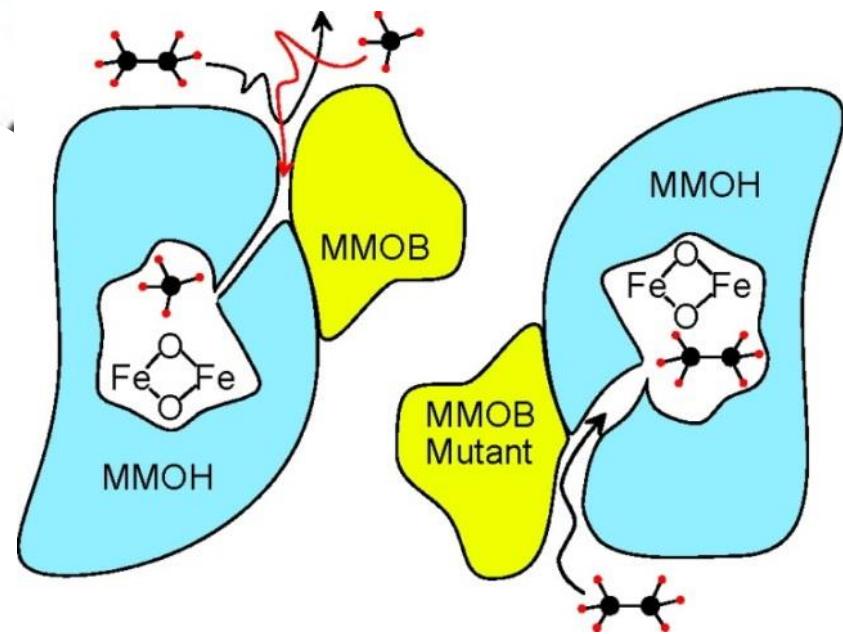
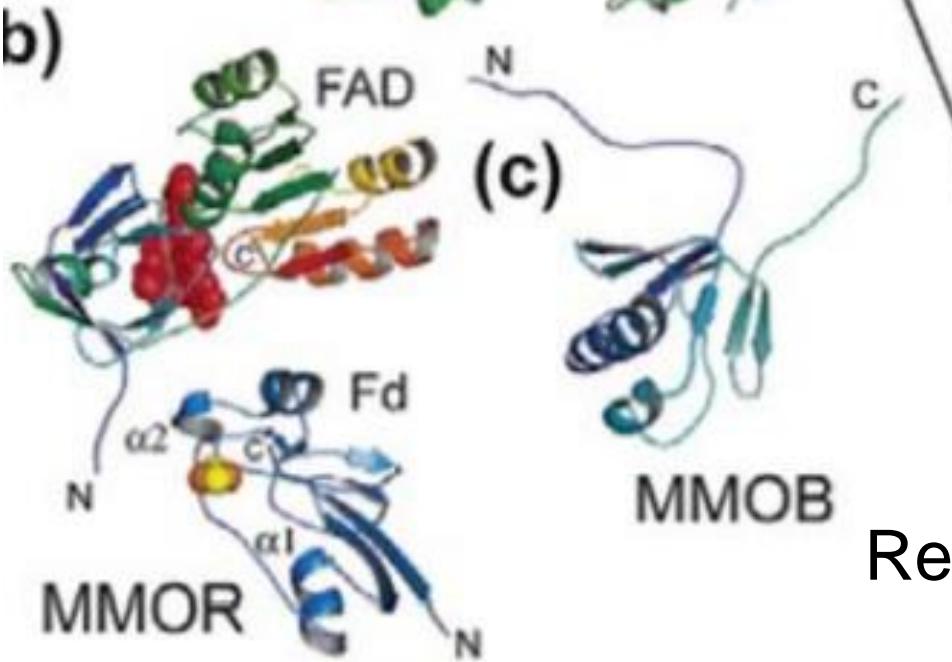
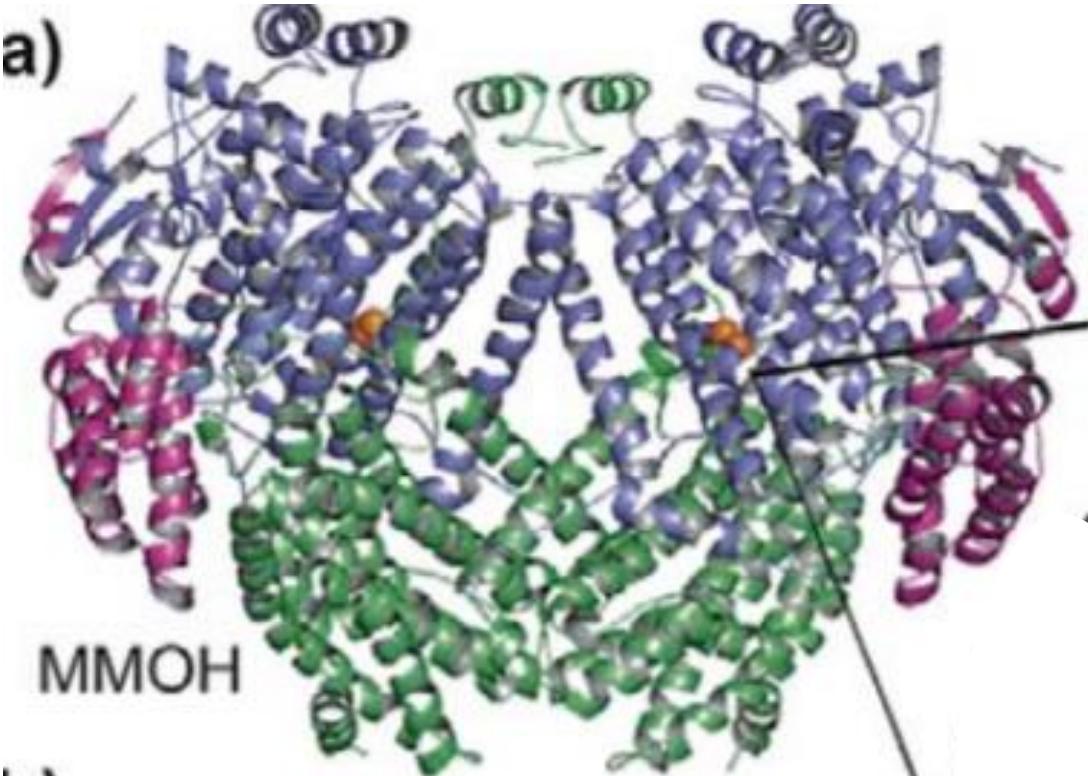
- Catalyzes conversion of CH_4 to CH_3OH in methanotrophic bacteria.
- A **di-iron monooxygenase**.
- Activate a very strong C-H bond ($\text{BDE} = 104 \text{ kcal/mol}$).



MMOH_{ox}

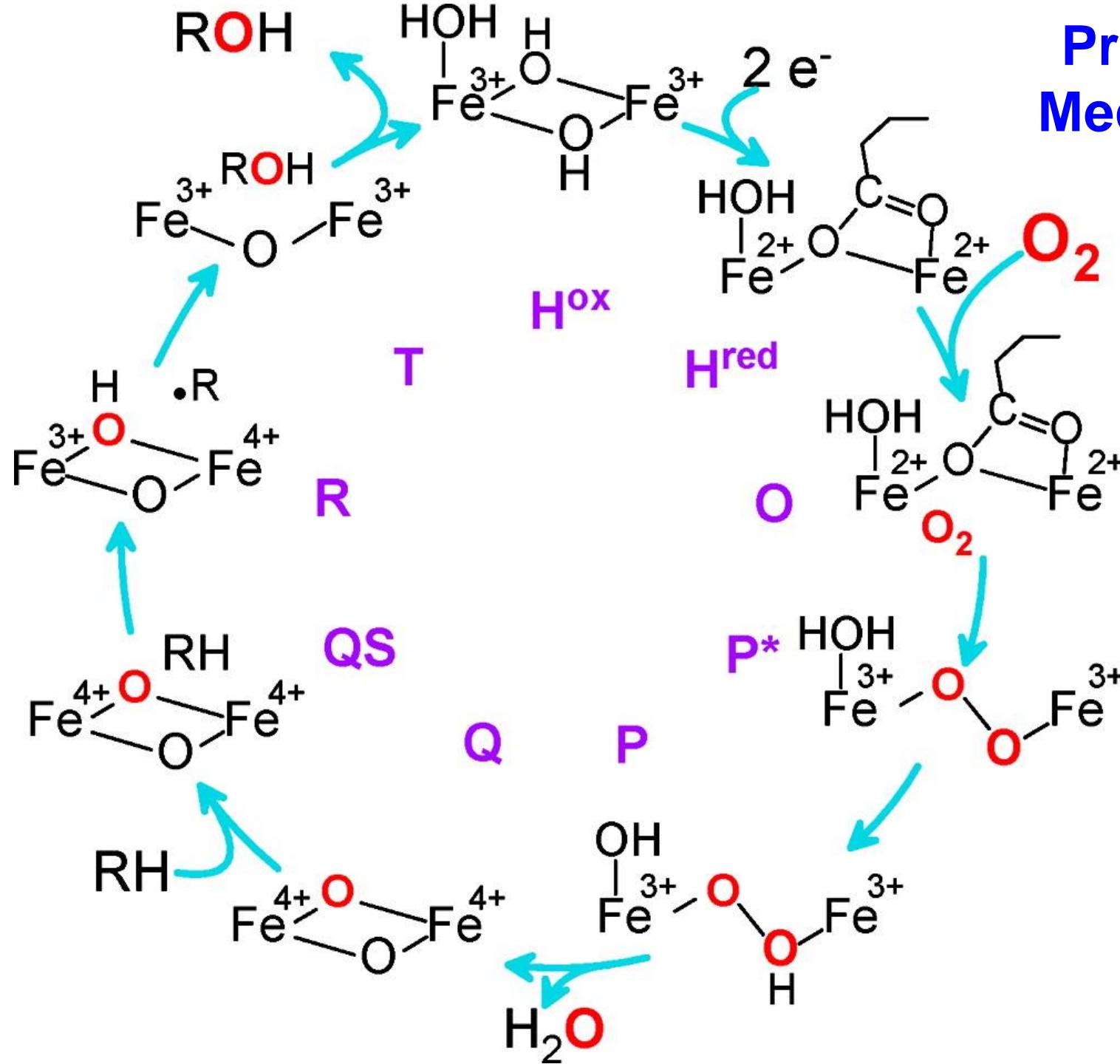


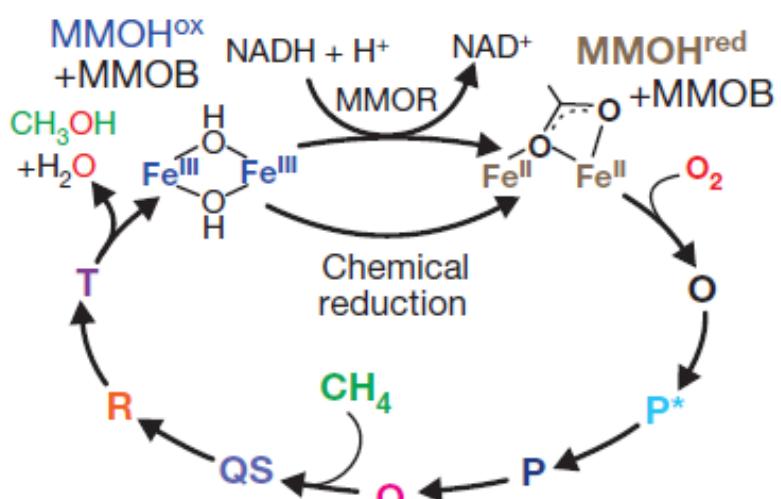
MMOH_{red}



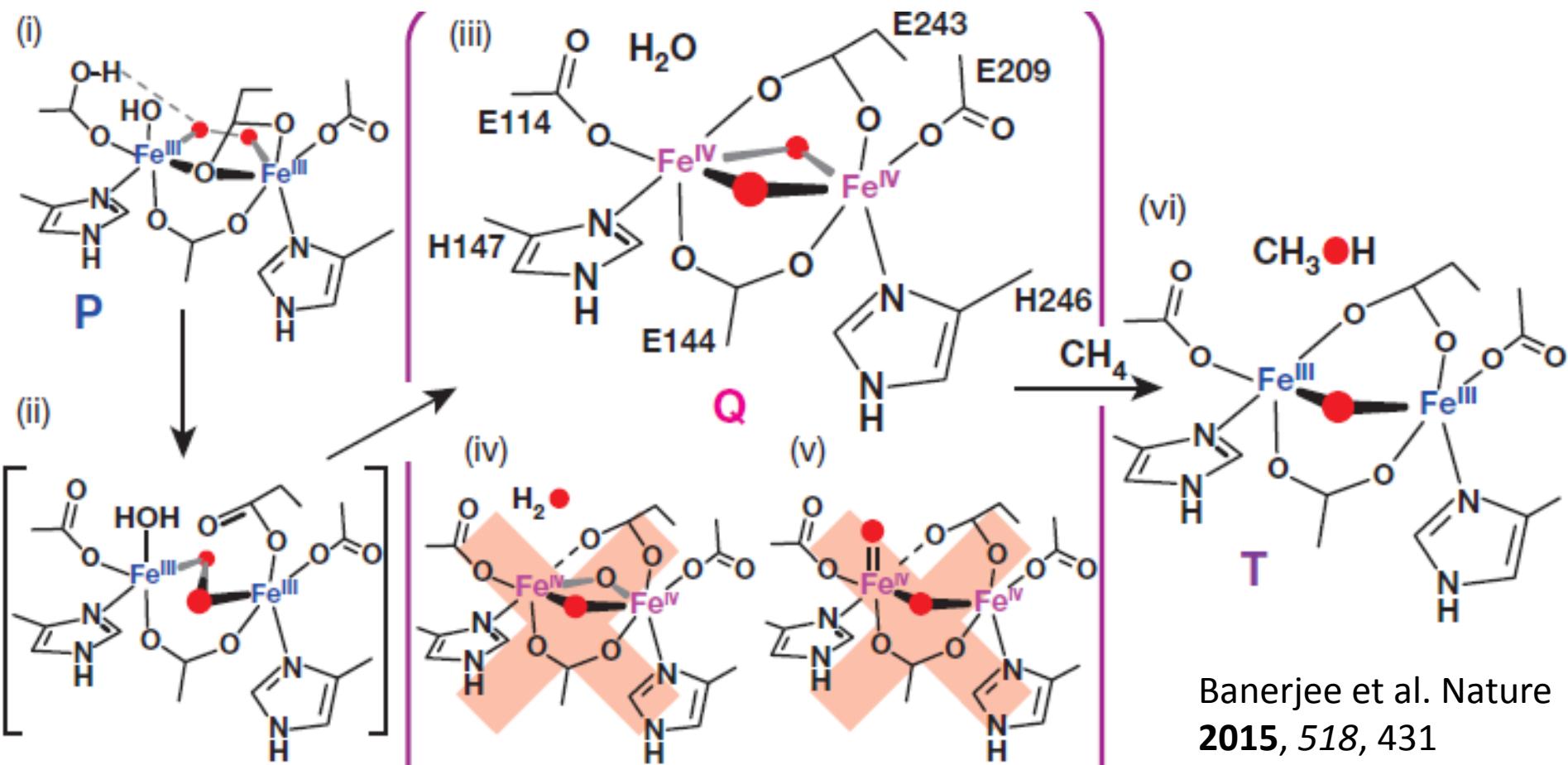
MMO hydroxylase (MMOH)
Regulatory B component (MMOB)
MMO reductase (MMOR)

Proposed Mechanism





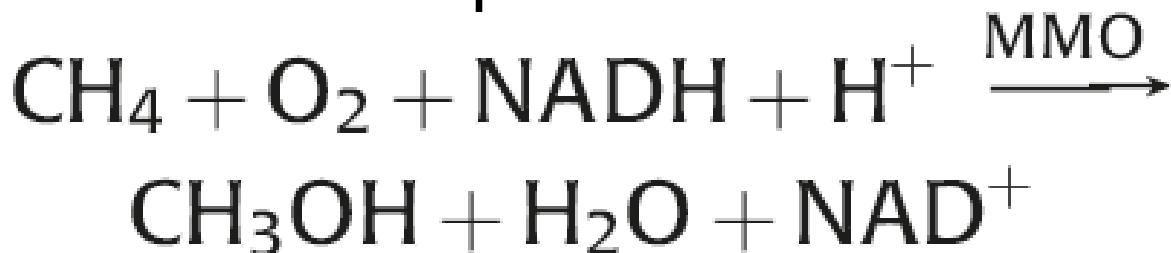
- Resonance Raman vibrational spectroscopy: the 2 O atoms from O₂ form the core structure in the key Compound Q.



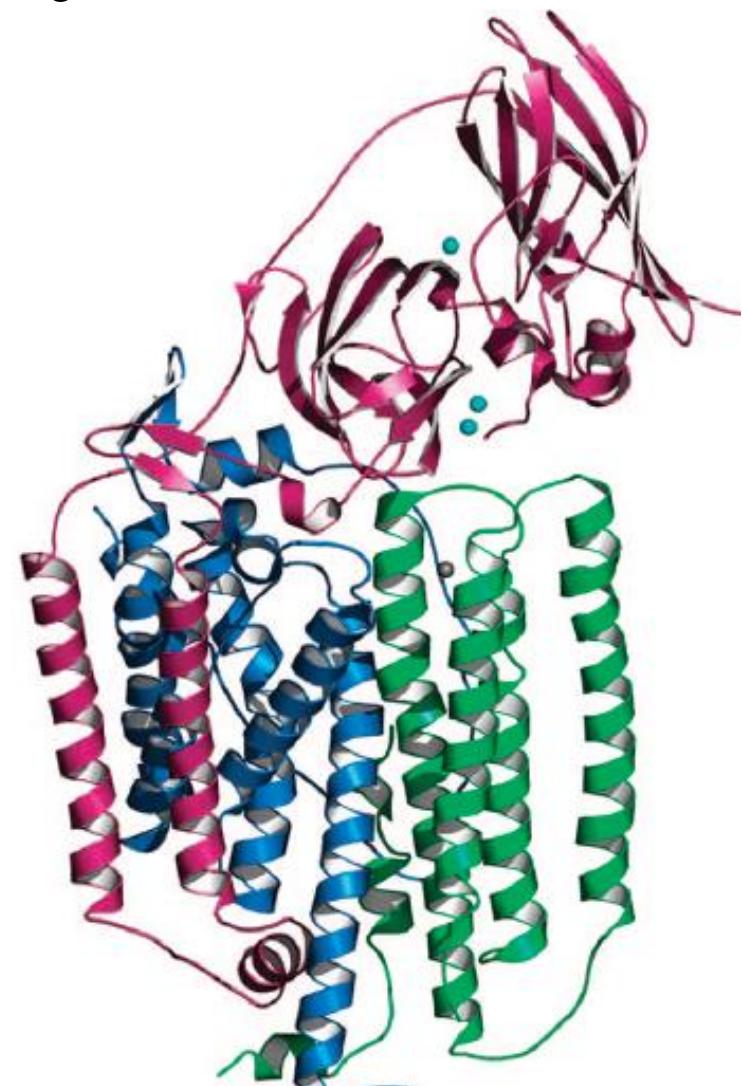
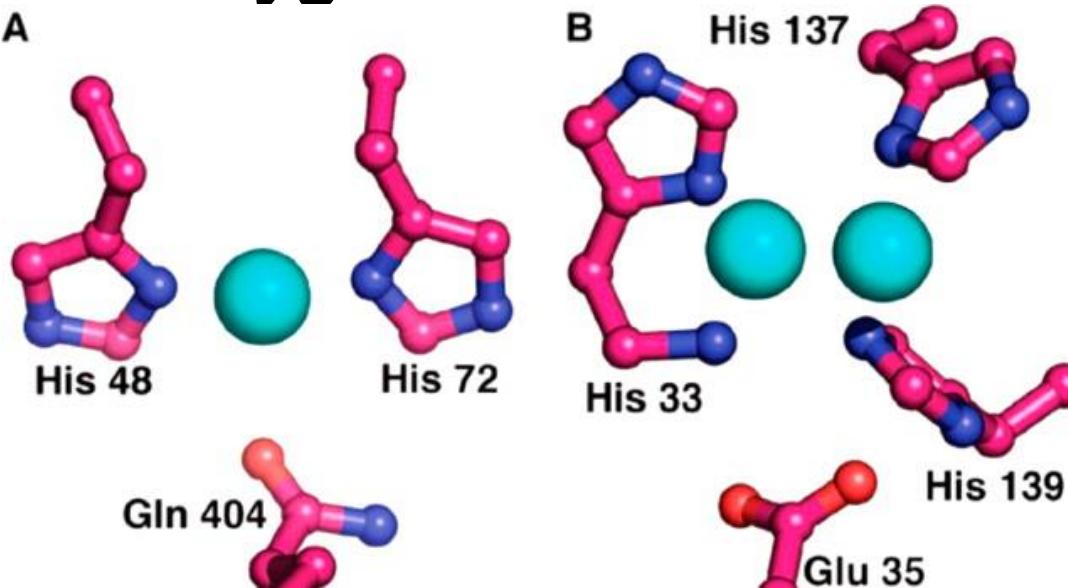
5c. Cu-containing Oxygenanse

Particulate Methane Monooxygenase (pMMO)

- Catalyzes conversion of CH_4 to CH_3OH in methanotrophic bacteria.

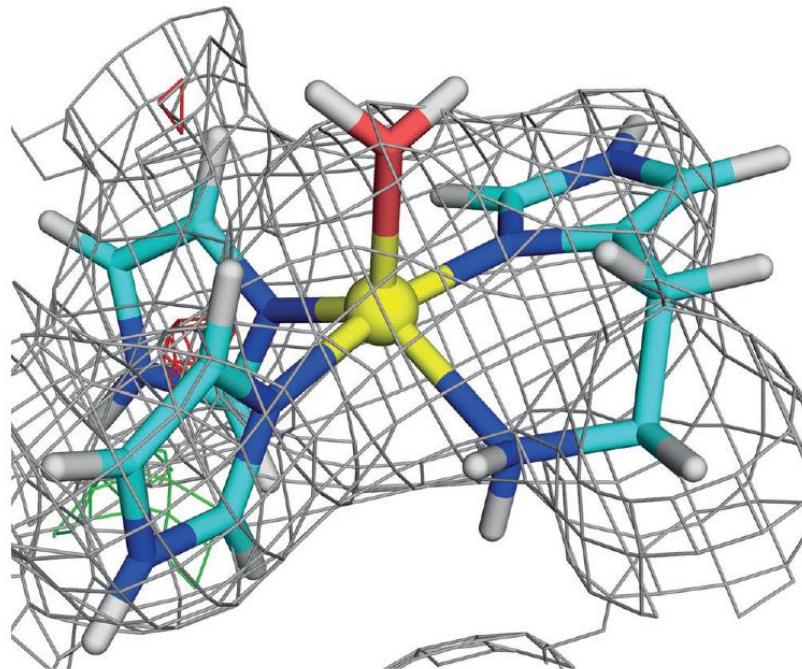
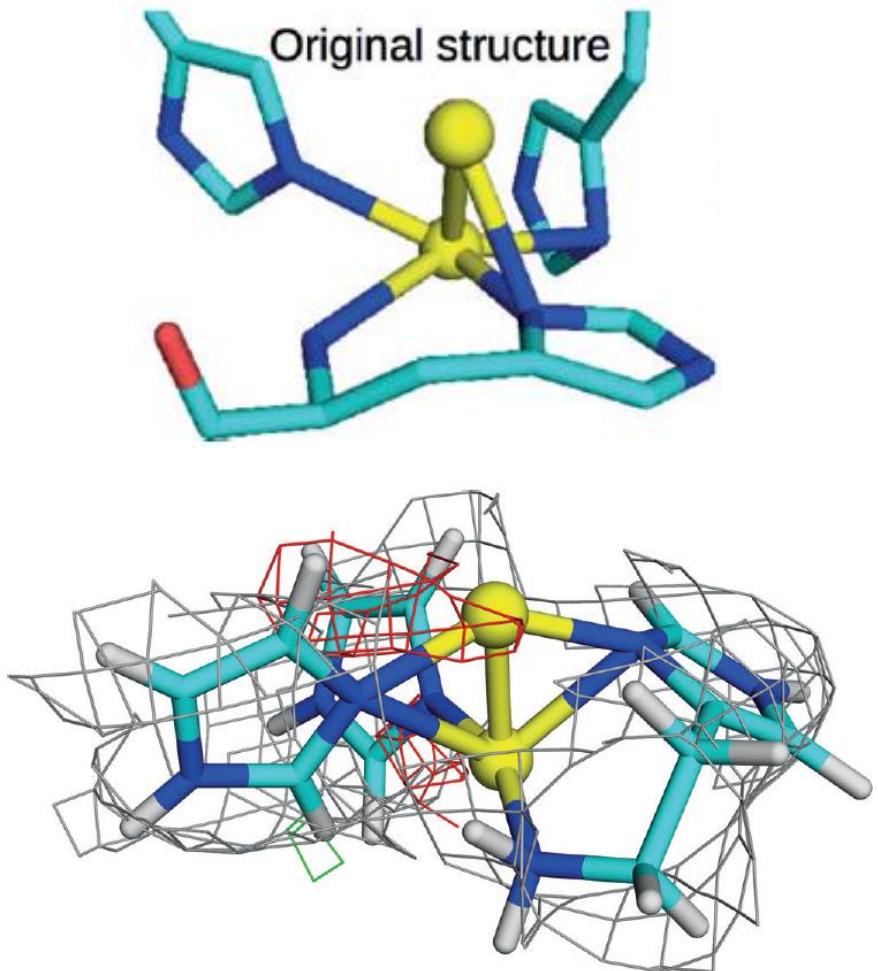


- A proposed **di-copper?** monooxygenase.



Quantum Refinement Does Not Support Dinuclear Copper Sites in Crystal Structures of Particulate Methane Monooxygenase

Lili Cao, Octav Caldararu, Amy C. Rosenzweig, and Ulf Ryde*



Quantum-refined
mono-Cu structure

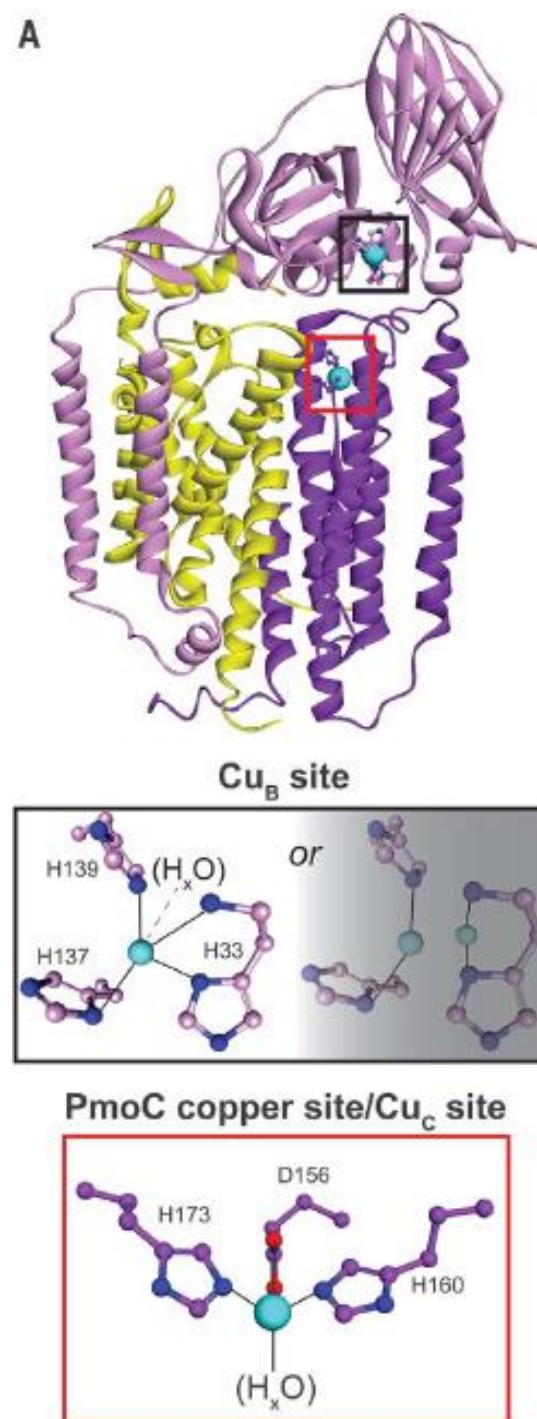
Particulate methane monooxygenase contains only mononuclear copper centers

Matthew O. Ross^{1,2}, Fraser MacMillan³, Jingzhou Wang^{4,5}, Alex Nisthal^{4,5*}, Thomas J. Lawton^{1,2†}, Barry D. Olafson⁶, Stephen L. Mayo^{4,5}, Amy C. Rosenzweig^{1,2‡}, Brian M. Hoffman^{1,2‡}

“EXAFS...initially modeled as **dicopper** in some, but not all, structures, with a later **quantum refinement** study supporting the **monocopper** assignment”;

“The nuclearity, ligation, and location of the pMMO copper active site have been **difficult to assign**. The pMMO **isolation and purification** procedure has been suggested to result in loss or alteration of the essential metallocofactor”;

“**Cu_C** may be the site of O₂ binding and methane oxidation...would be consistent with the suggested presence of a displaceable solvent ligand on Cu_C, as needed for O₂-binding/activation, binding of the nitrite inhibitor to Cu_C.”

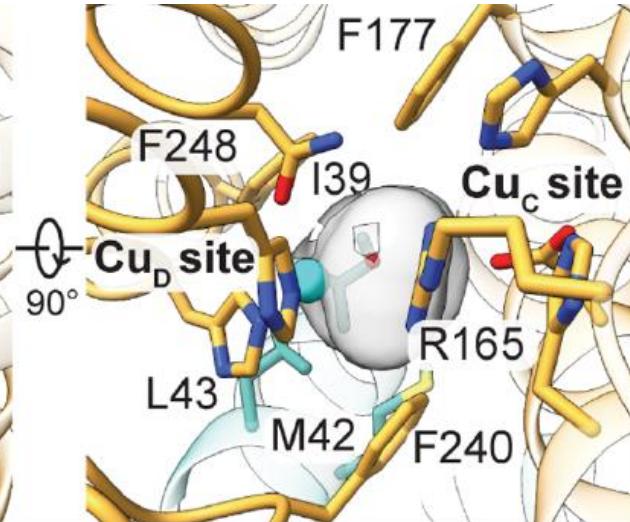
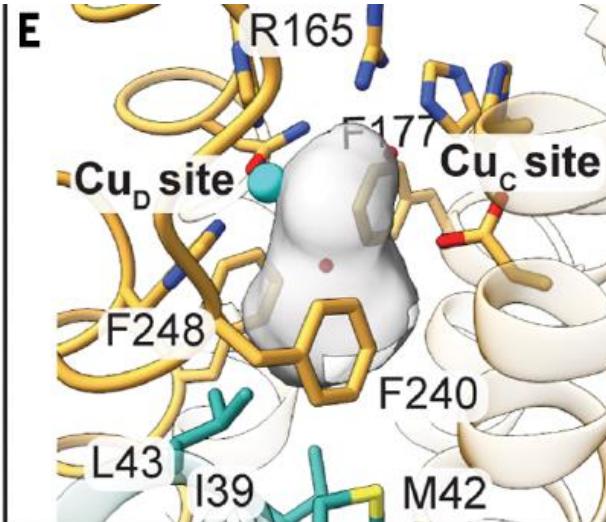
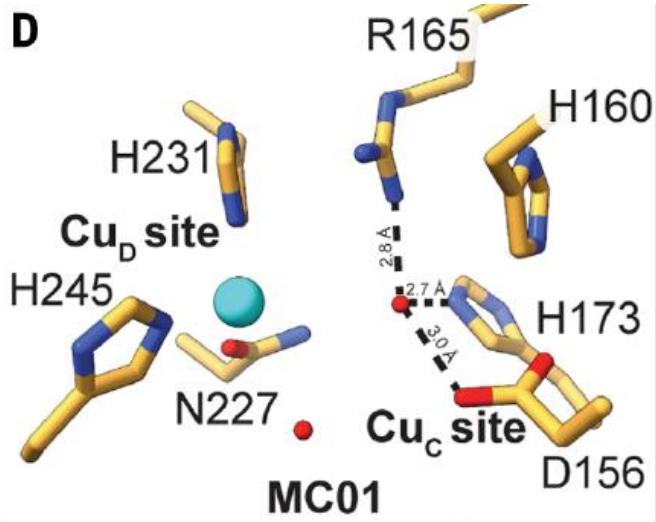
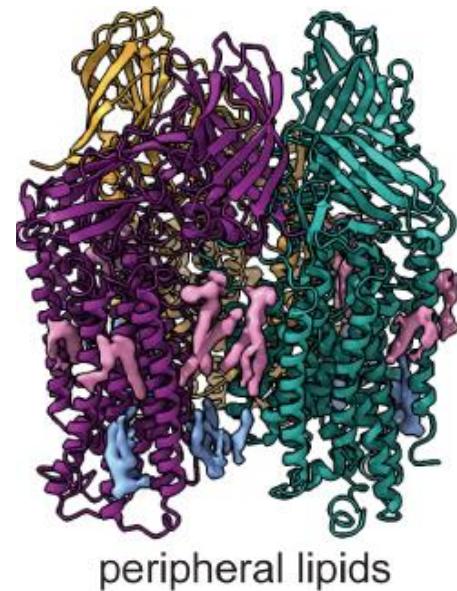


Recovery of particulate methane monooxygenase structure and activity in a lipid bilayer

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Bacterial methane oxidation using the enzyme particulate methane monooxygenase (pMMO) contributes to the removal of environmental methane, a potent greenhouse gas. Crystal structures determined using inactive, detergent-solubilized pMMO lack several conserved regions neighboring the proposed active site. We show that reconstituting pMMO in nanodiscs with lipids extracted from the native organism restores methane oxidation activity. Multiple nanodisc-embedded pMMO structures determined by cryo-electron microscopy to 2.14- to 2.46-angstrom resolution reveal the structure of pMMO in a lipid environment. The resulting model includes stabilizing lipids, regions of the PmoA and PmoC subunits not observed in prior structures, and a previously undetected copper-binding site in the PmoC subunit with an adjacent hydrophobic cavity. These structures provide a revised framework for understanding and engineering pMMO function.



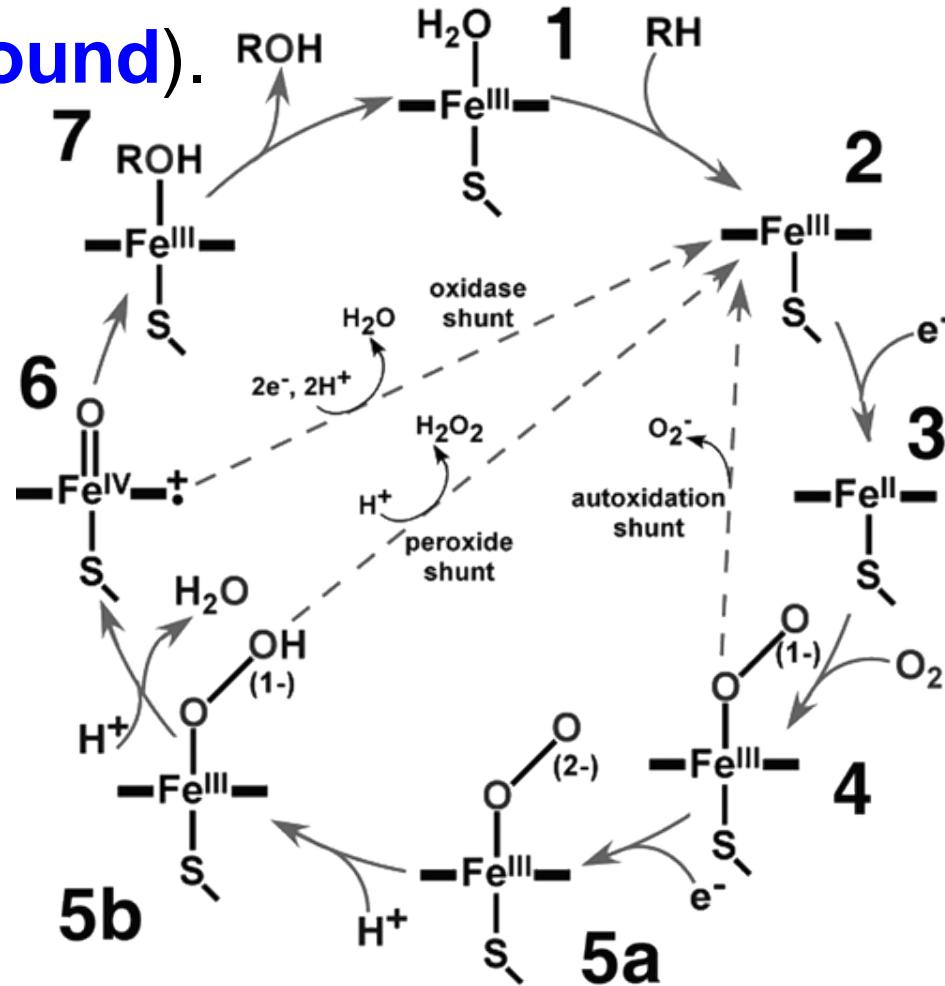
Key Summary

- Most non-photosynthetic systems gain **energy** from enzymatic **reduction** of O_2 to H_2O .
- Reactions of **triplet** O_2 with many (**close-shell singlet**) molecules are generally **thermodynamically favorable**, but require **high barriers** in the absence of the catalyst or radical initiators, due to **spin-restriction** or the energetically **unfavorable first reduction** of O_2 .
- **Metalloenzymes** catalyze the reactions with O_2 in an **effective, well-controlled & selective** manner by overcoming the spin restriction & unfavorable 1st reduction problems.

- Two common types of reactions with O_2 in enzymes: electron-transfer & oxygen atom transfer reactions (**oxidases** & **oxygenases**, respectively).
- Some side products from non-enzymatic & enzymatic reactions of O_2 , e.g. O_2^- , H_2O_2 , $HO\bullet$ (so-called reactive oxygen species, **ROS**) cause **oxidative damage**.
- Both small **anti-oxidant molecules** & **anti-oxidant enzymes** defend against such oxidative stress.
- Major anti-oxidant enzymes: **superoxide dismutase (SOD)** & **superoxide reductase (SOR)** enzymes for O_2^- ; **catalase** & **peroxidase** enzymes for H_2O_2 .
- **Anti-oxidant molecules**: e.g. Vitamins C & E.

- 2 classes of O_2^- detoxification enzymes: superoxide dismutase (SOD) & superoxide reductase (SOR).
- **SODs** catalyze the **disproportionation of O_2^-** to give O_2 & H_2O_2 ; 3 classes of **SODs**: (1) **CuZnSOD**, (2) **FeSOD & MnSOD**, & (3) **NiSOD**.
- **SORs** catalyze the **1e^- reduction of O_2^-** to give H_2O_2 : a **non-heme** enzyme.
- **Peroxidases** use H_2O_2 as the **oxidant** to oxidize different molecules by **heterolytic O-O cleavage** (**not homolytic** one to form toxic $\text{HO}\bullet$).
- Peroxidase is a **heme** enzymes, involving formation of compound I & compound II.
- **Catalases (heme)** catalyze **disproportionation of H_2O_2** to give O_2 & H_2O .

- Cytochrome P450: important heme-containing monooxygenases to catalyze many reactions with O_2 . A **proximal Cys ligand** for the heme; the formation of the key **reactive Compound I** for the stepwise hydroxylation (**H atom abstraction** with the oxo followed by **rebound**).



- Various non-heme oxygenases catalyze many different reactions with O_2 : e.g. α -ketoglutarate (KG) dependent oxygenases & soluble methane monooxygenase (sMMO).

- **sMMO** is a **di-iron monooxygenase** & catalyzes challenging conversion of CH_4 to CH_3OH .
- The formation of **Fe(IV)-oxo** was proposed as the key for H atom abstraction.
- Apart from sMMO, pMMO (possibly **mono-copper mono-oxygenase?**) also catalyzes challenging conversion of CH_4 to CH_3OH .

**Thank You for Your
Attention!
Any Questions?**