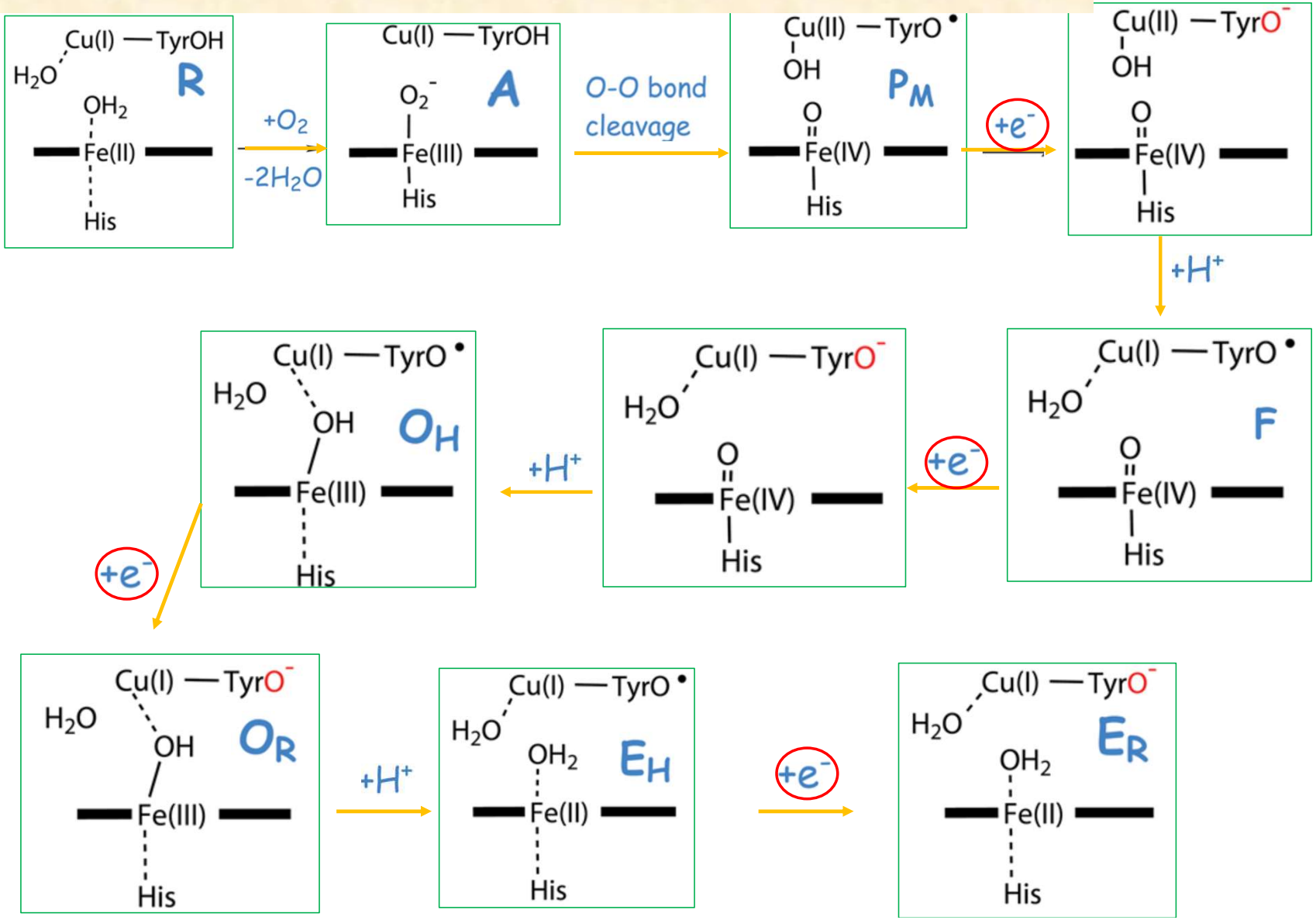
Cytochrome c Oxidase



Three slides for your reading only – in case it helps to better understand

- The resting oxidized state is suggested to have an extra proton in the active site, as compared to the situation during catalytic turnover, and the uptake of this extra proton occurs slowly when there are no more electrons supplied. This state, labled $\mathbf{O}_P^+$, has a neutral protonated tyrosine, Cu(II) and Fe(III) with a bridging hydroxyl ligand (NOT SHOWN IN PREVIOUS SLIDE).

- The proton coupled Cu(II) reduction potential for this state is found to be low, in agreement with equilibrium experiments of 0.28–0.35 V. Because of the extra proton there should be no proton pumping coupled to the initial reduction of this state. In contrast, the oxidized state during catalytic turnover, labled $\mathbf{O}_H$, has an unprotonated tyrosine with a tyrosyl radical, Cu(I) and Fe(III), which means that in the two reduction steps from the oxidized to the reduced state, $\mathbf{O}_H \rightarrow \mathbf{R}$, it is the tyrosyl radical and Fe(III) that are reduced.

- A major result from the computational studies is that the intermediate state, which is referred to as the one electron reduced state, and labled $\mathbf{E}_H$, also has an unprotonated tyrosine; i.e., the proton has entered the center of the **binuclear active centre** (BNC). This state is a mixture of two electronic configurations, the main one with a tyrosyl radical and Fe(II) and a secondary one with tyrosinate and Fe(III). In this way the reduction energy of the high potential tyrosyl radical is shared with the low reduction energy of heme a_3 Fe(III), and both reduction steps become exergonic enough to allow proton pumping also with a significant gradient.

- Molecular oxygen binds reversibly to the reduced state with Fe(II) and Cu(I), and after the O–O bond cleavage there is a tyrosyl radical, Cu(II)–OH and Fe(IV)=O ($\mathbf{P}_M$ state).

- According to the computational studies, the first reduction step after the O–O bond cleavage involves reduction of Cu(II) to Cu(I) and formation of a water molecule, and it leaves a tyrosyl radical also in the **F** state. At this stage the proton coupled reduction potential of Cu_B is high, on the order of 1 V.

- In the next reduction step Fe(IV) is reduced to Fe(III), and there is still an unprotonated tyrosyl with radical character in the $\mathbf{O}_H$ state. In these two reduction steps, $\mathbf{P}_M \rightarrow \mathbf{F}$ and $\mathbf{F} \rightarrow \mathbf{O}_H$, it is found to be thermodynamically more favorable to leave the tyrosine unprotonated and let the proton go to the center of the BNC.

- In contrast, in the $\mathbf{O}_H \rightarrow \mathbf{E}_H$ reduction step it has to be assumed that there is a kinetic effect that causes the avoidance of the lower lying **E** state with a protonated tyrosine.

- The K-channel, ending at the active site tyrosine, is only used in the $\mathbf{E}_H \rightarrow \mathbf{R}$ step, in which the tyrosine is reprotonated.

- The presence of the tyrosine in the active site, and the reduction mechanism presented here with an unprotonated tyrosine with radical character in all intermediate steps, are essential for the proton pumping.

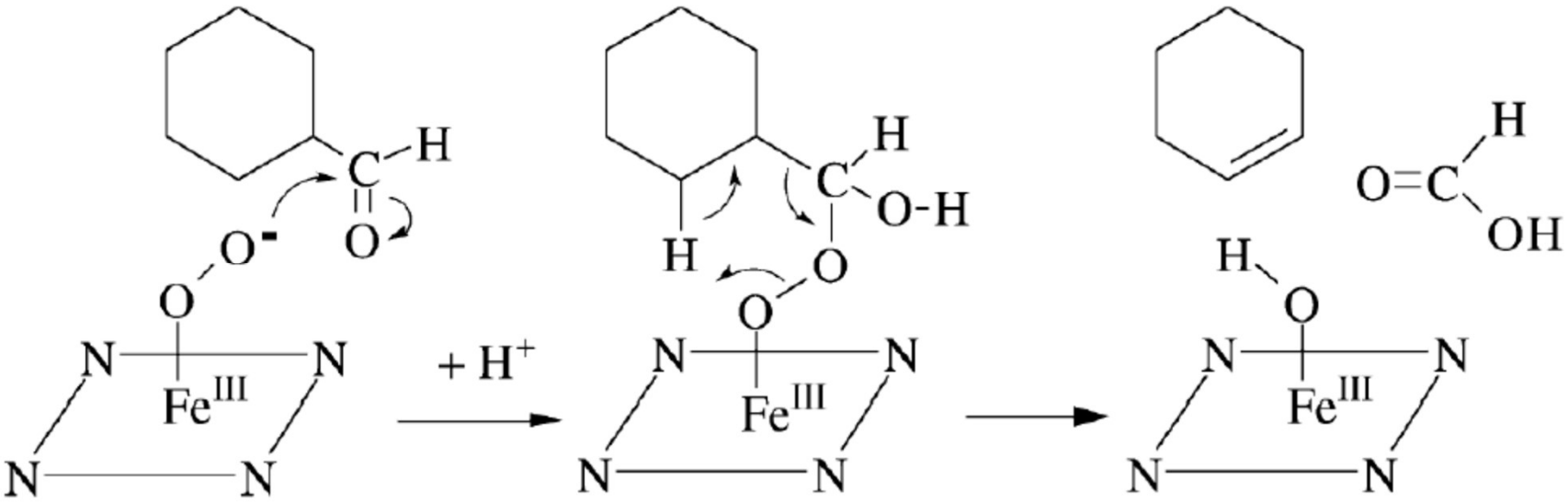
- Most likely the uptake of the pump-proton to a temporary loading site, the PLS, is driven by an electrostatic coupling to the electron transfer into the active site. Therefore, it is important that the product of each reduction step has a large enough affinity for uptake of the next electron to the active site already before the chemical proton has arrived.

- The calculations show that the intermediates with a proton in the center of the BNC and the tyrosine unprotonated (radical) secure such a high electron affinity, and essentially the electron goes initially to a tyrosyl radical in all reduction steps.

- The alternative form of the intermediate states where the proton instead is delivered to the tyrosine is found to have a significantly lower electron affinity, most likely requiring uptake of the chemical proton before electron transfer to the active site. Thus, the presence of the tyrosine in the active site enables the uptake of two protons per electron in cytochrome c oxidase.

- It is finally suggested that the tyrosyl radical is not easily observed experimentally because it is erased by the uptake of an extra proton to the active site when the flow of electrons is stopped.

Oxidative decarbonylation



Evidence for a Nonheme Fe(IV)=O Species in the Intramolecular Hydroxylation of a Phenyl Moiety

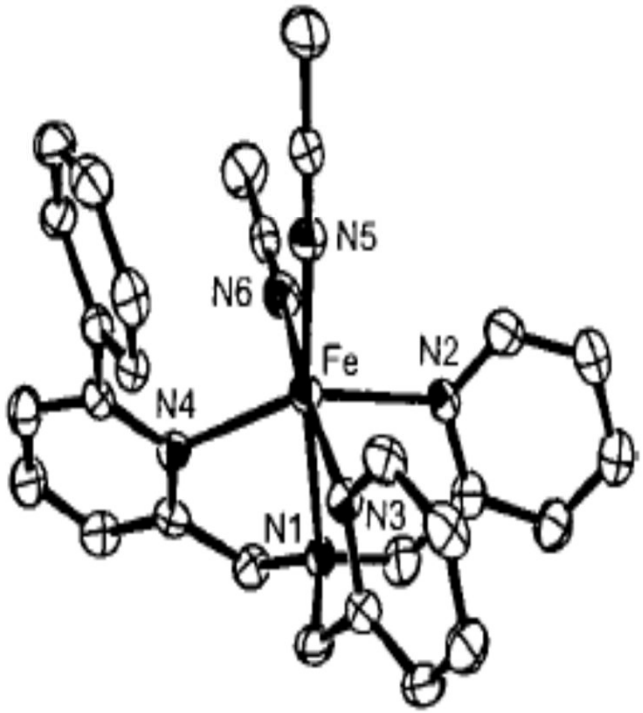
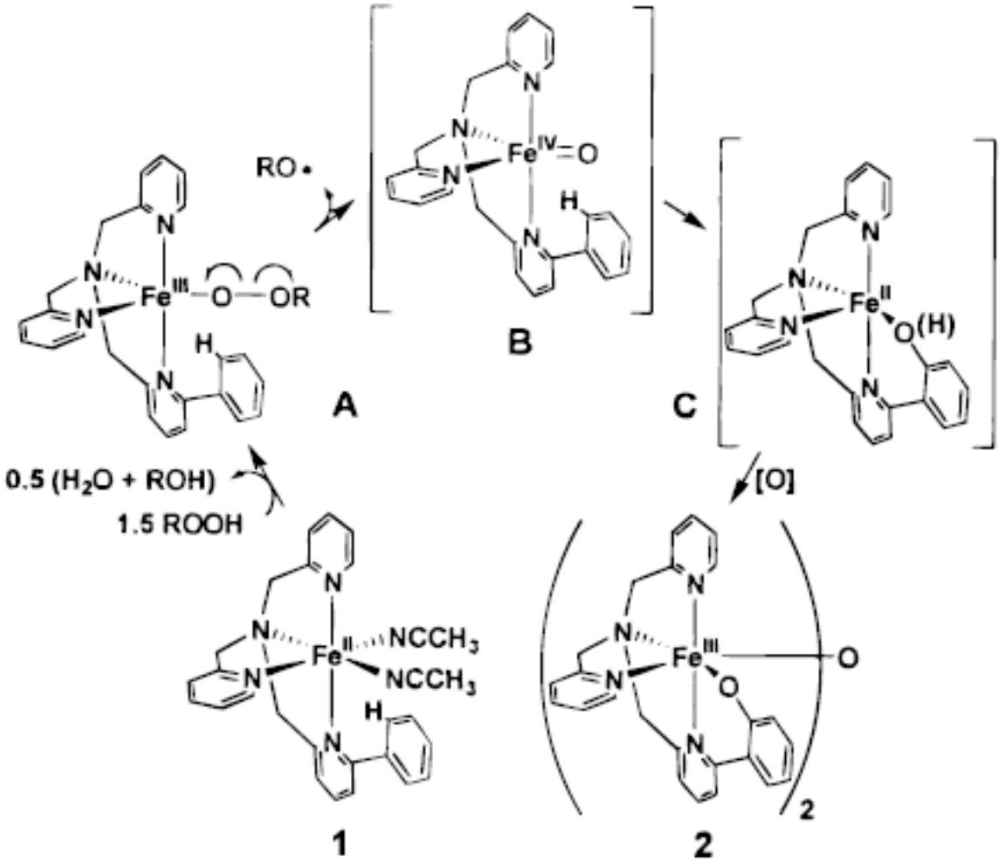
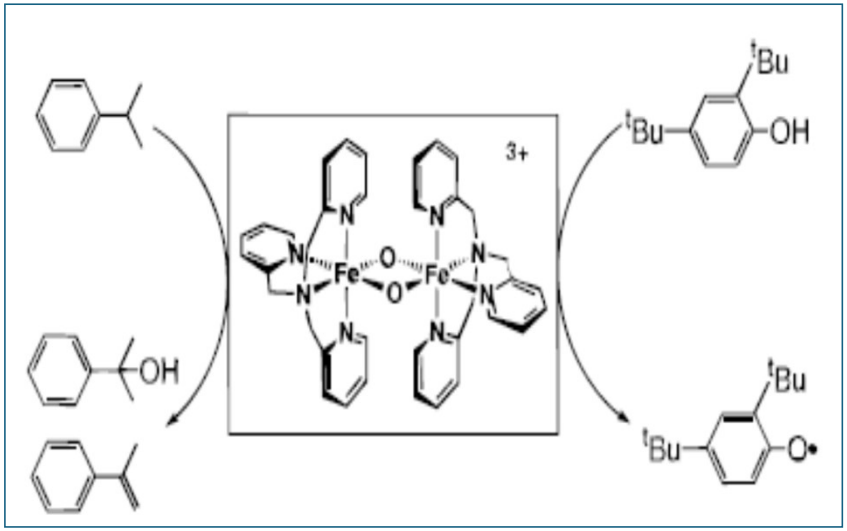


Figure 1. Thermal ellipsoid (50%) plot of **1**. Hydrogen atoms have been omitted for clarity. Selected interatomic distances (Å): Fe−N1 = 2.217 (3), Fe−N2 = 2.193 (3), Fe−N3 = 2.211 (3), Fe−N4 = 2.207 (3), Fe−N5 = 2.108 (4), Fe−N6 = 2.159 (4).

Scheme 1. Proposed Mechanism





Another work: *J. Am. Chem. Soc.* 2005, 127, 45, 15672–15673

The complex $[Fe_2(\mu\text{-}O)_2(tpa)_2]^{3+}$ also oxidizes cumene and affords $PhC(OH)Me_2$ and α-methylstyrene in excellent yield (88%).[43] The observed products respectively derive from hydroxylation and desaturation of the substrate, analogous to reactions catalysed by MMO and fatty acid desaturases. Since $[Fe_2(\mu\text{-}O)_2(tpa)_2]^{3+}$ is only a one-electron oxidant, 2 equivalents are required per product molecule formed, and substrate oxidation must occur in two steps, *i.e.* equations (2) and (3). The

$$[Fe_2(\mu\text{-}O)_2(tpa)_2]^{3+} + PhCH(Me)_2 \longrightarrow [Fe_2(\mu\text{-}O)(\mu\text{-}OH)(tpa)_2]^{3+} + PhC^{\bullet}Me_2 \quad (2)$$

$$[Fe_2(\mu\text{-}O)_2(tpa)_2]^{3+} + PhC^{\bullet}(Me)_2 \longrightarrow [Fe_2(\mu\text{-}O)(tpa)_2]^{4+} + PhC(OH)Me_2$$
$$\text{or } [Fe_2(\mu\text{-}O)(\mu\text{-}OH)(tpa)_2]^{3+} + PhC(Me){=}CH_2 \quad (3)$$

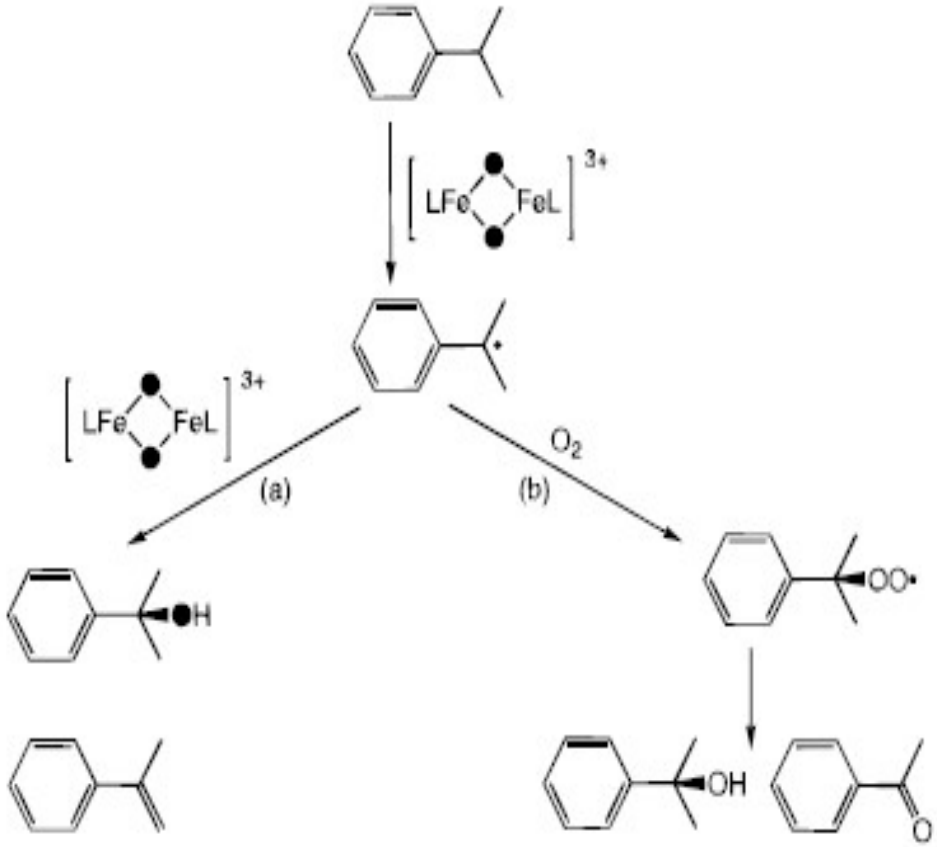
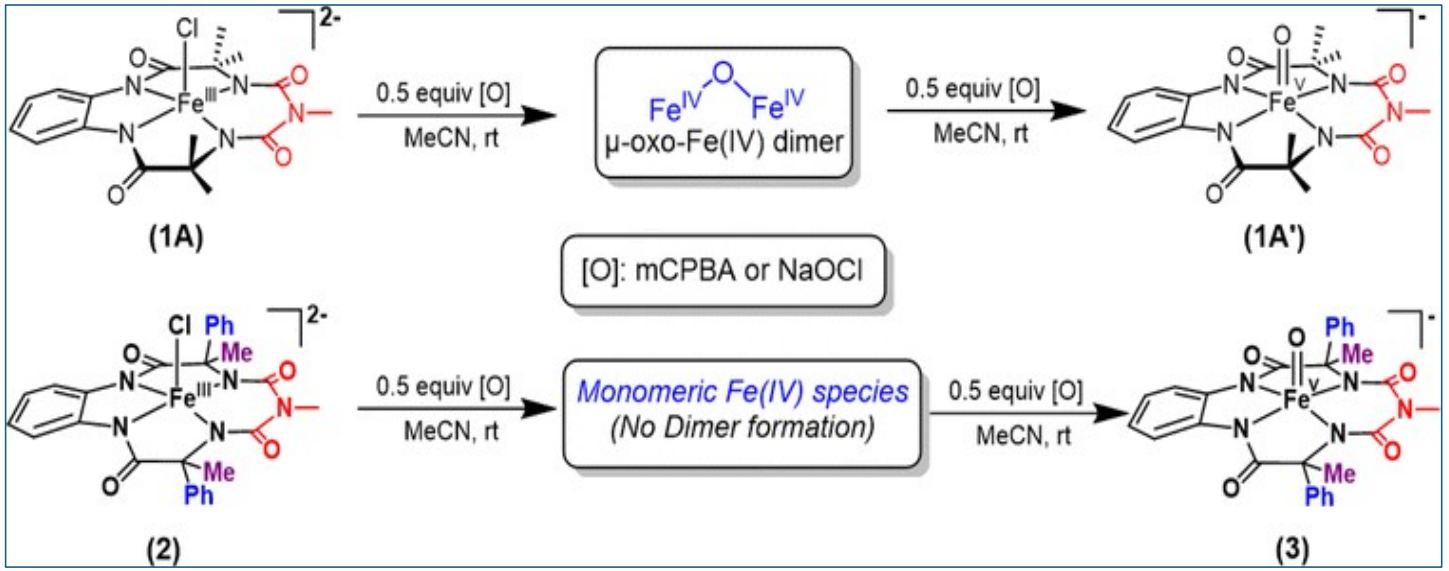
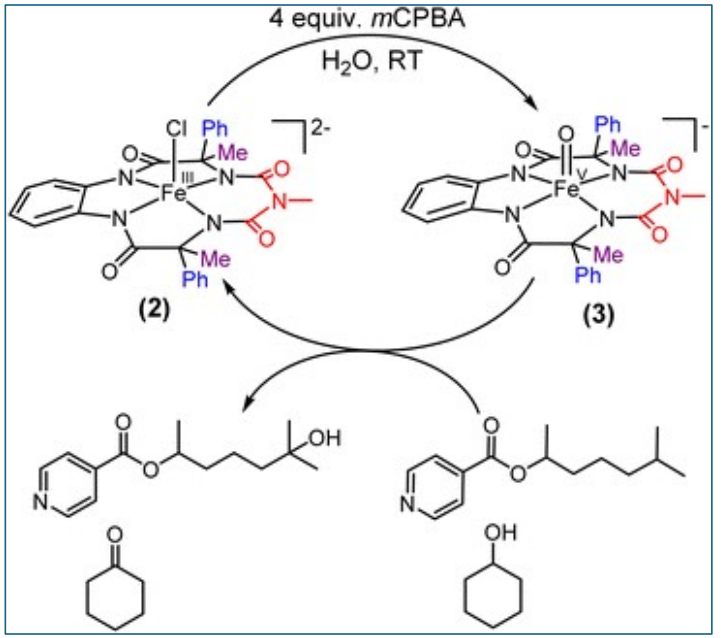


Figure 3. Proposed mechanism for alkane oxidation by **1**.

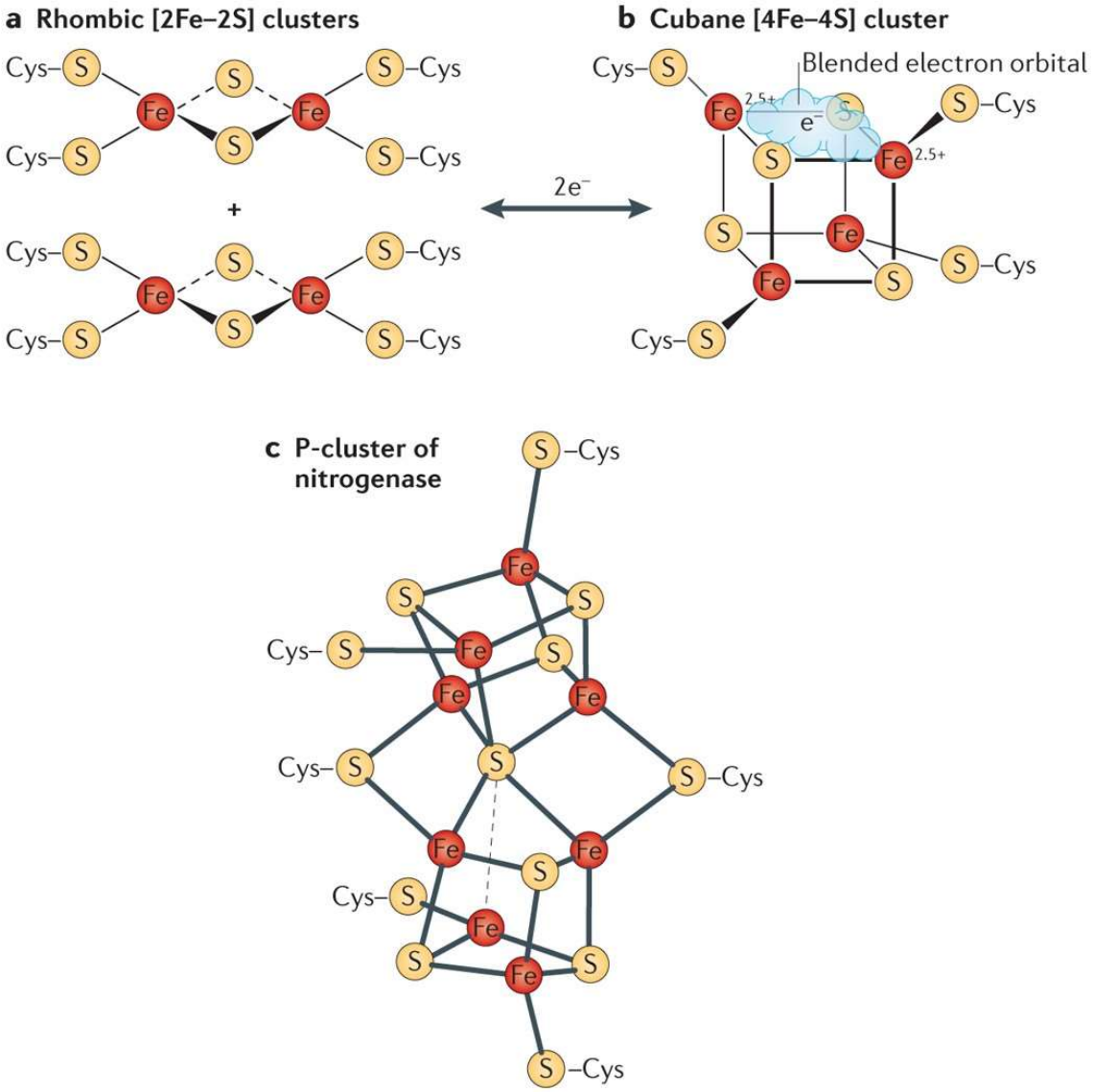
Highly regioselective oxidation of C–H bonds in water using hydrogen peroxide by a cytochrome P450 mimicking iron complex – Sayam SenGupta, IISER-K





Two-electron oxidation of **2** in acetonitrile by either meta-chloroperbenzoic acid (mCPBA) or aqueous sodium hypochlorite (NaOCl) led to the formation of oxoiron(V) species (**3**) whose UV spectral features (green colour, λmax 442 nm and 625 nm) are reminiscent of **1A'**

Iron-sulfur (Fe-S) proteins



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Localization and delocalization patterns in Fe-S clusters

Fe^{3+} (red)

Fe^{2+} (blue)

$Fe^{2.5+}Fe^{2.5+}$ pairs (green)

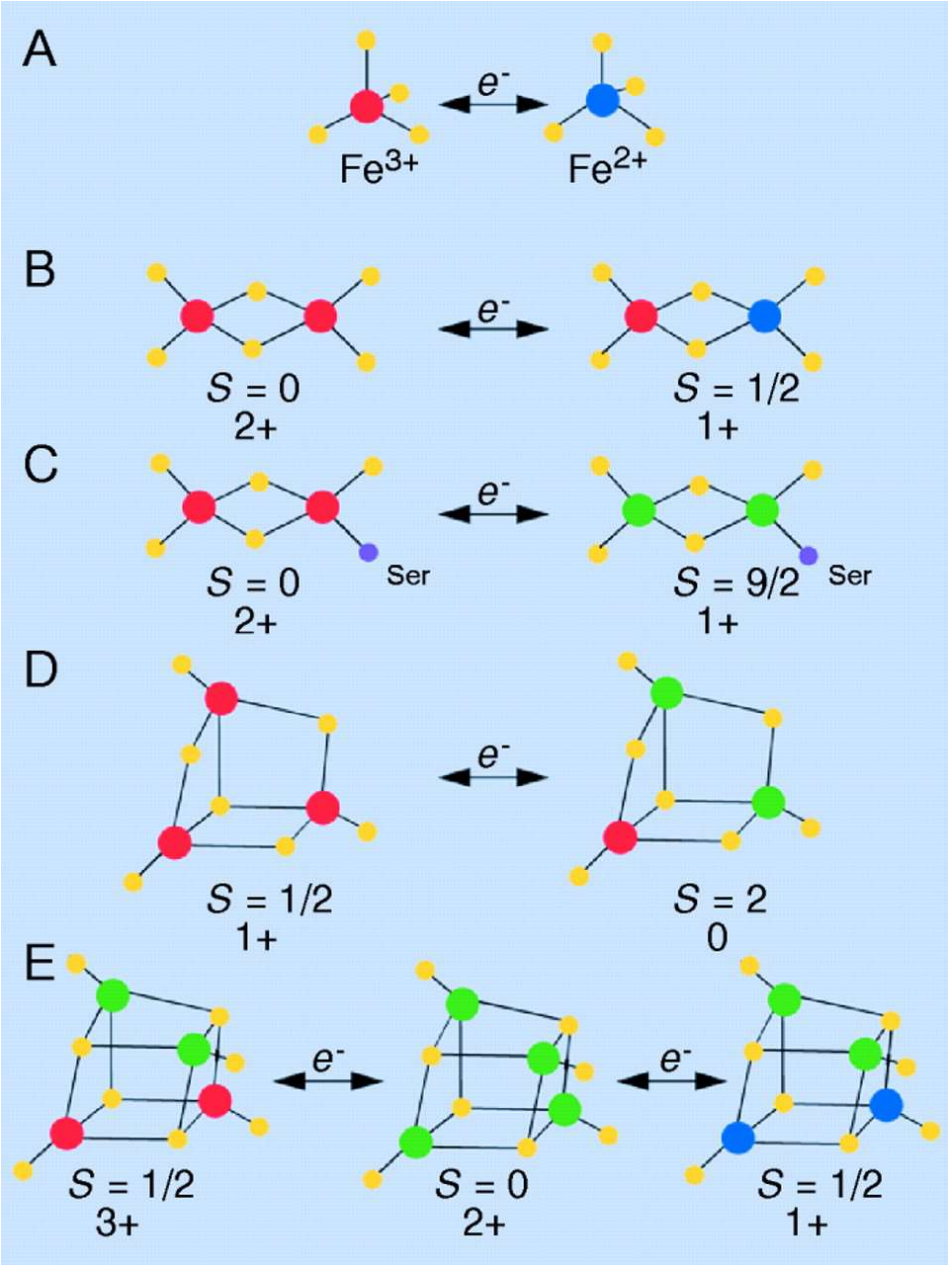

Table IV.3.
Spin Distribution within Iron–Sulfur Clusters

Cluster	Individual Spins	Subspins[a]	Total S
$[Fe_2S_2]^{2+}$	5/2, 5/2	5/2, 5/2	0
$[Fe_2S_2]^{+}$	2, 5/2	2, 5/2	1/2
$[Fe_3S_4]^{0}$	2, 5/2, 5/2	9/2*, 5/2	2
$[Fe_3S_4]^{+}$	5/2, 5/2, 5/2	2, 5/2 (3, 5/2)	1/2
$[Fe_4S_4]^{0}$	2, 2, 2, 2	0, 0 (?)	0
$[Fe_4S_4]^{+}$	2, 2, 2, 5/2	9/2*, 4 (?)	1/2
$[Fe_4S_4]^{2+}$	2, 2, 5/2, 5/2	9/2*, 9/2* (?)	0
$[Fe_4S_4]^{3+}$	2, 5/2, 5/2, 5/2	9/2*, 4 (7/2*, 3) (9/2*, 4 + 7/2*, 4)	1/2

1. One electron at a time is normally required, which implies that the electron-transfer protein must be capable of one-electron oxidation–reduction. Many redox active organic molecules, with a few exceptions, do not meet this requirement. On the other hand, metal-containing proteins solve the problem quite naturally because redox active metals often have accessible redox states differing by only one electron.
2. Electron transfer is faster when the free energy change for the electron to be transferred from the donor to the acceptor is negative, albeit not too much so. Therefore, the reduction potential must be fine-tuned to be in between those of the donor (the electron-transfer protein must be able to accept the electron) and the acceptor, when the same protein (in an electron-transfer chain) donates and accepts the electron. Although the biologically relevant redox metals are relatively few, they are used in a large variety of biological systems because their reduction potentials can be regulated over a wide range by their coordination chemistry and by the electrostatics of the metal ion environment.
3. There must be efficient electron-transfer pathways that connect the donor–acceptor interaction site(s) with the redox site. These pathways are often shortened by the fact that the metal is either ligated by a large "conducting" cofactor like a heme or is part of a "conducting" metal cluster.
4. The reorganization energy on passing from one redox state to another should be small. For the metal, this implies minimizing the geometric changes that accompany the change in oxidation state. This minimization may be accomplished by special coordination geometries that are in between those preferred in the two oxidation states (or possibly by distributing the electron over several metal ions, as in the case of metal clusters, see Chapter IV). Rules for a good electron-transfer protein are summarized in Fig. X.1.1.

Iron-Sulfur Electron-Transfer Proteins

How ferredoxin becomes suitable for an electron transfer

Ferredoxin, the iron–sulfur protein in the photosynthesis system, accepts electrons from an ionized chlorophyll and transfers them to the oxidized form of nicotinamide adenine dinucleotide phosphate ($NADP^+$). The reduction potential of the ionized chlorophyll system is estimated to be about -0.6 V and that of $NADP^+$ is about -0.3 V. Hence the ferredoxins must have reduction potentials in the range of -0.3 to -0.6 V. Ferredoxins from both plants and bacteria have indeed been found to have reduction potentials around -0.4 V.

The reduction potential of Fe_2S_3/FeS is about -0.7 V.

Iron-Sulfur Electron-Transfer Proteins

At pH 7 ($E^{o\prime}$), *the Fe–S protein associated* with FAD in the mitochondrial electron transfer system functions at a reduction potential in the range of -0.2 to -0.1 V, because the $E^{o\prime}$ *of FAD is around -0.12 V.*

In many Mo--enzymes, an [Fe_2S_2] unit transfers electrons between a Mo entity and a FAD. Hence the $E^{o\prime}$ values of these iron–sulfur cores need to be between the $E^{o\prime}$ *of Mo(VI)/Mo(IV) (-0.3* V) and the $E^{o\prime}$ *of FAD (-0.12 V). The* $E^{o\prime}$ *values are in fact* in this range.

So one thing is clear that the Fe-S proteins do vary in terms of their reduction potential in order to be commensurate with its functions

Iron-Sulfur Electron-Transfer Proteins

The reduction potential of an $[Fe_4S_4]$ cluster can be influenced by a number of factors, important among which are

(i) the oxidation stages involved,

(ii) the structure of the cluster, and

(iii) the cluster's environment.

$[Fe(III)_4S_4(cys)_4]$ or $[Fe_4S_4(cys)_4]^0$

$[Fe(III)_3Fe(II)S_4(cys)_4]$ or $[Fe_4S_4(cys)_4]^-$

$[Fe(III)_2Fe(II)_2S_4(cys)_4]$ or $[Fe_4S_4(cys)_4]^{2-}$

$[Fe(III)Fe(II)_3S_4(cys)_4]$ or $[Fe_4S_4(cys)_4]^{3-}$

$[Fe(II)_4S_4(cys)_4]$ or $[Fe_4S_4(cys)_4]^{4-}$

Iron-Sulfur Electron-Transfer Proteins

Only one particular pair of two oxidation states is in use in any given biological situation.

For example, the regular bacterial ferredoxin uses the
$[Fe(III)_2Fe(II)_2]$–$[Fe(III)Fe(II)_3]$ pair

whereas the *Chromatium ferredoxin is* believed to operate with the
$[Fe(III)_3Fe(II)]$-$[Fe(III)_2Fe(II)_2]$ pair.

Obviously, the reduction potential of the latter pair will be much higher than that of the former, because an electron is to be added to a more electron-poor entity (the $[Fe(III)_3Fe(II)]$ unit) rather than to the more electron-rich $[Fe(III)_2Fe(II)_2]$ as in the regular ferredoxin.

A ferredoxin from the genus *Chromatium contains two* $[Fe_4S_4]$ units but has an unusually high reduction potential of +0.35 V. This protein is also known as *"high potential iron* sulfur *protein", or HiPIP. The reduction potential of* rubredoxin $[Fe(cys)_4]$ is around 0 V.