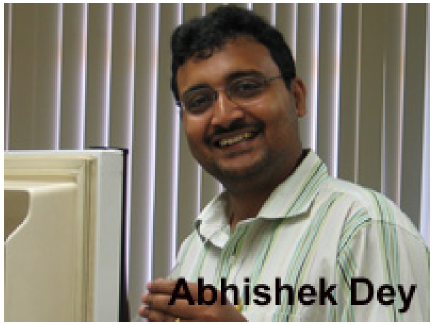
Three Gases, O_2, NO and H_2S

Meet in the Mitochondria

James P. Collman

Department of Chemistry
Stanford University
Stanford, California 94305

Acknowledgements

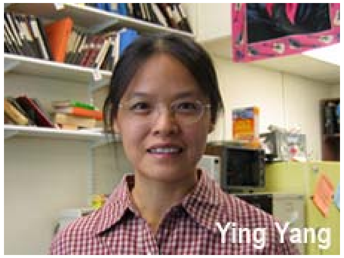


Abhishek Dey
Spectroscopy, Electrochemistry, Mechanism

Richard Decreau
Synthesis

Somdatta Ghosh Dey
Spectroscopy, Kinetics, Mechanism

Neal Devaraj
E-chem, Surface chemistry



Ying Yang
Synthesis

Roman Boulatov
E-chem, Mechanism

Christopher Chidsey	E-chem, Surface chemistry
Edward Solomon	EPR, Raman Spectroscopy

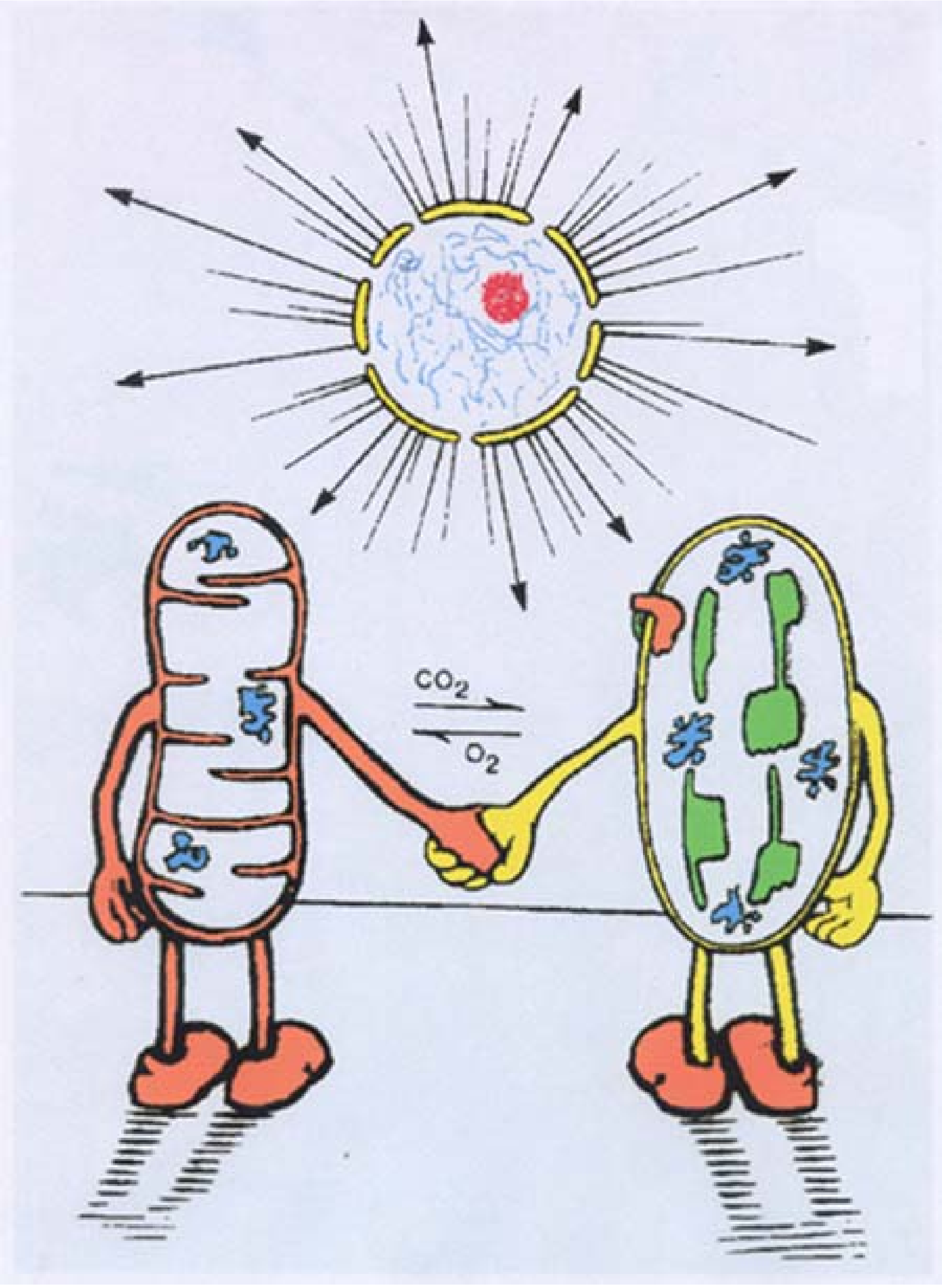
Multielectron Redox Processes

photosynthesis $H_2O \rightarrow$ Oxygen

fuel cells Oxygen $\rightarrow H_2O$

respiration Oxygen $\rightarrow H_2O$

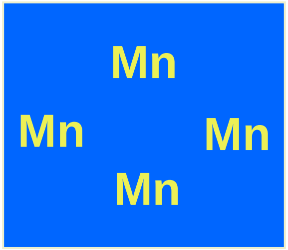
ammonia production $N_2 \rightarrow NH_3$


CO_2
O_2

Redox Enzymes

- Often possess **multiple** metal centers
- Metals play multiple roles
  1. **Bind substrate**
  2. **Increase reactivity of substrate**
  3. **Prevent side reaction**
  4. **Provide electrons quickly**

- Couple a **multielectron** process to several **single** electron processes



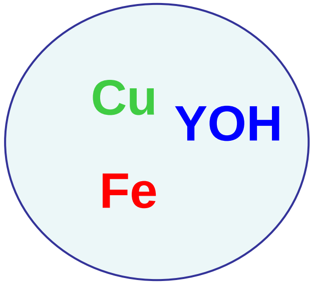
Mn

Mn Mn

Mn

Oxygen Evolving Complex

H_2O → Oxygen

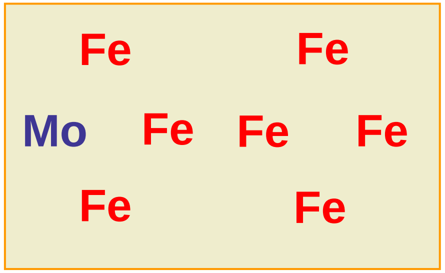


Cu YOH

Fe

Cytochrome *c* Oxidase

Oxygen → H_2O



Fe Fe

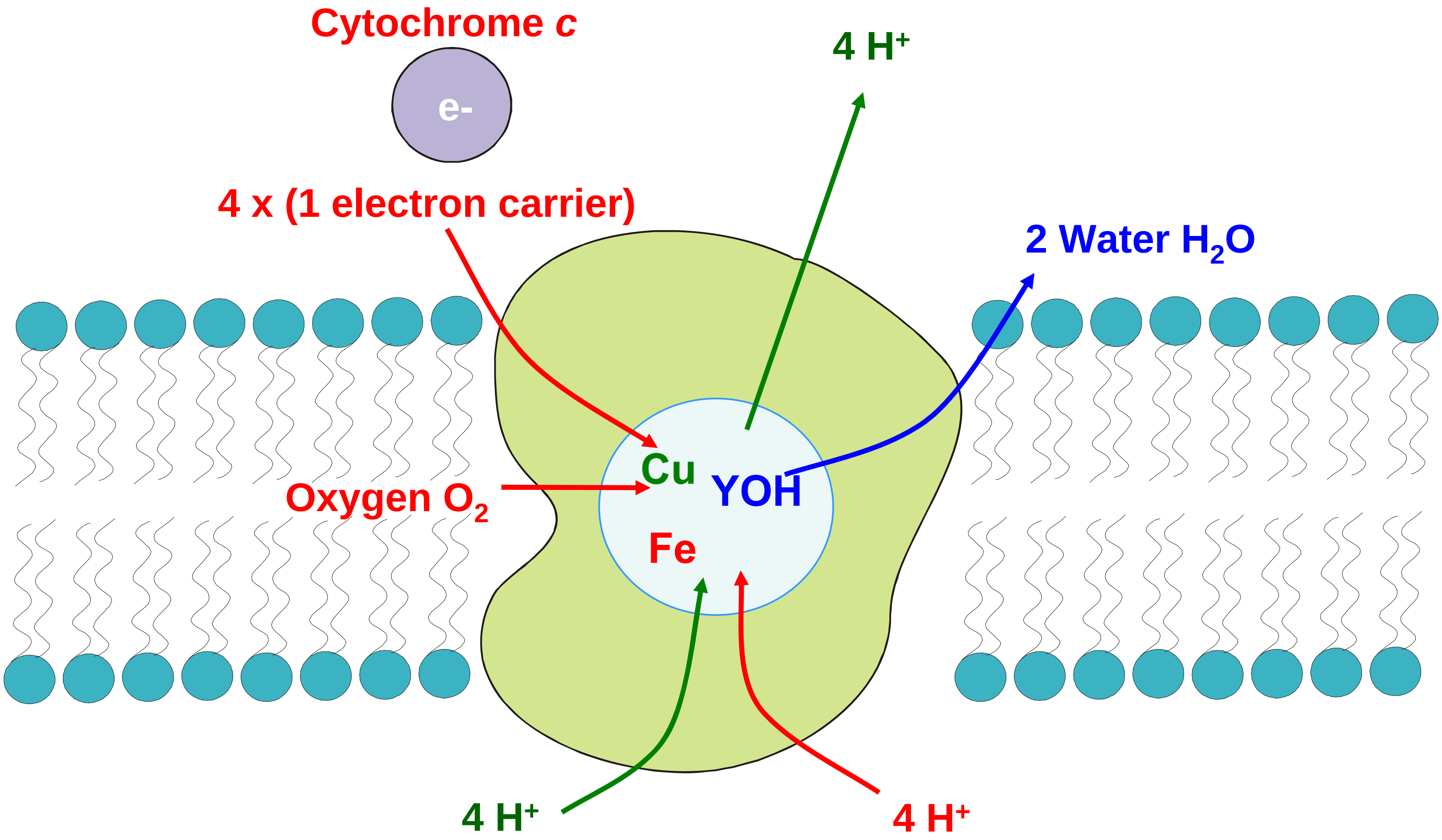
Mo Fe Fe Fe

Fe Fe

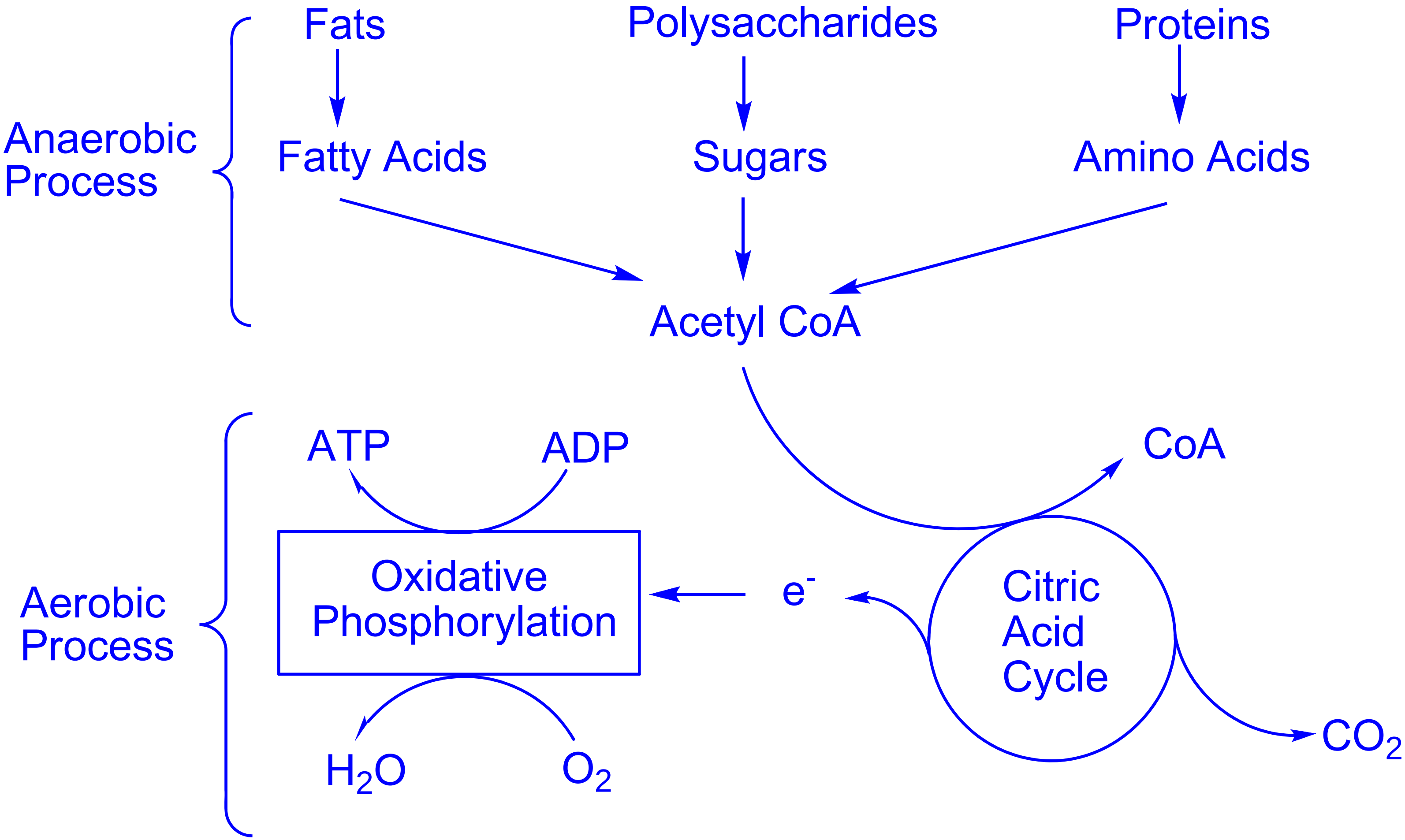
Nitrogenase

N_2 → NH_3

Cytochrome *c* Oxidase (CcO)



A Metabolic Overview



Anaerobic Process

Fats

Fatty Acids

Polysaccharides

Sugars

Proteins

Amino Acids

Acetyl CoA

CoA

Aerobic Process

ATP

ADP

Oxidative Phosphorylation

H_2O

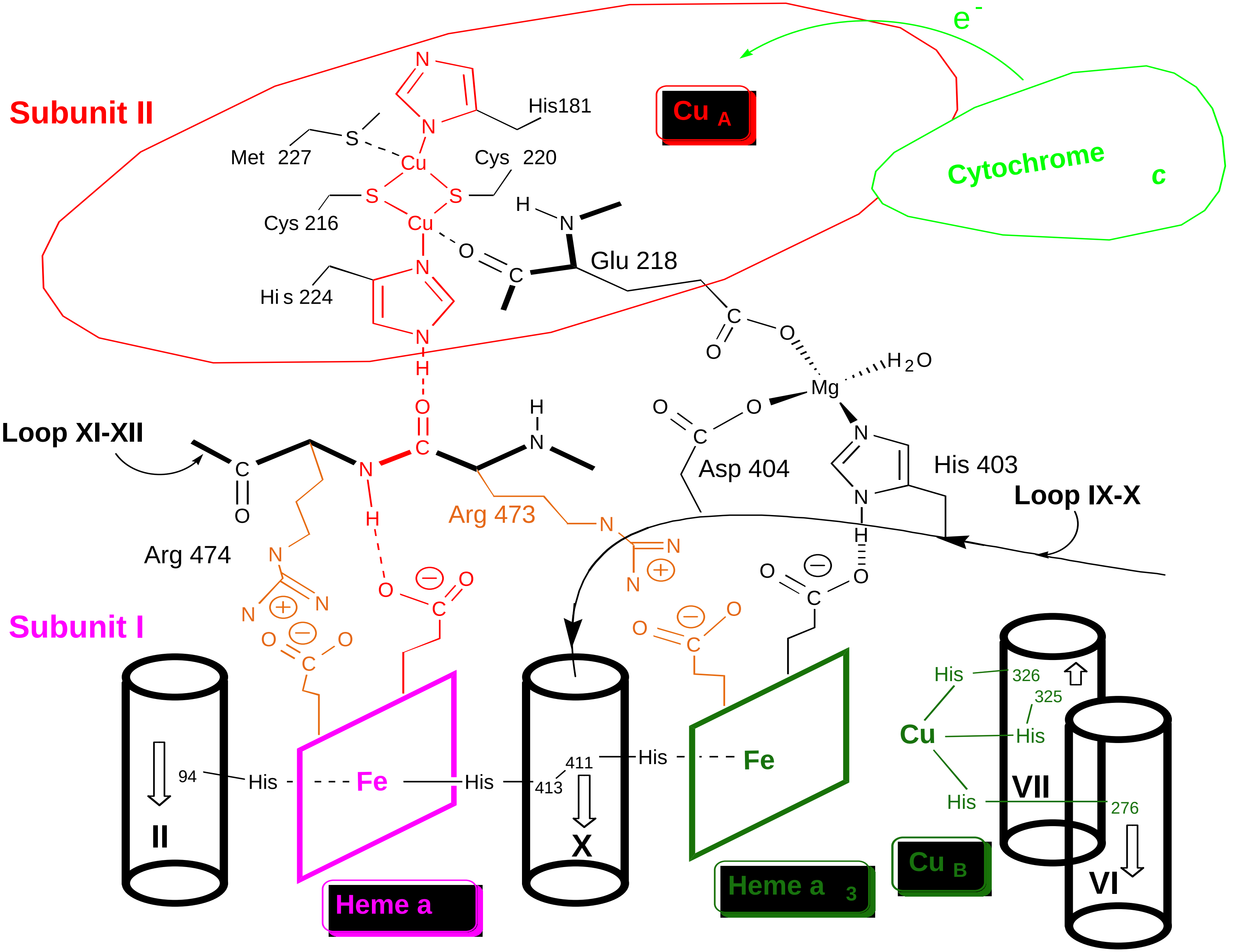
O_2

e^-

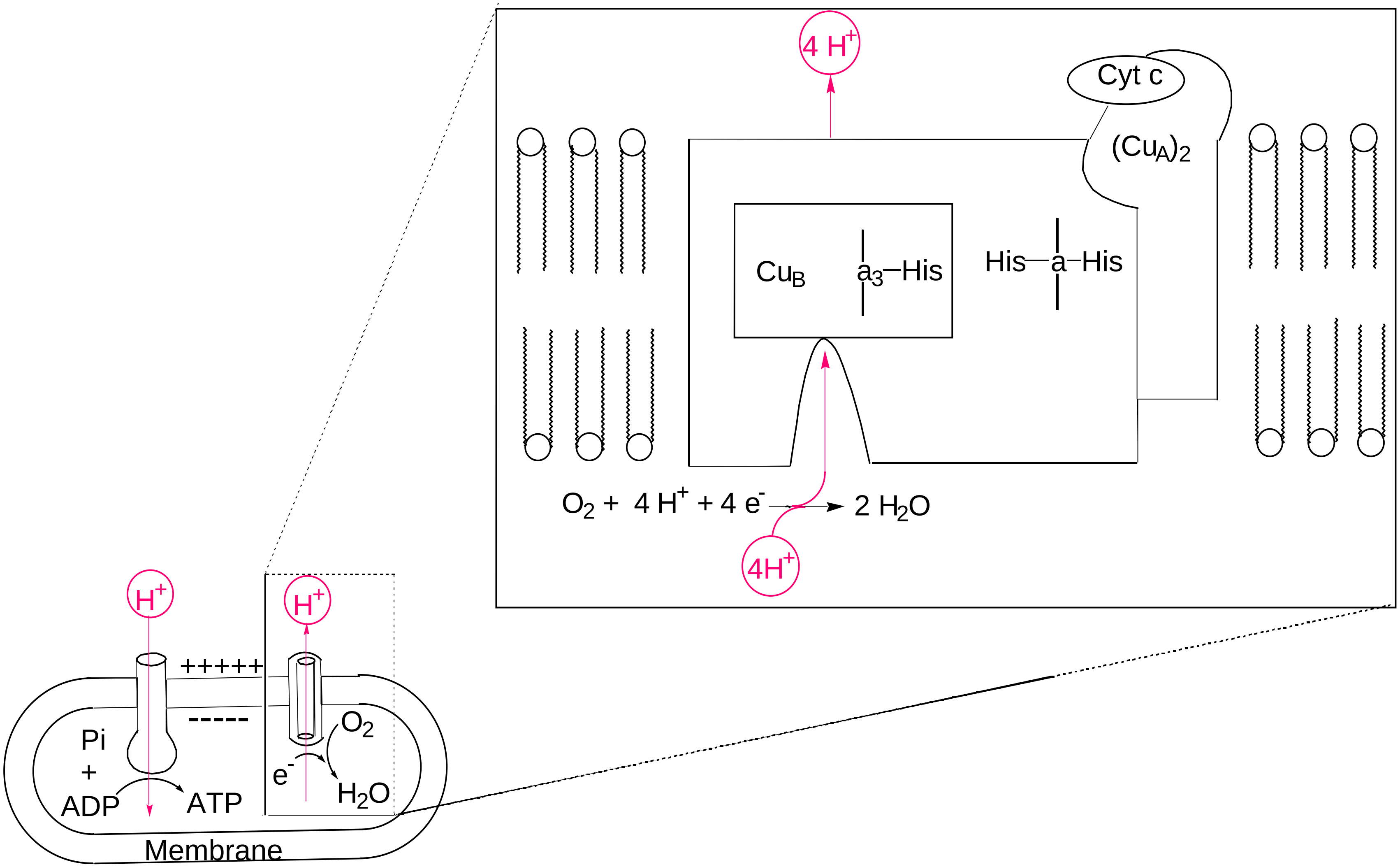
Citric Acid Cycle

CO_2

Schematic View of CcO in the Mitochondrial Inner Membrane

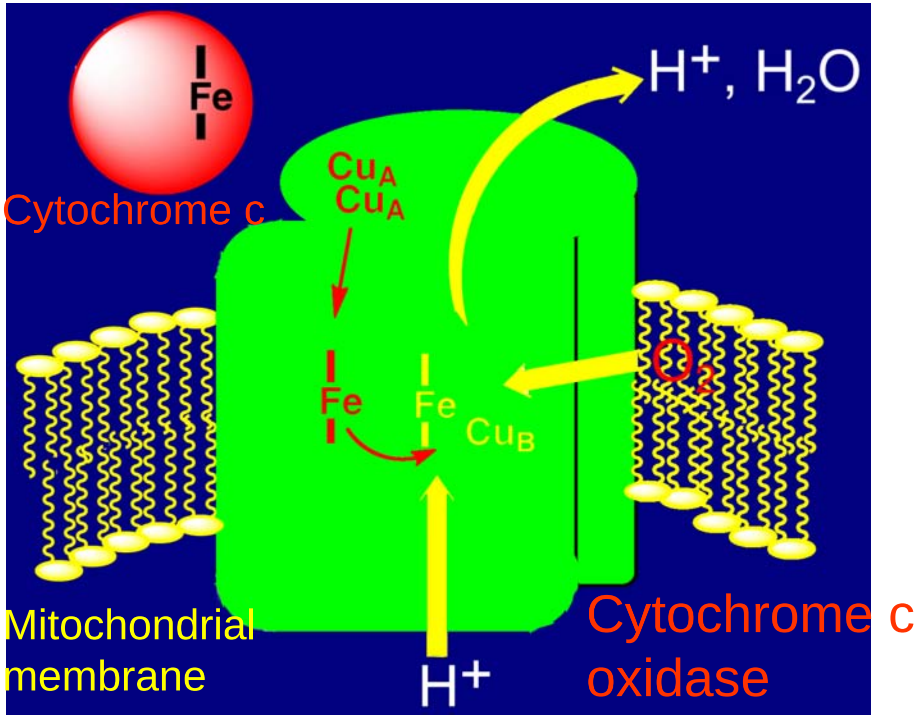


Schematic Diagram of Cytochrome c Oxidase

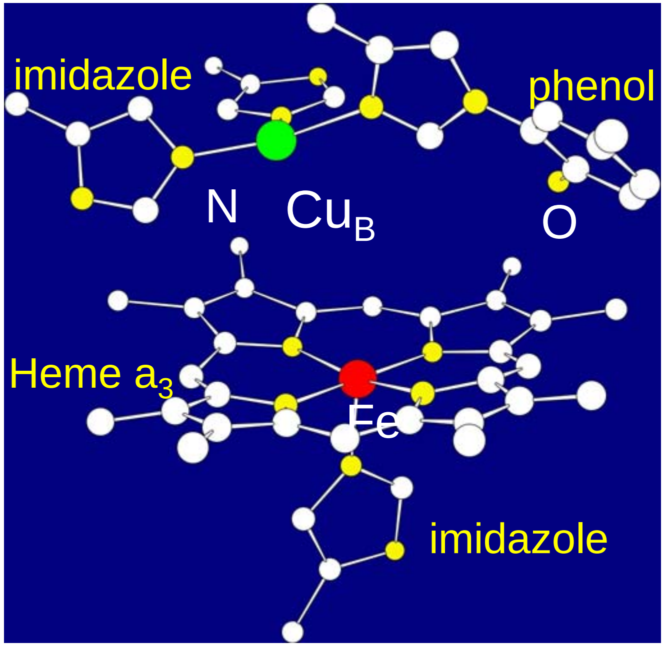


Cytochrome c Oxidase Couples Diffusional *1e* Oxidation of Ferrocytochrome c to Rapid *4e* Reduction of O_2

$$O_2 + 4H^+ + 4e^- \rightarrow 2\,H_2O$$

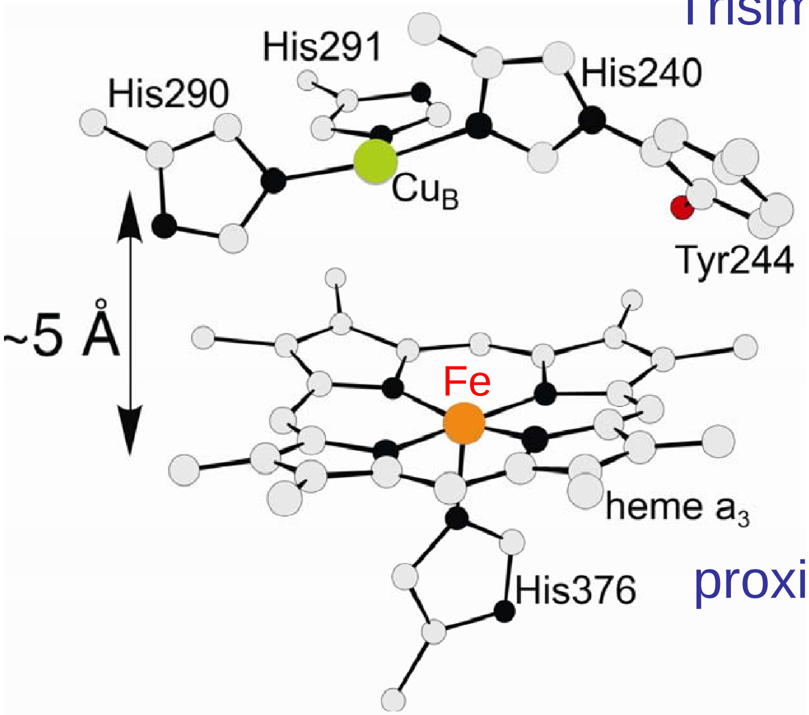


Catalytic heme/Cu site



Reduction level	Cu_A	Fe_a	Fe_{a3}	Cu_B	
Oxidized:	+2	+3	+3	+2	Aerobically stable
1e reduced:	mixed		+3	+2	
2e reduced:	+2	+3	**+2**	**+1**	Reduce O_2 by 4e
3e reduced:	mixed		**+2**	**+1**	
4e reduced:	**+1**	**+2**	**+2**	**+1**	

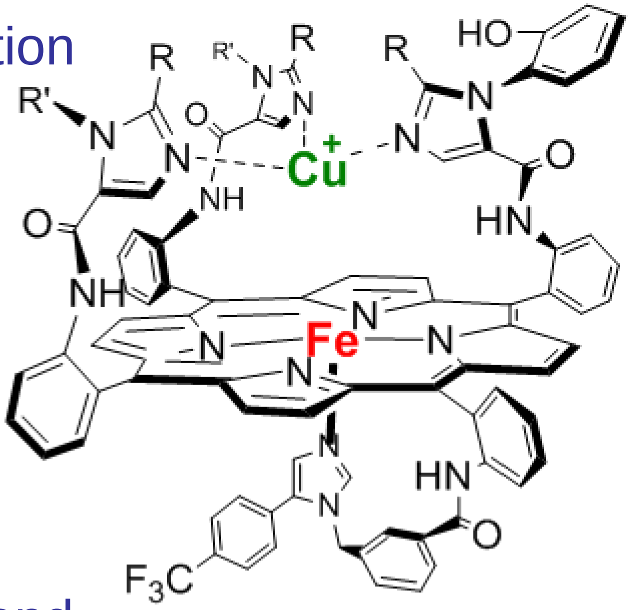
Our Complexes Reproduce Key Structural Features of the Heme a_3/Cu_B Site



Trisimidazole coordination sphere of Cu

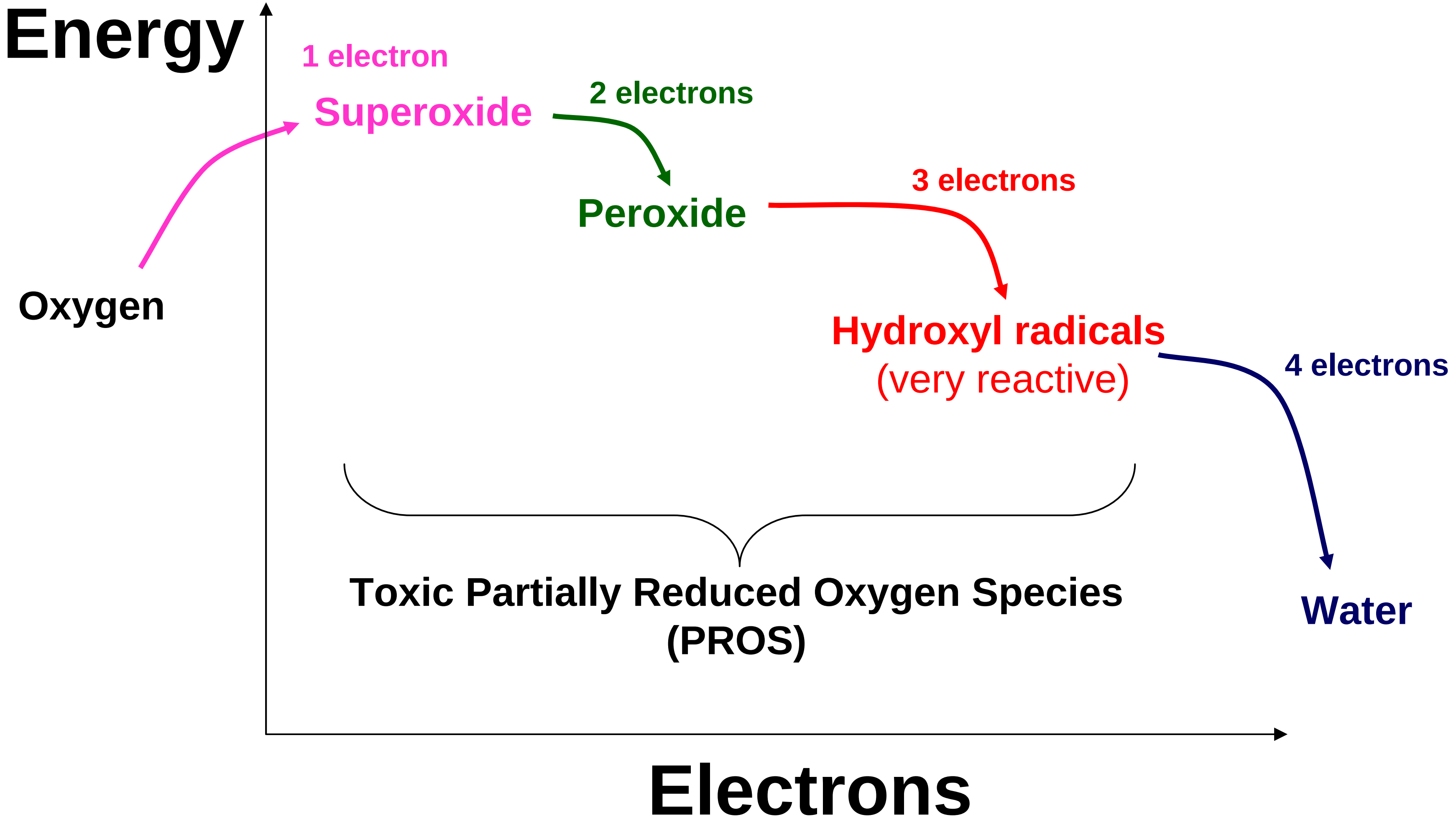
FeCu distance

proximal imidazole ligand



R = H, R’ = Me
R = Pr, R’ = H
R = Pr, R’ = Me

The Challenge of Using Oxygen



Energy

Oxygen

1 electron

Superoxide

2 electrons

Peroxide

3 electrons

Hydroxyl radicals
(very reactive)

4 electrons

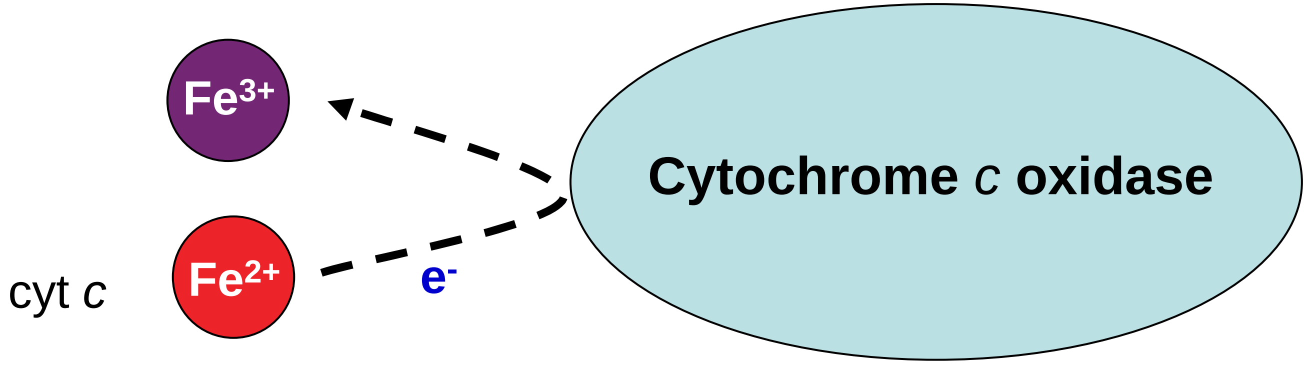
Water

Toxic Partially Reduced Oxygen Species
(PROS)

Electrons

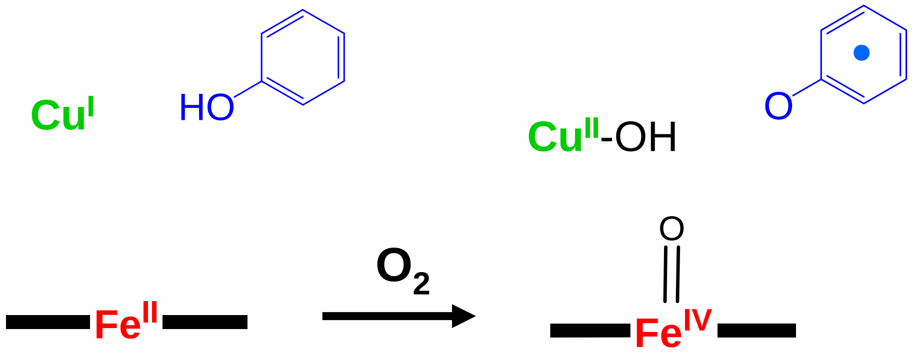
Organisms needed to develop a catalyst that can take oxygen to water without releasing reactive oxygen species

Difference in Electron Transfer Rates



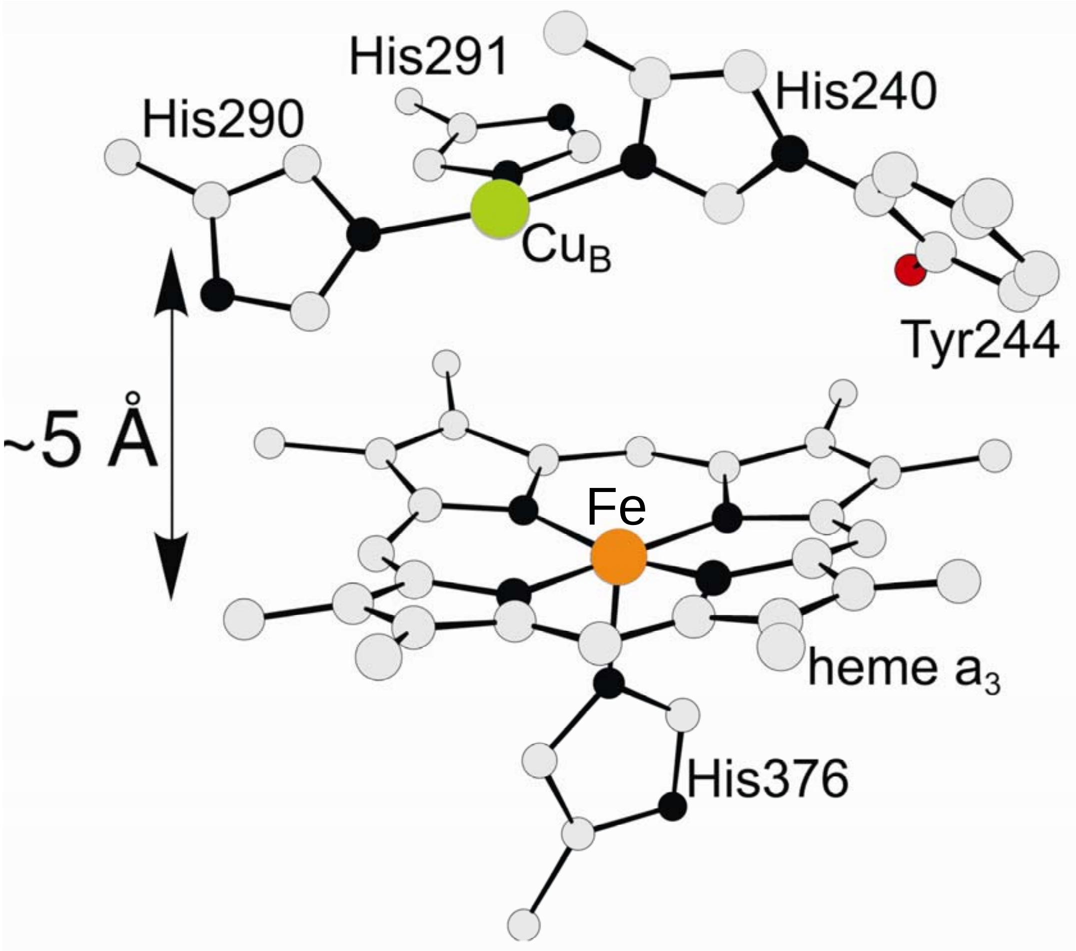
CcO can accept electrons 1 at a time from cytochrome c slowly (1 every 5-20 msec)

Cytochrome c limits the turnover frequency



In contrast, the active site can reduce oxygen by four electrons in less than 200μsec

CcO Active Site

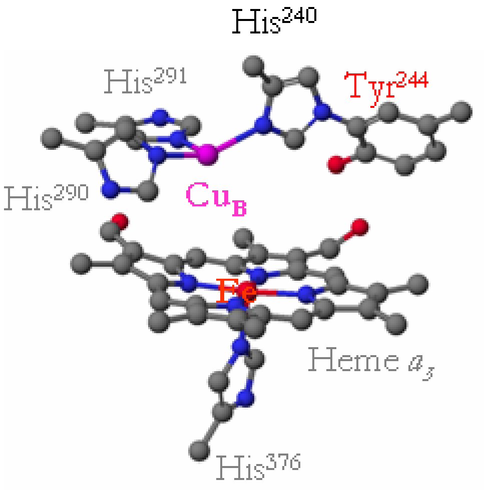


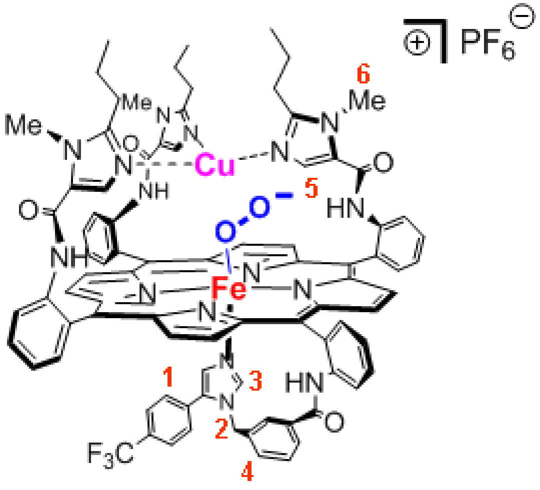
Redox active groups

Iron: 2 electrons
Copper: 1 electron
Tyrosine: 1 electron

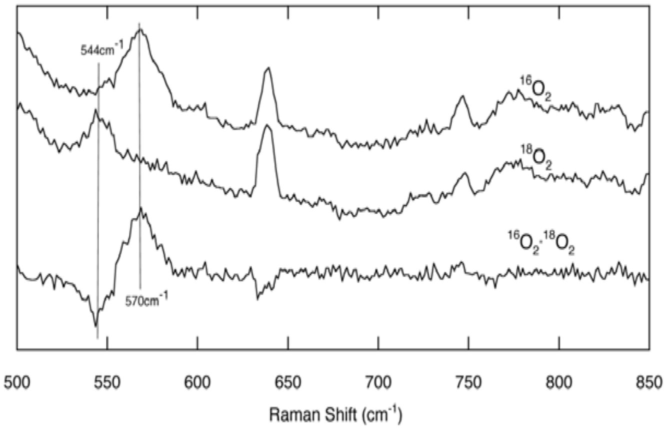
What are the roles of the redox centers?

Spectroscopic Evidence for a Heme-Superoxide/Cu(I) Intermediate



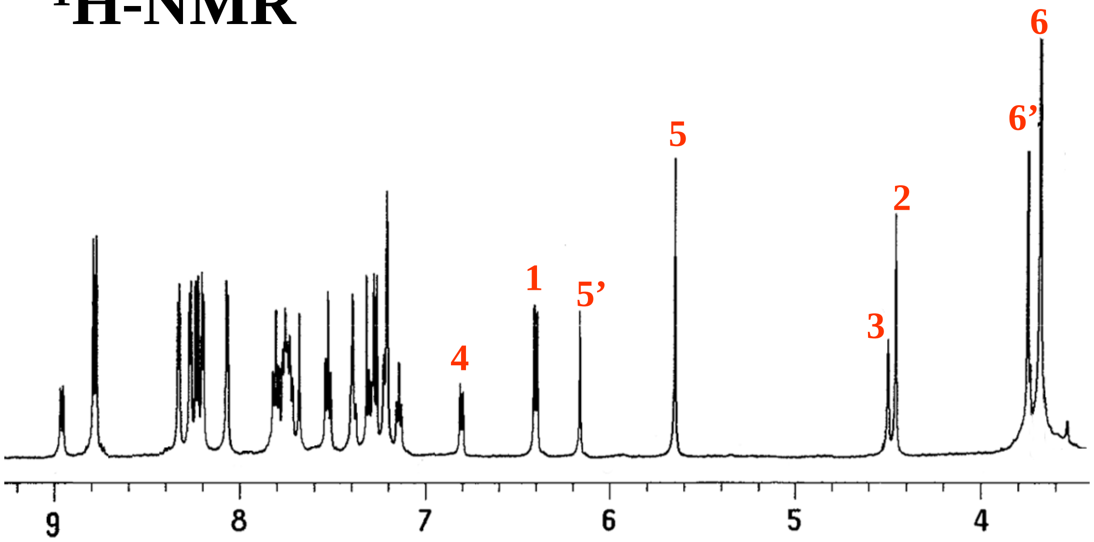


Oxy-1



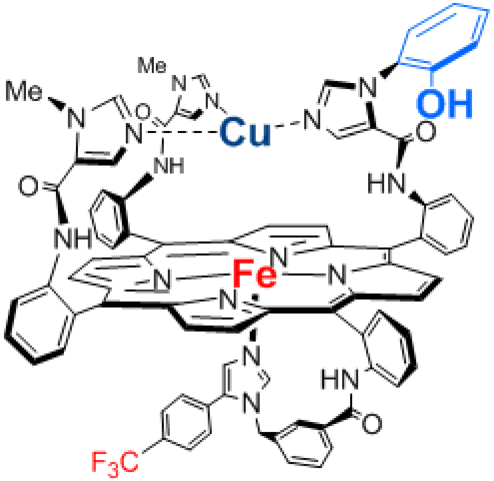
RR	Fe-$^{16}O_2$ stretch (cm^{-1})
Oxy-1	**570**
Oxy-Mb	569
Oxy-PFP	572
Oxy-C*c*O	571

^{1}H-NMR



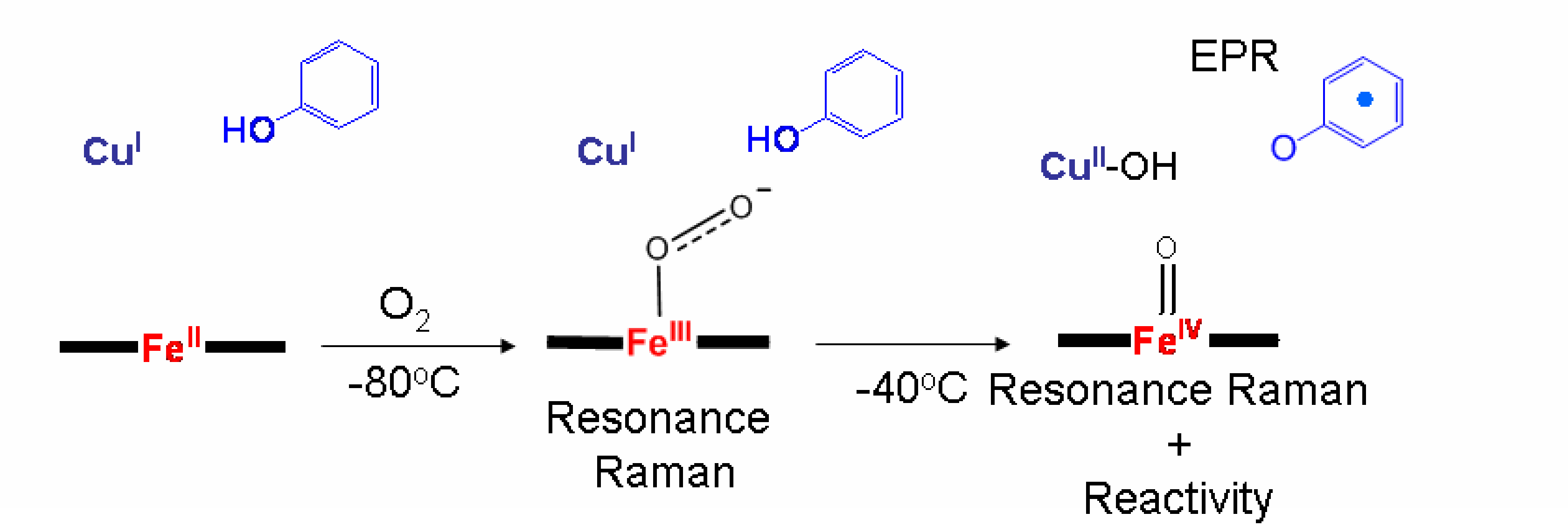
Collman, J. P. ; Sunderland, C. J.; Berg, K.; Vance, M.; Solomon, E. I. *J. Am. Chem. Soc.* **2003**, *125,* 2649

Single Turnover Studies



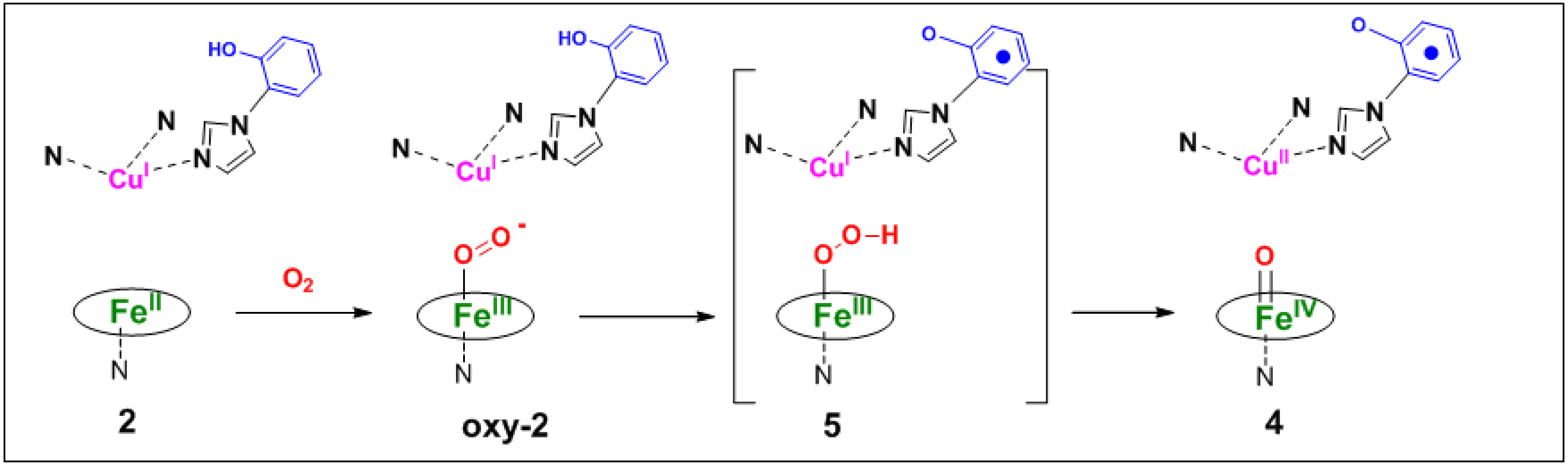
Model reacts with oxygen in a manner similar to that of the native enzyme (reduces oxygen by four electrons)

Demonstrates that the phenol can donate an electron/proton to bound oxygen

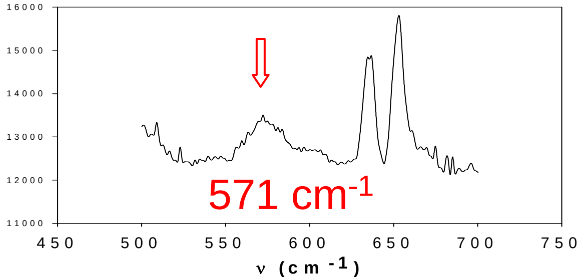


Collman, J. P.; Decréau, R. A.; Yan, Y.-L.; Yoon, J.; Solomon, E. I. *J. Am. Chem. Soc.* **2007**, *129*, 5794

Intramolecular Reaction in a Heme-Superoxide/Cu(I)



Superoxo (oxy-2) (-80° C)



	Fe-$^{16}O_2$ stretch (cm^{-1})
Oxy-2	**571**
Oxy-Mb	569
Oxy-PFP	572
Oxy-C*c*O	571

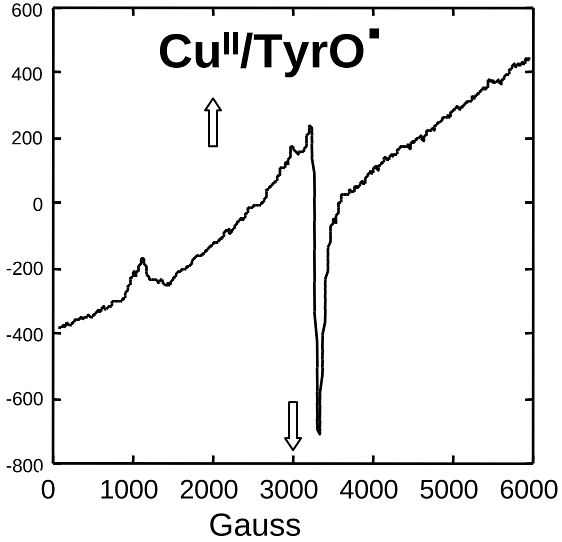
Ferryloxo (4) (-40° C) Cu^{II}/Tyrosyl Radical (4) (-40° C)

Reactivity:

$$PPh_3 \xrightarrow[>50\%]{4} PPh_3O$$

RR:
new bands at 770, 812 cm^{-1}

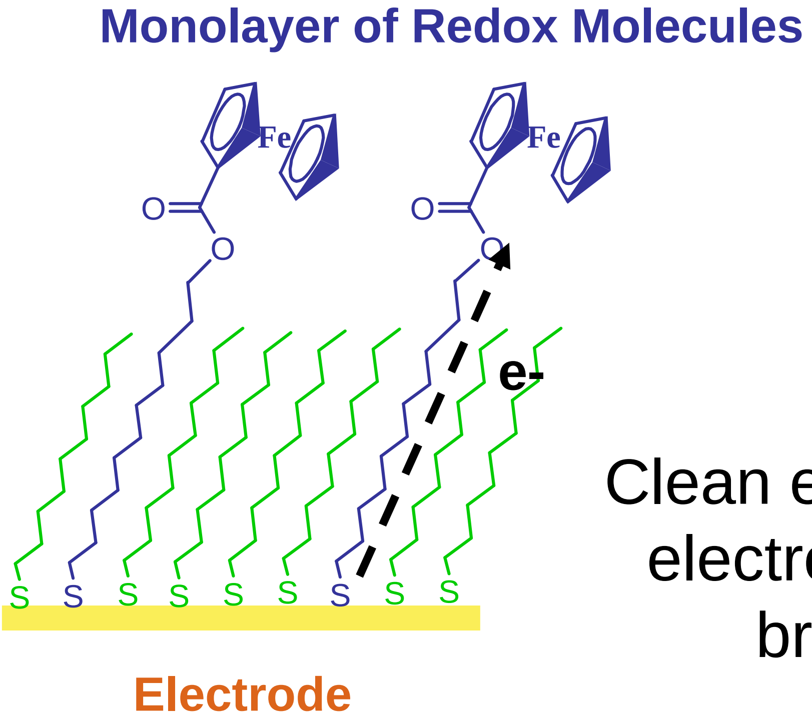
Mass Spec:
Calcd for $C_{82}H_{58}CuF_3FeN_{16}O_7$ + NaCl
1613.2936, Found 1613.2871

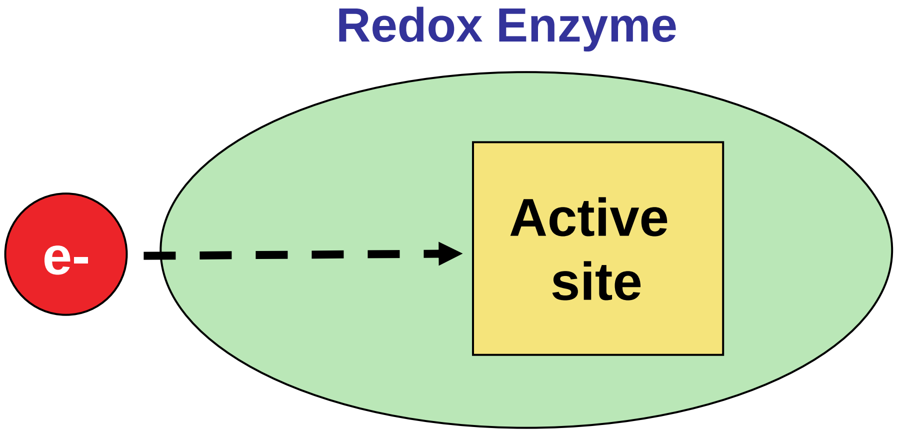


Collaboration with Ed. Solomon

Advantages of SAMs

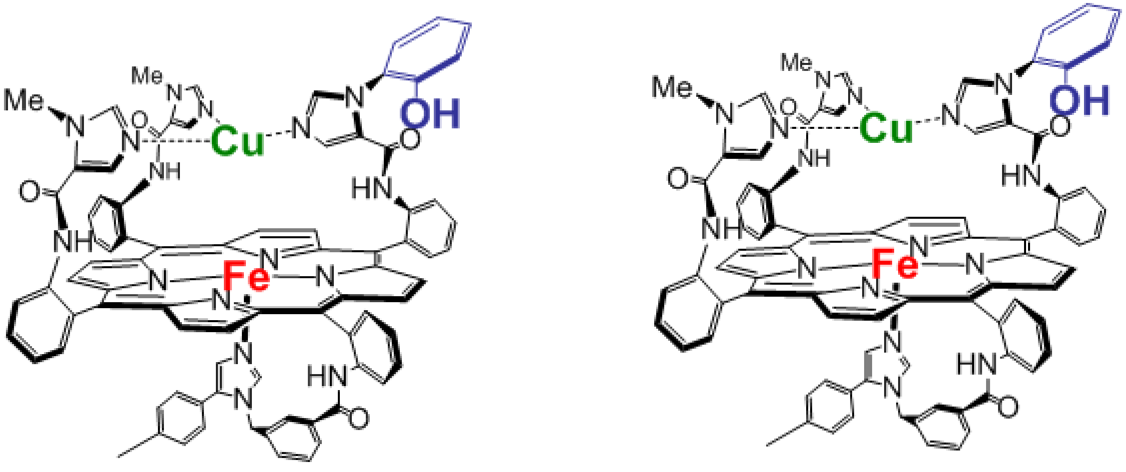
1. Well-defined and “easily” characterized surface (IR, XPS, etc)
2. Isolation of redox molecules using diluents
3. Control over the rate of electron transfer
4. Monolayers passivate bare electrode (barrier)



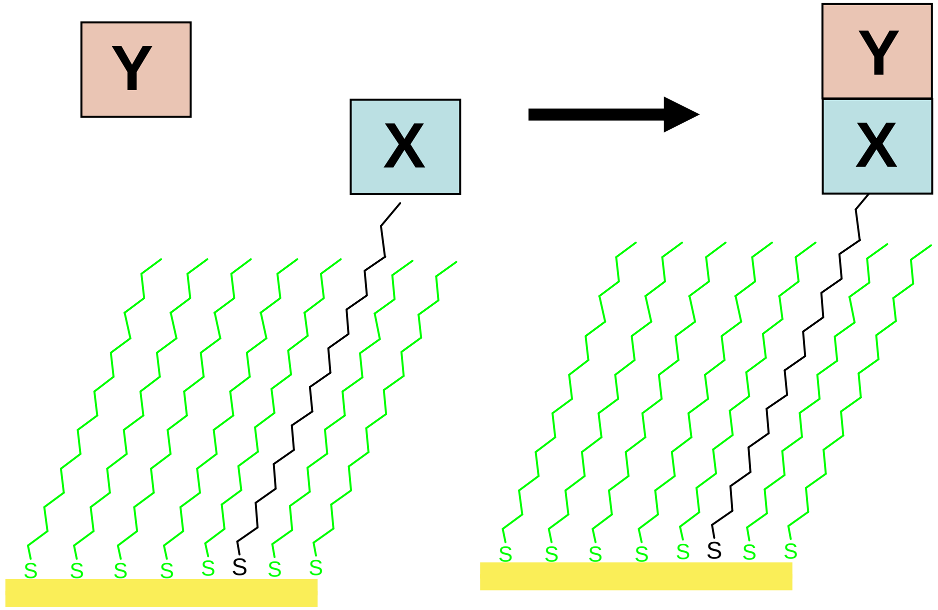


Clean electron transfer from the electrode through an organic bridge to a redox site

Post-coupling on Monolayers



Superior to Direct Absorption



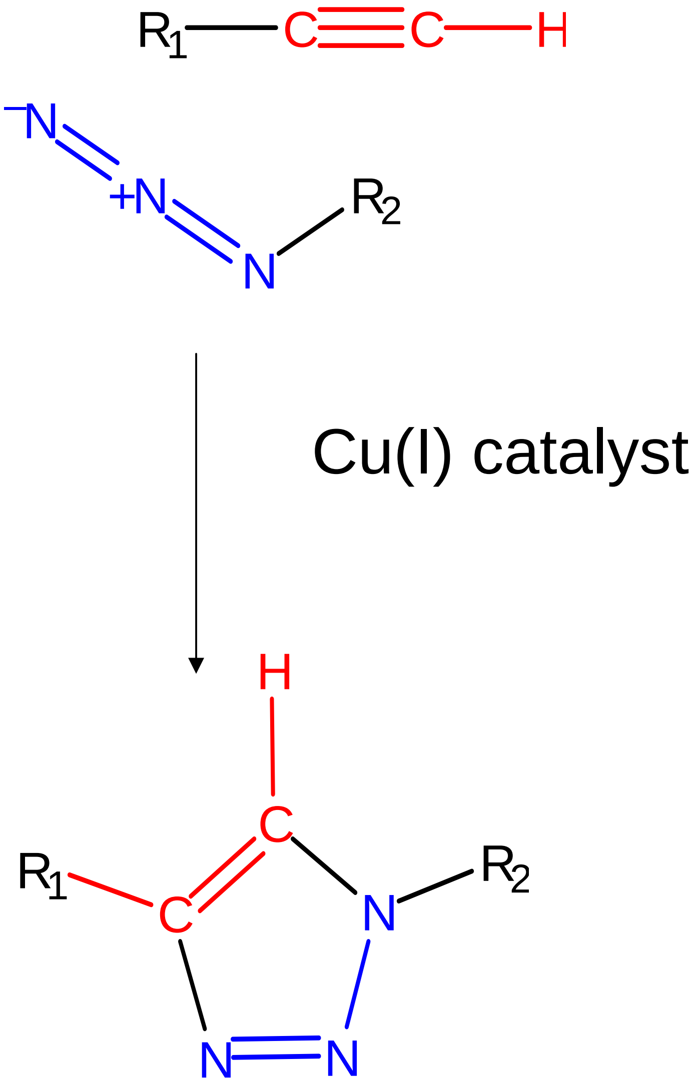
Past methods suffer from

1. **Incomplete coupling**
2. **Complexity**
3. **Harsh Conditions**

Required a better method

“Click” Chemistry

Copper(I) catalyzed azide-alkyne cycloaddition (Sharpless, Meldal)



High Yielding

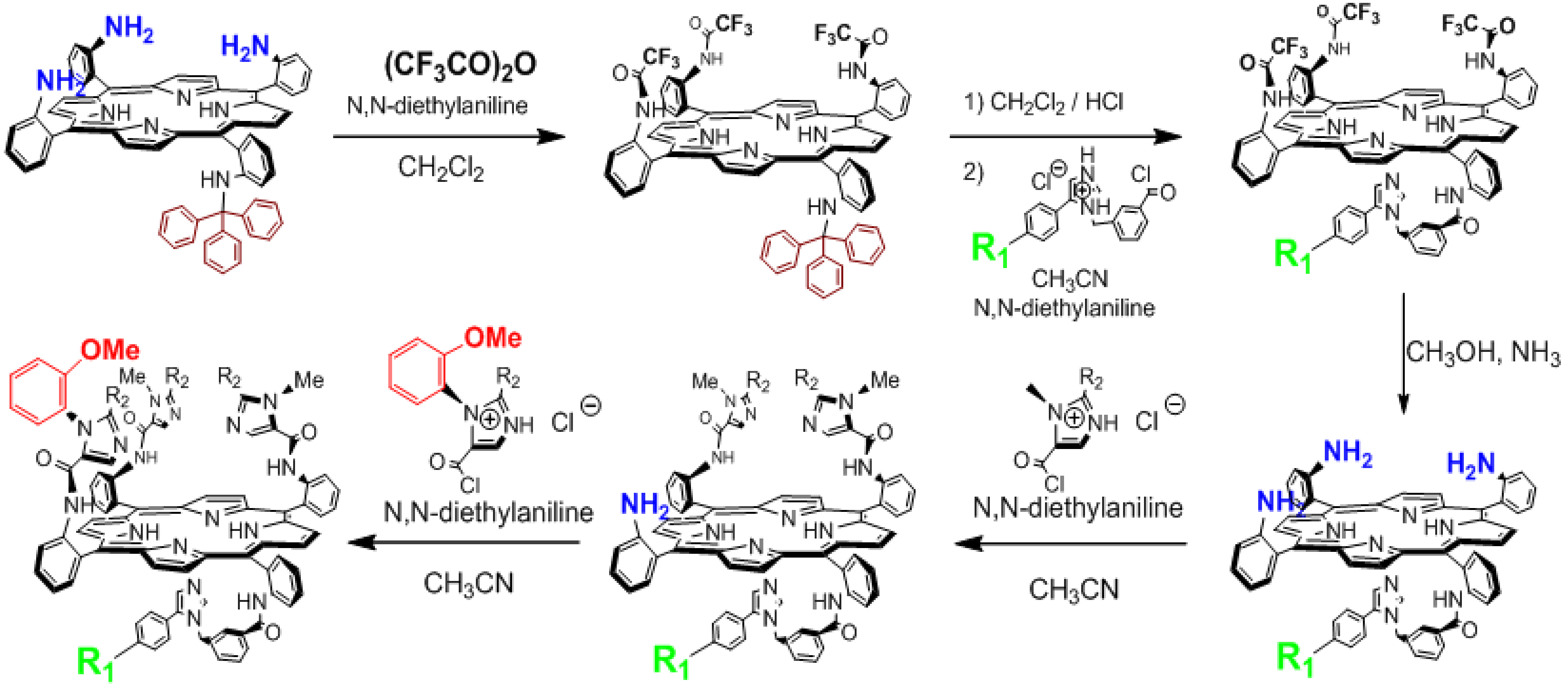
Extremely Selective

Room Temperature

Works Best in Aqueous Systems!

Catalyzed

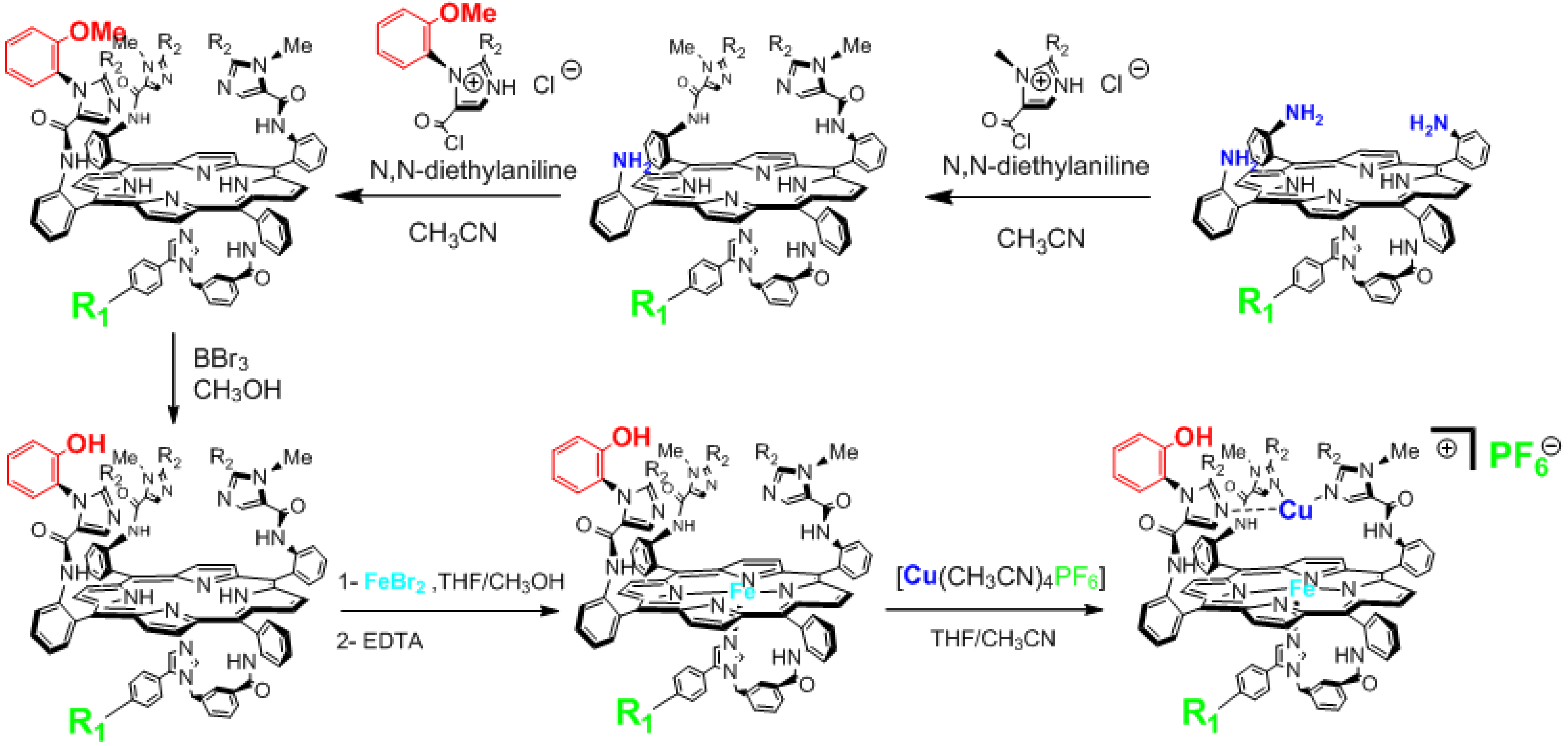
Syntheses of Cytochrome *c* Oxidase Models



R_1 = CF_3, —≡—H ; R_2 = H, Pr

Collman, J. P.; Broring, M.; Fu, L.; Rapta, M.; Schwenninger, R.; Straumanis, A. *J. Org. Chem.* **1998**, *63*, 8082
Collman, J. P.; Broring, M.; Fu, L.; Rapta, M.; Schwenninger, R. *J. Org. Chem.*, **1998**, *63*, 8084
Collman, J. P.; Decréau, R.A.; Zhang, C. *J. Org. Chem.*, **2004**, *69*, 3546.
Decréau, R. A.; Collman, J. P.; Yang, Y.; Yan, Y.-L.; Devaraj, N. K. *J. Org. Chem.* **2007**, *72*, 2794

Syntheses of Cytochrome *c* Oxidase Models



R_1 = CF_3, —≡—H; **R_2** = H, Pr

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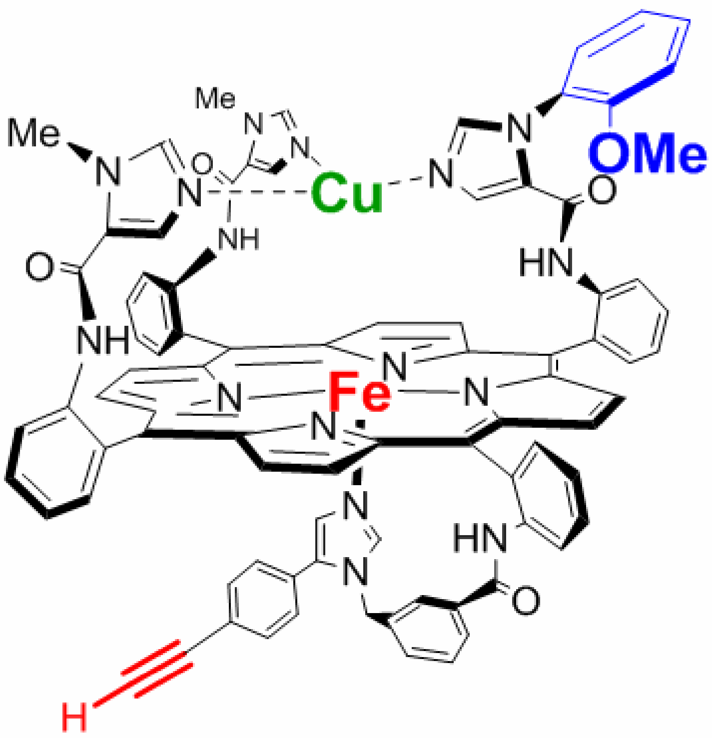
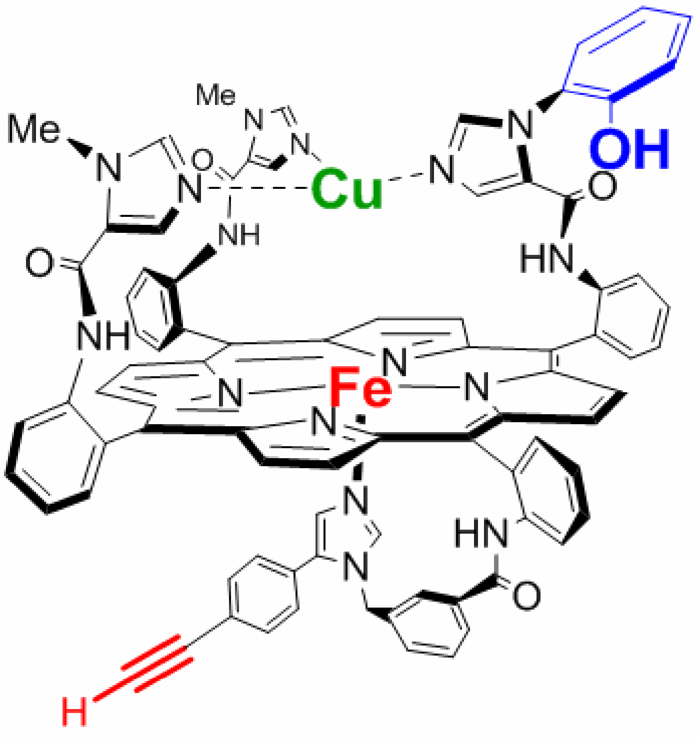
Collman, J. P.; Decréau, R.A.; Zhang, C. *J. Org. Chem.*, **2004**, *69*, 3546.
Decréau, R. A.; Collman, J. P.; Yang, Y.; Yan, Y.-L.; Devaraj, N. K. *J. Org. Chem.* **2007**, *72*, 2794

Models of Cytochrome c Oxidase Bearing a Phenol (Tyr 244 mimic)

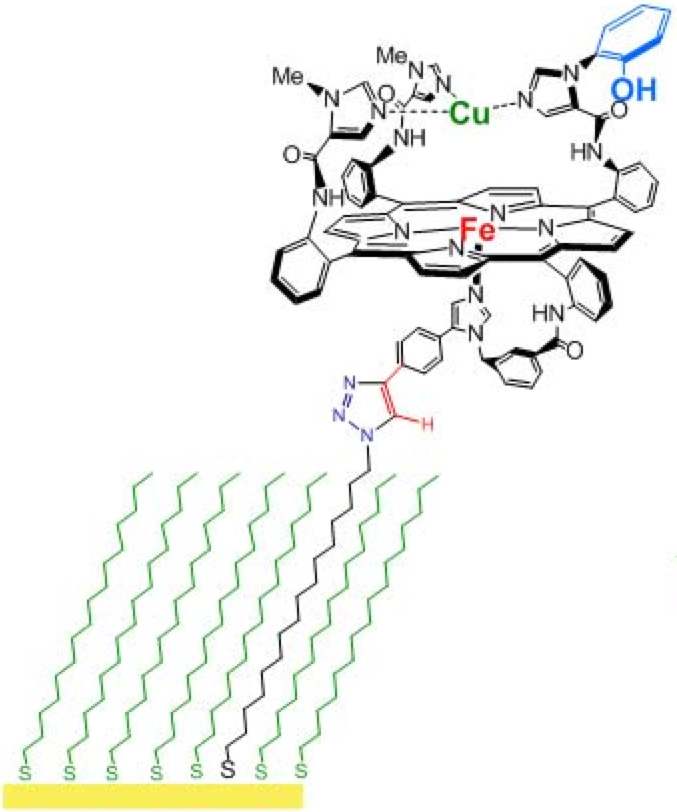




wash with acid to yield iron only model

Collman, J. P.; Devaraj, N. K.; Decréau, R. A.; Yang, Y.; Yan, Y.;
Ebina, W.; Eberspacher, T. A.; Chidsey, C. E. D., *Science*, **2007**, *315*, 1565

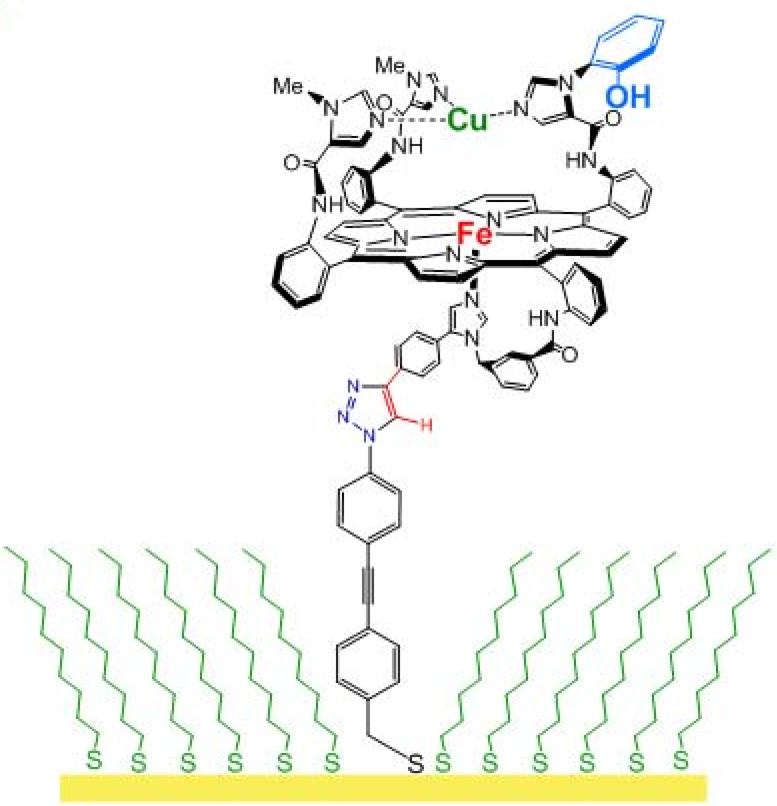
CcO Mimics on SAMs



Slow SAM

k^0 = 6 sec^{-1}

sluggish electron transfer



Fast SAM

$k^0 > 10^4 sec^{-1}$

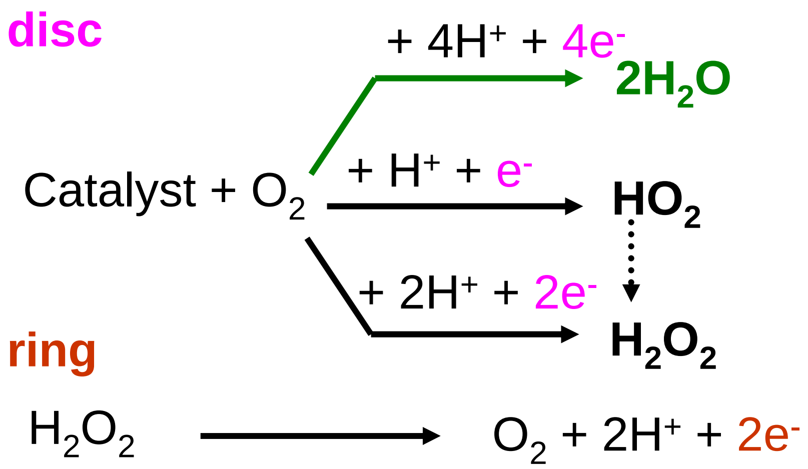
rapid electron transfer

pH 7 air-saturated 100mV vs. NHE
use platinum ring to detect peroxide (PROS)

Collman, J. P.; Devaraj, N. K.; Decréau, R. A.; Yang, Y.; Yan, Y.; Ebina, W.; Eberspacher, T. A.; Chidsey, C. E. D., *J. Phys. Chem. B*, **2006**, *110*, 15955

Detection of side reactions: RRDE

If the catalyst produces partially reduced oxygen species, they can be detected using a rotating ring disc electrode:



disc

$$\text{Catalyst} + O_2 \xrightarrow{+ 4H^+ + 4e^-} \mathbf{2H_2O}$$

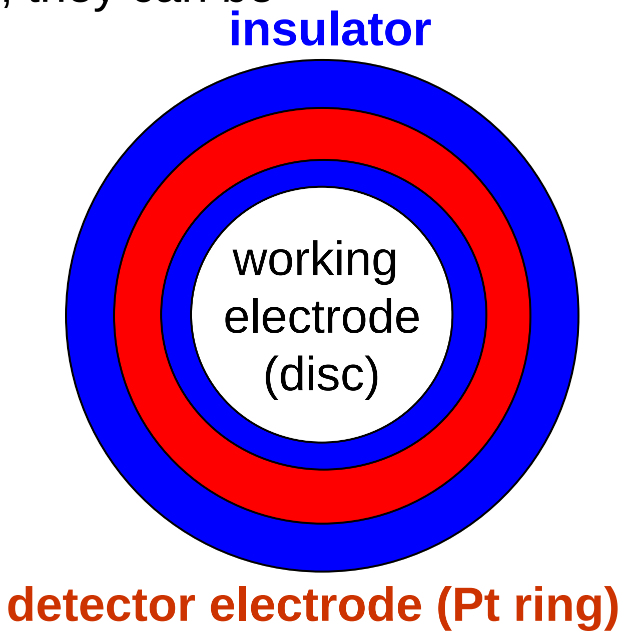
$$\text{Catalyst} + O_2 \xrightarrow{+ H^+ + e^-} \mathbf{HO_2}$$

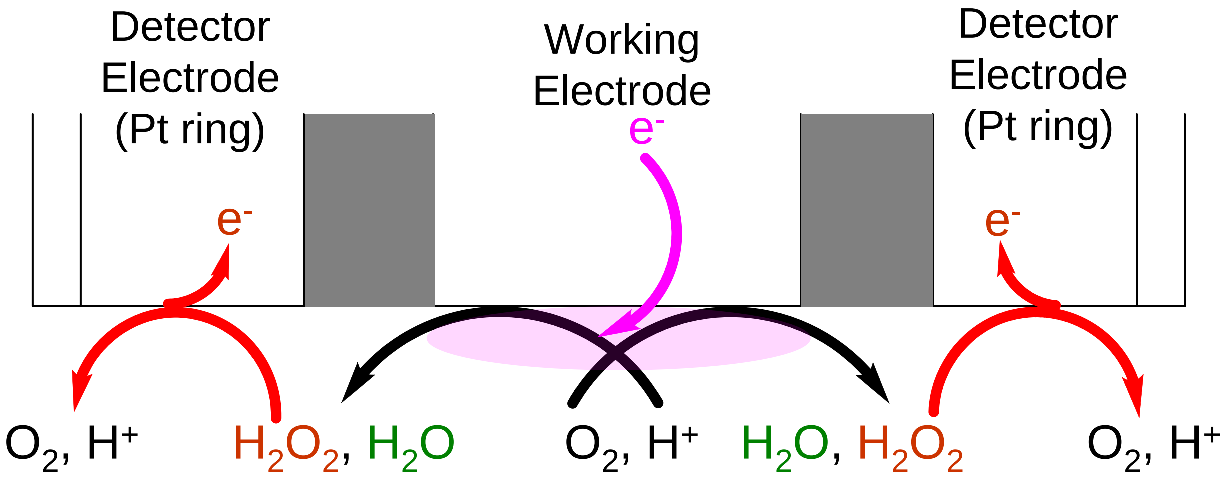
$$\text{Catalyst} + O_2 \xrightarrow{+ 2H^+ + 2e^-} \mathbf{H_2O_2}$$

$$\mathbf{HO_2} \dashrightarrow \mathbf{H_2O_2}$$

ring

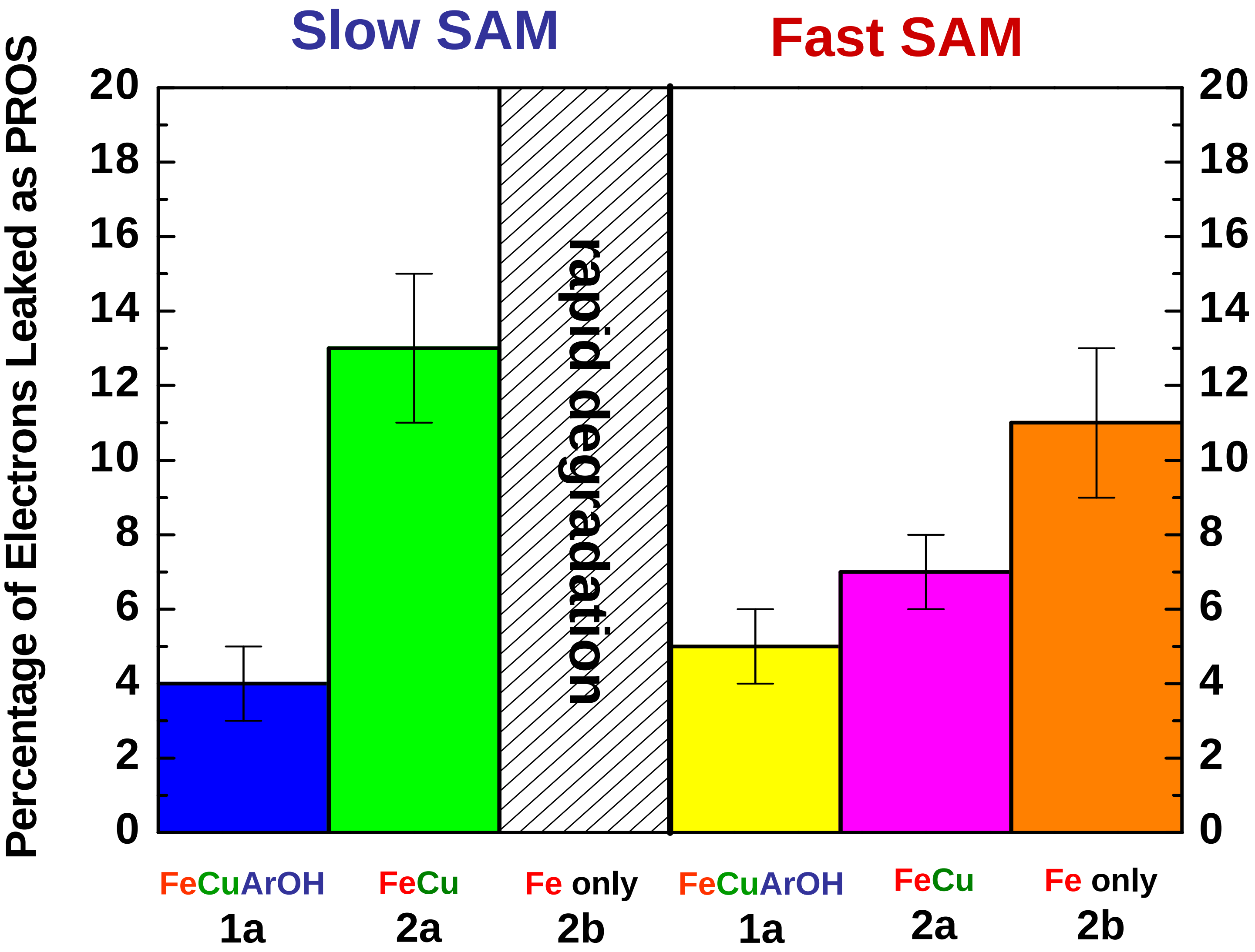
$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^-$$





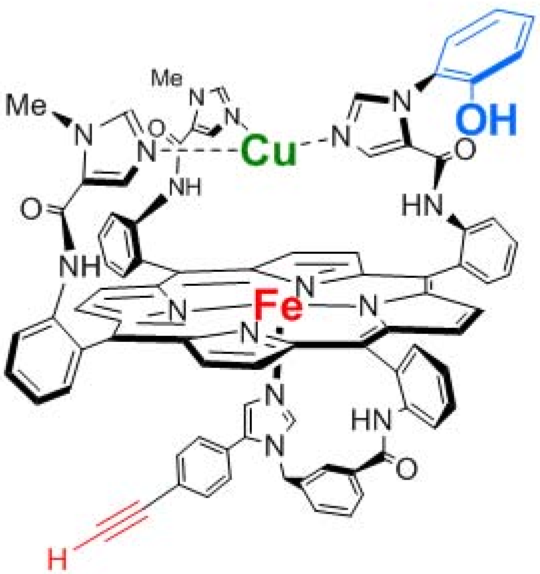
The ratio of the working electrode current (**I_{disc}**) to the detector electrode current (**I_{ring}**) allows one to estimate the proportion of the 4-electron pathway at any potential of the working electrode (how the rate of the redox steps effects the efficiency of the catalysis)

Partially Reduced Oxygen Leakage



Collman, J. P.; Devaraj, N. K.; Decréau, R. A.; Yang, Y.; Yan, Y.;
Ebina, W.; Eberspacher, T. A.; Chidsey, C. E. D., *Science*, **2007**, *315*, 1565

A Functional Model of CcO



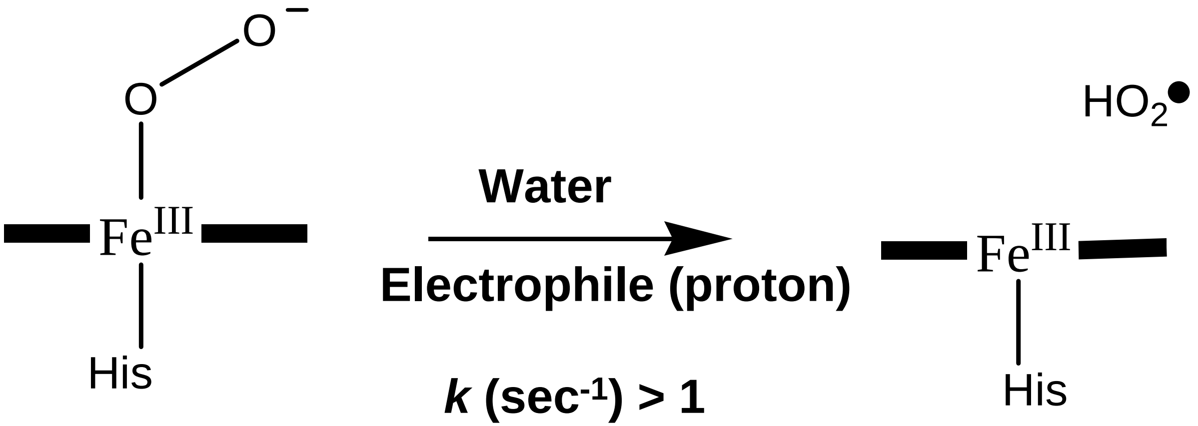
Model reproduces structure of the CcO active site:
active site contains four electron equivalents

Role of phenol during turnover is to lower release of partially reduced species under *rate-limiting electron flux*

~96% selectivity under rate-limiting electron-flux

Not Perfect: Why?

- Redox Cooperativity
  - – in enzyme Fe/Cu are either both reduced or both oxidized. Short circuiting (Fe(II)Cu(II)) prevented
- Heterogeneity in the Film?
  - – damaged catalyst? defects?
- Hydrolysis of the superoxide complex?

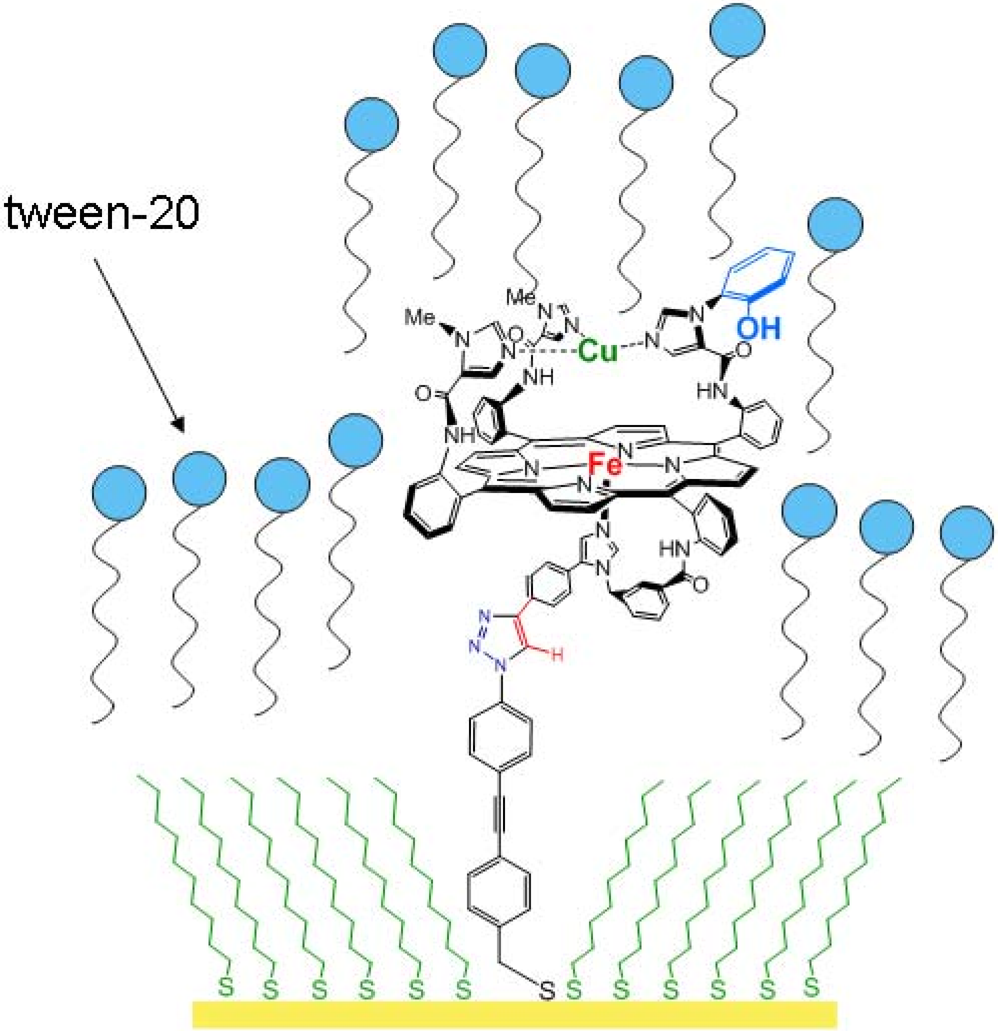


Typical oxyheme

k **(sec^{-1}) > 1**
$t_{1/2}$ < 1 sec
pH 8.5 25^{o}C

$2HO_2\bullet \longrightarrow H_2O_2 + O_2$

Improving Selectivity: toward >99%



Hydrophobic burying

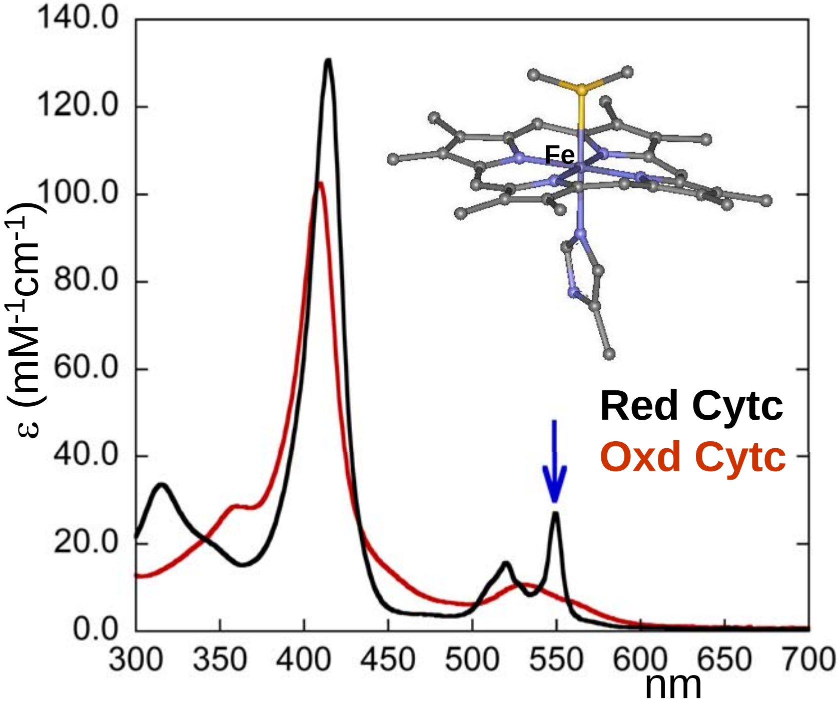
Preliminary results demonstrate that this can reduce PROS leakage by a factor of 2-3

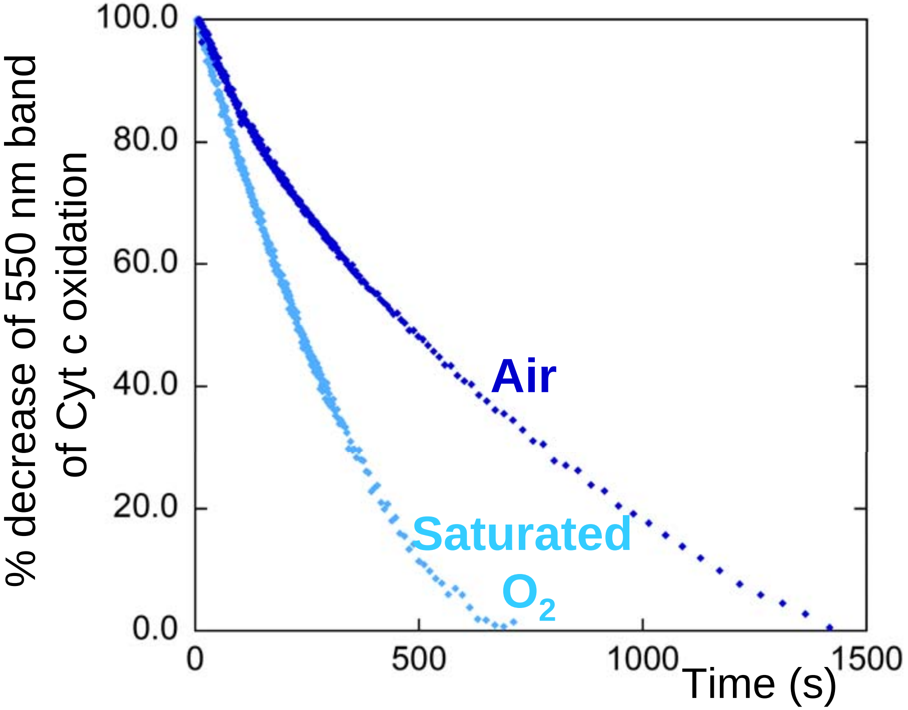
Raising the pH also can lower PROS

Catalytic Reduction of O_2 by Cytochrome c using the Functional CcO Model

$$4\text{Cytc}^{II}\text{ (horse heart)} + O_2 + 4H^+ \xrightarrow{\textit{2 mol\% CcO model}} 4\text{Cytc}^{III} + 2H_2O$$

50:50 aqueous buffer, CH_3CN solvent





Collman, J. P.; Ghosh, S.; Dey, A.; Decreau, R. A.; Yang, Y.J. Am. Chem. Soc. 2009, 131, 5034.

How Does C*c*O Tolerate NO?

Nitric Oxide is beneficial to CcO:
Lessons Learned from
"Functional" Models

Nitric Oxide (NO)

A critical regulator and a unique messenger molecule

Molecule of the year 1992

Nobel Prize in Chemistry 1998

Over 3000 publications a year

Collman, J. P.; Dey, A.; Decréau, R. A.; Yang, Y.; Hosseini, A.; Solomon, E. I. S.; Eberspacher, T. A. *Proc. Natl. Acad. Sci. U. S. A.*, **2008**, *105*, 9892-9896

Nitric Oxide: Potent Inhibitor of CcO

Mitochondrial NO synthase (mNOS) → produces a steady flux of NO

Involved in blood vessel modulation, neurotransmission, respiratory regulation

A stable but reactive free-radical, readily diffusible (50 μs^{-1} in biological systems)

$[NO]/[O_2] = 0.001$ in mitochondria

NO is a competitive inhibitor of CcO ($K_I = 0.27\ \mu M$)

Ferrous hemes strongly bind NO: $Fe^{II} + NO \rightarrow Fe\text{-}NO \quad K_{eq} = 10^9$

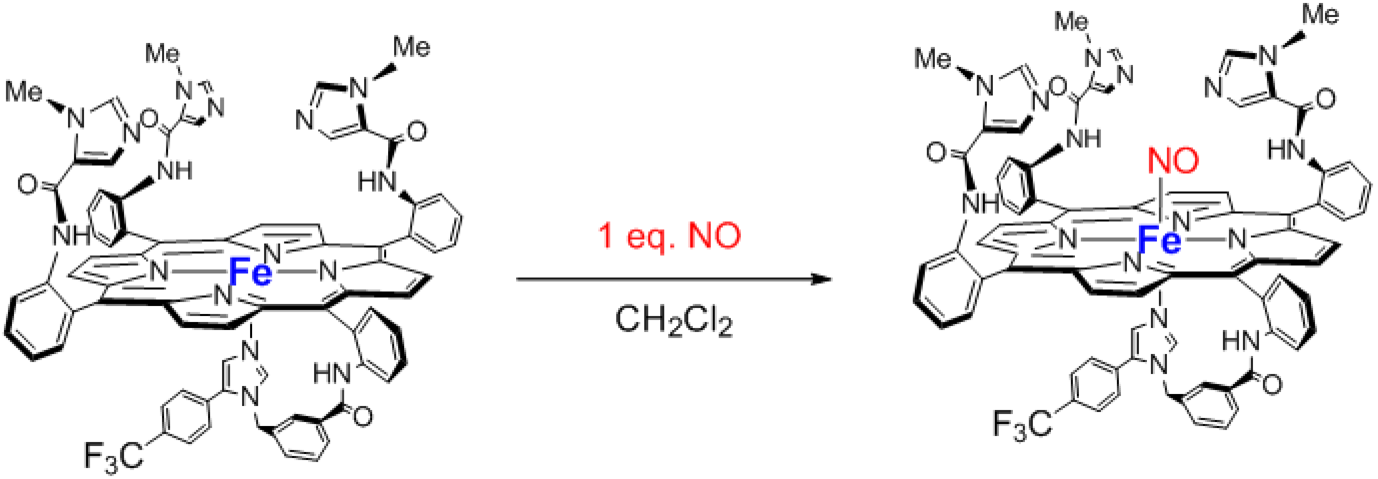
The dioxygen affinity is much lower: $Fe^{II} + O_2 \rightarrow Fe\text{-}O_2 \quad K_{eq} = 0.1$

Comparable k_{on} rates for both $10^{7\text{-}8}\ M^{-1}s^{-1}$

There’s a conundrum:
CcO should be permanently inhibited by NO in mitochondria

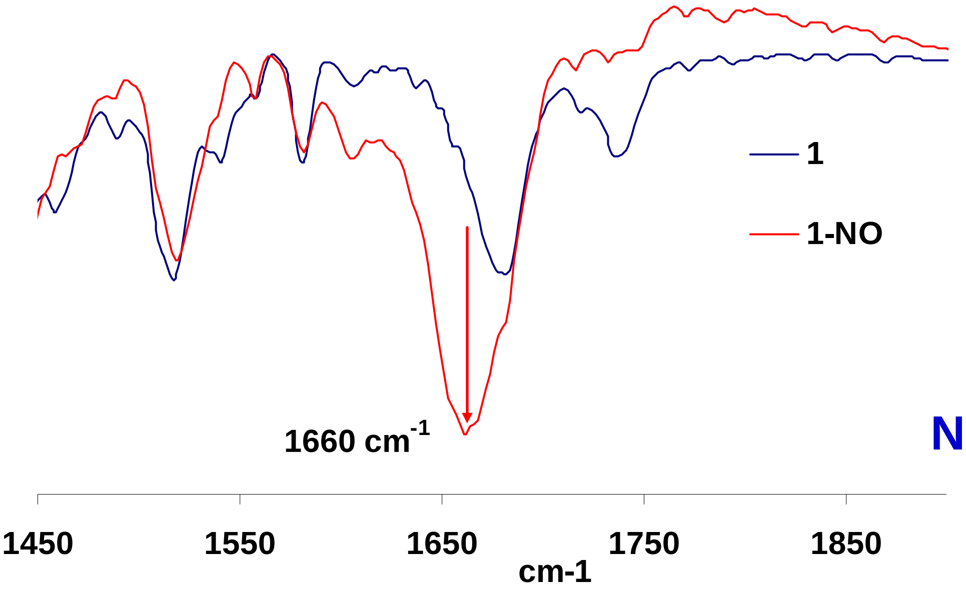
Ford, P. C.; Lorkovic, I. M. *Chem. Rev.* **2002,** *102,* 993
Stamler, J. S.; Singel, D. J.; Loscalzo, J. *Science*, **1992**, *258*, 1898

Nitrosyl Adducts: Spectroscopy FTIR/EPR

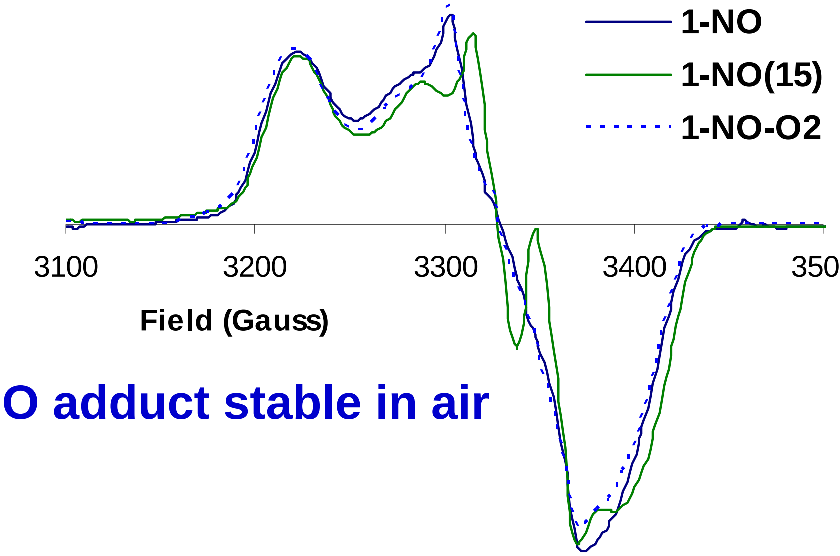


1

1-NO



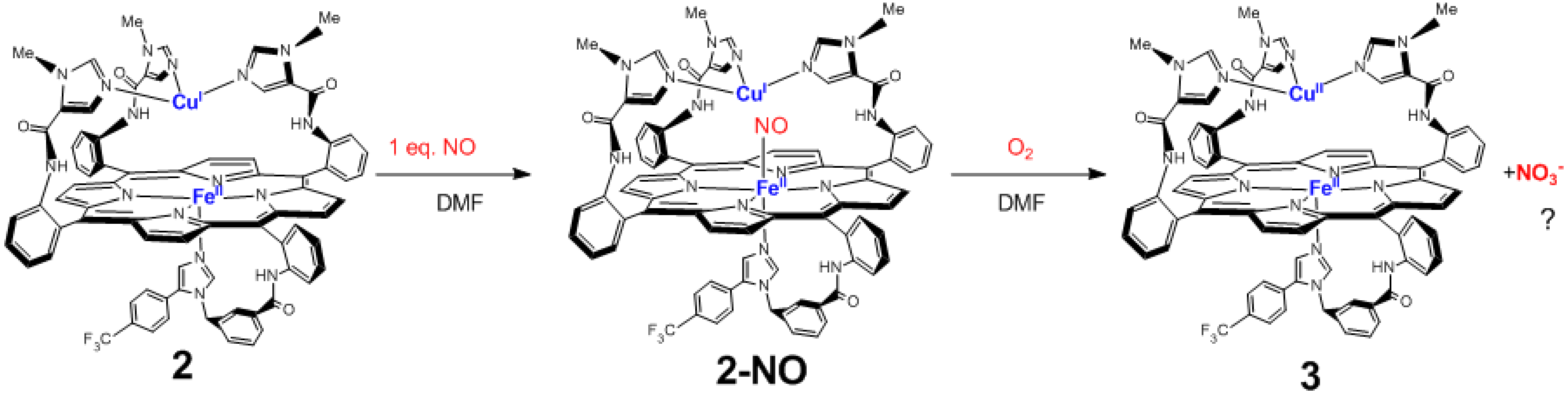
FTIR indicates $\nu_{N\text{-}O}$ at 1660 cm^{-1}
(confirmed by N^{15} isotope shift)



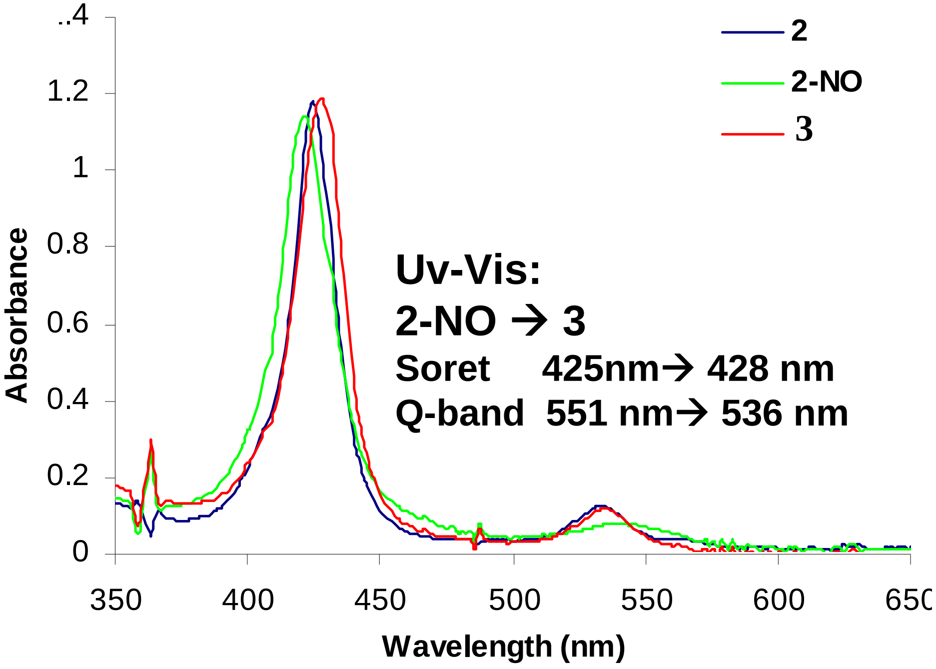
NO adduct stable in air

EPR ligand super-hyperfines show both NO and Imidazole are coordinated

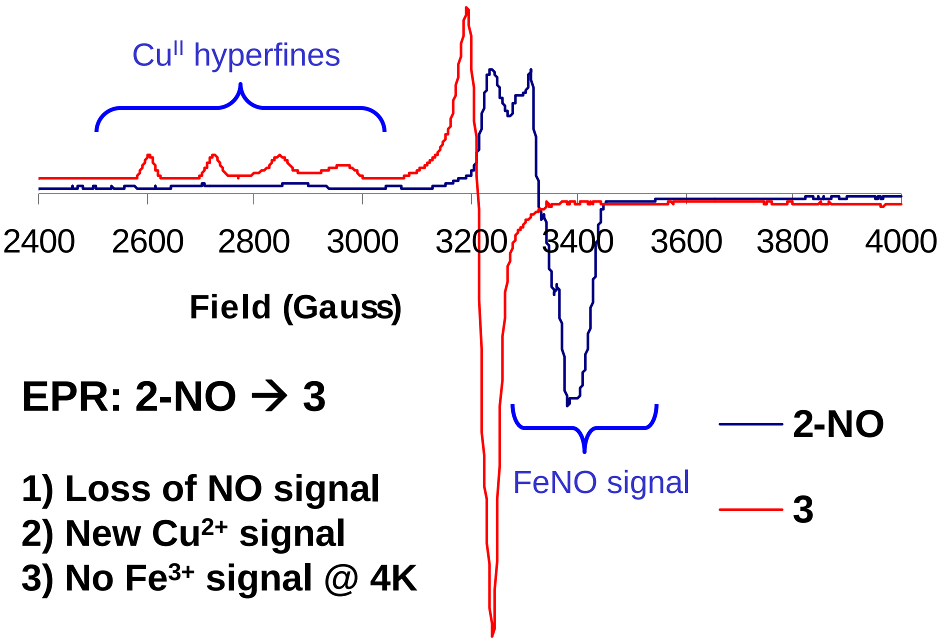
Reactivity of the Functional CcO Model with NO and O$_2$



2 → Fe^{II} “picket fence” porphyrin with covalently attached imidazole tail and **Cu^{I}** in the “distal pocket”
2-NO → NO adduct of **2**; Addition of O$_2$ to **2-NO** leads to **3** which is an Fe^{II} “picket fence” porphyrin with covalently attached imidazole tail and **Cu^{II}** in the “distal pocket”



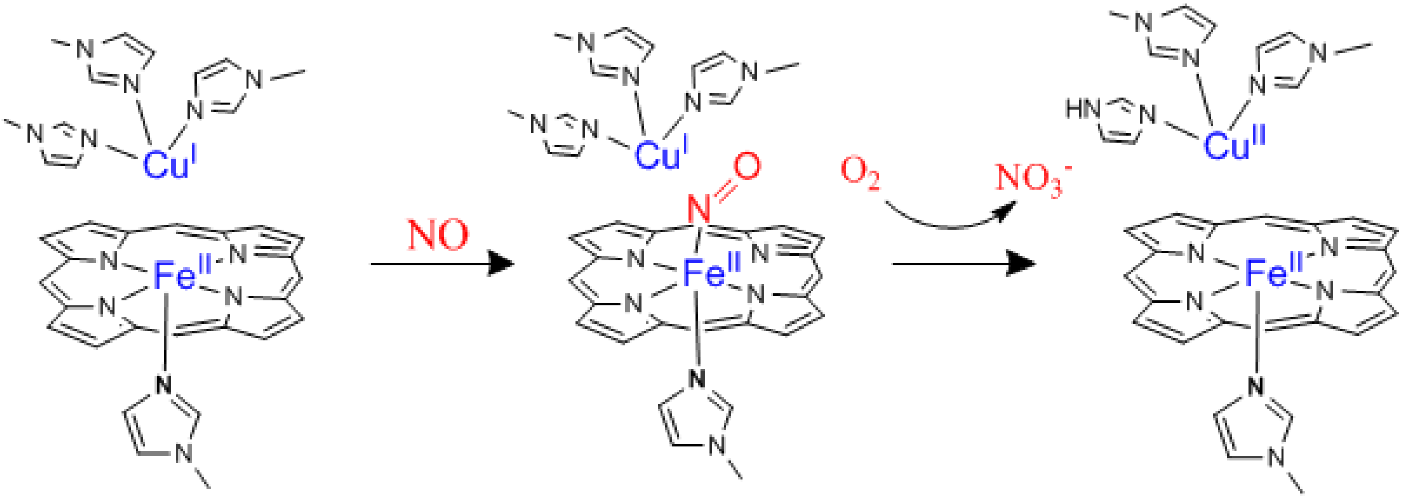
Uv-Vis:
2-NO → 3
Soret 425nm→ 428 nm
Q-band 551 nm→ 536 nm



EPR: 2-NO → 3

1) Loss of NO signal
2) New Cu^{2+} signal
3) No Fe^{3+} signal @ 4K

Recovery from NO Inhibition by the Functional CcO Model



428nm, 536nm

2

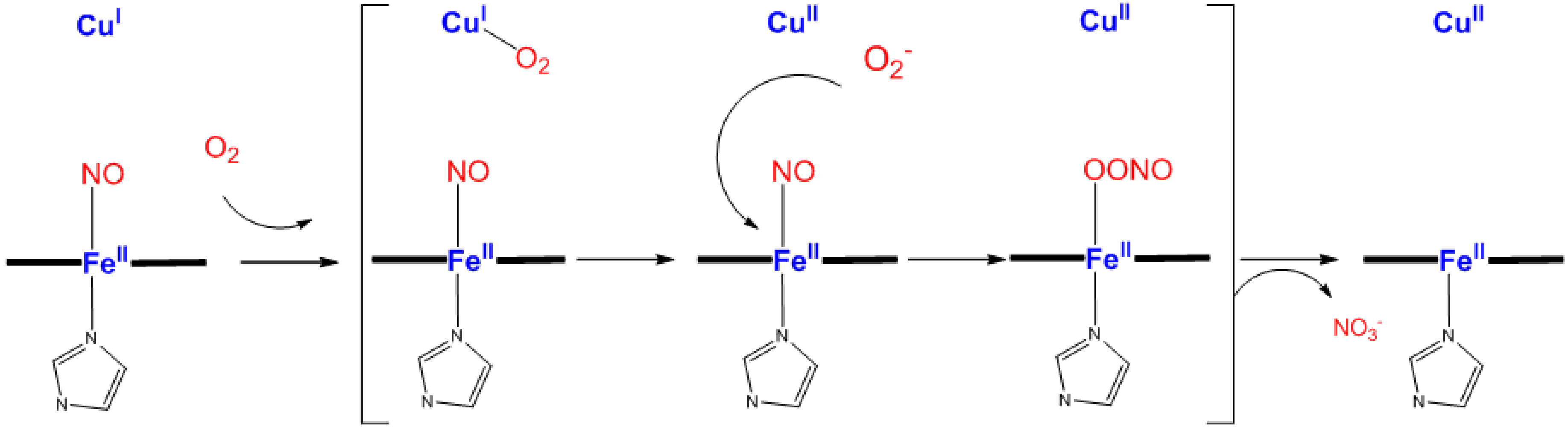
S=1/2 425nm, 548nm

2-NO

S=1/2 430nm, 537nm

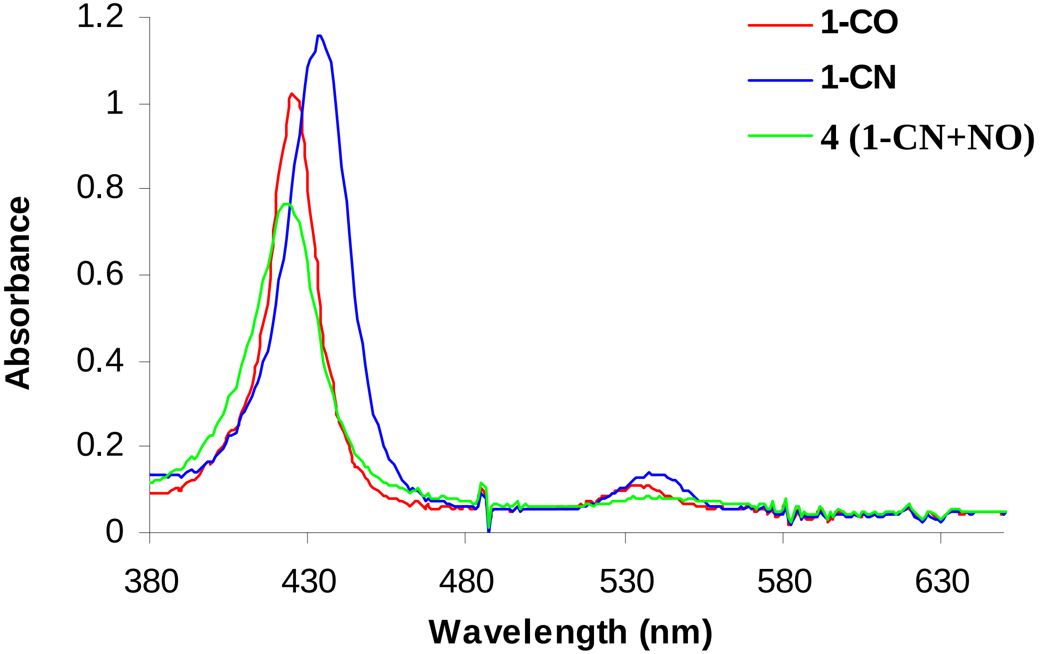
3

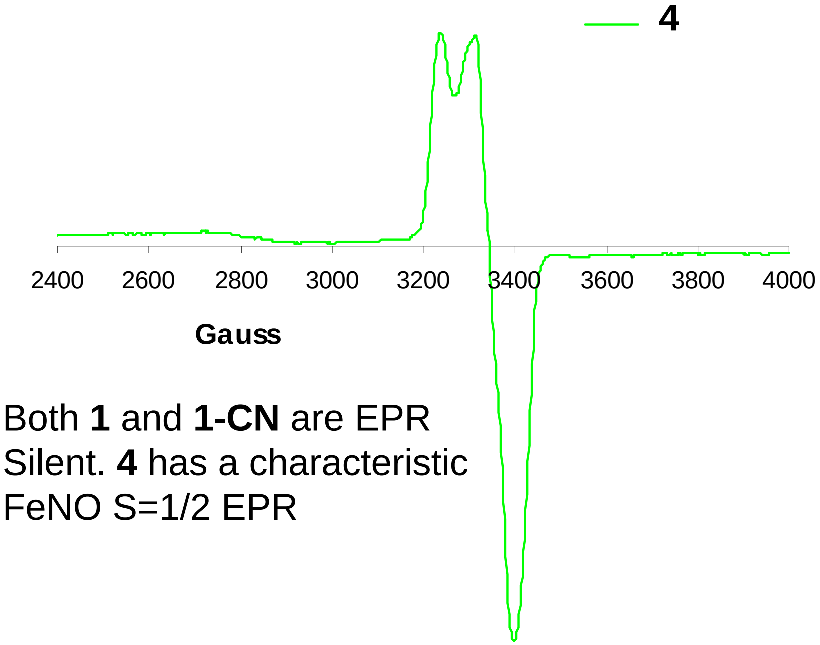
Proposed Mechanism



peroxynitrite?

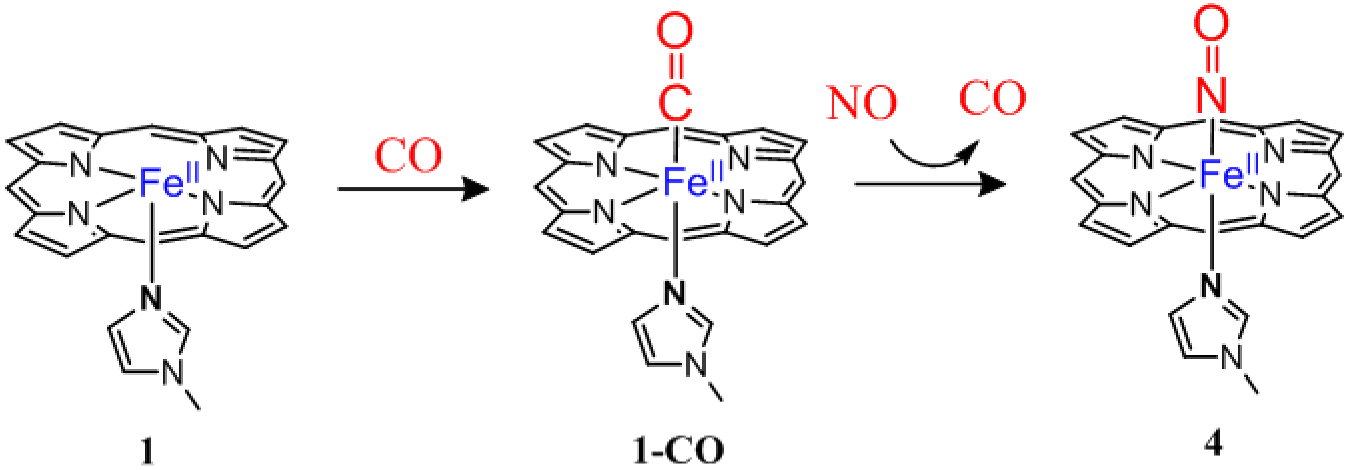
NO Generated Near CcO by NOS can Replace CO and CN^-



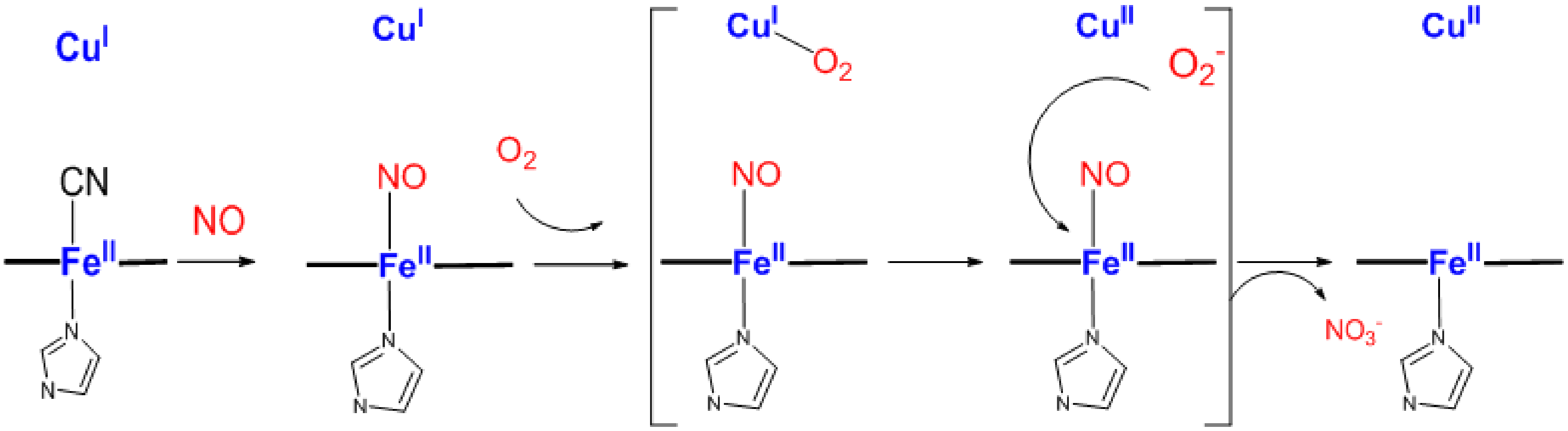


Both **1** and **1-CN** are EPR Silent. **4** has a characteristic FeNO S=1/2 EPR

1-CO → CO bound Fe^{II} “picket fence” porphyrin with covalently attached imidazole tail
1-CN → CN^- bound Fe^{II} “picket fence” porphyrin with covalently attached imidazole tail
4 → Results from NO addition to both **1-CN** and **1-CO** which is NO bound Fe^{II} “picket fence” porphyrin with covalently attached imidazole tail i.e. **1**



A Proposed NO Assisted Defense Mechanism in CcO



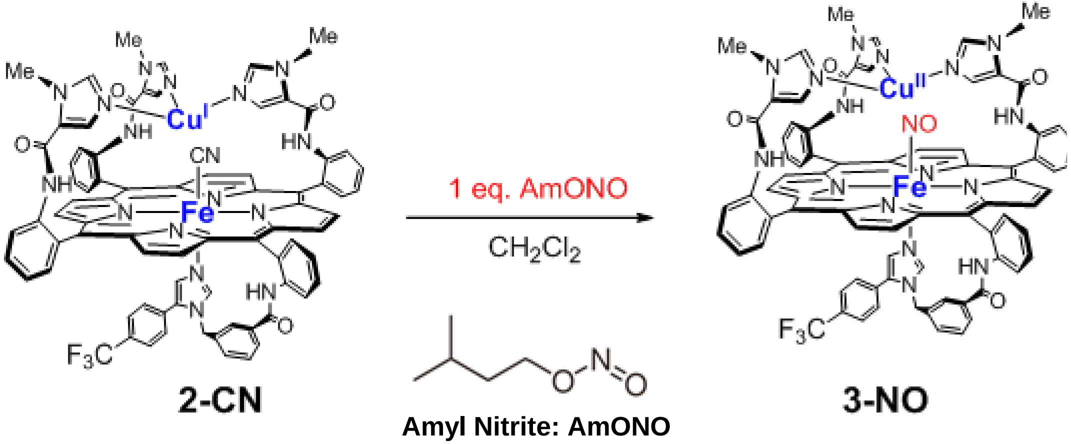
2-CO **2-NO**

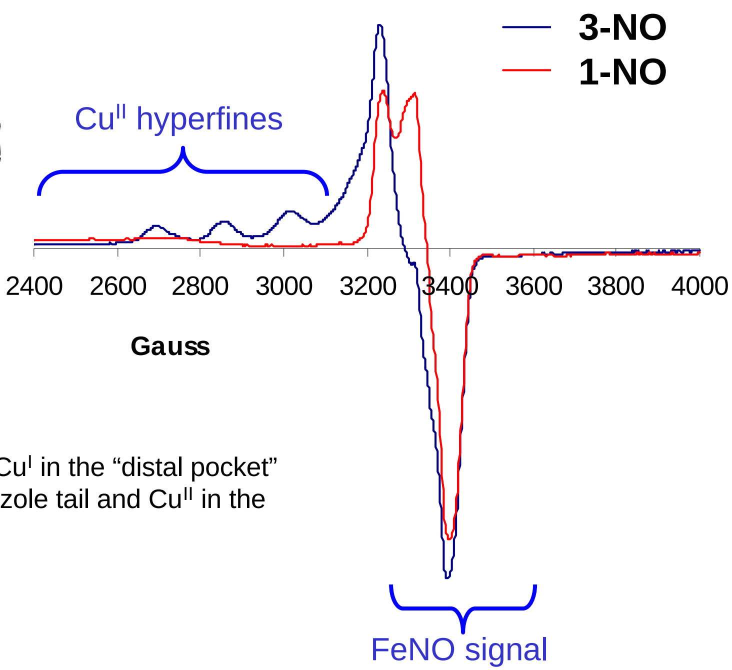
NO replacement of CO/CN$^-$

Recovery from NO inhibition by CcO

Need NO → Provided by mNOS which is present in mitochondria
Need O_2 → Substrate for CcO which is present in mitochondria
Need Electrons to reduce Cu_B provided by the electron transfer chain

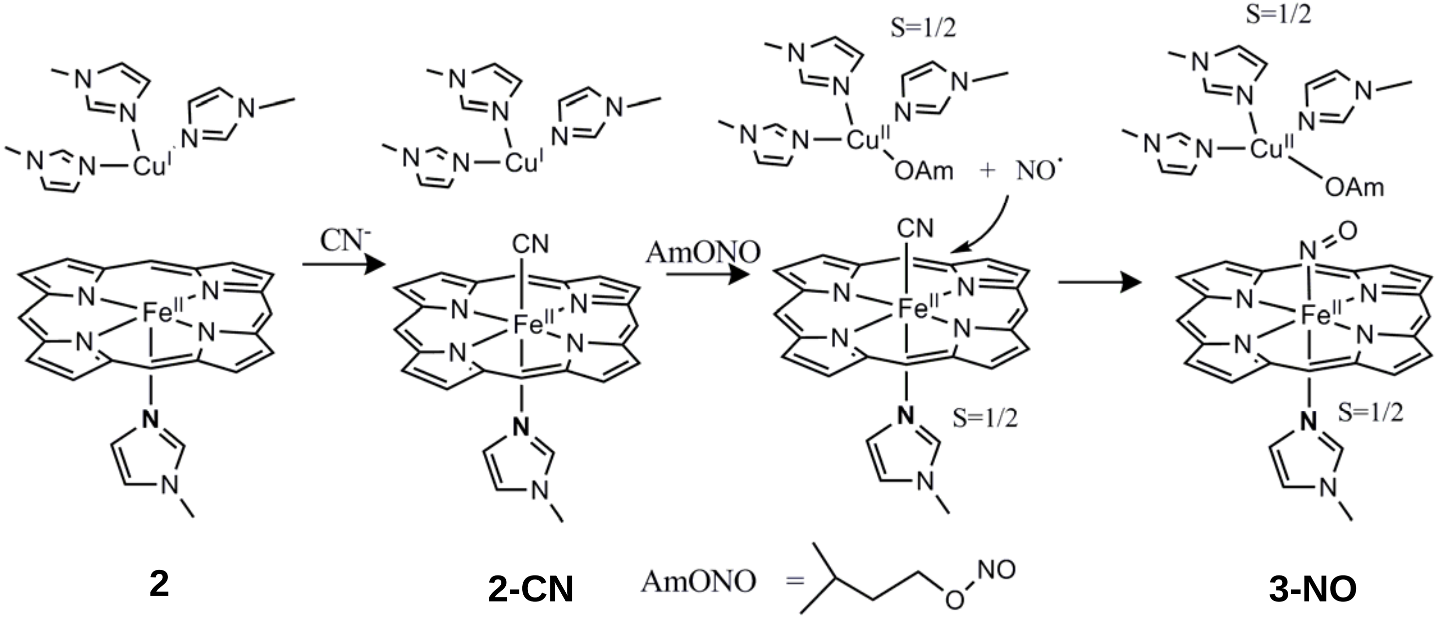
Amyl Nitrites: A Surrogate NO source for CcO





2-CN → CN^- bound Fe^{II} “picket fence” porphyrin with covalently attached imidazole tail and Cu^I in the “distal pocket”
3-NO → NO adduct of **3** which is Fe^{II} “picket fence” porphyrin with covalently attached imidazole tail and Cu^{II} in the “distal pocket”

Proposed Mechanism



A third gas, Hydrogen Sulfide (H_2S) may be encountered in the mitochondria

- H_2S is produced in mammals (including humans) from cysteine by two enzymes

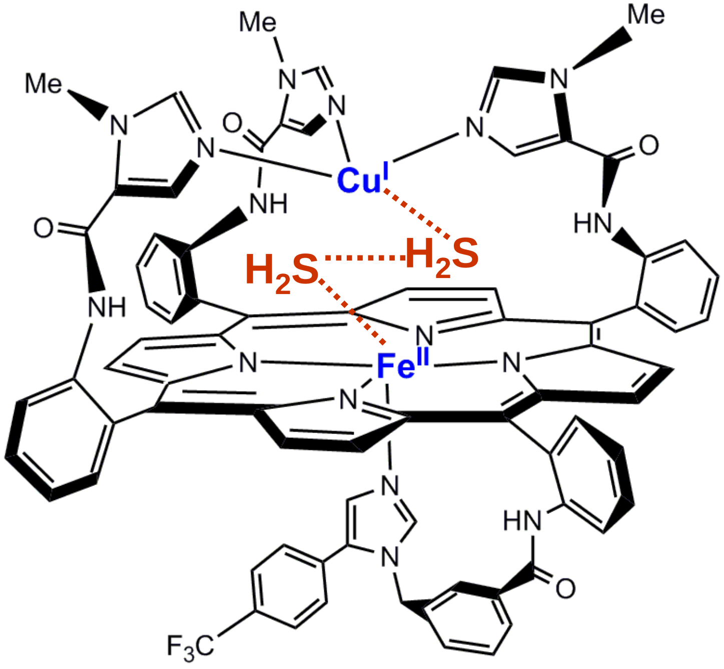
- At 600 ppm H_2S is lethal

- At 80 ppm H_2S slows respiration and produces hypothermia inducing a state resembling hibernation. This is reversible.

- H_2S is said to reversibly inhibit CcO

Key Reference: E. Blackstone, M. Morrison, and M. B. Roth, *Science*, 2005, *308*, 518.

H_2S Reversibly Binds to the Reduced Catalyst



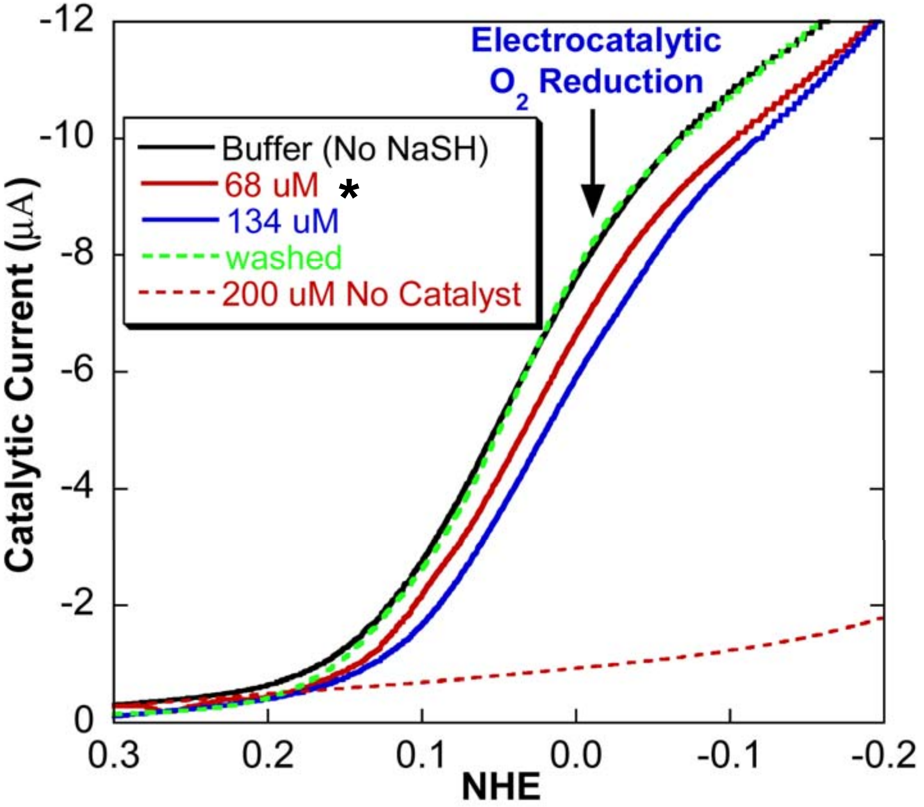
Evidence: UV-Vis, Mass spectrometry, ^{1}H NMR

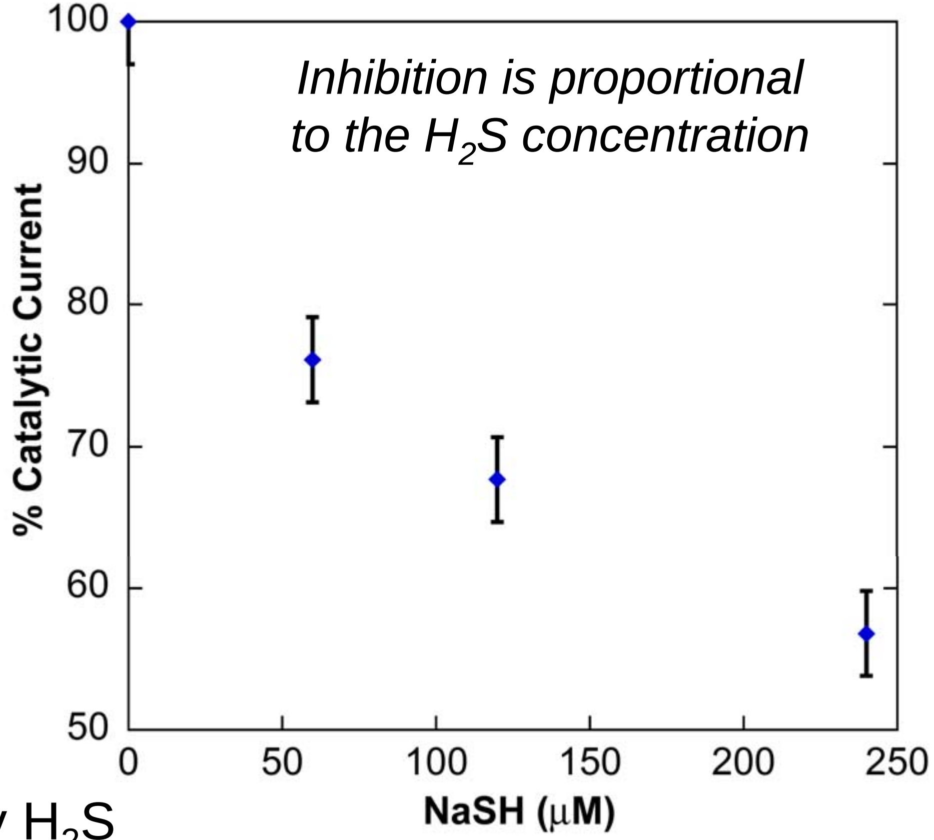
Estimated binding constants (K) = 0.5, 0.1 (much lower than O_2 binding)

In submission

H_2S Reversibly Inhibits the Electrochemical Catalytic Reduction of O_2

$$O_2 + 4H^+ + 4e^- \xrightarrow{\textit{CcO model}} 2H_2O$$





Inhibition is proportional to the H_2S concentration

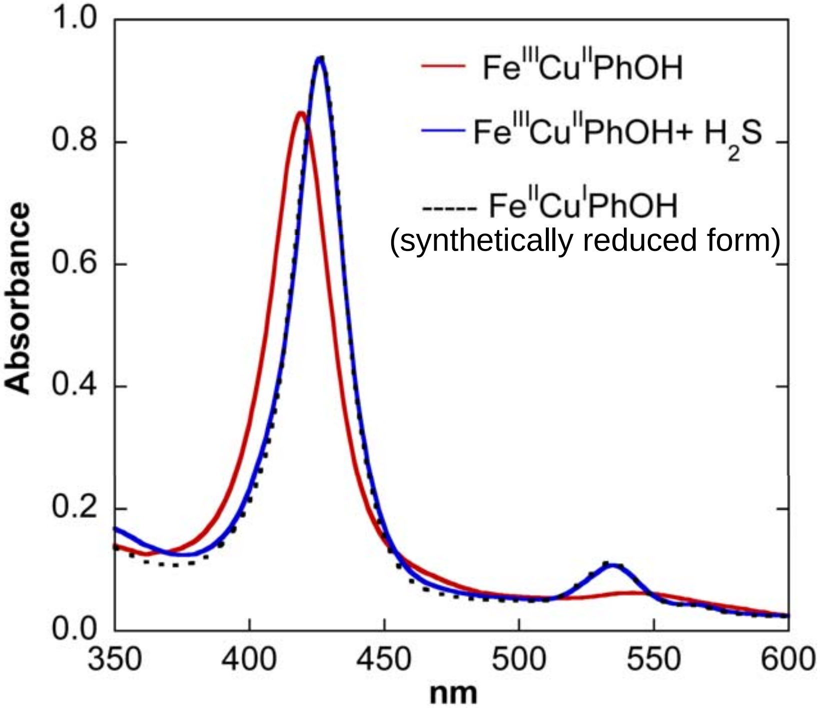
Reversible competitive inhibition by H_2S

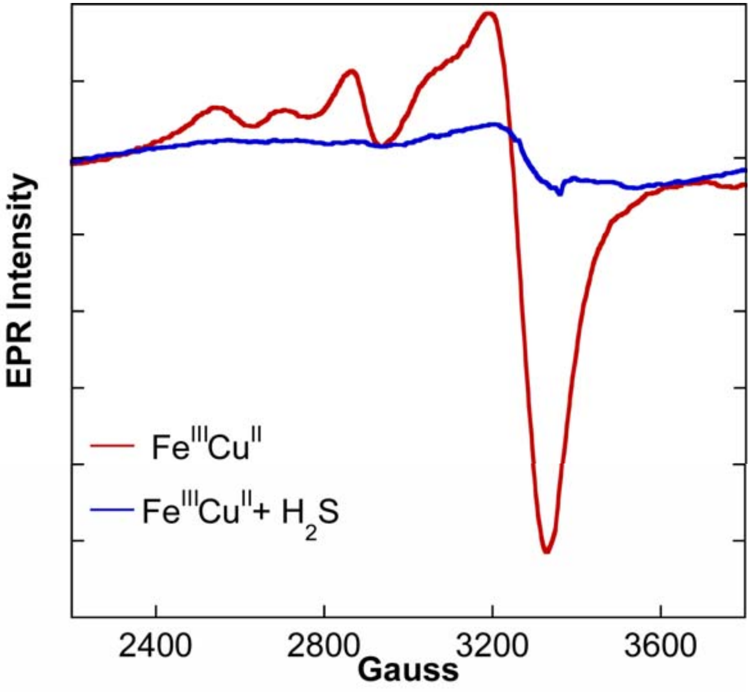
* Comparable amounts to those reported in literature to affect mice

In submission

H_2S is a Potent Two-Electron Reducing Agent

$Fe^{III}\ \ Cu^{II} + H_2S \rightarrow Fe^{II}\ \ Cu^{I} + (S)$





H_2S also reduces Cytochrome c

$Cytc^{III} + H_2S \rightarrow Cytc^{II} + (S)$

These results indicate that at low H_2S and moderate O_2 concentration, our model will catalytically reduce O_2 to H_2O

In submission