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# Free-Radicals: Chemistry and Biology

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http://iscamap.chem.polimi.it/citterio/education/free-radical-chemistry/

# Content

# Metals in Free Radical Reactions

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- Versatility of oxidation state, reduction potential, coordination number, spin state, ligand type, ligand equilibria, ligand dynamics and structure give to metals special characteristics.

- Metals play in several reactions a central role with their ability to display catalytic activity.

- Metals are frequently present in biological fluids and their concentration is strongly regulated by enzymes, moreover they are key components of the catalytic center of several enzymes.

- Metals are frequently involved in the generation of radicals, they bound efficiently radicals as ligant and show efficient oxidant or reducing power depending on the oxidation state.

**oxidation of alkyl radicals**

$CH_3CH_2\bullet$ → $H_2C{=}CH_2$ (via $Cu^{II}$)

$CH_3CH_2\bullet$ → $CH_3CH_2X$

**radical mechanism of oxidative addition**

$$L-Pd^{II}-I \ (Br) \xrightarrow{Ar^1\bullet} L-Pd^{III}-I \ (Br, Ar^1)$$
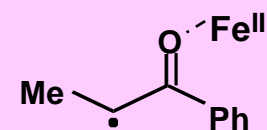
**$\alpha$-carbonyl radicals**

Me–$\dot{C}$(O···$Fe^{II}$)–Ph
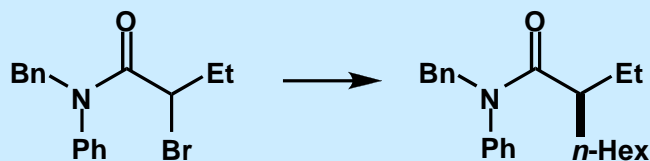
**metal-radical interactions in asymmetric catalysis**

Bn–N(Ph)–C(O)–$\overset{\bullet}{C}$(Et)(Br) → Bn–N(Ph)–C(O)–$\overset{*}{C}$(Et)(n-Hex)
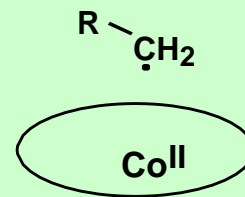
**coenzyme vitamin $B_{12}$**

R–$\dot{C}H_2$

$Co^{II}$

Three main mechanisms:

$$R\bullet \ + \ M^n X_m$$

addition to the metal → $R\text{-}M^{n+1}X_m$

ligand transfer → $R\text{-}X \ + \ M^{n-1}X_{m-1}$

electron transfer → $R^{\pm} \ + \ M^{n\pm1}X_m$
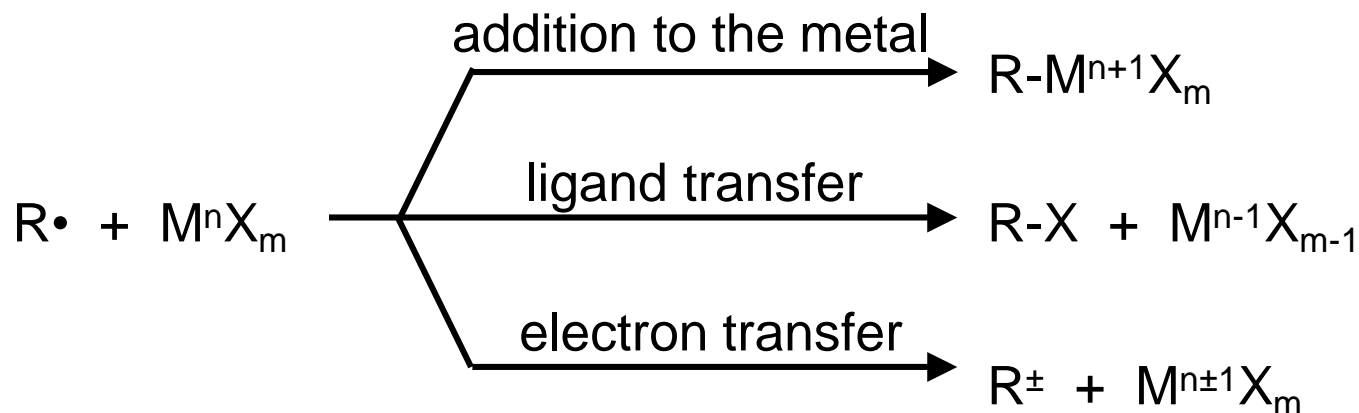
For example:

Different $Cu^{II}$ sources show divergent reactivity with respect to ethyl radical:

$$CH_3CH_2\bullet \ \xrightarrow{CuSO_4} \ H_2C{=}CH_2 \qquad\qquad CH_3CH_2\bullet \ \xrightarrow{CuCl_2} \ CH_3CH_2Cl$$

Kochi, J. K. Science. 1967, 27, 415-424

Minisci, Citterio JCS. Perkin II, 1978, pp. 519-24; Advances in Free-Radicals, (J.H. Williams Ed.), vol. VI, pp. 65-153 (1980)

- Oxidation by $CuSO_4$ is an electron transfer processes (outer-sphere)

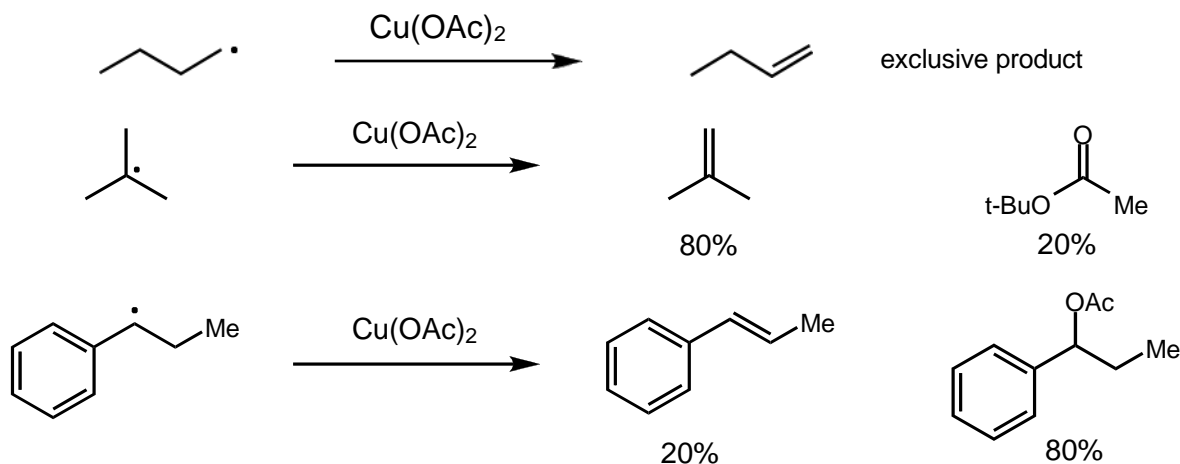$$Cu^{II} + CH_3CH_2^{\bullet} \longrightarrow Cu^{I} + CH_3CH_2^{+} \longrightarrow H_2C=CH_2 + + H^{+}$$

- Radicals which would give more stabilized carbocations give more substitution product



- Stabilities of the oxidation products do not control the selectivity of oxidative elimination
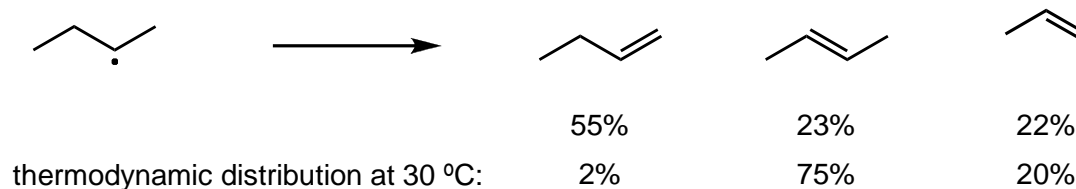


|  | 55% | 23% | 22% |
|---|---|---|---|
| thermodynamic distribution at 30 ºC: | 2% | 75% | 20% |

Kochi, J. K. *Science.* **1967**, *27,* 415-424.

**Reaction rate increased 200 time by the use of microwaves ***

**300 Kg/week
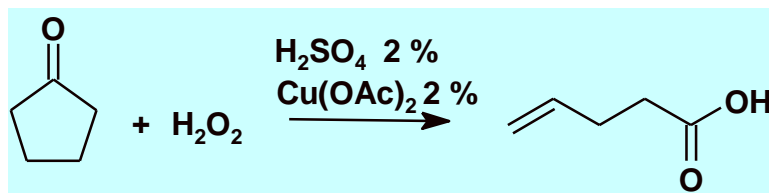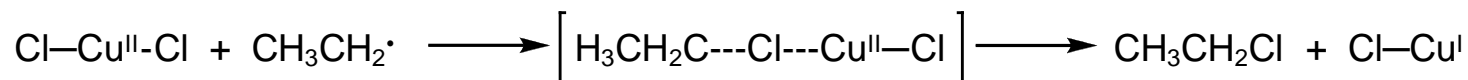(CWR reactor
200 ml, P = 10 atm)**
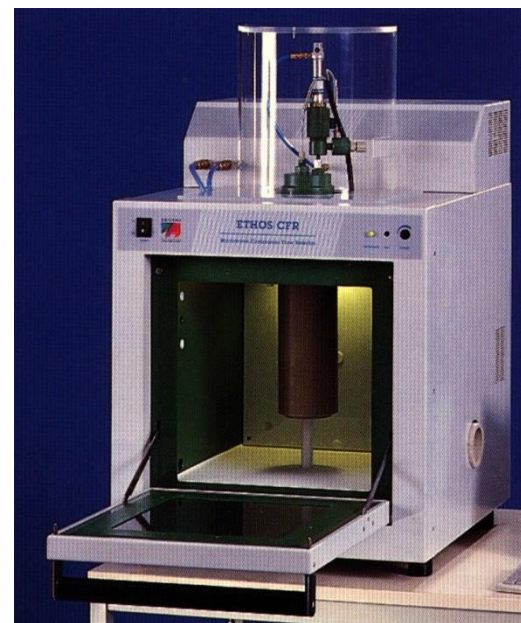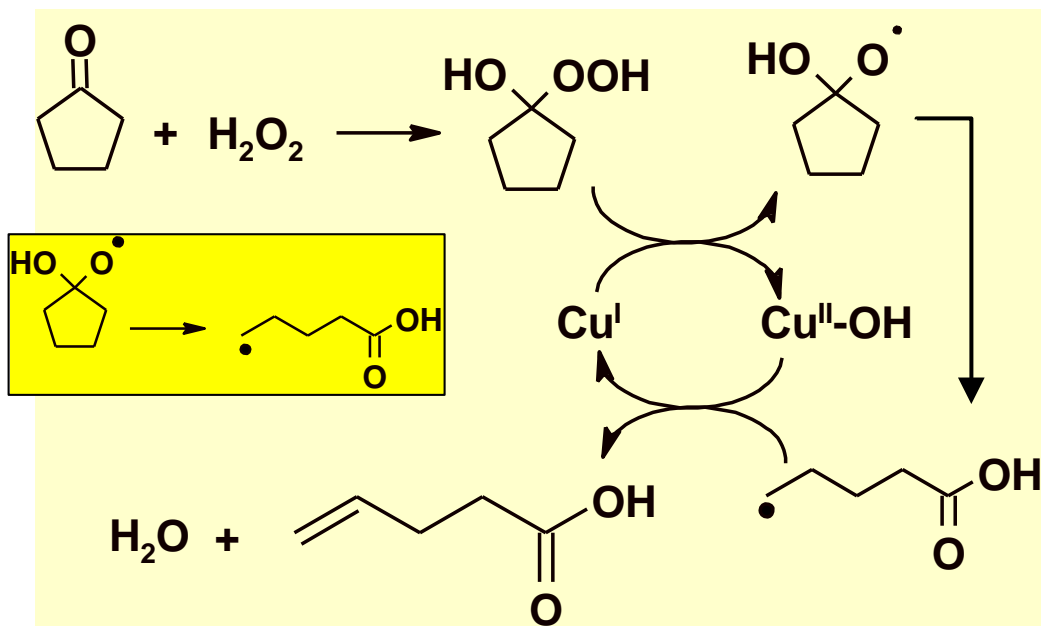
# Oxidation of Alkyl Radicals by Cu$^{II}$

- Oxidation by CuCl$_2$ is a ligand transfer processes (inner-sphere)

$$Cl-Cu^{II}\text{-}Cl + CH_3CH_2\cdot \longrightarrow \left[ H_3CH_2C\text{---}Cl\text{---}Cu^{II}-Cl \right] \longrightarrow CH_3CH_2Cl + Cl-Cu^{I}$$

- Oxidation of neopentyl radical gives no rearranged products with CuCl$_2$



carbocation rearrangement products

exclusive product
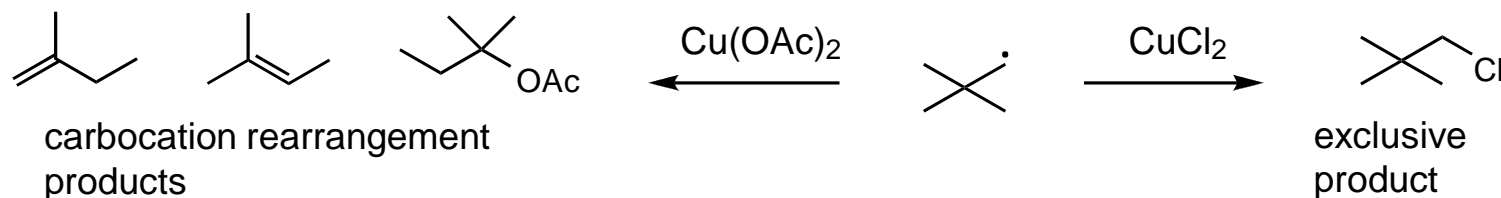
- Product ratios match those obtained with atom transfer reagents



|  | | |
|---|---|---|
| CuCl$_2$: | 70% | 30% |
| *t*-BuOCl: | 70% | 30% |
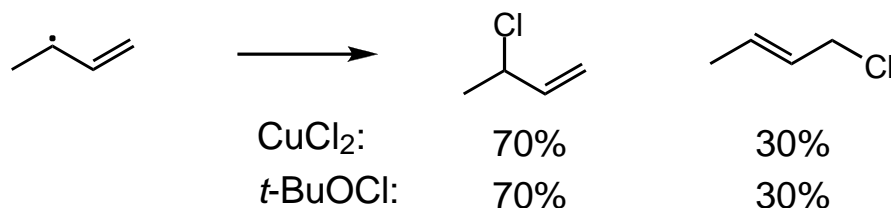
- Analogy with Taube inorganic ligand transfer process

$[Cr^{II}(H_2O)_5]^{2+}$

$[Co^{III}(NH_3)_5Cl]^{2+}$

$\longrightarrow$ $[(H_2O)_5Cr^{II}-Cl-Co^{III}(NH_3)_5]^{4+}$ $\longrightarrow$ $[Cr^{III}(H_2O)_5Cl]^{2+}$

$[Co^{II}(NH_3)_5]^{2+}$

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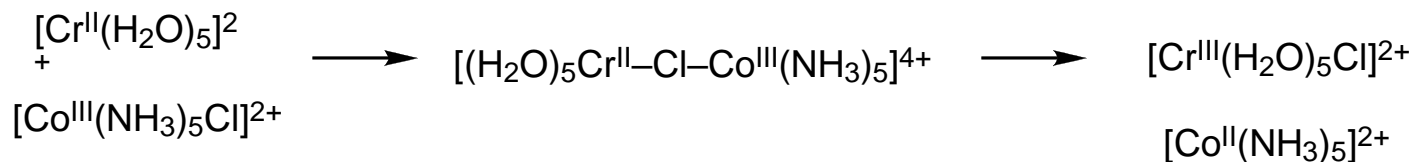
- *Metal-alkyl bonds are characteristically weak, correlate with degree of steric crowding*

bond dissociation energy (kcal·mol$^{-1}$)

| | |
|---|---|
| py)(SALOPH)Co–$CH_2CH_2CH_3$ | 25 |
| (py)(SALOPH)Co–$CH(CH_3)_2$ | 20 |
| (py)(SALOPH)Co–$CH_2C(CH_3)_3$ | 18 |
| (py)(SALOPH)Co–$CH_2C_6H_5$ | 22 |
| ($PMe_2Ph$)(DH)$_2$Co–$CH(CH_3)C_6H_5$ | 24 |
| ($PEtPh_2$)(DH)$_2$Co–$CH(CH_3)C_6H_5$ | 19 |
| ($PPh_3$)(DH)$_2$Co–$CH(CH_3)C_6H_5$ | 17 |
| | |
| $(CO)_5$Mn–$CH_3$ | 37 |
| $(CO)_5$Mn–$CF_3$ | 41 |
| $(CO)_5$Mn–$C_6H_5$ | 41 |
| $(CO)_5$Mn–$CH_2C_6H_5$ $(CO)_5$Mn– | 21 |
| $COC_6H_5$ | 21 |
| | |
| $(CO)_5$Re–$CH_3$ | 53 |

SALOPH = N,N'-disalicylidene-o-phenylenediamine, (DH)2 = dimethylglyoxime
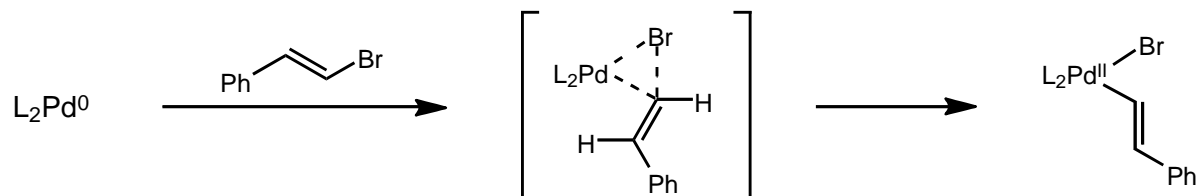
Halpern, J. Inorg. Chim. Acta. 1985, 100, 41-48.

Brown, D. L. S.; Connor, J. A.; Skinner, H. A. J. Organomet. Chem. 1974, 81, 403-409

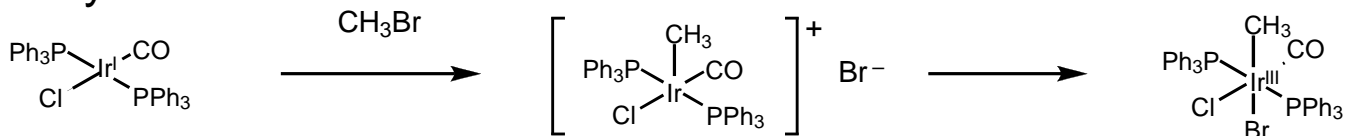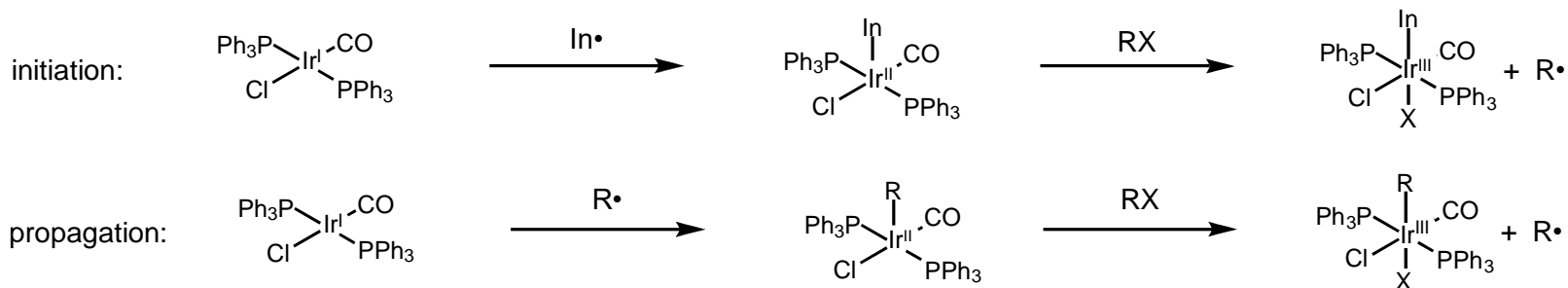Connor, J. A. et al. Organometallics. 1982, 1, 1166-1174

- Concerted pathway: *cis* insertion via a three-center, two-electron bond



- $S_N2$-type substitution: highly nucleophilic metal complexes attack primary or secondary halides



- Radical pathway (two inner sphere one-electron processes)

initiation:



propagation:



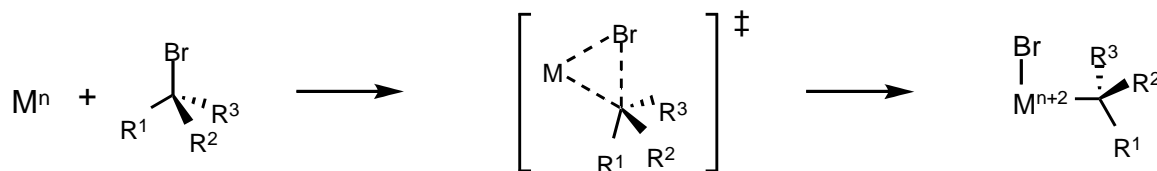- Electron-transfer mechanism (outer sphere one-electron process, then inner sphere one-electron process)  (see the specific powerpoint)

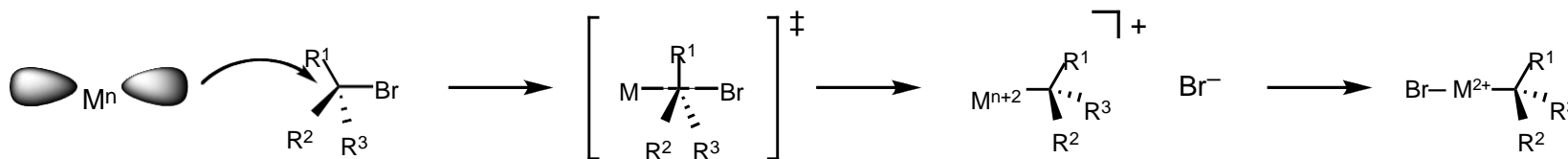Tsou, T. T.; Kochi, J. K. *J. Am. Chem. Soc.* **1979**, *101,* 6319-6332

- **Concerted pathway: requires retention of configuration**



- **$S_N2$-type substitution: requires inversion of configuration**



- **Radical pathways: likely to proceed with loss of stereochemistry**
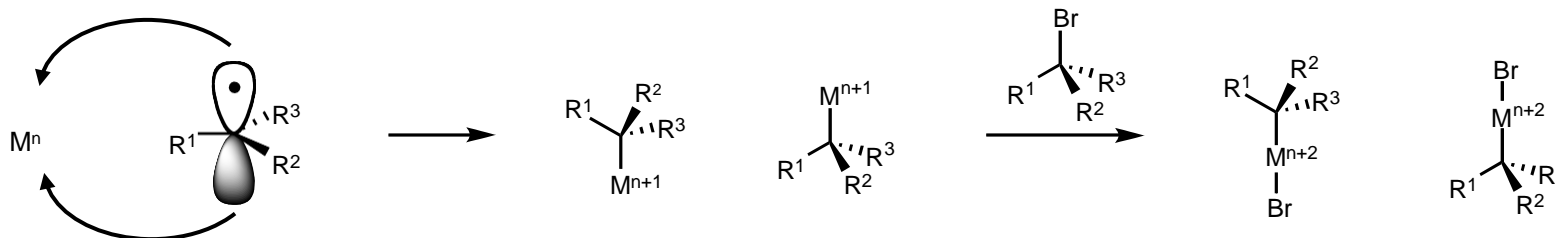
- Oxidative addition of alkyl halides to an $Ir^I$ complex proceeds with loss of stereochemistry



1:1 mixture
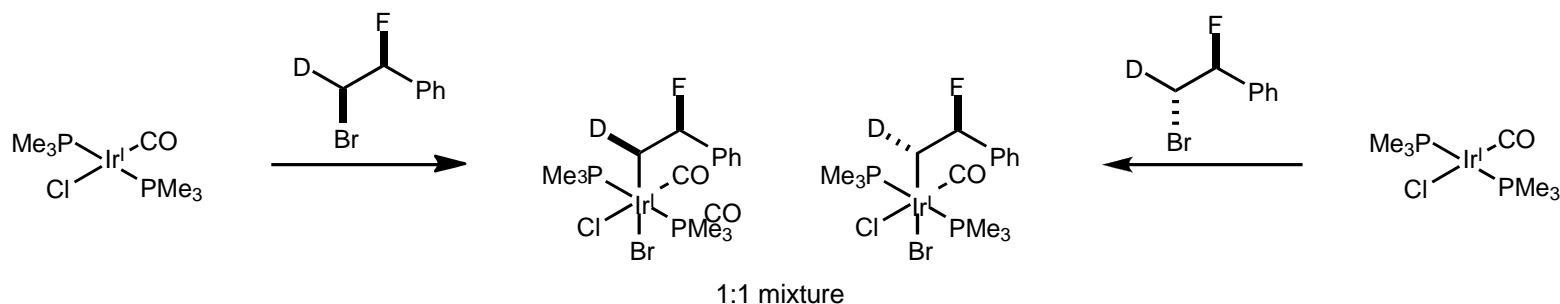
Labinger, J. A.; Osborn, J. A. Inorg. Chem. 1980, 19, 3230-3236.

- Cross-coupling of *endo*- and *exo*-2-norbornane leads to the same *exo* product



6% $NiI_2$

6% *trans*-2-aminocyclohexanol

NaHMDS (2 equiv.)
isopropanol, 60 ºC

84%

91%
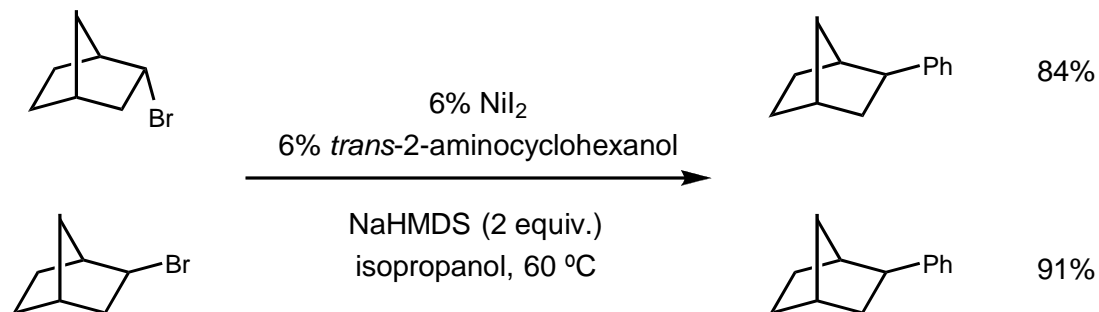
González-Bobes, F.; Fu, G. C. *J. Am. Chem. Soc.* **2006***, 5360-5361

- *Cis*- and *trans*-1,2-dichloroethylene give the same isomeric mixture of oxidative addition product



40:60 *cis:trans*
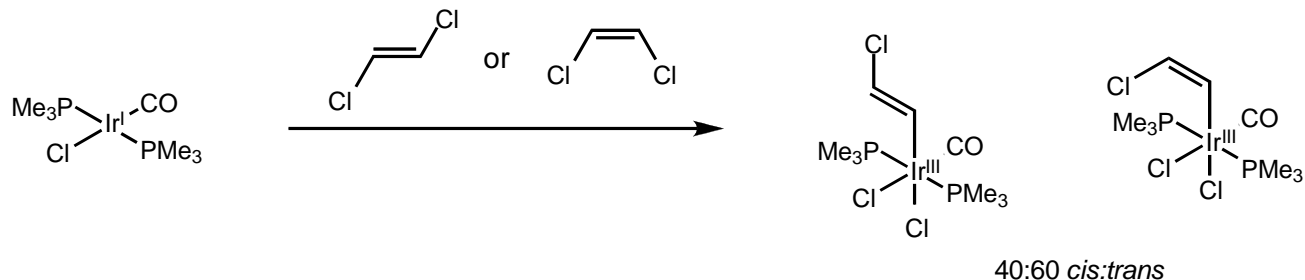
Labinger, J. A.; Osborn, J. A.; Coville, N. J. *Inorg. Chem.* **1980**, *19*, 3236-3243.

- ! Complete retention of configuration is observed in the oxidative addition to Pd(PPh₃)₄
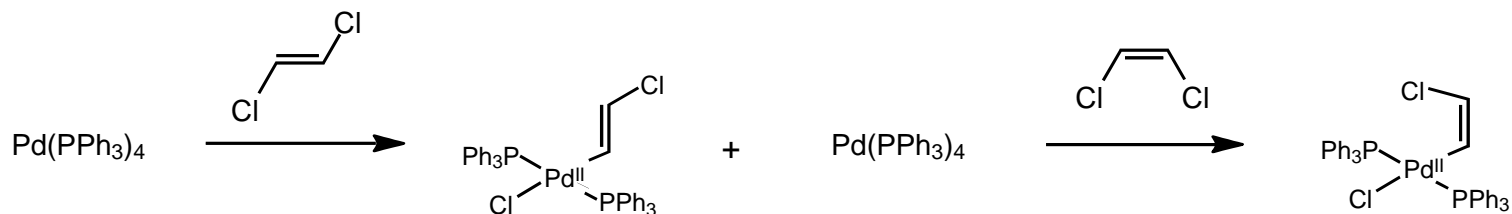


Fitton, P.; McKeon, J. E. *Chem. Commun.* **1968**, 4-6.

- Radical initiators promote the oxidative addition of alkyl halides to Ir(I) complexes



| initiator | conversion (%) | |
|---|---|---|
| | after 100 min | after 46 h |
| none | 11 | 36 |
| AIBN | 34 | 100 |
| benzoyl peroxide | | 100 |
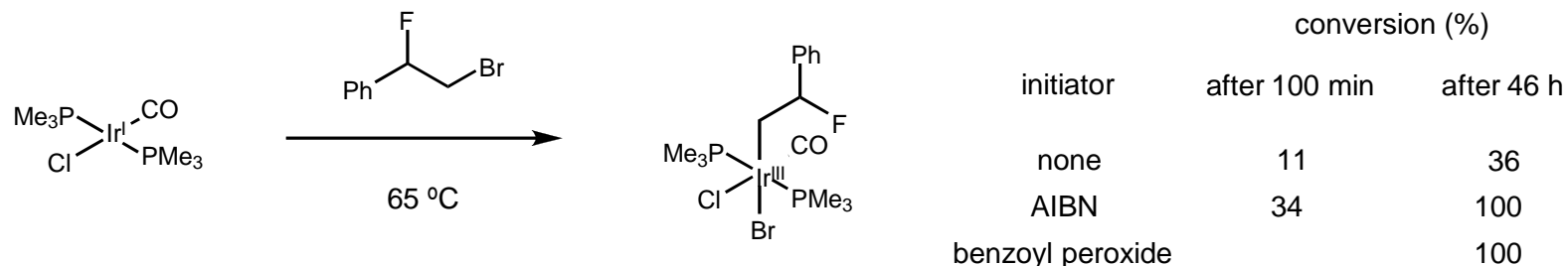
- Radical inhibitors depress the oxidative addition of alkyl halides to Ir(I) complexes
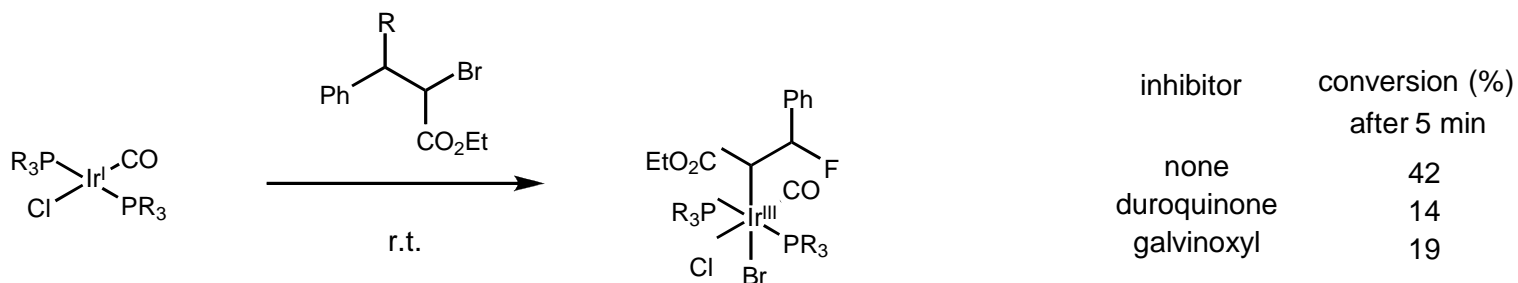


| inhibitor | conversion (%) after 5 min |
|---|---|
| none | 42 |
| duroquinone | 14 |
| galvinoxyl | 19 |

Labinger, J. A.; Osborn, J. A.; Coville, N. J. *Inorg. Chem.* **1980***, 19,* 3236-3243.

- Rate of reactivity of alkyl halides is consistent with radical process, inconsistent with $S_N2$

| RX | relative reactivity |
|---|---|
| n-BuBr | 1.0 (defined) |
| s-BuBr | 5.4 |
| t-BuBr | 6.8 |
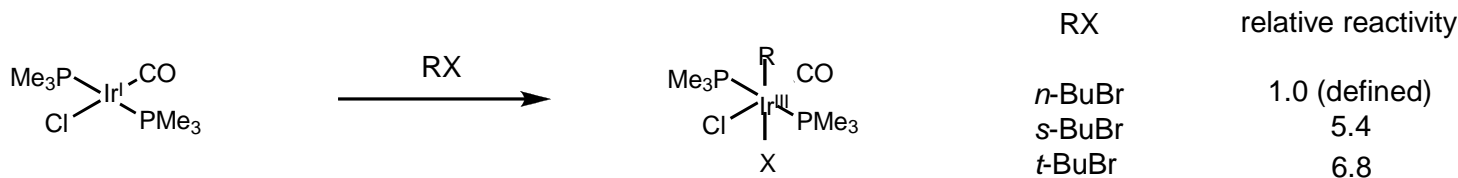
- Trapping of radical intermediates with acrylonitrile
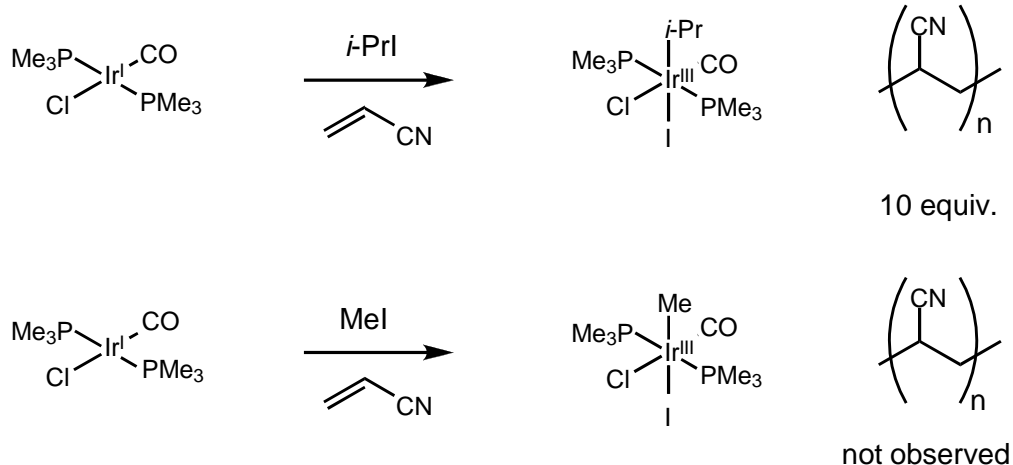
10 equiv.

not observed

Labinger, J. A.; Osborn, J. A.; Coville, N. J. *Inorg. Chem.* **1980***, 19,* 3236-3243

- Alkyl iodides bearing tethered alkenes undergo radical cyclization concomitant with oxidative addition



79%

R =
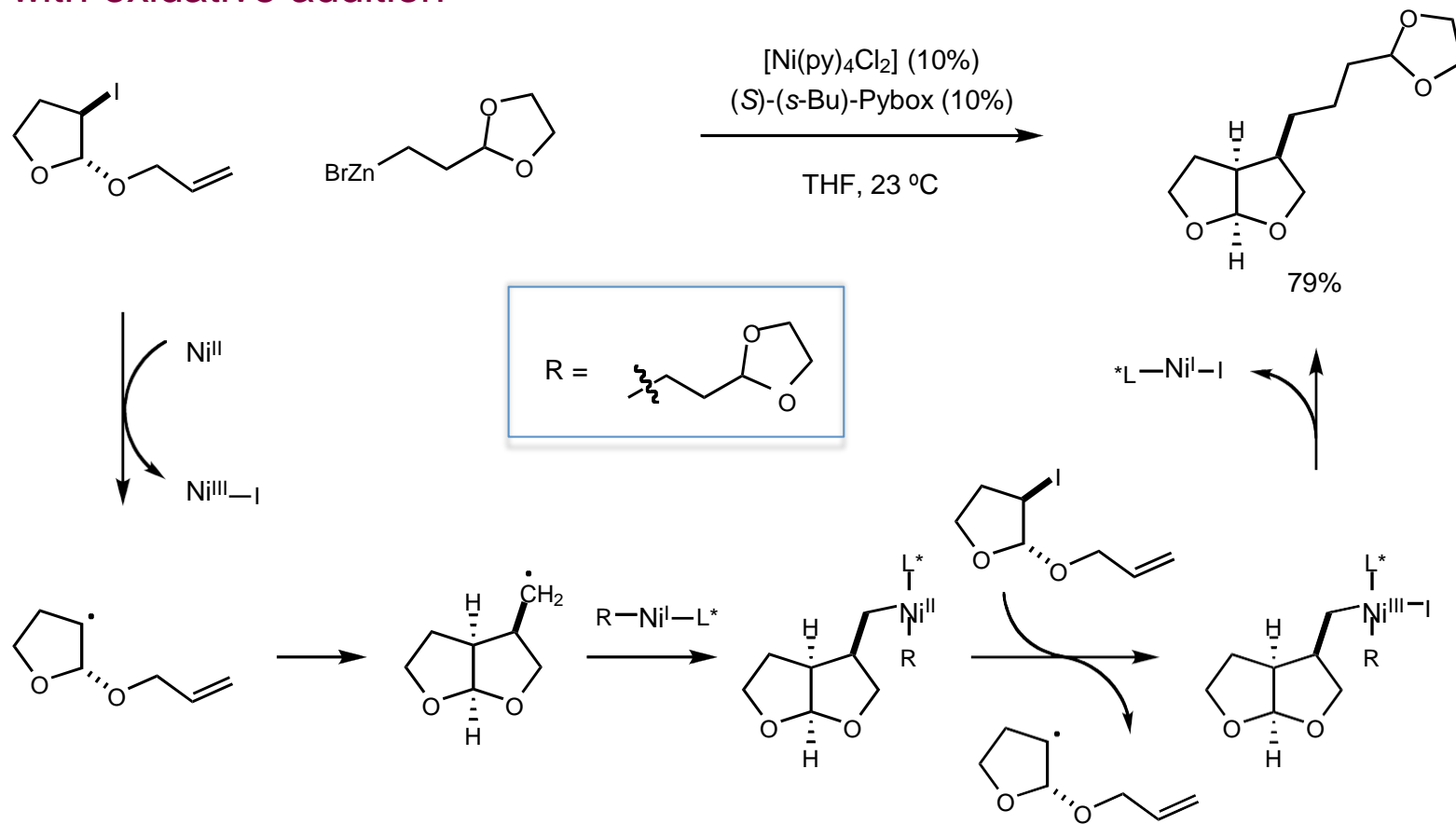
Phapale, V. B.; Buñuel, E.; García-Iglesias, M.; Cárdenas, D. J. *Angew. Chem. Int. Ed.* **2007***, 46,* 8790-8795.

# Oxidative Reactions with Manganese(III) Acetate

- Synthesis of $\gamma$-lactones by reaction of Mn(OAc)$_3$ with olefins



Mn(OAc)$_3$·2H$_2$O (2 equiv.)

HOAc, KOAc
135 °C, 1 h

60%

proposed intermediate:
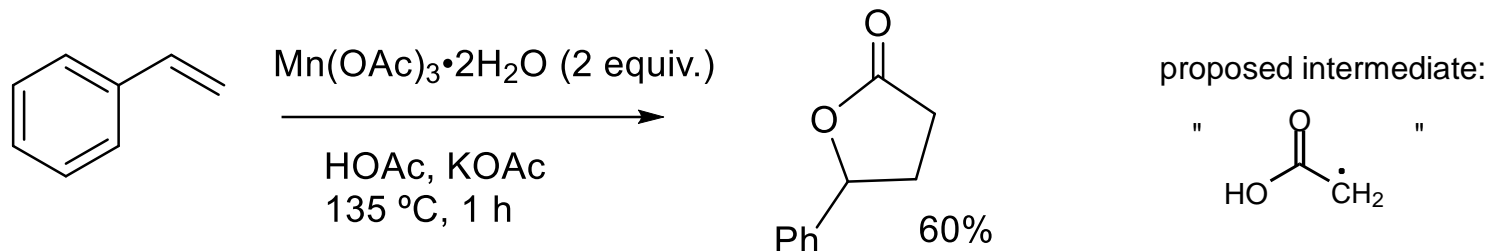
Heiba, E. I.; Dessau, R. M.; Koehl, W.J. *J. Am. Chem. Soc.* **1968**, *90,* 5905-5906.
Heiba, E. I.; Dessau, R. M.; Rodewald, P. G. *J. Am. Chem. Soc.* **1974**, *96*, 7977-7981.

- Key mechanistic question: Is the species that adds to the olefin a free or metal-complexed radical?

outer-sphere oxidation

styrene

inner-sphere oxidation

styrene

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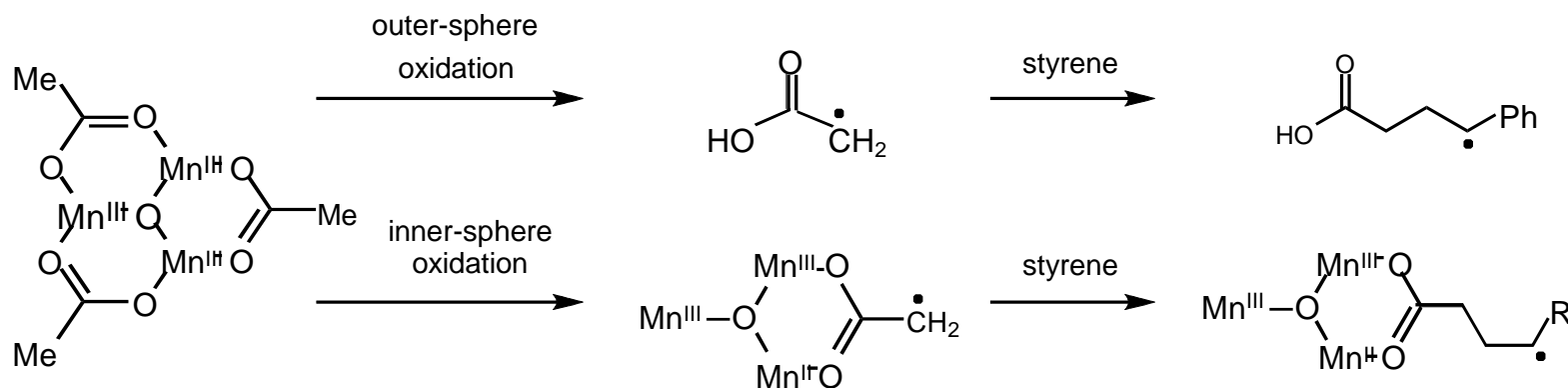
■ No polymerization of styrene observed

$$\text{styrene} \xrightarrow[\text{HOAc, Ac}_2\text{O} \;\; \text{reflux}]{\text{Mn(OAc)}_3\bullet 2\text{H}_2\text{O}} \text{no polymerization observed}$$

■ Acetate esters add to olefins upon initiation with ($t$-BuO)$_2$, but not Mn(OAc)$_3$•2H$_2$O

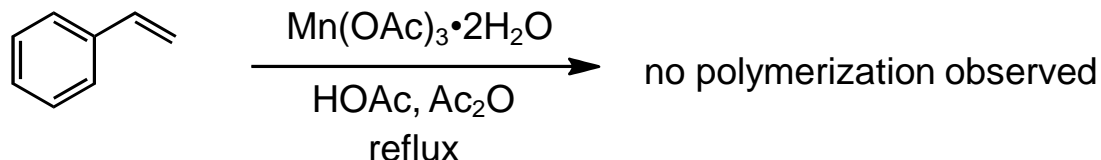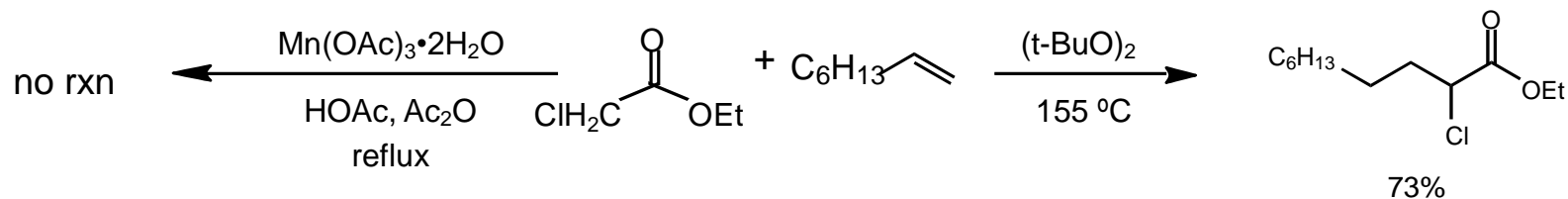$$\text{no rxn} \xleftarrow[\text{HOAc, Ac}_2\text{O} \;\; \text{reflux}]{\text{Mn(OAc)}_3\bullet 2\text{H}_2\text{O}} \text{ClH}_2\text{C}\text{—CO—OEt} + \text{C}_6\text{H}_{13}\text{—CH=CH}_2 \xrightarrow[155\;^\circ\text{C}]{(t\text{-BuO})_2} \text{product}$$

73%

Bush, J. B., Jr.; Finkbeiner, H. J. *J. Am. Chem. Soc.* **1968**, *90,* 5903-5905.
Allen, J. C.; Cadogan, J. I. G.; Hey, D. H. *J. Chem. Soc.* **1965**, 1918-1932

■ No dimerization of acetic acid radicals observed

$$\text{H}_3\text{C}\text{—CO—OH} \xrightarrow[\text{Ac}_2\text{O, reflux} \;\; \textit{no alkene}]{\text{Mn(OAc)}_3\bullet 2\text{H}_2\text{O}} \text{succinic acid} \quad \text{succinic acid not observed}$$

Fristad, W. E.; Peterson, J. R.; Ernst, A. B.; Urbi, G. B. *Tetrahedron.* **1986**, *42,* 3429-3442.

- H/D exchange experiments show no rate dependence on the metal

A: 0.25 M $Mn(OAc)_3 \cdot 2H_2O$
B: no metal

$$H_3C-C(=O)-OH \; + \; D_3C-C(=O)-OD \xrightarrow{\text{KOAc, reflux}} DH_2C-C(=O)-OD$$

$rate_A = rate_B$
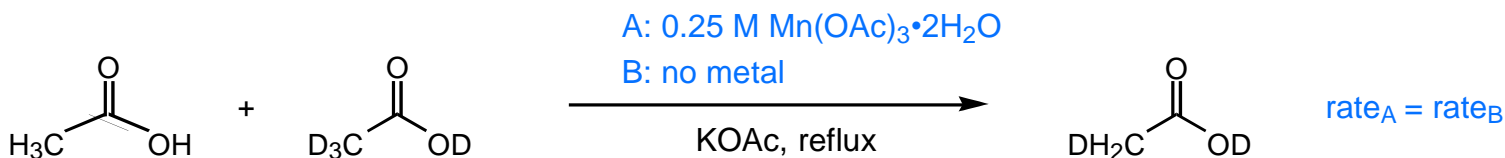
- $\gamma$-lactone formation exceeds total solution H/D exchange



□ Olefin    △ Lactone    ▽ Solvent Enolization

- Results are only consistent with rate-determining enolization of complexed acetate

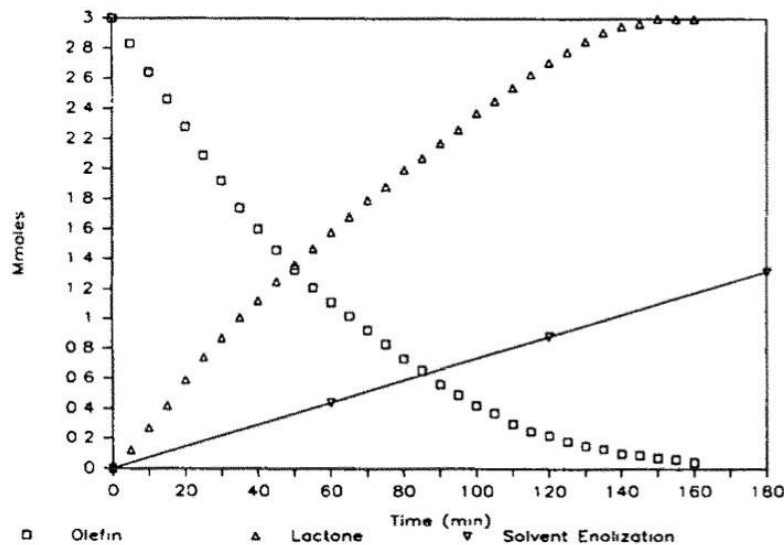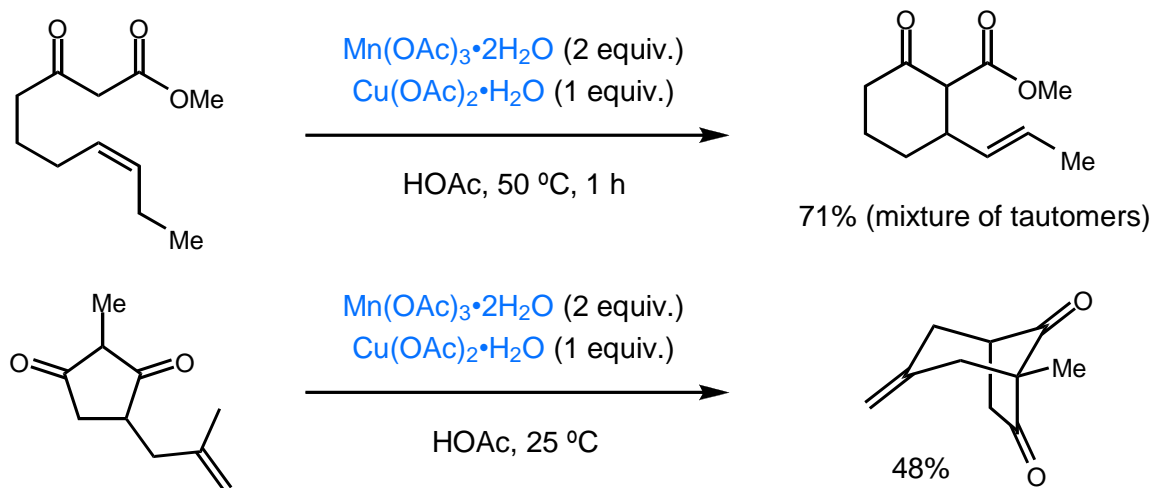Fristad, W. E.; Peterson, J. R.; Ernst, A. B.; Urbi, G. B. *Tetrahedron.* **1986**, *42,* 3429-3442.
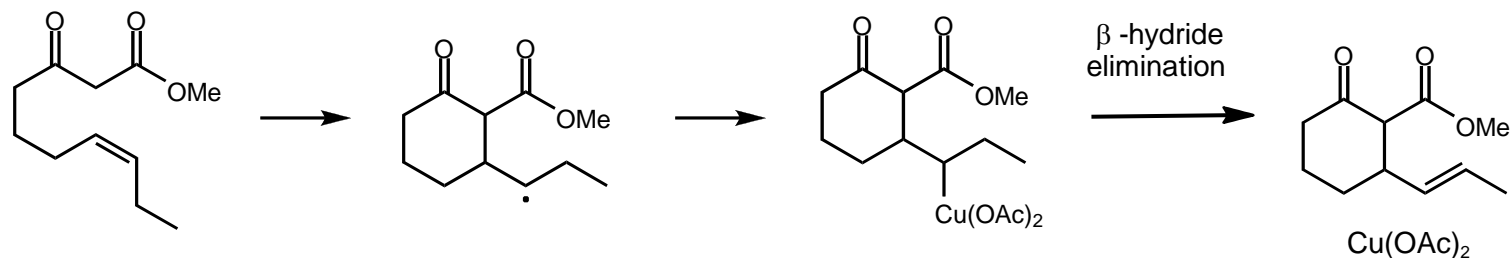
- Mn(OAc)$_3$ initiates radical cyclizations of $\beta$-keto esters and 1,3-diketones



Mn(OAc)$_3$•2H$_2$O (2 equiv.)
Cu(OAc)$_2$•H$_2$O (1 equiv.)

HOAc, 50 ºC, 1 h

71% (mixture of tautomers)

Mn(OAc)$_3$•2H$_2$O (2 equiv.)
Cu(OAc)$_2$•H$_2$O (1 equiv.)

HOAc, 25 ºC

48%

- Cu(OAc)$_2$ is used to oxidize the alkyl radical to the alkene



$\beta$-hydride elimination

Kates, S. A.; Dombroski, M. A.; Snider, B. B. *J. Org. Chem.* **1990**, *55*, 2427-2436

- Mn(OAc)$_3$ can initiate radical cascade cyclizations
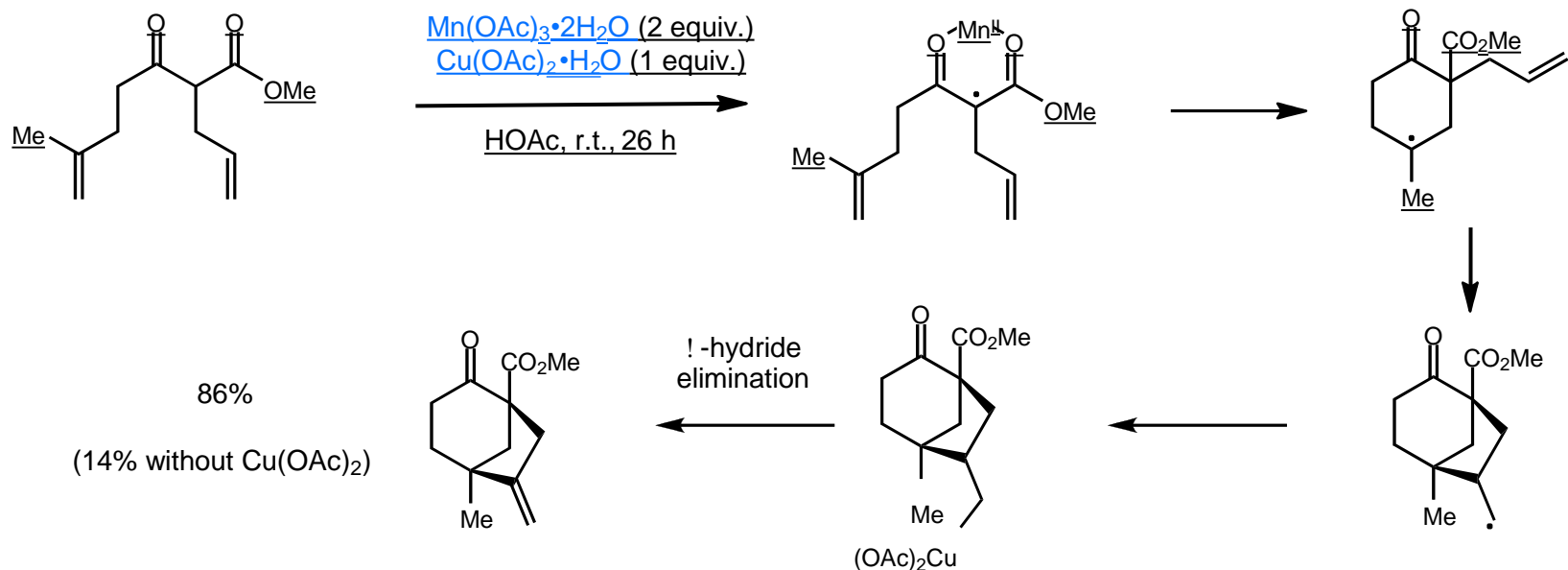
Dombroski, M. A.; Kates, S. A.; Snider, B. B. *J. Am. Chem. Soc.* **1990**, *112,* 2759-2767.
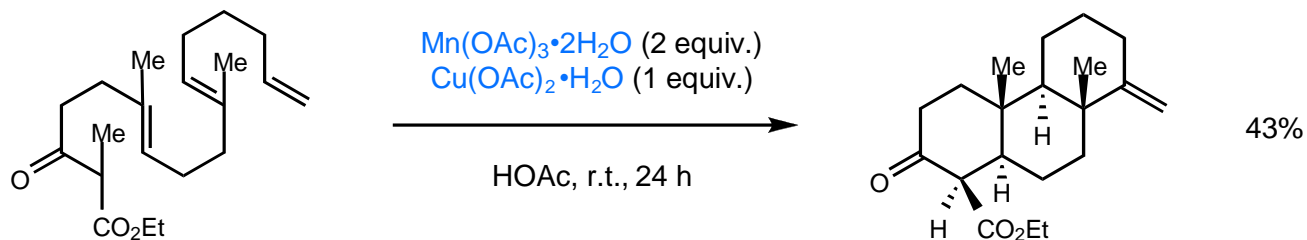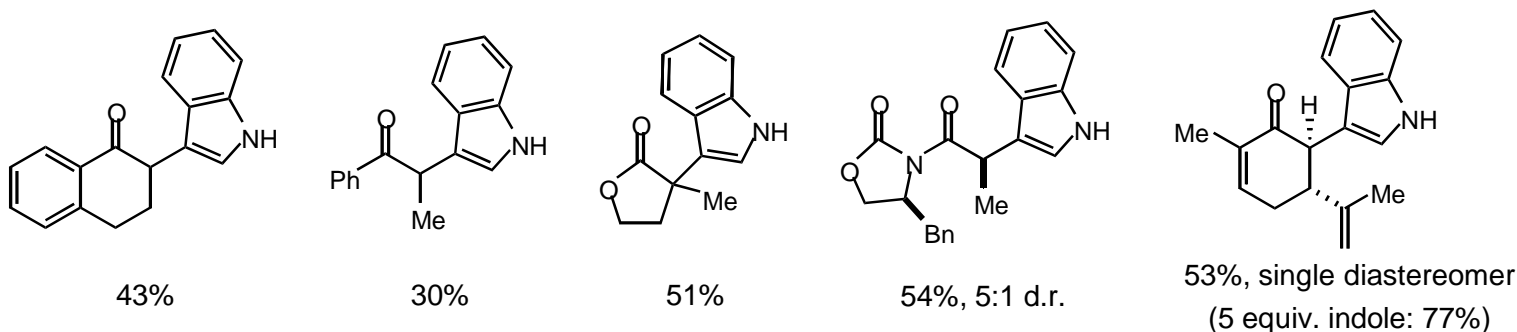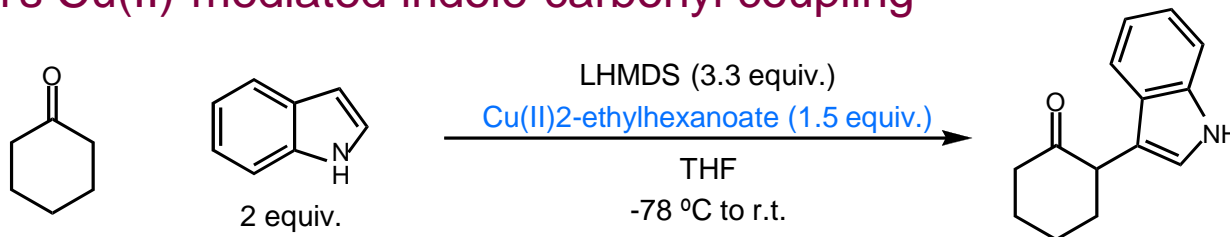
Zoretic, P. A.; Shen, Z.; Wang, M.; Riberio, A. A. *Tetrahedron Lett.* **1995**, *36,* 2925-2928.
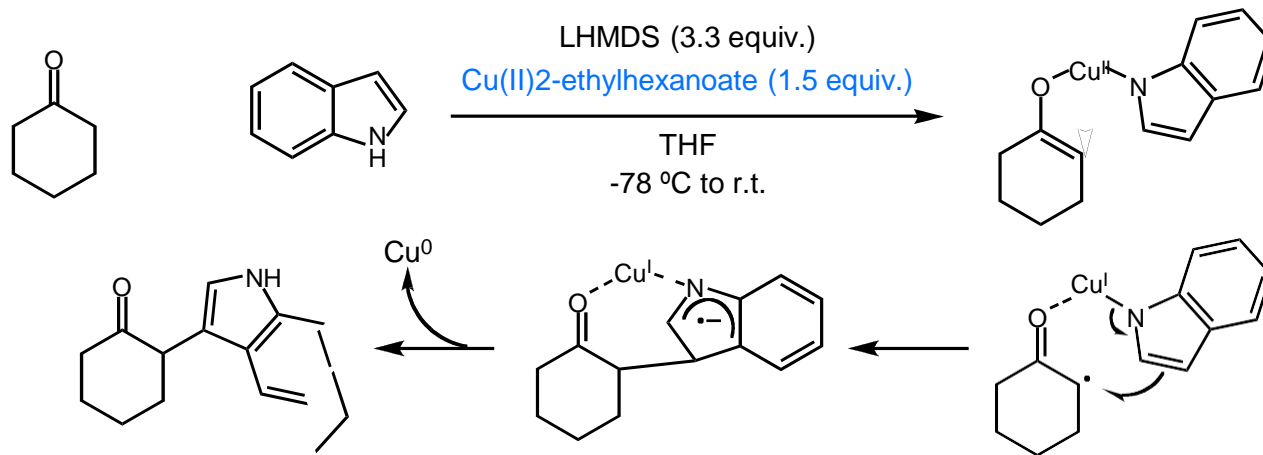
- **Baran's Cu(II)-mediated indole-carbonyl coupling**



43%  30%  51%  54%, 5:1 d.r.  53%, single diastereomer
(5 equiv. indole: 77%)

MECHANISM

- Free N-H is required for reactivity under copper conditions



LHMDS
Cu(II)2-ethylhexanoate

-78 ºC to r.t.
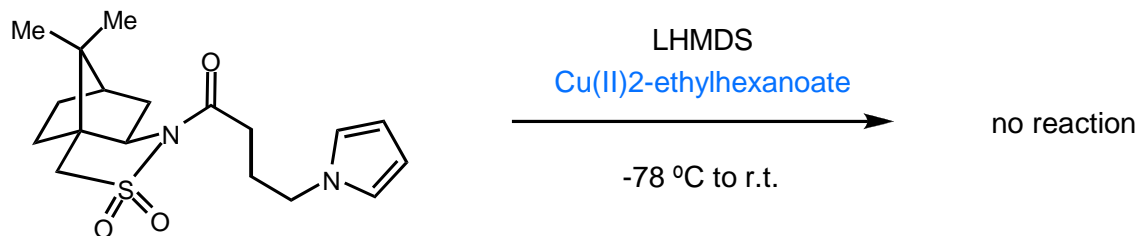
no reaction

- Reaction proceeds with a known outer-sphere oxidant



$Fe^+$  $PF_6^-$

LHMDS, NEt$_3$

discrete α-carbonyl radical

11%, 4.5:1 d.r.
(after benzoylation)
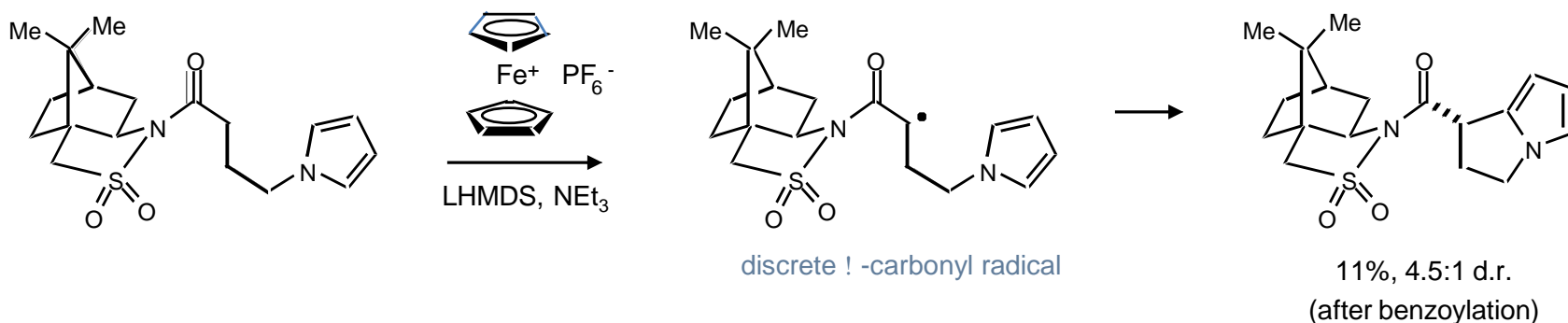
*implicates inner-sphere oxidation*
*via indole-bound copper enolate:*



Richter, J. M.; Whitefield, B. W.; Maimone, T. J.; Lin, D. W.; Castroviejo, M. P.; Baran, P. S. *J. Am. Chem. Soc.* **2007**, *129*, 12857-12869.
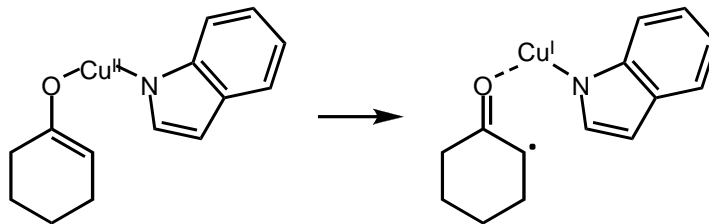
- Enables the synthesis of 1,4-dicarbonyl compounds via $\alpha$-carbonyl radicals

lithium amide base
$CuBr_2$ (2 equiv.)

85%
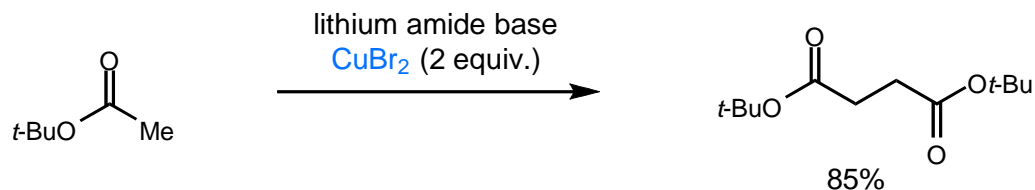
- Reaction does not proceed via formation of the $\alpha$-bromoester

10%
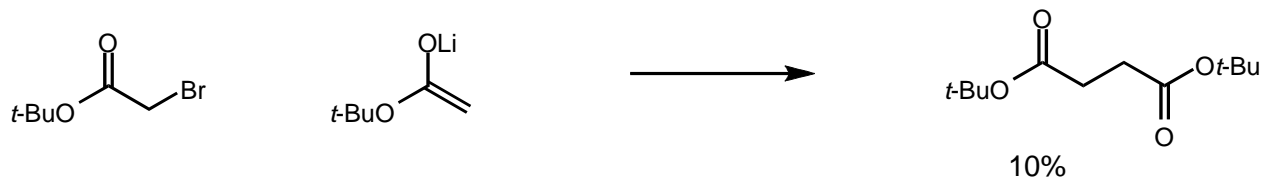
- Proposed to proceed via single-electron oxidation of enolate to α- carbonyl radical
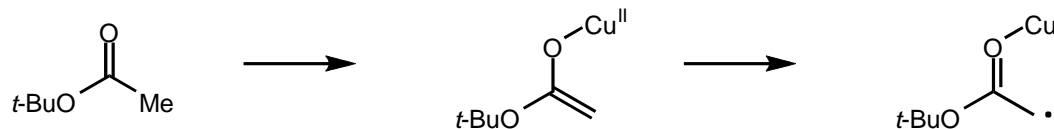
Rathke, M. W.; Lindert, A. J. *J. Am. Chem. Soc.* **1971**, *93*, 4605-4606.

- Heterocoupling can be achieved in the coupling of imides or amides with ketones or esters
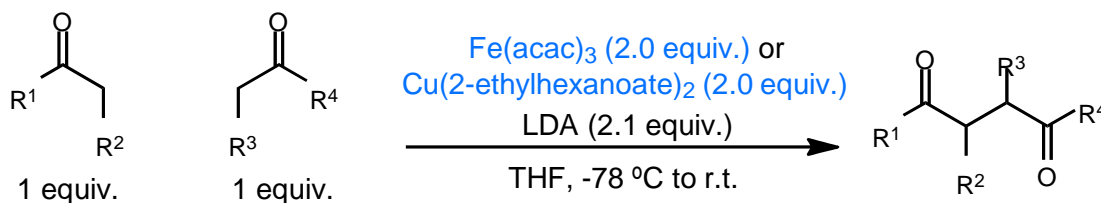


Baran, P. S.; DeMartino, M. P. *Angew. Chem. Int. Ed.* **2006**, *45*, 7083-7086.

DeMartino, M. P.; Chen, K.; Baran, P. S. *J. Am. Chem. Soc.* **2008**, *130,* 11546-11560

Baran, P. S.; DeMartino, M. P. *Angew. Chem. Int. Ed.* **2006**, *45*, 7083-7086.

DeMartino, M. P.; Chen, K.; Baran, P. S. *J. Am. Chem. Soc.* **2008***, 130,* 11546-11560.
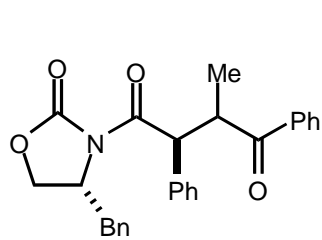
Fe(III): 57%, 1.8:1 d.r.
Cu(II): 55%, 1:1.6 d.r.
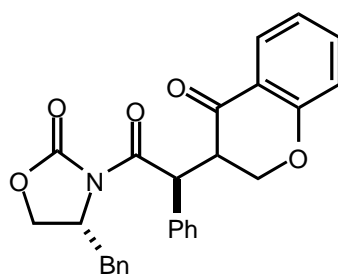
Fe(III): 60%, 2.6:1 d.r.
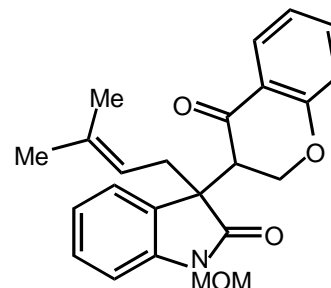
Fe(III): 73%, 2:1 d.r.
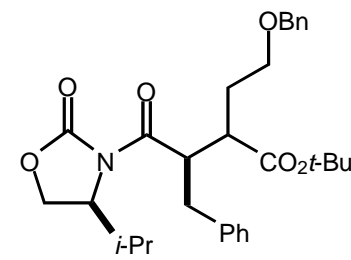
Cu(II): 62%, 1.2:1 d.r.
(1.75 equiv. ester)

MECHANISM
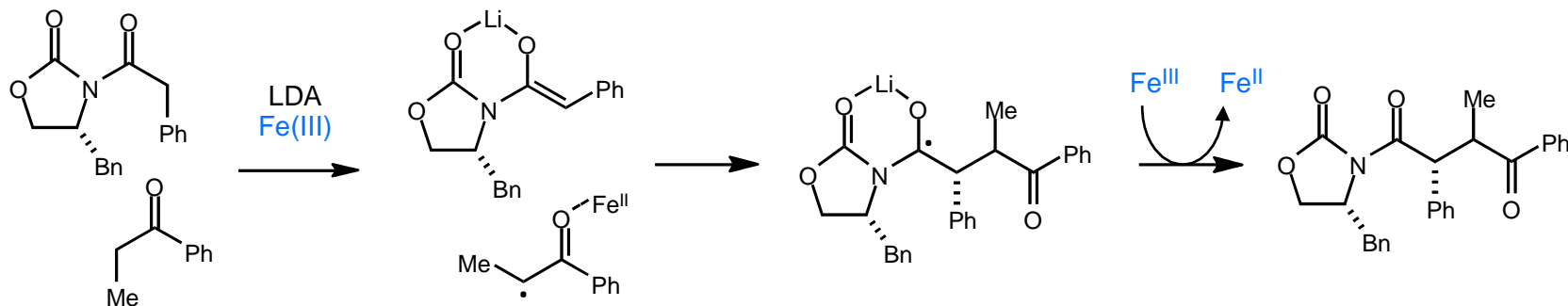
- Fe(III) and Cu(II) show divergent reactivity in cyclopropane radical clock studies
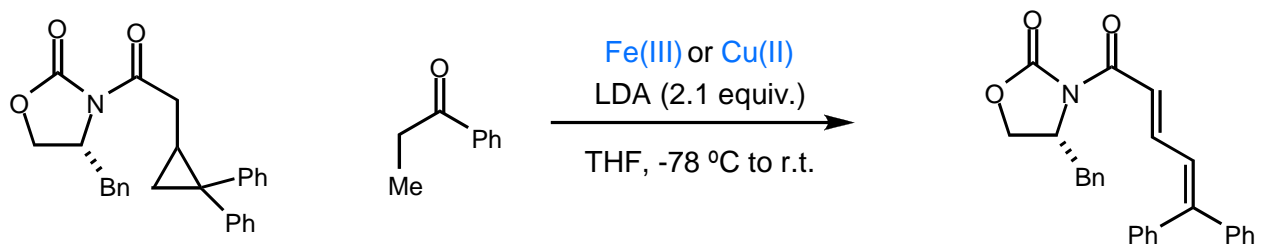


Fe(III): trace
Cu(II): 20% (mixture
of ring-opened products)
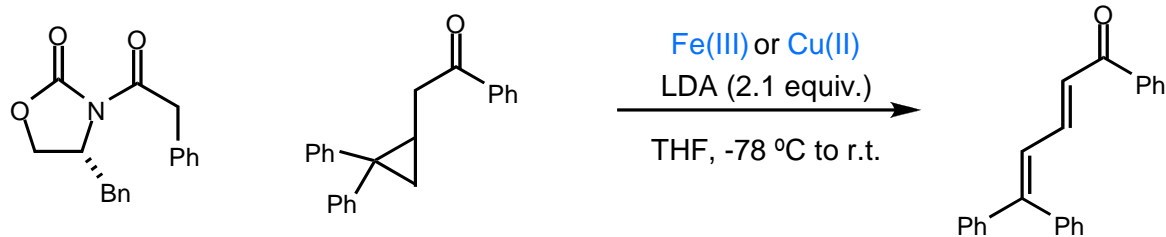


Fe(III): trace
Cu(II): 14%

- Fe(III) and Cu(II) promote cyclization with tethered olefins
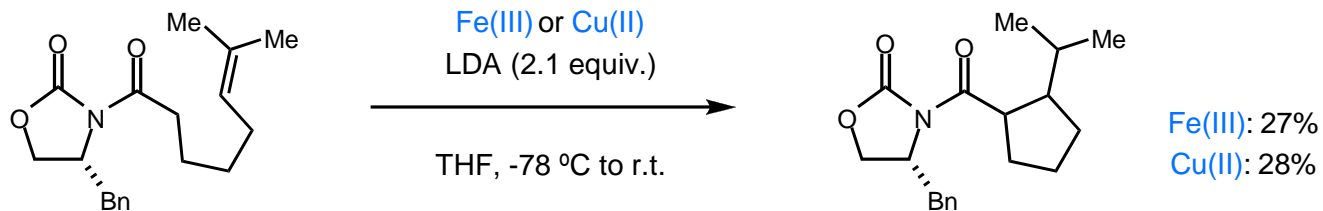


Fe(III): 27%
Cu(II): 28%

- Racemic starting material is converted to single enantiomer via radical intermediate
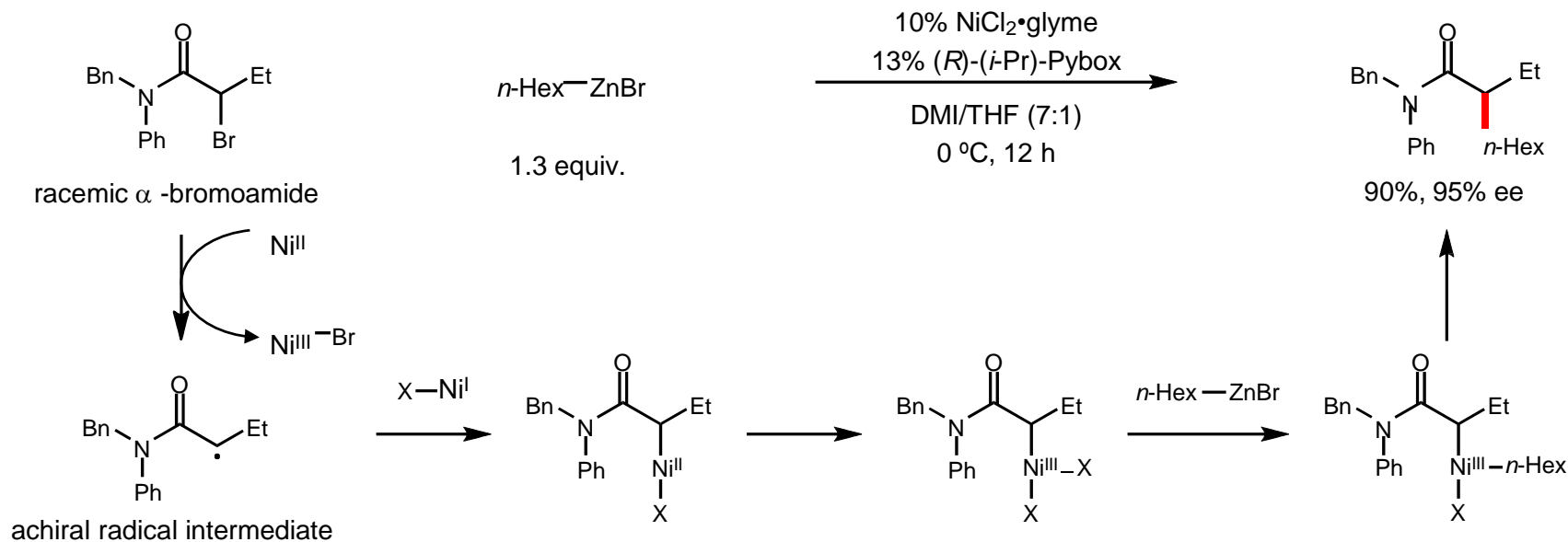


Fischer, C. F.; Fu, G. C. *J. Am. Chem. Soc.* **2005**, *127*, 4594-4595
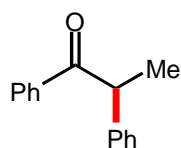


81%, 92%

! -bromoketones
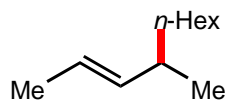
95%, 87% ee

secondary allylic chlorides
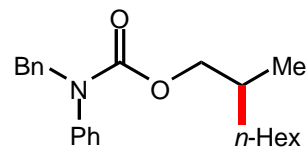
80%, 94% ee

acylated halohydrins
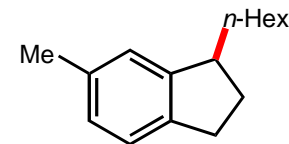
89%, 96% ee

secondary benzylic halides

*Angew. Chem. Int. Ed.* **2009**, *48,* 154-156.
*J. Am. Chem. Soc.* **2010**, *132*, 1264-1266.

*J. Am. Chem. Soc.* **2008**, *130,* 2756-2757.

*J. Am. Chem. Soc.* **2010**, *132,* 11908-11909.

*J. Am. Chem. Soc.* **2005**, *127,* 10482-10483.

- A copper/chiral diamine catalyst controls the dimerization of 2-naphthol derivatives



85%, 93% ee

enolization and catalyst release
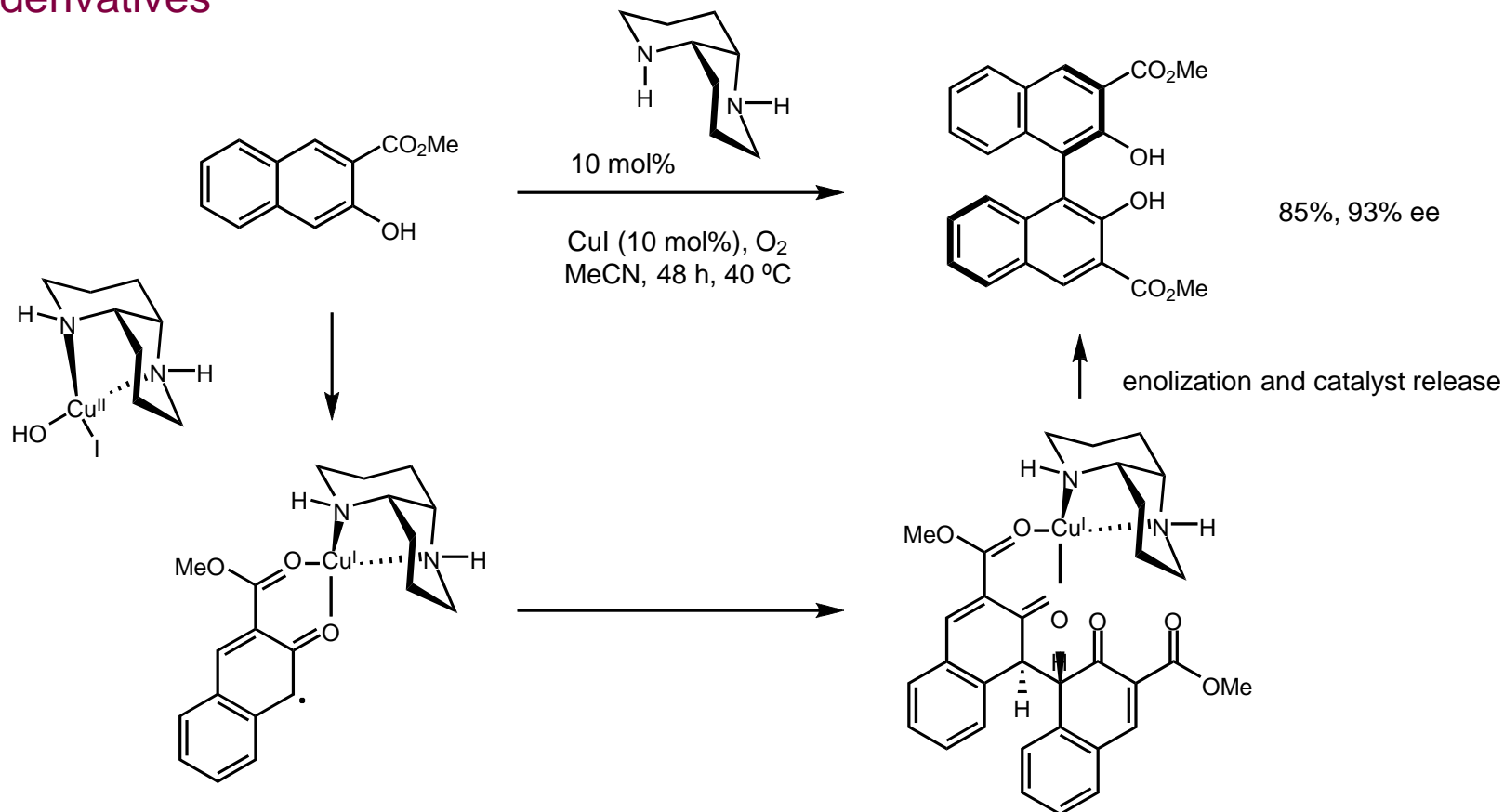
Li, X.; Yang, J.; Kozlowski, M. C. *Org. Lett.* **2001**, *3*, 1137-1140

Hewgley, J. B.; Stahl, S. S.; Kozlowski, M. C. *J. Am. Chem. Soc.* **2008**, *130*, 12232-12233.

- Homolysis of cobalt-carbon bond generates carbon-centered radical capable of performing catalysis

5'-deoxyadenosyl radical
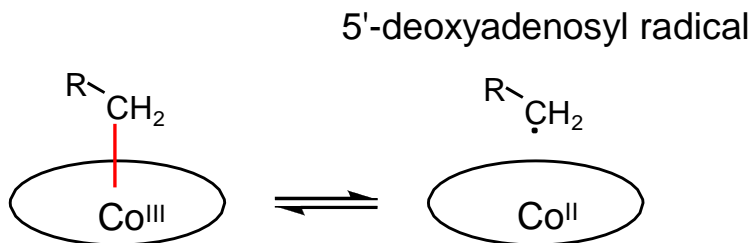
BDE = 30 kcal·mol$^{-q}$

"latent radical reservoir"

Buckel, W.; Golding, B. T. *Annu. Rev. Microbiol.* **2006**, *60,* 27-49.
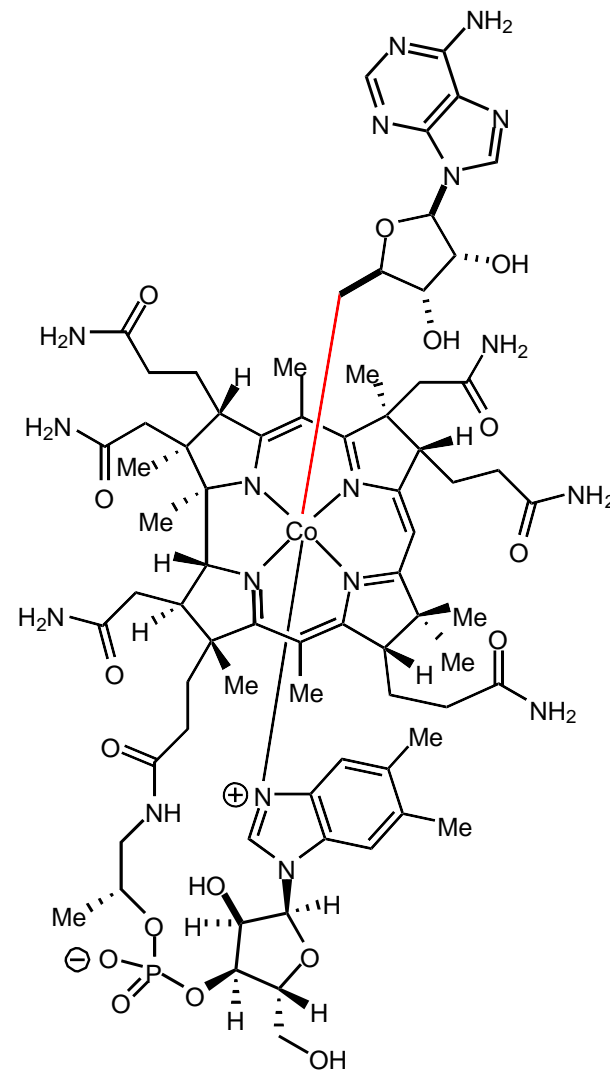
- Glutamate mutase converts (*S*)-glutamate to (2*S*,3*S*)-3-methylaspartate
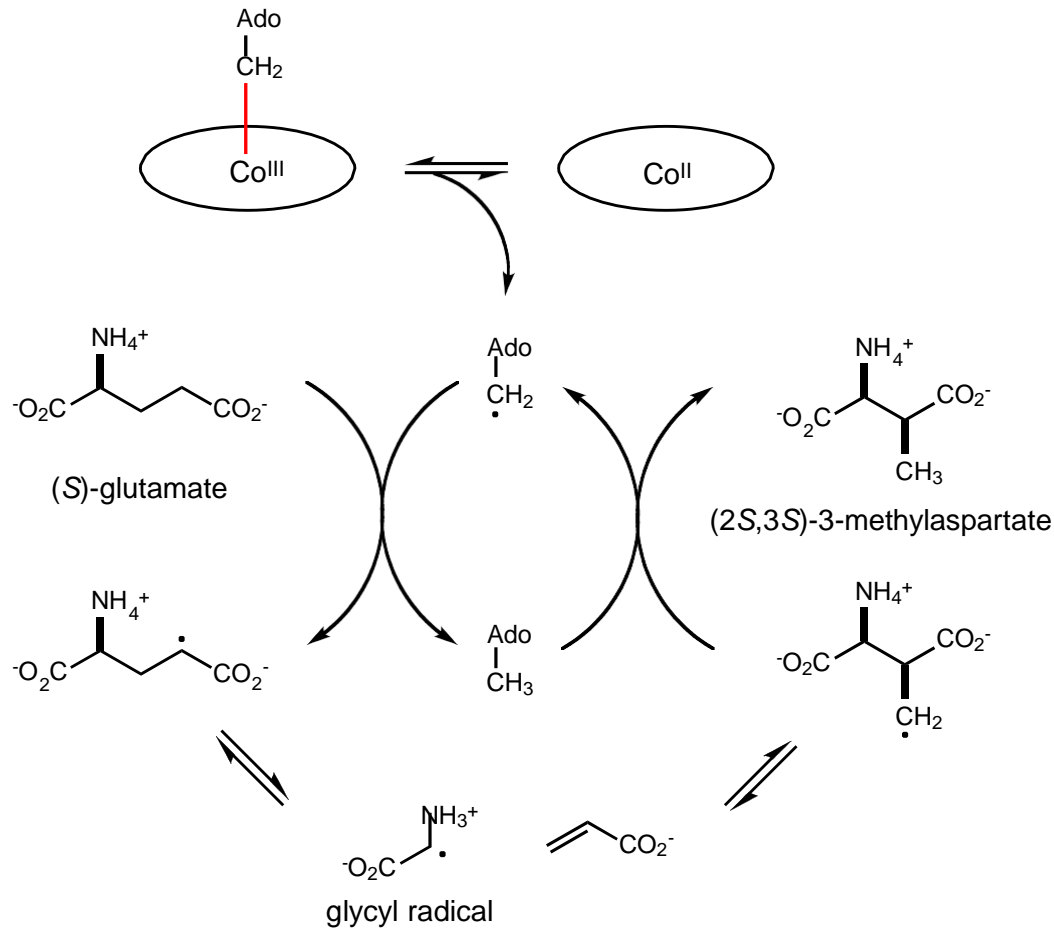


Buckel, W.; Golding, B. T. *Annu. Rev. Microbiol.* **2006***, 60,* 27-49.

- Methylmalonyl CoA mutase converts L-methylmalonyl CoA to succinyl CoA
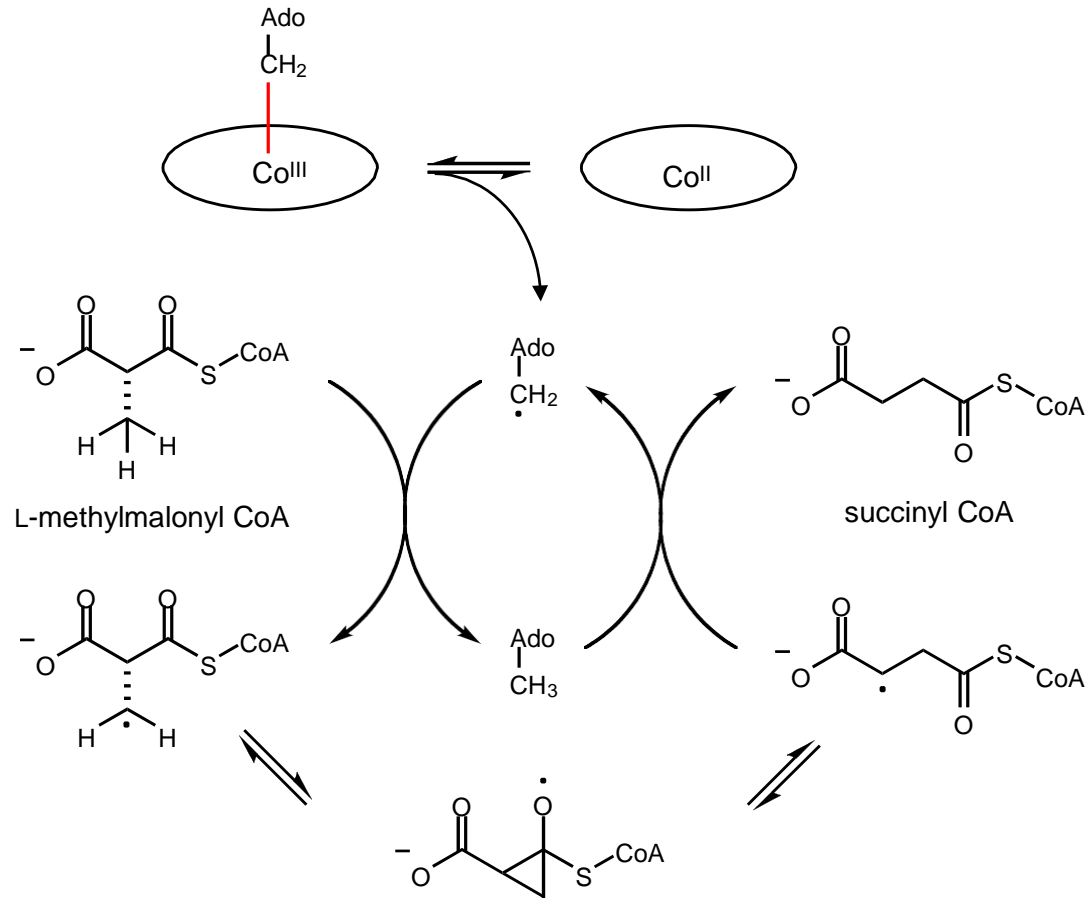


L-methylmalonyl CoA

succinyl CoA

Banerjee, R.; Ragsdale, S. W. *Annu. Rev. Biochem.* **2003**, *72,* 209-247.

- D-ornithine aminomutase converts D-ornithine to (2*R*,4*S*)-2,4-diamino-pentanoic acid



R = pyridoxal phosphate

D-ornithine

2,4-diaminopentanoic acid
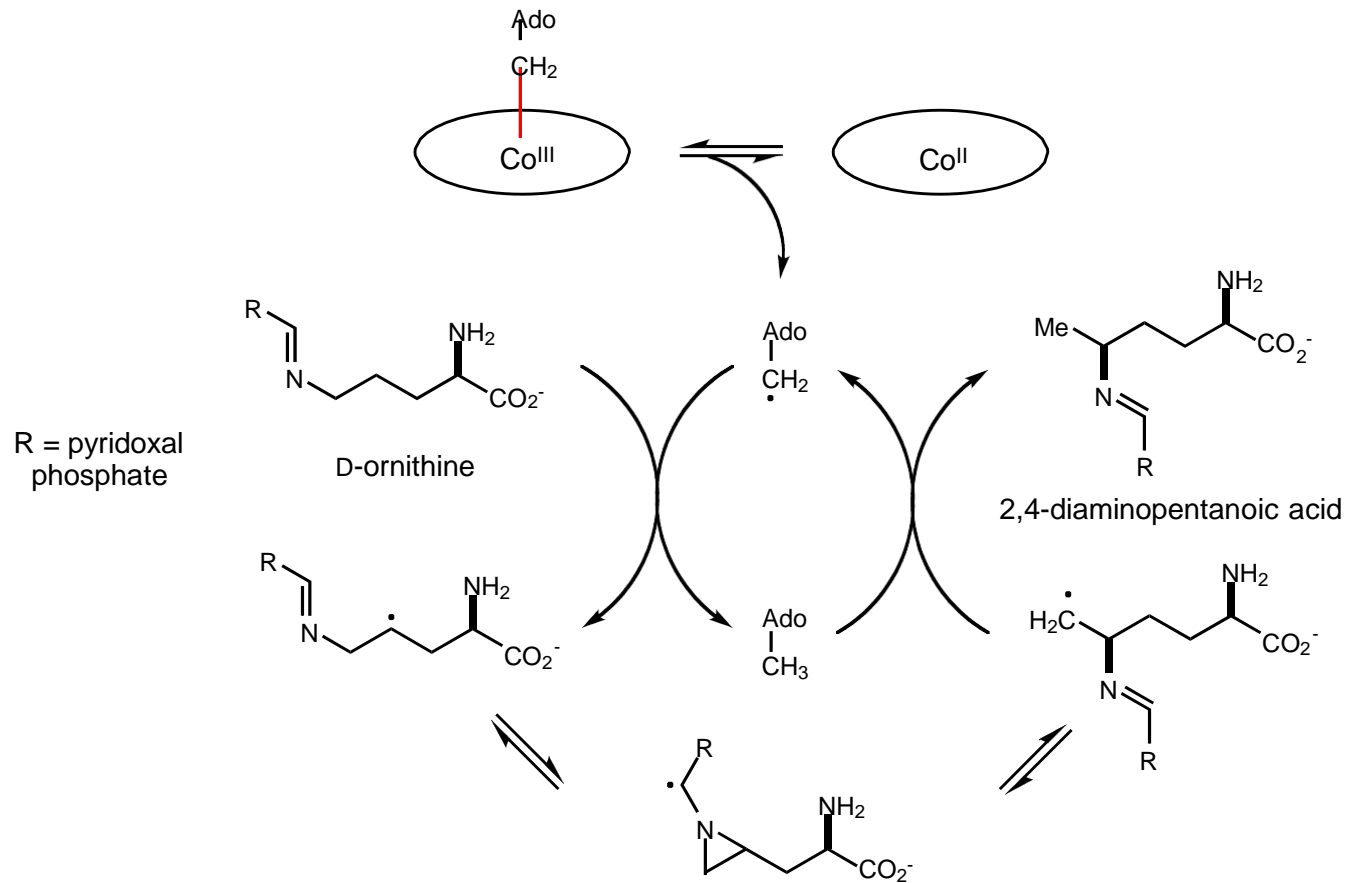
Banerjee, R.; Ragsdale, S. W. *Annu. Rev. Biochem.* **2003**, *72,* 209-247.
Chen, H.-P.; Wu, S.-H.; Lin, Y.-L.; Chen, C.-M. *J. Biol. Chem.* **2001**, *276*, 44744-44750.
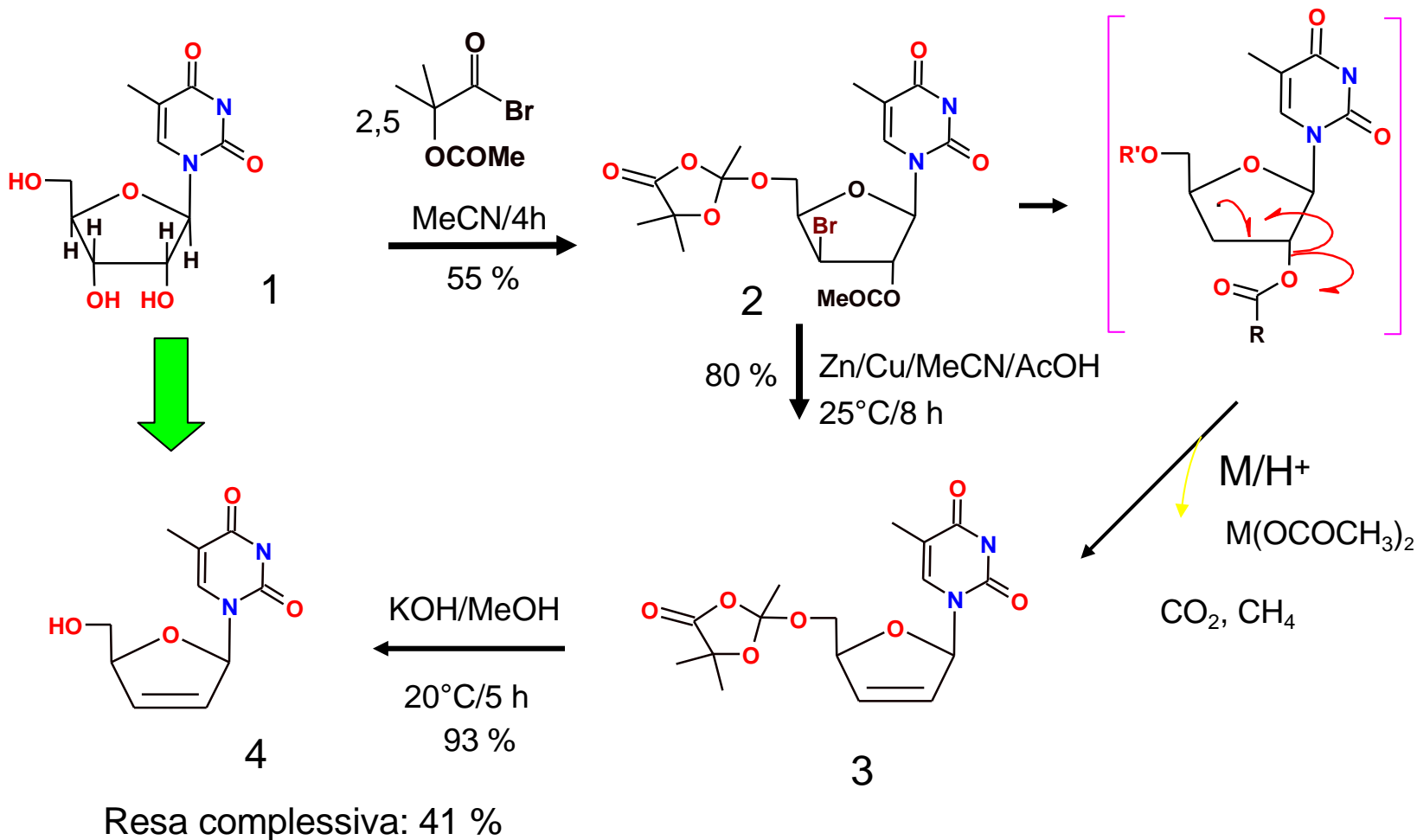
2,5

MeCN/4h

55 %

1

2

80 %  Zn/Cu/MeCN/AcOH
25°C/8 h

M/H$^+$

M(OCOCH$_3$)$_2$

CO$_2$, CH$_4$

KOH/MeOH

20°C/5 h
93 %

4

3

Resa complessiva: 41 %

70-95 %

1,2-shift
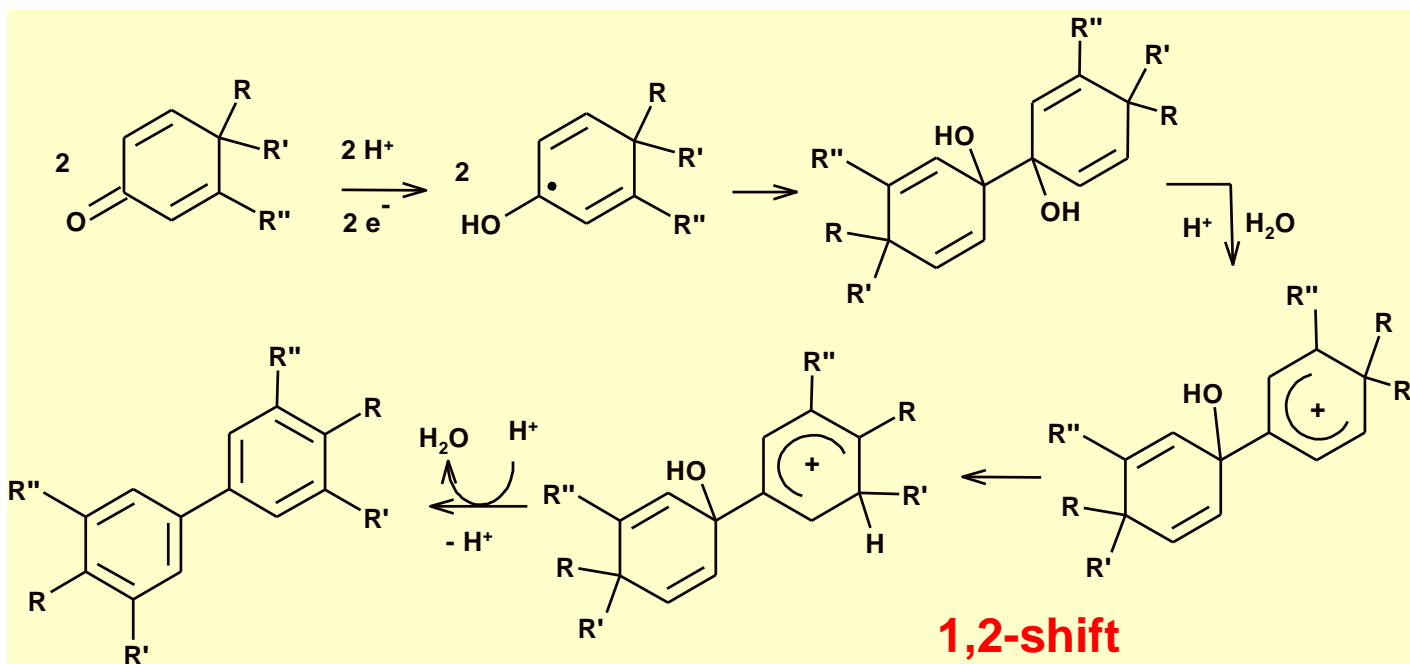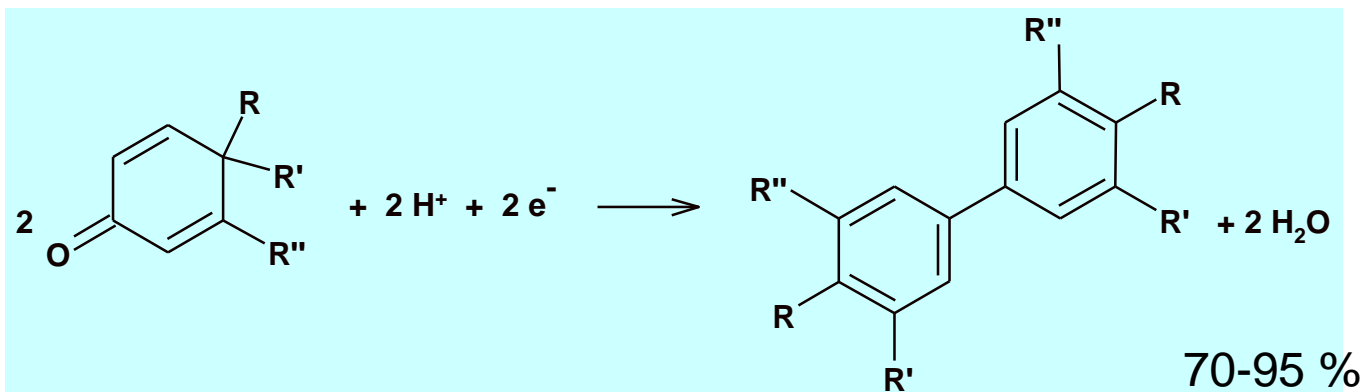
# Iron and Free Radical Chemistry: Reactions

Prof. Attilio Citterio

Dipartimento CMIC "Giulio Natta"

Iron is an integral biological cofactor for many proteins: heme moieties, iron-sulfur clusters, di-iron centers, etc..

The chemistry of iron in the generation of free and complexed radicals is quite versatile, and has been exploited from a synthetic point of view.

Iron presents a significant paradox to the field of oxygen radicals in biology.  It is required for many cellular functions, yet it can also catalyze the deleterious oxidation of biomolecules.

Examples of the extensive use of iron in biological systems, all of which are controlled or mediated by chelation, are as follows:

- redox chemistry involved in simple electron-transfer reactions;

- redox chemistry involved in reactions with $O_2$, ranging from $O_2$ transport and storage to $O_2$ reduction by cytochrome oxidase, and O atom insertion catalyzed by cytochrome P450; and

- substrate activation by the electrophilic behavior of iron;  for example, hydrolase enzymes such as purple acid phosphatase.

# Iron Electronic Configuration: $3d^6 4s^2$

- *Minerals* :

  Hematite ($Fe_2O_3$), siderite ($FeCO_3$), pyrite ($FeS_2$), magnetite ($Fe_3O_4$), limonite ($Fe_2O_3 \cdot 3H_2O$).

- *Properties of element* :

  Structural soft metal, used in alloy with Carbon and other Metals (steel), easily rusting.  (4th for abundance)

- *Oxidation states* :

  (- 2, - 1, 0, + 1) very common

  + 2, + 3,  + 4 e + 5 , + 6

- *Presence in water* :

  Ions $Fe^{2+}$ ($3d^6$, green, r = 0.78 Å) and $Fe^{3+}$ ($3d^5$, yellow, r = 0.645 Å) in acids, and hydroxides in bases.
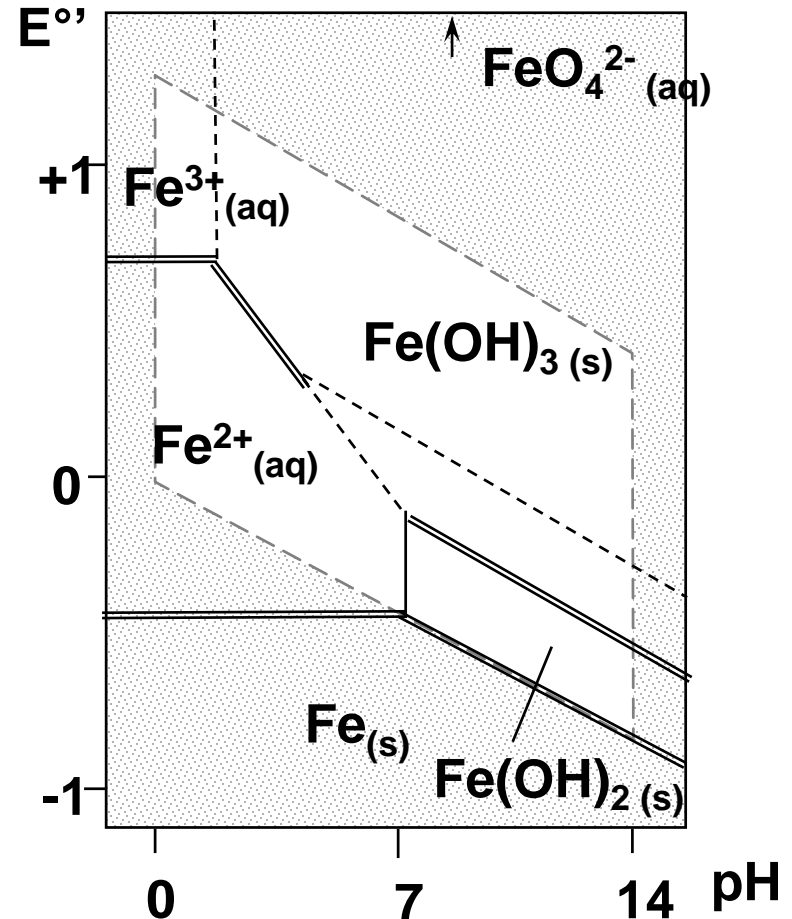


*Eh-pH diagram in water solution (T = 25°C, $10^{-4}$M)*

The $[Fe(H_2O_6]^{2+}$ ion is present in the Mohr $(NH_4)_2[Fe(H_2O)_6]_2SO_4$ salt, stable to $O_2$, used in volumetric analysis and in magnetic measurements. $FeSO_4 \cdot 7H_2O$ is efflorescent and becomes dark yellow by oxidation.

Addition of $HCO_3^-$ or $SH^-$ to aqueous solutions of $Fe^{2+}$ allows to precipitate $FeCO_3$ and FeS. The Fe(II)-Fe(III) couple show a remarkable effect of ligands and solvents on the relative stability of oxidation states :

$$[Fe(Oxquin)_3] + e^- = [Fe(Oxquin)_2] + oxquin.^- \qquad E° = -0.30\ V \qquad \textit{Fe(II) red.}$$

$$[Fe(EDTA)]^- + e^- = [Fe(EDTA)]^{2-} \qquad E° = -0.12\ V$$

$$[Fe(C_2O_4)_3]^{3-} + e^- = [Fe(C_2O_4)_2]^{2-} + C_2O_4^{2-} \qquad E° = +0.02\ V$$

$$[Fe(CN)_6]^{3-} + e^- = [Fe(CN)_6]^{4-} \qquad E° = +0.36\ V$$

$$[Fe(H_2O)_6]^{3+} + e^- = [Fe(H_2O)_6]^{2+} \qquad E° = +0.77\ V$$

$$[Fe(Bipy)_3]^{3+} + e^- = [Fe(Bipy)_3]^{2+} \qquad E° = +0.96\ V$$

$$[Fe(phen)_3]^{3+} + e^- = [Fe(phen)_3]^{2+} \qquad E° = +1.12\ V \qquad \textit{Fe(III) ox.}$$
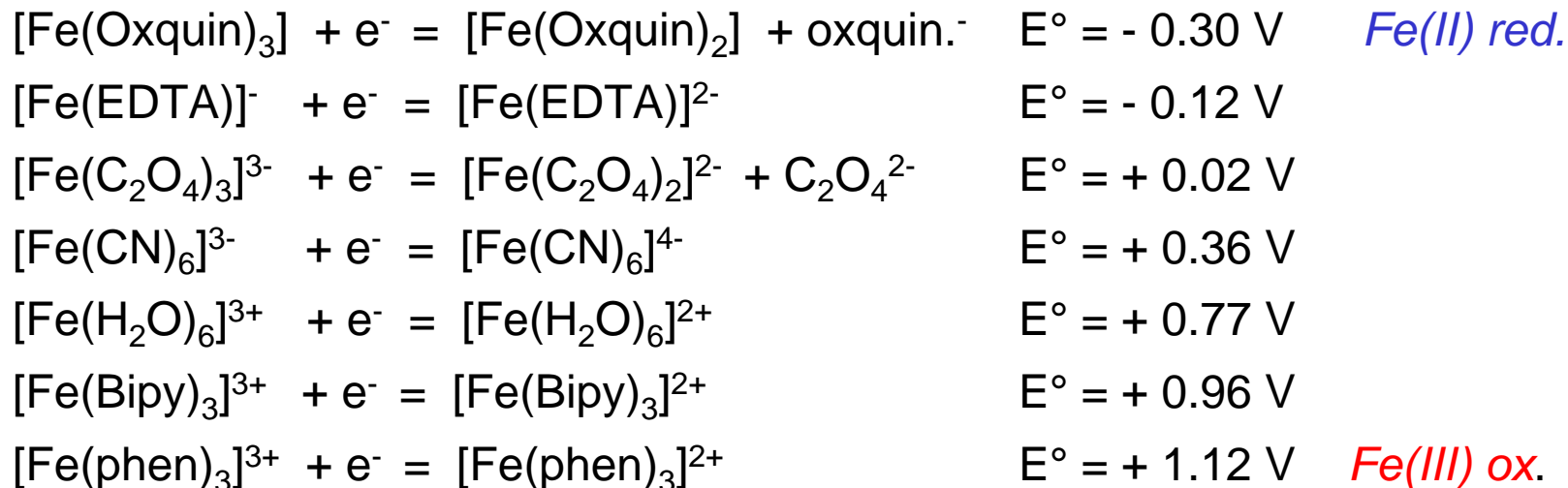
Miller, D.M., Buettner, G.R., and Aust, S.D. (1990) *Free Radic. Biol. Med.* **8**, 95-108.

## Iron metabolism:

Recommended dietary allowance 10-15 mg

10-50 mg in the diet (only 10-15% is normally absorbed)

## Iron distribution:

|  | g | % |
|---|---|---|
| Hemoglobin | 2.5 | 68 |
| Myoglobin | 0.15 | 4 |
| Transferrin | 0.003 | 0.1 |
| Ferritin, tissue | 1.0 | 27 |
| Ferritin, serum | 0.0001 | 0.004 |
| Enzymes | 0.02 | 0.6 |
| **Total** | **3.7** | **100** |

- Heme = $Fe^{2+}$ surrounded by porphyrin group, four N act as ligands.

- As $O_2$ carrier:  $O_2$ binds to $Fe^{2+}$ as a ligand

- Reversible process

- CO and $CN^-$ bind irreversible to $Fe^{2+}$
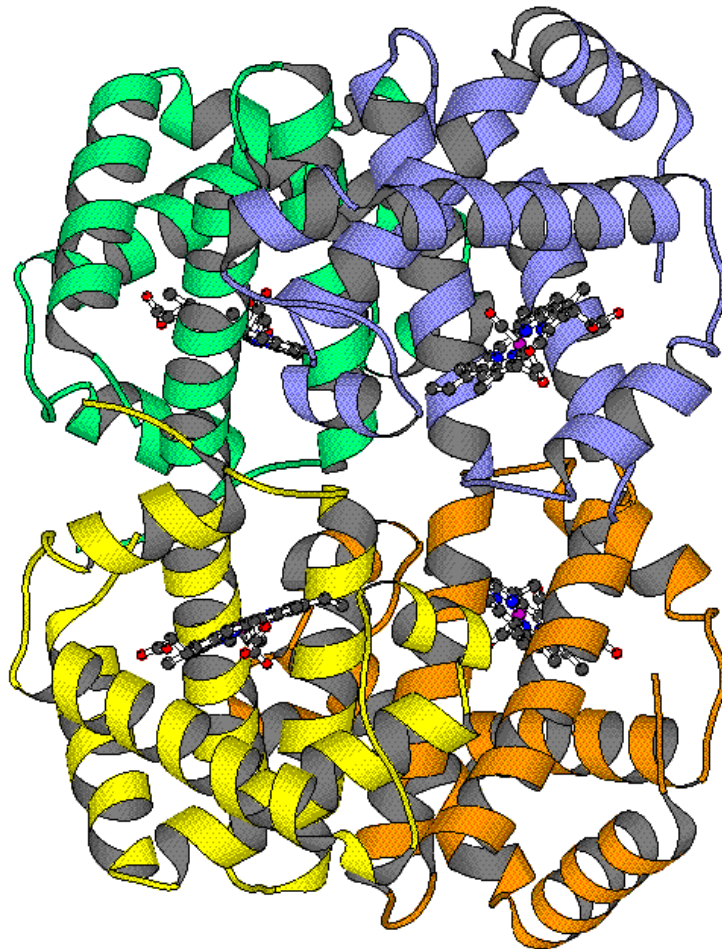
Tetrameric structure of **hemoglobin**: 4 heme prosthetic groups

Easy
to
reduce

$E^o$
volts

n+

L  L  L  **Fe**  L  L  L
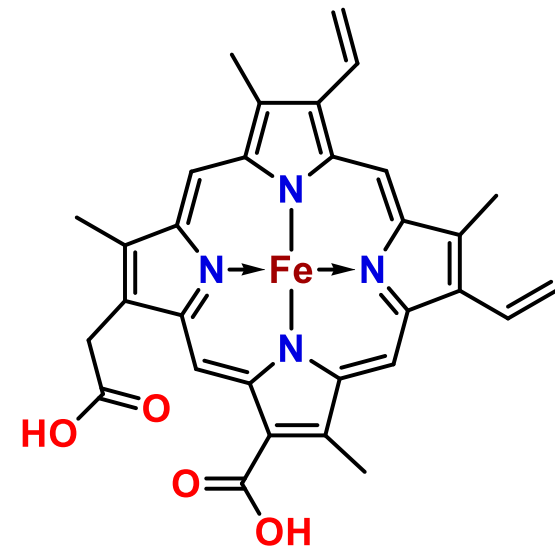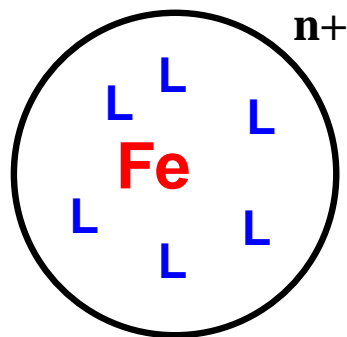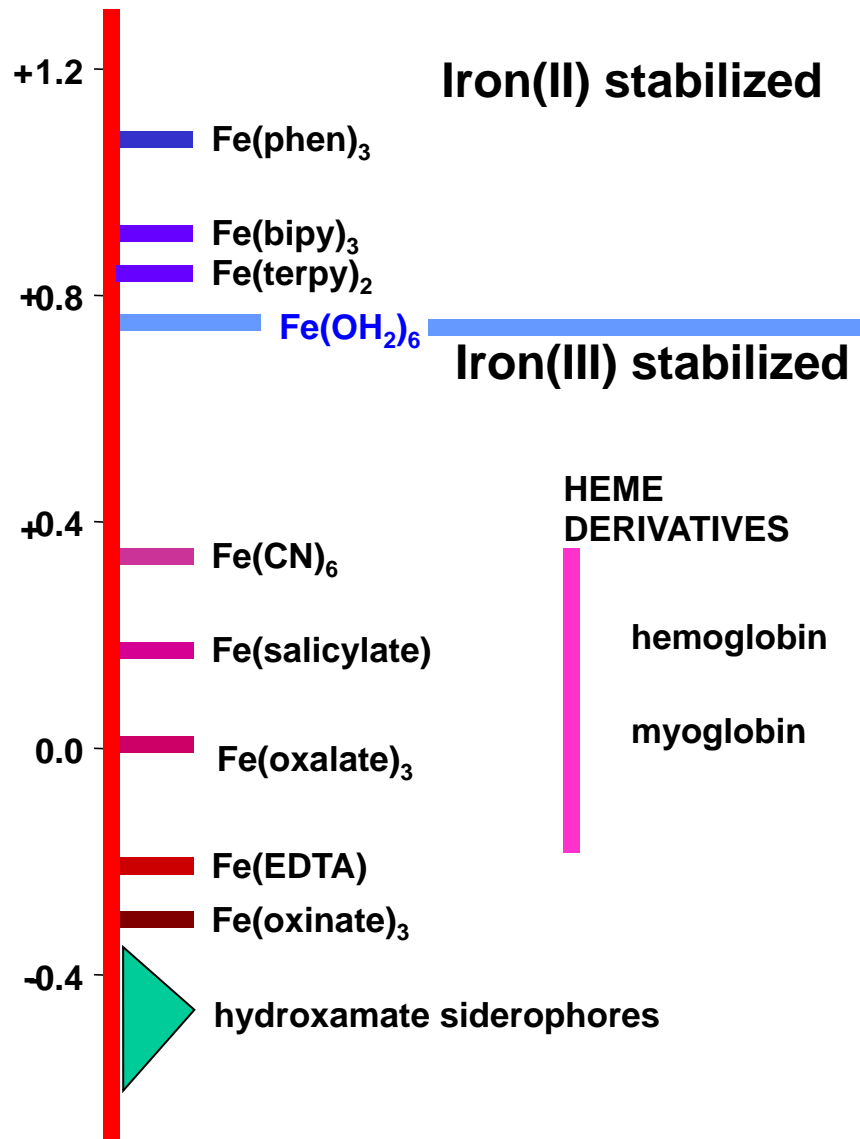
Fe(III/II) redox potential varies significantly with ligands in 1st coordination shell

**Iron(II) stabilized**

+1.2 — Fe(phen)$_3$

Fe(bipy)$_3$
+0.8 — Fe(terpy)$_2$

Fe(OH$_2$)$_6$

**Iron(III) stabilized**

**HEME DERIVATIVES**

+0.4 — Fe(CN)$_6$

Fe(salicylate)

**hemoglobin**

0.0 — Fe(oxalate)$_3$

**myoglobin**

Fe(EDTA)
Fe(oxinate)$_3$

−0.4 —

**hydroxamate siderophores**

$$Fe(S)^{3+} + e^- \rightleftharpoons Fe(S)^{2+}$$

| 0.4 | 0.6 | 0.8 | 1.0 | 1.2 | 1.4 | 1.6 | 1.8 |

$E°(V)$ *vs. NHE*

DMSO DMF  H$_2$O  MeOH  MeCOOH  MeNO$_2$  MeCN

(MeCO)$_2$O
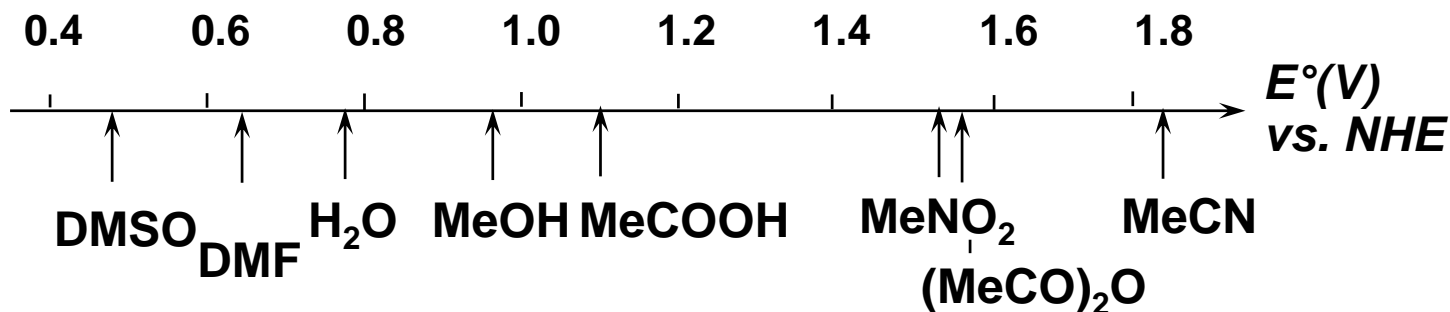
In general, all basic ligands (both neutral molecules or anions) affect strongly the redox reactivity of iron cations. The following was observed:

Ligation of iron by chelators that stabilize the ferrous form of iron, such as phenanthrolines, results in an increase in the reduction potential of the iron ($\approx$+1.1 V).  Conversely, ligation of iron by chelators that stabilize the ferric form of iron, such as deferroxamine, results in a decrease in the reduction potential of the iron ($\approx$ -0.4 V).

- Several salts with different anions are known, unless the reducing (i.e. iodide)

$$Fe^{3+} + I^- \rightleftarrows Fe^{2+} + I_2$$
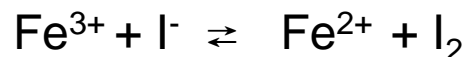
- Salts containing the $[Fe(H_2O)_6]^{3+}$ ion (pale pink), i.e. $Fe(ClO_4)_3 \cdot 10H_2O$, show colors from pink to white. $Fe^{3+}$ hydrolyze in $H_2O$ at pH > 2-3 giving yellow hydroxo species owing CT bands in UV-Vis spectra.

$$[Fe(H_2O)_6]^{3+} \rightleftarrows [Fe(H_2O)_5(OH)]^{2+} + H^+ \qquad K= 10^{-3.05}$$

$$[Fe(H_2O)_5(OH)]^{2+} \rightleftarrows [Fe(H_2O)_4(OH)_2]^+ + H^+ \qquad K= 10^{-3.26}$$

$$2\,[Fe(H_2O)_6]^{3+} \rightleftarrows [Fe(H_2O)_4(OH)_2Fe(H_2O)_4]^{4+} + 2H^+ \qquad K = 10^{-2.91}$$
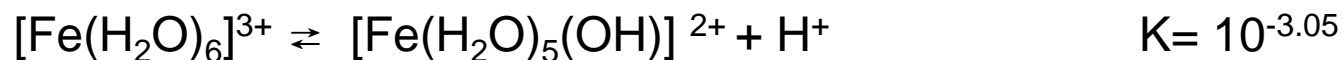
- The binuclear ions further condense to binuclear oxoions and to polinuclear ions, with final formation of $Fe(OH)_3$ gel or hydrated oxides.
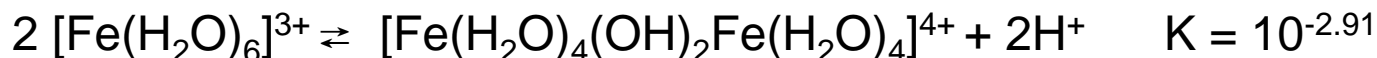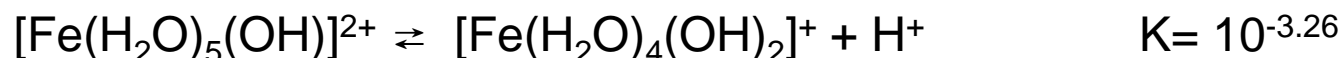- Iron(III) ion has a strong affinity for $F^-$ (with $Cl^-$ complexes have K= 10, 3, 0,1).

$$F^{3+} + F^- \rightleftarrows FeF^{2+} \qquad FeF^{2+} + F^- \rightleftarrows FeF_2^+ \qquad Fe_2^+ + F^- \rightleftarrows FeF_3$$
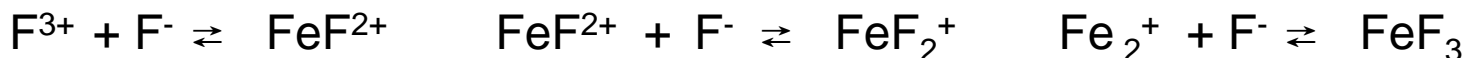
$$K_1 = 10^5 \qquad\qquad K_2 = 10^5 \qquad\qquad K_3 = 10^3$$

- $FeCl_3$ (red brown crystals, nearly black easily hydrolyzing in $H_2O$) can be obtained from chlorine and iron by heating. It is soluble in ethers and in polar solvents and a catalyst in Friedel-Craft electrophilic substitutions.

- Fe insoluble due to hydrolysis



Higher insoluble polymers

$[Fe^{3+}]_{tot} = 10^{-10}$ M at pH 7
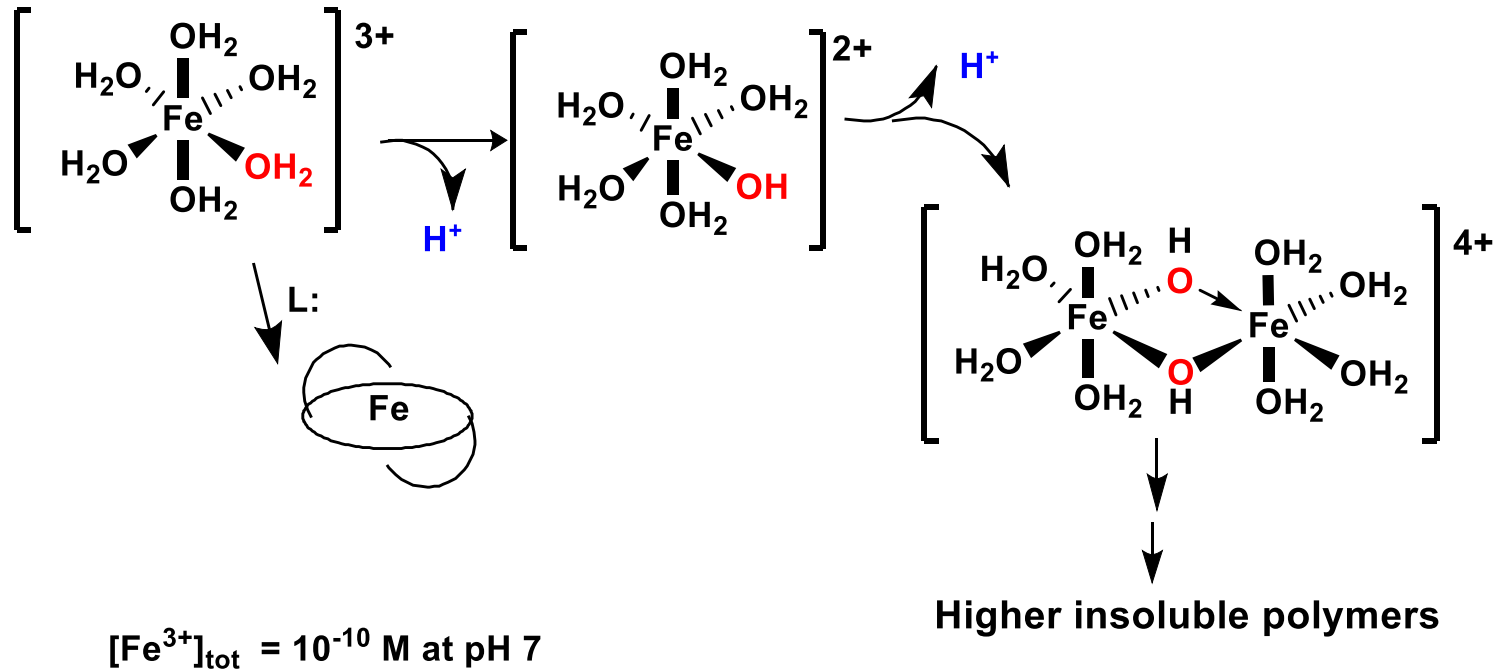
- Strong chelators prevent hydrolysis and precipitation

Common iron ligand donor groups in biology include amino acid side chains, such as amine (I), carboxylate (II), imidazole (III), phenol (IV), and thiol (V). Other ligating groups include $\alpha$-hydroxy carboxylate (VI), catecholate (VII), hydroxamate (VIII) and porphyrin (IX).

(I)  (II)  (III)  (IV)  (V)  (VI)  (VII)  (VIII)  (IX)
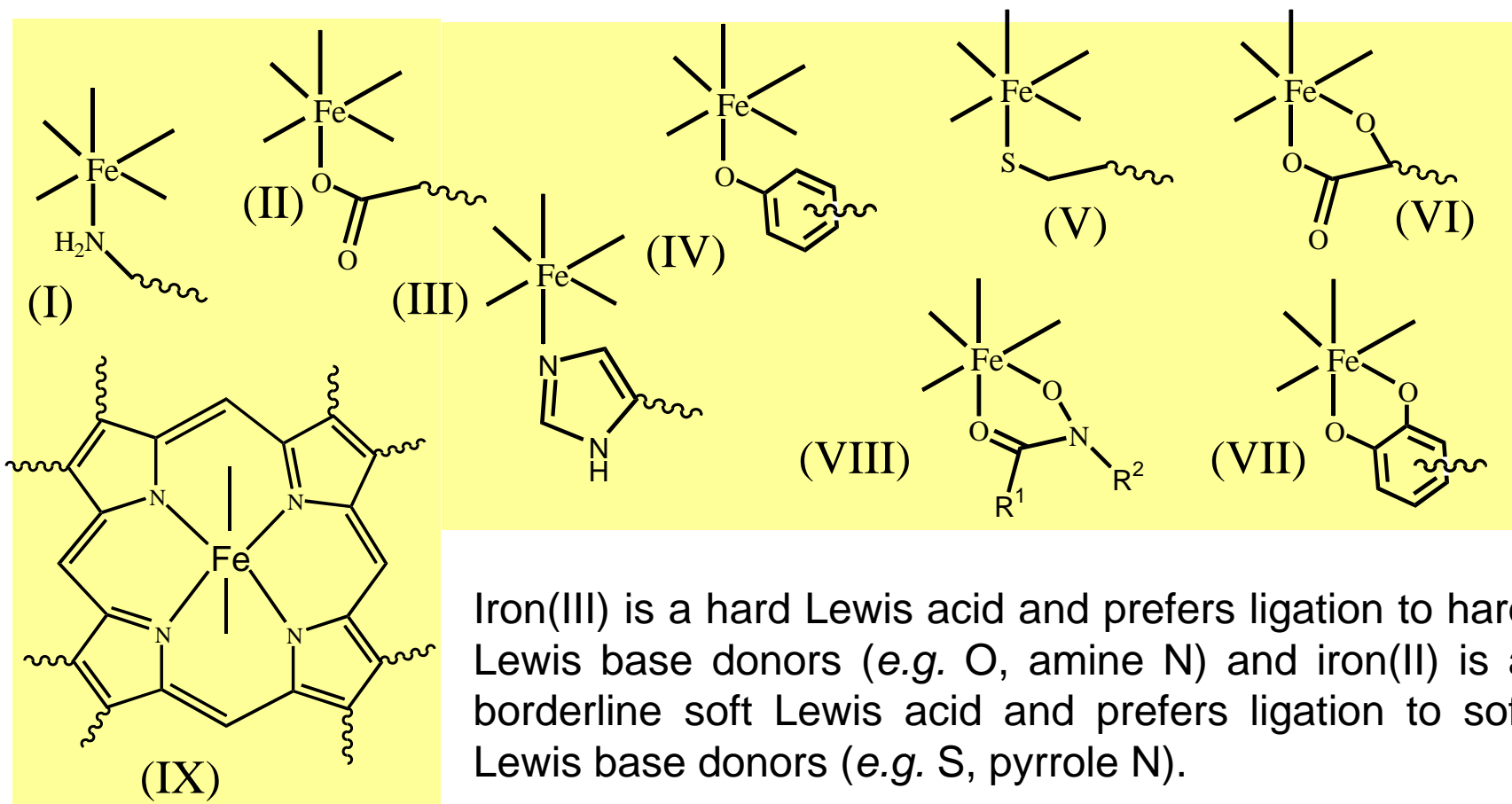
Iron(III) is a hard Lewis acid and prefers ligation to hard Lewis base donors (*e.g.* O, amine N) and iron(II) is a borderline soft Lewis acid and prefers ligation to soft Lewis base donors (*e.g.* S, pyrrole N).

*Why control $E^0$?*

- **Prevent redox cycling & ROS production**
- **Fe(III) selectivity**
- **Control stability**
- **Control ligand exchange kinetics**
- **Control "switch" sensitivity**



$+940$ — $O_2^{\cdot-}/H_2O_2$

$+770$ — $Fe(H_2O)_6^{3+}/Fe(H_2O)_6^{2+}$ — $10^0$

$+460$ — $H_2O_2/HO^{\cdot}, HO^{-}$

E (mV vs NHE)

$RH \rightarrow ROS$

$HO^{\cdot}$  $Fe^{3+}$  $O_2^{\cdot-}$
$HO^{-}$
$H_2O_2$  $Fe^{2+}$  $O_2$

$-160$ — $O_2/O_2^{\cdot-}$

*Haber-Weiss Cycle* — $10^{10}$

$-320$ — $NAD(P)^+/NAD(P)H$

$-480$ — Ferrioxamine B
$-500$ — Transferrin — $10^{20}$

$b^{III}/b^{II}$
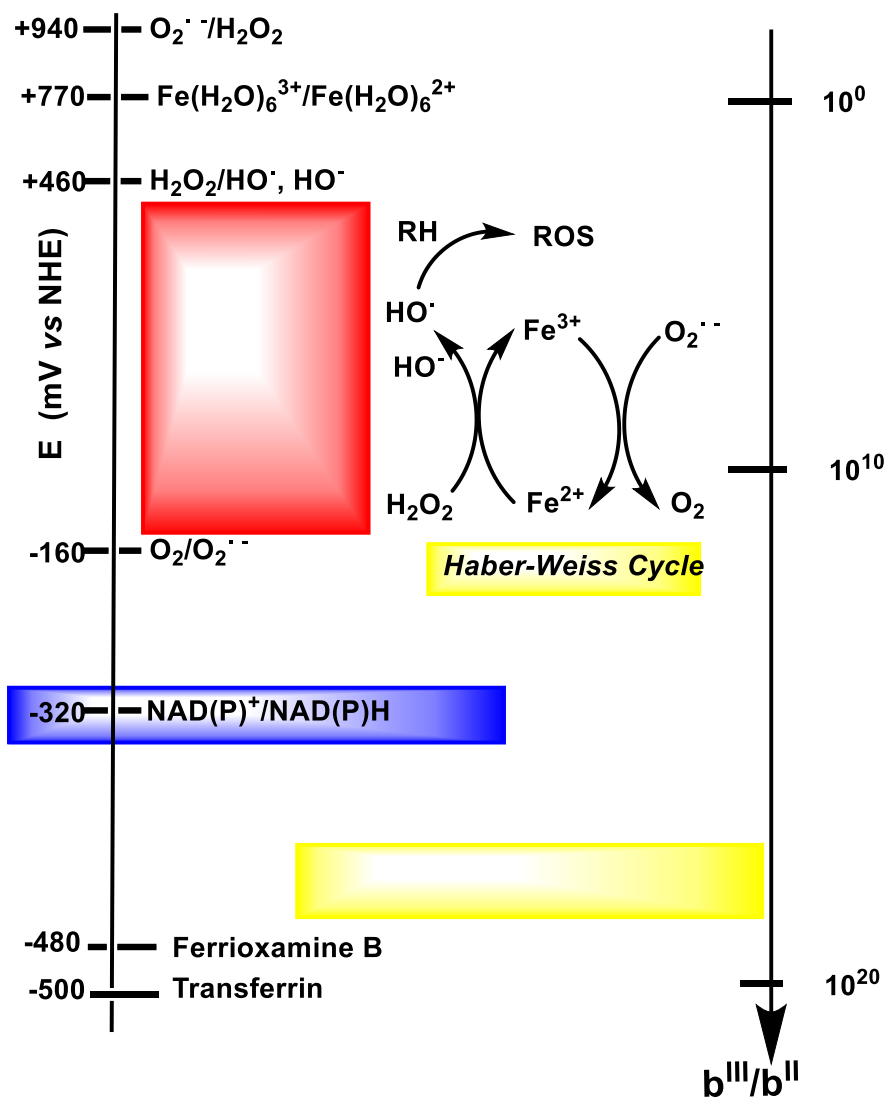
Illustration of the loss of several orders of magnitude of stability on reduction of high spin Fe(III) complex to Fe(II).
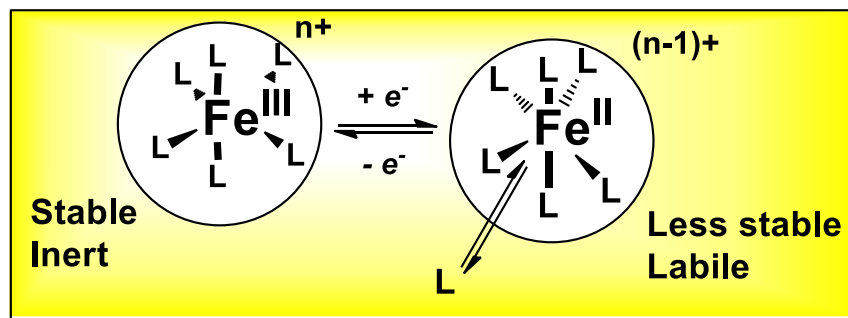
| | |
|---|---|
| **Fe(III)transferrin** | **log K at pH 7.4 = 20** |
| Fe(II)transferrin | log K at pH 7.4 = 3 |
| Fe(III)ferrioxamine B | log $b_{110}$ = 30.6 |
| Fe(II)ferrioxamine B | log $b_{110}$ = 10.3 |

*Kinetics*

$$Fe(OH_2)_6^{3+} + {}^*OH_2 \xrightleftharpoons{\quad t_{1/2} = 4\ ms \quad} Fe(OH_2)_5({}^*OH_2)^{3+} + OH_2$$

$$+e^- \big\updownarrow -e^-$$

$$Fe(OH_2)_6^{2+} + {}^*OH_2 \xrightleftharpoons{\quad t_{1/2} = 0.2\ \mu s \quad} Fe(OH_2)_5({}^*OH_2)^{2+} + OH_2$$
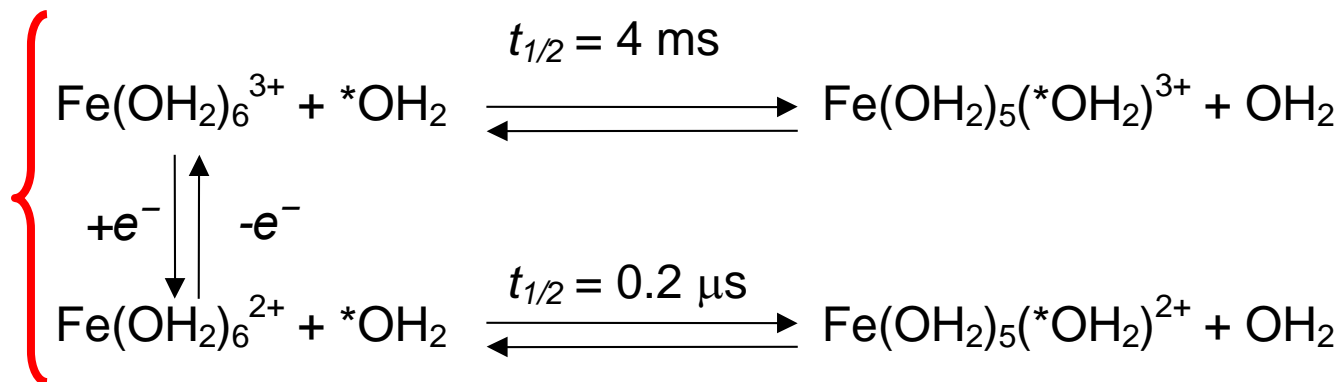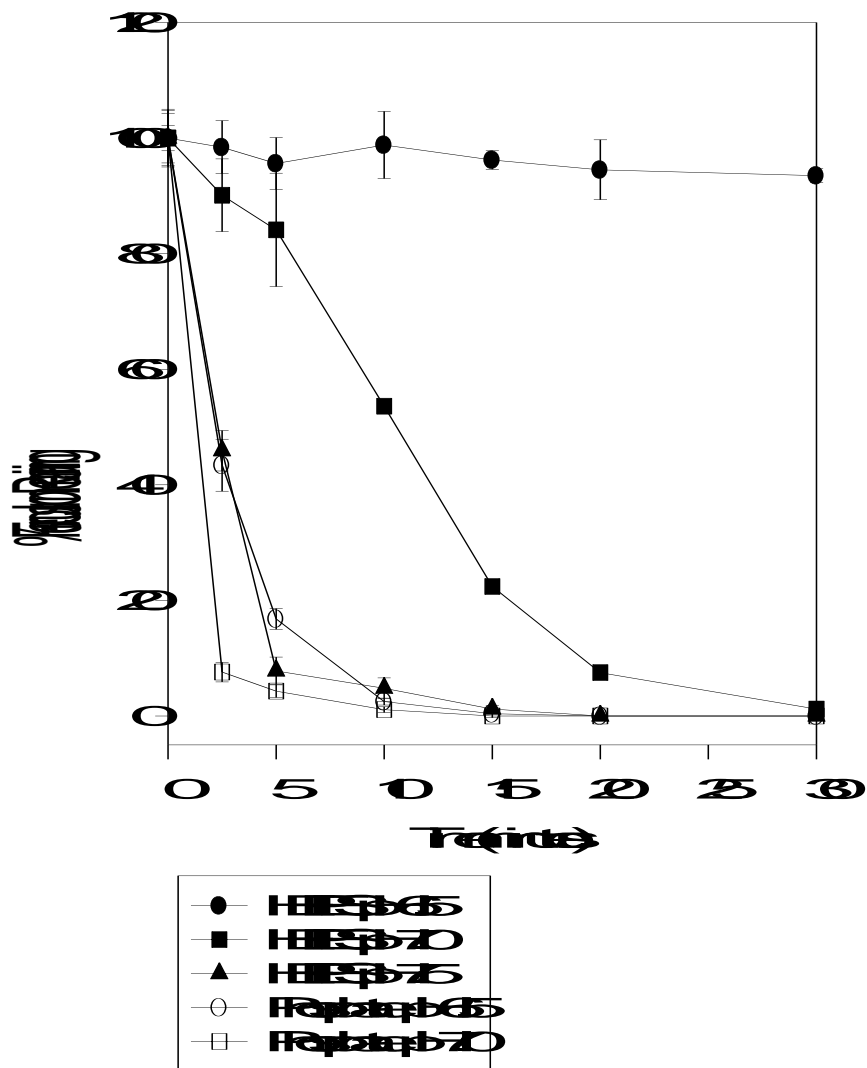
In general the rate of Fe(II) autoxidation in an aqueous solution (in the absence of any other chelators) is proportional to the square of [OH⁻].

$$rate = [Fe(II)][O_2][OH^-]^2$$

Harris, D.C. and Aisen, P. (1973)
*Biochim. Biophys. Acta* **329**, 156-158.

However, relatively strong chelators such as phosphate, can override the effect of pH on the rate of autoxidation.

For example, almost no Fe(II) autoxidizes in a HEPES buffered solution at pH 6.5, conversely, Fe(II) autoxidizes very rapidly in phosphate buffer, at pH 6.5.

Welch, K.D., Davis, T.Z., and Aust, S.D. (2002)
*Arch. Biochem. Biophys.* **397**, 360-369.

It has been shown experimentally that chelators, in general, affect the stability of Fe(II) as expected, *i.e.,* chelators that ligate Fe(II) *via* oxygen ligands promote the autoxidation of Fe(II), whereas the autoxidation of Fe(II), ligated by chelators with nitrogen ligands, is slower. Interestingly, the stoichiometry of the autoxidation reaction appears to be inversely related to the rate, *i.e.,* the faster the rate of Fe(II) autoxidation the lower the stoichiometry.

Welch, K.D., Davis, T.Z., and Aust, S.D.  (2002)  *Arch. Biochem. Biophys.*  **397**, 360-369.
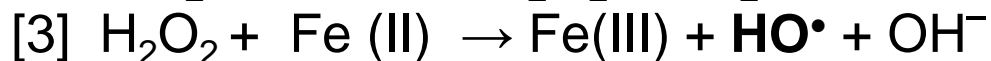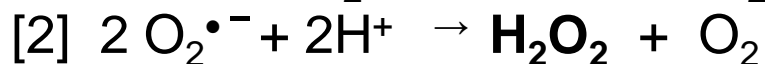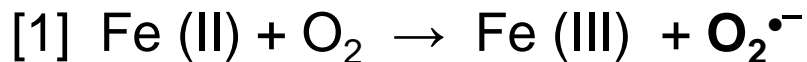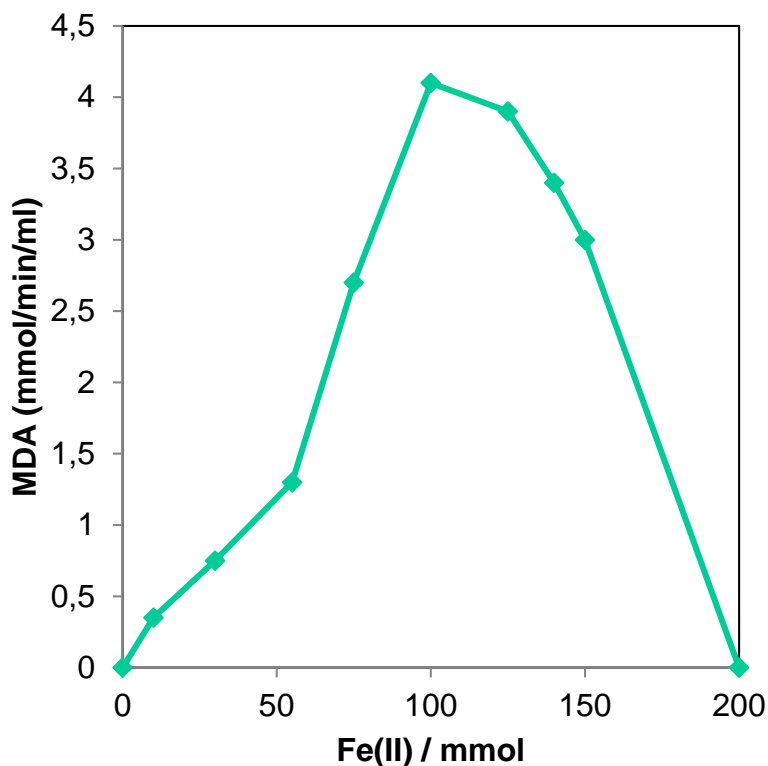
Iron can mediate the deleterious oxidation of biomolecules, which can have serious consequences for an organism, resulting in various diseases, *e.g.,* cancer, atherosclerosis, and diabetes.

Iron can oxidize numerous biomolecules indirectly *via* partially reduced oxygen species that can be produced in the presence of iron.  One of the most commonly accepted mechanisms of iron-mediated oxygen radical production is described by the Haber-Weiss series of reactions shown below.

Iron can also oxidize numerous biomolecules *via* direct transfer of an electron from the molecule to an iron complex, *e.g.,* ascorbate and dopamine.

[1]  $Fe(II) + O_2 \rightarrow Fe(III) + \mathbf{O_2^{\bullet-}}$

[2]  $2\,O_2^{\bullet-} + 2H^+ \rightarrow \mathbf{H_2O_2} + O_2$

[3]  $H_2O_2 + Fe(II) \rightarrow Fe(III) + \mathbf{HO^{\bullet}} + OH^-$

It has been shown that maximal rates of lipid peroxidation are observed when the the ratio of Fe(II):Fe(III) is 1:1.

The addition of Fe(II), chelated by any one of a number of different iron chelators, in a liposomal system often results in a short lag period before lipid peroxidation starts. The addition of a second chelator results in an increase in the lag period or inhibition of lipid peroxidation.

The degree of inhibition depends on the stability constant of the iron:chelator complex. The most pronounced effects were observed for the chelators with higher stability constants, which supports the hypothesis that a 1:1 ratio of Fe(II):Fe(III) is responsible for iron-mediated lipid peroxidation.



Minotti, G. and Aust S.D.  (1987) J. Biol. Chem.  262, 1098-1104.

Tang, L.X.  *et al.*  (1997)  *J. Inorg. Biochem.*  **68**, 265-272.
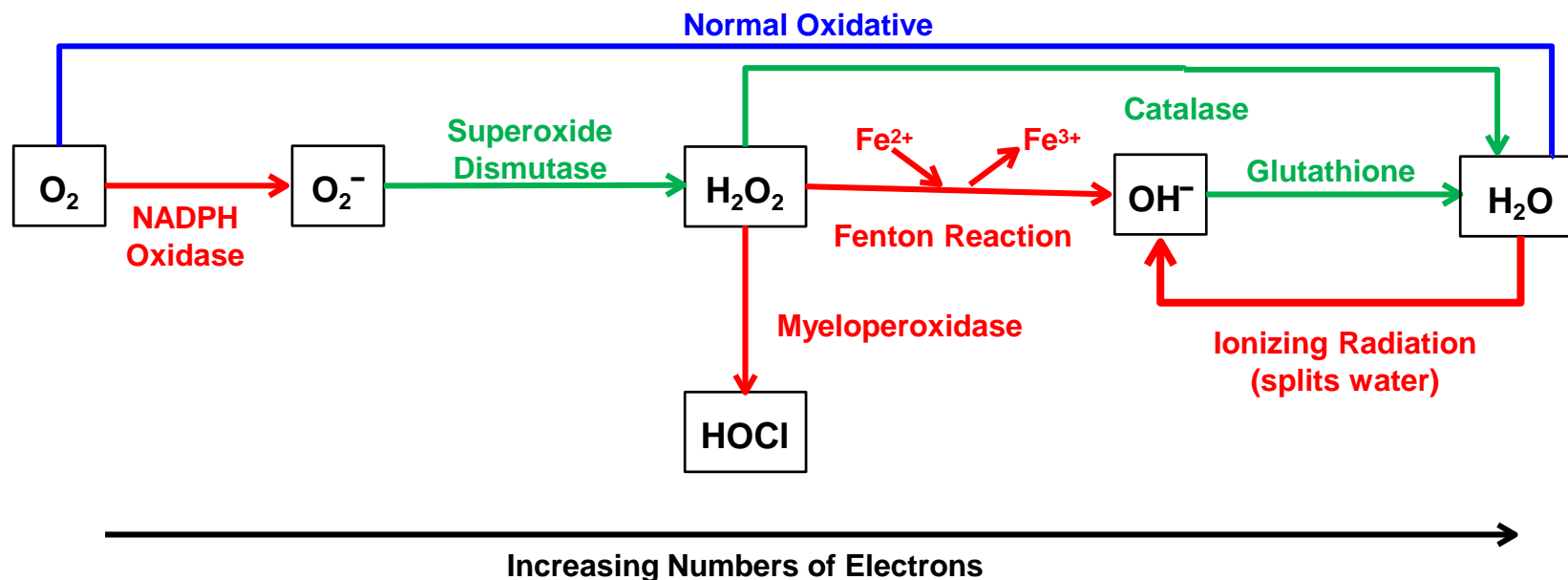
**Hydroxyl radicals formation from reducing metal cations and hydrogen peroxide:**

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet$$

$$Cu^+ + H_2O_2 \rightarrow Cu^{2+} + OH^- + OH^\bullet$$

$$Ti^{3+} + H_2O_2 \rightarrow Ti^{4+} + OH^- + OH^\bullet$$

$$Fe^{+++} + O_2^{\bullet-} \rightarrow Fe^{++} + O_2$$

$$Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + HO^- + HO^\bullet$$

$$\overline{\rule{0pt}{1em}\hspace{9cm}}$$

$$O_2^{\bullet-} + H_2O_2 \rightarrow O_2 + HO^- + HO^\bullet$$

**Haber-Weiss Reaction**

# Enzymes Involved in the Redox Oxygen Chain

Normal Oxidative

$O_2$ → $O_2^-$ (NADPH Oxidase) → (Superoxide Dismutase) → $H_2O_2$ → (Fenton Reaction, $Fe^{2+}$ → $Fe^{3+}$) → $OH^-$ → (Glutathione) → $H_2O$

Catalase

$H_2O_2$ → (Myeloperoxidase) → HOCl

Ionizing Radiation (splits water)

**Increasing Numbers of Electrons**

| Enzymes that Generate Free Radicals | Enzymes that Neutralize Free Radicals |
|---|---|
| ¤ **NAPDH Oxidase** converts oxygen to Super Oxide ($O_2^-$) mainly in neutrophils | ¤ **Superoxide Dismutase (SOD)** converts Super Oxide ($O_2^-$) to peroxide ($H_2O_2$) |
| ¤ **Myeloperoxidase (MPO)** converts peroxide ($H_2O_2$) into Hypochlorous acid (HOCl) mainly in neutrophils | ¤ **Glutathione** convert Hydroxide ($OH^-$) to water |
| ¤ Fenton Reaction generates Hydroxide ($OH^-$) from peroxide ($H_2O_2$) using iron | ¤ **Catalase** converts Peroxide ($H_2O_2$) to water |

**Ferric haem protein + peroxide:**

$$Fe^{3+} + H_2O_2 + R_{en} \rightarrow Fe^{4+}=O + H_2O + R_{en}^{\bullet+}$$
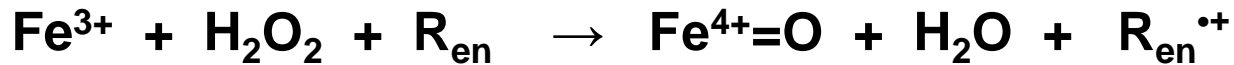
**Products are ferryl haem and protein-bound free radical.**

**Use of ferryl iron and haem radicals in defence and biosynthesis**
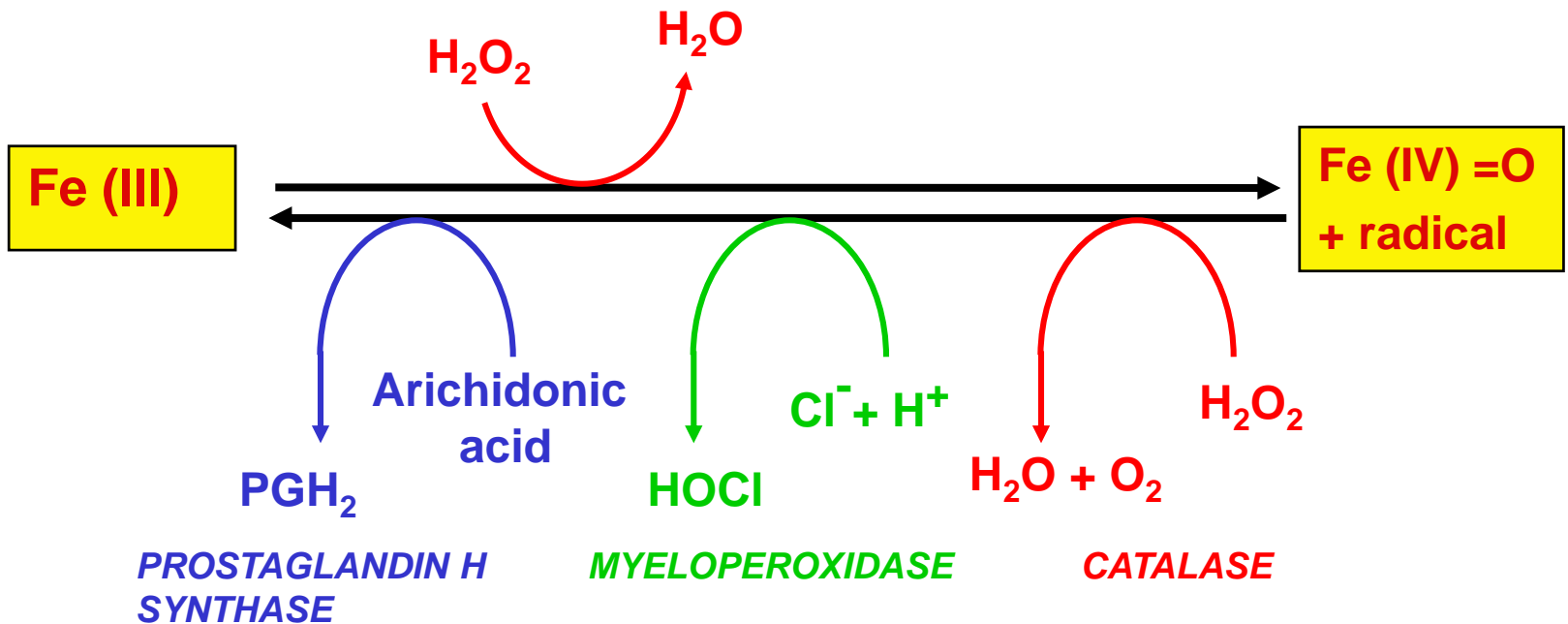
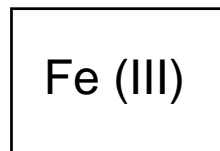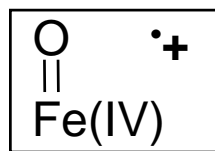## RADICAL FORMING          ENZYME

| Fe (III) | $\xrightarrow{\text{Peroxide}}$ | $\underset{Fe(IV)}{\overset{O}{\parallel}}$ $\cdot^{+}$ | Horseradish peroxidase Catalase Chloroperoxidase |

Fe (III) → Peroxide → O=Fe(IV) —— $-O\bullet$ + $H^+$
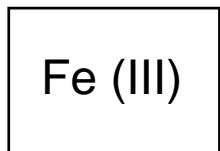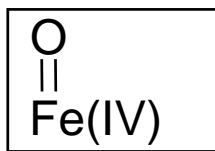
Prostaglandin H synthase
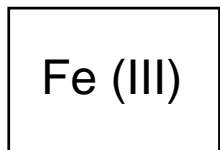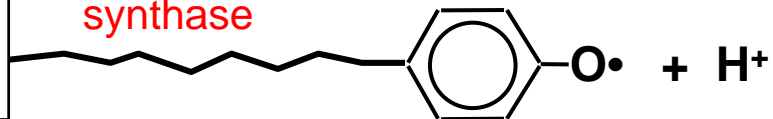
Fe (III) → Peroxide → O=Fe(IV) —— indole $\cdot^{+}$
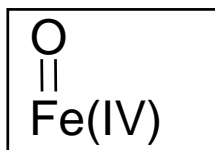
Cytochrome C peroxidase (Yeast)

Fe (III) — Fe (III) → Peroxide → O=Fe(IV) — Fe (III)
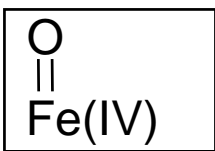
Cytochrome C Peroxidase
*Pseudomonas aeruginosa*
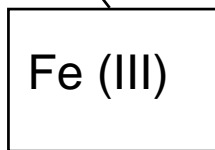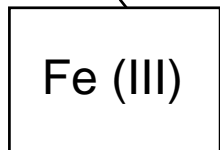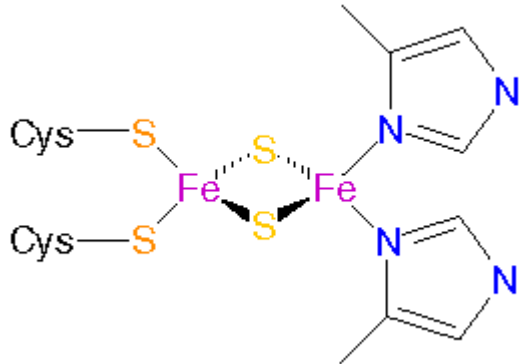
Rieske iron-sulfur proteins
[2Fe-2S]
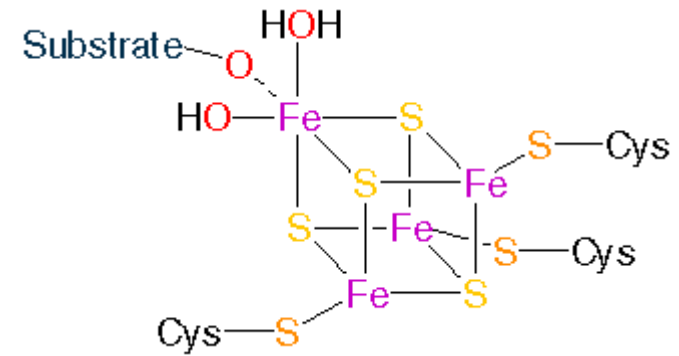


Aconitase family
[4Fe- 4S] cluster
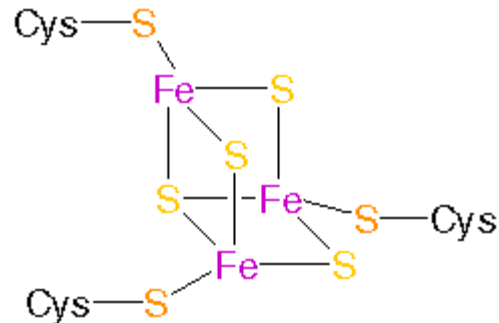


[3Fe-4S] cluster

## Catalyzes isomerization of citrate to isocitrate



**Citrate**  **cis-Aconitate**  **Isocitrate**
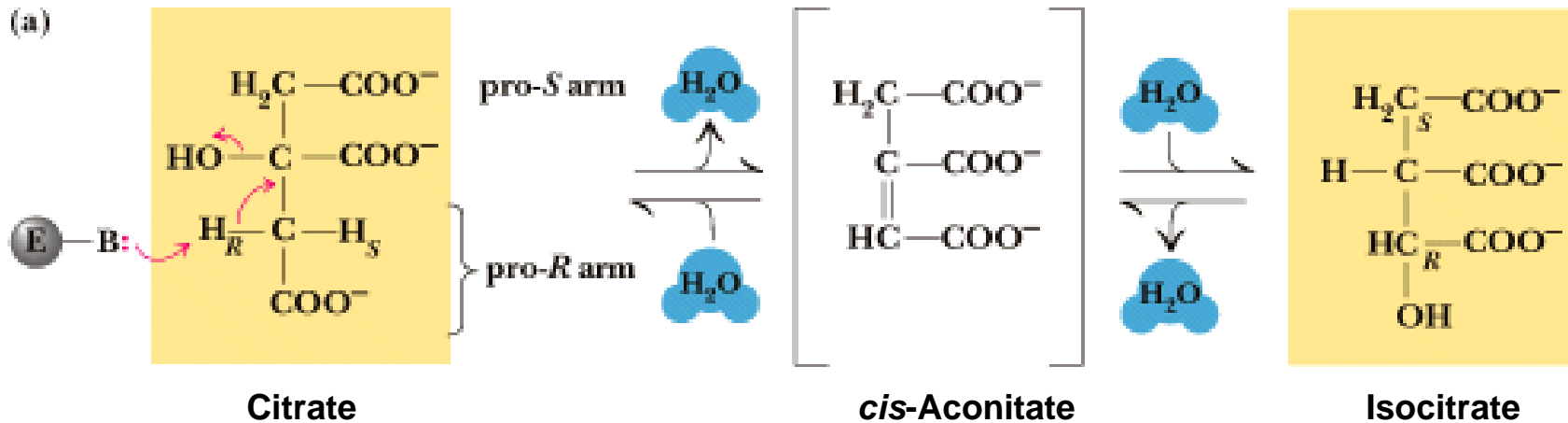
**Aconitase removes the pro-*R* H
of the pro-*R* arm of citrate**

**Iron-sulfur center
in aconitase**

Basic residue
(keeps the citrate
in active site)

Current Opinion in Chemical Biology
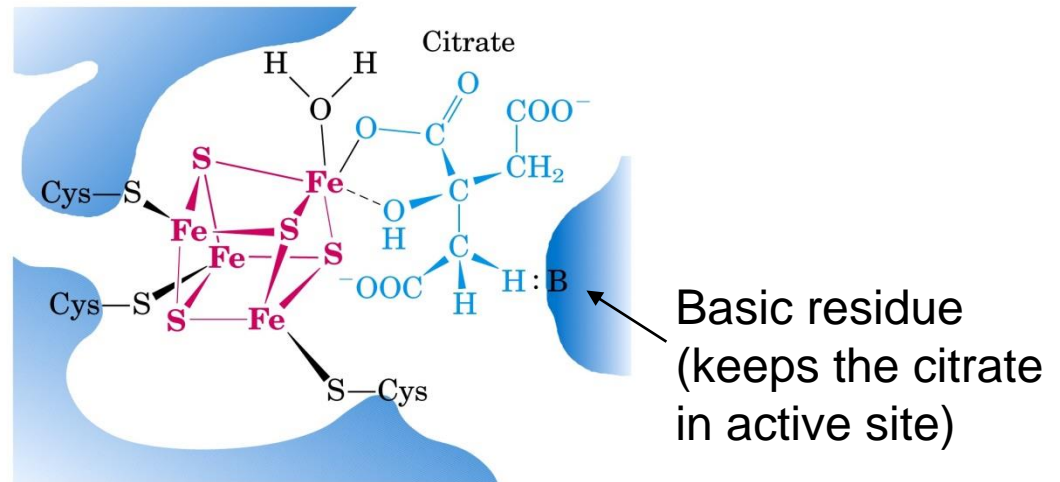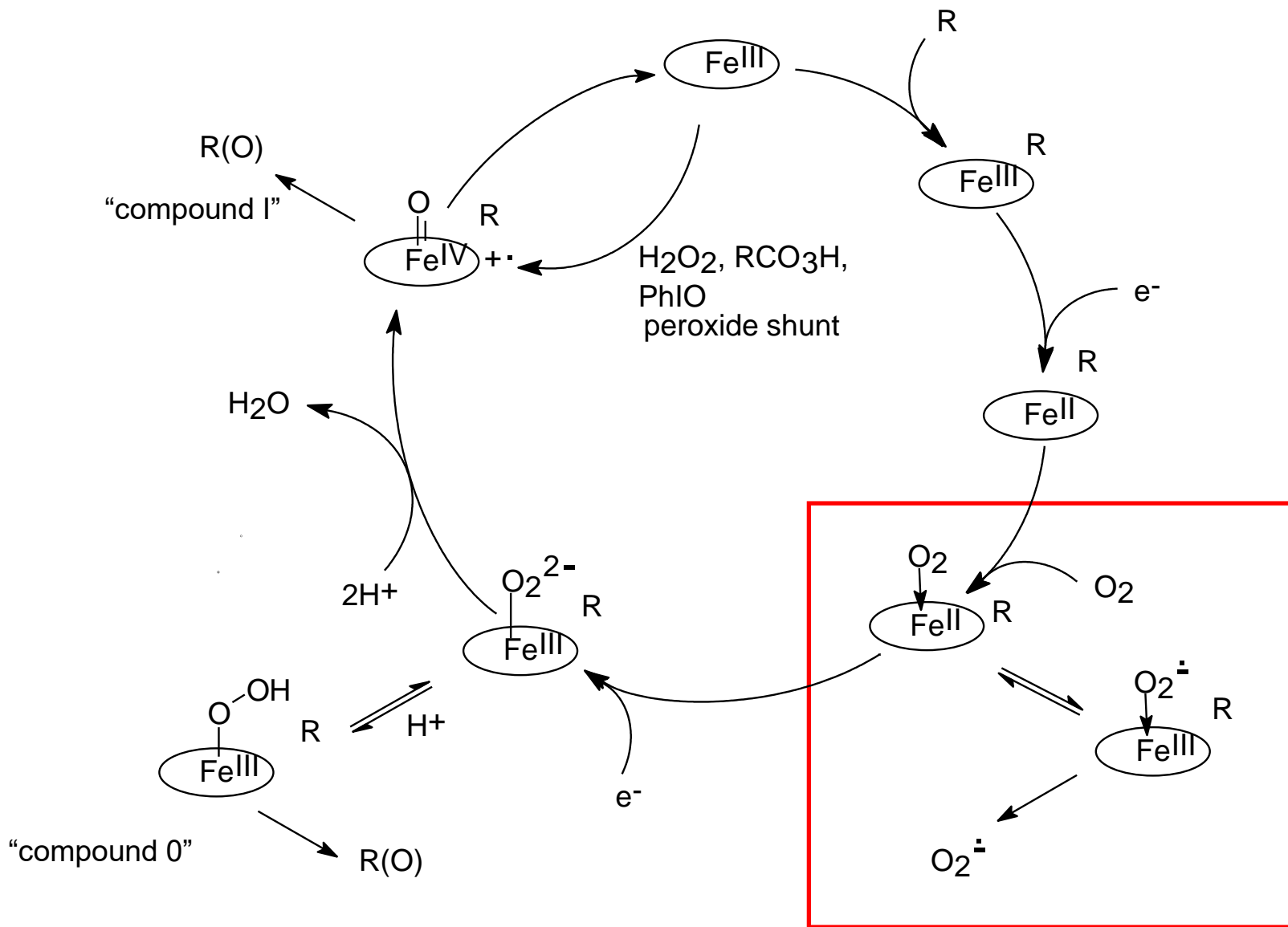
# Protein Oxidation

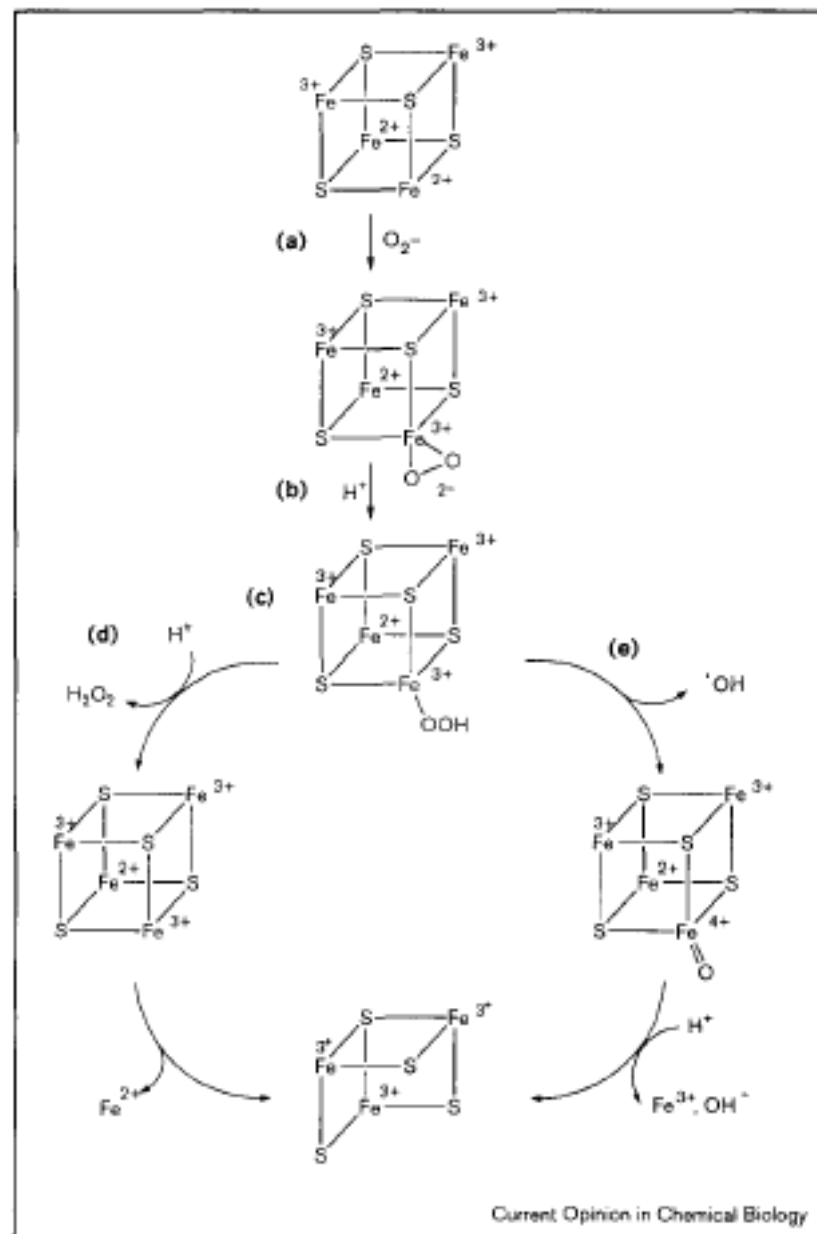- Molecular oxygen, Fe(III), and an appropriate electron donor can catalyze the oxidative modification of proteins. Only a few amino acids are modified and relatively little peptide bond cleavage occurs when proteins are exposed to iron-mediated oxidation systems. The available data indicate that iron-mediated oxidation systems catalyze the reduction of Fe(III) to Fe(II) and of $O_2$ to $H_2O_2$ and that these products react at metal-binding sites on the protein to produce active strong oxidants ($^\bullet$OH, ferryl ion) which attack the side chains of amino acid residues at the metal-binding site.

<div align="right">

Stadtman, E.R. (1990)
*Free Radic. Biol. Med.* **9**, 315-325.

</div>

- Iron-binding sites on proteins serve as centers for repeated production of $^\bullet$OH that are generated via the Fenton reaction. Prevention of the site-specific free radical damage can be accomplished by using selective chelators for iron, by introducing high concentrations of HO$^\bullet$ scavengers, and by adding enzymes that remove $O_2^{\bullet-}$ to $H_2O_2$. Histidine, for example, is a compound that can intervene in free radical reactions in a variety of modes.

<div align="right">

Chevion, M. (1988)
*Free Radic. Biol. Med.* **5**, 27-37.

</div>

- It is thought that the genotoxicity of many chemicals is enhanced by their ability to decompartmentalize iron.

  Li, A.S. et al. (2001)
  *Free Radic. Biol. Med.* **30**, 943-946.

- One mechanism by which iron could be involved in the initiation or promotion of cancer is through the oxidation of DNA. The species responsible for oxidizing DNA is believed to be the $HO^\bullet$. Superoxide radicals have no effect on the oxidation of DNA in the absence of adventitious metals. This suggests that the role of $O_2^{\bullet-}$ in DNA oxidation is simply as a constituent of the Haber-Weiss reactions to produce the $HO^\bullet$.

- The addition of any chemical that will act as an alternate reactant for the $HO^\bullet$, such as organic-based buffers or $HO^\bullet$ scavengers, inhibits the oxidation of DNA. Conversely, the presence of chemicals which increase the iron-mediated production of $HO^\bullet$ will promote the oxidation of DNA.

  Djuric, Z. *et al.* (2001)
  *J. Biochem. Mol. Toxicol.* **15**, 114-119.