What is a Free Radical?

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or, What is a Radical?

A free radical is an atom or group of atoms possessing <u>one or more unpaired</u> electrons [1,2].

The word "free" in front of "radical" is, in this era, considered unnecessary [1,2].

Historically: What is a Free Radical?

Historically, radical and free radical had different, but related meanings. For example, Linus Pauling defined them as [3]:

"Free Radicals. An atom or group of atoms with one or more unshared electrons, which may enter into chemical-bond formation, is called a free radical. (The same group in a molecule is called a radical; for example, the methyl radical in methyl cyanide or other molecules.)"

Thus, when reading older literature be aware of this nuance in meaning. We now realize that not all free radicals will react to make covalent bonds.

Free Radical Notation?

A. Superscript dot to the right, usually

B. Examples (Note: dot, then charge)

$H^{\bullet}$, $Cl^{\bullet}$, $HO^{\bullet}$, or $(HO)^{\bullet}$

$O_2^{\bullet\bullet}$ or $O_2^{2\bullet}$ dioxygen, the O_2 you are breathing now.

$H_3C^{\bullet}$

$O_2^{\bullet-}$, $CO_2^{\bullet-}$, $Asc^{\bullet-}$, $PQ^{\bullet+}$

Common Notations and Abbreviations

Species	Systematic IUPAC Name	Alternative/Comments
O^{-}	oxide(1-)	hydroxyl radical without proton
$O_2^{\bullet -}$	dioxide(1-)	superoxide
O_3	trioxygen	ozone
O_3^{-}	trioxide(1-)	ozonide
$HO^{\bullet}$	hydroxyl	not hydroxy, hydroxide is OH^{-}
$HO_2^{\bullet}$	hydrogen dioxide	hydrodioxyl, or hydroperoxyl, but perhydroxyl does not make sense
HO_2^{-}	hydrogen dioxide(1-)	hydrogenperoxide(1-)
H_2O_2	hydrogen peroxide	
$RO^{\bullet}$	alkoxyl	not alkoxy
$ROO^{\bullet}$	alkyldioxyl	alkylperoxyl not peroxy
ROOH		alkyl hydroperoxide
$ONOO^{-}$	oxoperoxonitrate (1-)	peroxynitrite
ONOOH	hydrogen oxoperoxonitrate	peroxynitrous acid
$NO^{\bullet}$	nitrogen monoxide	nitric oxide

Types of radicals; we have:

Sigma, σ

pi-delocalized, π

Mixture of sigma and pi

Carbon-centered, $H_3C^\bullet$

O_2–centered, $H_3COO^\bullet$

Sulfur-centered, $GS^\bullet$

Nitrogen-centered, $R_2NO^\bullet$

Reducing radicals, $CO_2^{\bullet -}$, $PQ^{\bullet +}$

Oxidizing radicals, $HO^\bullet$, $LOO^\bullet$, $CO_3^{\bullet -}$

Reactivity, wide range

k = very, very slow at RT

$O_2^{2\bullet}$ + CH_4 ⟶ [Intermediates] → …

$k = 5 \times 10^9\ M^{-1}\ s^{-1}$

$O_2^{2\bullet}$ + $H_3C^\bullet$ ⟶ $H_3COO^\bullet$

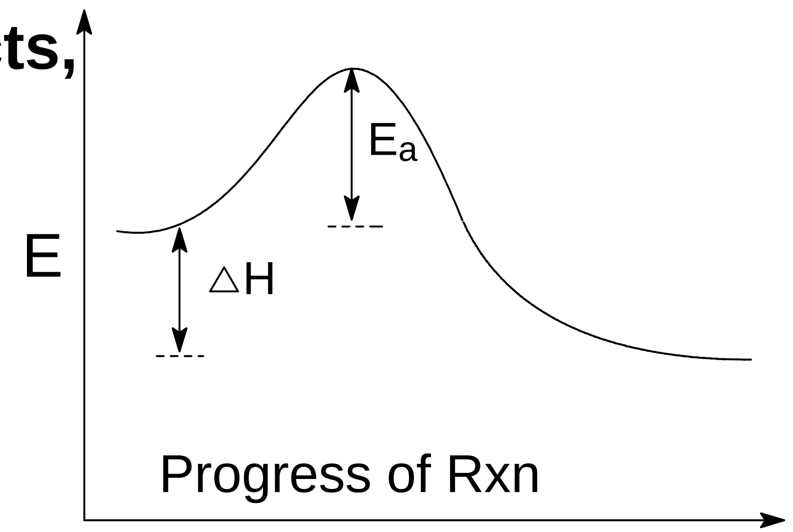
Radical + Radical rxns typically very fast

Radical + non-radical → wide range

Why is ground state O_2 ($O_2^{2\bullet}$: $^3\Sigma^-_g$) so reactive—yet unreactive?

The Spin Restriction [4]

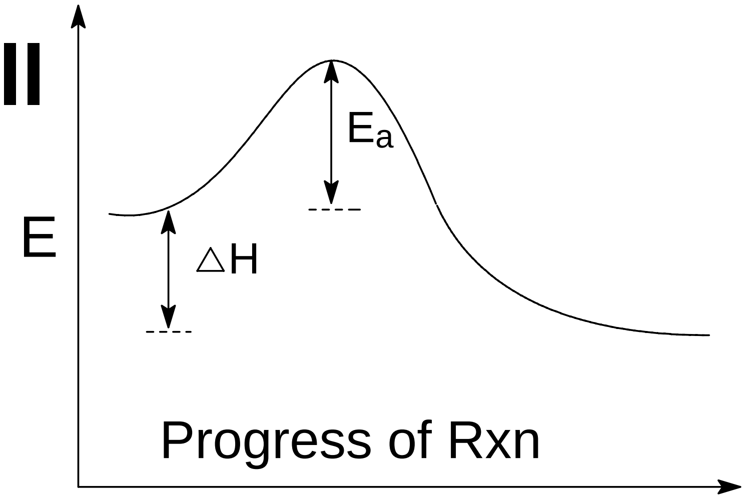
1. Can orbitals overlap to form a reasonable transition state?
2. Activation energy of oxygen!
3. $E_a \geq 23$ kcal/mole for $^3O_2$ reactions, *i.e.* $^1O_2$
4. $^3O_2$ + ^{1}(carbon) $\longrightarrow$ Products, but very slow!



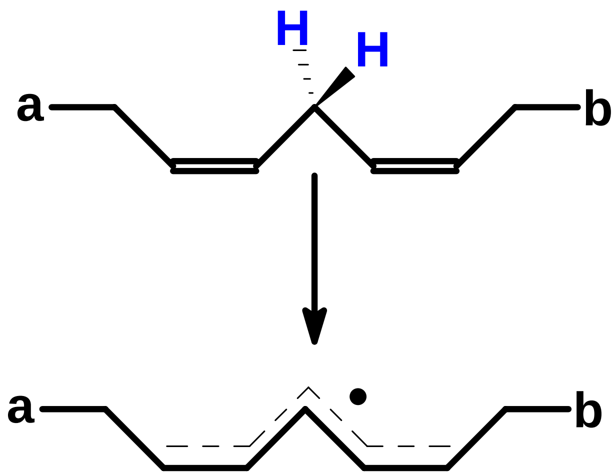
Why does ground state O_2 react so fast with many radicals?

There is no spin restriction [4].

1. **Radical-radical reactions will not have to overcome the spin restriction.**
2. **E_a typically very small**



Example Rxns 1

ROO• +  (PUFA)*

pentadienyl radical

+ ROOH

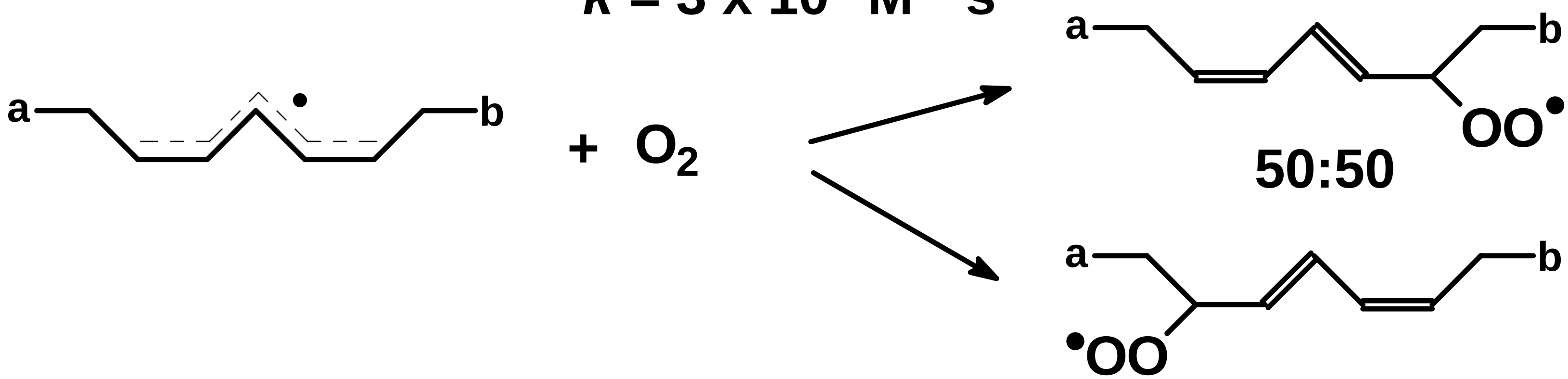
Slow, $k \approx 50\ M^{-1}\ s^{-1}$ (for *bis*-allylic hydrogens)

***It is only the PUFA in lipids that are oxidizable.**
Oxidizability $\propto$ number of double bonds [13]

Example Rxns 2

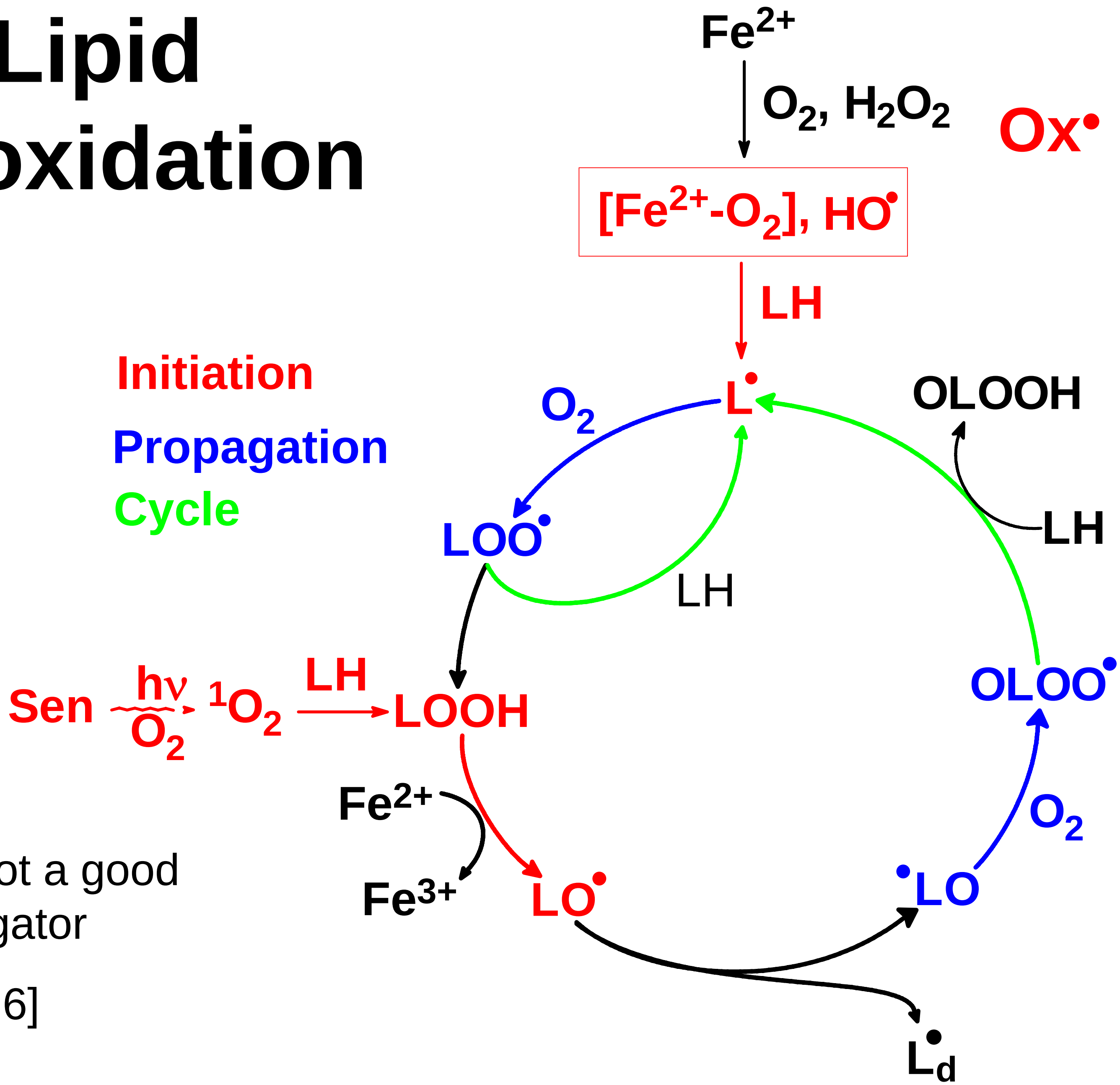
$H_3C^\bullet + O_2 \rightarrow H_3COO^\bullet$ $\quad k = 2 \times 10^9\ M^{-1}\ s^{-1}$

$k = 3 \times 10^8\ M^{-1}\ s^{-1}$



$NO^\bullet + O_2^{\bullet -} \rightarrow O{=}NOO^-$ $\quad k = 1 \times 10^{10}\ M^{-1}\ s^{-1}$

Lipid Peroxidation



LO• not a good propagator

Ref [5,6]

Kinetics rule

The competition

$$L^\bullet + O_2 \xrightarrow{3 \times 10^8\ M^{-1}\, s^{-1}} LOO^\bullet$$ *Propagation*

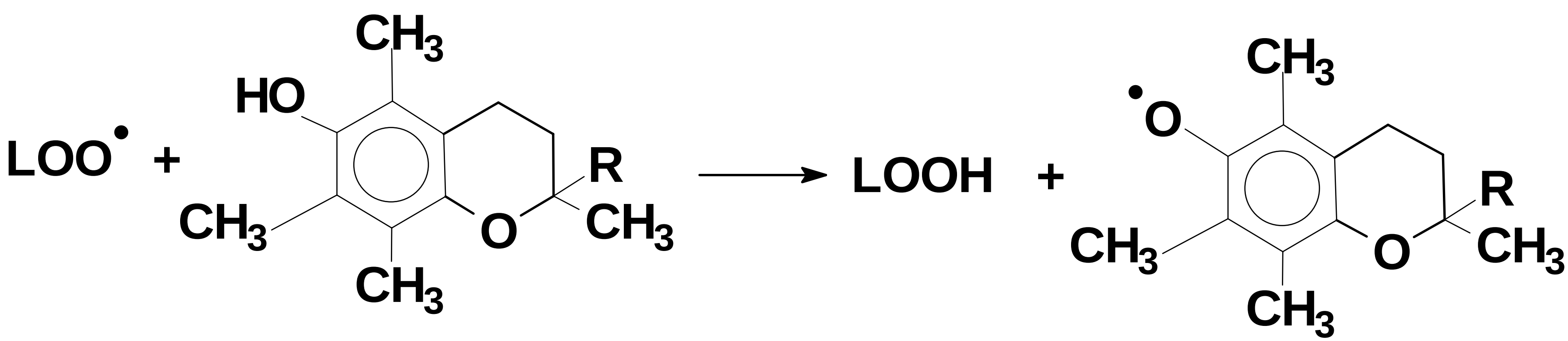
$$LOO^\bullet + L\text{-}H \xrightarrow{10 - 50\ M^{-1}\, s^{-1}} L^\bullet + LOOH$$ *Cycle*

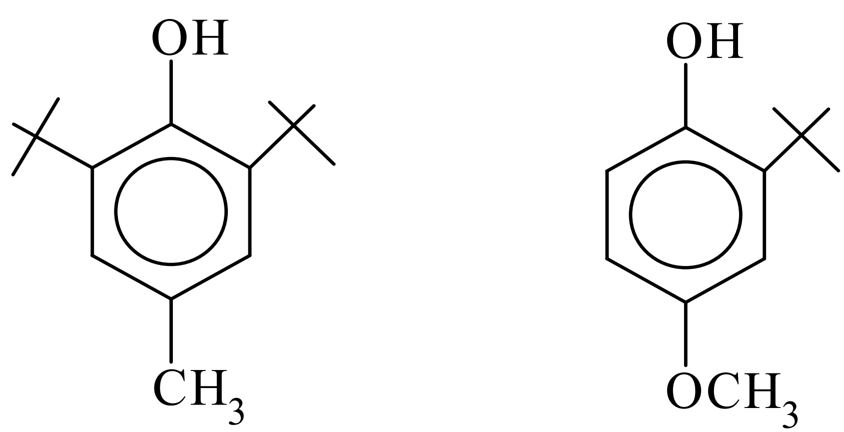
Vitamin E

$\approx 10^5\ M^{-1}\, s^{-1}$ in membranes or LDL

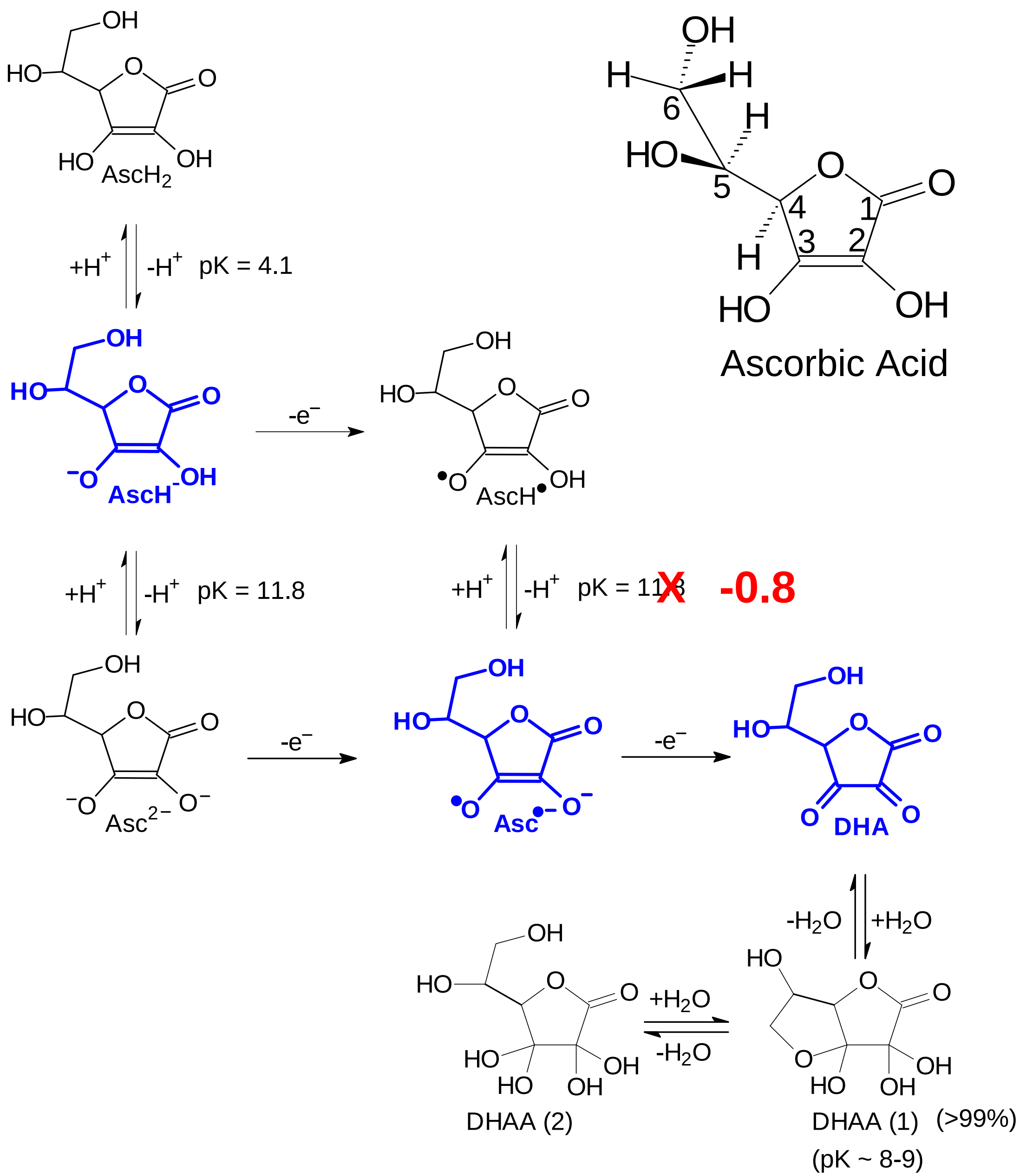
$$LOO^\bullet + TOH \longrightarrow TO^\bullet + L\text{-}H$$

Tocopherol in Action

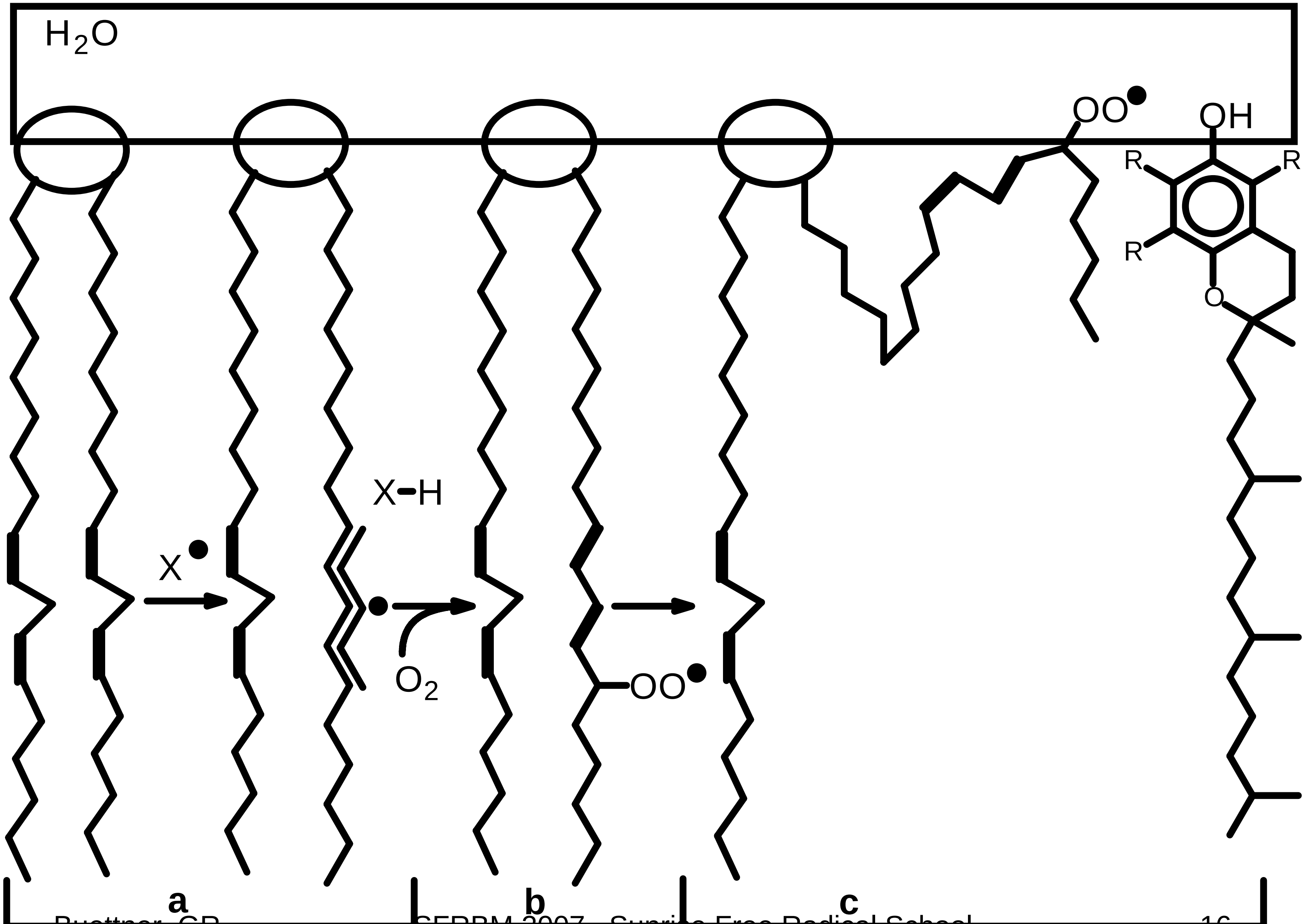




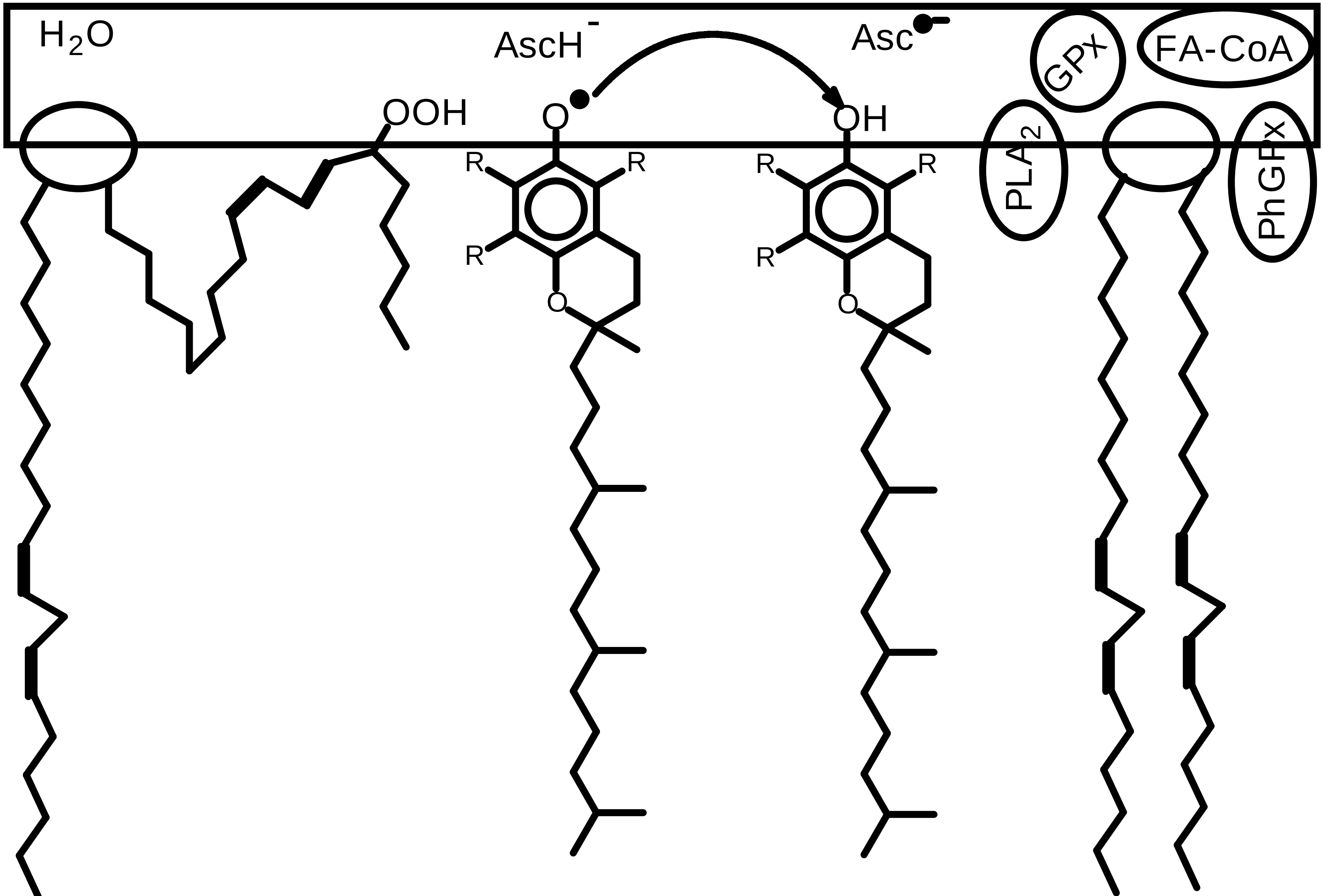
Ascorbate a Donor Antioxidant



C and E as Co-Antioxidants



C and E as Co-Antioxidants



Thermodynamics

Both, kinetics and thermodynamics are involved in the control of antioxidant reactions.

The Pecking Order [7]

Redox Couple (one-electron reductions)	E°'/mV
$HO^{\bullet}, H^+/H_2O$	+ 2310
$RO^{\bullet}, H^+/ROH$ (aliphatic alkoxyl radical)	+ 1600
$ROO^{\bullet}, H^+/ROOH$ (alkyl peroxyl radical)	+ 1000
$GS^{\bullet}/GS^-$ (glutathione)	+ 920
$PUFA^{\bullet}, H^+/PUFA\text{-}H$ (*bis*-allylic-H)	+ 600
$TO^{\bullet}, H^+/TOH$	**+ 480**
$H_2O_2, H^+/H_2O, HO^{\bullet}$	+ 320
$Asc^{\bullet -}, H^+/AscH^-$	**+ 282**
$CoQH^{\bullet}, H^+/CoQH_2$	+ 190
Fe(III) EDTA/ Fe(II) EDTA	+ 120
$O_2/ O_2^{\bullet -}$	- 160
$CoQ/CoQ^{\bullet -}$	- 230
$Paraquat^{2+}/ Paraquat^{\bullet +}$	- 448
Fe(III)DFO/ Fe(II)DFO	- 450
$RSSR/ RSSR^{\bullet -}$ (GSH)	- 1500
H_2O/ e^-_{aq}	- 2870

Jumping to the top, Fenton Rxn

Redox Couple (one-electron reductions)	E°'/mV
$HO^{\bullet}$, H^{+}/H_2O	**+ 2310**
$RO^{\bullet}$, H^{+}/ROH (aliphatic alkoxyl radical)	+ 1600
$ROO^{\bullet}$, $H^{+}/ROOH$ (alkyl peroxyl radical)	+ 1000
$GS^{\bullet}/GS^{-}$ (glutathione)	+ 920
$PUFA^{\bullet}$, H^{+}/PUFA-H (*bis*-allylic-H)	+ 600
$TO^{\bullet}$, H^{+}/TOH	**+ 480**
H_2O_2, H^{+}/H_2O, $HO^{\bullet}$	**+ 320**
$Asc^{\bullet-}$, $H^{+}/AscH^{-}$	**+ 282**
$CoQH^{\bullet}$, $H^{+}/CoQH_2$	+ 190
Fe(III) EDTA / Fe(II) EDTA	+ 120

Jumping up in lipid peroxidation

Redox Couple (one-electron reductions)	E°'/mV
HO•, H^+/H_2O	**+ 2310**
RO•, H^+/ROH (aliphatic alkoxyl radical)	+ 1600
ROO•, H^+/ROOH (alkyl peroxyl radical)	**+ 1000**
GS•/$GS^–$ (glutathione)	+ 920
PUFA•, H^+/PUFA-H (*bis*-allylic-H)	**+ 600**
TO•, H^+/TOH	**+ 480**
H_2O_2, H^+/H_2O, HO•	**+ 320**
Asc•–, $H^+/AscH^-$	**+ 282**
CoQH•, $H^+/CoQH_2$	**+ 190**
Fe(III) EDTA/ Fe(II) EDTA	+ 120

Trouble, trouble, trouble …

When a reaction produces a product that "jumps up" in the Pecking Order.

$HO^{\bullet}$, H^+/H_2O	+ 2310
$ROO^{\bullet}$, $H^+/ROOH$ (alkyl peroxyl radical)	+ 1000
$PUFA^{\bullet}$, H^+/PUFA-H (*bis*-allylic-H)	+ 600
H_2O_2, H^+/H_2O, $HO^{\bullet}$	+ 320

Note: the reaction of $L^{\bullet}$ ($PUFA^{\bullet}$) with O_2 will result in a species higher in the Pecking Order ($ROO^{\bullet}$ above); likewise with the Fenton Rxn, $HO^{\bullet}$.

Iron, a bit of history

1. **Iron contaminates buffers, 0.1 – 1 or more μM;**
2. **Choice of chelating agent can change observations;**
3. **DETAPAC (DTPA) introduced to free radical community;**
4. **Iron a big player in spin trapping;**
5. **Everything goes better with DETAPAC (DTPA).**

Buettner, G.R. and Oberley, L.W. (1978) "Considerations in the spin trapping of superoxide and hydroxyl radicals in aqueous systems using 5,5-dimethyl-1-pyrroline-1-oxide." ***Biochem. Biophys. Res. Commun*****. 83: 69-74. (and the Pinawa Meeting, 1977)**

Buettner, G.R., Oberley, L.W., and Leuthauser, S.W.H.C. (1978) "The effect of iron on the distribution of superoxide and hydroxyl radicals as seen by spin trapping and on the superoxide dismutase assay." ***Photochem. Photobiol*****. 28: 693-695. (and the Pinawa Meeting, 1977)**

Iron from Syringes

Treatment, pH 7.4 PO_4	[Fe]/μM
Untreated	≈1
Chelex 100® (See [12])	≤ 0.01 probably < 1 nM
Hamilton, 705-N	5.0 ± 2.9
Gas-Tight, Hamilton 1705-TEF (22S Steel needle)	0.18 ± 0.12
1705-TEF (Teflon needle)	0.14 ± 0.03
1725-TEF LL (Steel needle)	0.061 ± 0.008
1725-TEF LL (Teflon needle)	0.015 ± 0.007

Buettner, G.R. (1990) Ascorbate oxidation: UV absorbance of ascorbate and ESR spectroscopy of the ascorbyl radical as assays for iron. *Free Rad Res Commns*, 10: 5-9.

Iron, mechanisms

$$Fe(III)chelate + O_2^{\bullet -} \longrightarrow Fe(II)chelate + O_2$$

or $$Fe(III)chelate + AscH^- \longrightarrow Fe(II)chelate + Asc^{\bullet -}$$

Then,

$$Fe(II)chelate + H_2O_2 \longrightarrow HO^{\bullet} + Fe(III)chelate$$

or $$LOOH \longrightarrow LO^{\bullet}$$

or

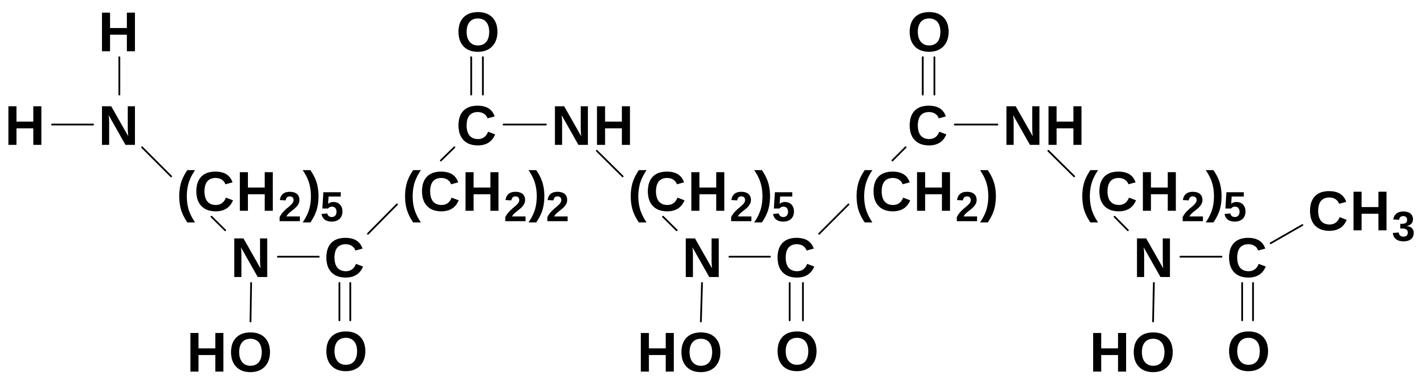
$$Fe(II) + O_2 \longrightarrow Oxidants$$ [8]

Chelates, can drive Fe(II) oxidation

E°′ (Fe(III)Desferal/Fe(II)Desferal) = - 450 mV

$K_{stability}$ Fe(III) ~ $10^{30.6}$

$K_{stability}$ Fe(II) ~ $10^{7.2}$

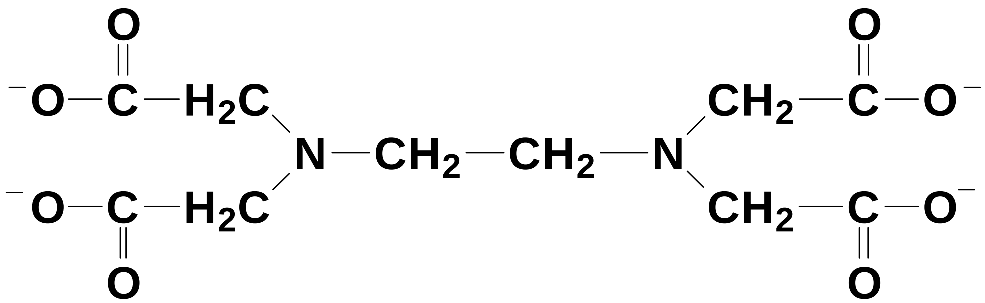


Deferrioximine

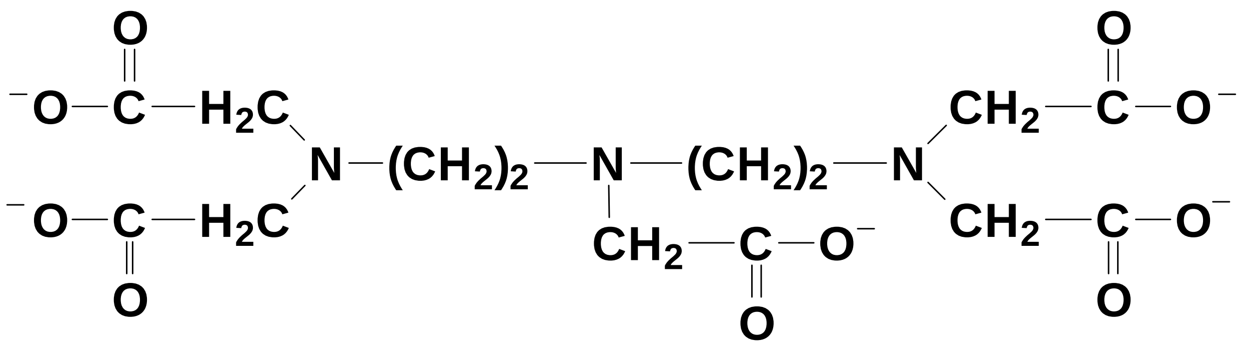
Chelates, can be great for Fenton rxn

$E^{\circ\prime}$ (Fe(III)EDTA/Fe(II)EDTA) = +120 mV

k (Fe(II)EDTA + H_2O_2) ~ 10^4 M^{-1} s^{-1}

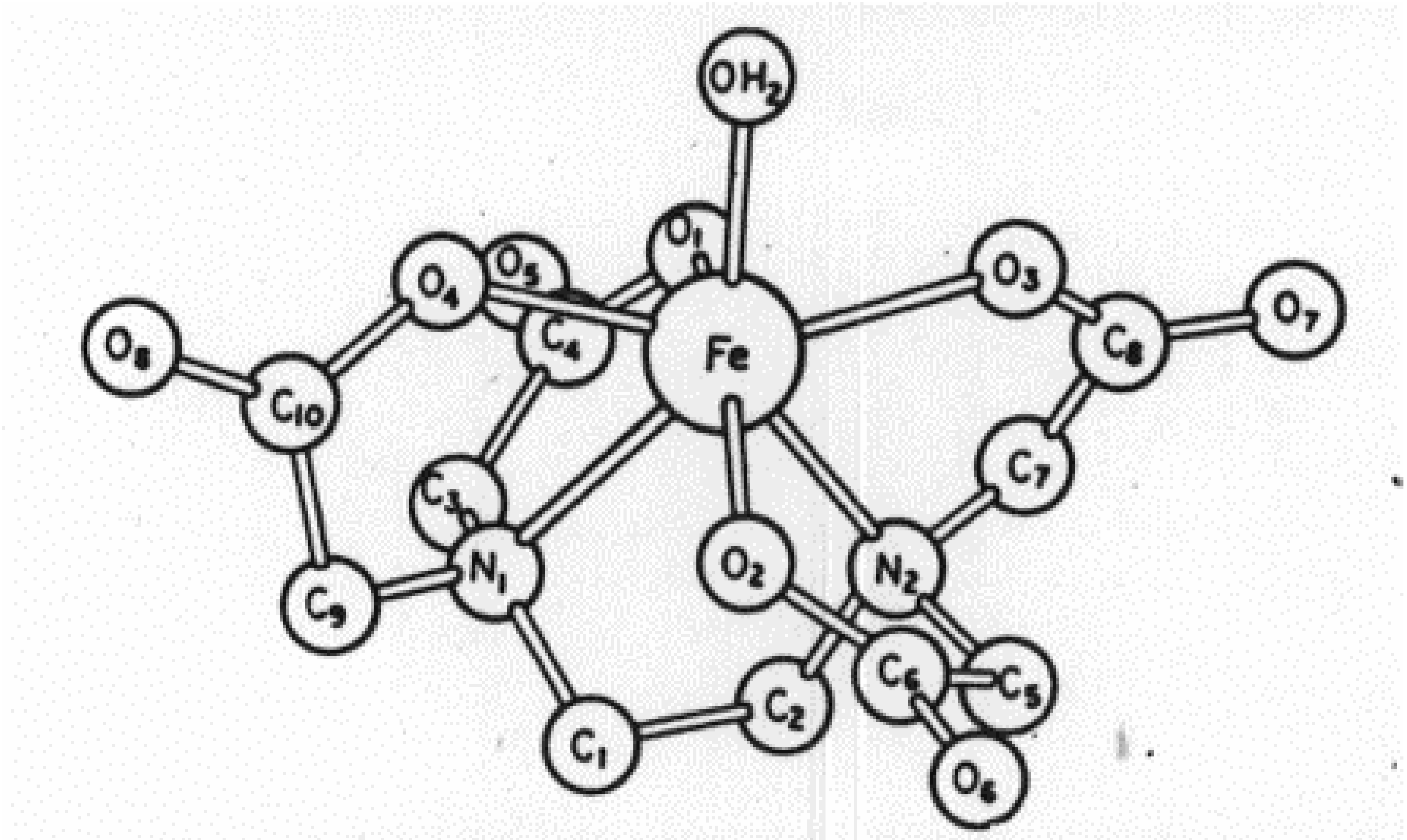


EDTA

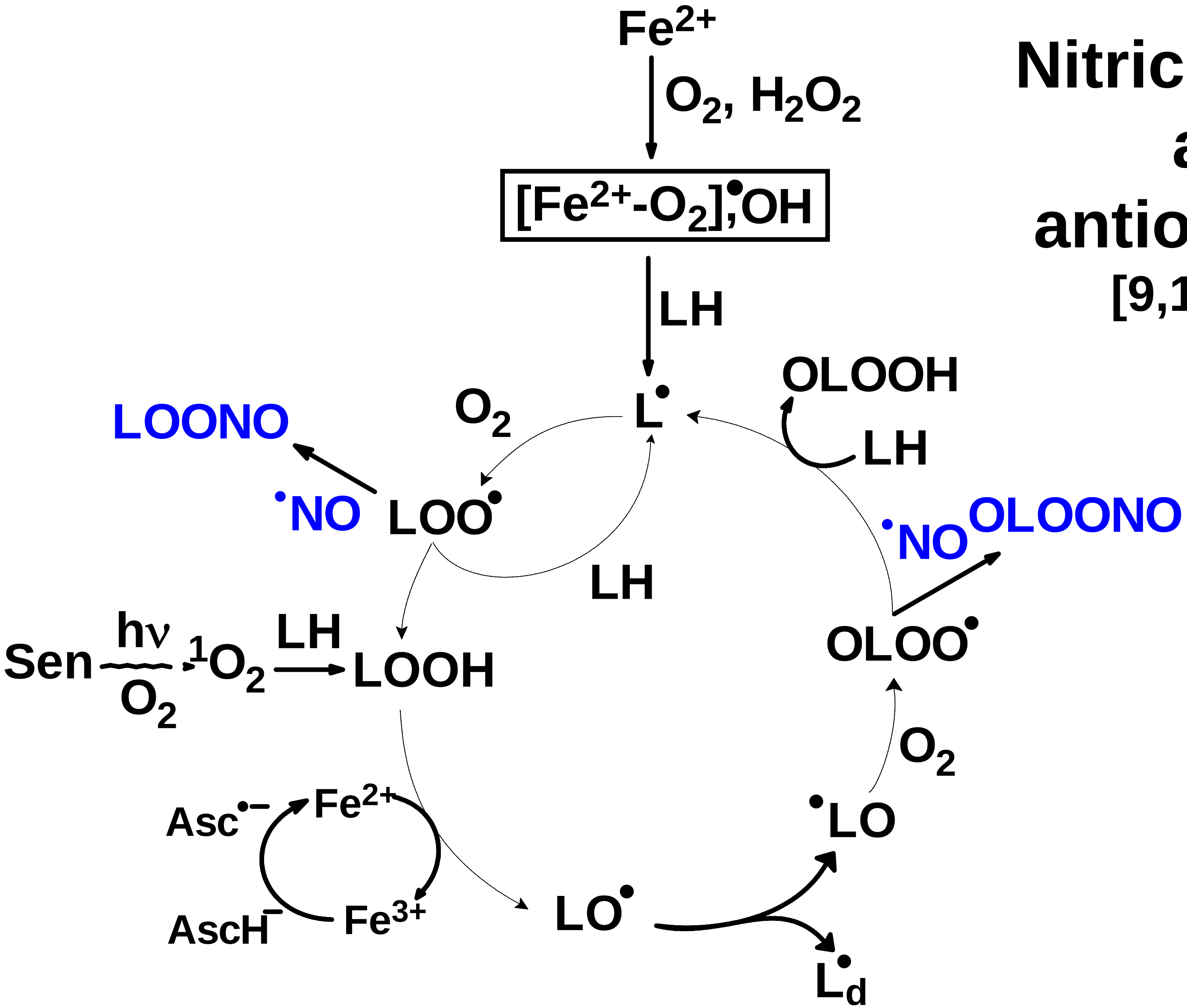


DTPA, DETAPAC

EDTA, Coordination of Fe(III)



Nitric Oxide, an antioxidant
[9,10,11]



Vit E *vs* NO• : Kinetics

$$k_{NO} \approx 2 \times 10^{9}\ M^{-1}s^{-1}$$

$$NO^{\bullet} + LOO^{\bullet} \rightarrow LOONO$$

$$k_{TOH} \approx 10^{5}\ M^{-1}s^{-1}$$

$$TOH + LOO^{\bullet} \rightarrow TO^{\bullet} + LOOH$$

$$\frac{k_{NO} \approx 5 \times 10^{9}\ M^{-1}s^{-1}}{k_{TOH} \approx 10^{5}\ M^{-1}s^{-1}}$$

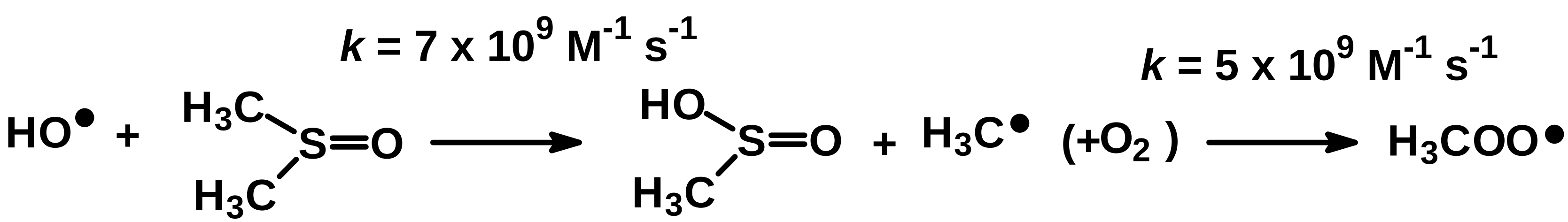
$$k_{NO} / k_{TOH} \approx 10^{4} - 10^{5}$$

Kinetics, rate constants

Many free radical reactions are controlled by second order rate laws:

Rate = *k* [Target]^1 x [Radical]^1

Consider possible reaction of peroxyl radicals, *e.g.* $H_3COO^\bullet$



Peroxyl radical rxns

$H_3COO^\bullet$ + D-H → H_3COOH + $D^\bullet$

k (PUFA) = 1×10^2 M^{-1} s^{-1}
k (GSH) = 2×10^4 M^{-1} s^{-1}
k (TOH) = 1×10^5 M^{-1} s^{-1}
k ($AscH^-$) = 2×10^6 M^{-1} s^{-1}
k ($NO^\bullet$) = 2×10^9 M^{-1} s^{-1} *

* $H_3COO^\bullet$ + $NO^\bullet$ → H_3COONO

Compare rates, not *k*

Rate = k [Target] x [$H_3COO^\bullet$] = k' [$H_3COO^\bullet$]

k (PUFA) = 1×10^{2} M^{-1} s^{-1} [1×10^{-3} M]

k (GSH) = 2×10^{4} M^{-1} s^{-1} [3×10^{-3} M]

k (TOH) = 1×10^{5} M^{-1} s^{-1} [1×10^{-3} M]

k ($AscH^-$) = 2×10^{6} M^{-1} s^{-1} [3×10^{-3} M]

k ($NO^\bullet$) = 2×10^{9} M^{-1} s^{-1} [5×10^{-8} M]

Easiest to compare *k'*

Rate = k [Target] x $[H_3COO^\bullet]$ = k' $[H_3COO^\bullet]$

	k		k'
k (PUFA) =	$1 \times 10^{2}\ M^{-1}\ s^{-1}$	$[1 \times 10^{-3}\ M]$ =	$1 \times 10^{-1}\ s^{-1}$
k (GSH) =	$2 \times 10^{4}\ M^{-1}\ s^{-1}$	$[3 \times 10^{-3}\ M]$ =	$2 \times 10^{1}\ s^{-1}$
k (TOH) =	$1 \times 10^{5}\ M^{-1}\ s^{-1}$	$[1 \times 10^{-3}\ M]$ =	$1 \times 10^{2}\ s^{-1}$
k ($AscH^-$) =	$2 \times 10^{6}\ M^{-1}\ s^{-1}$	$[3 \times 10^{-3}\ M]$ =	$2 \times 10^{3}\ s^{-1}$
k ($NO^\bullet$) =	$2 \times 10^{9}\ M^{-1}\ s^{-1}$	$[5 \times 10^{-8}\ M]$ =	$2 \times 10^{1}\ s^{-1}$

Fun, but serious science

Free Radicals and related oxidants. Understanding their reactions assists in understanding their biology.

Future: The need is to progress to more quantitative approaches in the biology of free radicals, related oxidants, and antioxidants, *i.e. quantitative redox biology*.

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