WHAT ARE FREE RADICALS?

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Sunrise Free Radical School Free Radical Biology: Back to the Basics
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What Are Free Radicals?

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Free Radical?

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

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

Historically?

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

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

k = 4.9 x 10^9 M^{-1} s^{-1}

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

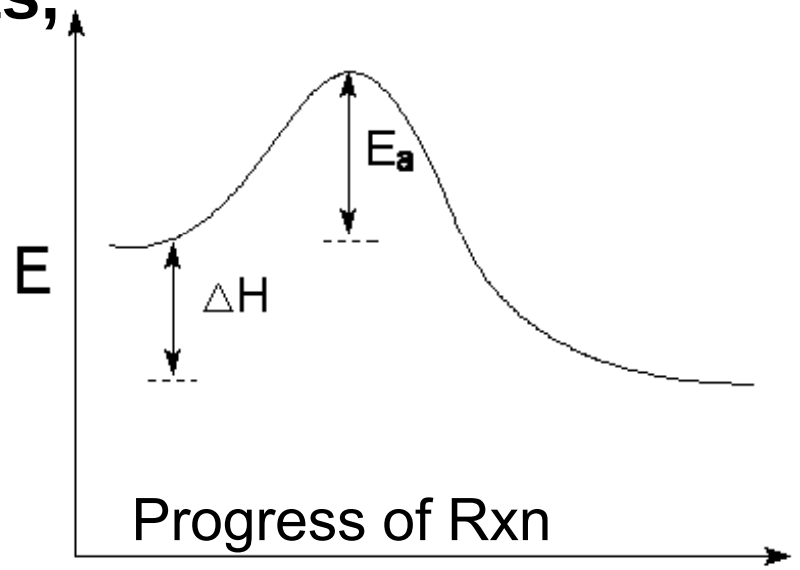
Radical + Radical rxns typically very fast

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]

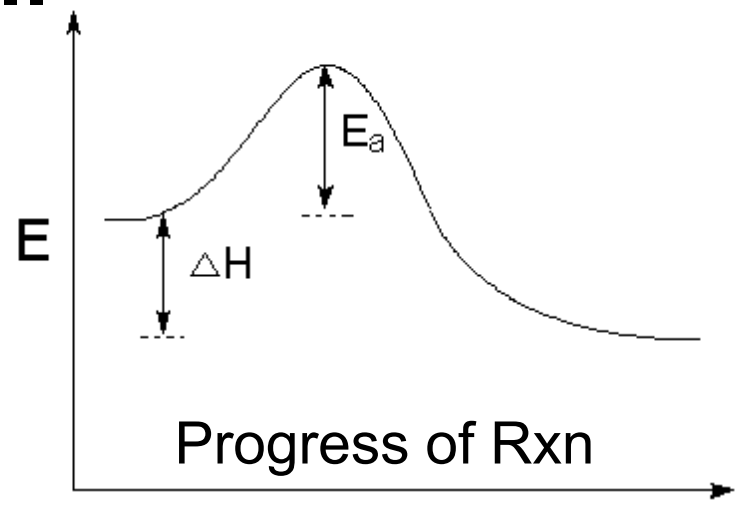
1. Can orbitals overlap to form a reasonable transition state?
2. Activation energy of oxygen!
3. $E_a \geq 23$ kcal/mole for $^3O_2$ reactions, *i.e.* $^1O_2$
4. $^3O_2$ + ^{1}(carbon) $\longrightarrow$ Products, but very slow!



Why Does Ground State O_2 React so Fast with Many Radicals?

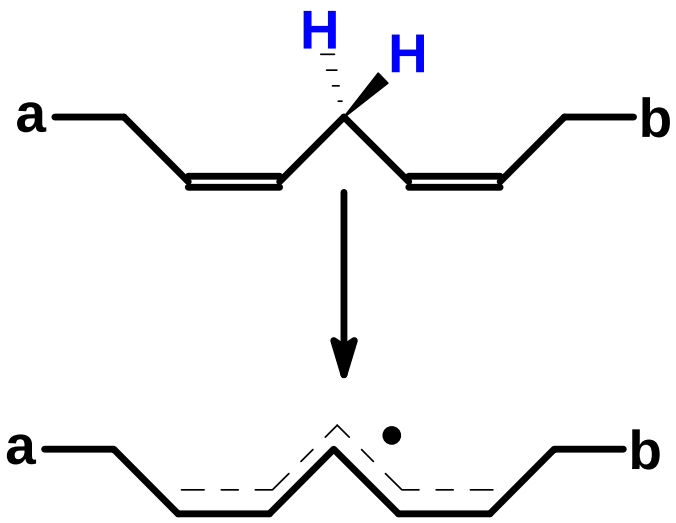
There is no spin restriction [4].

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

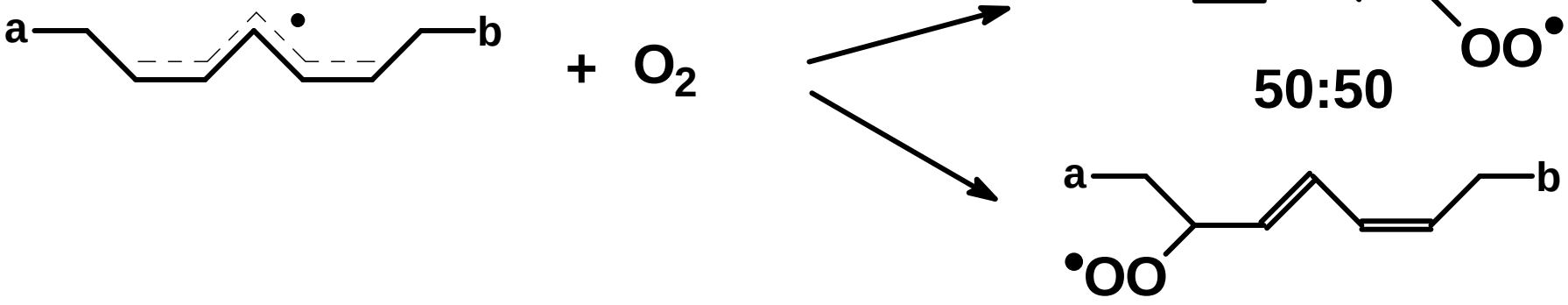
Slow, $k \approx 50\ M^{-1}\ s^{-1}$ (for the *bis*-allylic hydrogens)

***It is only the PUFA in lipids that are oxidizable.**
Oxidizability ∝ number of double bonds [5]

Example Rxns 2

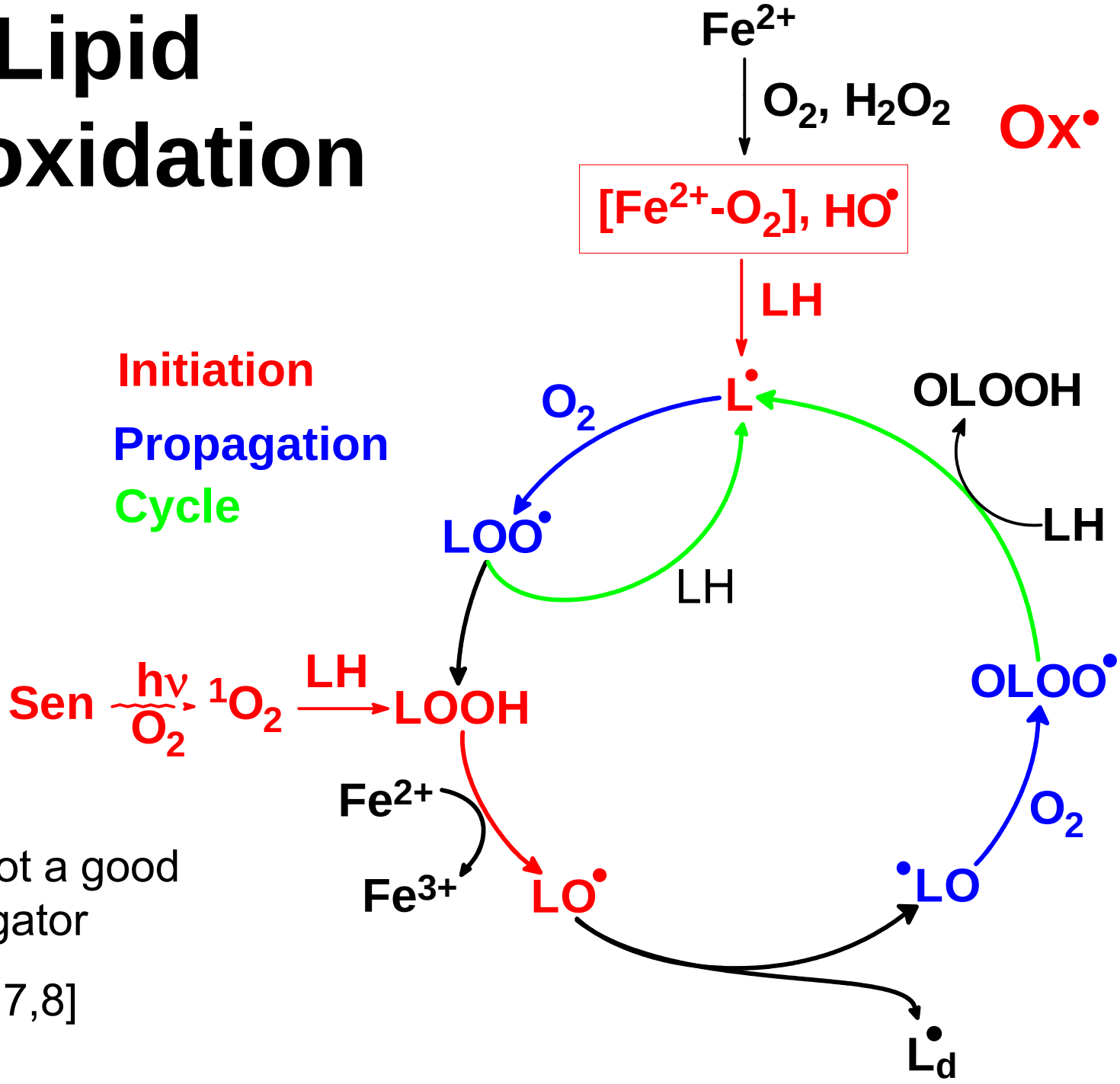
$H_3C^{\bullet} + O_2 \rightarrow H_3COO^{\bullet}$ $\quad k = 4.9 \times 10^9\ M^{-1}\ s^{-1}$

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



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

Lipid Peroxidation



LO• not a good propagator

Ref [6,7,8]

Kinetics rule

The competition

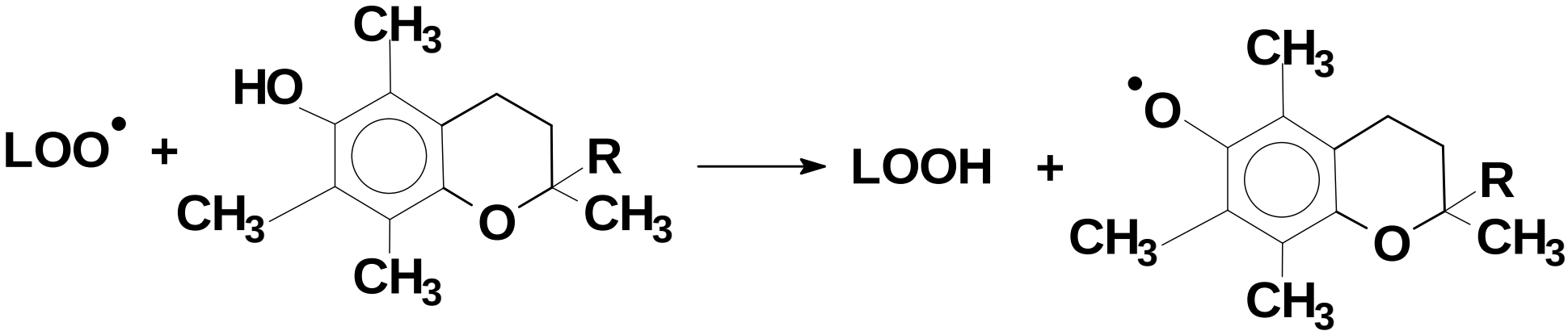
$$L^{\bullet} + O_2 \xrightarrow{3 \times 10^8\ M^{-1} s^{-1}} LOO^{\bullet}$$ ***Propagation***

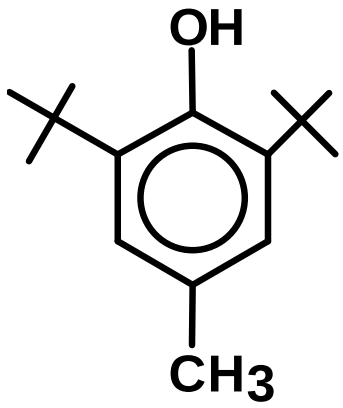
$$LOO^{\bullet} + L\text{-}H \xrightarrow{10 - 50\ M^{-1} s^{-1}} L^{\bullet} + LOOH$$ ***Cycle***

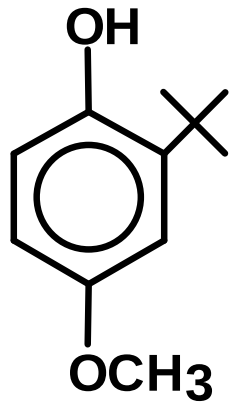
Vitamin E

$$LOO^{\bullet} + TOH \xrightarrow{\approx 10^5\ M^{-1} s^{-1}\ \text{in membranes or LDL}} TO^{\bullet} + L\text{-}H$$

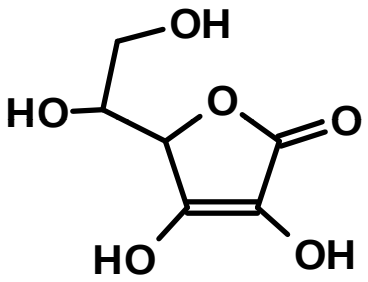
Tocopherol in Action



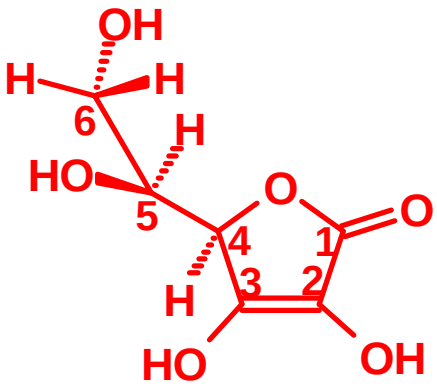




Ascorbate a Donor Antioxidant

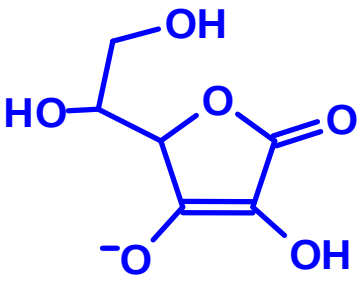


$AscH_2$



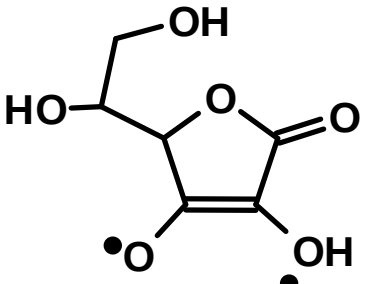
Ascorbic Acid

$+H^+$ $-H^+$ $pK = 4.1$



$AscH^-$

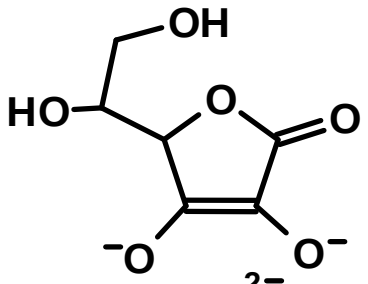
$-e^-$



$AscH^{\bullet}$

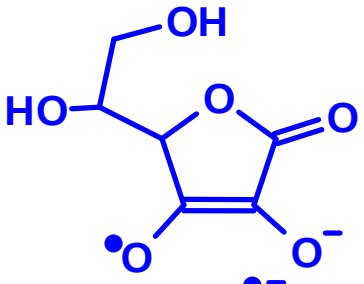
$+H^+$ $-H^+$ $pK = 11.8$

$+H^+$ $-H^+$ $pK = -0.86$



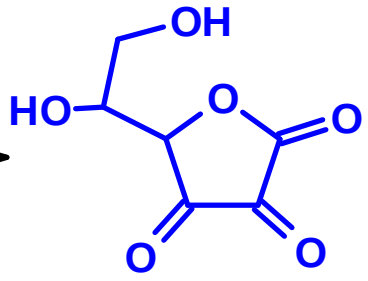
Asc^{2-}

$-e^-$



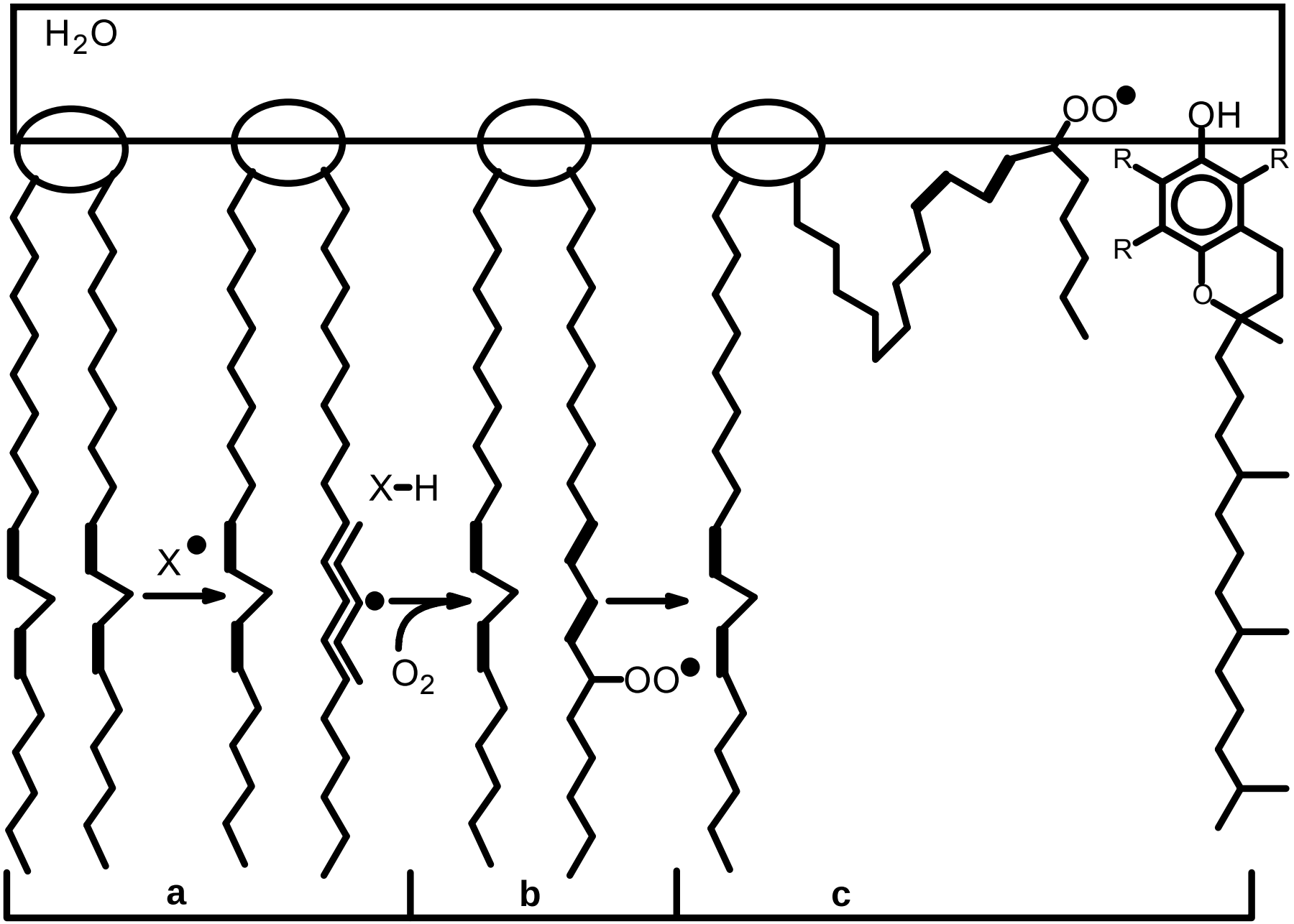
$Asc^{\bullet -}$

$-e^-$

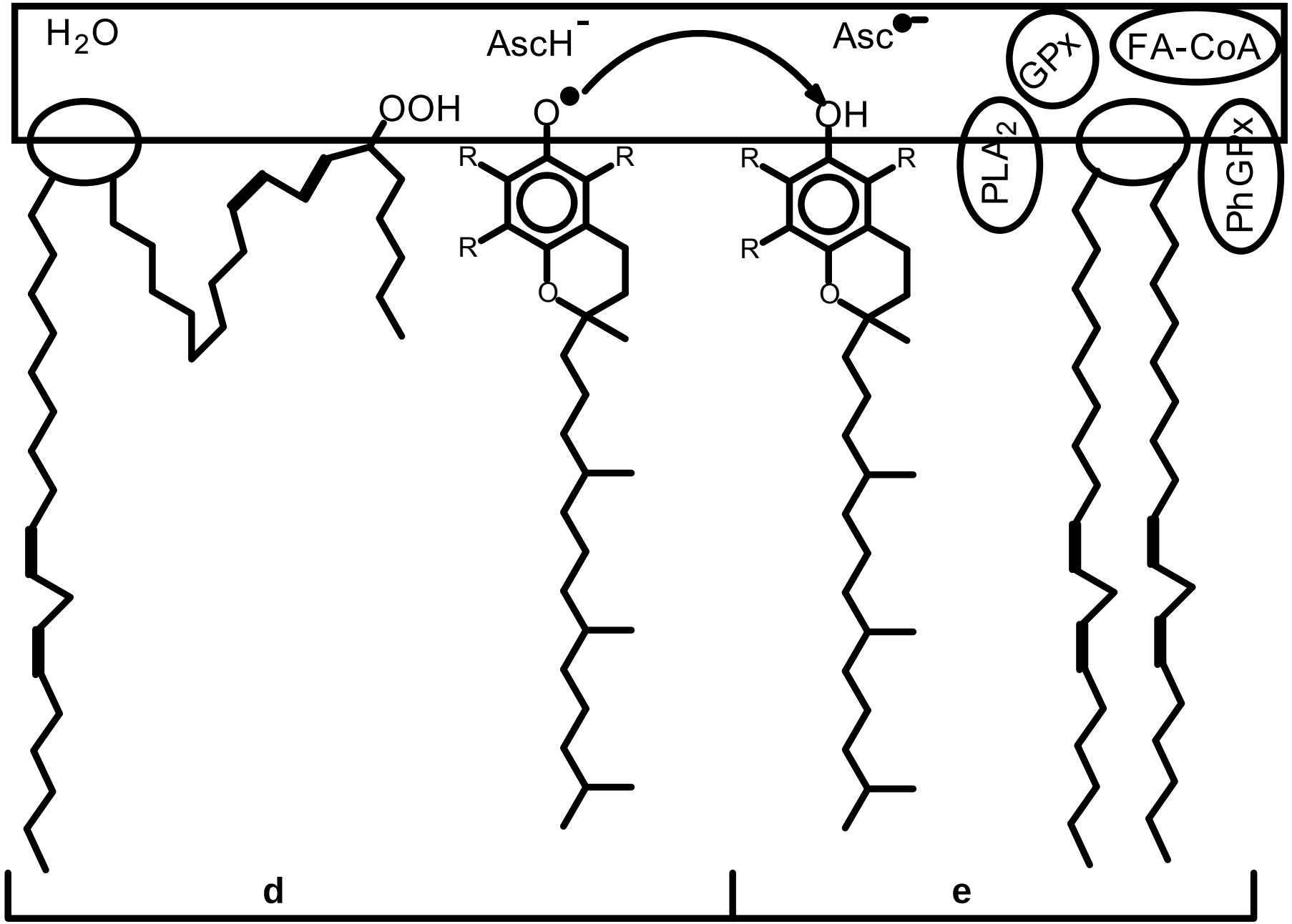


DHA

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 [9]

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
$O_2/ O_2^{\bullet-}$	- 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
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$Asc^{\bullet-}$, $H^{+}/AscH^{-}$	**+ 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
$HO^{\bullet}$, 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

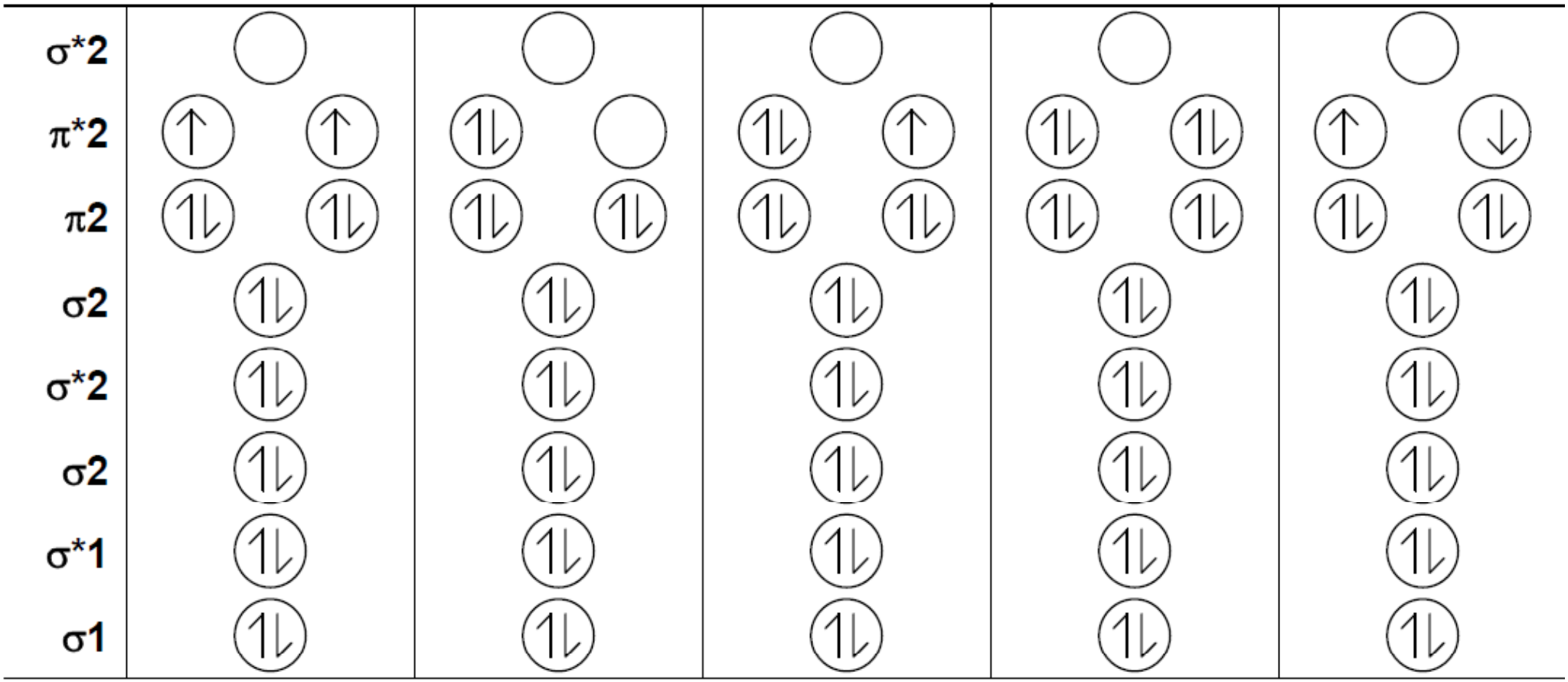
Trouble, Trouble, Trouble …

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, $HO^{\bullet}$.

A closer look at some of these radicals and related ROS



Singlet Oxygen

Singlet oxygen, $^1O_2$ or $^1\Delta_g O_2$ ($t_{1/2}$ = 3/40 μs in H_2O/D_2O)

Member of Reactive Oxygen Species (ROS) family

But, not a free radical (extra energy)

Electrophilic

β-carotene (physical quenching) $k \approx 10^{10}\ M^{-1}\ s^{-1}$

Cys-SH $k \approx 10^6\ M^{-1}\ s^{-1}$; Cys-$S^-$ $k \approx 10^8\ M^{-1}\ s^{-1}$

Cys-SOOH → products

$^1O_2$ + PUFA → PUFA-OOH $k \approx 10^5\ M^{-1}\ s^{-1}$

Hydroxyl Radical

pK_a ($HO^{\bullet} \leftrightarrow O^{\bullet -} + H^+$) = 11.9

Reaction rate constants with nearly all organics are in the range of $k = 10^9 – 10^{10}$ M^{-1} s^{-1};

High electron density sites → 10^{10} M^{-1} s^{-1}
Low electron density → 10^9 M^{-1} s^{-1}

These high reactivities and the lack of selectivity make it a poor species to initiate specific signaling pathways.

Superoxide

pK_a ($HO_2^\bullet$ ←→ $O_2^{\bullet-}$ + H^+) = 4.8

$O_2/O_2^{\bullet-}$ E° ’ = -160 mV; $HO_2^\bullet,H^+/H_2O_2$ E° ’ = +1060 mV

Rxn with only a very, very limited set of organics making a covalent bond -- spin traps, DHE-radical.

$O_2^{\bullet-}$ + $NO^\bullet$ → O=NOOH → NO_3^- + H^+

(-1/2,-1/2) (+2,-2) (-2, +3,-1,-1,+1) (+5,-2)

Rxn with transition metals, k = 0 – 10^9 M^{-1} s^{-1}; don’t forget, $O_2^{\bullet-}/HO_2^\bullet$ can reduce or oxidize metals, but very selective.

These reactivities, or lack thereof, make it selective and ideal for signaling

Hydrogen Peroxide

pK_a (H_2O_2 ←→ HO_2^- + H^+) = 11.6

Reaction rate constants with nearly all organics are very small;

$DCFH_2$, DHE, DFH_2 + H_2O_2 → essentially no rxn [10]

DNA + H_2O_2 → essentially no rxn [11]

Cys-SH + H_2O_2 $k \sim 10^1\ M^{-1}\ s^{-1}$;

For most oxidative reactions of H_2O_2 in biology, metals (iron) are involved in its "activation", *e.g.*

Fe, in heme peroxidases, labile iron, . . .

Fe^{2+} - O_2 – H_2O_2

Activation of iron through Fenton Rxn is classically thought[2b]

$Fe(III)L_n + O_2^{\bullet -} \longrightarrow Fe(II)L_n + O_2$ then Fenton Rxn:

$Fe(II)L_n + H_2O_2 \longrightarrow HO^{\bullet} + Fe(III)L_n + OH^-$

How about?

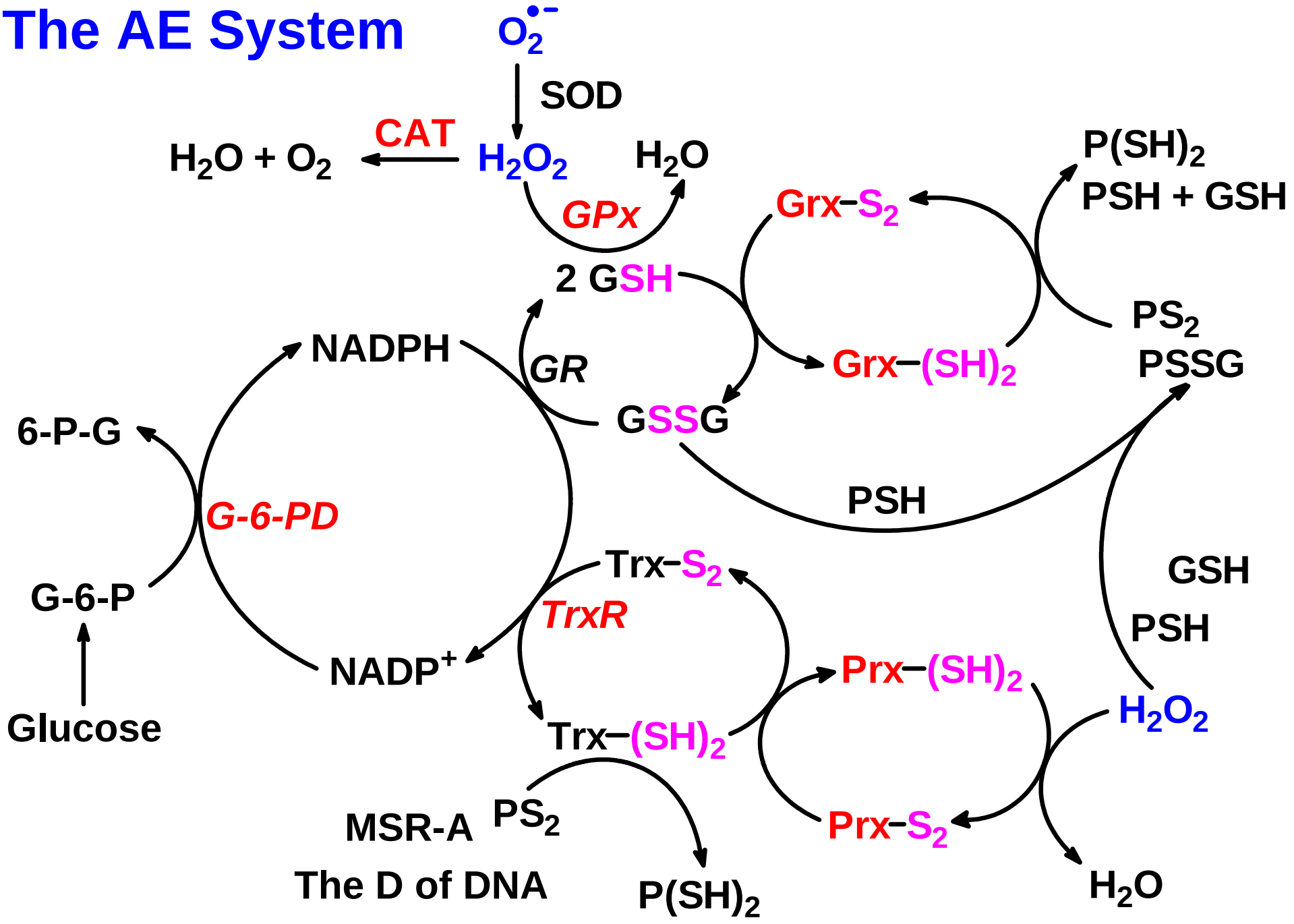
$Fe(III)L_n + AscH^- \longrightarrow Fe(II)L_n + Asc^{\bullet -}$

Or -- how about?

$Fe(II) + O_2 \longrightarrow$ Oxidants, a route often overlooked

An important role of $O_2^{\bullet -}$ is rather as an oxidant of Fe^{2+}, not a reductant for Fe^{3+}, releasing iron, *e.g.* aconitase.

The AE System



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