# WHAT ARE FREE RADICALS?


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# What Are Free Radicals?

## Sunrise Free Radical School

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**Garry R. Buettner, Ph.D.**

**Free Radical & Radiation Biology and**
**ESR Facility**
**The University of Iowa**
**Iowa City, IA 52242-1101**

# 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, $\sigma$**

**pi-delocalized, $\pi$**

**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 $10^9$ $M^{-1}$ $s^{-1}$

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

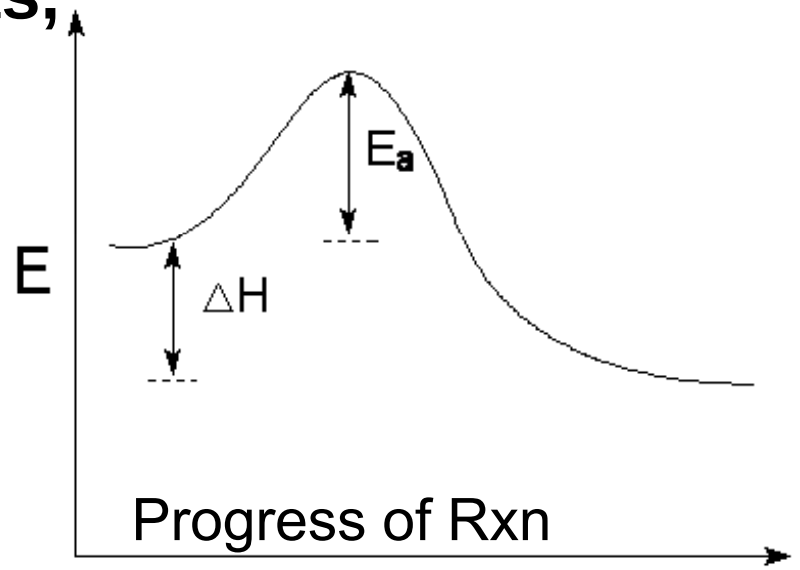
**Radical + Radical rxns typically very fast**

**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]

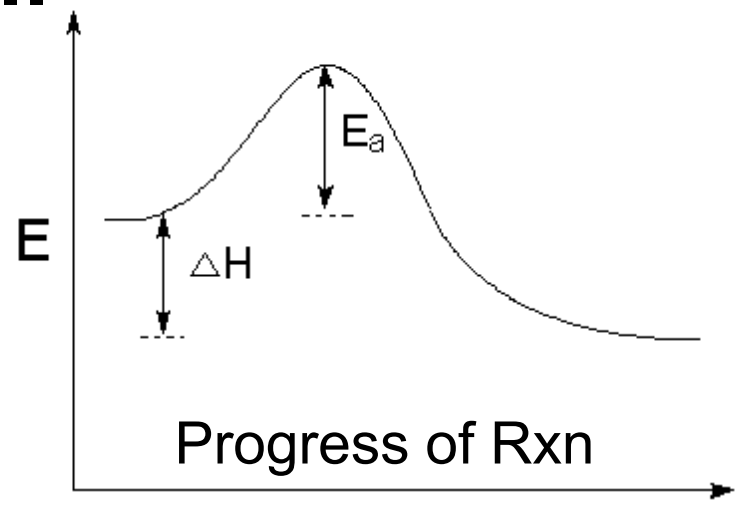
1. Can orbitals overlap to form a reasonable transition state?
2. Activation energy of oxygen!
3. $E_a \geq 23$ kcal/mole for $^3O_2$ reactions, *i.e.* $^1O_2$
4. $^3O_2$ + $^1$(carbon) $\longrightarrow$ Products, but very slow!



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

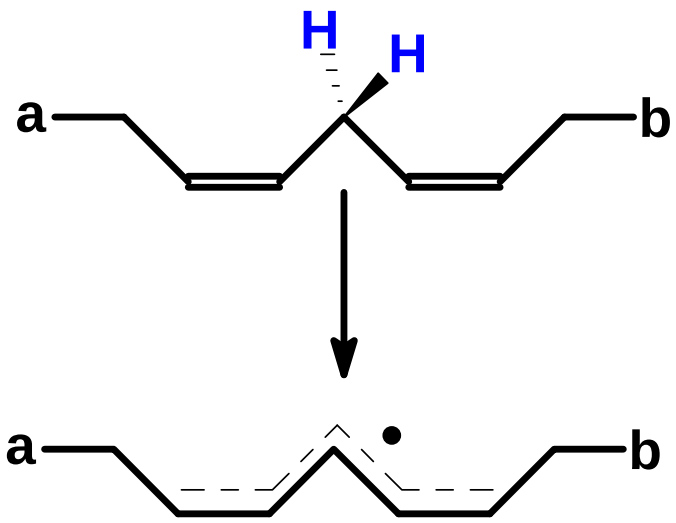
## There is no spin restriction [4].

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**

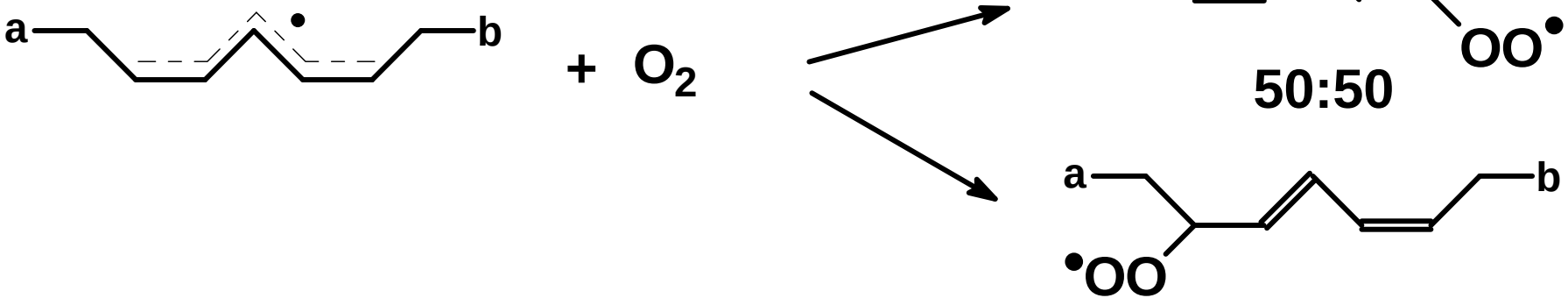
**Slow, $k \approx 50\ M^{-1}\ s^{-1}$ (for the *bis*-allylic hydrogens)**

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

# Example Rxns 2

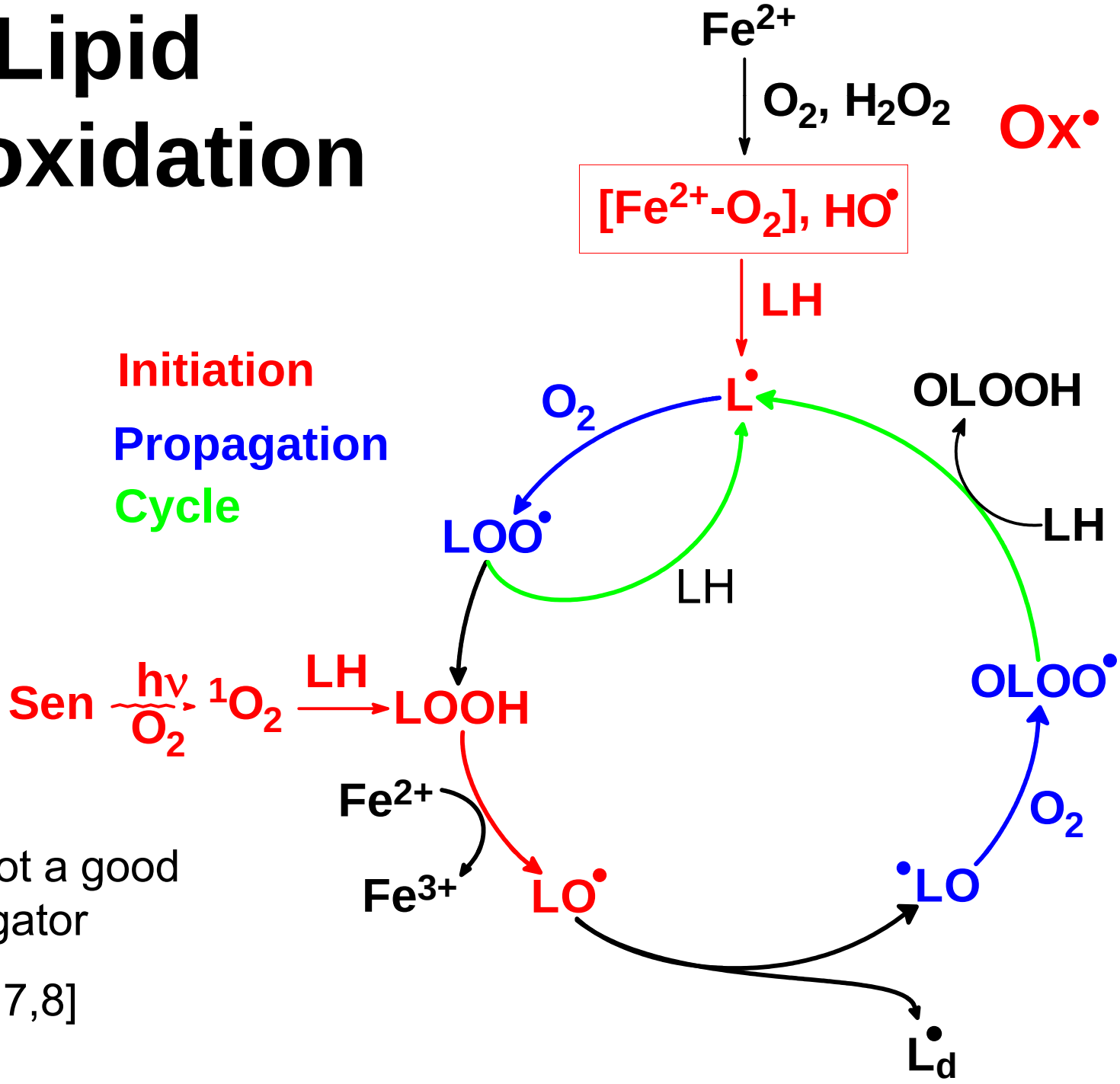
$H_3C^{\bullet} + O_2 \rightarrow H_3COO^{\bullet}$ $\quad k = 4.9 \times 10^9\ M^{-1}\ s^{-1}$

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




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

# Lipid Peroxidation




LO• not a good propagator

Ref [6,7,8]

# Kinetics rule

## The competition

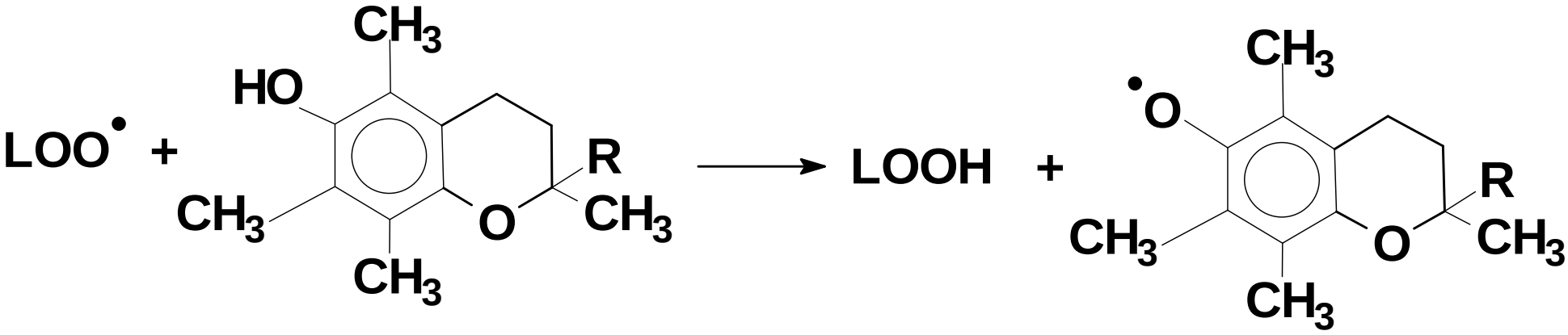
$$L^{\bullet} + O_2 \xrightarrow{3 \times 10^8\ M^{-1} s^{-1}} LOO^{\bullet}$$ ***Propagation***

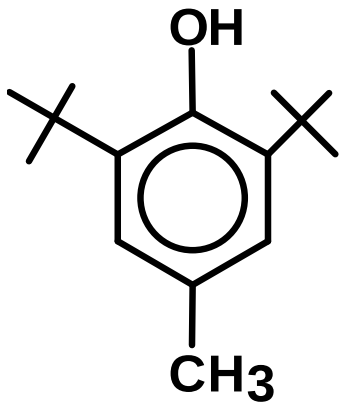
$$LOO^{\bullet} + L\text{-}H \xrightarrow{10 - 50\ M^{-1} s^{-1}} L^{\bullet} + LOOH$$ ***Cycle***

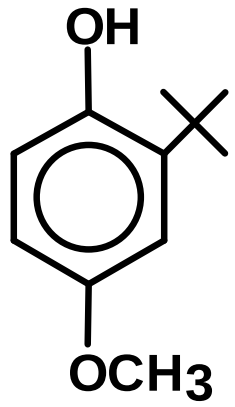
## Vitamin E

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

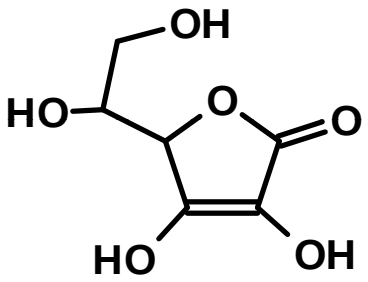
# Tocopherol in Action



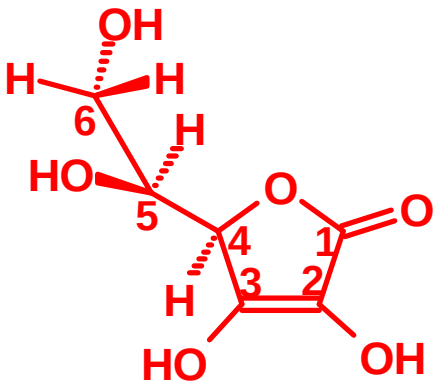



# Ascorbate a Donor Antioxidant

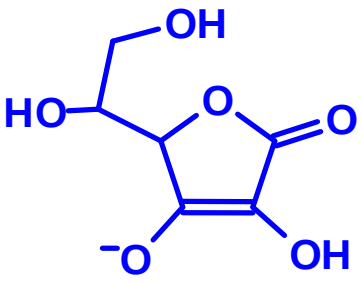



$AscH_2$



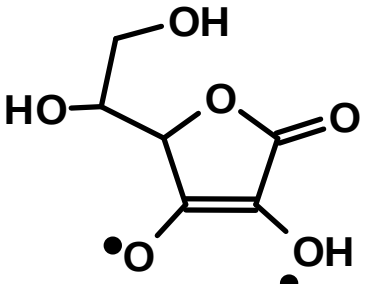

Ascorbic Acid

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




$AscH^-$

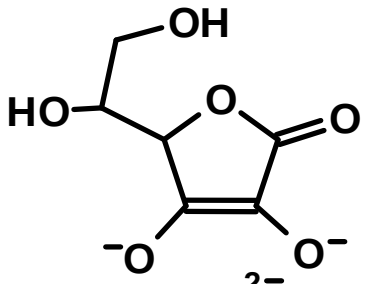
$-e^-$




$AscH^{\bullet}$

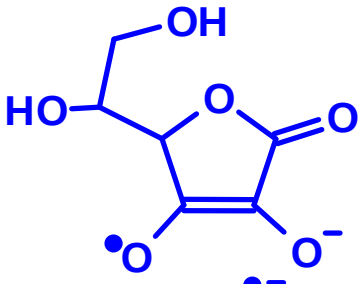
$+H^+$ $-H^+$ $pK = 11.8$

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



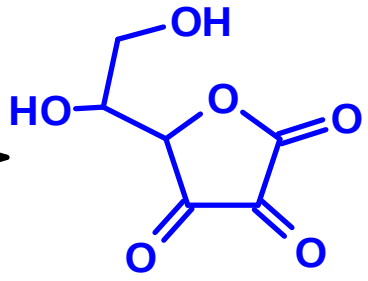

$Asc^{2-}$

$-e^-$



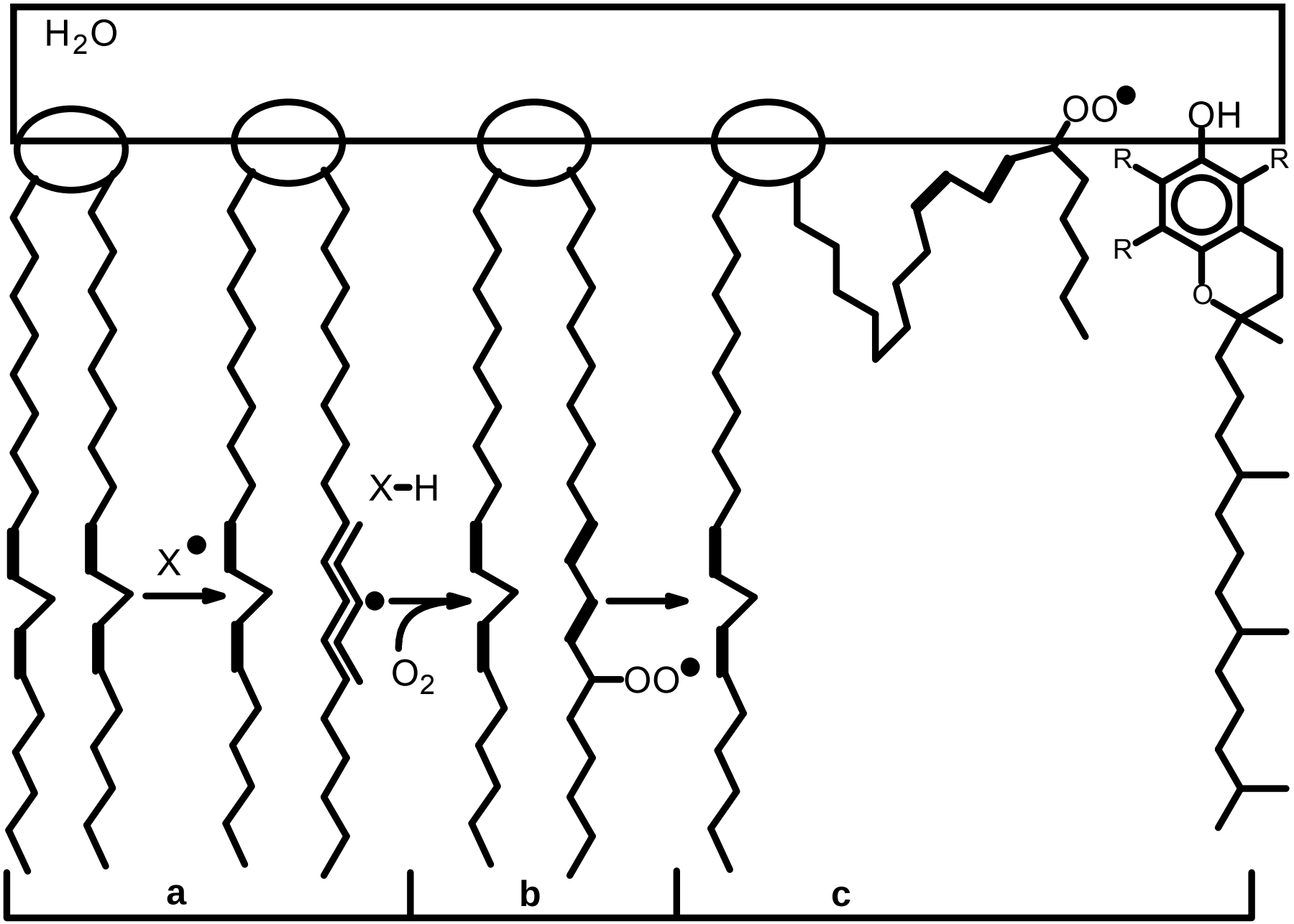

$Asc^{\bullet -}$

$-e^-$

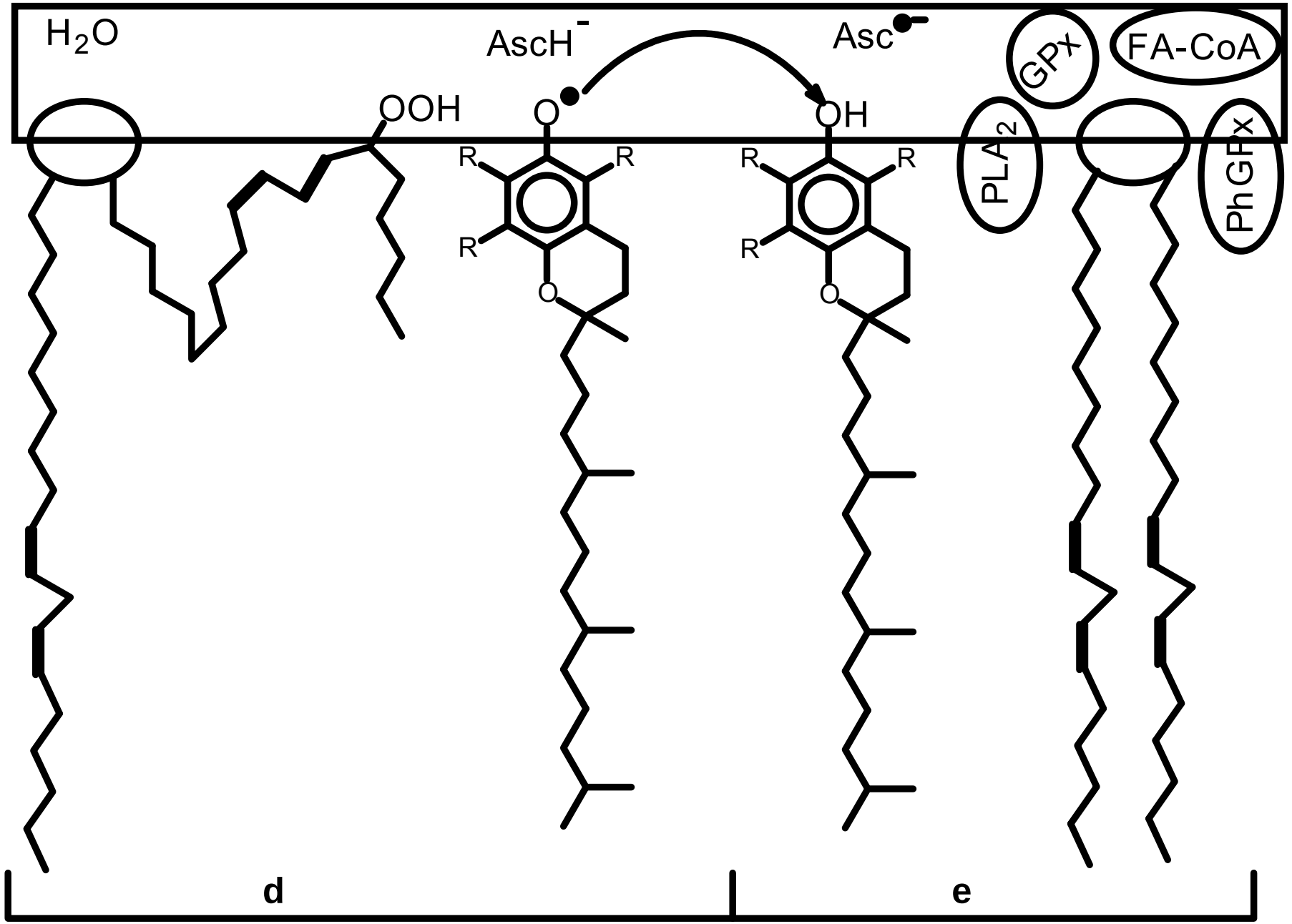



DHA

# 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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| **$CoQH^{\bullet}$, $H^{+}/CoQH_2$** | **+ 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 |

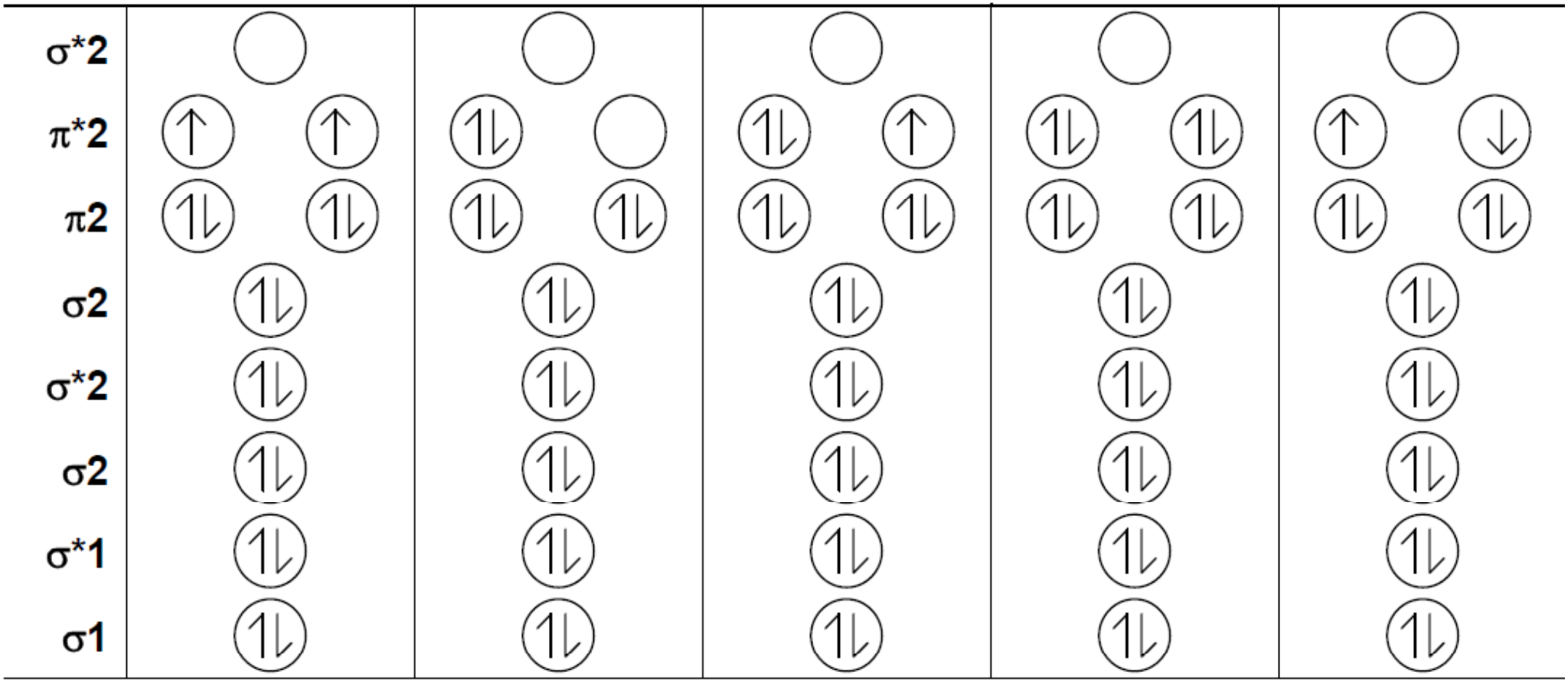
# Trouble, Trouble, Trouble …

**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

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

p$K_a$ ($H_2O_2$ ←→ $HO_2^-$ + $H^+$) = 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?**

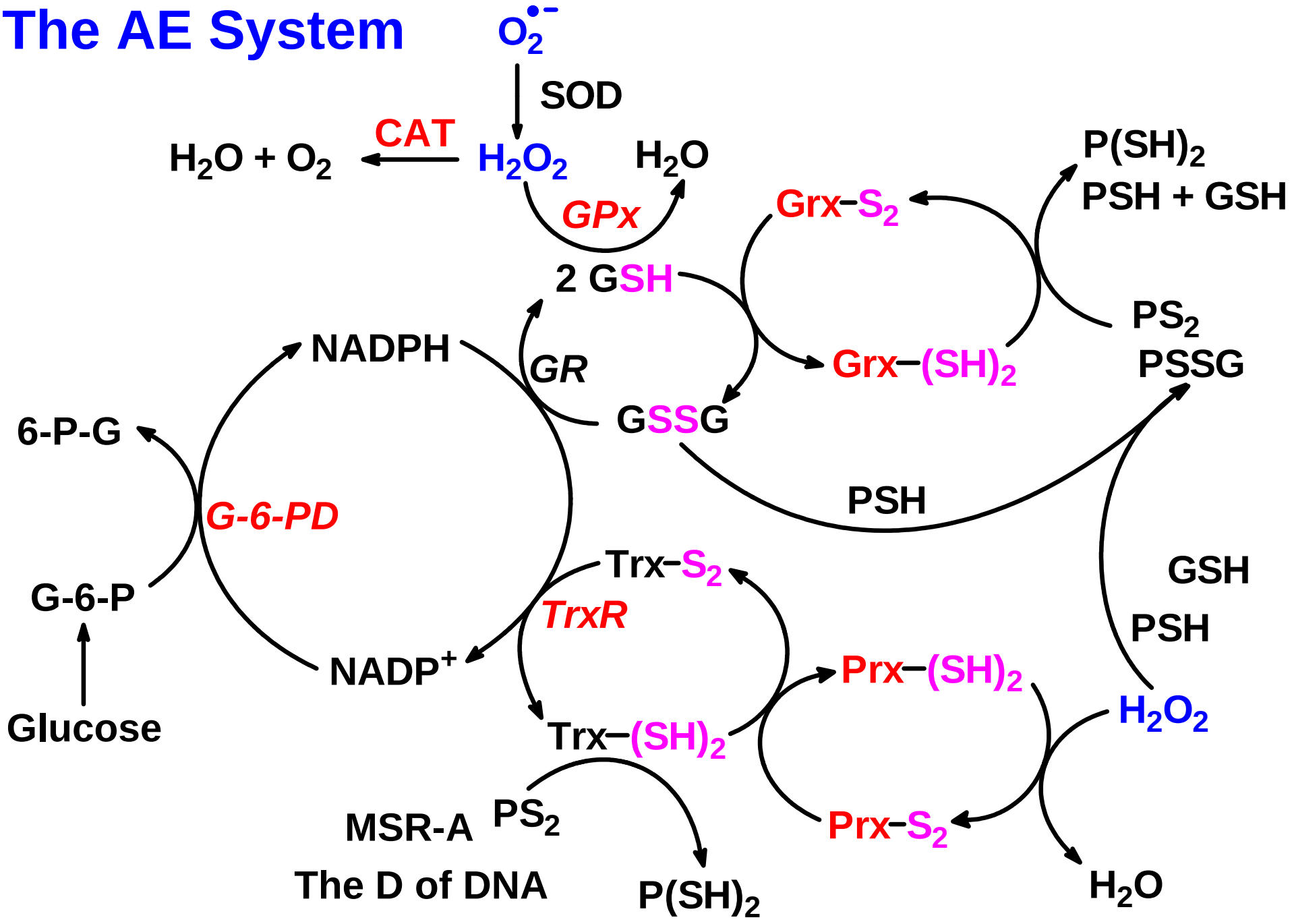
**$Fe(III)L_n + AscH^- \longrightarrow Fe(II)L_n + Asc^{\bullet -}$**

**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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