What is an antioxidant?
How do antioxidants work?

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Antioxidants, the road ahead

This presentation focuses on the action/reaction of small molecule antioxidants.

1. **Overview and vocabulary**
2. **Preventive**
3. **Chain-breaking**
4. **Retarder *vs* true antioxidant**

Antioxidants: A definition

A substance when present in trace (small) amounts inhibits oxidation of the bulk.

OR

A little bit goes a long way.

So, what is a little bit?

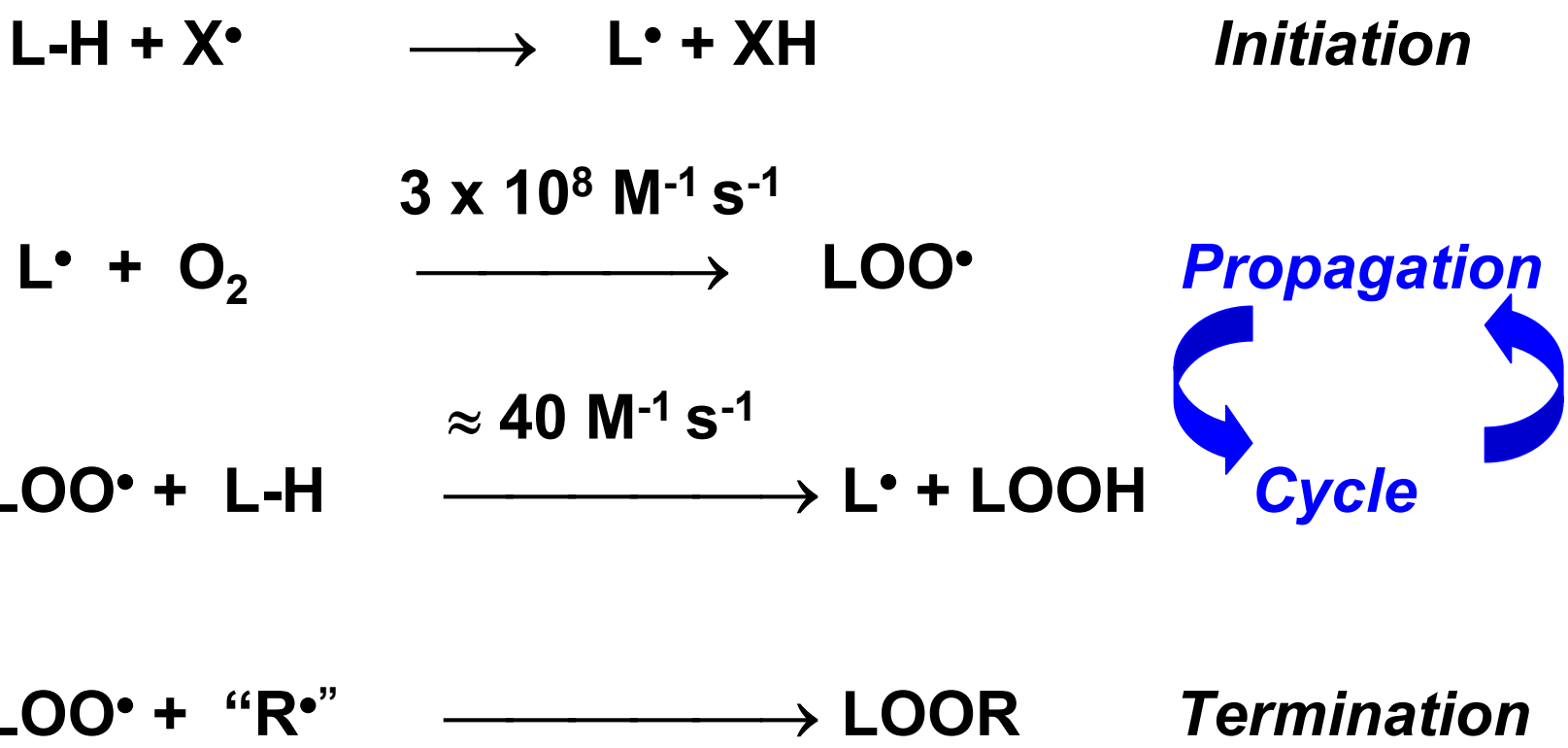
Antioxidants: two broad classes

Preventive and Chain-Breaking

Preventive antioxidants intercept oxidizing species before damage can be done.

Chain breaking antioxidants slow or stop oxidative processes after they begin, by intercepting the chain-carrying radicals.

Elementary Lipid Peroxidation



Preventive Antioxidants

Don’t let it get started.

Preventive antioxidants act by:

A. Deactivating metals, *e.g.* transferrin, ferritin, Desferal, DETAPAC, EDTA, …

B. Removing hydroperoxides, *e.g.* catalase, glutathione peroxidases, pyruvate, …

C. Quenching singlet oxygen, *e.g.* β-carotene, lycopene, bilirubin, …

Preventive Antioxidants:
Targeting Metals

Fe & Cu are the principal metals targeted– loosely bound*

Proteins & metals –

Transferrin / Hemoglobin / Ceruloplasmin

Chelates –

Fe^{3+} – EDTA, DETAPAC (DTPA), Desferal

Fe^{2+} – Phenanthrolines, …

*** "Loosely" bound iron on proteins, DNA as well as iron in hemes can be dangerous.**

Preventive Antioxidants:
Why target metals?

Because they promote oxidant production.

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

or

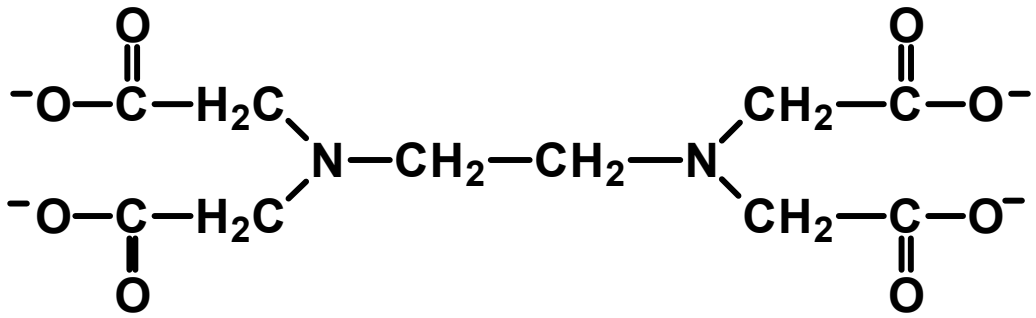
$$Fe(II)chelate + LOOH \rightarrow LO^{\bullet} + Fe(III)chelate + LOH$$

and

$$Fe(II)chelate + O_2 \rightarrow Oxidants^a$$

[a] Qian SY, Buettner GR. (1999) Iron and dioxygen chemistry is an important route to initiation of biological free radical oxidations: An electron paramagnetic resonance spin trapping study.*Free Radic Biol Med*, **26**: 1447-1456.

Metal Deactivation



EDTA Ethylenediaminetetraacetic acid anion

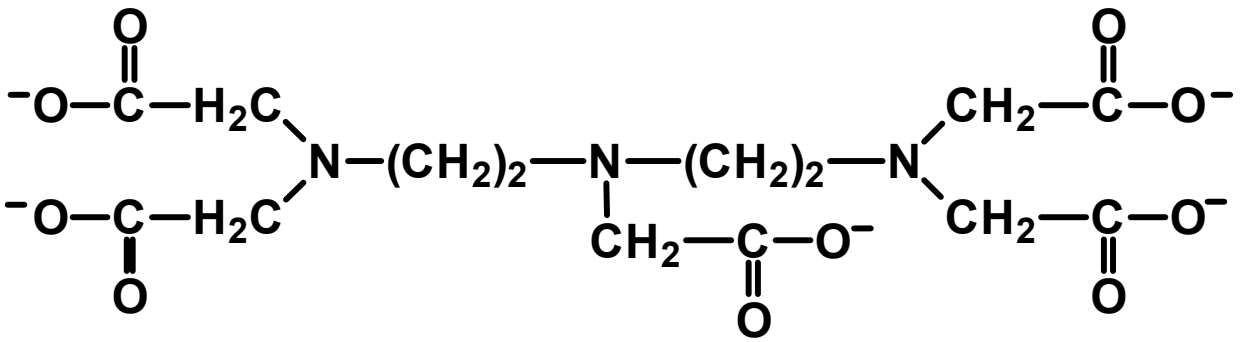
Fe(III)/Fe(II)

EDTA

E = + 120 mV

Rxn with $O_2^{\bullet-}$

k **= 10^6 M^{-1} s^{-1}**



DETAPAC Diethylenetriaminepentaacetic acid anion

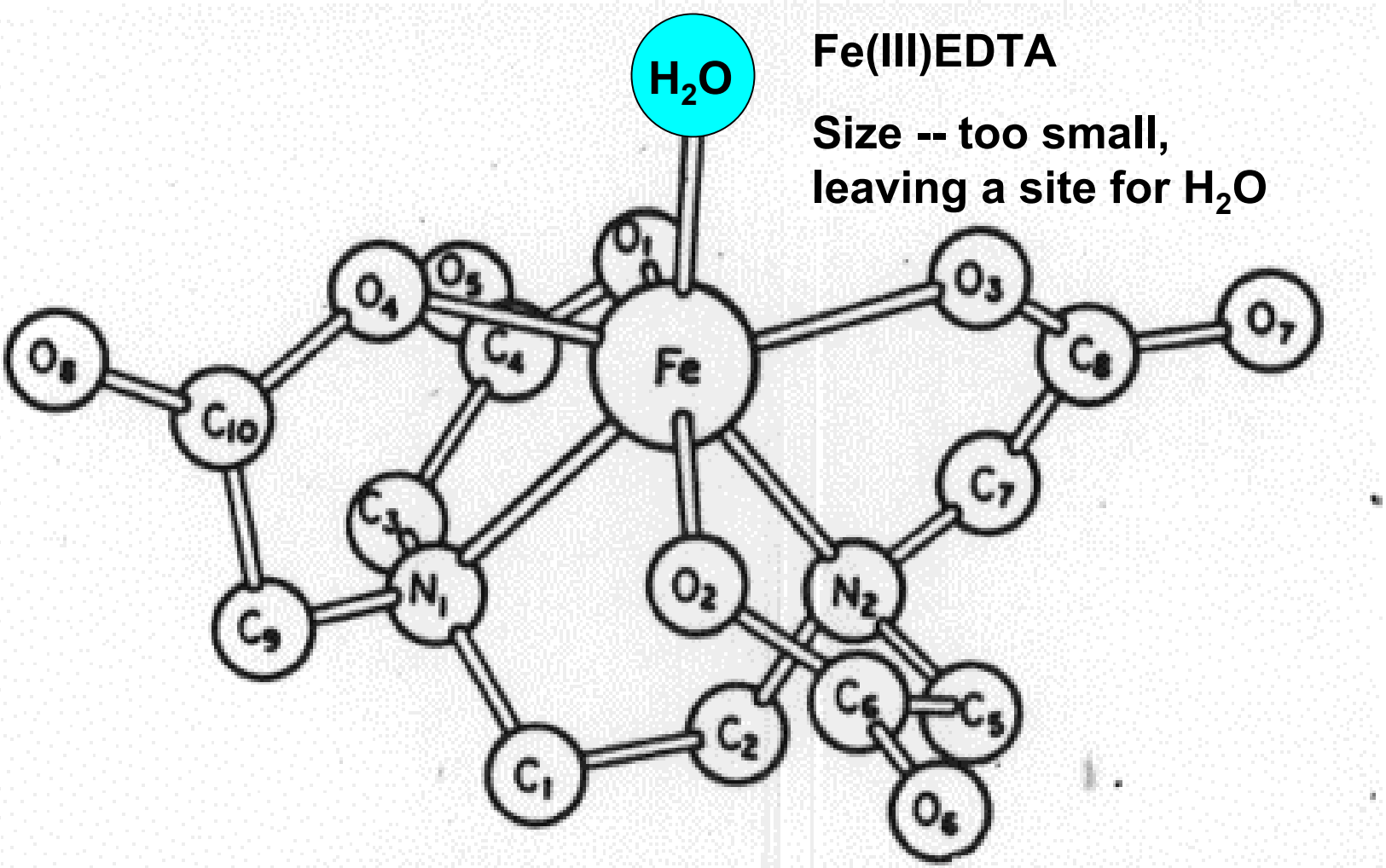
DETAPAC

E = + 30 mV

Rxn with $O_2^{\bullet-}$

k **< 10^2 M^{-1} s^{-1}**

Metal Deactivation: Why the difference?



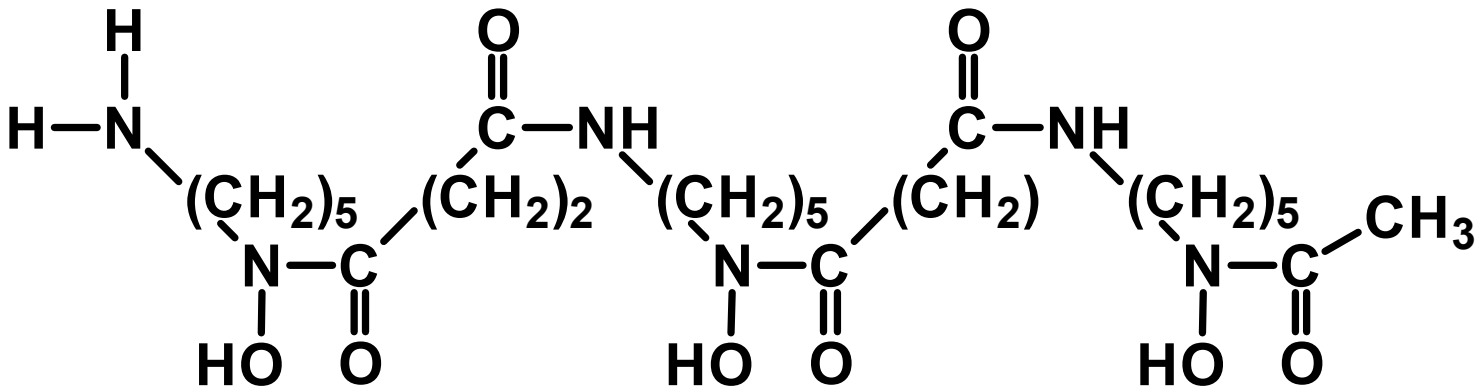
Metal Deactivation: Desferal®

$E^{\circ\prime}$ (Fe(III)DFO/Fe(II)DFO) = - 450 mV

$K_{stability}$ Fe(III) $\approx 10^{30.6}$ k (with $O_2^{\bullet -}$) $< 10^3$ M^{-1} s^{-1}

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

De-activates Fe(III) kinetically (no H_2O of coordination) and thermodynamically.



Deferrioximine

Preventive antioxidants act by:

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B. Removing hydroperoxides, *e.g.* catalase, glutathione peroxidases, pyruvate, …

C. Quenching singlet oxygen, *e.g.* β-carotene, lycopene, bilirubin, …

Enzymes targeting peroxides: H_2O_2 , LOOH

Catalase: $2H_2O_2 \rightarrow 2H_2O + O_2$

GPx (GPx1): $H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$ or

$ROOH + 2GSH \rightarrow H_2O + ROH + GSSG$

PhGPx (GPx4): $PLOOH + 2GSH \rightarrow PLOH + GSSG + H_2O$

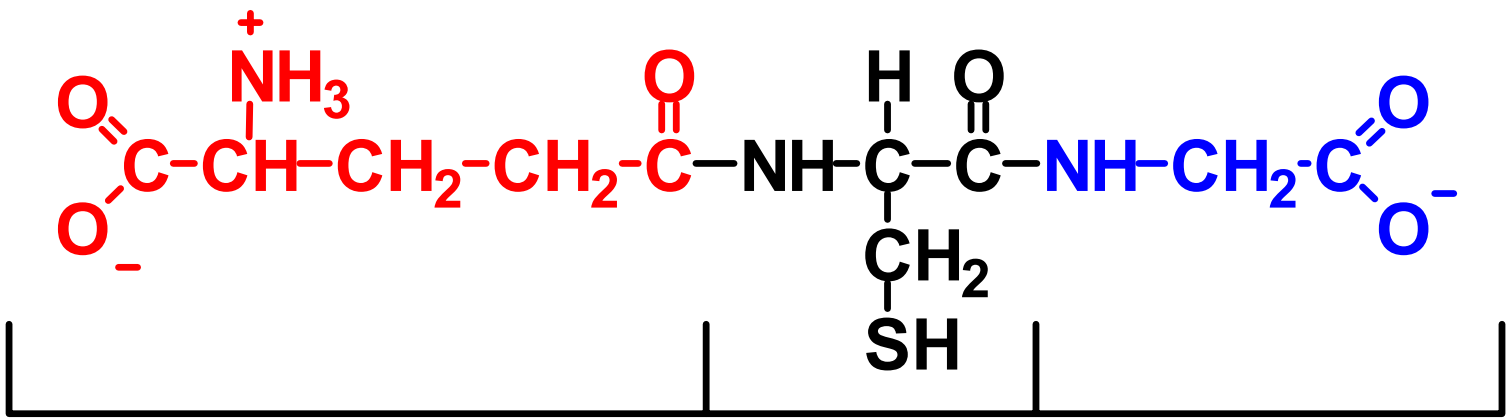
Prx (peroxidredoxins): $H_2O_2 + Trx(SH)_2 \rightarrow 2H_2O + Trx(SS)$

1-cysPrx: $PLOOH + 2GSH \rightarrow PLOH + GSSG + H_2O$

Non-enzymatic rxns $H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$ or

$ROOH + 2GSH \rightarrow H_2O + ROH + GSSG$

Glutathione (GSH)



Glutathione is a tri-peptide

Preventive Antioxidants:
Removing Hydroperoxides

GSH will react directly with H_2O_2,

albeit very slowly.

$2\ GSH + H_2O_2 \rightarrow 2\ H_2O + GSSG$

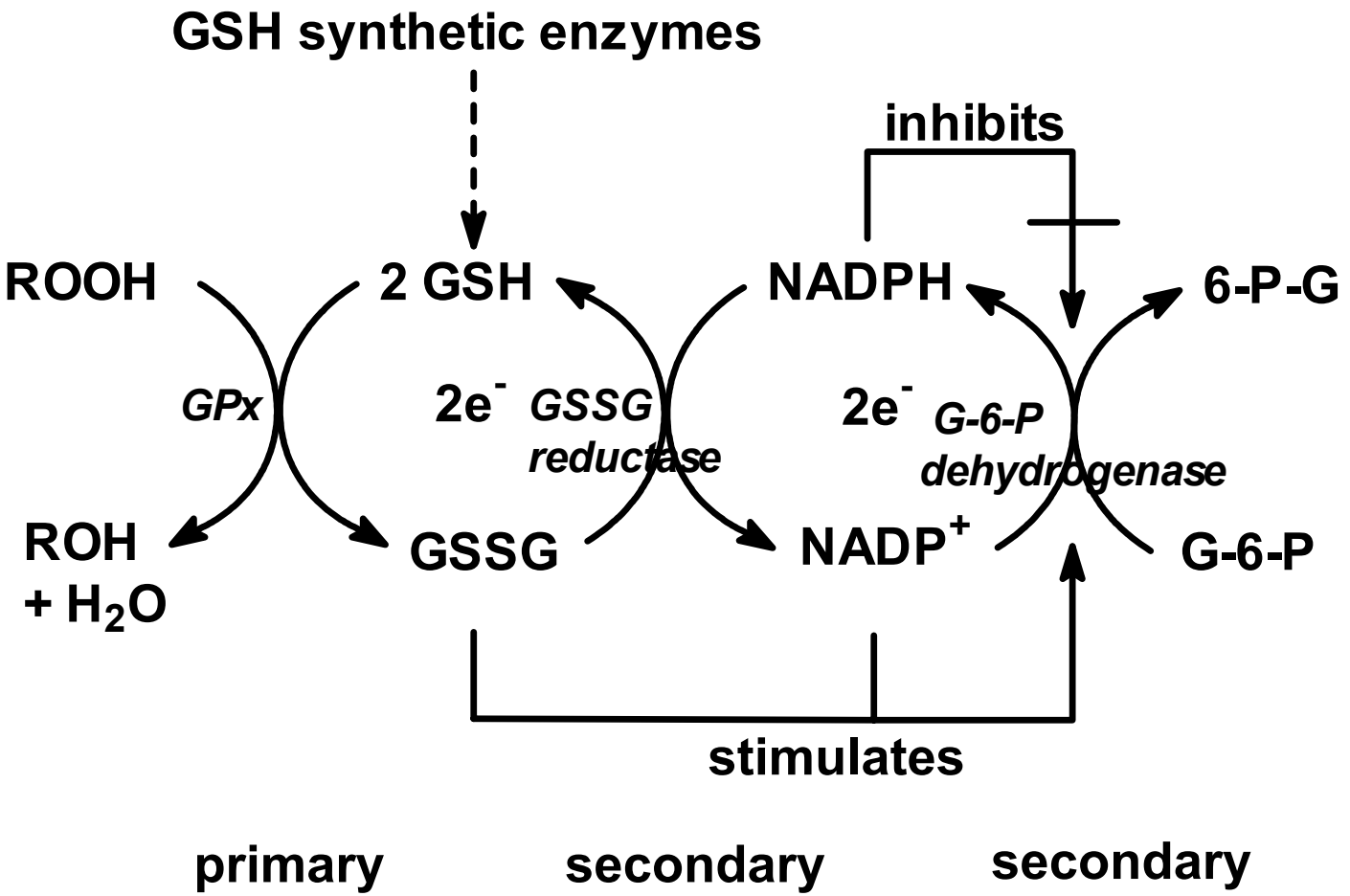
k_{obs} (7.4) $\approx$ 1 M^{-1} s^{-1} *

Appears to be too slow for biological significance.

* Estimated from: Radi *et al.* (1991) *J Biol Chem.* **266:**4244-4250.

Hydroperoxide removal by GSH is mainly *via* coupled enzyme reactions

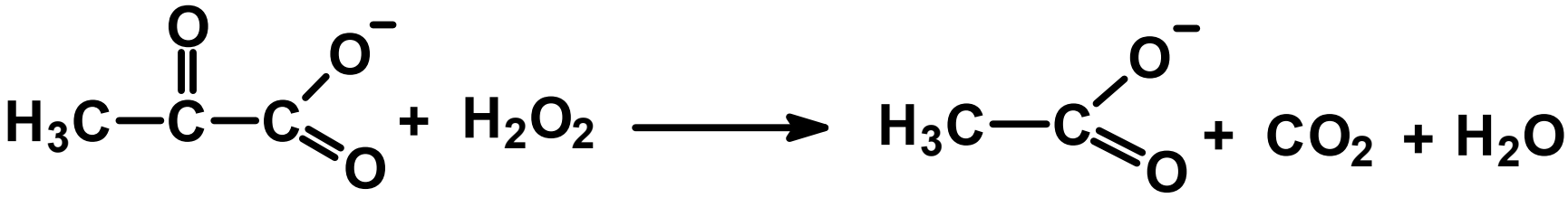
$$2\ GSH + ROOH \rightarrow GSSG + H_2O + ROH$$



Pyruvate and H_2O_2

Pyruvate is a three-carbon ketoacid produced during glycolysis.

Pyruvate can remove H_2O_2 by a stoichiometric chemical reaction.



Pyruvate **Acetate**

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C. Quenching singlet oxygen, *e.g.* β-carotene, lycopene, bilirubin, …

Singlet oxygen quenching, avoiding peroxides

Singlet Oxygen $^1O_2$, *i.e.* oxygen with extra energy

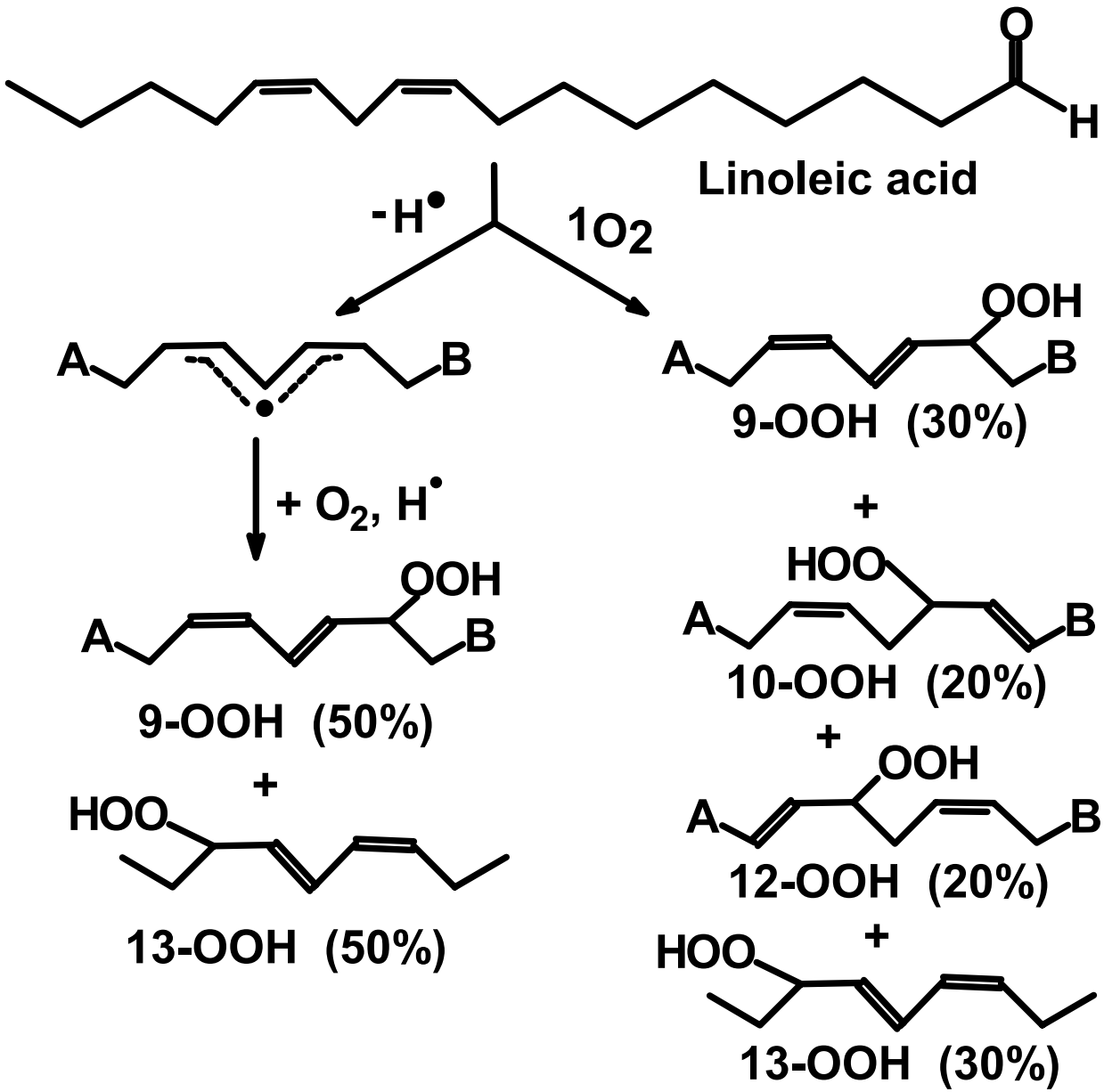
$^1\Delta_g O_2$ 23.4 kcal mol^{-1} above the ground state

Singlet oxygen is electrophilic, thus it reacts with the double bonds of lipids.

(No free radicals; hydroperoxides formed.)

$$k \approx 2 \times 10^5\ M^{-1}\ s^{-1}$$

$$^1O_2 + PUFA \longrightarrow PUFA\text{-}OOH$$



LOOHs: $^1O_2$ *vs* radicals

Quenching of $^1O_2$

Chemical quenching is a term used to signify that an actual chemical reaction has occurred. Hydroperoxide formation is chemical quenching.

$$^1O_2 + LH \rightarrow LOOH$$

Physical quenching is the removal of the excitation energy from $^1O_2$ without any chemical changes.

$$^1O_2 + \beta\text{-carotene} \rightarrow O_2 + \beta\text{-carotene}^*$$

$$\beta\text{-carotene}^* \rightarrow \beta\text{-carotene} + heat$$

Antioxidants, the road ahead

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3. Chain-breaking
4. Retarder *vs* true antioxidant

Chain-Breaking Antioxidants

In general chain breaking antioxidants act by reacting with peroxyl radicals,

$ROO^{\bullet}$

Chain breaking Antioxidants can be:

A) **Donor antioxidant**, *e.g.* tocopherol, ascorbate, uric acid, …

B) **Sacrificial antioxidant**, *e.g.* nitric oxide

Peroxyl Radicals as Targets[#]

$$RH + ROO^{\bullet} \rightarrow ROOH + R^{\bullet}$$

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$

Peroxyl radicals, $ROO^{\bullet}$, are often the chain-carrying radical.

[#] The chain reaction can also be broken by intercepting $R^{\bullet}$. In biology this is rare, but in the polymer industry it can be very important.

Terminating the Chain, Peroxyl Radicals as Targets

Tocopherol, a donor antioxidant -

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

Nitric oxide, a sacrificial antioxidant -

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

Characteristics of a Good Chain-breaking Antioxidant

a. Both Antioxidant & Antiox• should be relatively UN-reactive

b. Antiox• - decays to harmless products

c. Does not add O_2 to make a peroxyl radical

d. Renewed (Recycled) – somehow

e. If the chain-breaking antioxidant is a hydrogen atom donor, it should be in the middle of the pecking order.

The Pecking Order

Antioxidants have reduction potentials that places them in the middle of the Pecking order.

This location in the pecking order provides antioxidants with enough reducing power to react with reactive oxidizing species. At the same time they are too weak to initiate reductive reactions.

$$LOO^{\bullet} + TOH \longrightarrow LOOH + TO^{\bullet}$$ ***Termination***

Buettner GR. (1993) *Arch Biochem Biophy*. **300:**535-543.

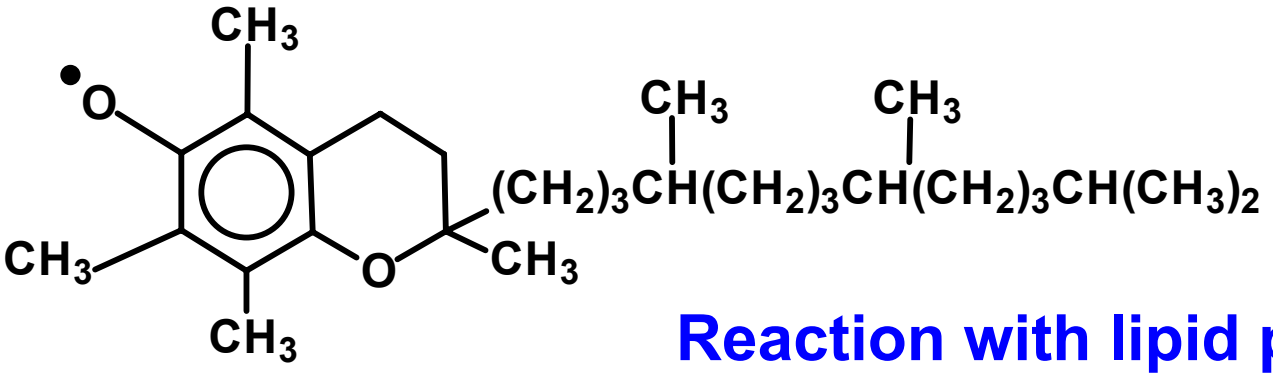
The Pecking Order

Depending on their reduction potential, antioxidants can recycle each other. For example, ascorbate with a reduction potential of +282 mV can recycle TO$^{\bullet}$ (+480 mV) and urate$^{\bullet-}$ (+590 mV).

Buettner GR. (1993) *Arch Biochem Biophy.* **300:**535-543.

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 (tocopherol)	**+480**
H_2O_2, H^{+}/H_2O, $HO^{\bullet}$	+320
$Asc^{\bullet-}$, H^{+}/AscH^{-} (Ascorbate)	**+282**
$CoQ^{\bullet-}$, $2H^{+}/CoQH_2$	+200
Fe(III) EDTA/Fe(II) EDTA	+120
$CoQ/CoQ^{\bullet-}$	-36
$O_2/O_2^{\bullet-}$	-160
Paraquat/Paraquat$^{\bullet-}$	-448
Fe(III)DFO/Fe(II)DFO	-450
RSSR/RSSR$^{\bullet-}$ (GSSG)	-1500
H_2O/e^{-}_{aq}	-2870

Donor Antioxidant - Vitamin E

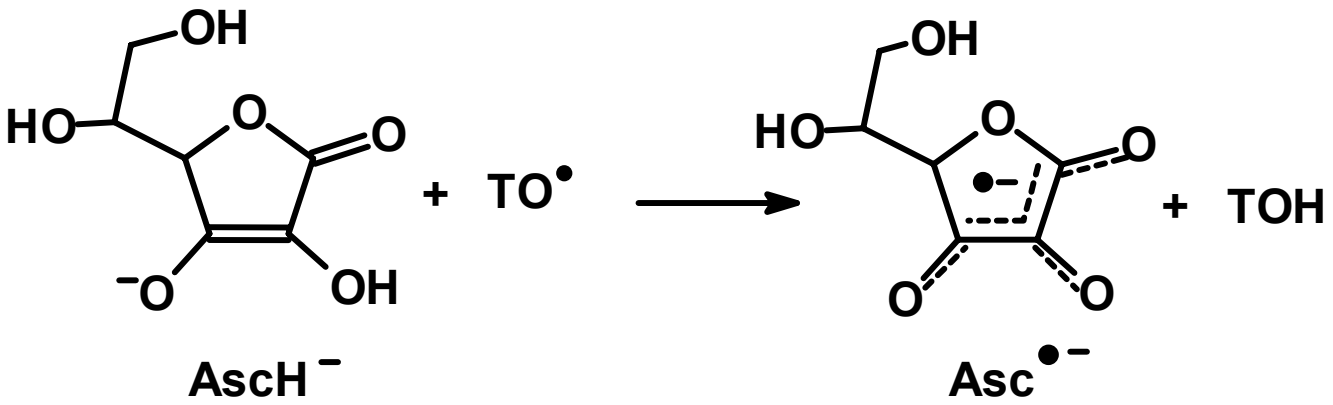


TO•

Reaction with lipid peroxides:

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

Recycling reaction with ascorbate

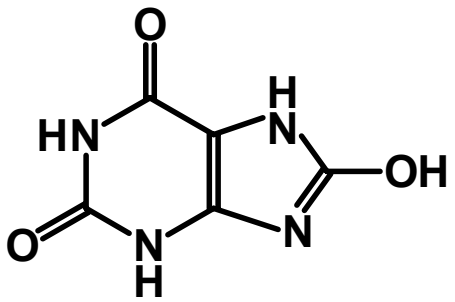


$AscH^-$ + TOH

$Asc^{\bullet -}$

Donor Antioxidant – Uric Acid

Uric acid is produced by the oxidation of xanthine by xanthine oxidase. At physiological pH it is ionized to urate.



Uric acid

$$UH_3 \underset{pK_{a1} = 5.4}{\rightleftharpoons} UH_2^- \underset{pK_{a2} = 9.8}{\rightleftharpoons} UH^{2-}$$

Normal urate concentrations in human plasma range from 0.2 – 0.4 mM.

Ames BN *et al.* (1981) Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer. A hypothesis. Proc. Natl Acad Sci. USA 78, 6858.

Uric Acid Reacts with Peroxyl Radicals

$$k = 3 \times 10^6\ M^{-1}\ s^{-1}$$

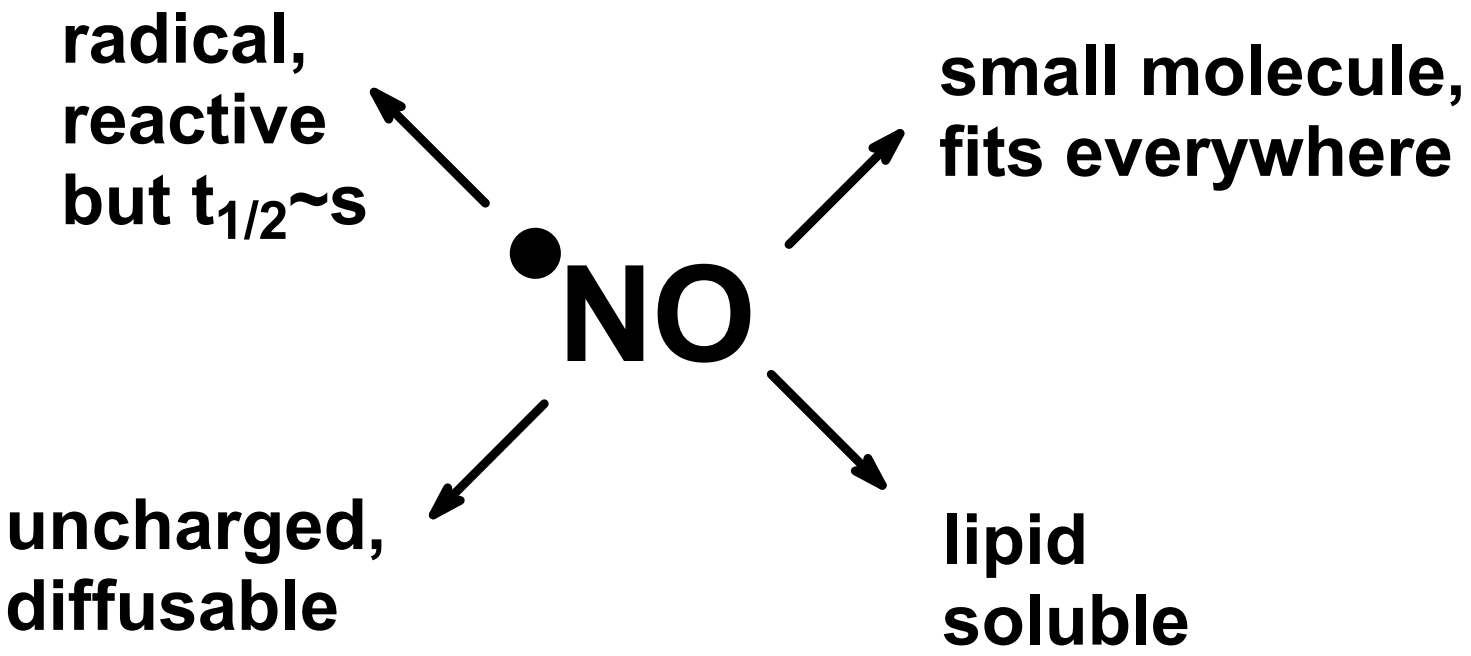
$$ROO^{\bullet} + UH_2^{-} \longrightarrow ROOH + UH^{\bullet -}$$

Recycling by Ascorbate:

$$k = 1 \times 10^6\ M^{-1}\ s^{-1}$$

$$UH^{\bullet -} + AscH^{-} \longrightarrow UH^{2-} + Asc^{\bullet -}$$

Chain Breaking Antioxidant
Sacrificial – Nitric Oxide



Nitric Oxide as Antioxidant

Preventive: •NO coordinates with heme-iron,

$$\text{heme-Fe}^{2+} + {}^{\bullet}\text{NO} \longrightarrow \text{heme-Fe}^{2+}\text{—NO}$$

We have used this for centuries in food preservation, the "sausage" effect.

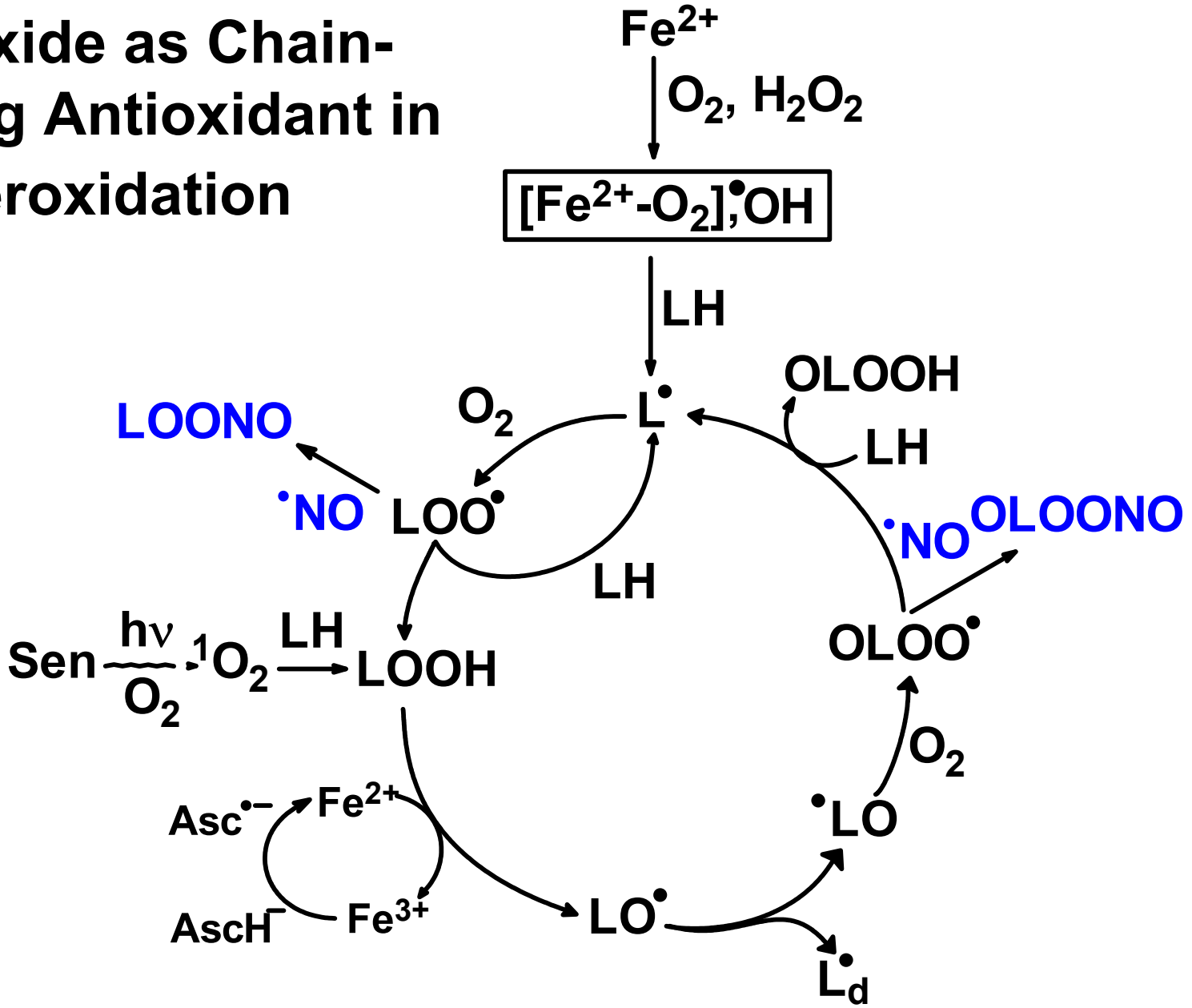
Chain-breaking: •NO can react with oxyradicals:

$$\text{ROO}^{\bullet} + {}^{\bullet}\text{NO} \longrightarrow \text{ROONO}$$

$$\text{RO}^{\bullet} + {}^{\bullet}\text{NO} \longrightarrow \text{RONO}$$

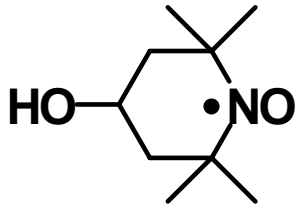
•NO upregulates systems that contribute to the antioxidant network:
heme oxygenase, ferritin, hsp70, and γ-glutamylcysteine synthetase

Nitric Oxide as Chain-Breaking Antioxidant in Lipid Peroxidation



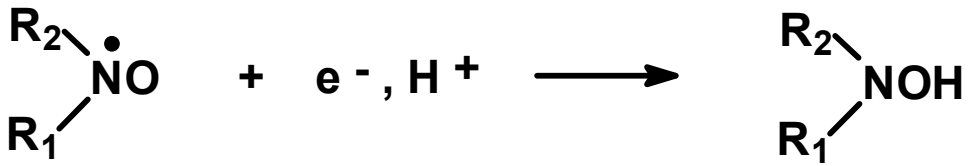
Chain Breaking Antioxidant – Nitroxide

Example nitroxide

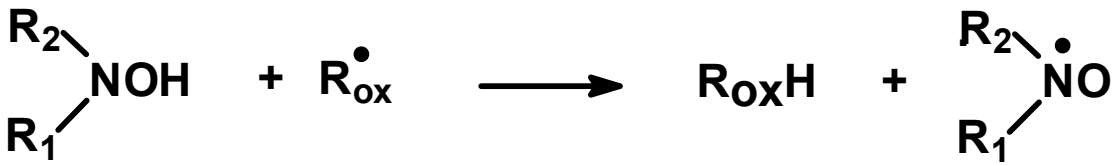


Tempol

A possible antioxidant cycle for a nitroxide



nitroxide **hydroxylamine**



Retarders *vs* Antioxidant

Retarders suppress oxidations only slightly compared to a true antioxidant.

A retarder is only able to make a significant change in the rate of oxidation of the bulk when present in relatively large amounts.

Retarders are often confused with antioxidants.

Kinetic Comparison of Antioxidant and Retarder

Theorem: There are no true antioxidants for $HO^{\bullet}$, only retarders.

Proof:

1. The **rate constants** for nearly all reactions of **$HO^{\bullet}$** in biology are **$10^9 - 10^{10}$ M^{-1} s^{-1}**. Thus, everything reacts rapidly with it and it will take a lot of a "antioxidant" to inhibit oxidation of the bulk.

2. **Comparing rates**:

$$\frac{\text{Rate } (HO^{\bullet} + \text{Bulk}) = k_b\ [\text{Bulk}]\ [HO^{\bullet}]}{\text{Rate } (HO^{\bullet} + \text{Antiox}) = k_a\ [\text{Antiox}]\ [HO^{\bullet}]}$$

Antioxidant *vs* Retarder

3. If we want 98% of the $HO^{\bullet}$ to react with an "antioxidant" AND have only a little bit of antioxidant (1% of bulk), then using

$$\frac{Rate_{Bulk} = k_b\ [Bulk]\ [HO^{\bullet}]}{Rate_{Antiox} = k_a\ [Antiox]\ [HO^{\bullet}]}$$

we have

$$\frac{2 = k_b\ [99\%]\ [HO^{\bullet}]}{98 = k_a\ [1\%]\ [HO^{\bullet}]}$$

then, $k_a = 5\,000\ k_b$

Antioxidant *vs* Retarder

4. If $k_a = 5\,000\ k_b$ and $k_b = 2 \times 10^9\ M^{-1}\ s^{-1}$,

   then k_a must be $1 \times 10^{13}\ M^{-1}\ s^{-1}$

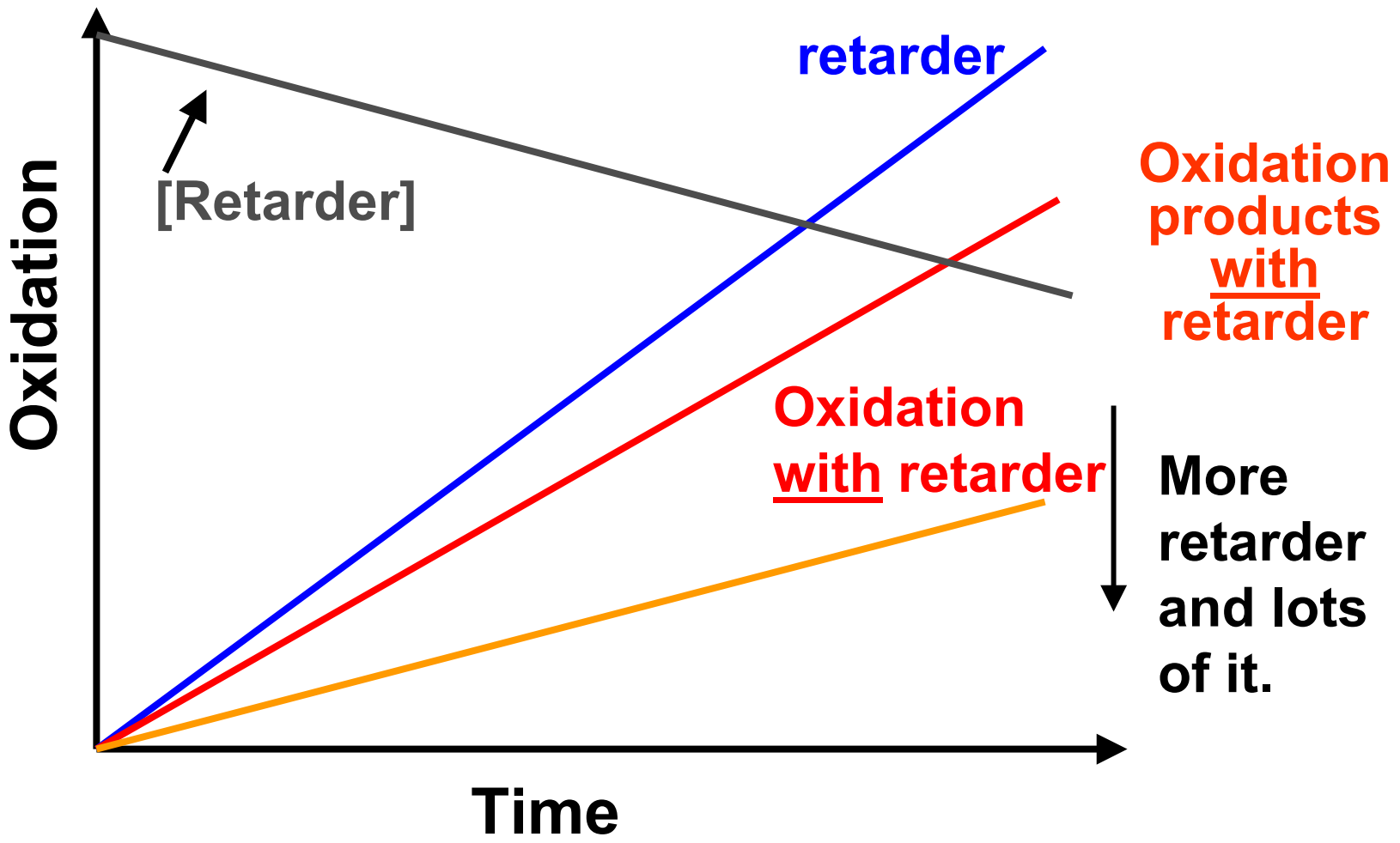
5. No way, not in water.

   In H_2O k must be $<\approx 10^{11}\ M^{-1}\ s^{-1}$

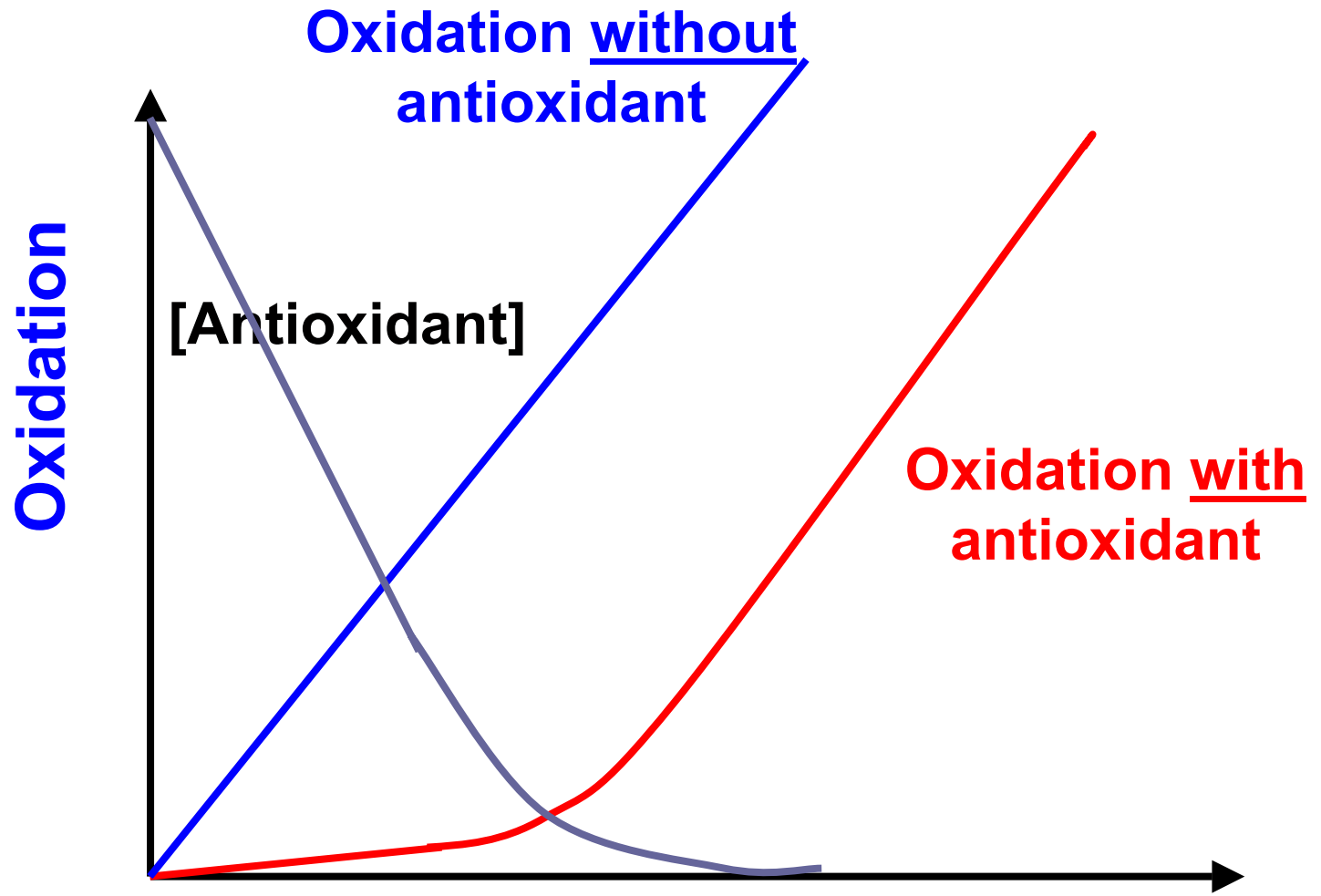
6. Because the a rate constant of $10^{13}\ M^{-1}\ s^{-1}$ in H_2O is not possible and is 100x larger than the upper limit for a rate constant in water, there are no true antioxidants for $HO^{\bullet}$, only retarders.

7. QED

Retarder


Oxidation products without retarder
Oxidation products with retarder
Oxidation with retarder
[Retarder]
Oxidation
Time
More retarder and lots of it.

Antioxidant - no recycling



Lag time due to kinetic advantage

What is a practical kinetic advantage?

Compare the pseudo first-order rate constants.

Rate ($LOO^{\bullet}$ + Antiox) = k_a [Antiox] [$LOO^{\bullet}$] = k_a' [$LOO^{\bullet}$]

Rate ($LOO^{\bullet}$ + Bulk) = k_b [Bulk] [$LOO^{\bullet}$] = k_b' [$LOO^{\bullet}$]

where $k_a' = k_a$ [Antiox] and $k_b' = k_b$ [Bulk]

If 1% "leakage" (damage) is acceptable,

then $k_a' = 100\ k_b'$

If 0.01%, then $k_a' = 10\ 000\ k_b'$

LDL and TOH

Compare the pseudo first-order rate constants.

Rate ($LOO^{\bullet}$ + PUFA) = 40 M^{-1} s^{-1} [PUFA] [$LOO^{\bullet}$]

Rate ($LOO^{\bullet}$ + TOH) = 10^5 M^{-1} s^{-1} [TOH] [$LOO^{\bullet}$]

If [PUFA] in LDL ≈ 1.5 M & [TOH] in LDL ≈ 0.02 M,*

then $k'_{TOH} = 30\ k'_{PUFA}$

Leakage about 3%

Estimated from: Bowery VW, Stocker R. (1993) J *Am Chem Soc.* **115:** 6029-6043

Parting Thoughts 1

To test a compound for possible efficacy as a donor, chain-breaking antioxidant, studying its reactions with $ROO^{\bullet}$ would be much more appropriate than with $HO^{\bullet}$.

Parting Thoughts 2

Antioxidants come in all colors and flavors.

Picture stolen from C. Rice-Evans.

Keep in mind that besides possible antioxidant activity, the primary bio-activity of the "antioxidant" may be very different. [C. Rice-Evans - next]