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Department CMIC

Lecture 10 – FR10





Free-Radicals: Chemistry and Biology

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Dipartimento CMIC “Giulio Natta”

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

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Antioxidants

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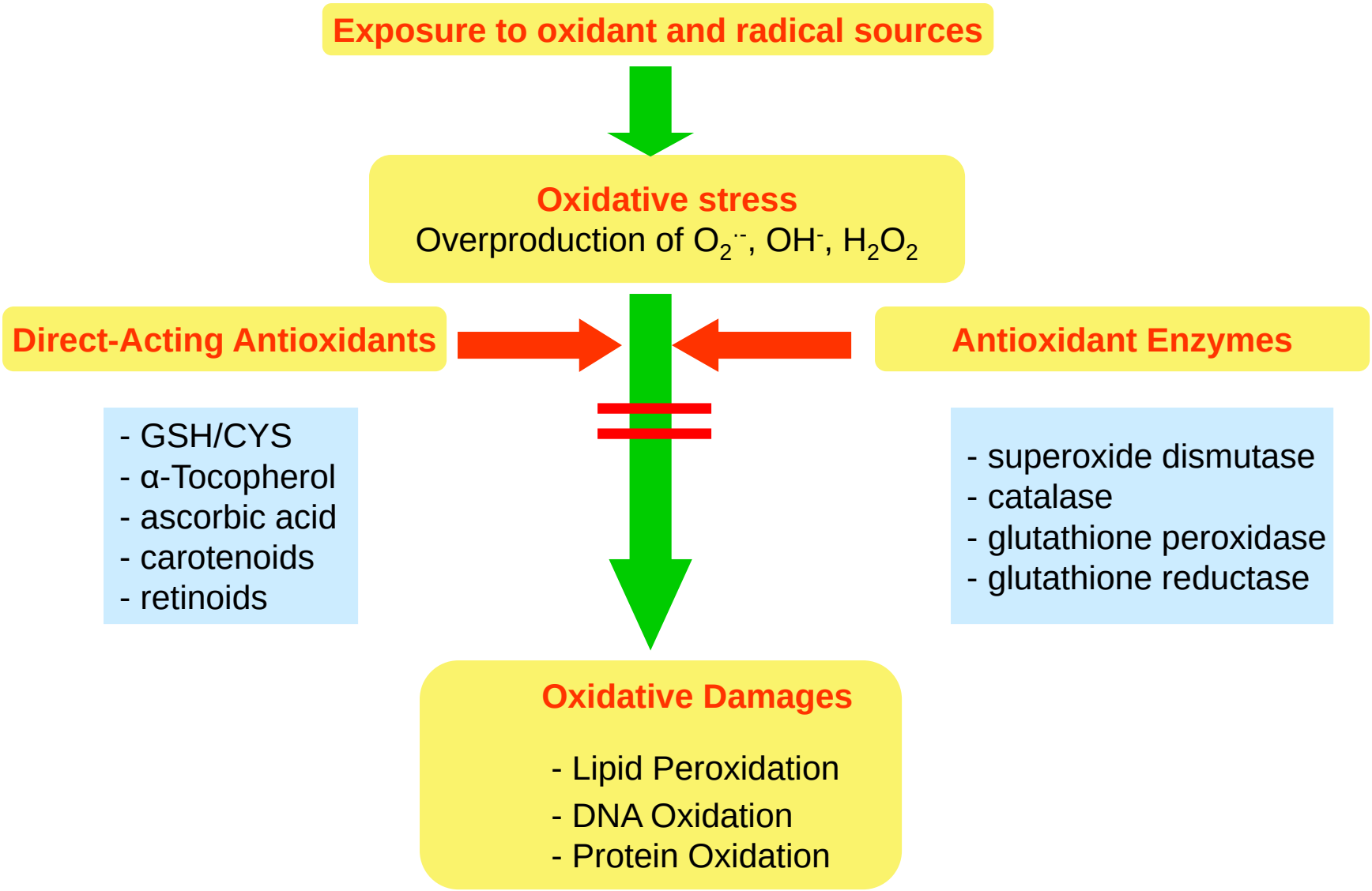
Role of Antioxidants in the Oxidative Stress



Defense Against Pro-oxidants

1. **Prevention of pro-oxidant formation**
2. **Interception of pro-oxidants**
3. **Breaking the chain of radical reactions**
4. **Repair of damage caused by pro-oxidants**

ANTIOXIDANT: a substance that is able, at relatively low concentrations, to compete with other oxidizable substrates and, thus, to significantly delay or inhibit the oxidation of other substrates

Resources:

Gordon, M. H., "Dietary Antioxidants in Disease Prevention," Natural Product Reports (1996) 13: 265-273; Pietta, P.-G., "Flavonoids as Antioxidants", Journal of Natural Products (2000) 63: 1035-1042; Scalbert, A., Johnson, I.T., Saltmarsh, M. "Polyphenols: antioxidants and beyond," American Journal of Clinical Nutrition (2005) 81: 215S-217S; Huang, D., Ou, B., Prior, R. "The Chemistry Behind Antioxidant Capacity Assays", J. Agric. Food Chem. (2005) 53:1841-56.

Prevention of Pro-oxidant Formation

Physical prevention:

Behavioral: - avoidance

Barriers: - organismal level

- organ level

- cellular level

Biochemical prevention:

Control of pro-oxidant molecules:

- transition metal chelators

- catalytic control of O_2 reduction

Control of pro-oxidant enzymes:

- blockade of stimuli

- inhibition of enzymes

Examples of Preventative ‘Antioxidants’

- **Anti-inflammatory agents**
- **Nitric oxide synthase inhibitors**
- **Metal chelators:**
  - Metallothionein
  - Transferrin
  - Lactoferrin
- **NADPH oxidase inhibitors**
- **Xanthine oxidase inhibitors**

Interception of Pro-oxidants

‘Classical’ antioxidant:

- Intercepts species, once formed
- Excludes from further damaging activity
- Transfers species from critical parts of cell

Important considerations for interception reactions:

- Speed of reaction (rate constant)
- Concentration of intercepting species *in vivo*
- Is reaction truly a detoxication pathway?
- Is reaction catalytically recyclable?

Chain Breaking Antioxidants

Example of radical chain-reaction: lipid peroxidation.

ROO• (peroxyl radicals) are often the chain-carrying radicals

Chain-breaking oxidants act by reacting with intermediate radicals:

- "Donor" antioxidants (tocopherol, ascorbate, uric acid,…)

$$LOO\bullet + TOH \rightarrow LOOH + TO\bullet$$

- "Sacrificial" antioxidants (Nitric oxide):

$$LOO\bullet + NO\bullet \rightarrow LOONO$$

Good chain-breaking antioxidant:

- both **ANT** and **ANT•** should be relatively UNreactive
- **ANT•** decays to harmless products
- does not add O_2 to make a new peroxyl radical
- is regenerated (recycled)

Cellular Antioxidants

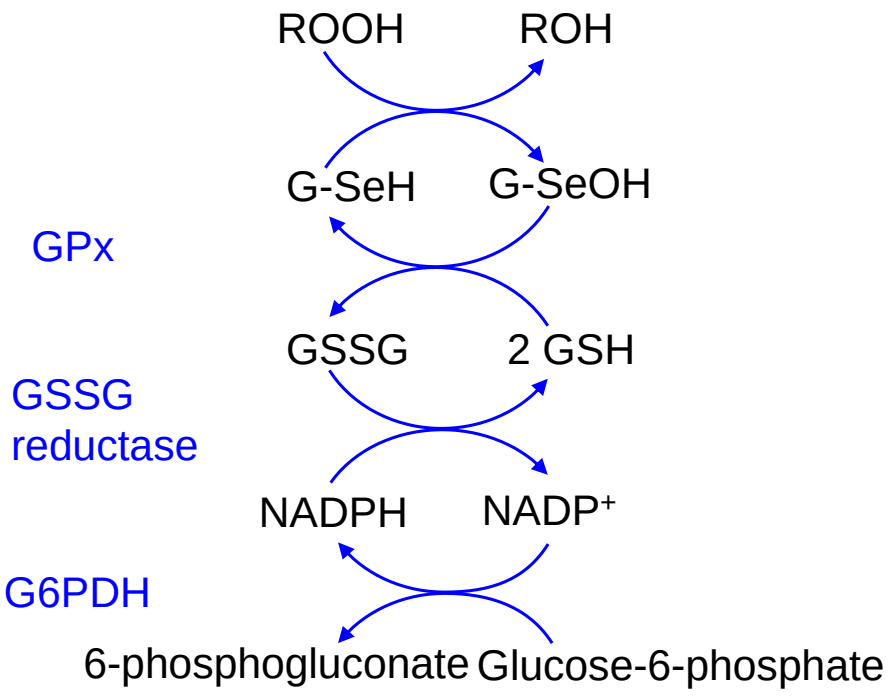
- **Small Molecules**
  - **Water soluble:** glutathione, uric acid, ascorbate (Vit. C)
  - **Lipid soluble:** α-tocopherol (Vit. E), β-carotene, coenzyme Q

- **Proteins**
  - **Intracellular:** SOD (I and II), glutathione peroxidase, catalase
  - **Cell membrane:** SOD (III), ecGPx, plasma proteins (e.g. albumin)
  - **Extracellular:** phospholipid hydroperoxide GPx (PHGPx)

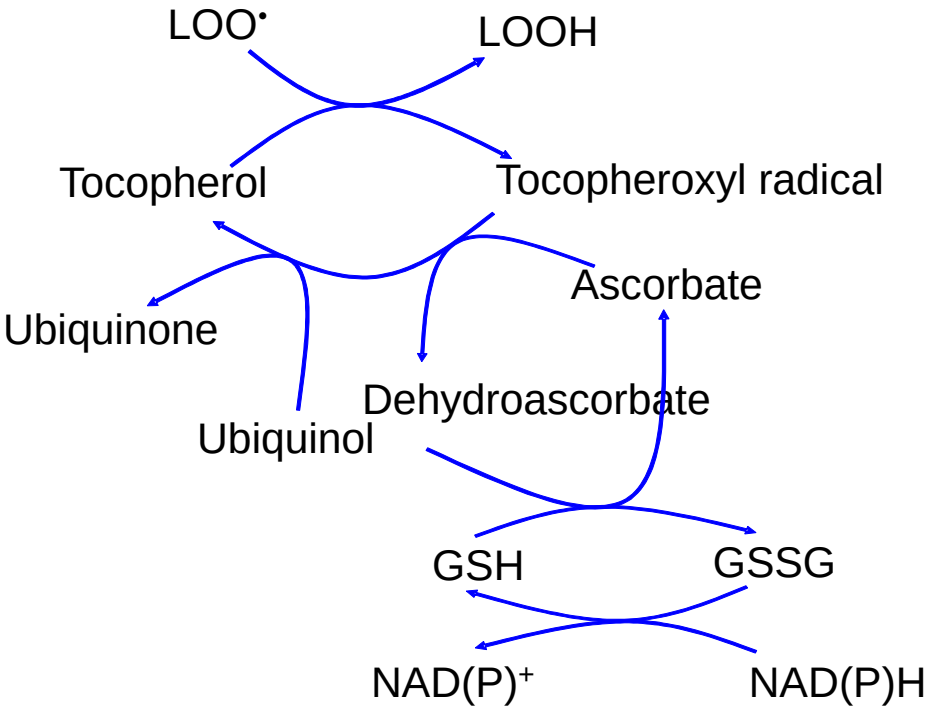
‘Antioxidant Network’

- Catalytic maintenance of antioxidant defense
- Non-scavenging enzymes (re-reduce antioxidants)
- Dependence on energy status of cell
- Glucose most important ‘antioxidant’

Catalytic reduction of peroxides



Catalytic reduction of lipid radicals



Repair of Damage Caused by Pro-oxidants

- **Protection not perfect**
- **Repair of damaged products**
  - **proteins and lipids**
    - reduction and degradation
  - **DNA**
    - repair enzymes
- **Cell death (apoptosis/necrosis)**

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Antioxidants: Preventive

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Preventive Antioxidants Act by:

Minimizing the formation of initiating radicals.

They intercept oxidizing species before damage can be done.

1. **Deactivating metals, *e.g.* transferrin, ferritin, Desferal, DETAPAC, EDTA, … (see also chelators)**

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

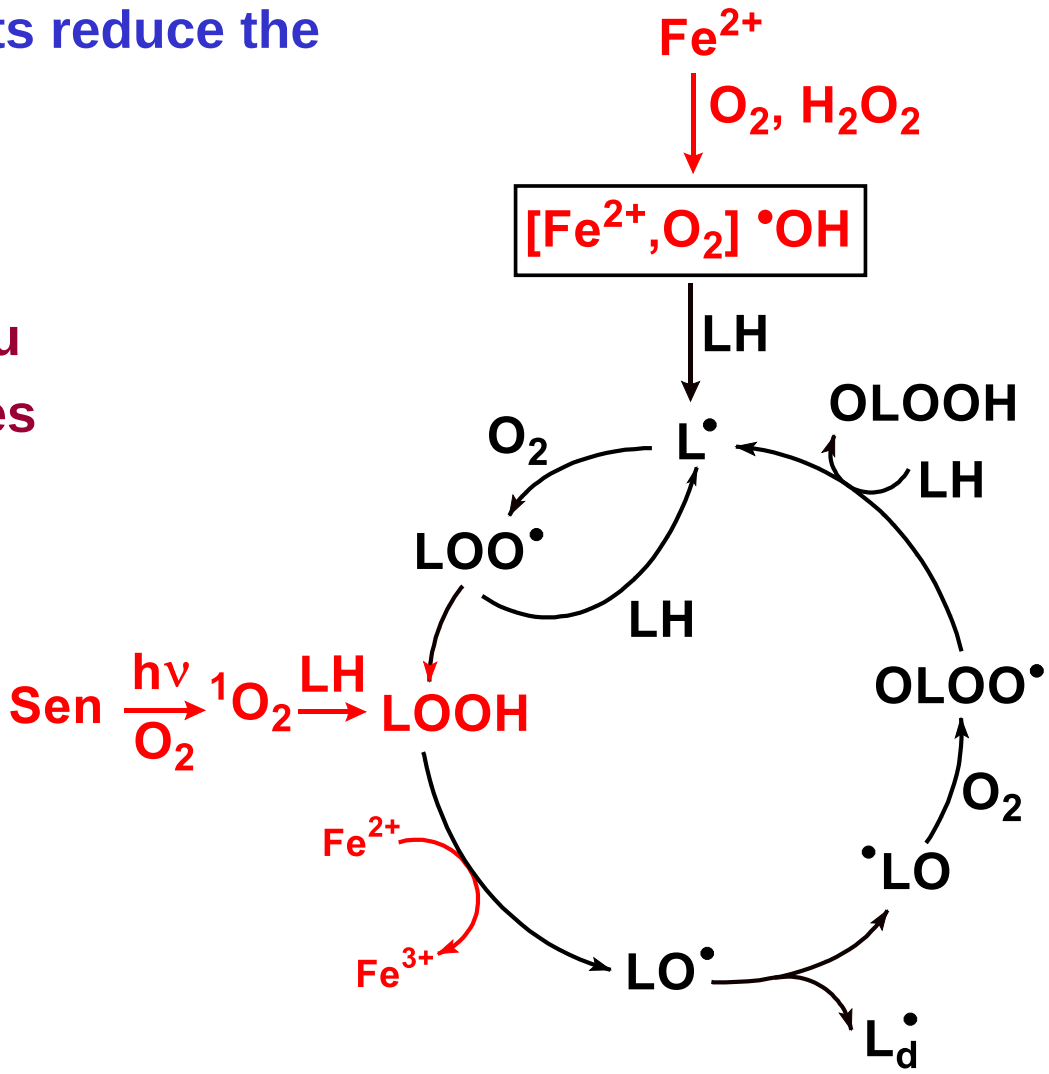
3. **Quenching singlet oxygen, *e.g.* β-carotene, lycopene, bilirubin, …**

Preventive Antioxidants

Preventative Antioxidants reduce the rate of chain initiation.

Targets are:

1. **Metals - Fe, Cu**
2. **Hydroperoxides**



Preventive Antioxidants: Why target metals?

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.**

Because they promote oxidant production.

- **Fe(II)chelate + H_2O_2 → $HO^{\bullet}$ + Fe(III)chelate + OH^- or**
- **Fe(II)chelate + LOOH → $LO^{\bullet}$ + Fe(III)chelate + LOH^-**

and

- **Fe(II)chelate + O_2 → 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.

Preventive Antioxidants Act by:

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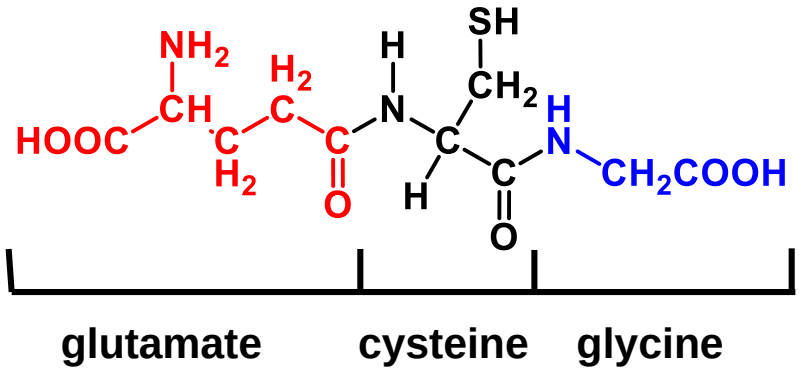
2. Removing hydroperoxides, *e.g.* catalase, glutathione peroxidases, pyruvate, …
3. Quenching singlet oxygen, *e.g.* β-carotene, lycopene, bilirubin, …

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

GSH will react directly with H_2O_2,

albeit very slowly.

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

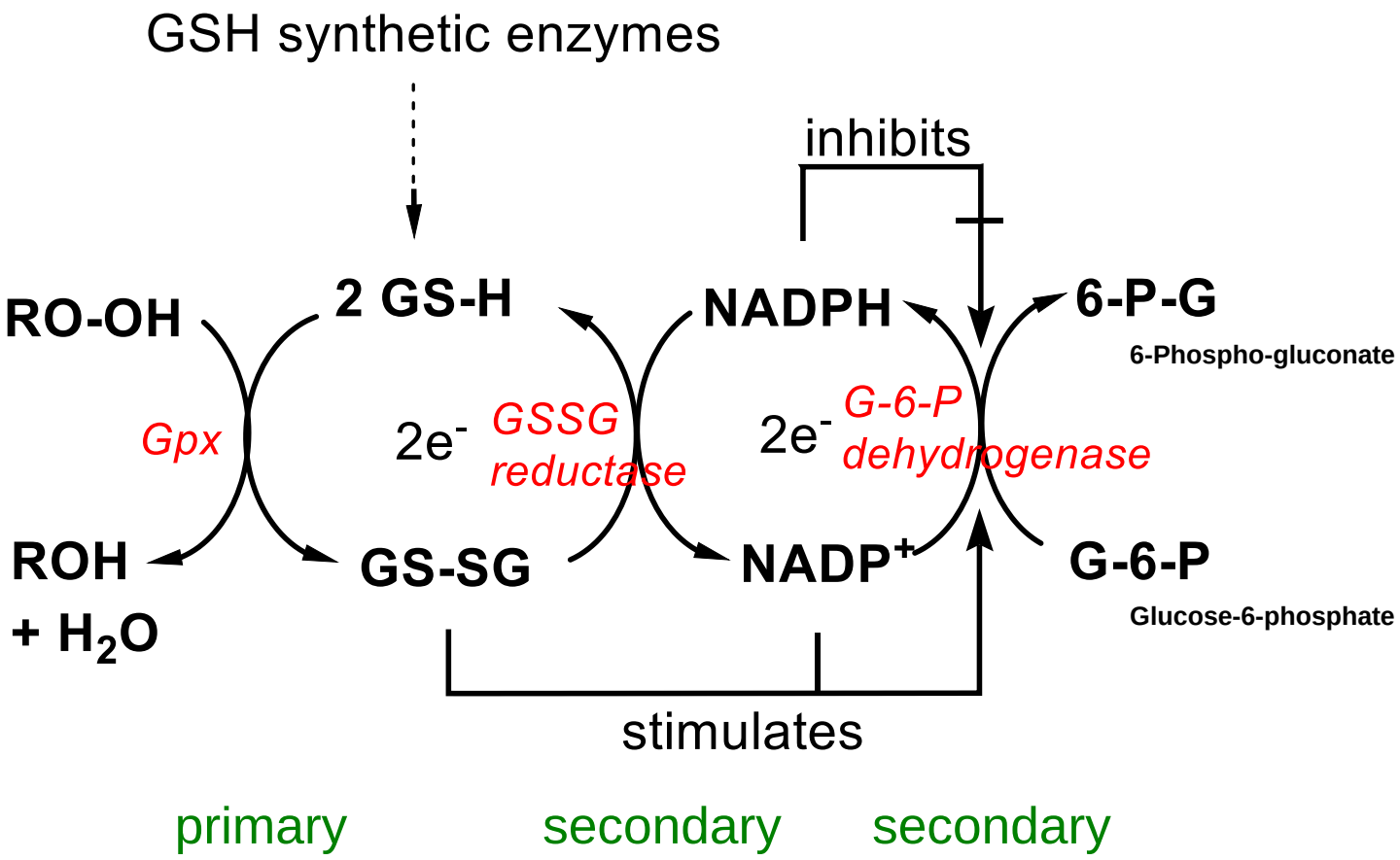
k_{obs} (7.4) ≈ 1 $M^{-1} \cdot s^{-1}$ *

Appears to be too slow for biological significance.

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

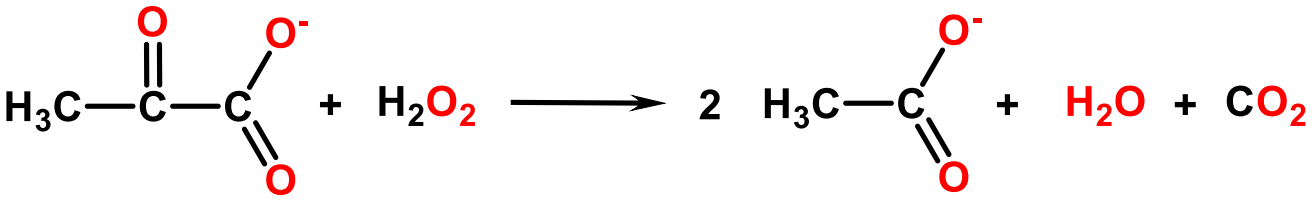
Hydroperoxide Removal by GSH is Mainly *via* Coupled Enzyme Reactions

2 GSH + ROOH → GSSG + H_2O + ROH

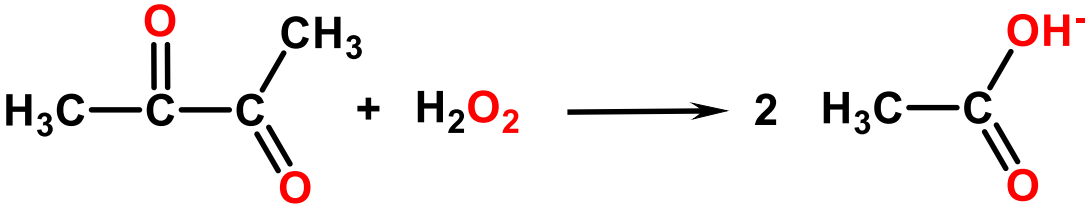


Pyruvate (and Other α-Dicarbonyl Compounds) and H_2O_2

- Pyruvate is a three-carbon ketoacid produced during glycolysis.
- Pyruvate can remove H_2O_2 by a stoichiometric chemical reaction via instable peroxide intermediate.



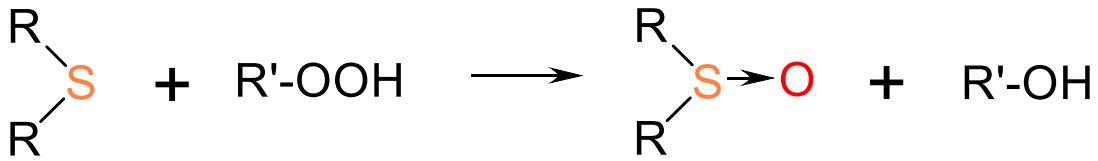
Pyruvate Acetate



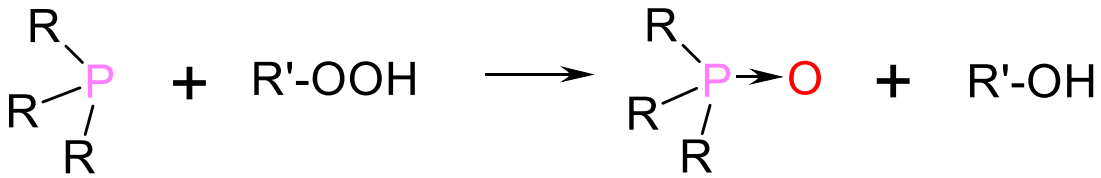
Biacetyl Acetic acid

Secondary Antioxidants

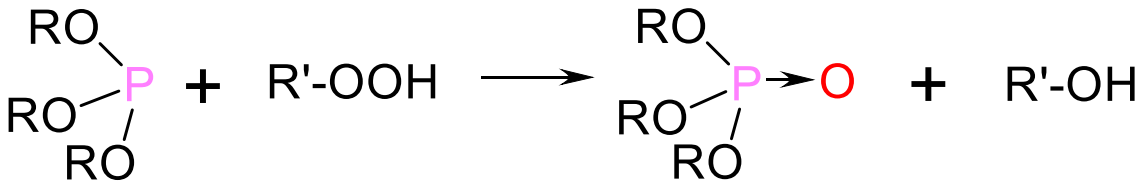
- Chemicals reacting fast with peroxidic products arising from autoxidation through non radical mechanisms.
  - **Sulfides**: give sulfoxides (Thioesters, i.e. $S(CH_2CH_2CO_2R)_2$)



  - **Phosphines**: give phosphine-oxide



  - **Phosphites**: give Phosphates



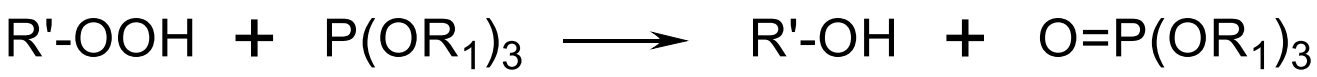
Often used as co-stabilizers with hindered phenols

Stabilization by Secondary (Preventive) Antioxidant Phosphites (with radical inter.)

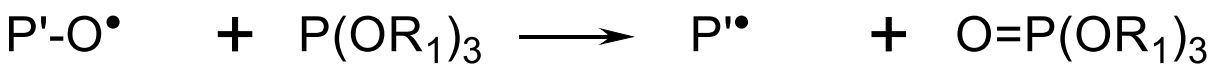
Decompose hydroperoxides without intermediate formation of free radicals.

- Phospites

  reduce hydroperoxides to the corresponding alcohol and are transformed into phospates:



  can react with peroxy and alkoxy radicals:

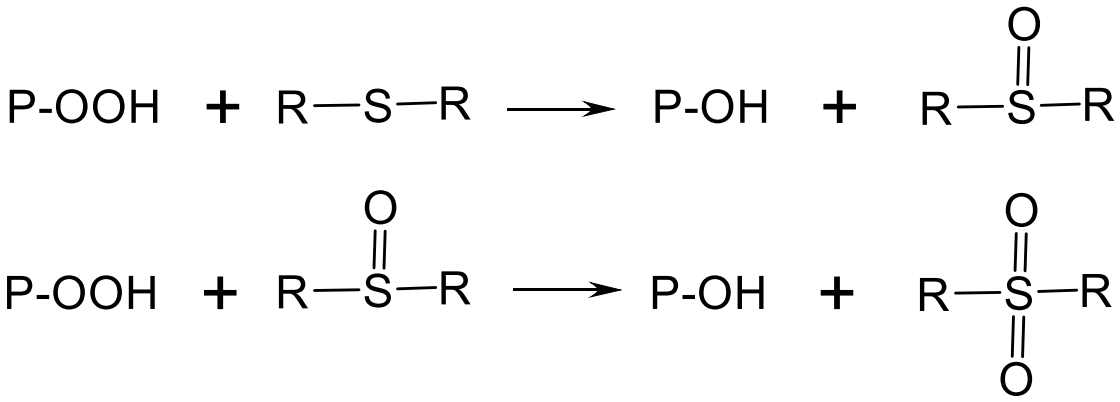


Stabilization by Secondary (Preventive) Antioxidant Sulfides (without radical inter.)

Decompose hydroperoxides without intermediate formation of free radicals.

- Organic sulfides

  transform one/two molecules of hydroperoxide into alcohols forming sulfoxides and sulfones:



Preventive Antioxidants Act by:

1. **Deactivating metals, *e.g.* transferrin, ferritin, Desferal, DETAPAC, EDTA, … (see chelators)**
2. **Removing hydroperoxides, *e.g.* catalase, glutathione peroxidases, pyruvate, …**
3. **Quenching singlet oxygen, *e.g.* β-carotene, lycopene, bilirubin, …**

Singlet oxygen quenching, avoiding peroxides

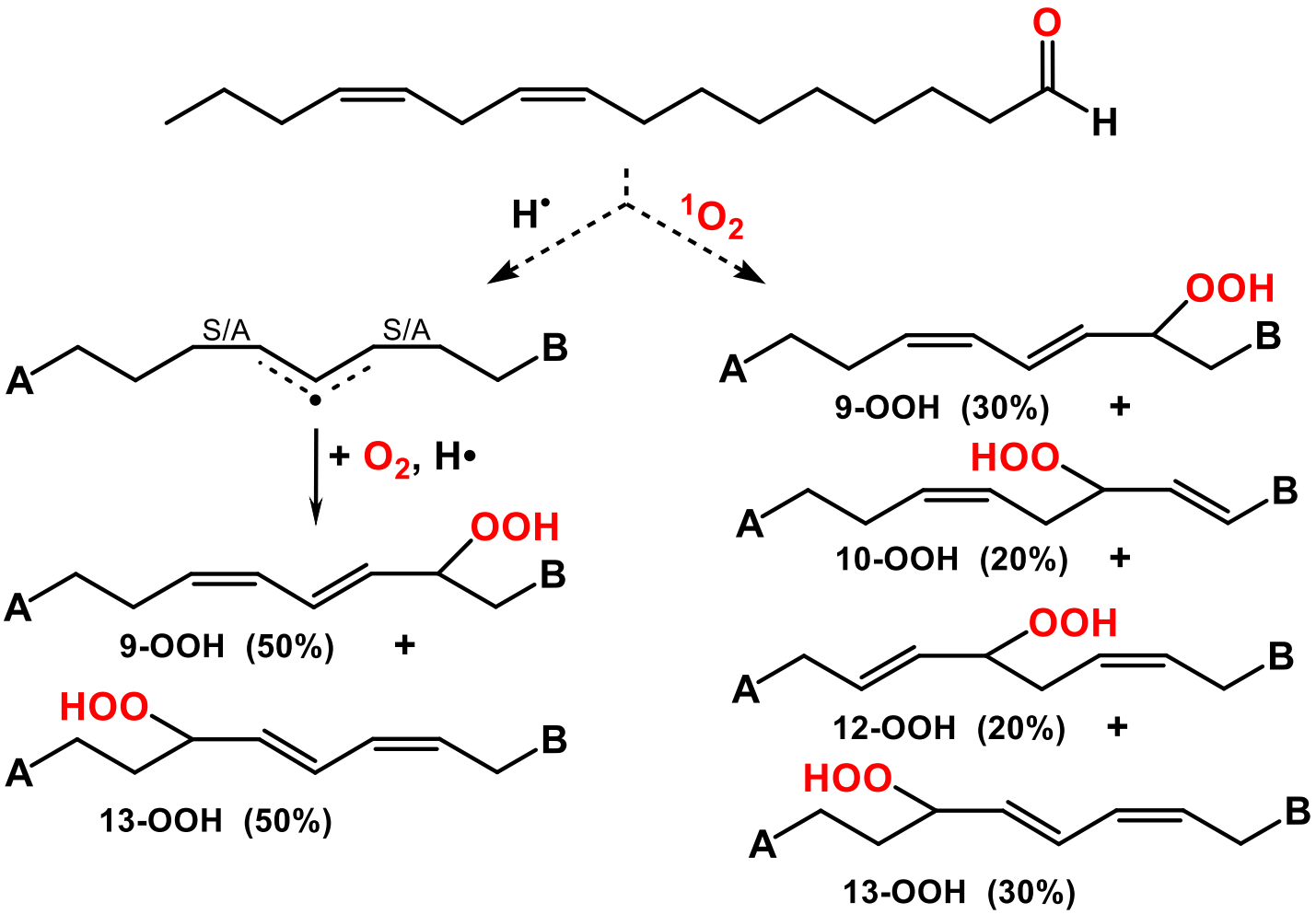
Singlet Oxygen $^1O_2$, *i.e.* excited oxygen with extra energy:

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

Singlet oxygen is electrophilic, reacts with double bonds of lipids.
(No free radicals; hydroperoxides formed!)

$$^1O_2 + \text{PUFA} \xrightarrow{k \approx 2\times10^5\ M^{-1}\cdot s^{-1}} \text{PUFA-OOH}$$

LOOHs: $^1O_2$ *vs.* Radicals



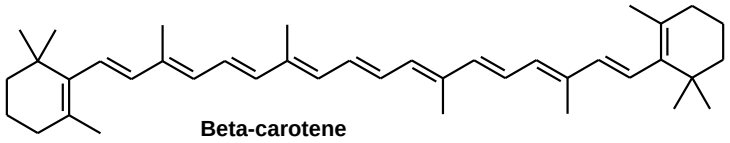
Quenching of $^1O_2$

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

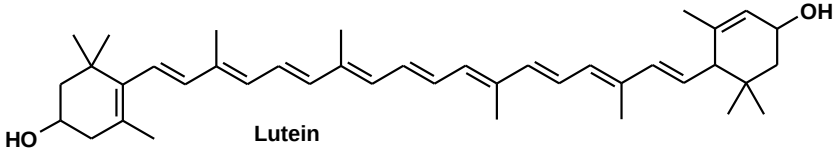
$$^1O_2 + LH \rightarrow LOOH$$

- **Physical quenching** is the removal of the excitation energy from $^1O_2$ without any chemical changes.
  - $^1O_2 + \beta\text{-carotene} \rightarrow O_2 + \beta\text{-carotene}^*$
  - $\beta\text{-carotene}^* \rightarrow \beta\text{-carotene} + heat$

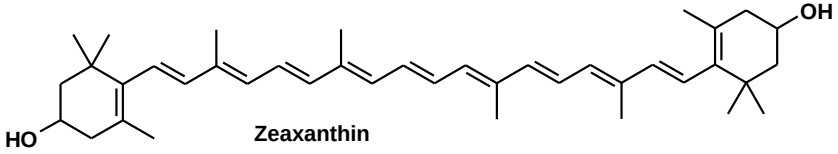
Carotenoids



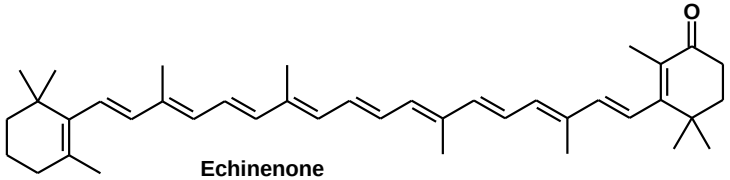
Beta-carotene



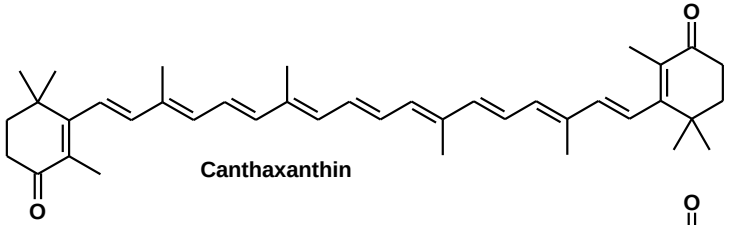
Lutein



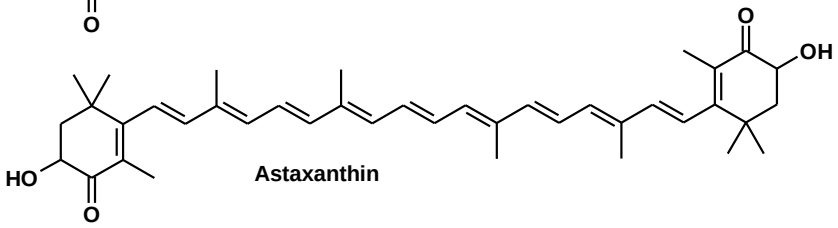
Zeaxanthin



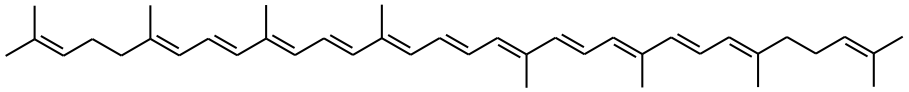
Echinenone



Canthaxanthin



Astaxanthin



Lycopene

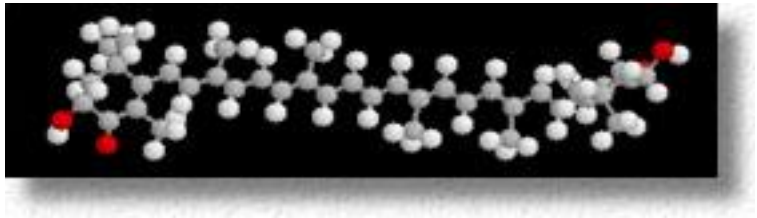
Molecular Weight =536,89
Exact Mass =536
Molecular Formula =C40H56
Molecular Composition =C 89.49% H 10.51%

Chains of conjugated double bonds are “electron rich”.

Loss of electrons more easily tolerated

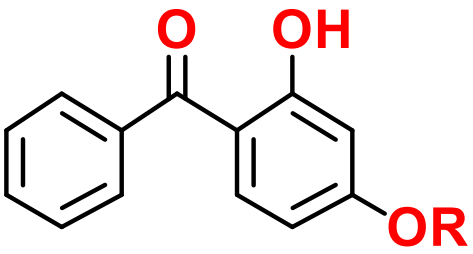
Carotenoids work by:

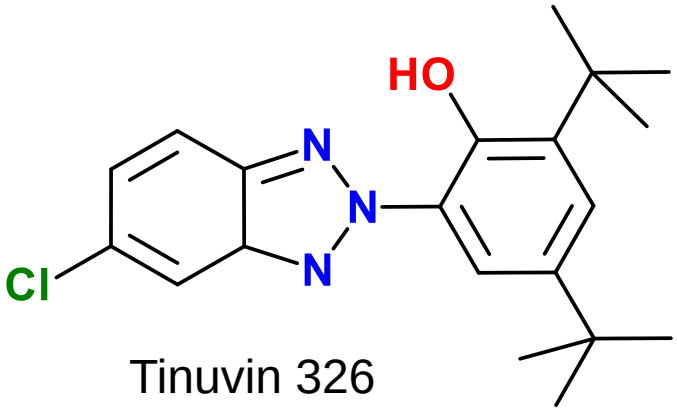
- quenching of singlet oxygen and dissipating the energy as heat
- scavenging of radicals to prevent or terminate chain reactions.



Preventive Antioxidants: Preferential UV absorbers

- A special class of preventive antioxidants are compounds able to absorb Visible or UV light without generation of free radicals, so preventing damage from radiation energy absorption.
- Several compounds are known to protect materials from Visible and UV radiations.
- The protection is particularly relevant for life cells and for humans because UV radiation causes cancer skin (melanoma, basal cells..).
- The compounds used to protect the human skin are known as sunscreen agents.





Tinuvin 326

UV Index

Factors Affecting:

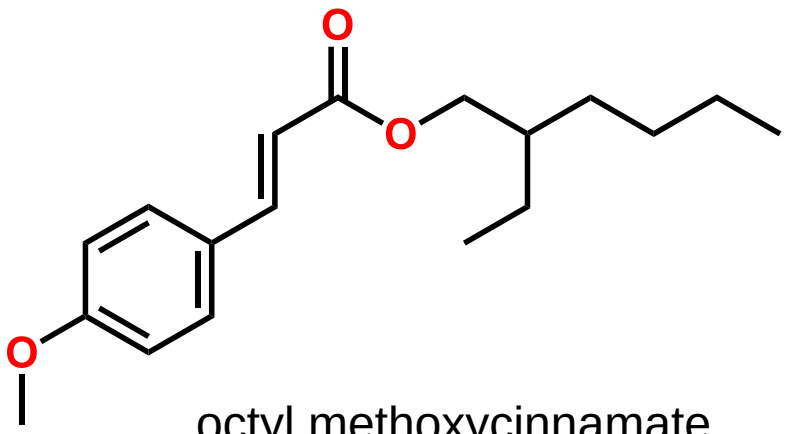
1. Measurements of current total ozone amounts for the entire globe
2. Amount of UV radiation reaching the ground
3. How human skin responds to UV wavelengths.
4. Actual incoming radiation level
5. Estimates are then adjusted for the effects of elevation and clouds
6. Value is scaled by a conversion factor of 25 and rounded to the nearest whole number

Exposure Category	Index Number	Sun Protection Messages
LOW	<2	Wear sunglasses on bright days. In winter, reflection off snow can nearly double UV strength. If you burn easily, cover up and use sunscreen.
MODERATE	3-5	Take precautions, such as covering up and using sunscreen, if you will be outside. Stay in shade near midday when the sun is strongest.
HIGH	6-7	Protection against sunburn is needed. Reduce time in the sun between 11 a.m. and 4 p.m. Cover up, wear a hat and sunglasses, and use sunscreen.
VERY HIGH	8-10	Take extra precautions. Unprotected skin will be damaged and can burn quickly. Try to avoid the sun between 11 a.m. and 4 p.m. Otherwise, seek shade, cover up, wear a hat and sunglasses, and use sunscreen.
EXTREME	11+	Take all precautions. Unprotected skin can burn in minutes. Beachgoers should know that white sand and other bright surfaces reflect UV and will increase UV exposure. Avoid the sun between 11 a.m. and 4 p.m. Seek shade, cover up, wear a hat and sunglasses, and use sunscreen.

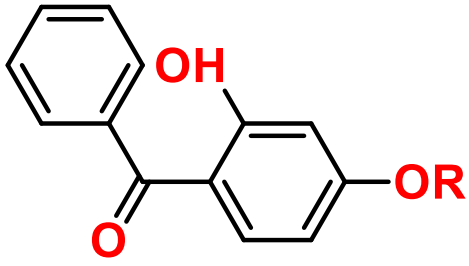
Sunscreens: How They Work

Combination of Organic and Inorganic Ingredients

- *Inorganic*: reflects or scatters UV radiation
  - i.e. ZnO_2 and TiO_2
- *Organic*: absorb UV radiation and dissipate as heat
  - i.e. OMC, oxybenzone

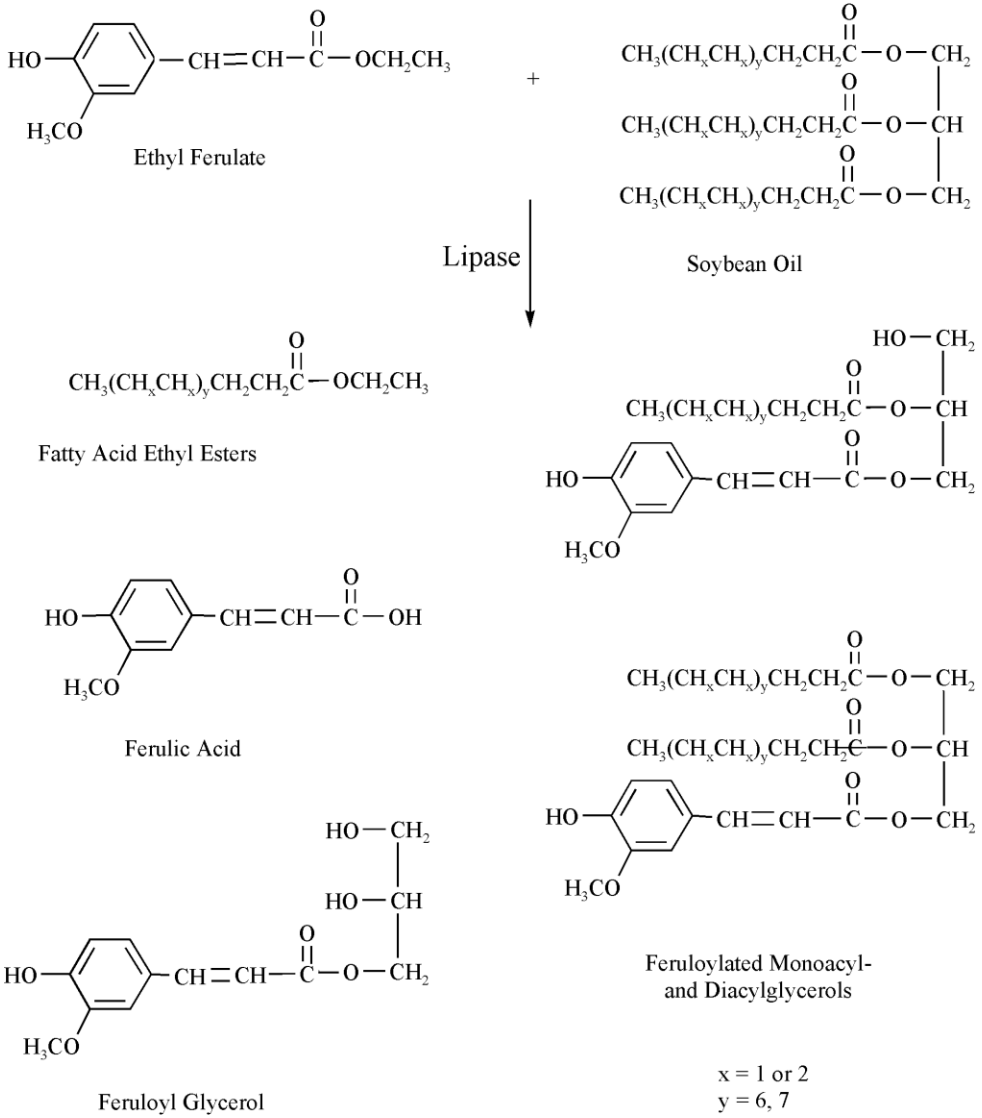


octyl methoxycinnamate



Oxybenzone

Green Sunscreen Process



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.

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

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

   Rate ($HO^{\bullet}$ + Antiox) = k_a [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

$$\mathbf{Rate_{Bulk} = k_b\,[Bulk]\,[HO^{\bullet}]} \quad \mathbf{Rate_{Antiox} = k_a\,[Antiox]\,[HO^{\bullet}]}$$

we have

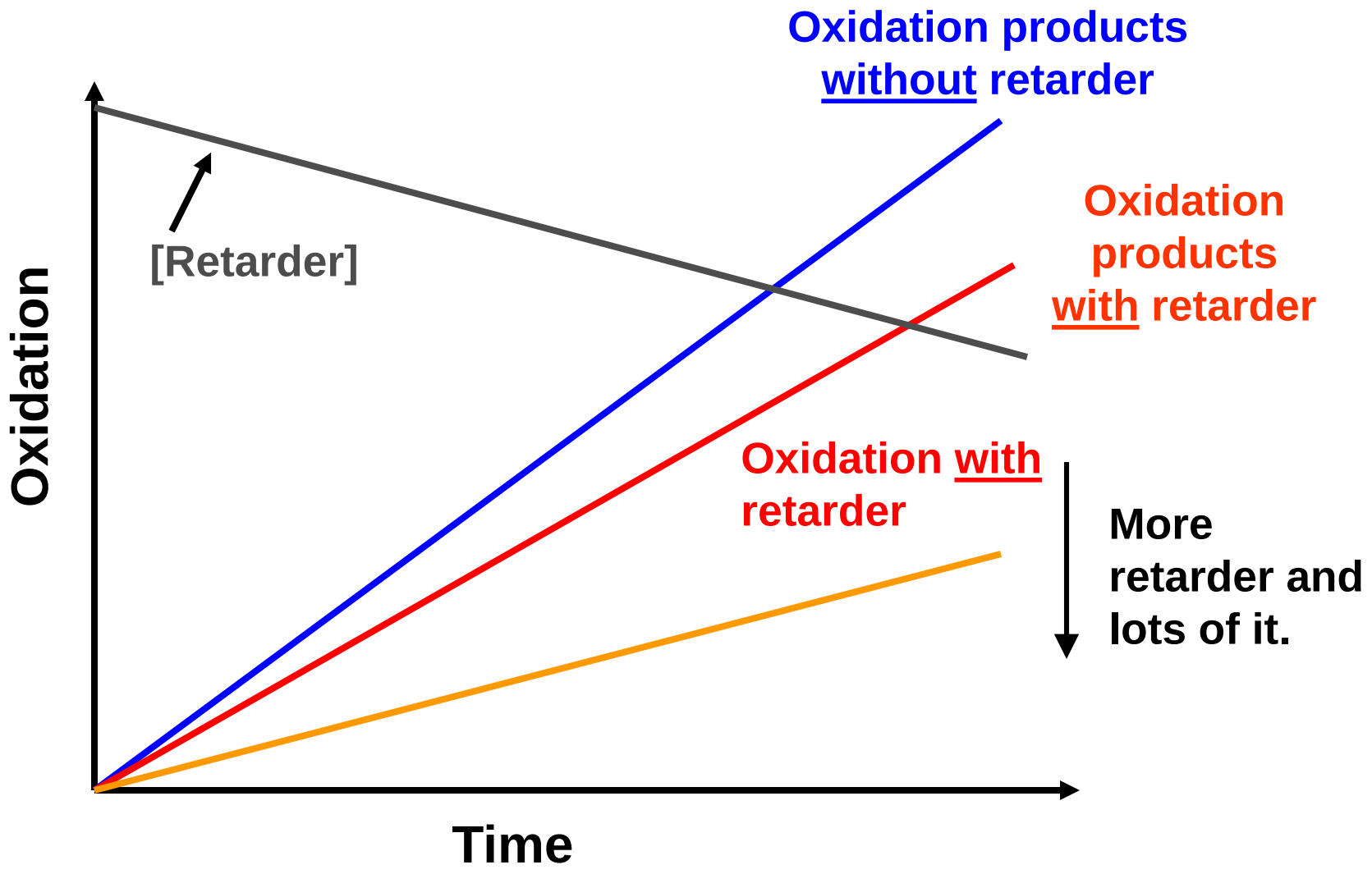
$$\mathbf{2 = k_b\,[99\%]\,[HO^{\bullet}]} \qquad \mathbf{98 = k_a\,[1\%]\,[HO^{\bullet}]}$$

then, $\mathbf{k_a = 5\,000\,k_b}$

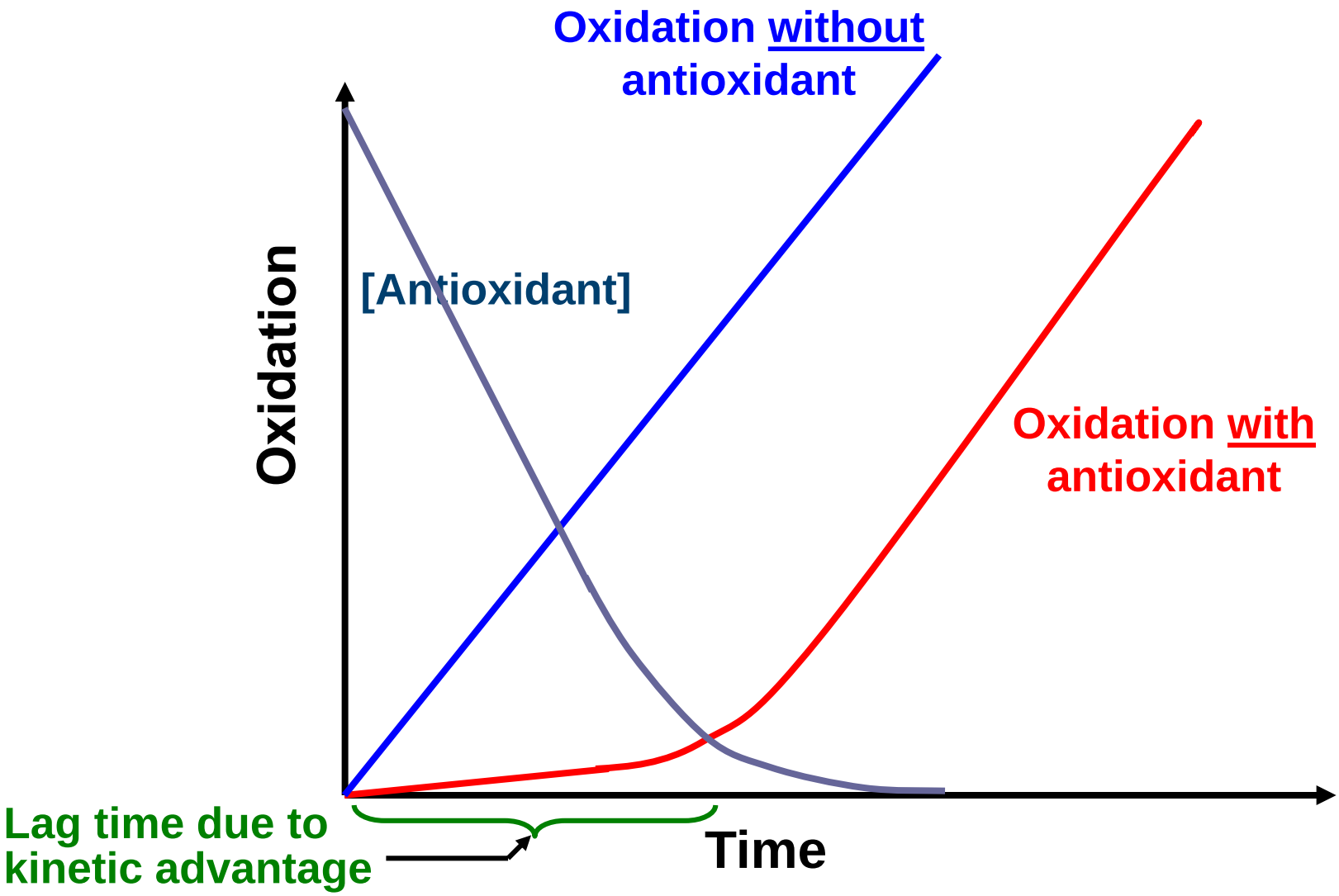
4. If $k_a = 5\,000\,k_b$ and $k_b = 2\times10^{9}\ M^{-1}\cdot s^{-1}$, then k_a must be $1\times10^{13}\ M^{-1}\cdot s^{-1}$
5. No way, not in water.

   In H_2O k must be $<\approx 10^{11}\ M^{-1}\cdot s^{-1}$
6. Because the a rate constant of $10^{13}\ M^{-1}\cdot s^{-1}$ 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 (there is no inhibition period!)



Antioxidant - No Recycling



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 $k_a' = 10\,000\ k_b'$

Example: LDL and TOH

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

Rate ($LOO^{\bullet}$ + TOH) = 10^5 $M^{-1} \cdot s^{-1}$ [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. *J. Am. Chem. Soc.* **1993**, *115*, 6029-6043

POLITECNICO DI MILANO





Antioxidants: Chain Breaking

Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”

Radical Scavenging Antioxidant

Radical scavenging antioxidants break free radical chain reaction. They slow or stop oxidative processes after they begin, by intercepting the chain-carrying radicals by cross dimerization with persistent radicals.

1) Synthetic
   - Phenols and Hindered Phenol
   - Aromatic Amines
   - Hindered Amines (HALS)

2) Naturals
   - Vitamin C
   - Tocopherol
   - Quercetin
   - Anthocyanin

Characteristics of a Good Chain-breaking Antioxidant

a. Both Antioxidant & Antiox$^{\bullet}$ should be relatively UN-reactive

b. Antiox$^{\bullet}$ - decays to harmless products

c. Does not add O_2 to make a peroxyl radical

d. Renewed (Recycled) – somehow, the regeneration can be quite complex!

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

Chain Breaking Antioxidants

Two main alternatives:

1) **Sacrificial Antioxidant**:

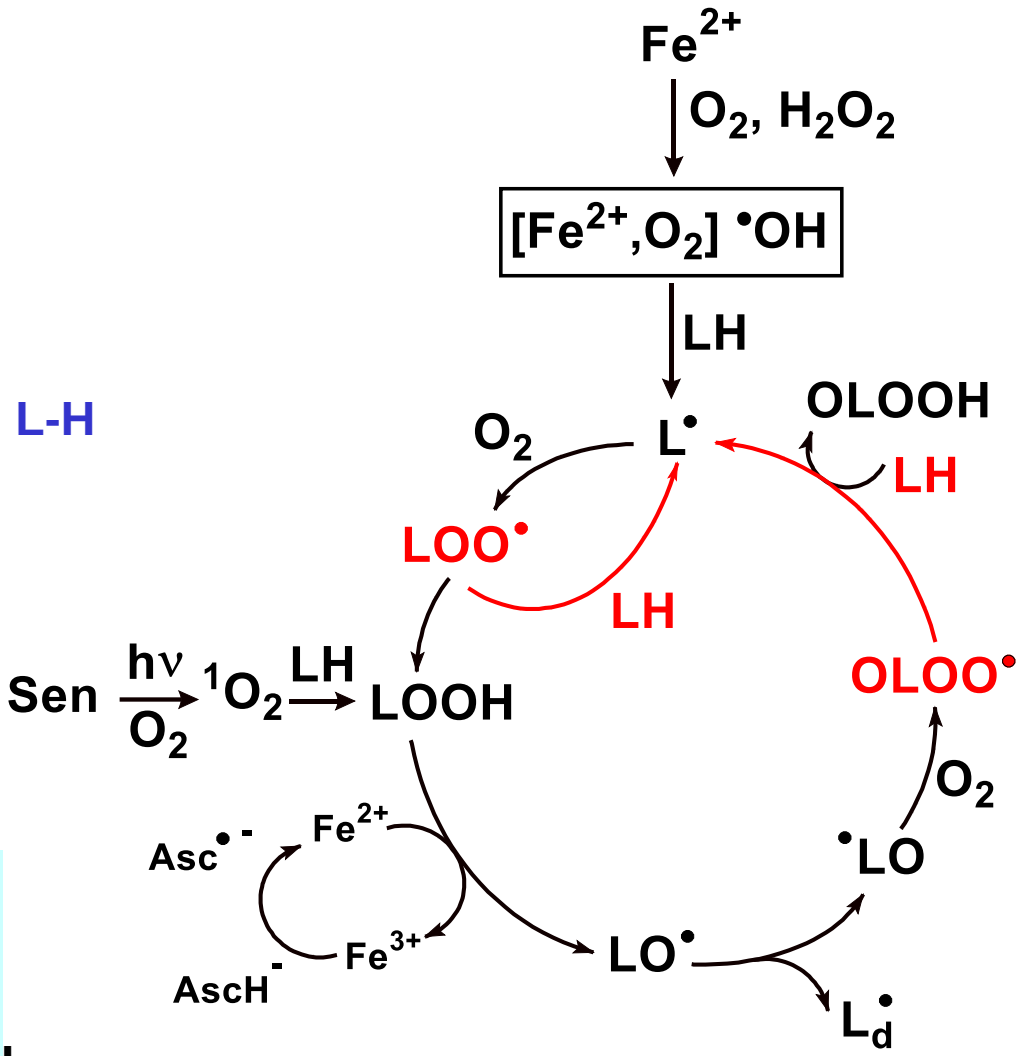
$L^{\bullet} + Antiox^{\bullet} \rightarrow Antiox\text{-}L$

2) **Donor antioxidant**

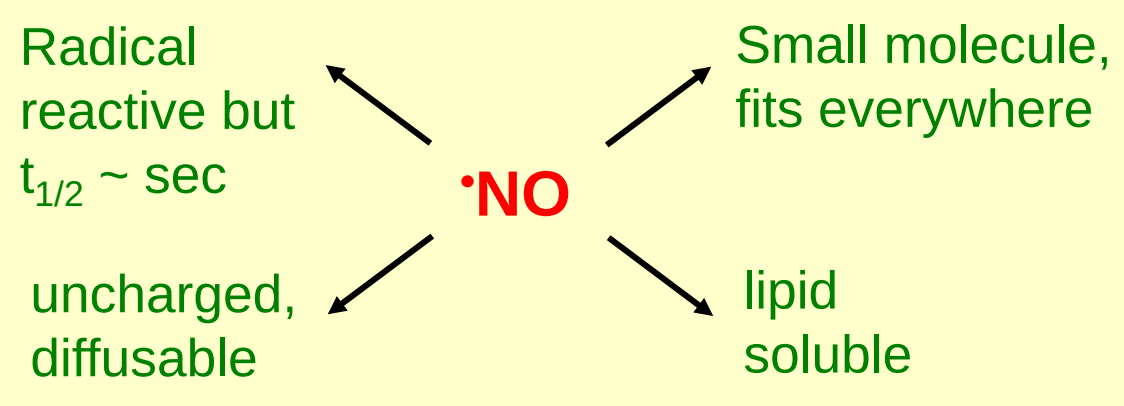
$L^{\bullet} + Antiox\text{-}H \rightarrow Antiox^{\bullet} + L\text{-}H$

$Antiox^{\bullet} + R^{\bullet} \rightarrow Antiox\text{-}R$

$$3 \cdot 10^{8}\ M^{-1} \cdot s^{-1}$$
$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$$
$$10\text{-}50\ M^{-1} \cdot s^{-1}$$
$$LOO^{\bullet} + L\text{-}H \rightarrow L^{\bullet} + LOOH$$



Chain Breaking Sacrificial Antioxidants: •NO



Preventive: **•NO coordinates with heme-iron,**

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

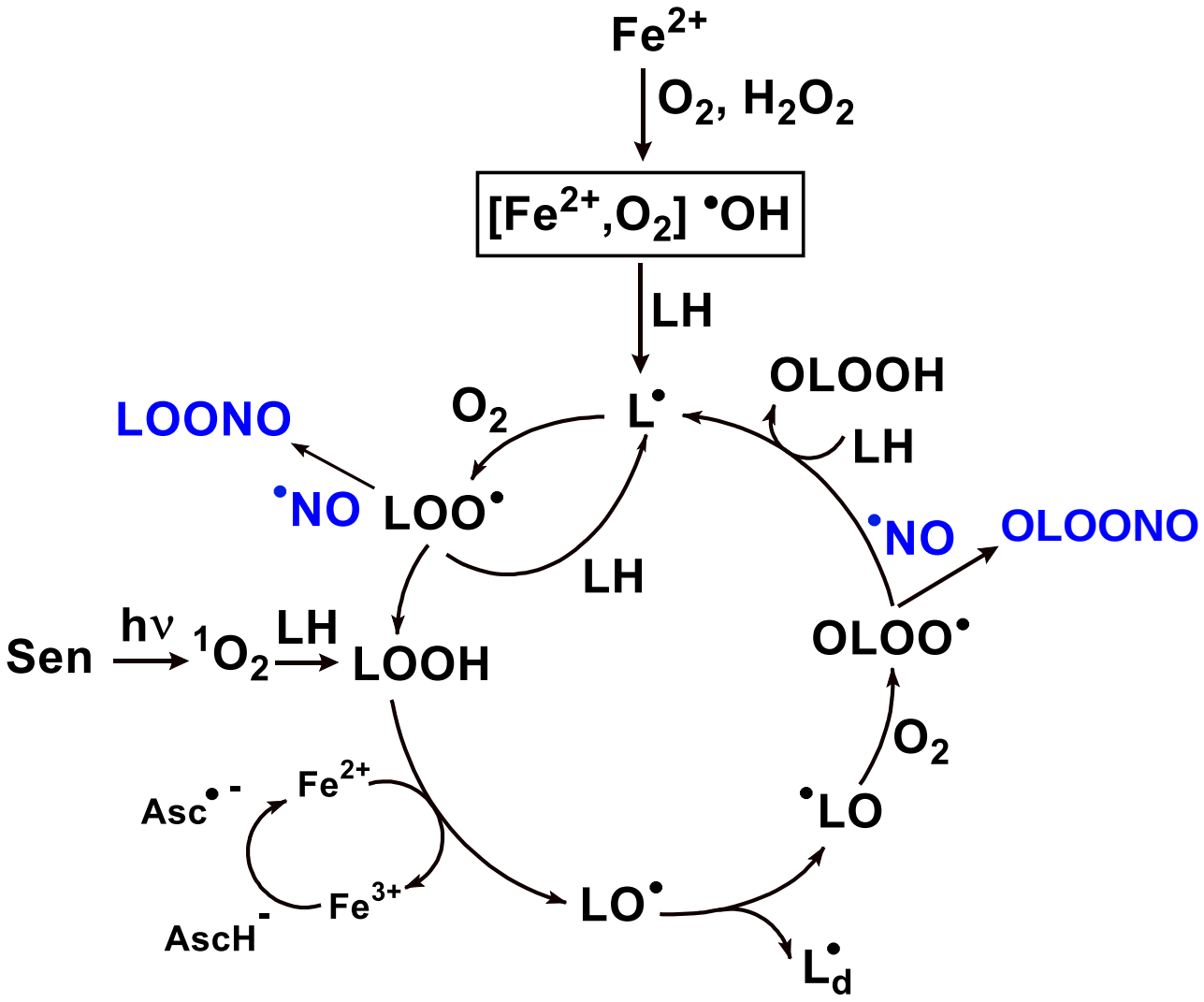
We have used this for centuries in food preservation, the "sausage" effect.

Chain-breaking: **•NO can react with oxyradicals:**

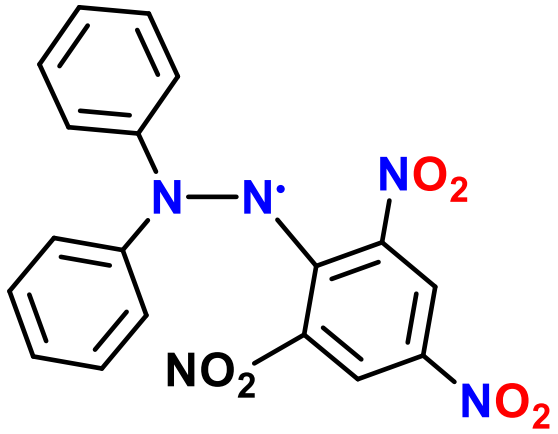
$$\text{ROO}^{\bullet} + {}^{\bullet}\text{NO} \rightarrow \text{ROONO}$$
$$\text{RO}^{\bullet} + {}^{\bullet}\text{NO} \rightarrow \text{RONO}$$

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

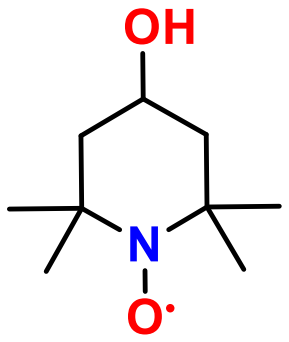
Nitric Oxide as Chain-Breaking Antioxidant in Lipid Peroxidation



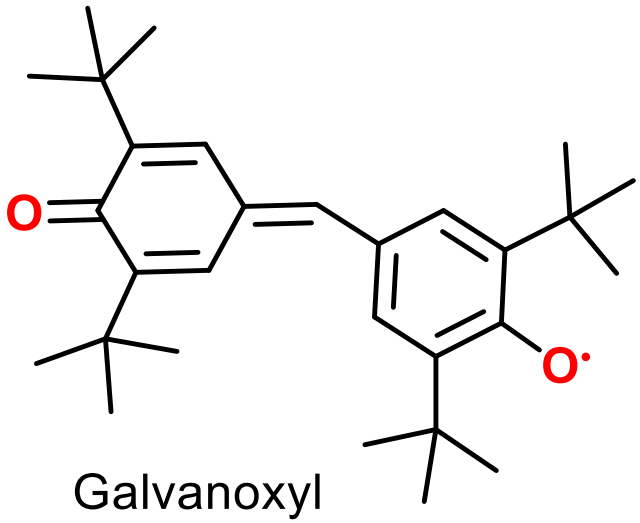
Stable Radical Inhibitors



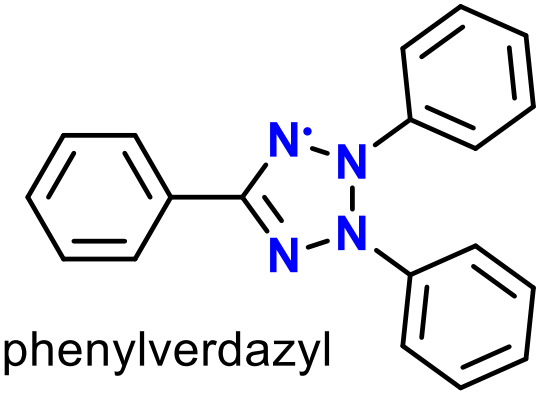
Diphenylpicrylhydrazyl, DPPH



TEMPO



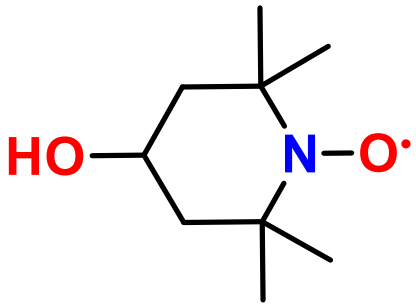
Galvanoxyl



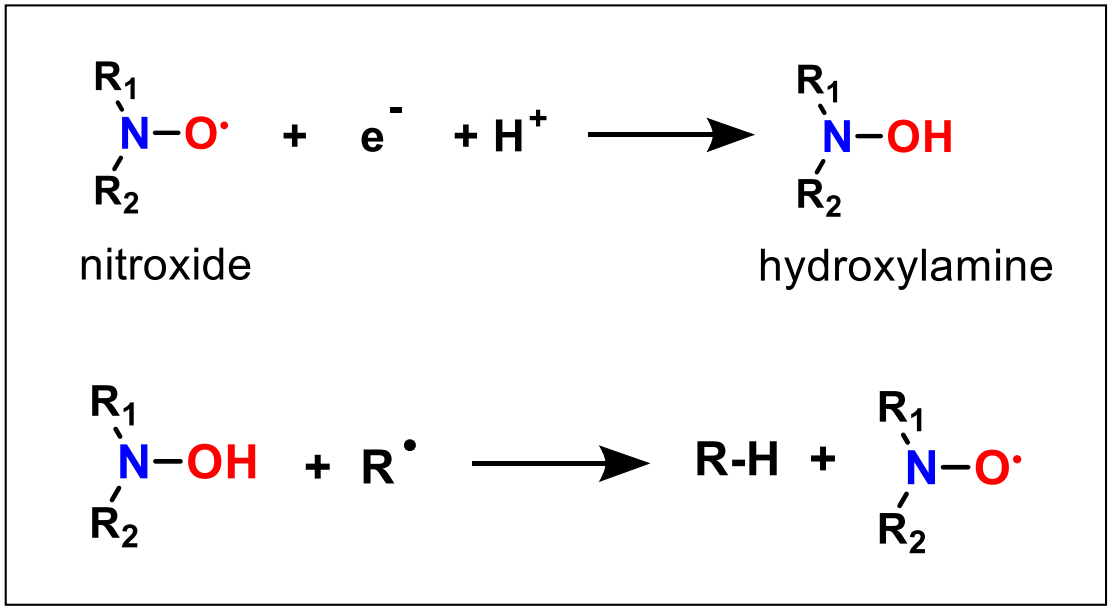
Triphenylverdazyl

Chain Breaking Antioxidant – Nitroxide

Nitroxide: Stable radicals owing to three electron bonds – example: Tempol



A possible antioxidant cycle for a nitroxyl radical



Radical Scavenging Antioxidant: Donor Antioxidants

- Trapping of carbon and oxygen-centered radicals occur with chain breaking antioxidants by hydrogen transfer to give persistent radicals (A•) which live enough in the media to trap by termination all reactive radicals forming stable products.

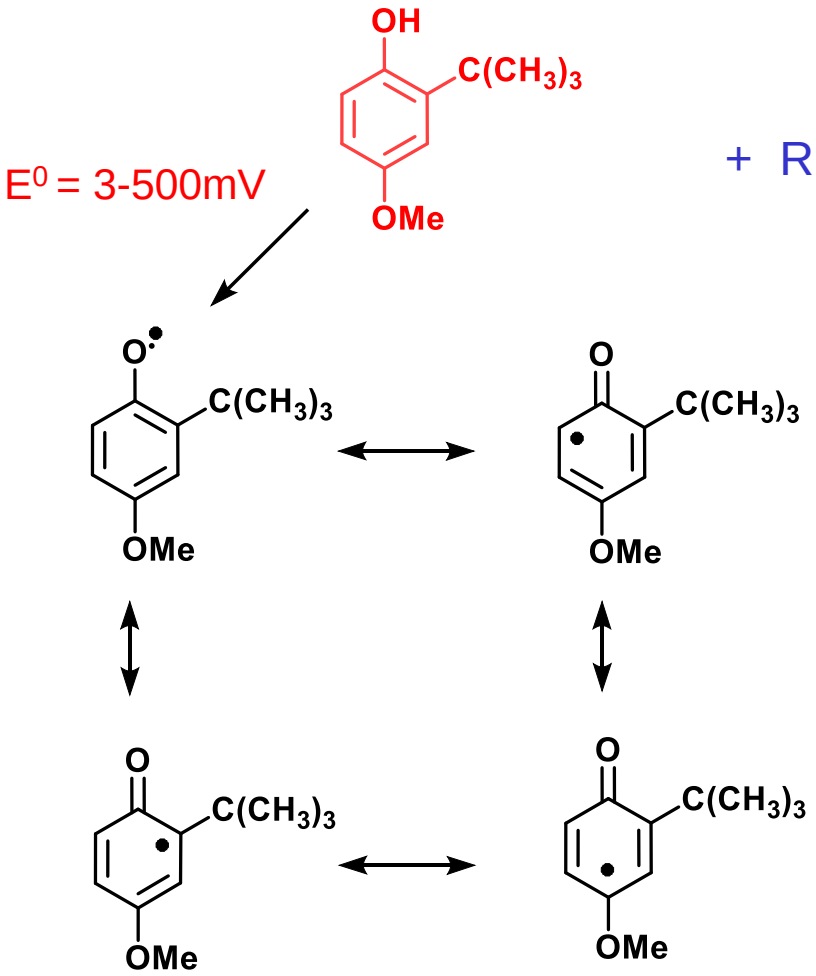
$$\left.\begin{array}{lcl} R^{\bullet} + AH & \longrightarrow & RH + A^{\bullet} \\ RO^{\bullet} + AH & \longrightarrow & ROH + A^{\bullet} \\ ROO^{\bullet} + AH & \longrightarrow & ROOH + A^{\bullet} \end{array}\right\}$$

$$\left.\begin{array}{lcl} R^{\bullet} + A^{\bullet} & \longrightarrow & RA \\ RO^{\bullet} + A^{\bullet} & \longrightarrow & ROA \\ ROO^{\bullet} + A^{\bullet} & \longrightarrow & ROOA \end{array}\right\}$$

$$\text{Antioxidant} + O_2 \longrightarrow \text{Oxidized antioxidant}$$

Phenol Antioxidants

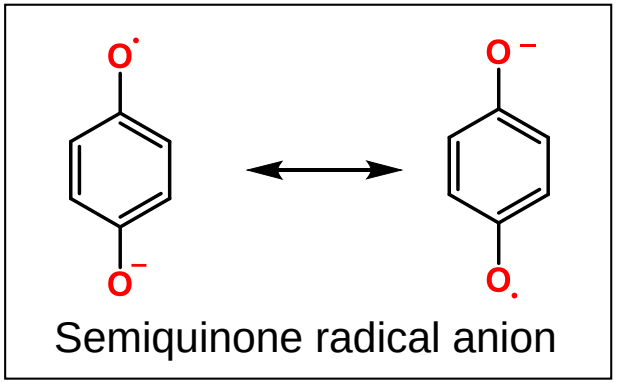
- Phenoxy Radicals are Resonance Stabilized.



E^0 = 1000 mV

+ R• , RO• , ROO• → RH, ROH, ROOH

($k = 10^7\ M^{-1}\cdot sec^{-1}$)

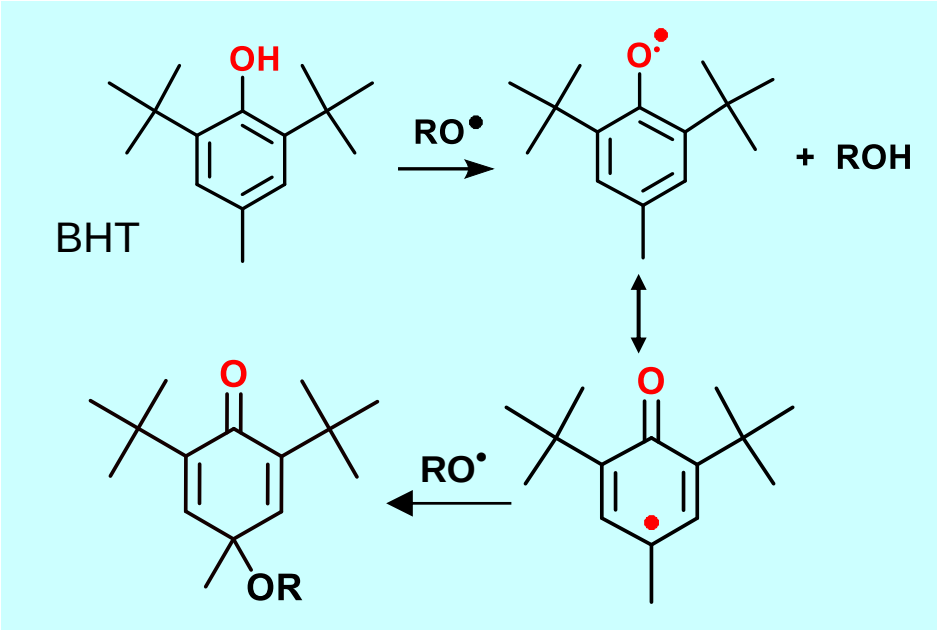


Hindered Phenols

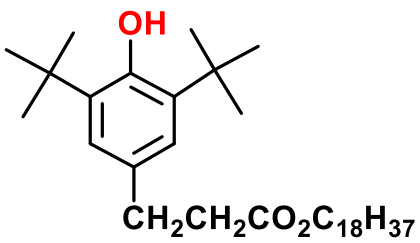
Steric hindrance make even more less reactive phenoxy radicals. Some of these compounds, i.e. galvinoxyl, can be stored for years.

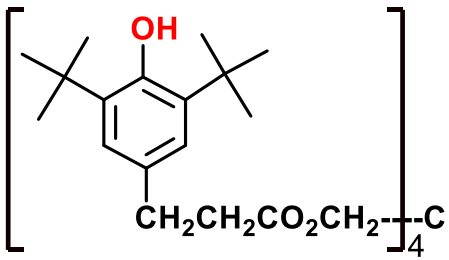
BHT has FDA approval and is relatively inexpensive

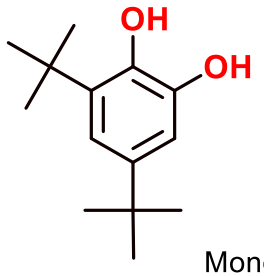
But volatile and may cause discoloration.



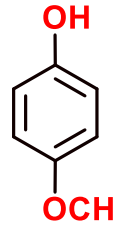
Less volatile HP:



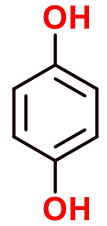




3,5-ditert-butyl catechol

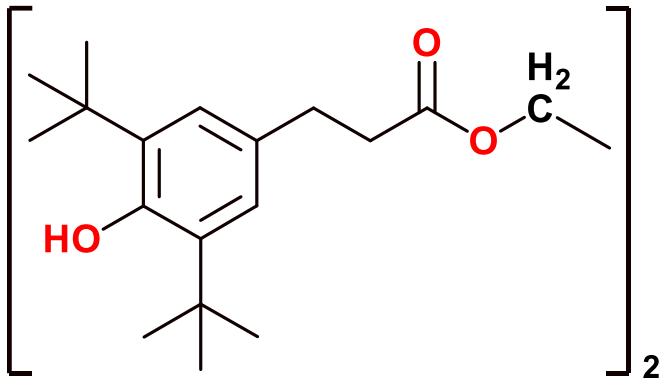


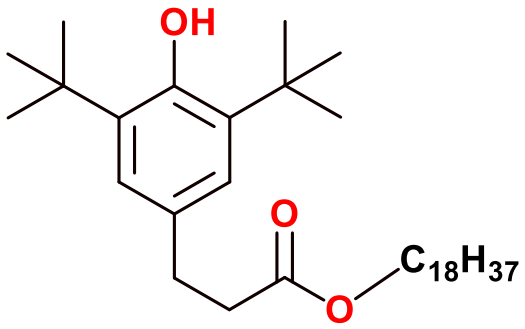
Monomethylhydroquinone

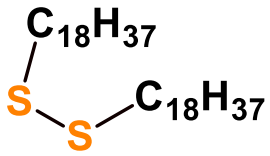


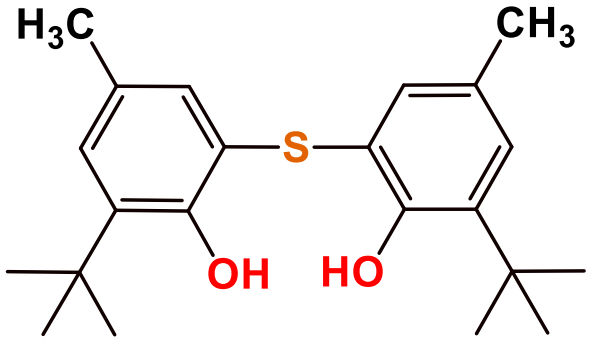
HQ

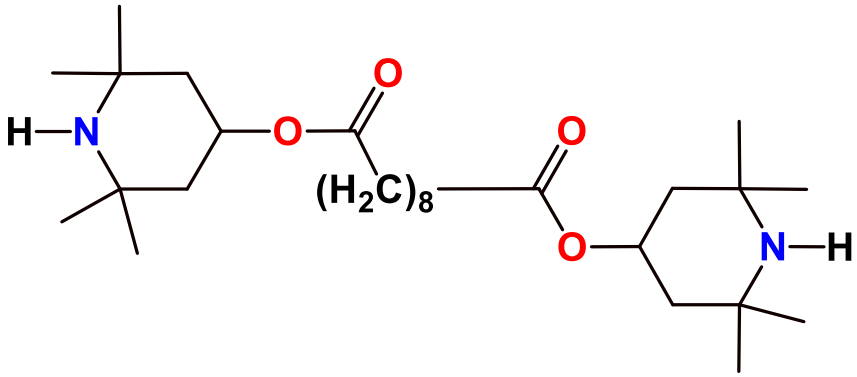
Other Synthetic Antioxidant Phenols



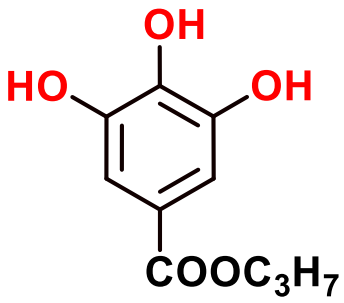




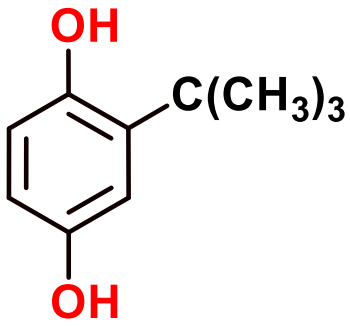




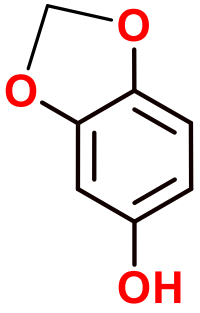
Other Natural Phenol Antioxidants



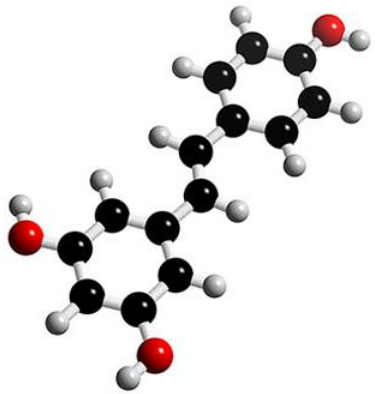
Propyl Gallate



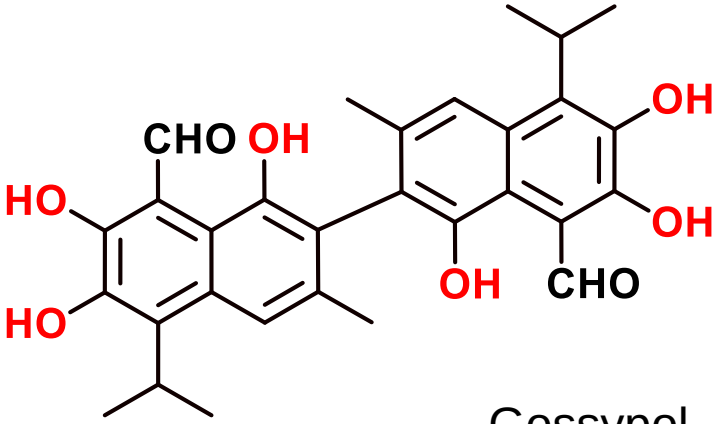
TBHQ



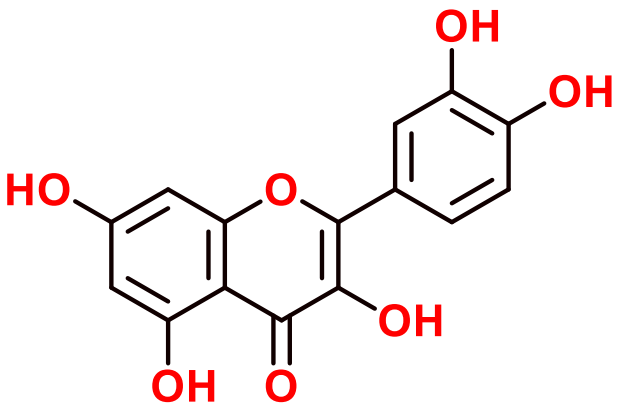
Sesamol



Resveratrol



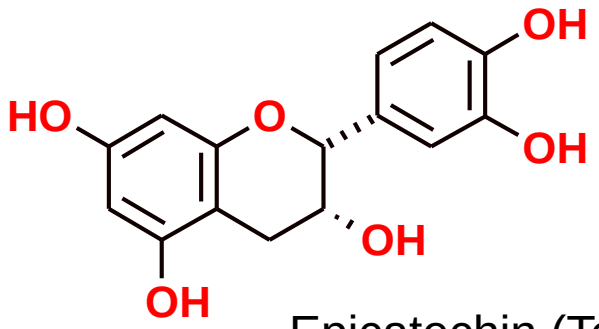
Gossypol



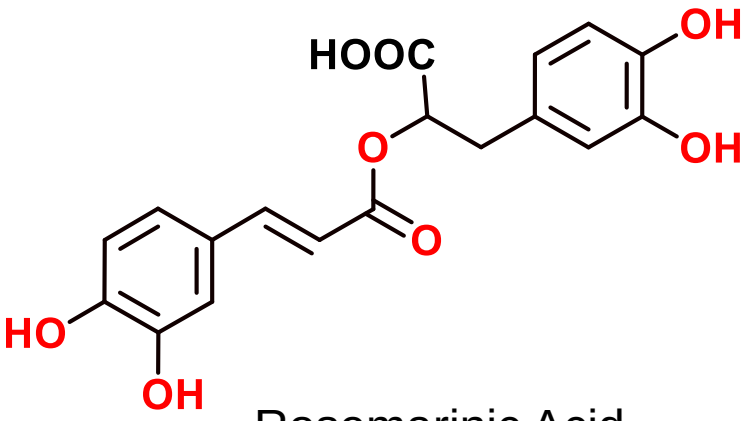
Quercetin

Other Antioxidant Phenols

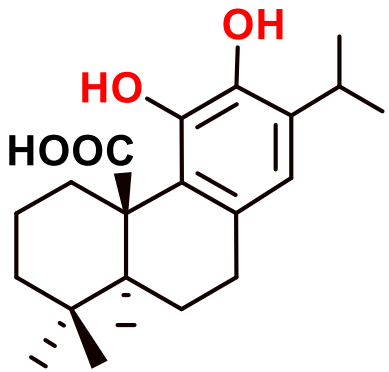
- Anthocyanins
- Polyphenolic compounds
- Isoflavones



Epicatechin (Tea)

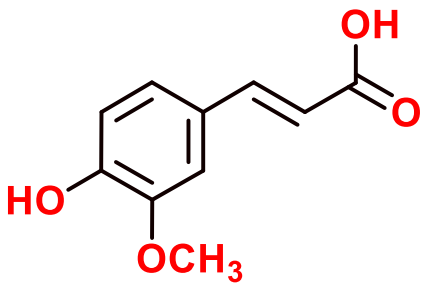


Rosemarinic Acid



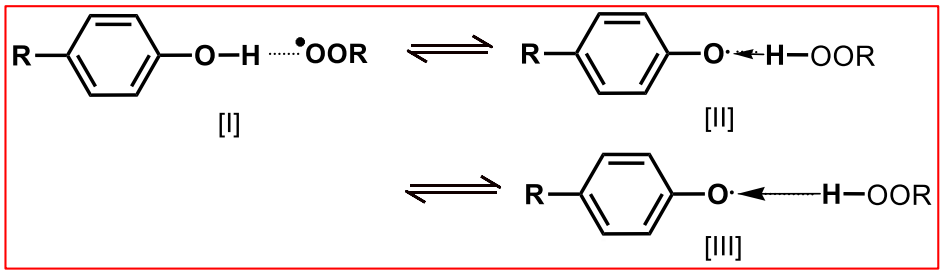
Carnosoic Acid (Ginger)

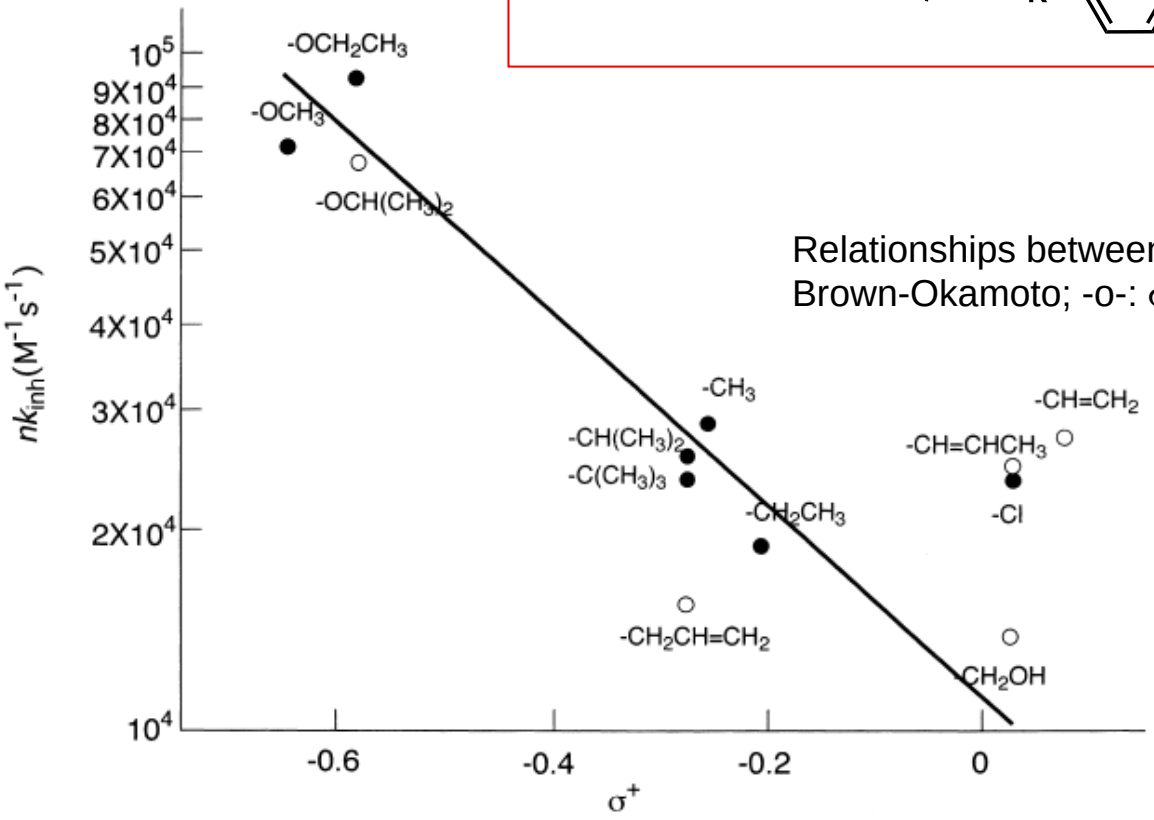
$3 \times 10^7\ M^{-1} \cdot s^{-1}$ (ROO•)



Ferulic acid (Ginseng)

Inhibition effect of Phenols and Acidity

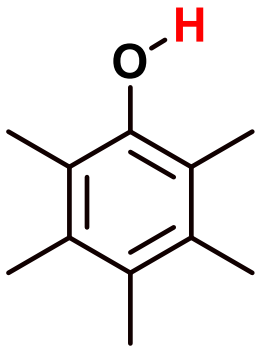




Relationships between nk_{inh} and σ^+; -•-: σ^+ value of Brown-Okamoto; -o-: σ^+ value estimated from ν_{OH}.

T. Kajiyama, Y. Ohkatsu / Polymer Degradation and Stability 71 (2001) 445±452

Effect of Type and Position of Substituents on Bond Dissociation Enthalpy of Antioxidant



87
(kcal·mol^{-1})

Group	Bond Dissociation Enthalpy (kcal·mol^{-1})		
	ortho	*meta*	*para*
OCH_3	86	86	81
OH	79	87	81
CH_3	85	87	85
tert-butyl	84	86	85
CHO	95	89	90
COOH	95	90	90

ROO• + Oleic Acid $\rightarrow$ 1 $M^{-1}\cdot sec^{-1}$

ROO• + Antioxidant $\rightarrow$ 10^7 $M^{-1}\cdot sec^{-1}$

Sources of antioxidants in the diet

Natural Antioxidants: 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.**

$$LOO^{\bullet} + TOH \xrightarrow{8\times10^{4}\ M^{-1}\cdot s^{-1}} LOOH + TO^{\bullet}$$

$$TO^{\bullet} + LOO^{\bullet} \xrightarrow{6\times10^{8}\ M^{-1}\cdot s^{-1}} T(O)\text{-}OOL$$

Termination

Buettner GR. *Arch Biochem Biophy*. **1993**, *300*, 535-543.

The Pecking Order of Redox Potentials

Note that the donor antioxidants are found in the middle of the "pecking order".

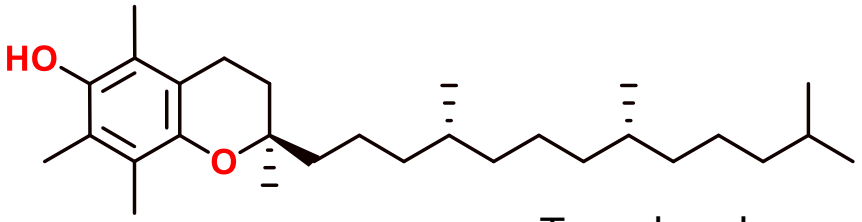
Redox Couple (one-electron reductions)	E°'/mV
$HO^{\bullet}, H^{+}/H_2O$	+ 2310
$RO^{\bullet}, H^{+}/ROH$ (aliphatic alkoxyl radical)	+ 1600
$ROO^{\bullet}, H^{+}/ROOH$ (alkyl peroxyl radical)	+ 1000
$GS^{\bullet}/GS^{-}$ (glutathione)	+ 920
$PUFA^{\bullet}, H^{+}/PUFA\text{-}H$ (*bis*-allylic-H)	+ 600
$TO^{\bullet}, H^{+}/TOH$ (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
$RSSR/RSSR^{\bullet-}$ (GSH)	- 1500
H_2O/e^{-}_{aq}	- 2870

Buettner GR. (1993) The pecking order of free radicals and antioxidants: Lipid peroxidation, α-tocopherol, and ascorbate. *Arch Biochem Biophys.* **300:**535-543**.** [PDF]

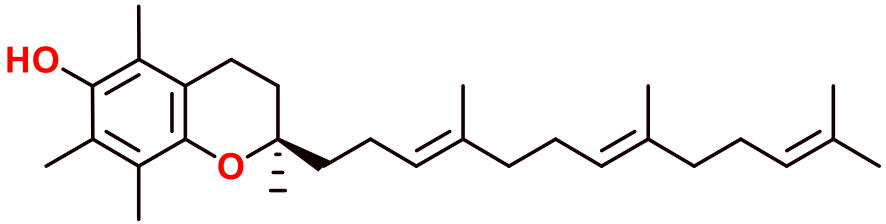
Importance in Biology & Medicine of Vitamin E

- The aromatic ring allows it to donate one or two electrons and still maintain a relatively stable structure

Vitamin E

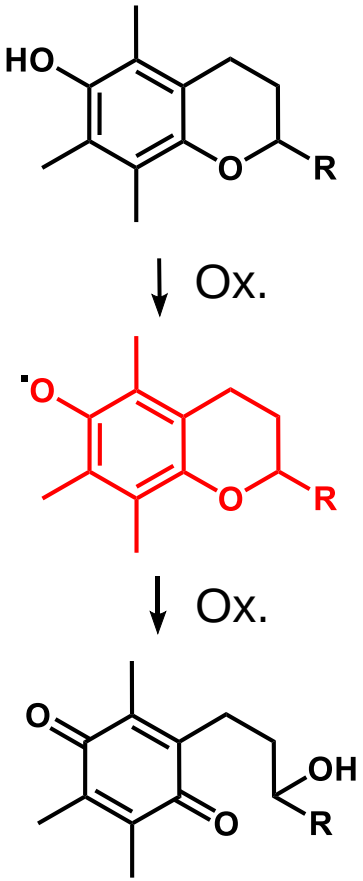


α-Tocopherol
(most common)

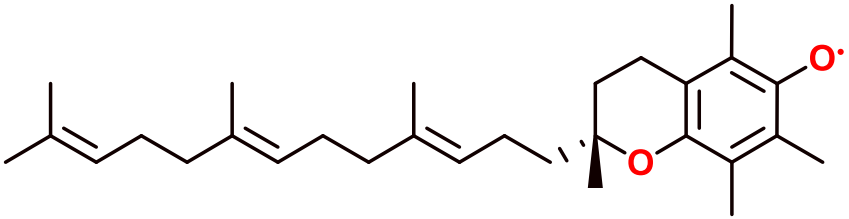


Tocotrienol

Source: http://www.biochem.wisc.edu/biochem510/slides/20antiox1.pdf



Donor Antioxidant - Vitamin E Regeneration

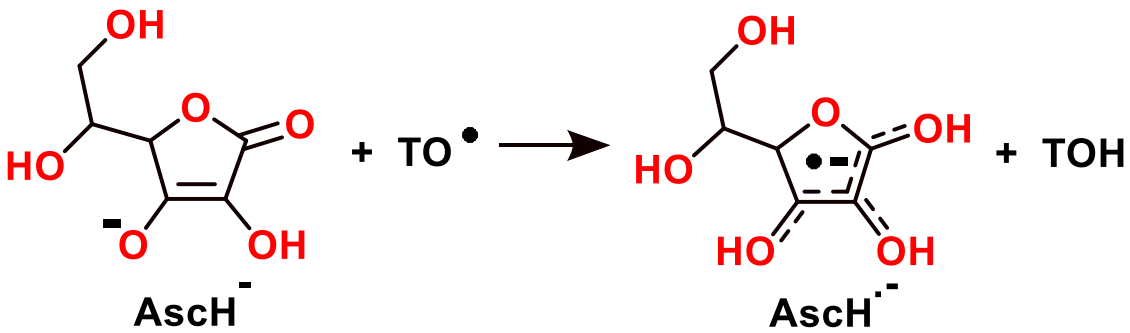


TO·

Reaction with lipid peroxides:

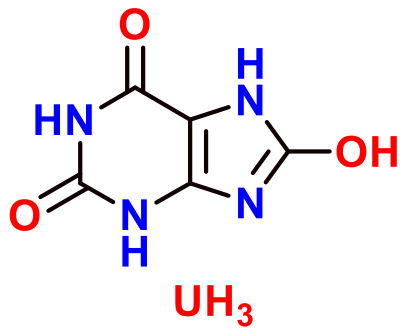
$$LOO^{\bullet} + TOH \longrightarrow LOOH + TO^{\bullet}$$

Recycling reaction with ascorbate



Donor Antioxidant – Uric Acid

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



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

Normal urate concentrations in 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.

$$ROO^{\bullet} + UH_2^{-} \xrightarrow{k = 1 \cdot 10^6\ M^{-1} \cdot s^{-1}} ROOH + UH^{\bullet -}$$

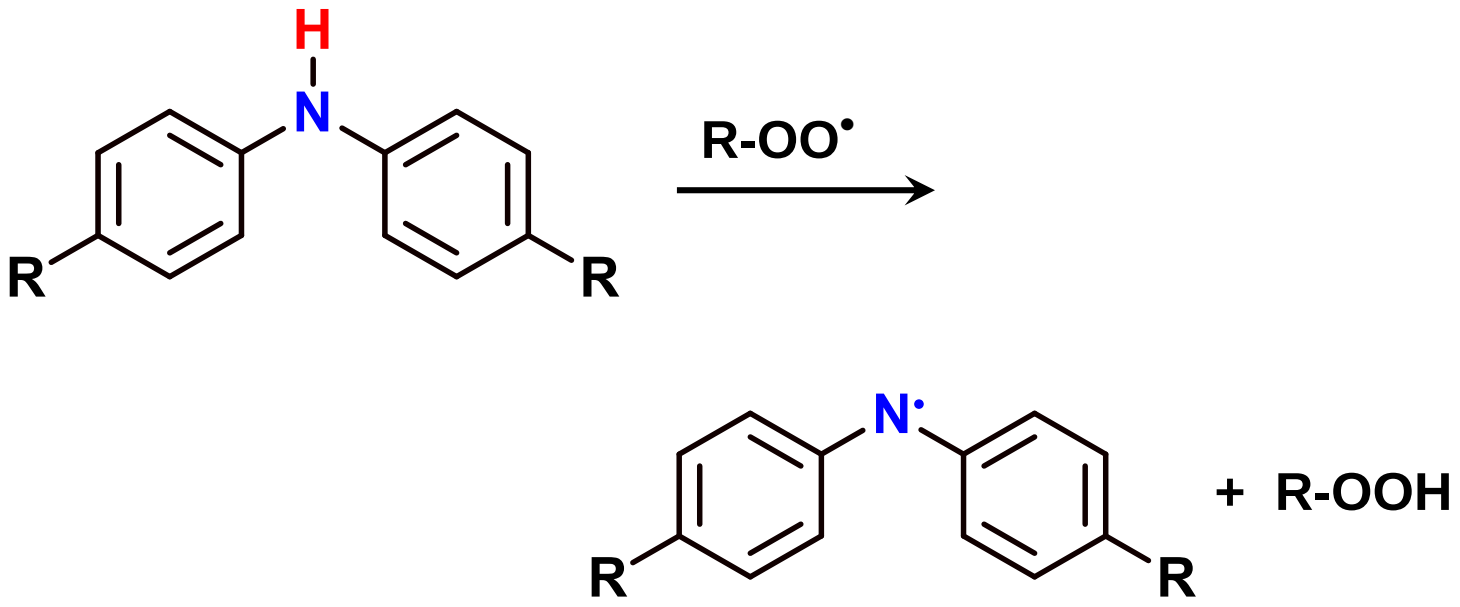
Recycling by Ascorbate:

$$UH^{\bullet -} + AscH^{-} \xrightarrow{k = 3 \cdot 10^6\ M^{-1} \cdot s^{-1}} UH^{2-} + Asc^{\bullet -}$$

Diaryl Amine Antioxidants

Hydrogen Donor to Conjugated Nitrogen

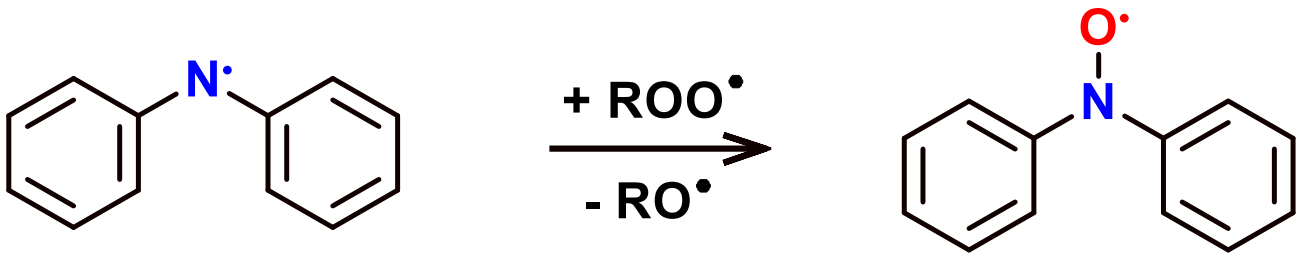
- Generates Aromatic Aminyl Radicals



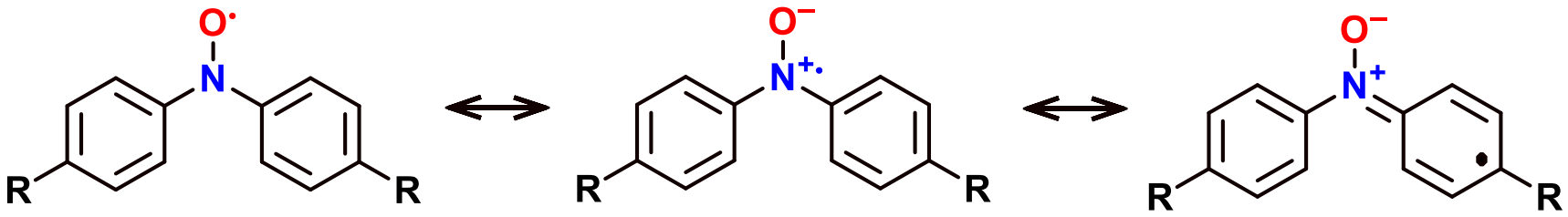
Diaryl Amine Antioxidants

Hydrogen peroxide decomposition

- Generates Nitroxyl Radical



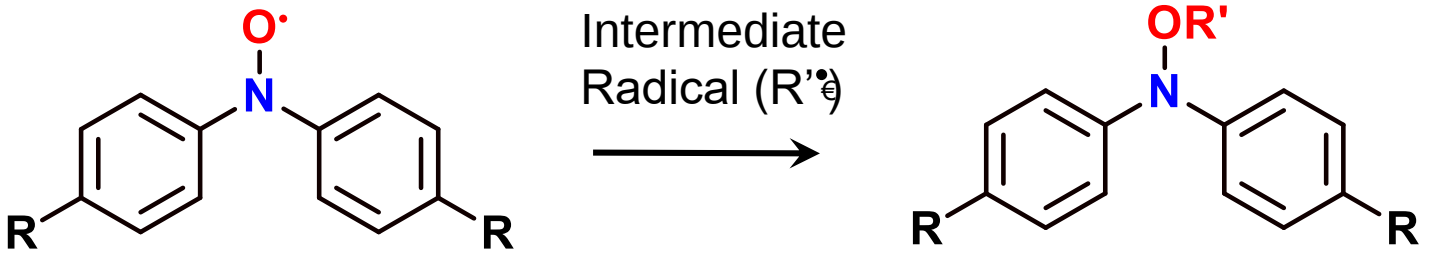
Nitroxyl Resonance Structures



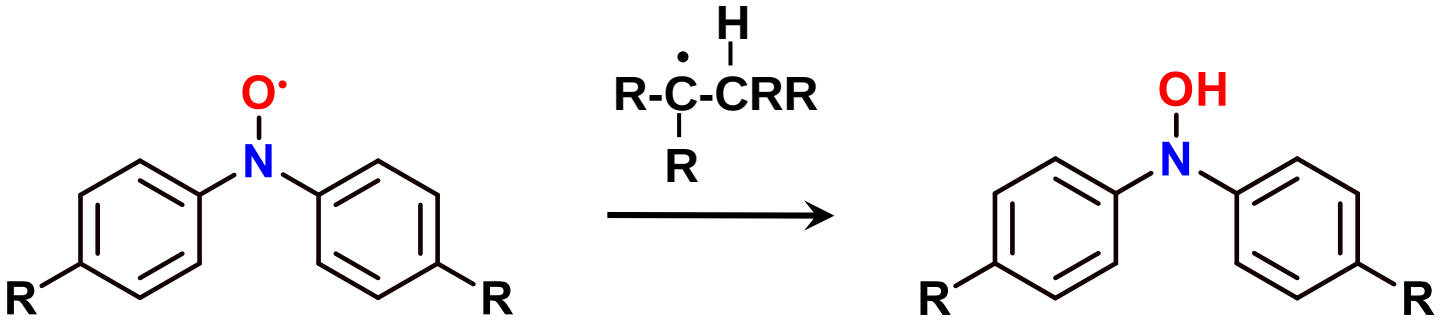
Diaryl Amine Antioxidants

Nitroxyl as Radical Traps:

- By dimerization

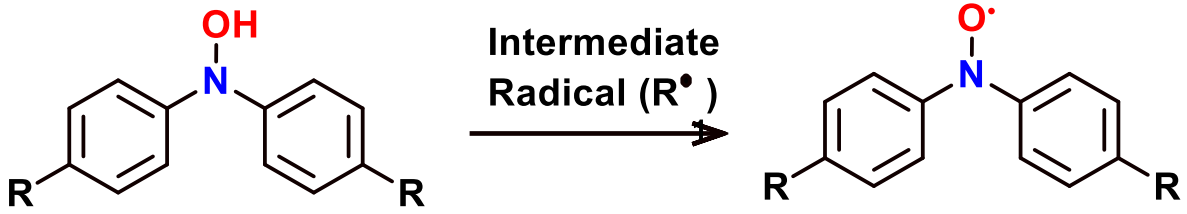


- By disproportionation

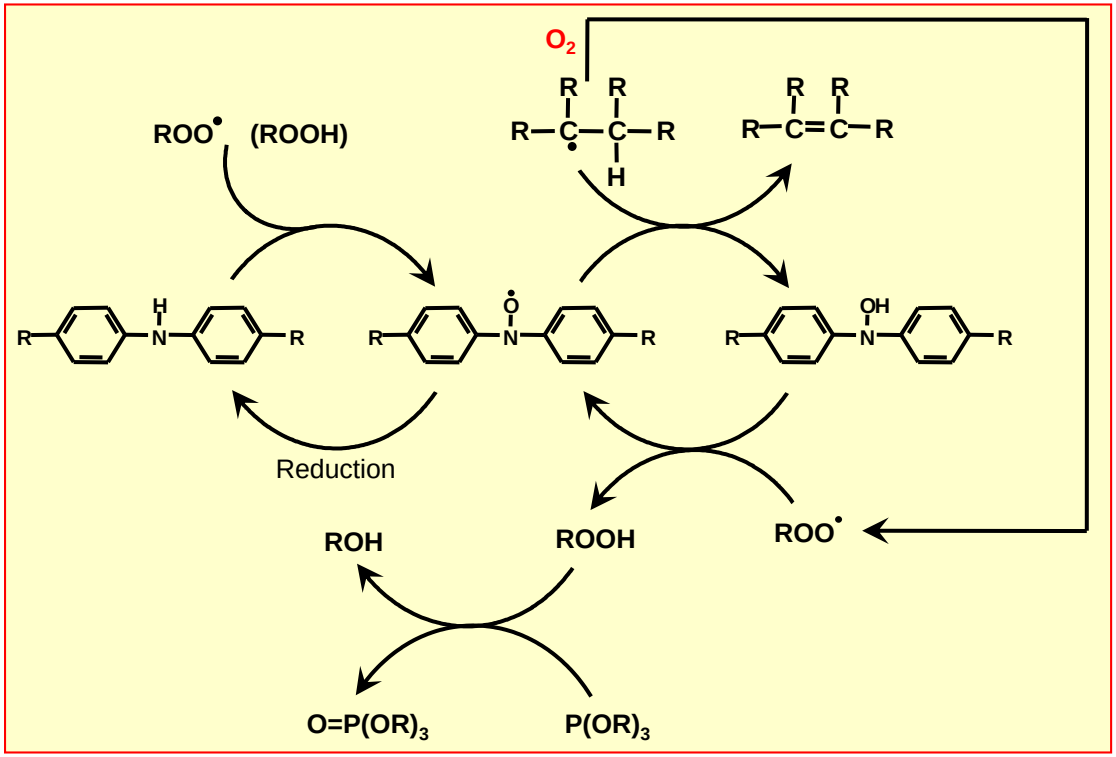


Diaryl Amine Antioxidants

Hydroxylamine as Hydrogen Donor - Nitroxyl Regeneration

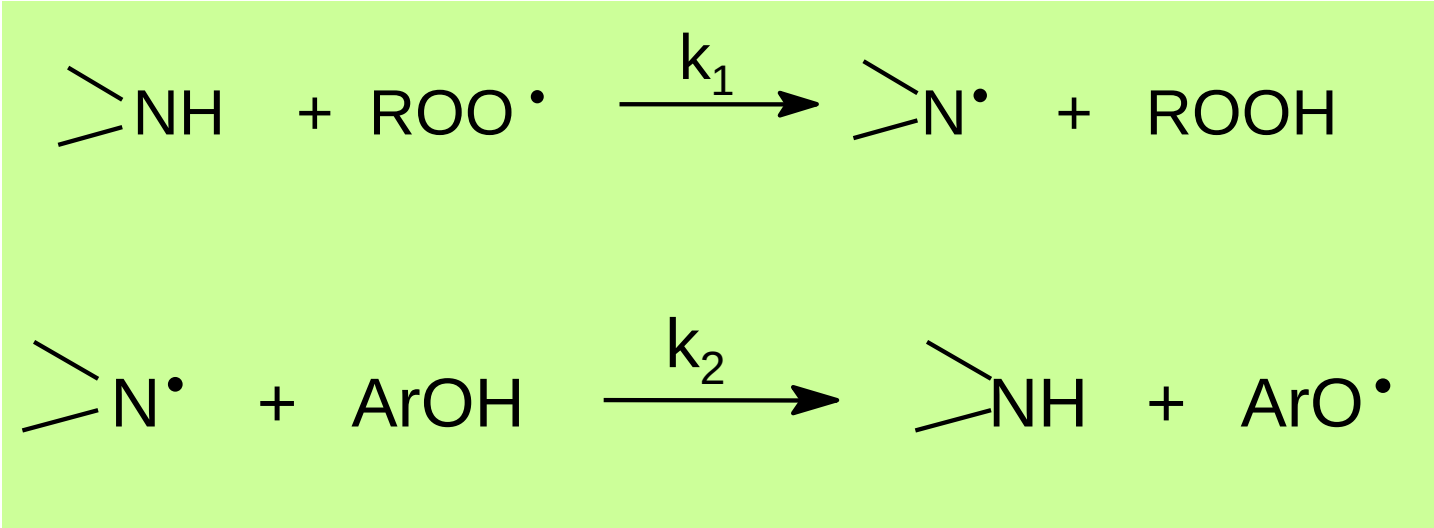


Chain processes in the presence of diaryl amine and trialkyl phosphite antioxidants



Diaryl Amine Antioxidants

- Act synergistically with phenols, and possibly other donor antioxidants, in that the latter regenerate aryl amine



$$k_2 \geq k_1$$

Donor Antioxidants: Mercaptans

- Mercaptans (R'S-H) are efficient donor of hydrogen atoms mainly carbon-centered radical, the resulting thiyl radical terminates commonly by dimerization, but addition to double bond is also known.

$$R\bullet + R'S\text{-}H \rightarrow R\text{-}H + R'S\bullet$$
$$2R'S\bullet \rightarrow R'S\text{-}SR'$$

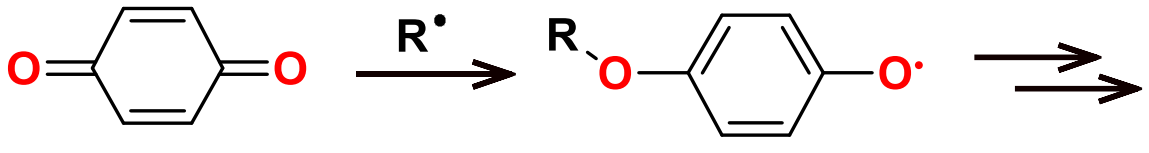
- Mercaptans are also oxidized by O_2 in the presence of catalytic amount of a base, generating superoxide radical anion and thiyl radicals. The reaction can be a possible source of ROS.

$$O_2 + R'S^- \rightarrow O_2^{\bullet -} + R'S\bullet$$

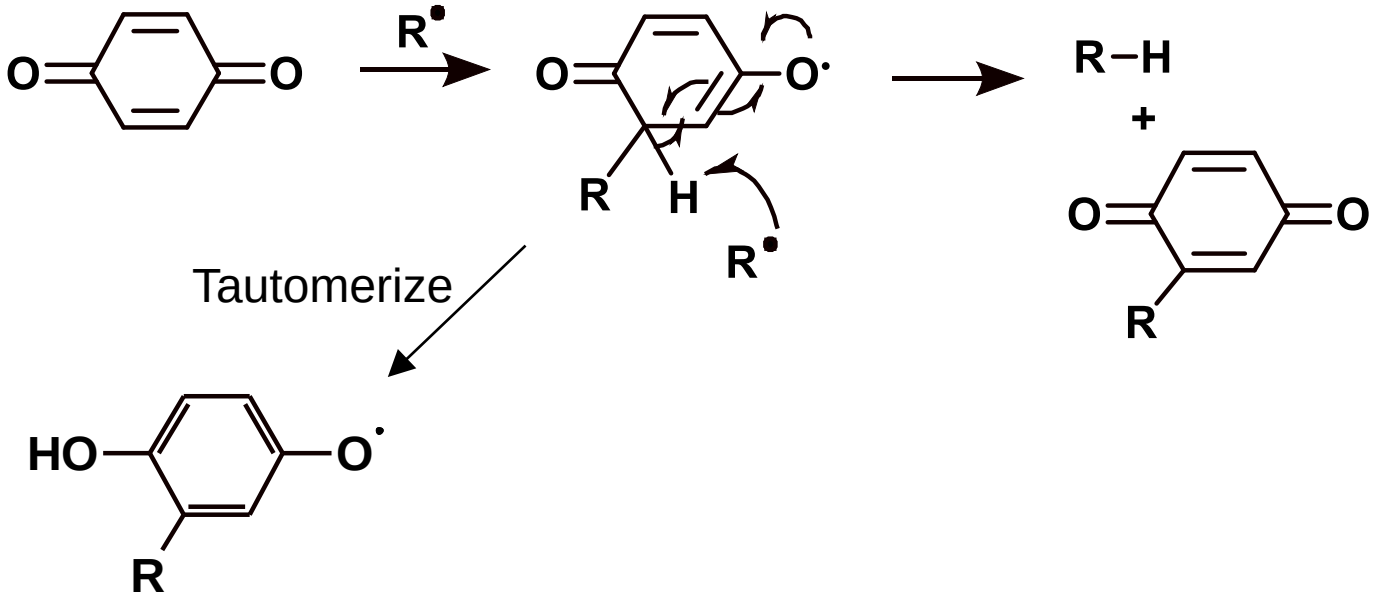
- Regeneration of thiols can be realized with several different reducing agent and, in biological systems, by NADPH and other reducing coenzymes.

Chain Breaking Antioxidants: Quinones

- Carbon centered radicals stopped by addition to oxygen and mainly to carbon:



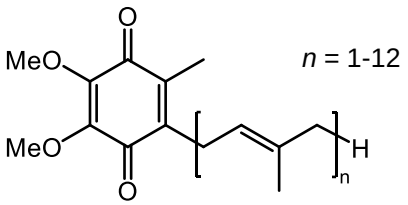
Benzoquinone



Antioxidants and more…Phylloquinones and tocopherols are part phenolic, part isoprenoid

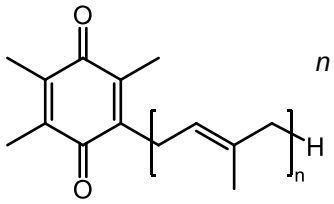
Coenzyme Q10:

Redox carrier for electrons in human mitochondrial ETS

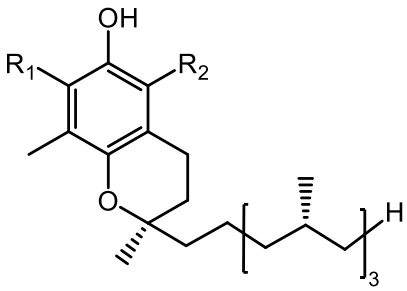


ubiquinone-*n*

(coenzyme Q_n)

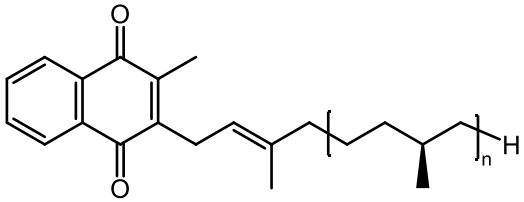


Plastoquinone-*n*

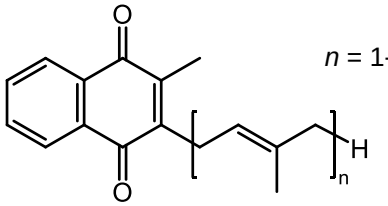


R_1=R_2= Me, α-tocopherol
R_1=R_2= Me, β-tocopherol
R_1=Me, R_2= H, γ-tocopherol
R_1=R_2= H, δ-tocopherol

(vitamin E)



phylloquinone
(vitamin K, phytomenadione)



menaquinone-*n*

(vitamin K_2)

Vitamin K1

Sources: plants, primarily green veggies

Role: blood clotting – needed for carboxylation of Glutamate residues in prothrombin

Inhibited by warfarin (Coumadin)

Vitamin E

Sources: cereals, seed oils eggs, soybean, corn oil, barley

--Free radical scavenger

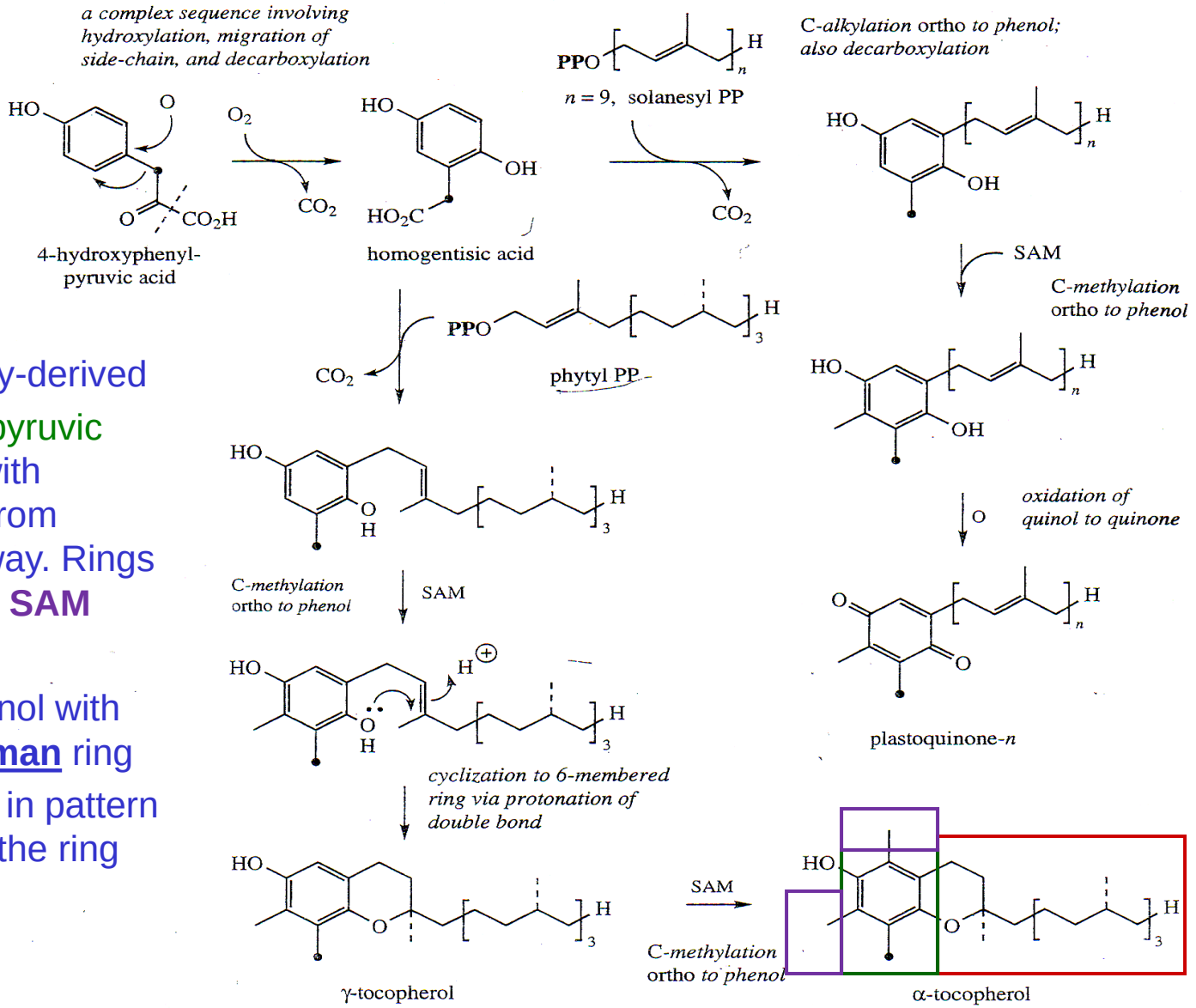
--Protects lipids in LDL and cell membranes from oxidation

Biosynthesis of Vitamin E:

Shikimate pathway-derived 4-hydroxyphenyl pyruvic acid is alkylated with isoprenoid chain from mevalonate pathway. Rings are methylated by **SAM**

Cyclization of phenol with chain forms **chroman** ring

Tocopherols differ in pattern of methylation on the ring

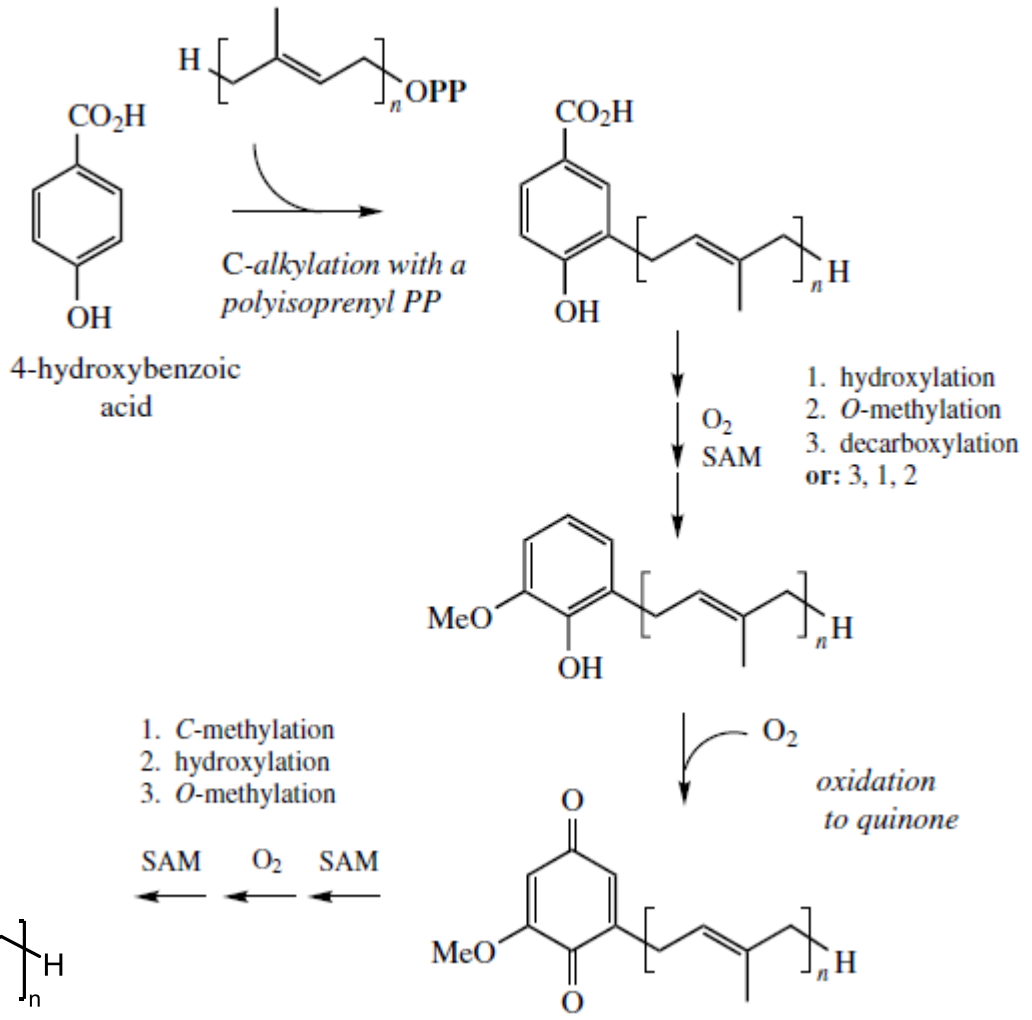


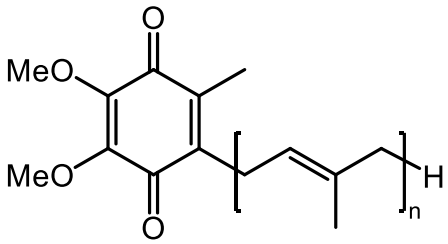
Oxidation of Phenolic Ring to Quinone Forms Plastoquinones, Ubiquinones.

Coenzyme Q10 comes from 4-hydroxybenzoic acid precursor.

After attachment of the isoprene chain, the ring is:

1. Decarboxylated
2. oxidized to quinone
3. Methylated
4. Hydroxylated
5. O-methylated





ubiquinone-*n*

POLITECNICO DI MILANO





Antioxidants: Small Molecules

Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”

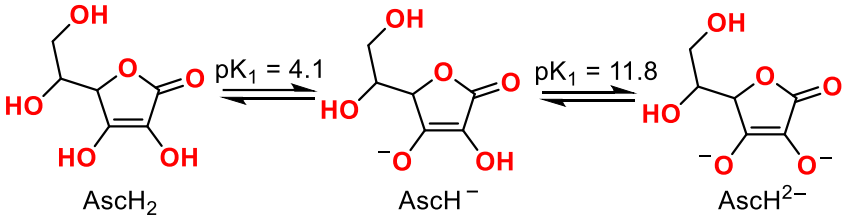
Small Antioxidant Molecules

- Ascorbic acid
- Vitamin E
- Vitamin C
- Coenzyme Q
- Uric Acid
- Carotenoids
- Lycopene
- Melatonin

β-carotene/vitamin A
SOD mimic
DTT
Taurine
“E”-analogues
α-Tocopherol
EDTA
γ-Tocopherol
Ergothionine
Trolox
Flavonoids
Urate
GPx mimics
Vitamin K
GSH
Zinc
Lactate

Lipoic acid
BHA/BHT
Lycopene
Bathocuproine
NAC
Bilirubin
NADPH
“C”-analogues
Nitroxides
CAT mimics
NO•
CoQH2
OTC
Cysteine
Pyruvate
Desferal□
Resveratrol

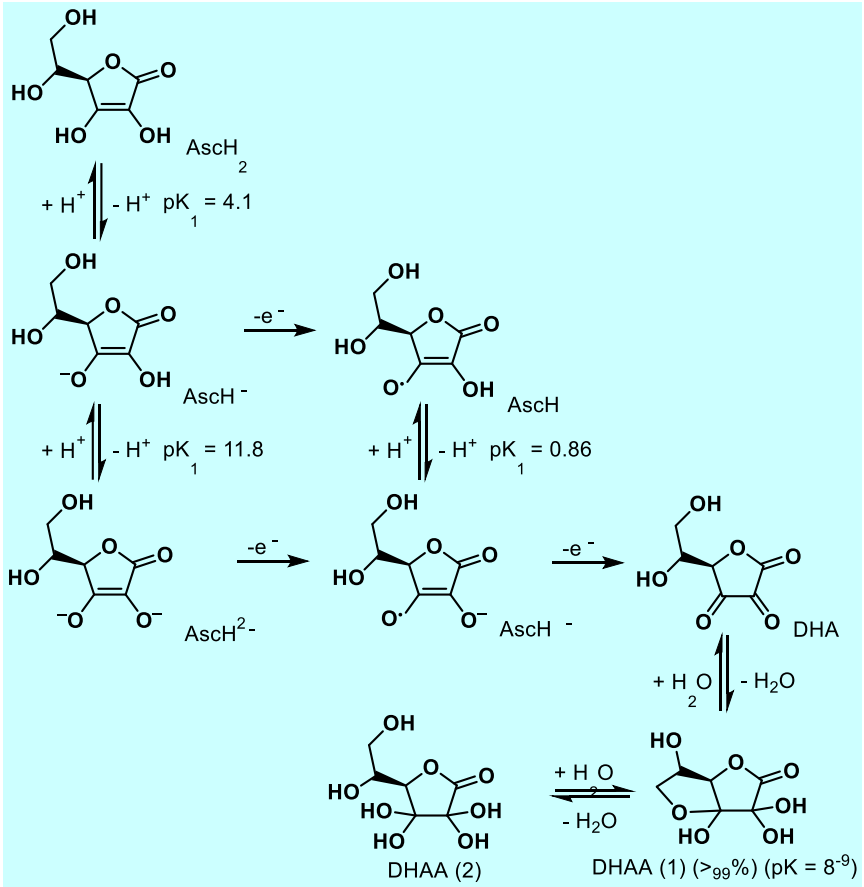
Ascorbic Acid



At pH 7.4, 99.95% of vitamin C will be present as $AscH^-$; 0.05% as $AscH_2$ and 0.004% as Asc^{2-}. Thus, the antioxidant chemistry of vitamin C is from $AscH^-$.

The unpaired electron of $Asc^{\bullet-}$ resides in the π-system that includes the tri-carbonyl moiety of ascorbate. This results in a weakly oxidizing and weakly reducing radical. Due to its π-character $Asc^{\bullet-}$ does not react with oxygen to form dangerously oxidizing peroxyl radicals. Thermodynamically, it is relatively unreactive with a one-electron reduction potential of only +282 mV. It is considered to be a terminal, small-molecule antioxidant.

Equilibria of Ascorbate



Kinetics of AscH$^-$ Reactions

Radical	K_{obs}/M^{-1}·s^{-1} (pH 7.4)[a]
HO$^\bullet$	1.1×10^{10}
RO$^\bullet$ (ter-butyl alkoxy radical)	16×10^{9}
ROO$^\bullet$ /alkyl peroxyl radical e.g. $CH_3OO^\bullet$	$1\text{-}2\times10^{6}$
$CCl_3COO^\bullet$	1.8×10^{8}
GS$^\bullet$ (glutathiyl radical)	6×10^{8} (5.6)
UH$^{\bullet-}$ (Urate radical)	1×10^{6}
TO$^\bullet$ (Tocopheroyl radical)	2×10^{5} [b]
Asc$^{\bullet-}$	2×10^{5}
CPZ$^{\bullet+}$	1.4×10^{9} (5.9)
Fe(III)EDTA / Fe(II)EDTA	$\approx10^{2}$
O$^{\bullet-}$ /$HO_2^\bullet$	2.7×10^{5}
Fe(III)Desferal© / Fe(II)Desferal©	Very slow

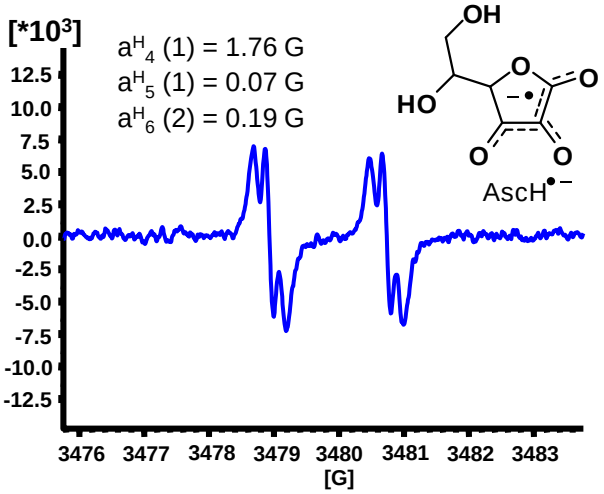
[a] Estimated k_{obs} for TO$^\bullet$ when in a biological membrane.
[b] k is pH dependent, thus this is k_{obs} at pH 7.4.

Adapted from: Buettner GR, Jurkiewicz BA. *Rad Research* **1996**, *145*, 532-541.

These rate constants are for the reaction:

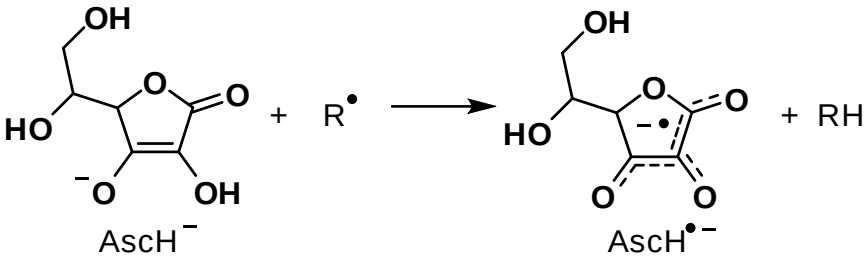
$$AscH^- + R^\bullet \rightarrow Asc^{\bullet-} + RH$$

AscH$^-$ reacts rapidly with these and similar oxidants making it an outstanding donor antioxidant.



With appropriate instrument settings a more detailed spectrum can be observed by EPR.

$AscH^-$ is a Donor Antioxidant



$AscH^-$ donates a hydrogen atom ($H^\bullet$ or $H^+ + e^-$) to an oxidizing radical to produce the resonance-stabilized tricarbonyl ascorbate free radical. $AscH^\bullet$ has a pK_a of -0.86; thus, it is not protonated in biology and will be present as $Asc^{\bullet-}$.

Bors W, Buettner GR. (1997) The vitamin C radical and its reactions in *Vitamin C in Health and Disease*, ed. by L. Packer and J. Fuchs, Marcel Dekker, Inc., New York, Chapter 4, pp75-94

Dismutation of Ascorbate Rad.

$2\ Asc^{\bullet-} + H^+ \rightleftarrows AscH^- + DHA$

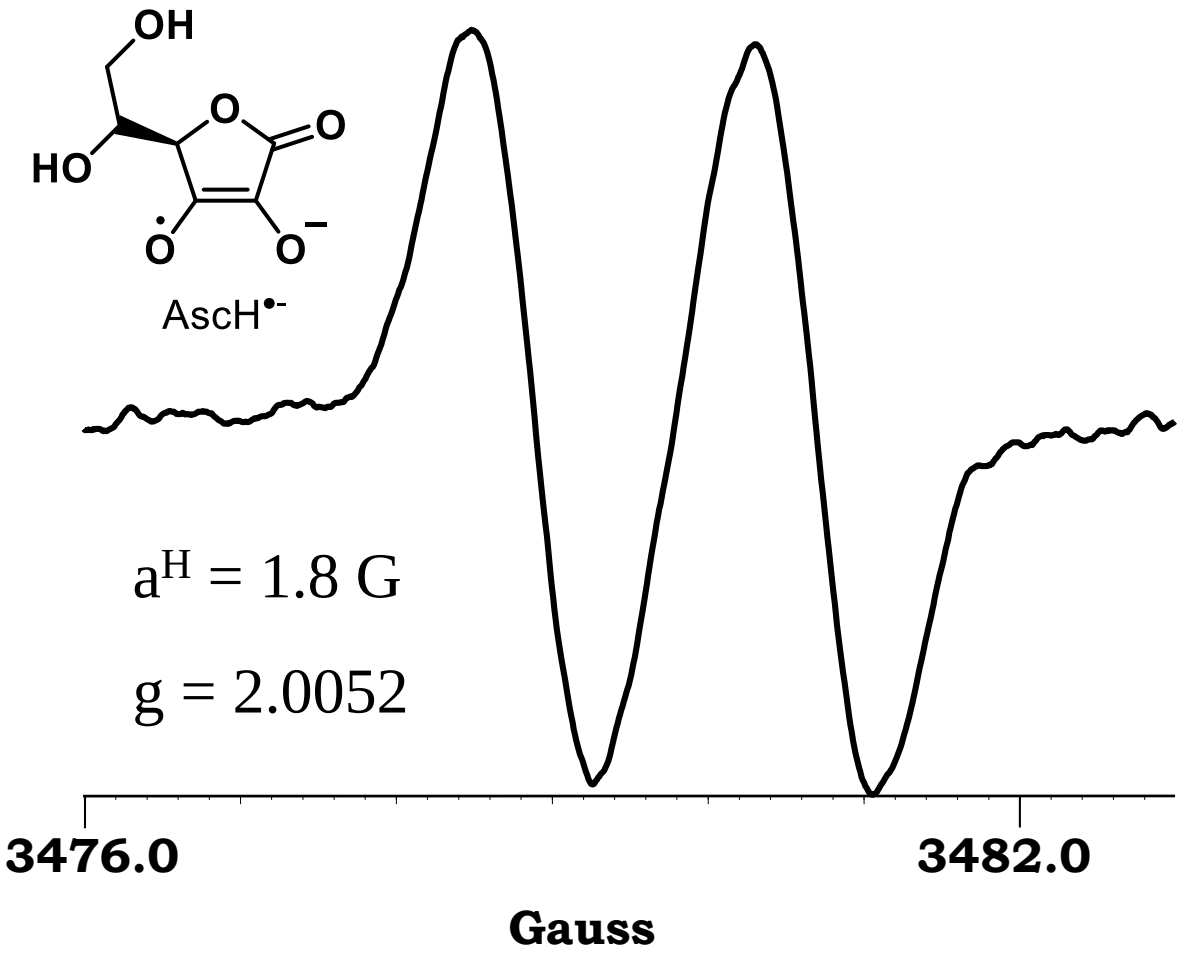
$k_{obs}\ (7.4) = 1.4\times10^5\ M^{-1}\cdot s^{-1}$

$K = 5\times10^{14}\ M^2$ (pH dependent)

This rate constant increases by a factor of ≈10 when phosphate is present.

This dismutation reaction is the principal route to the elimination of the $Asc^{\bullet-}$ *in vitro.* However, *in vivo* it is thought that reducing enzymes are involved in the removal of this radical, resulting in the recycling of ascorbate

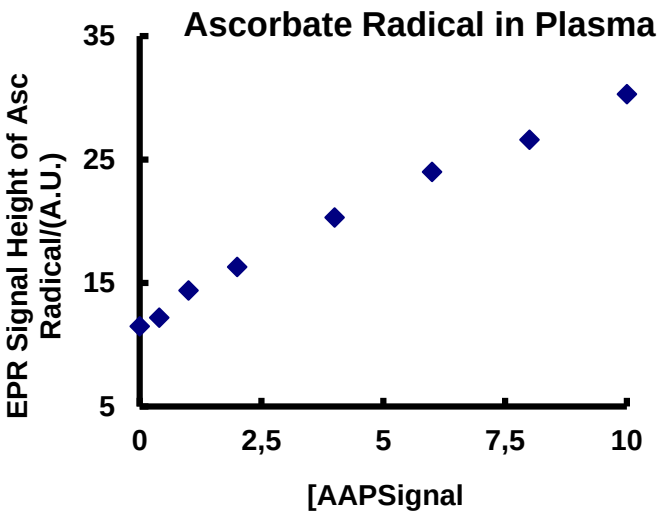
EPR Detection of $Asc^{\bullet-}$

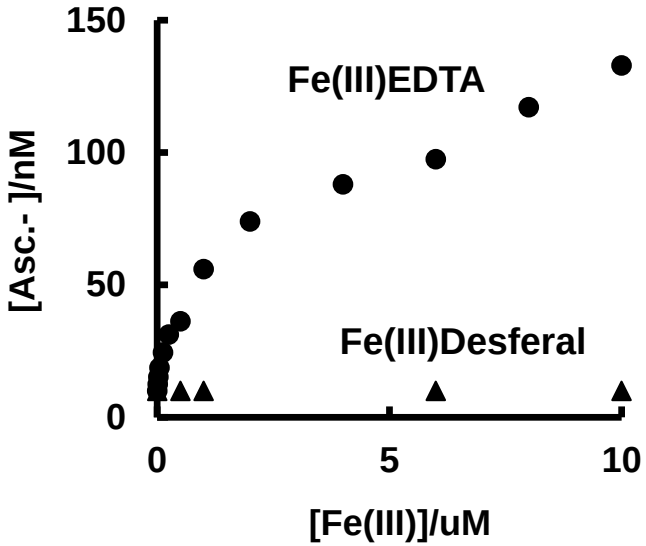


The ascorbate radical is usually observed as a simple doublet species by EPR.

The intensity of the EPR spectrum of $Asc^{\bullet-}$ can be used as an indicator of oxidative stress *in vitro* and *in vivo*.

$Asc^{\bullet-}$, Real Time Marker of Oxidative Stress and Indicator for Adventitious Transition Metals





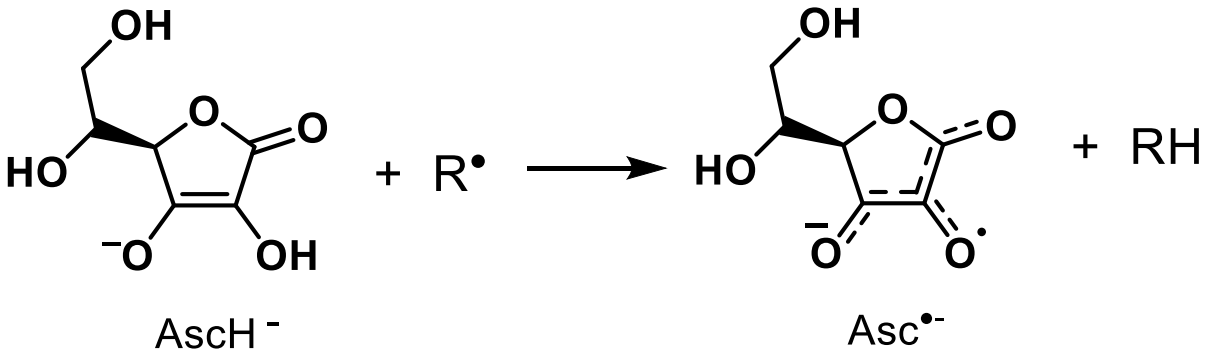
$[Asc^{\bullet-}]_{ss}$ is proportional to the rate of ascorbate oxidation.

In the absence of catalytic metals, ascorbate does not autoxidize at pH 7: Ascorbate as a test for catalytic metals

$[Asc^{\bullet-}]_{ss}$ in plasma is directly proportional to oxidative flux: EPR signal height of $Asc^{\bullet-}$ (arbitrary units) *versus* AAPH concentration. The solutions contained 58 µM ascorbate in plasma and various amounts of the free radical-generator AAPH. Buettner GR, Jurkiewicz BA. *Free Radic Biol Med* **1993**, *14*, 49-55.

Thermodynamics of Ascorbate

- The unpaired electron of $Asc^{\bullet -}$ resides in the π-system that includes the tri-carbonyl moiety of ascorbate.
- This results in a weakly oxidizing and weakly reducing radical.
- Due to its π-character $Asc^{\bullet -}$ does not react with oxygen to form dangerously oxidizing peroxyl radicals.
- Thermodynamically, it is relatively unreactive with a one-electron reduction potential of only +282 mV.
- It is considered to be a terminal, small-molecule antioxidant.



The Ascorbate Test for Fe and Cu Contaminants

- **Ascorbic acid solution (3.5 μL of 0.100 M) is added to 3.00 mL of near-neutral buffer solution;**
- **Absorbance is followed for 15 min at 265 nm ($AscH^-$ ε_{265} = 14,500 $M^{-1} \cdot cm^{-1}$);**
- **A loss of more than 0.5% in this time indicates significant metal contamination; goal <0.05%.**

Tips : use $AscH_2$, not Na-$AscH^-$

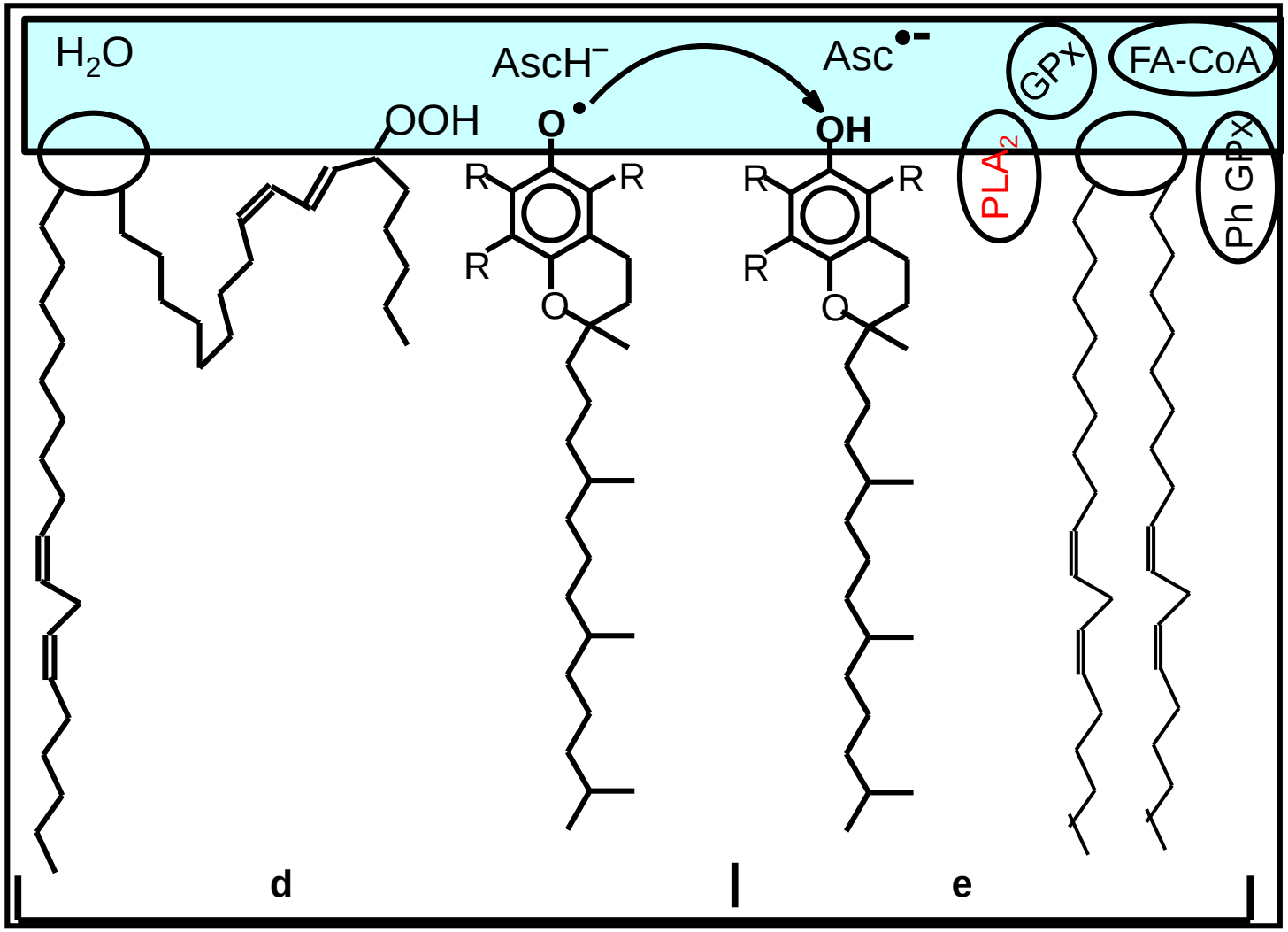
Do not interrogate the solution continuously, photochemistry

Clean, clean, clean

ground glass is a disaster

Buettner GR. *J Biochem Biophys Meth* **1988**, *16*, 20-40.

Vitamin C and E as Co-Antioxidants



Vitamins C and E as Co-antioxidants

As seen in the thermodynamic pecking order, the tocopherol radical, $TO^{\bullet}$, is more oxidizing then $Asc^{\bullet -}$. It is thought that ascorbate contributes to the recycling of $TO^{\bullet}$ back to TOH.

$$\mathbf{TO^{\bullet} + AscH^{-} \rightarrow TOH + Asc^{\bullet -}}$$

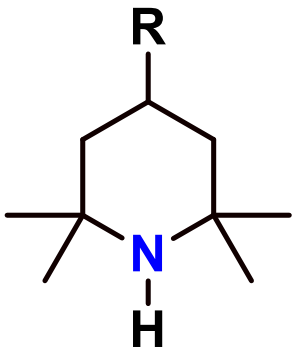
This mechanism is clearly important in protecting LDL from unwanted oxidations, because LDL lacks enzymes that could recycle $TO^{\bullet}$. But its importance in cells and tissues is still being debated.

Once oxygen reacts with the lipid chain, the change in dipole moment will cause the peroxyl radical to “float” to the interface.

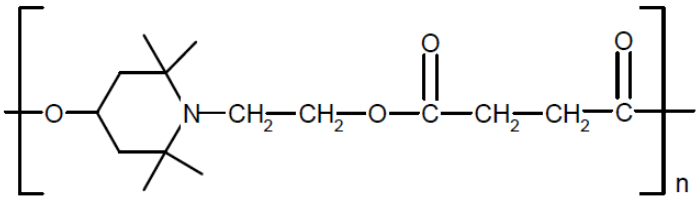
Vitamin E removes the peroxyl radical; ascorbate can recycle E; enzymes then remove the damaged fatty acid and insert a new one, repairing the lipid.

The recycling of ascorbate appears to be an enzyme-dependent process. The two electrons required can come from GSH.

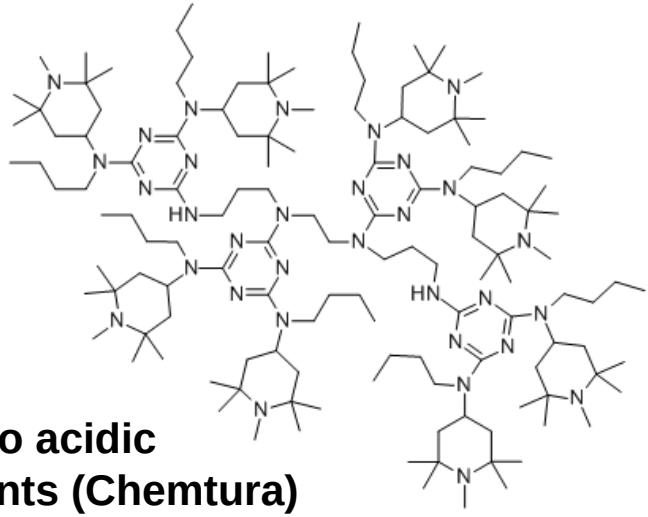
Hindered Amine Light Stabilizers (HALS)



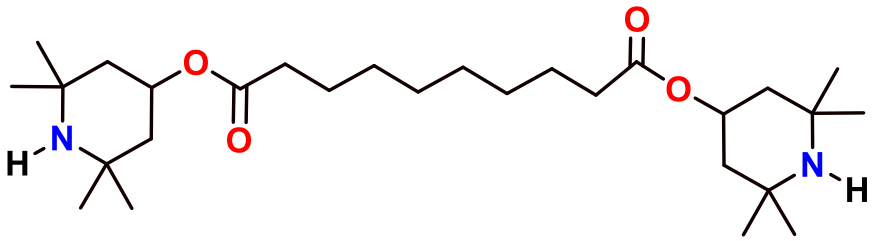
R = H, OH, OR', =O



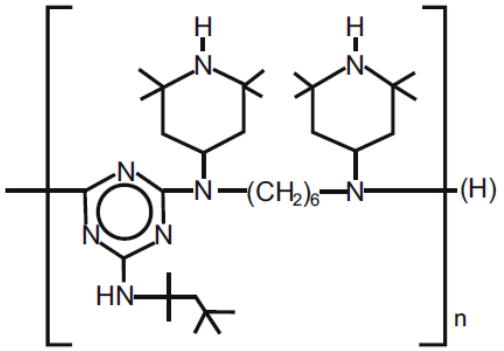
Light stabilizer (Ciba)



Resistant to acidic environments (Chemtura)

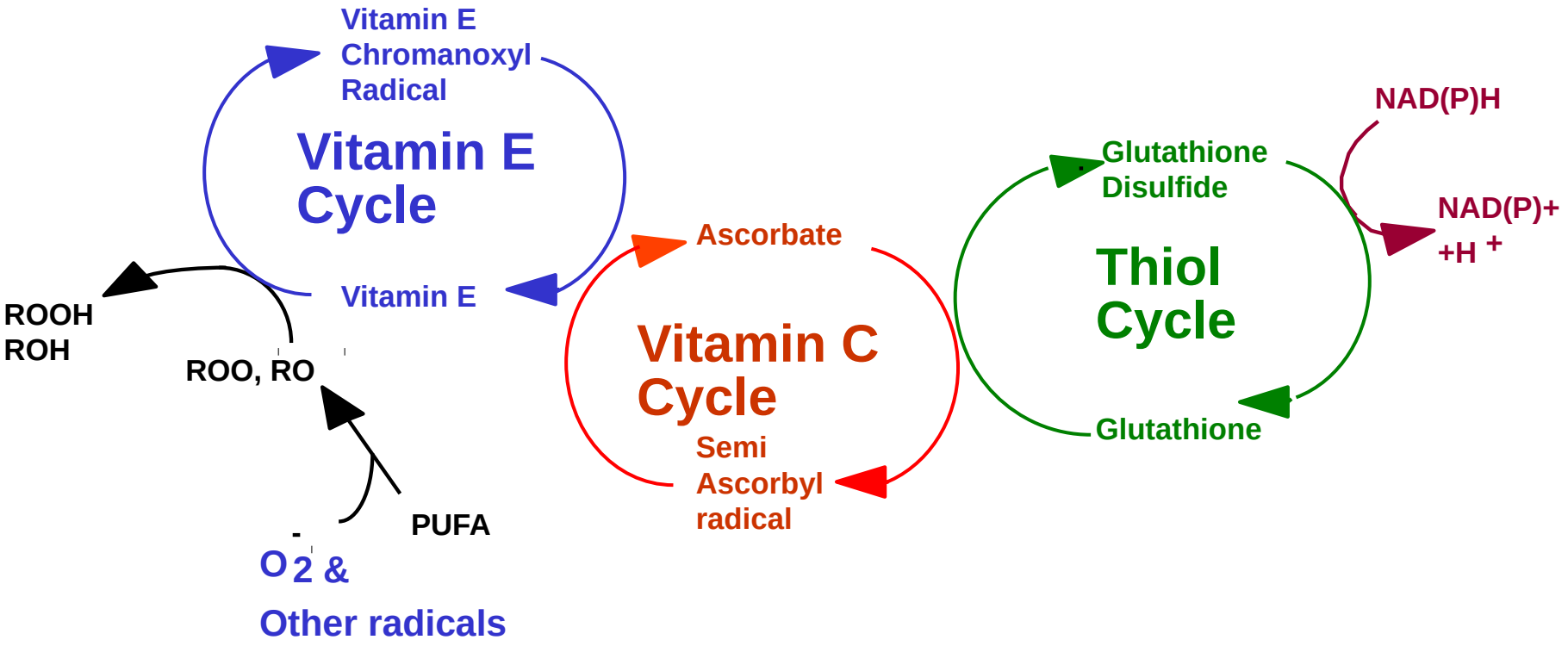


Radical and Light stabilizer (Ciba)



UV and long term heat stabilizer (Vanderbilt)

Oxidative Stress Activates Network Antioxidants



POLITECNICO DI MILANO





Antioxidants: Enzymes

Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”

Enzymes Antioxidant

Enzymes	**Antioxidant**	**Role**	**Remarks**
	Superoxide dismutase (SOD) Mitochondrial Cytoplasmic Extracellular	Dismutates $O_2^{\bullet -}$ to H_2O_2	Contains Manganese (Mn-SOD) Contains Copper & Zinc (CuZn-SOD) Contains Copper (Cu-SOD)
	Catalase	Dismutates H_2O_2 to H_2O	
	Glutathione peroxidase (GSH.Px)	Removes H_2O_2 and lipid peroxides	Selenoproteins (contains Se^{2+}) Primarily in the cytosol also mitochondria Uses GSH

Specific Antioxidant Enzymes

- Superoxide dismutase

$$O_2^{\bullet -} + O_2^{\bullet -} + 2\ H^+ \rightarrow O^{2-} + H_2O_2$$

- Catalase

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

- Glutathione Peroxidases

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

- Glutathione Transferases

All reactive centers are main or transition metal species!

Metals in Enzymes and in Biology

Copper (Cu) – SOD, Ceruloplasmin, Cyt c oxidase + more

Iron (Fe) – heme/non-heme (Hb, Mb, peroxidases, aconitase)

Zinc (Zn) – SOD, Zn fingers, Metallothionen, Metalloproteinases

Cobalt (Co) – Vitamin B_{12}

Molybdenum (Mo) – xanthine dehydrogenase, aldehyde oxidase

Manganese (Mn) – Mitochondrial SOD

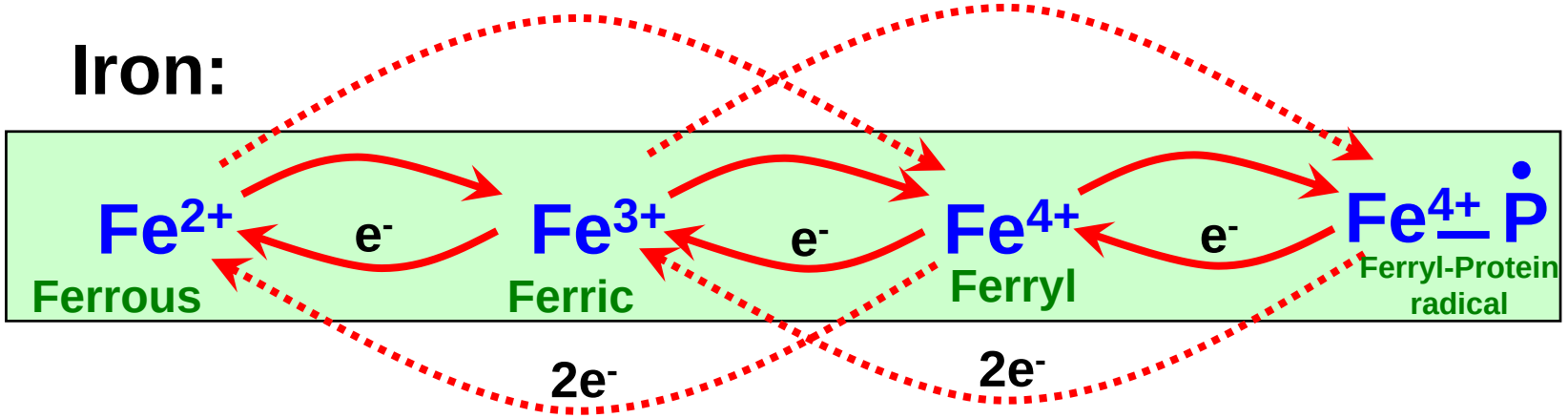
Selenium – Glutathione Peroxidase

Tungsten (W) – anaerobic bacterial proteins

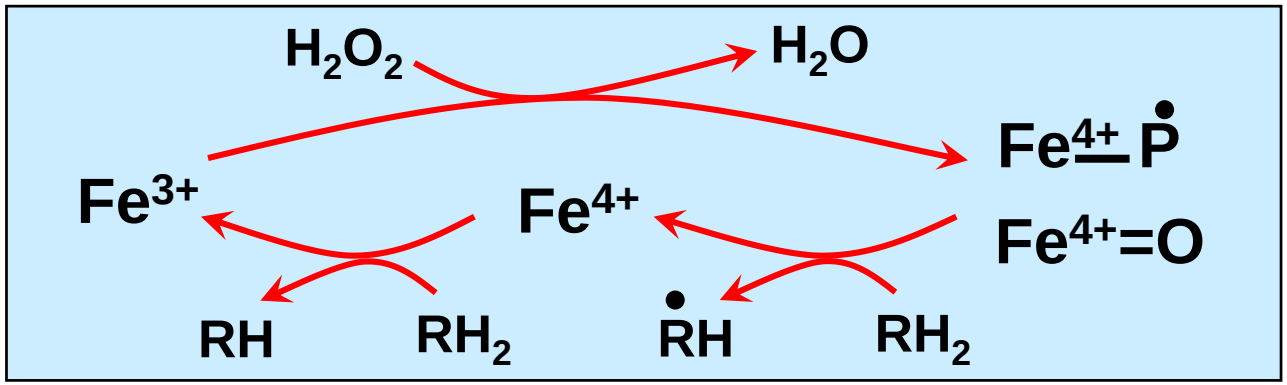
Vanadium (V) – inhibition of phosphatases

Chromium (Cr) – possible role in glucose metabolism

Complex Chemistry and Oxidation States

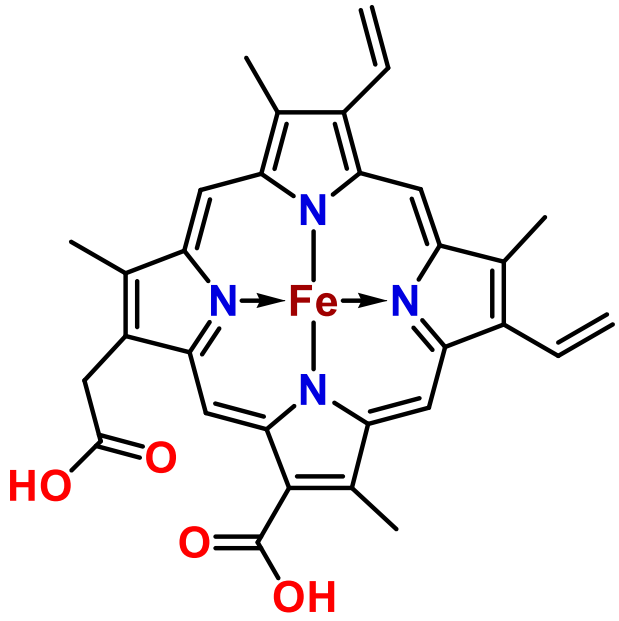


e.g. Heme Peroxidases



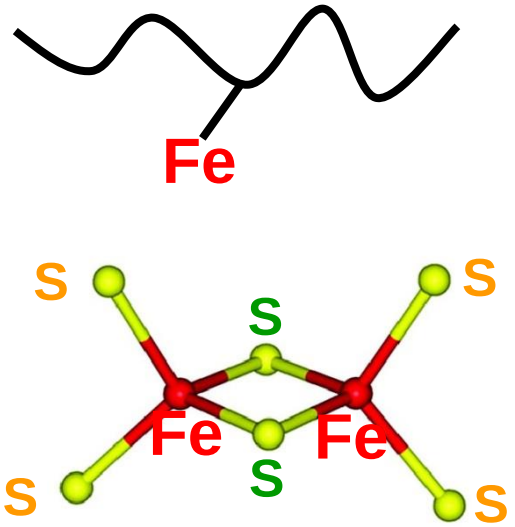
Iron Containing Metalloproteins- Heme and Non-heme

Heme



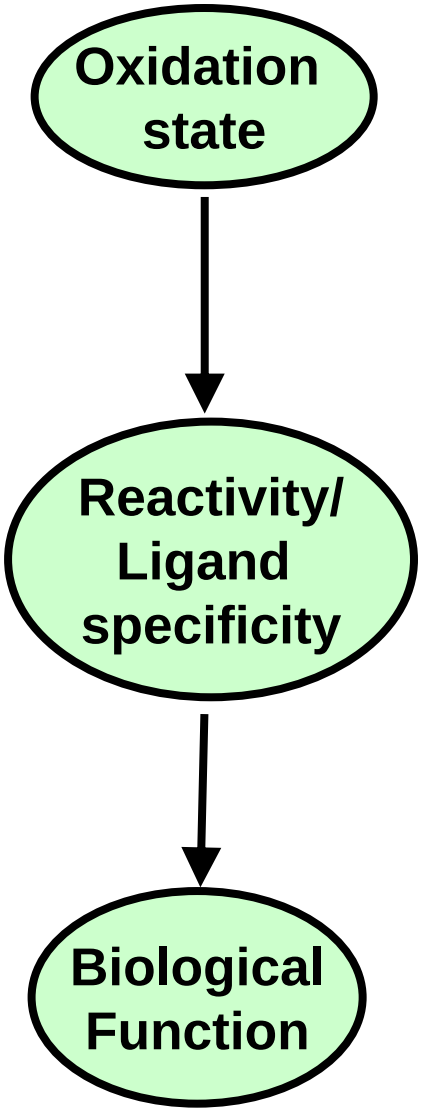
- peroxidases (MPO, COX, HRP, CYP450)
- O_2 metabolism (Hb, Mb, Cyt. c ox)
- NO metabolism (NOS, sGC)

Non-Heme



- Lipoxygenase
- Ribonucleotide reductase
- Aconitase
- Ferritin, Transferrin

Importance of Ligand and Oxidation State



$Fe^{2+} + O_2 \rightarrow Fe^{2+}\text{-}O_2$
$Fe^{2+} + CO \rightarrow Fe^{2+}\text{-}CO$
$Fe^{2+} + NO \rightarrow Fe^{2+}\text{-}NO$
} Ferrous Ligands

$Fe^{2+}\text{-}O_2 \rightarrow Fe^{3+} + O_2^{\bullet -}$
$Fe^{2+}\text{-}O_2 + NO \rightarrow Fe^{3+} + NO_3^-$
$Fe^{2+}\text{-}O_2 + NO_2^- \rightarrow Fe^{3+} + NO_3^-$
} Ferrous oxidation

$Fe^{3+} + CN^- \rightarrow [FeCN]^{2+}$
$Fe^{3+} + N_3^- \rightarrow [FeN_3]^{2+}$
} Ferric Ligands

$Fe^{3+} + H_2O_2 \rightarrow Fe^{4+}{=}O + H_2O$
$Fe^{3+} + LOOH \rightarrow Fe^{4+} + LO$
$Fe^{3+} + ONOO(H) \rightarrow Fe^{4+}{=}O + NO_3^-$
} Ferric Oxidation

$Fe^{3+} + 2\ NO \rightarrow Fe^{2+}\text{-}NO$
$Fe^{3+} + \text{strong reductant} \rightarrow Fe^{2+}\text{-}NO$
} Ferric Reduction

$Fe^{4+} + \text{reductant} \rightarrow Fe^{3+}$
$(H_2O_2, LOOH, RSH, asc, NO)$
} Ferryl Reduction

Superoxide Anion Radical Dismutation by SOD

- ***Spontaneous, non-enzymatic dismutation***

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2 \qquad 10^5\ M^{-1}\cdot s^{-1}$$

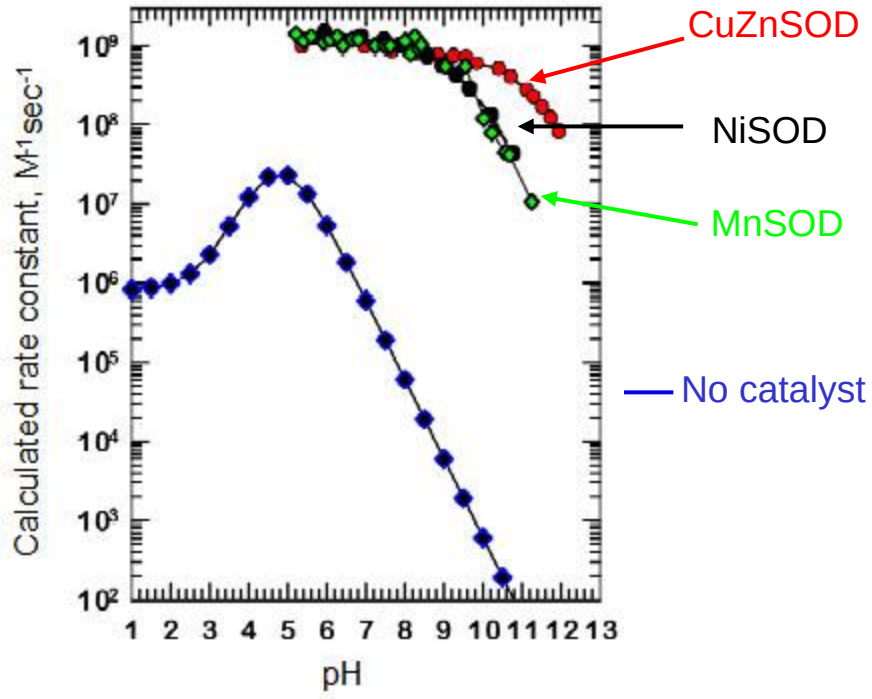
- ***Enzyme-catalyzed dismutation***

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2 \qquad \sim 10^9\ M^{-1}\cdot s^{-1}$$

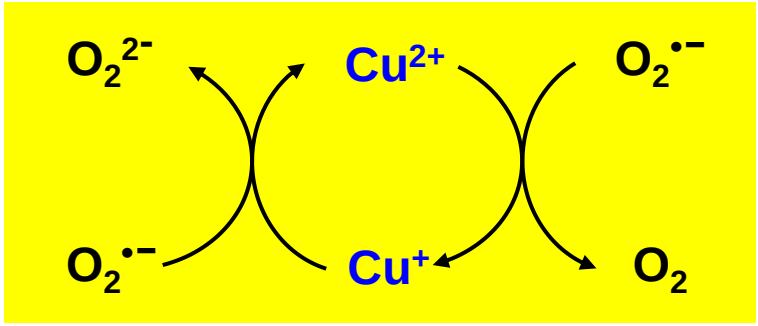
Superoxide dismutase (SOD)

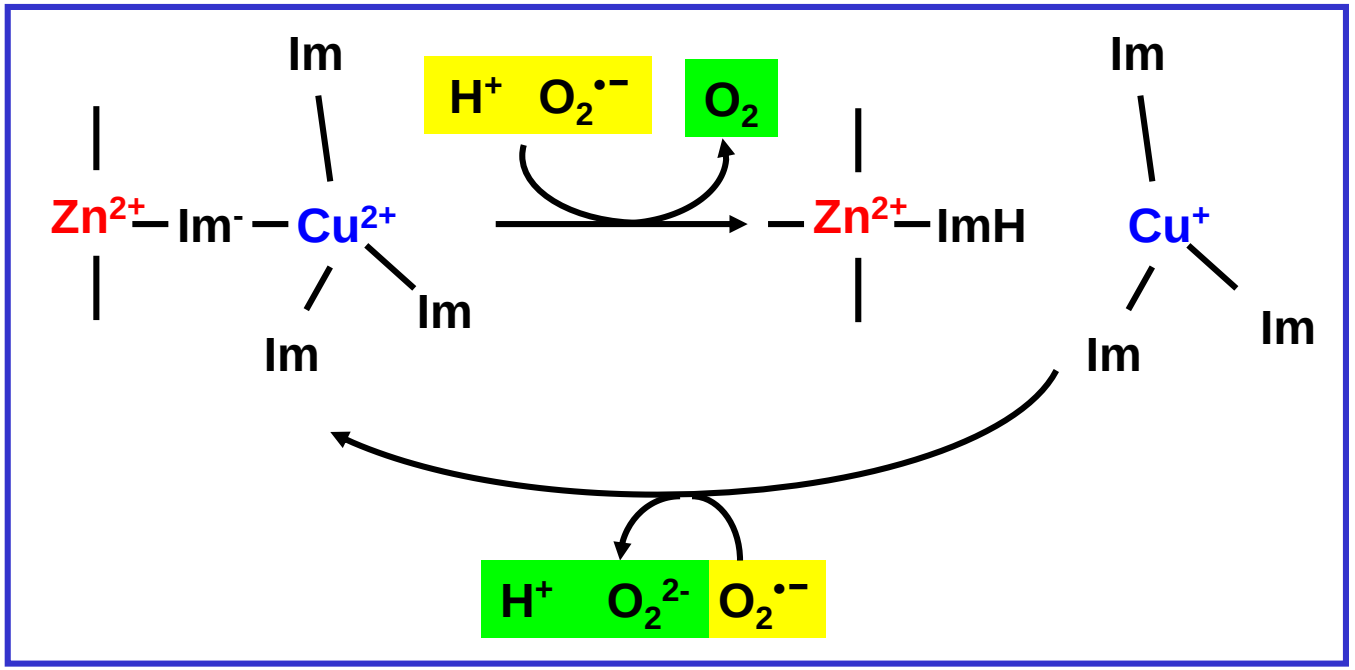
Cu,Zn–SOD	cytosol
Mn–SOD	mitochondria
Cu,Zn–SOD	extracellular

	Cu,Zn-SOD (μg/mg protein)
liver	4.7
brain	3.7
testis	2.2
renal cortex	1.9
cardiac muscle	1.8
lung	0.5



Superoxide Dismutase - Mechanism





Specific Antioxidant Enzymes

- ***Catalase* — peroxisomes (**a tetramer; each subunit contains a heme group and a NADP group)

$$H_2O_2 + H_2O_2 \rightarrow 2\,H_2O + O_2$$

- ***Glutathione peroxidase* — cytosol, mitochondria**

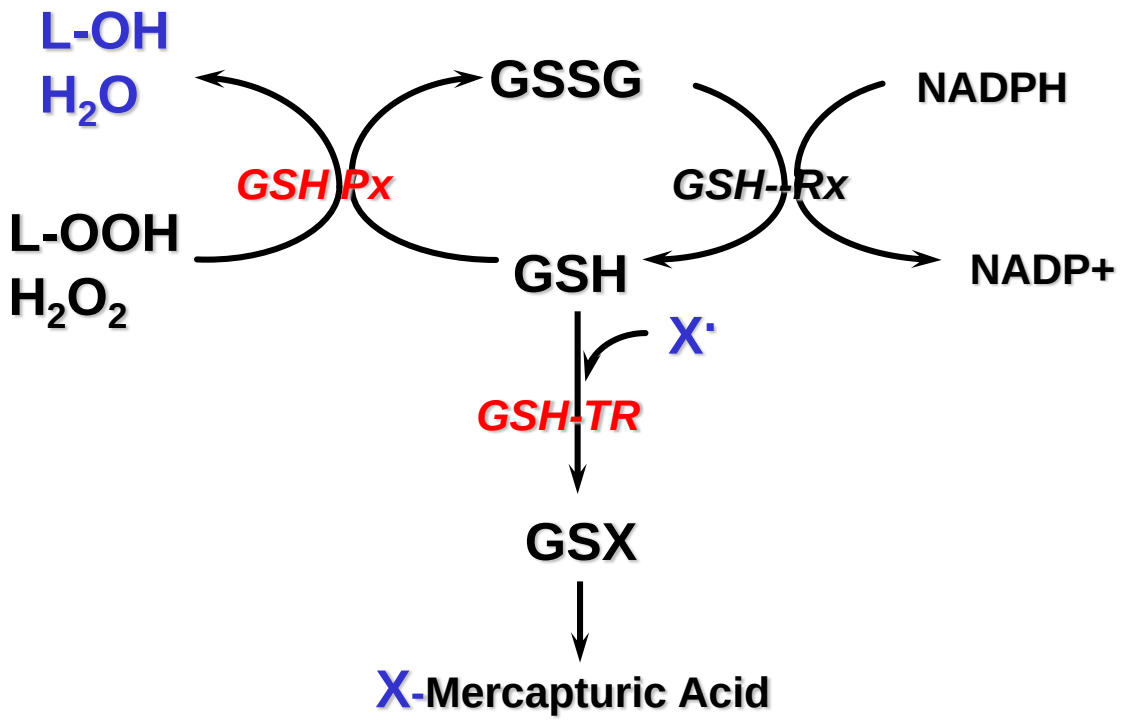
$$H_2O_2 + 2GSH \rightarrow H_2O + GSSG$$

	Catalase (units / mg protein)	*GPx*
liver	1300	190
erythrocytes	1000	19
kidney cortex	430	140
lung	210	53
pancreas	100	43
heart	54	69
brain	11	79

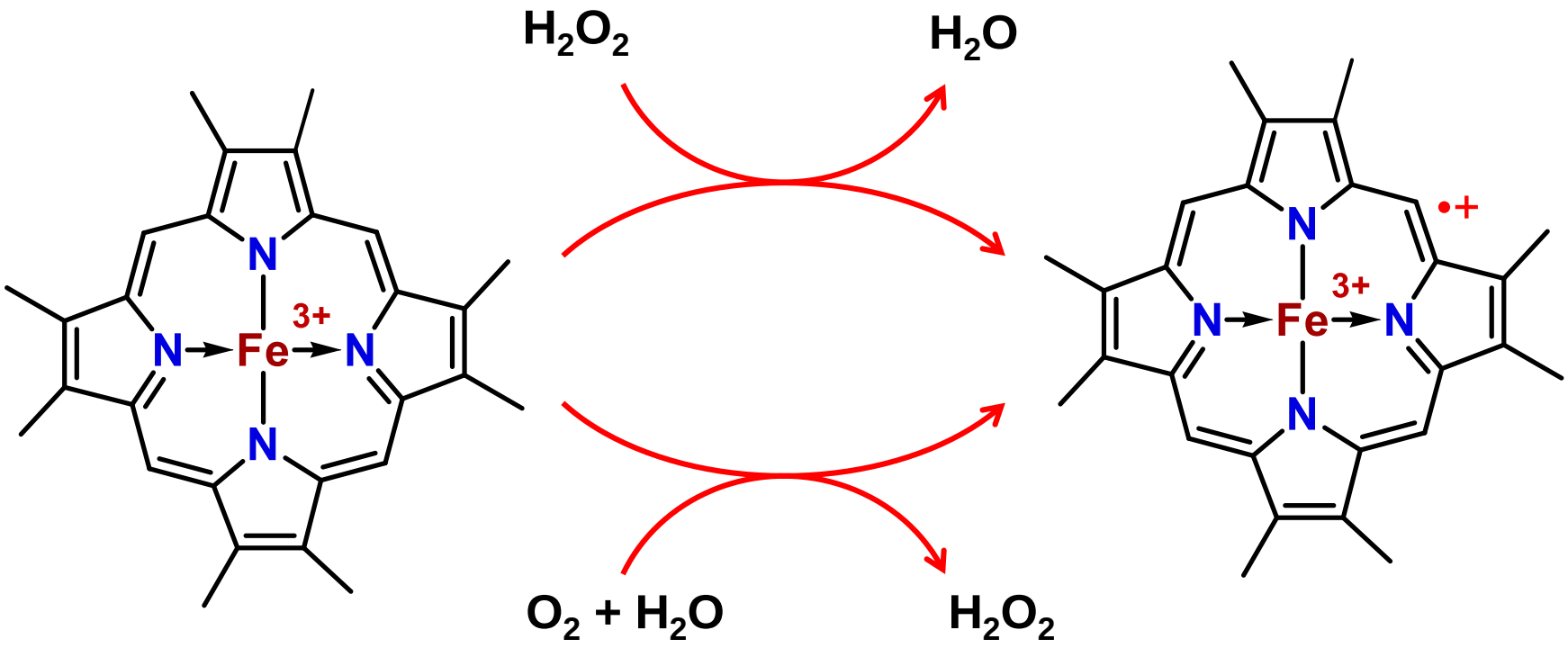
Antioxidant Enzymes - 3

Glutathione Peroxidase (GSH PX) [a tetramer; each subunit has a selenocysteine residue in its active site]

- to get rid of hydrogen peroxide (H_2O_2) and some lipid peroxide. It requires reduced glutathione (GSH) as substrate and produces oxidized glutathione (GSSG) as product. A cytosolic enzyme.



ROS Metabolism- Decomposition of H_2O_2 by Catalase

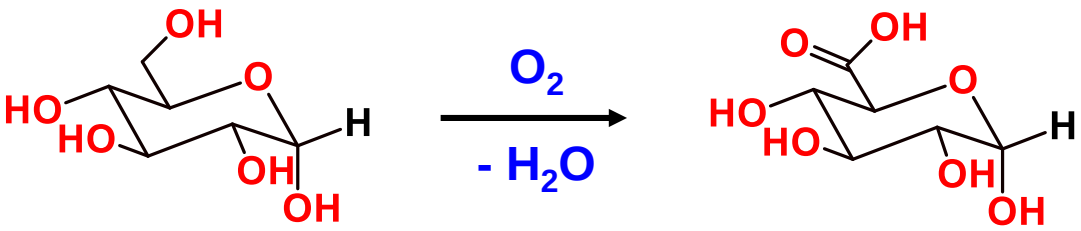


Glucose Oxidase/Catalase

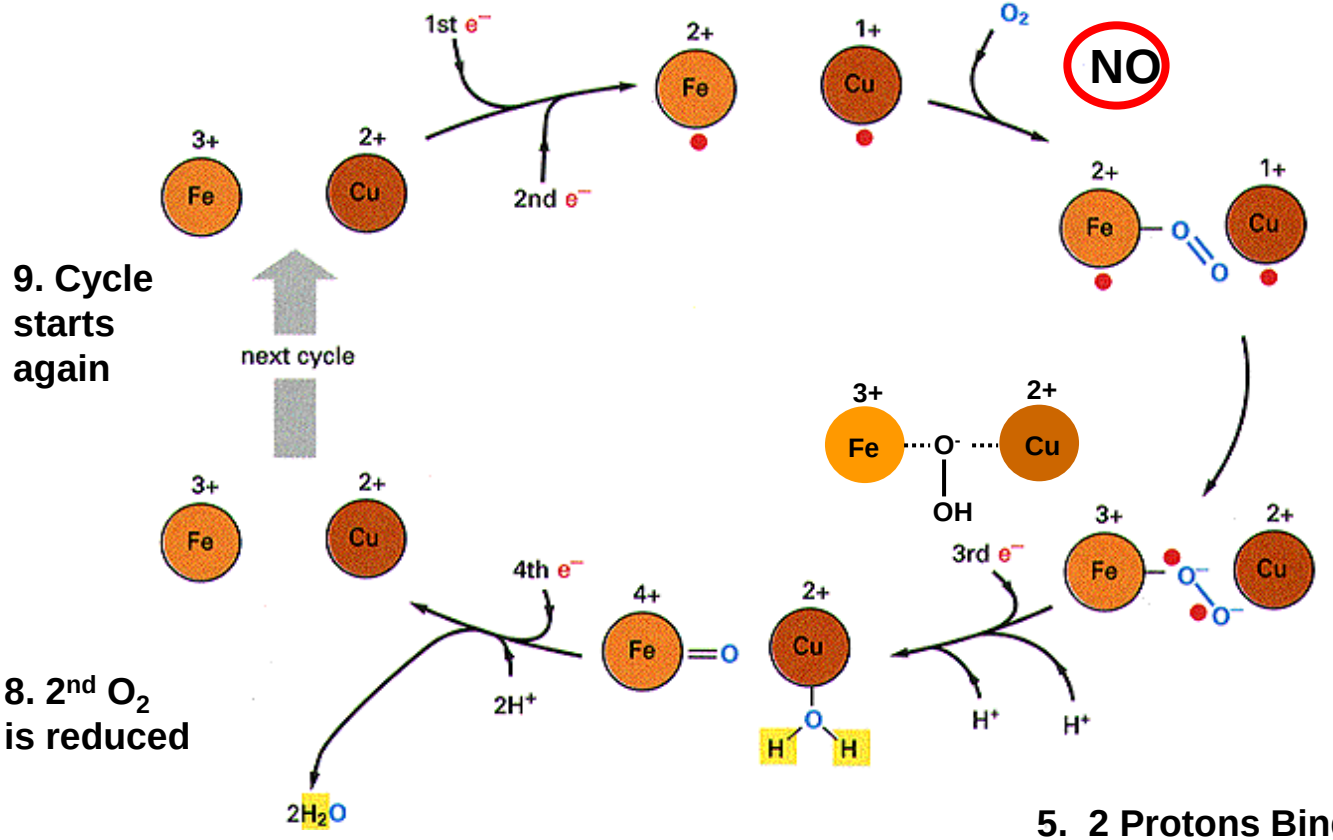
$$2\ \text{Glucose} + 2O_2 + 2H_2O \xrightarrow{\text{Glucose Oxidase}} 2\ \text{Gluconic acid} + 2H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

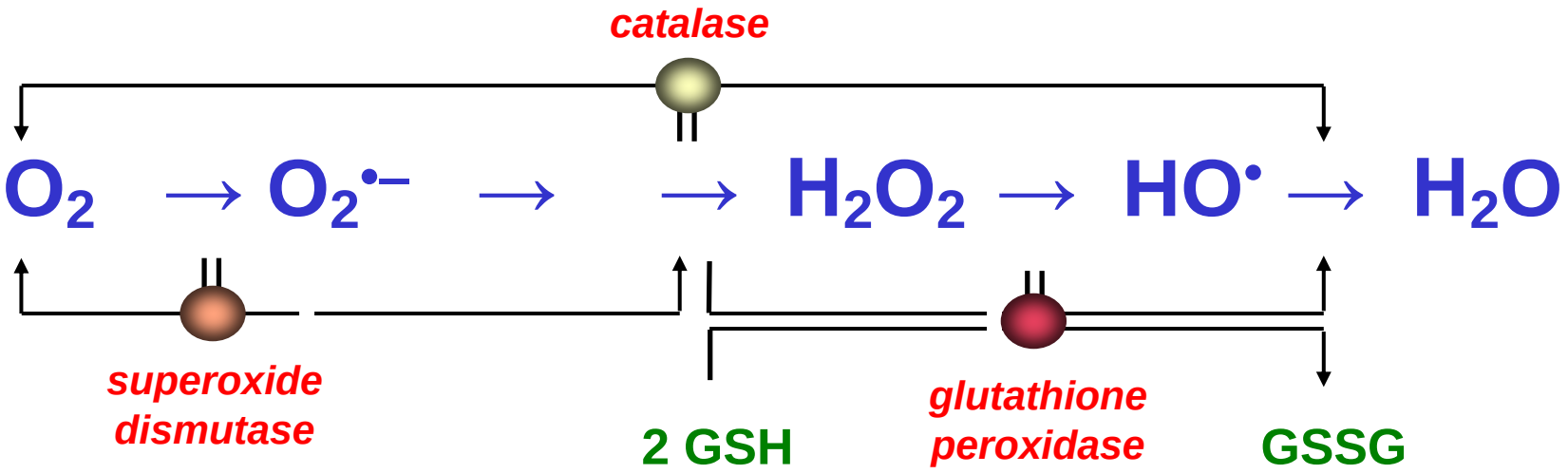
$$2\ \text{Glucose} + O_2 \xrightarrow[\text{Catalase}]{\text{Glucose Oxidase}} 2\ \text{Gluconic acid}$$



Reduction of Oxygen in Cytochrome C Oxydase



Summary of Specific Enzymatic Antioxidant Defenses



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Antioxidants: Chelates

Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”

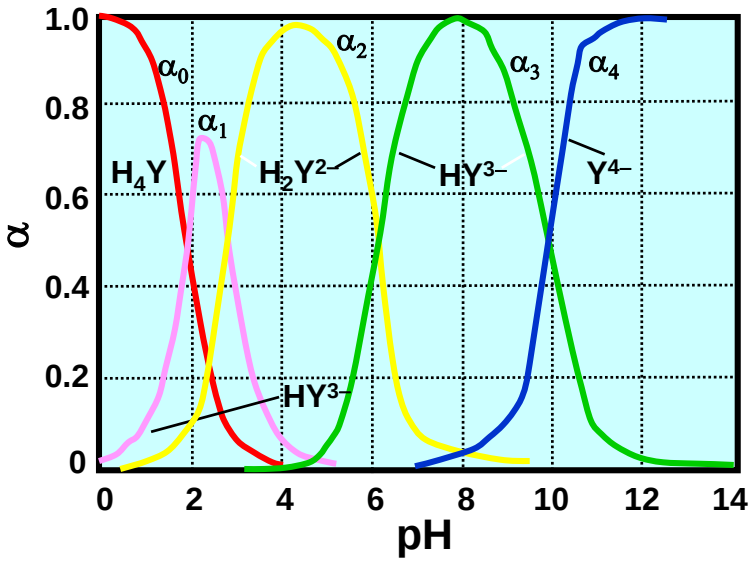
Mechanism of Metal Chelators

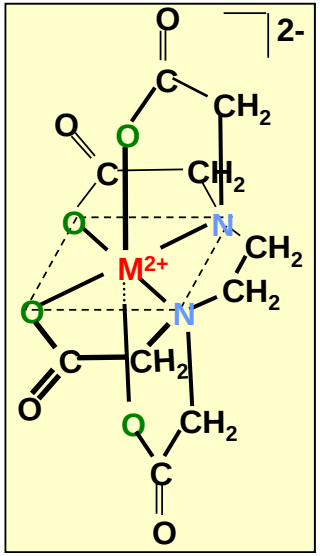
- Formation of complex ions or coordination compounds with metals
  - Prevention of metal redox cycling
  - Formation of insoluble metal complexes
  - Steric hindrance of interactions between metals and lipid intermediates

- Metal coordination cannot prevent in general the pro-oxidant properties of transition metal ions unless the first solvation shell is complete and not easy accessible to oxidants. Therefore, exadentate ligand (i.e. EDTA, deferioximine, some proteins ….) are particularly efficient inhibitor of metal promoted oxidations in a limited pH range owing the acid-base properties of ligand.
- Moreover, it must be remembered that the thermodynamic stability constant of complex depends on the charge of the ion – $K(Fe^{3+}) > K(Fe^{2+})$).

Kinds of Metal Chelator Antioxidants

- Metal chelators deactivate trace metals that are free or salts of fatty acids by the formation of complex ion or coordination compounds.
  - 1. Phosphoric acid
  - 2. Citric acid
  - 3. Ascorbic acid
  - 4. Ethylene-Diamine-Tetra-Acetate (EDTA)





Speciation of EDTA from pH in water solution.

Stability Constants log K

Ion	Ca^{+2}	Fe^{+2}	Fe^{+3}	Cu^{+2}
EDTA	10.7	14.3	25.7	18.8
Citric Acid	3.5	3.2	11.8	6.1
Pyrophosphate	5.0		22	6.7

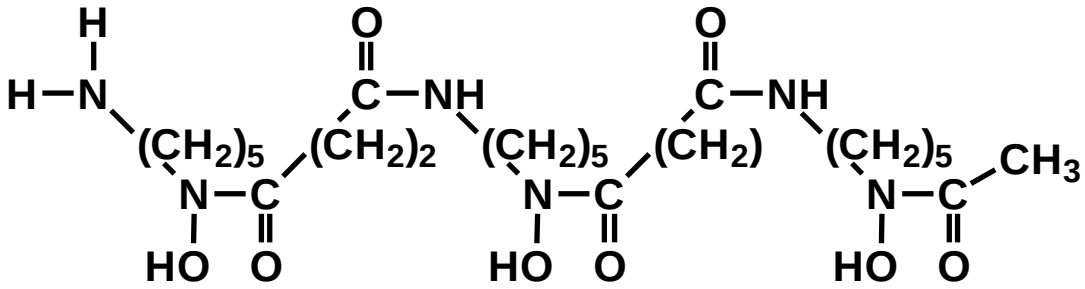
DESFERAL®

E°′ (Fe(III)DFO/Fe(II)DFO) = - 450 mV

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

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

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



Deferrioximine

ORAC Assay

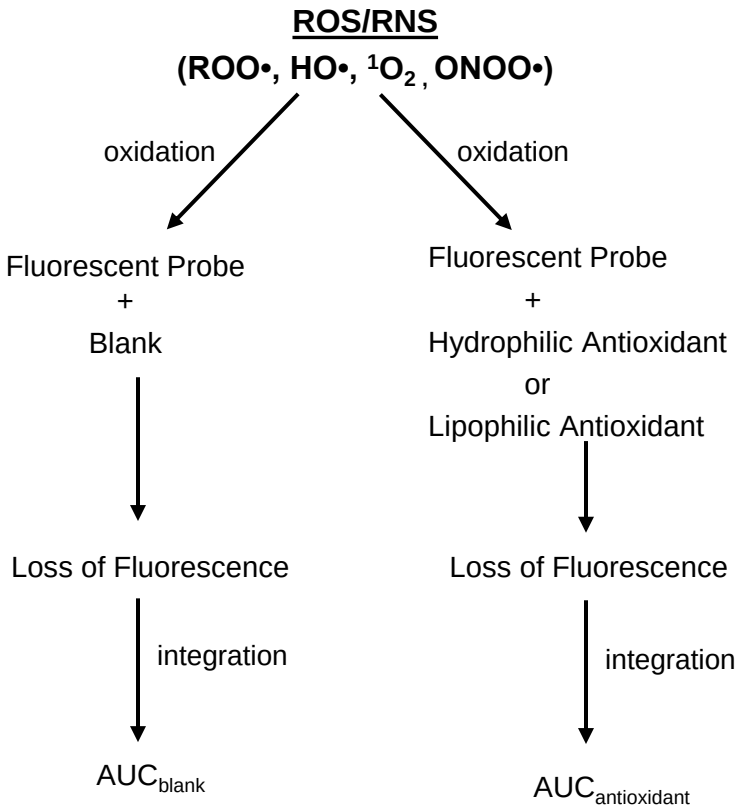
ORAC (oxygen radical absorbance capacity) assay is used extensively to compare antioxidant activities of foods, beverages, and antioxidant capacity of human blood samples in a clinical setting.

ORAC is based on the inhibition of **peroxyl-radical-induced oxidation** initiated by thermal decomposition of azo-compounds such as 2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH)

Free radical damage to a fluorescent probe is quantified by measuring the change in its **fluorescence intensity**.

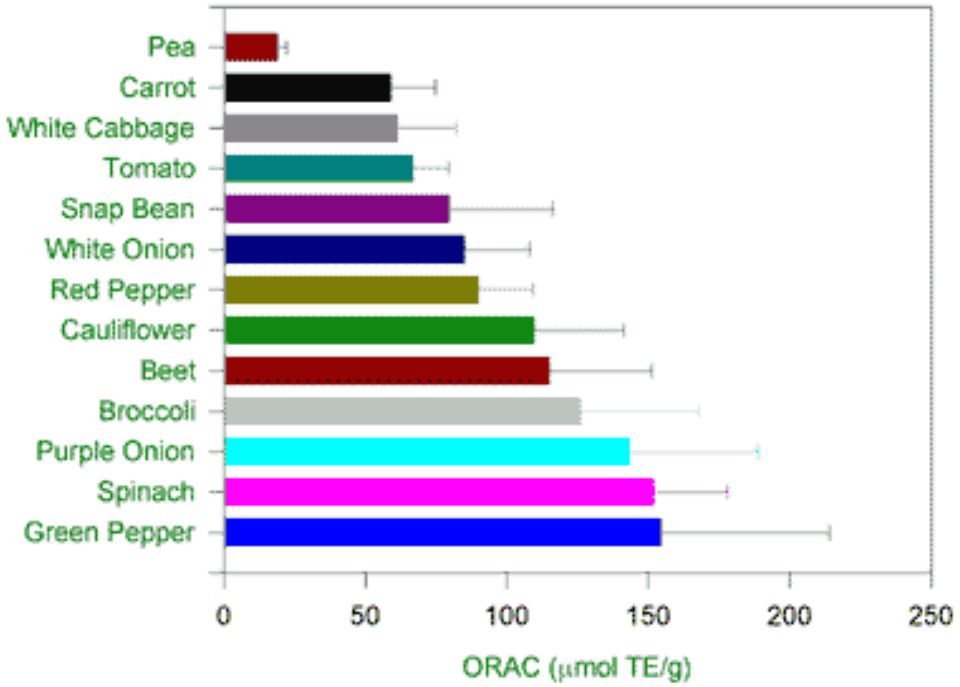
The inhibition of free radical damage by an antioxidant is assessed by comparing probe fluorescence in presence or absence of the antioxidant.

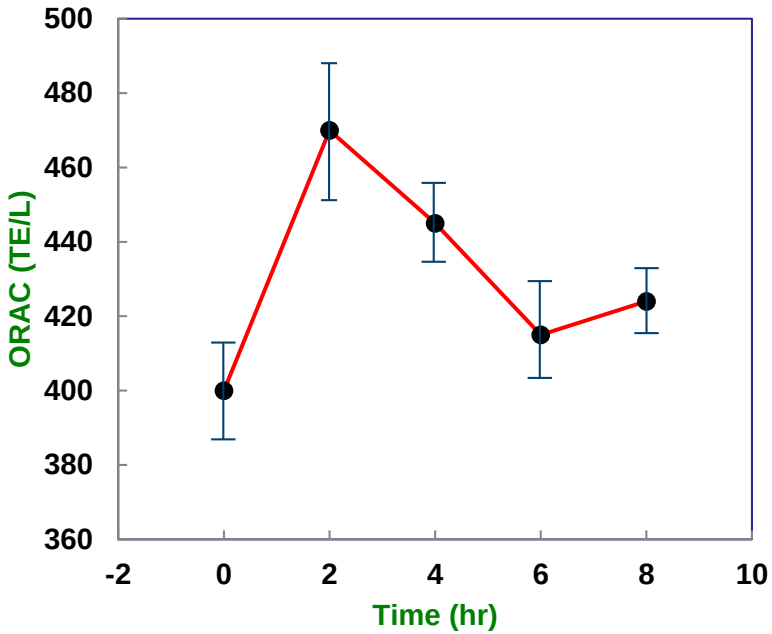
Grandfathers of ORAC: method was developed by G. Cao in 1992. In 1995, Dr. Ronald, L. Prior's group at Jean Mayer USDA Human Nutrition Research Center on Aging to develop a semi-automated ORAC assay.



Antioxidant Capacity = $AUC_{antioxidant}$ - AUC_{blank}

Use of ORAC to compare antioxidant power of foods or change in plasma antioxidant capacity





ORAC values are expressed as mmoles of Trolox equivalents per unit mass or volume

Trolox = water-soluble Vitamin E analog

Source: Brunswick labs (http://brunswicklabs.com/app_orac.shtml)