Intracellular and extracellular antioxidants

Novi Sad, 13-14 December 2016

What are antioxidants?

- Substances that are able to neutralize reactive molecules and reduce oxidative damage.
- An antioxidant is present at lower concentrations respect to the oxidizable substrate and *significantly delays or reduces oxidation of the substrate*.
  - They reduce the effect of dangerous oxidants by *binding together with these harmful molecules*, decreasing their destructive power.
  - They may be considered as *the scavengers of free radicals*.

Antioxidants: two broad classes

Preventive antioxidants intercept oxidizing species before damage can be done.

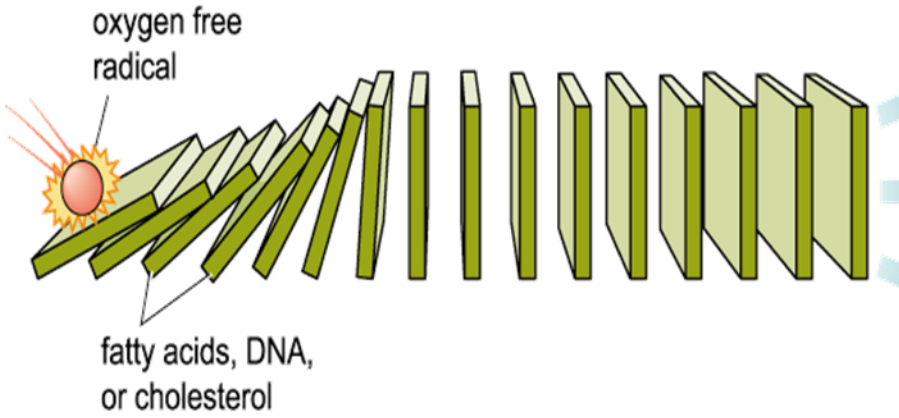
- Deactivating metals, *e.g.* transferrin, ferritin, EDTA, …
- Removing radicals, *e.g.* catalase, glutathione peroxidases, …
- Quenching singlet oxygen, *e.g.* β-carotene, lycopene, bilirubin

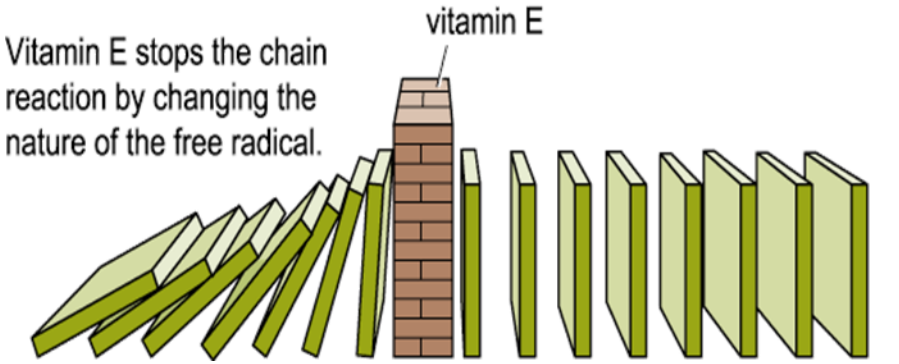
Chain breaking antioxidants slow or stop oxidative processes after they begin, by intercepting the chain-carrying radicals.

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

Chain breaking

1. A chemically reactive oxygen free radical attacks fatty acid, DNA, protein, or cholesterol molecules forming other free radicals.
2. This initiates a rapid, destructive chain reaction.
3. The result is injury to tissues and the formation of more free radicals:
   - damage to cell membrane lipids and proteins, disabling them
   - precancerous changes in DNA
   - oxidation of blood cholesterol initiating steps leading to heart disease
4. And ultimately, diseases and tissue aging:
   - cancer
   - heart disease
   - macular degeneration
   - other diseases
   - aging





How to classify antioxidants?

Antioxidants are a heterogeneous family of molecules and enzymes, difficult to classify by common shared structural properties. Several classifications have been attempted taking into account the

origin (endogenous or exogenous),

nature (enzymatic or non-enzymatic),

chemical-physical properties (hydrophilic or lipophilic),

structure (flavonoids, polyphenols, etc.),

mechanism (preventive, chain-breaking, etc.),

distribution (intracellular or extracellular).

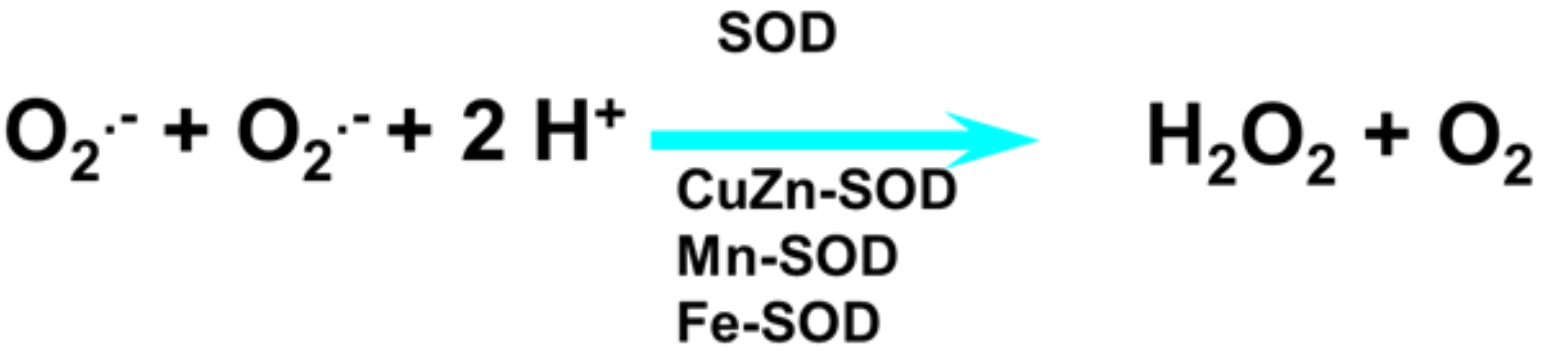
Some of them act **directly** by quenching or neutralizing ROS, others act **indirectly** by regulating the biosynthesis of antioxidant proteins, promoting their synthesis and/or availability.

Current Pharmaceutical Design, 2004, *10*, 1677-1694

Intracellular antioxidants

- Superoxide dismutase (SOD)
- Catalase (CAT)
- Glutathione and its related enzymes (GPx, GR, GST)
- Thioredoxin (Trx) and Trx reductase

Superoxide dismutase (SOD)



SOD is the first line of defense in the antioxidant enzyme repertoire, catalyzing $O_2^{\bullet -}$ anion dismutation into O_2 and H_2O_2

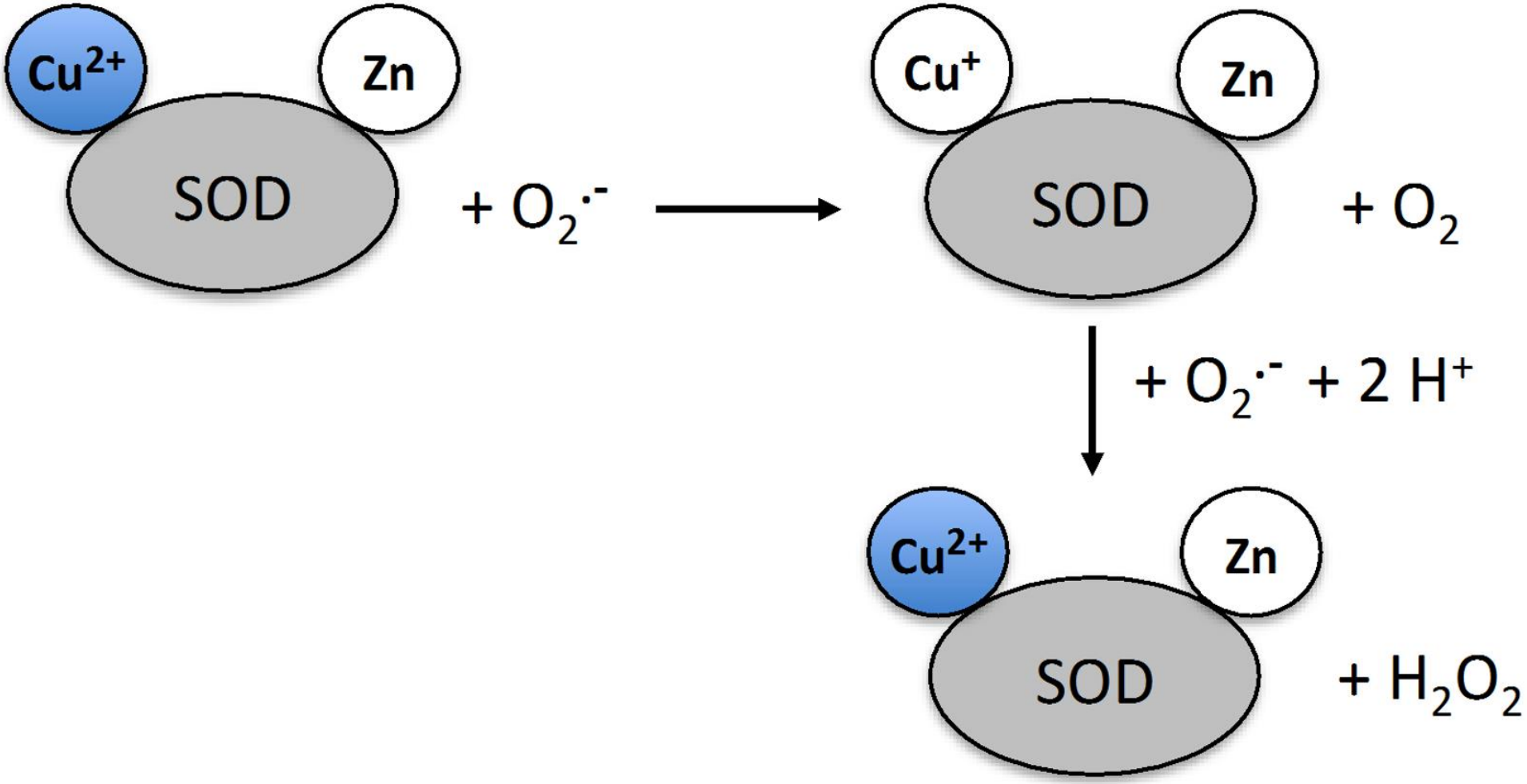
Its final product, H_2O_2, is less reactive

SOD isoforms

SOD is a metal-dependent enzyme, many isoforms have been described among eukaryotic and prokaryotic organisms and at least three of them are present in the human cells and tissue:

- **CuZnSOD** (SOD1) an omodimeric protein located in the cytosol, nucleus, peroxisomes.
- **MnSOD** (SOD2) a tetrameric protein mainly located in the mitochondria.
- **Extracellular CuZnSOD** (SOD3) a tetrameric protein located in the extracellurar fluids.
- FeSOD and NiSOD have been described in prokaryotic cells

SOD reaction



CuZnSOD (SOD1 and SOD3)

SOD1, located in the cytosol, nucleus, peroxisomes, and intermembrane space of the mitochondria is essential for antioxidant defense and mutations of this enzyme have been linked to neurodegenerative disorders such as amyotrophic lateral sclerosis.

The **SOD3** isoform is produced by smooth muscle cells and released outside. Due to its extracellular location, it has been claimed as the principal regulator of endothelium-derived NO• bioactivity through its $O_2^{\bullet -}$ scavenging activity.

MnSOD (SOD2)

- **MnSOD**, the only isoform present in mitochondria, is considered essential for aerobic survival. Genetic studies have revealed that the null homozygous mutation ($MnSOD^{-/-}$) is lethal, whereas knockouts of Cu/ZnSOD is not. MnSOD-knockout mice have severe mitochondrial damage, decreased GSH, increased 8-hydroxy-2 deoxyguanosine (8-OhdG), and die shortly after birth. The short lifespan in MnSOD knockouts is likely related to the enzyme's role in maintaining nanomolar or lower $O_2^{\bullet -}$ concentration.

Even if H_2O_2 is less reactive and dangerous than $O_2^{\bullet -}$ it must be neutralized to prevent oxidative damage to macromolecules.

Two enzymes are able to eliminate H_2O_2

Catalase (CAT) and Gluthatione peroxidase (GPx)

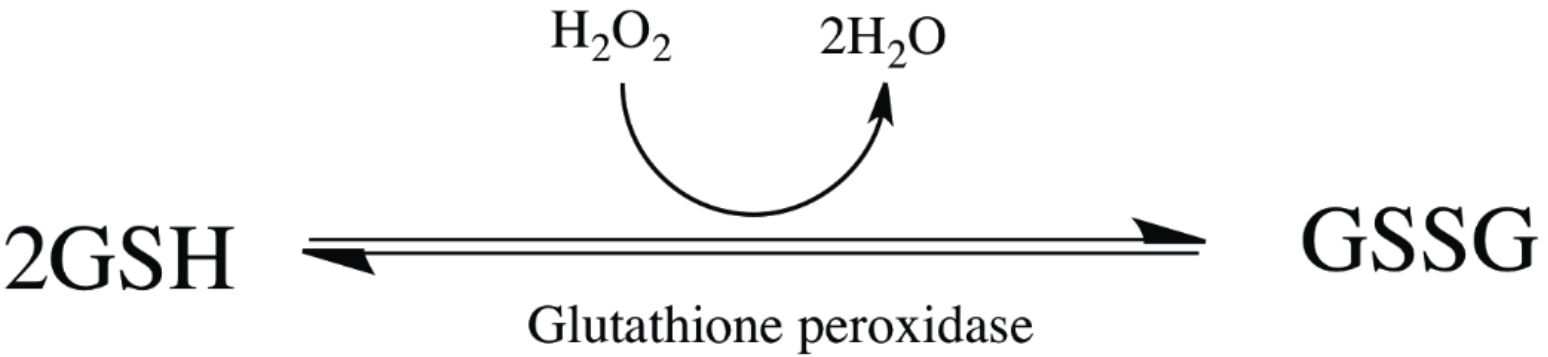
Catalase (CAT)

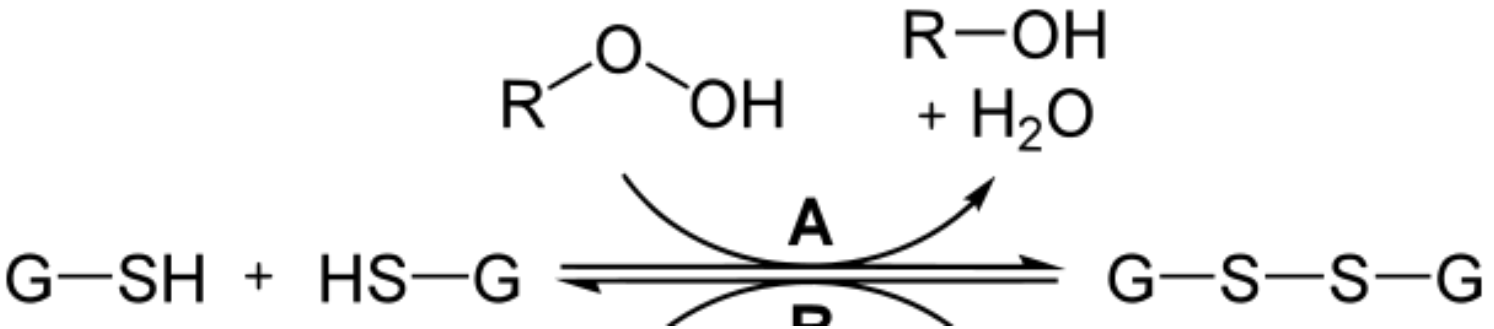
CAT is one of the most abundant peroxisomal proteins in mammalian cells and converts H_2O_2 into H_2O and O_2

CAT is composed of four identical subunits and uses a heme/iron group to perform the reaction.

Glutathione peroxidase (GPx)

GPx is a Selenium dependent enzyme, it catalize the reduction of 2 molecules of H_2O_2 at the expences of 2 molecules of GSH





Glutathione peroxidase (GPx)

GPx is located in the cytosol, nucleus, mitochondria and also in plasma. It detoxifies H_2O_2 and lipidhydroperoxides (ROOH) into H_2O and alcohols (ROH), respectively.

GPx isoforms

Isoform	Location	Cofactor	Substrate
GPx1	cytosol	Se	H_2O_2
GPx2	gastrointestinal	Se	H_2O_2
GPx3	plasma	Se	H_2O_2
GPx4	Membr-bound	Se	ROOH
GPx5			
GPx6		selenocysteine	
GPx7			
GPx8			

GPx4 is a monomeric protein and is the only considered essential for life. GPx4 null mice die during embryonic development, while GPx1-3 knockout mice develop normally.

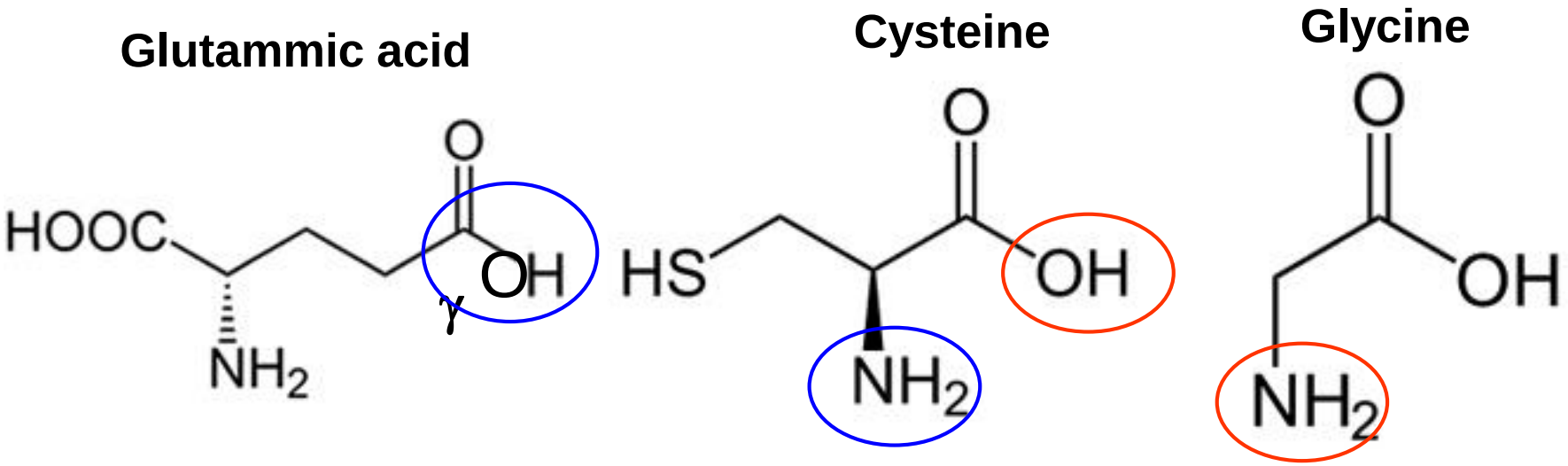
CAT and GPx

Why do we need 2 enzymes to catalize the detoxification of H_2O_2?

- CAT is often segregated into the peroxisomes, it has a low affinity for H_2O_2, this mean that CAT is active when [H_2O_2] is high (ie in peroxisomes).
- GPx has higher affinity to H_2O_2 than CAT and is active also when [H_2O_2] is low (ie outside peroxisomes).

Physiol Rev 88: 1243–1276, 2008

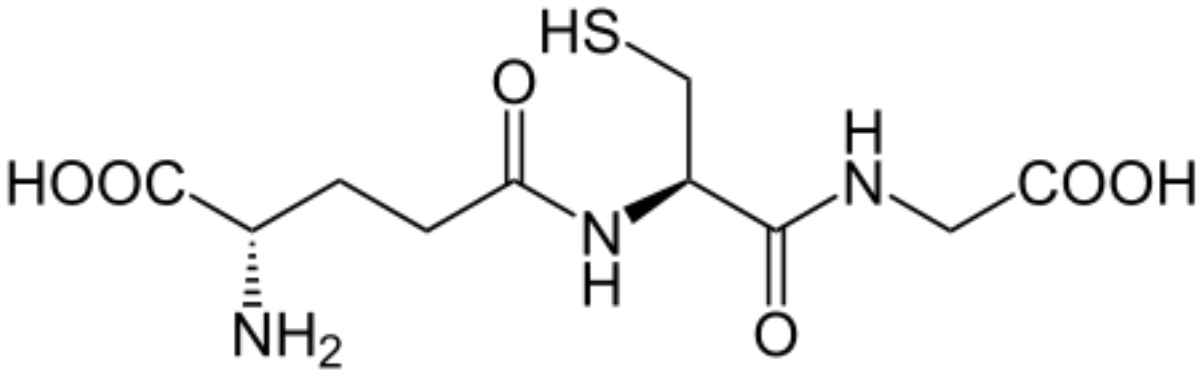
Glutathione



Reaction 1: catalysed by gamma-glutamylcysteine synthetase, requires 1 ATP

Reaction 2: glutathione synthetase adds a glycine to the C-terminal of *gamma*-glutamylcysteine, requires 1 ATP

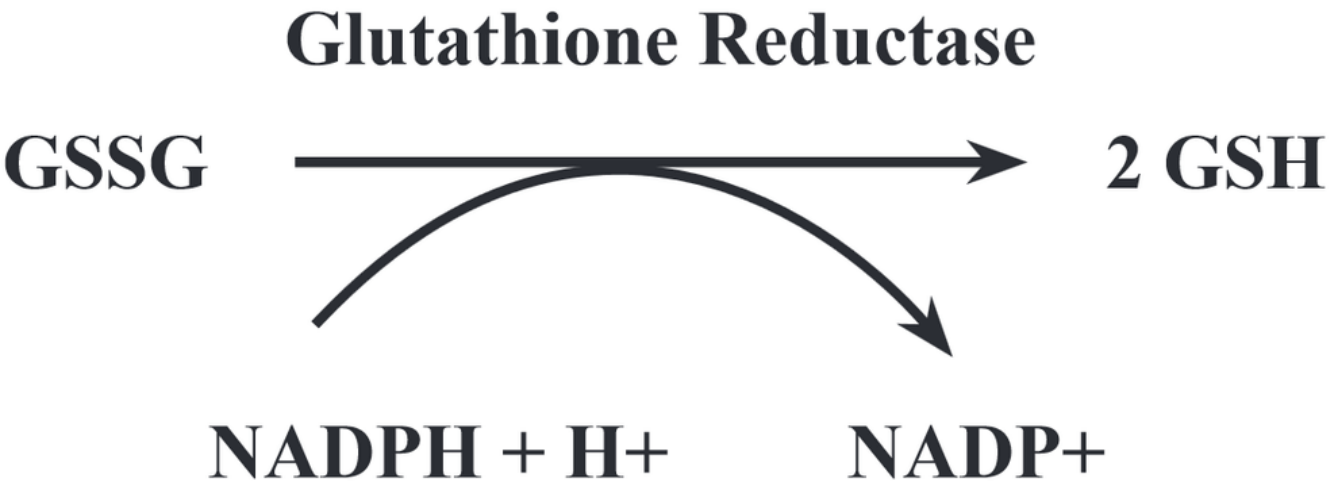
Glutathione (GSH)



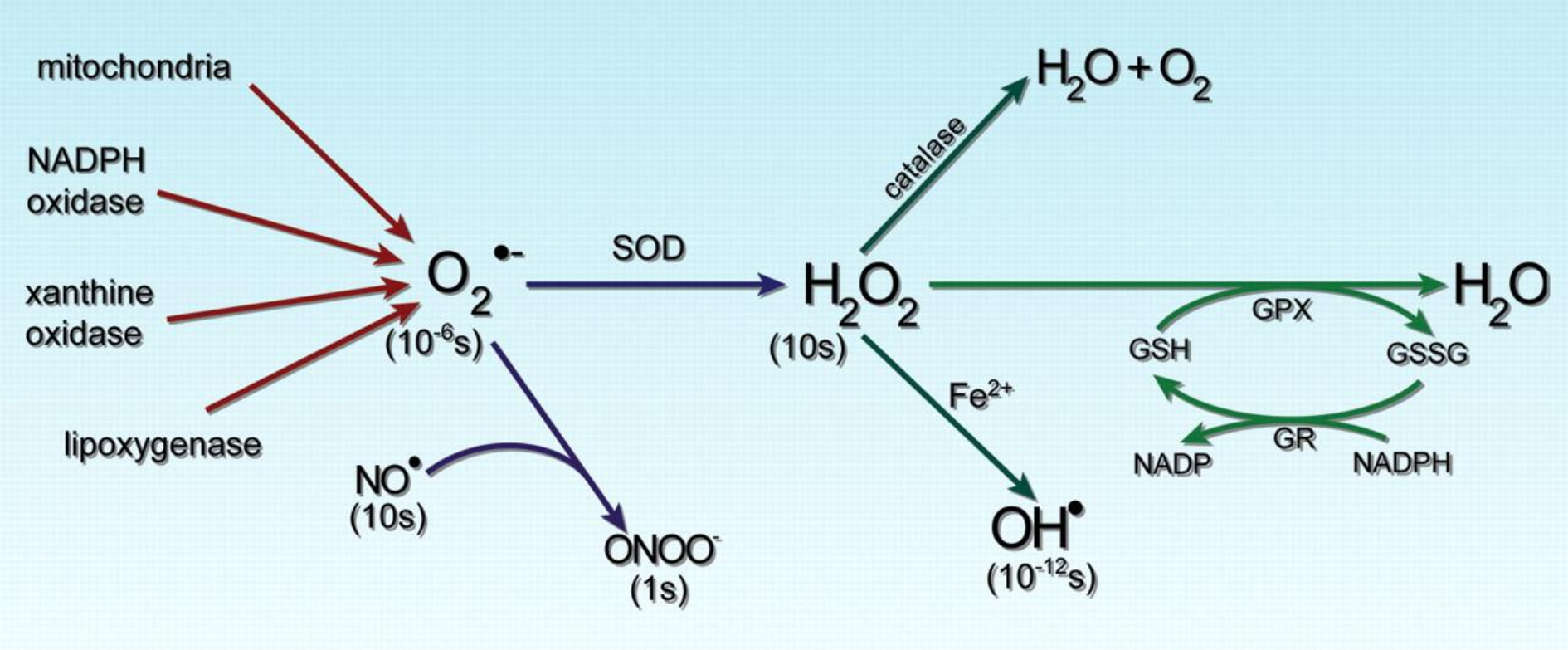
The thiol group is the reducing center of GSH it donates reducing equivalent to ROS or to highly electrophilic molecules. Once GSH donates its electron it reacts quickly with another GS• to form oxidized glutathione (GSSG).
GSH is the most important intracellular antioxidant thanks to its mM concentration.

Glutathione (GSH)

Once oxidized GSH can be rigenerated by consuming reducing equivalents donated by NADPH:

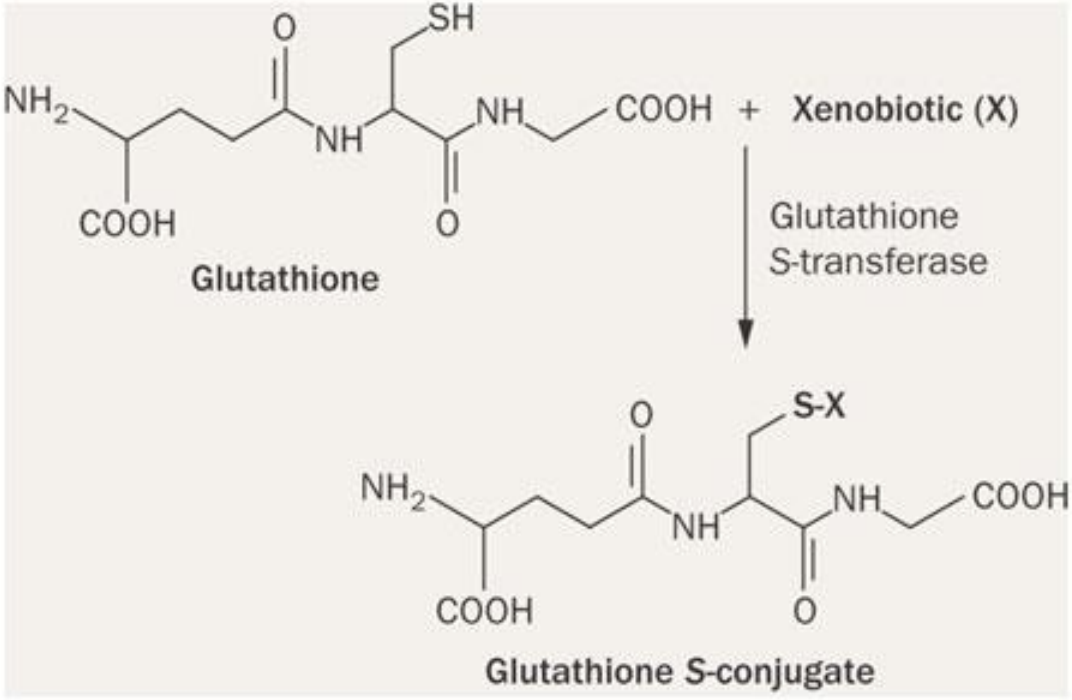


The Glutathione cycle



Other reactions involving GSH

GSH is often employed in detoxification reaction. It is conjugated to electrophilic xenobiotics to reduce their toxicity and eliminate them. Glutathione-S-transferase is a superfamily of enzymes responsibole for the conjugation of GSH with xenobiotics.



Bound to GSH, X is less reactive and more hydrophilic

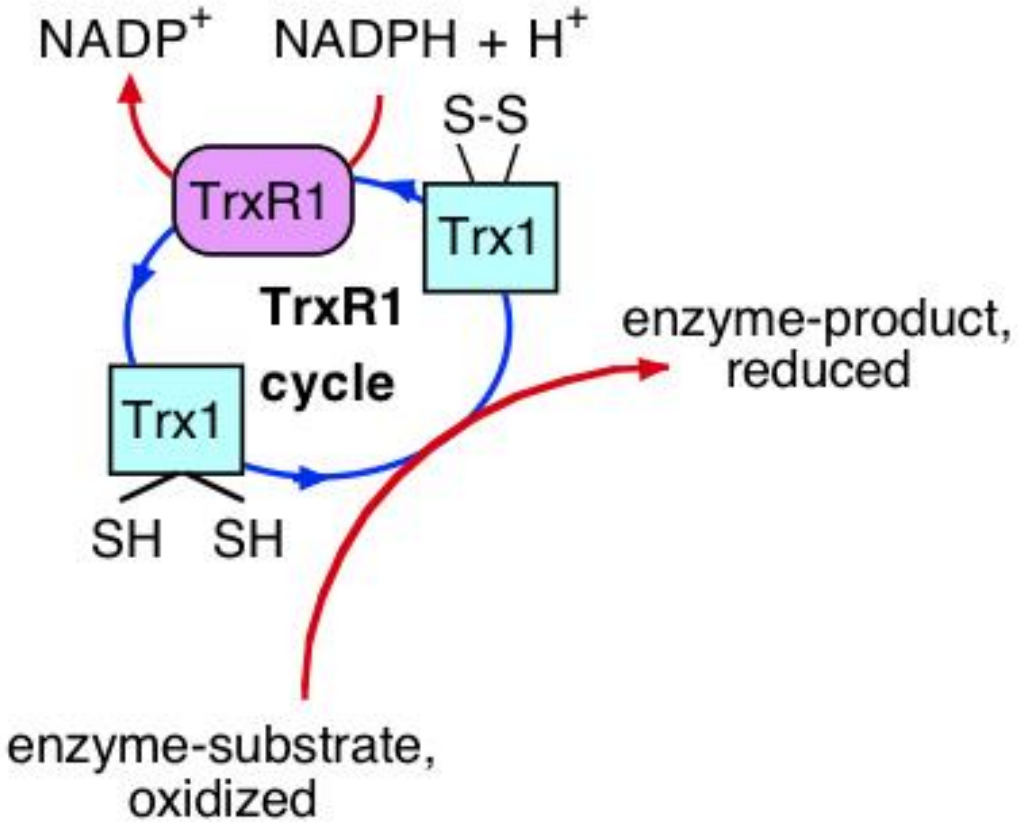
Thioredoxin and Thioredoxin reductase

Oxidized Trx is regenerated by TrxR, an antioxidant enzyme that uses reducing equivalents from NADPH to reduce Trx.

- Trx is a small protein (12,5 KD) which catalytic site contains 2 Cys able to reduce oxidized proteins becoming oxidized itsself.

$Trx(SH)_2 + Prot(S\text{-}S) \rightarrow Trx(S\text{-}S) + Prot(SH)_2$

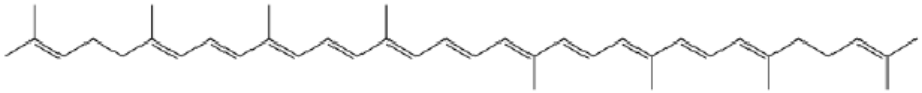
Thioredoxin and Thioredoxin reductase



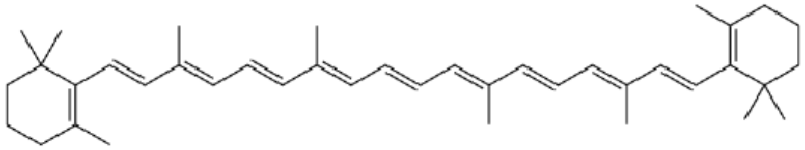
Carotenoids

- Carotenoids, are liposoluble organic pigments of plant origin. At least 600 different carotenoids are known, divided in two classes: xanthophylls (contain oxygen) and carotenes (no oxygen).
- Most carotenoids absorb light from 400-550 nm and protect plant from photodamage.
- In the retina they protect from UV damage
- They act as antioxidants by quenching singlet oxygen or as chain bracker.

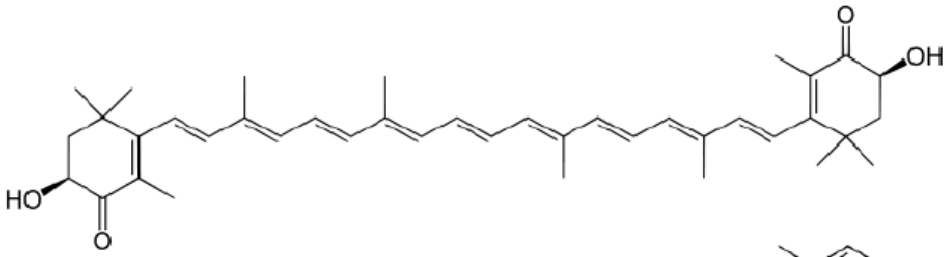
Carotenoids



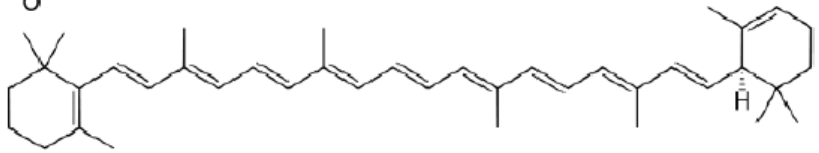
Lycopene



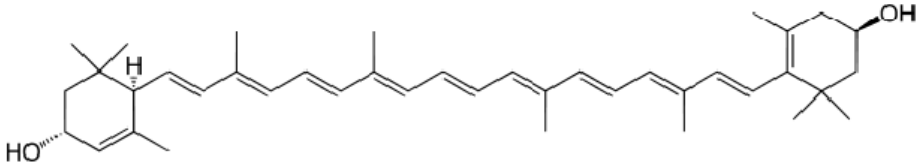
β-carotene



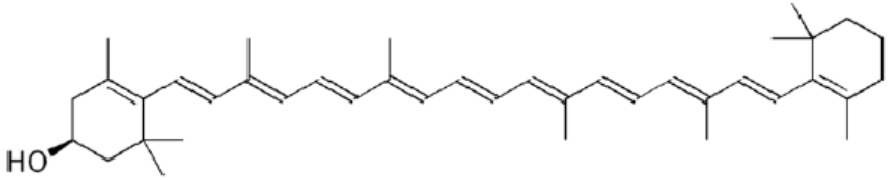
Astaxanthin



α-carotene

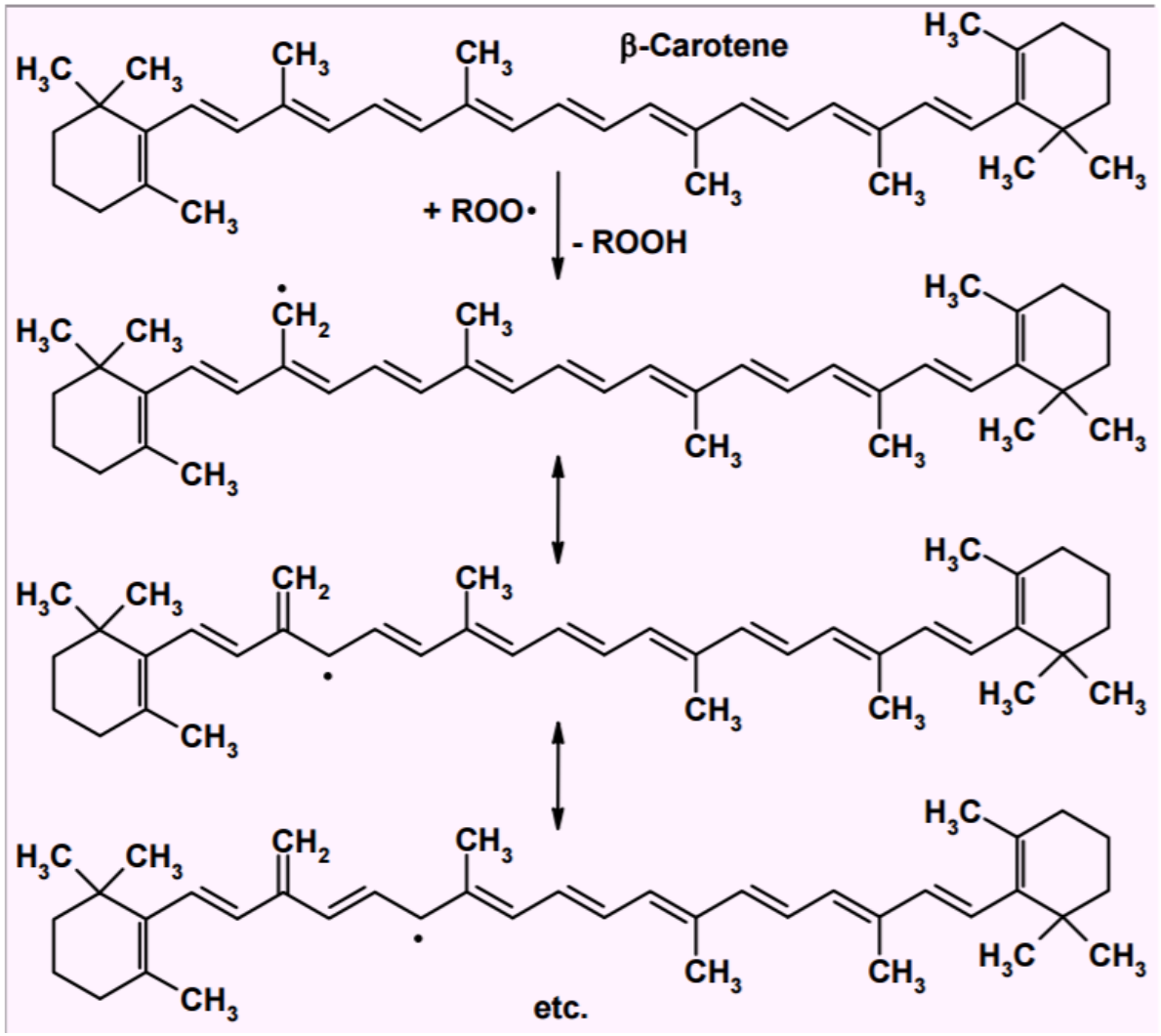


Lutein



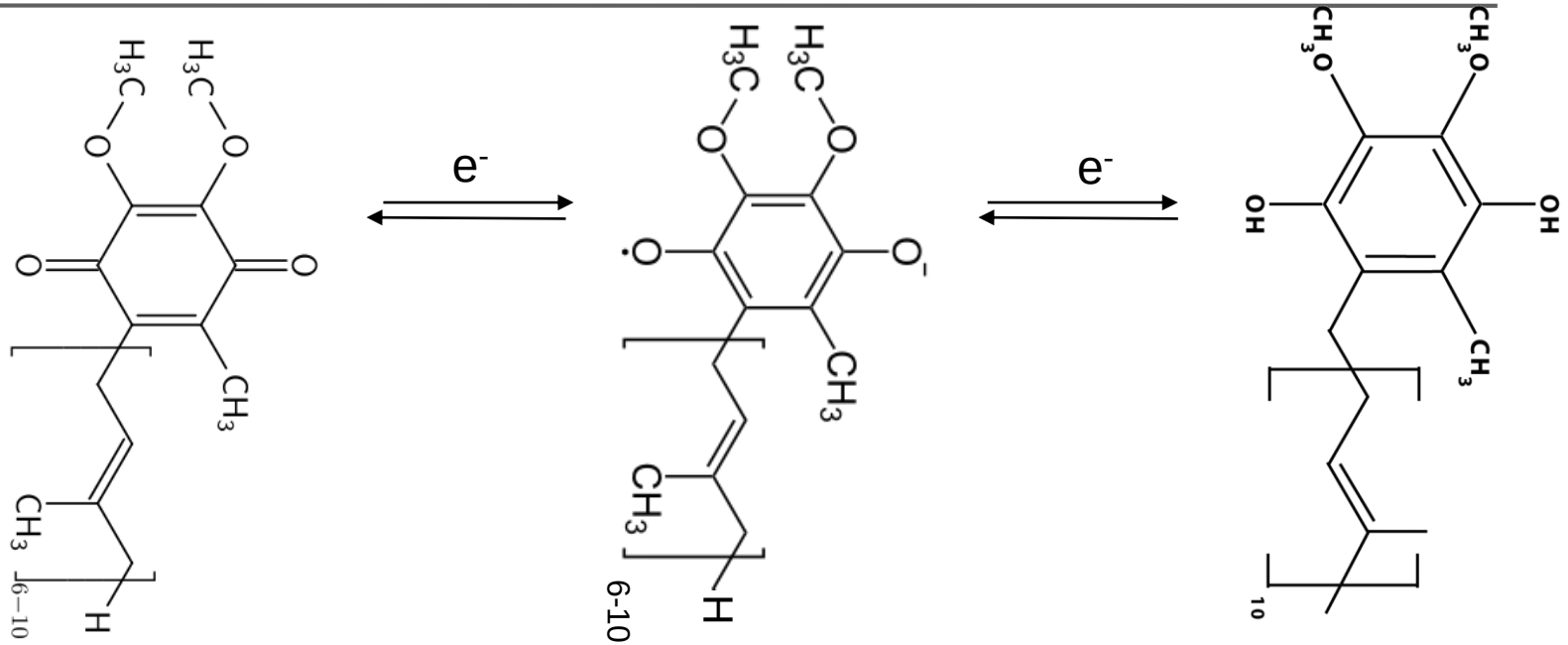
β-crytoxanthin

Carotenoids as chain bracker



Carotenoids act as chain bracker by donating an e-, the CAR• is a stable radical with many resonance structures

Coenzyme Q_{10}



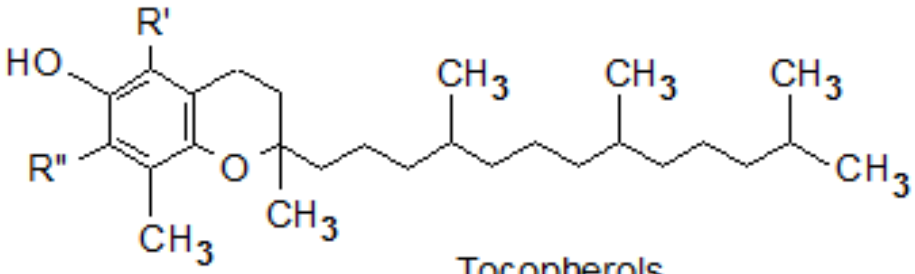
Ubiquinone Ubisemiquinone Ubiquinol

Q_{10} is known for its role in the respiratory chain, it functions as an electron carrier from enzyme complex I and II to complex III, beside this key role it act as an antioxidant, in the reduced status (ubiquinol) it gives up one or both electrons quite easily to electrophilic compounds.

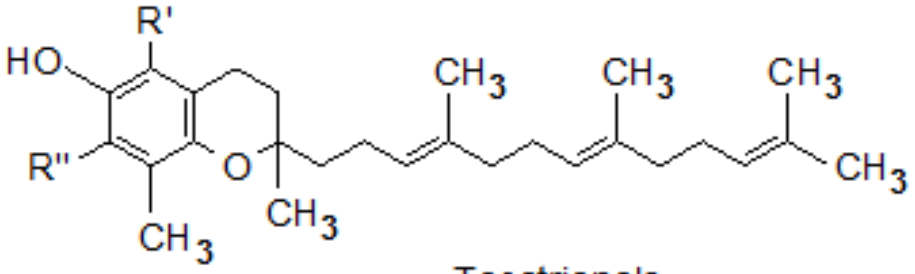
Coenzyme Q_{10}

- Q_{10} is distributed among lipid membrane especially at mitochondrial level, but it is also found in plasmatic and nuclear membrane.
- Q_{10} act as a scavenger and inhibits lipid peroxidation by preventing the production of lipid peroxyl radicals

Tocopherol (Vit E)



Tocopherols



Tocotrienols

α: R' = CH_3, R'' = CH_3

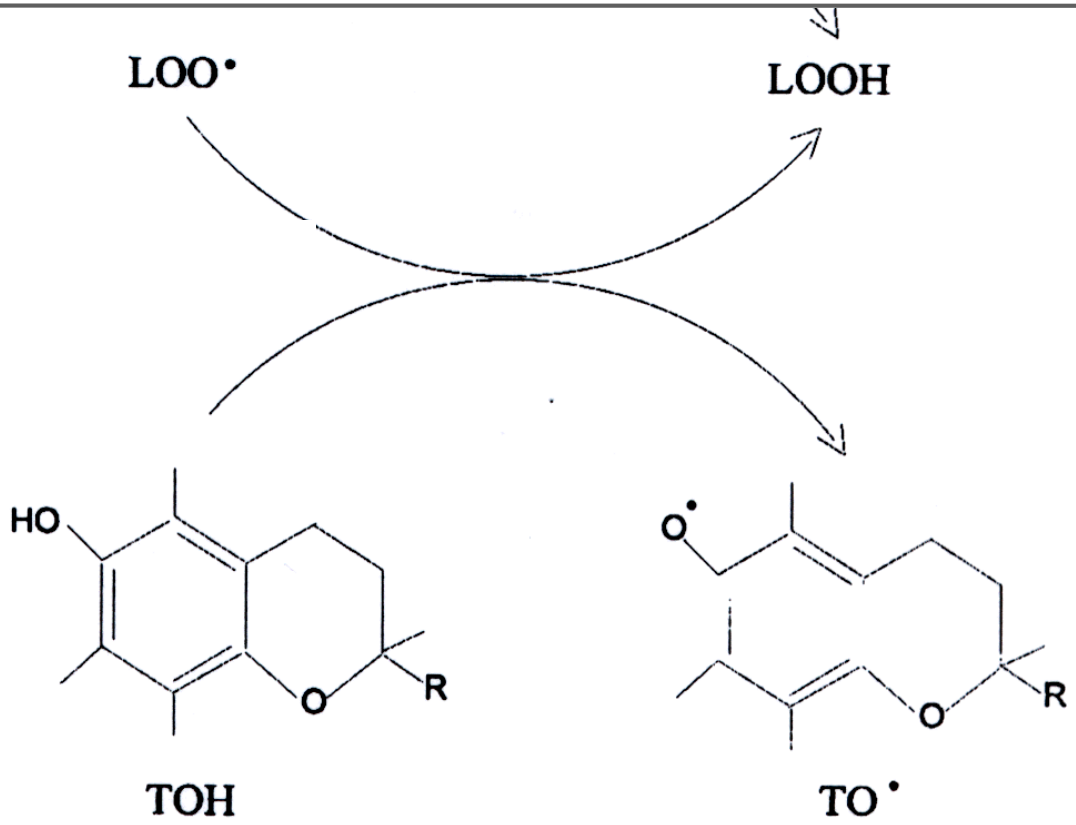
β: R' = CH_3, R'' = H

γ: R' = H, R'' = CH_3

δ: R' = H, R'' = H

They are distributed in the membranes due to their lipophilic structure, and protect them from lipid peroxidation acting as chain breacker.

Tocopherol (Vit E)

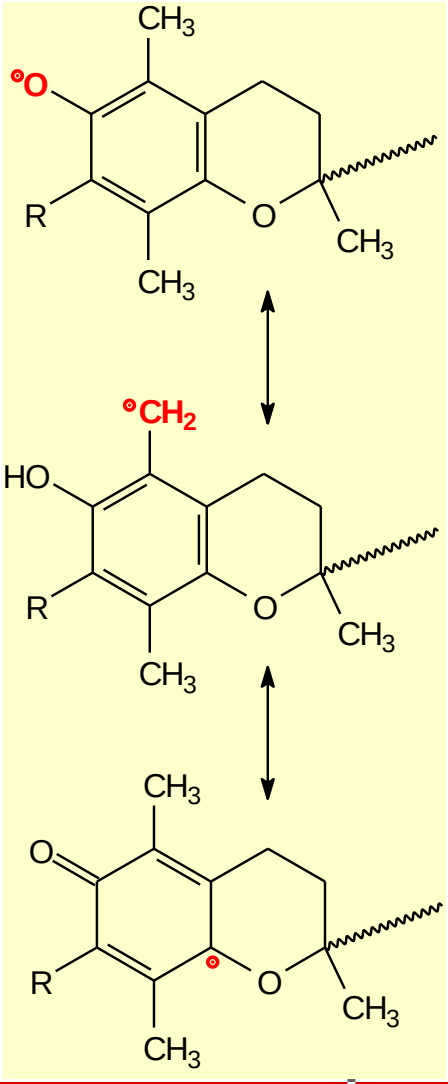


Tocopheryl radical is stable and not reactive, more important is that it can be regenerated

Tocopherol (Vit E)

Tocopheryl radical can delocalize the unpaired electron among many different resonance structures resulting in a quite stable radical.

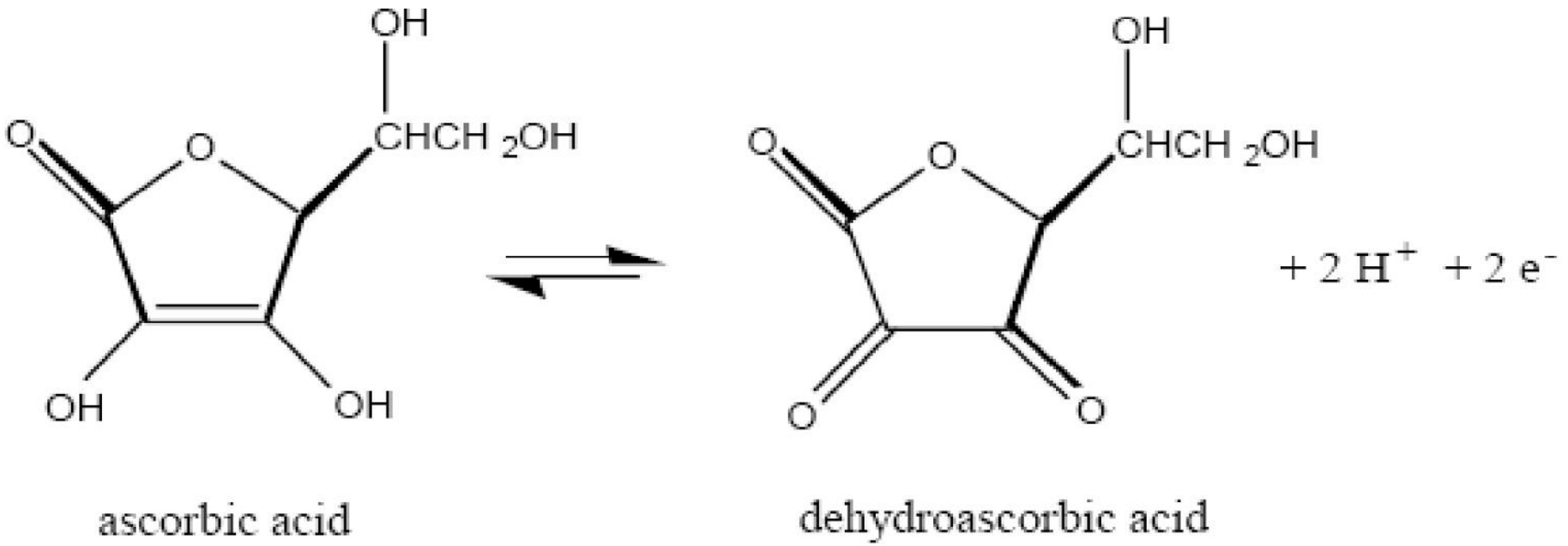
Tocopheryl radical can also be regenerated by ubiquinol, ascorbate or GSH.



Ascorbic acid

Hydrophilic antioxidant distributed in cell cytosol. As well as Vit E it is an essential nutrient, it can not be synthesized by human cells so we need to introduce it with foods, other mammals can produce ascorbate by themselves.

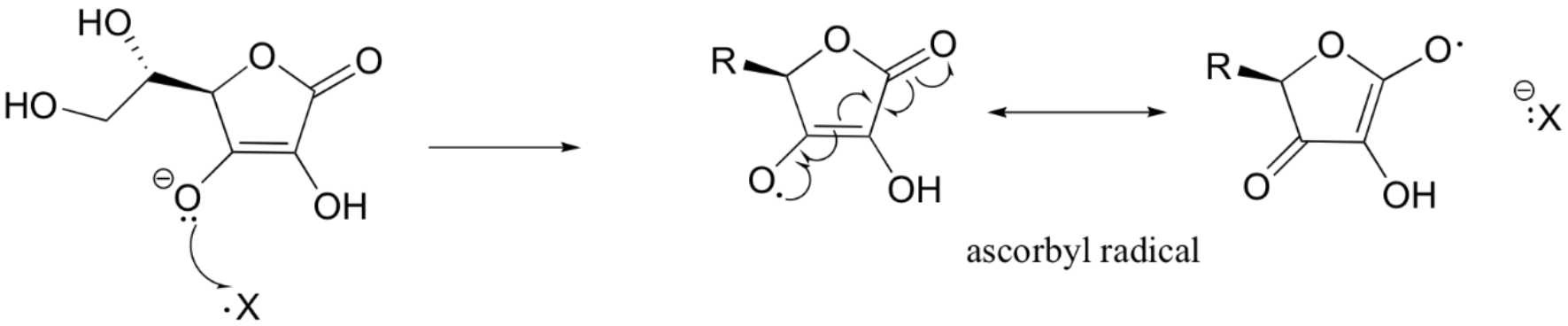
Ascorbic acid



ascorbic acid

dehydroascorbic acid

Ascorbyl radical

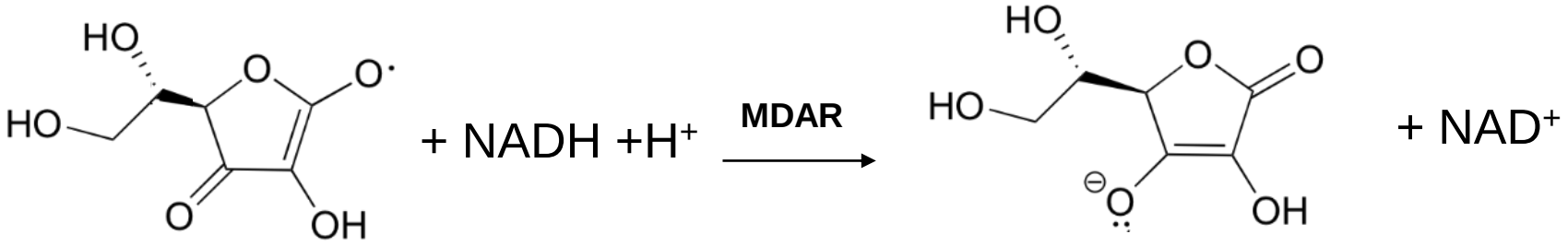


Ascorbyl radical (monodehydroascorbate) can delocalize the unpaired electron among different resonance structures resulting in a quite stable radical.
Ascorbate act as a scavenger of $O_2^{\bullet -}$ OH•, Tocopheril radical

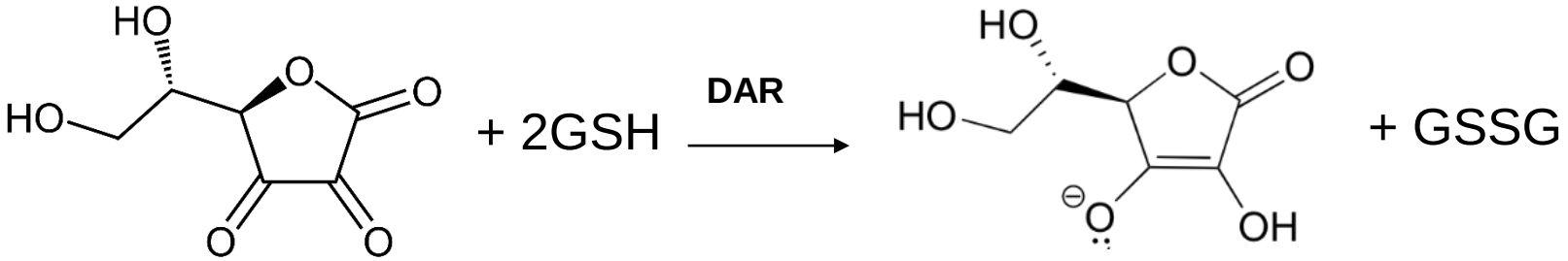
Recycling oxidized ascorbate

Two enzymes can regenerate ascorbate:

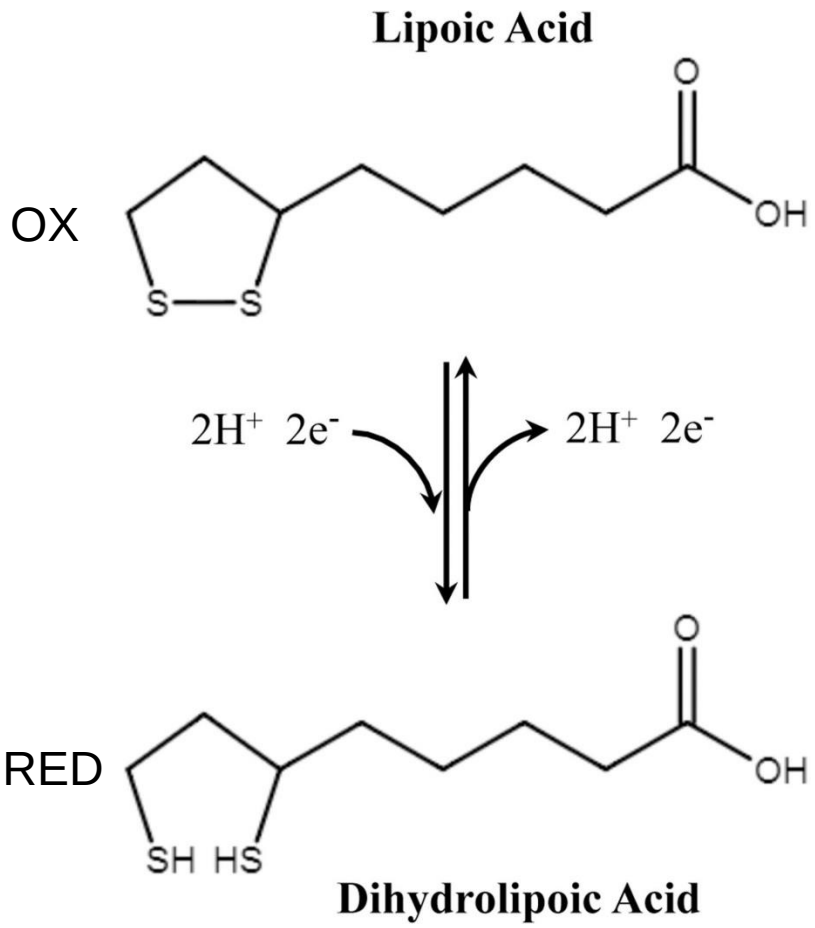
1.Monodehydroascorbate reductase, a NADH dependent enzyme



2.dehydroascorbate reductase, a GSH dependent enzyme

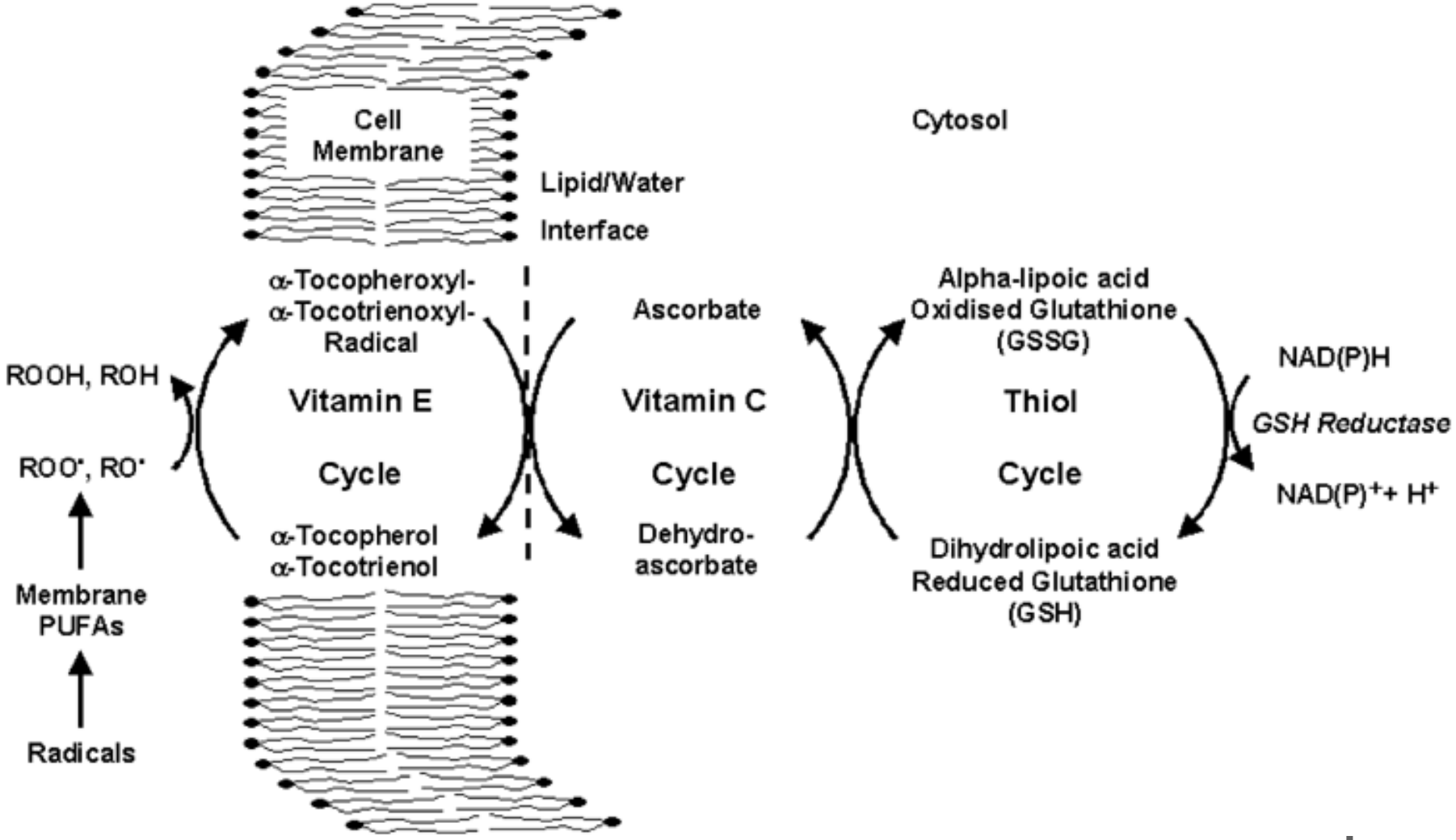


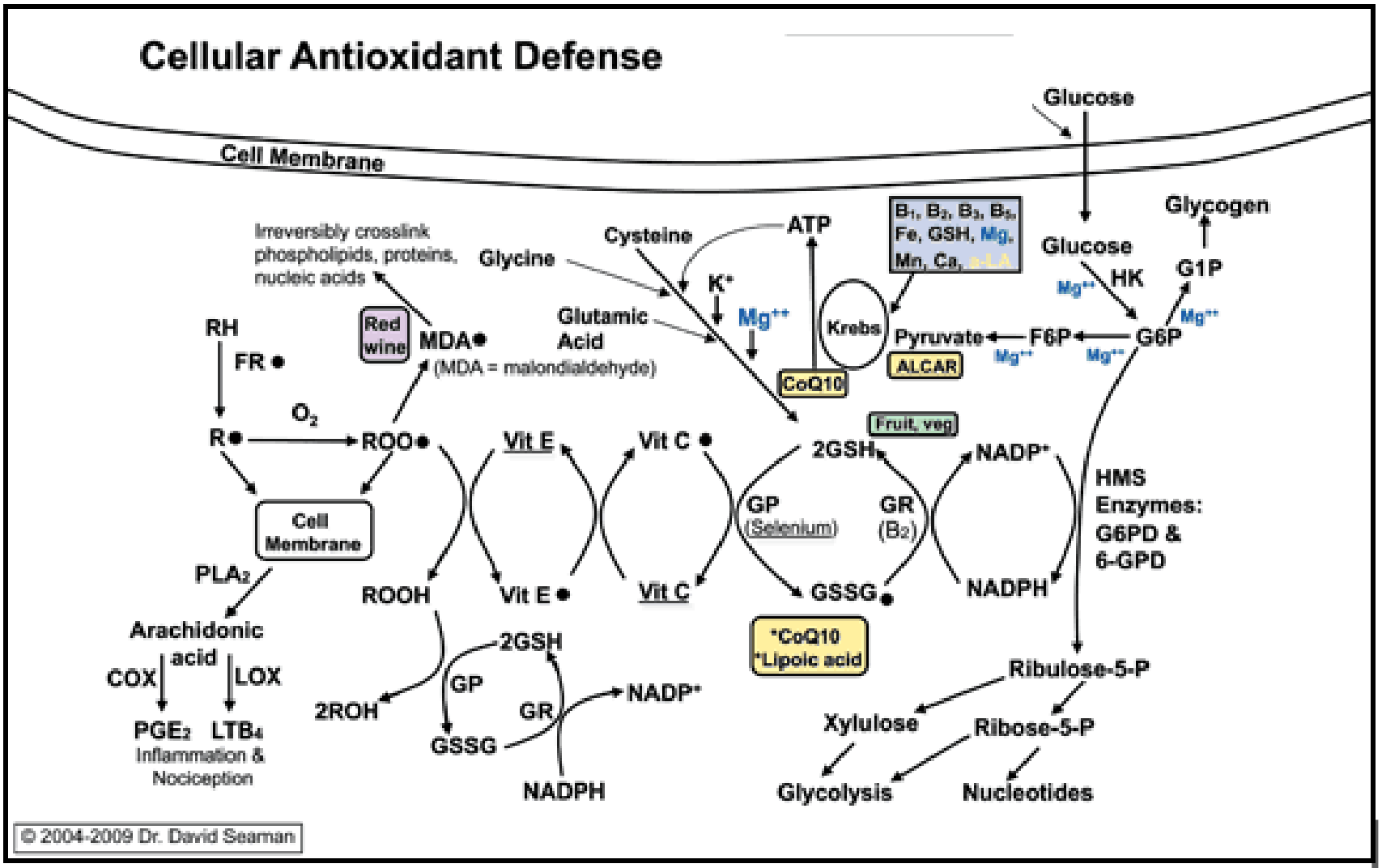
Lipoic acid



LA acts as cofactor of many enzymes, mainly dehydrogenases, but it is also a strong antioxidant able to donate 2 e^-

The antioxidants work together




Cellular Antioxidant Defense
Cell Membrane
Glucose
Irreversibly crosslink phospholipids, proteins, nucleic acids
Glycine
Cysteine
ATP
K+
Mg++
Glutamic Acid
Krebs
B1, B2, B3, B5, Fe, GSH, Mg, Mn, Ca, a-LA
Glucose
HK
G1P
Glycogen
Mg++
RH
FR ●
Red wine
MDA●
(MDA = malondialdehyde)
Pyruvate
F6P
G6P
ALCAR
CoQ10
O2
R●
ROO●
Vit E
Vit C ●
Fruit, veg
2GSH
NADP+
HMS Enzymes: G6PD & 6-GPD
Cell Membrane
GP (Selenium)
GR (B2)
PLA2
ROOH
Vit E ●
Vit C
GSSG●
NADPH
Arachidonic acid
COX
LOX
*CoQ10
*Lipoic acid
Ribulose-5-P
2GSH
GP
2ROH
GR
NADP+
Xylulose
Ribose-5-P
PGE2
LTB4
Inflammation & Nociception
GSSG
NADPH
Glycolysis
Nucleotides
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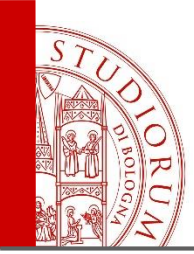
The take home message

Whenever possible our cells regenerate antioxidants (Q_{10}, Vit C, Vit E and GSH), most reducing equivalents needed to scavenge ROS and free radicals come from NAD(P)H produced in the cell metabolism.

Extracellular antioxidants

- Extracellular isoforms of antioxidant enzymes (Ec-SOD, GPx3…)
- Non enzimatic antioxidants
- Transition metals chelating molecules
  - Transferrine (binds Fe^{3+})
  - Ceruloplasmin (binds Cu and keeps Fe as Fe^{3+})
  - Urate (final prodact of purine breackdown, it is a strong antioxidants)



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