Sunrise Free Radical School, 2007

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Antioxidants

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Issues for consideration

- 1- Kinetics and concentrations - relation to bioavailability
- 2- Will the antioxidant get to the right place?
- 3- Specificity for scavenging target ROS/RNS
- 4- Potential interactions with co-antioxidants
- 5- Can antioxidants function as pro-oxidants?
- 6- Modes of action - scavengers or cell signaling regulators
- 7- The “specific hydroxyl radical scavenger syndrome”

1- Kinetics and concentrations

$$Antioxidant + Oxidant \text{ É } Oxidized\ Antioxidant + Detoxified\ Oxidant$$

$$\Delta G^o = - RT \ln K_{eq}$$

$$\Delta G = \Delta G^o + RT \ln \frac{[Oxidized\ Antioxidant]_i [Detoxified\ Oxidant]_i}{[Antioxidant]_i [Oxidant]_i}$$

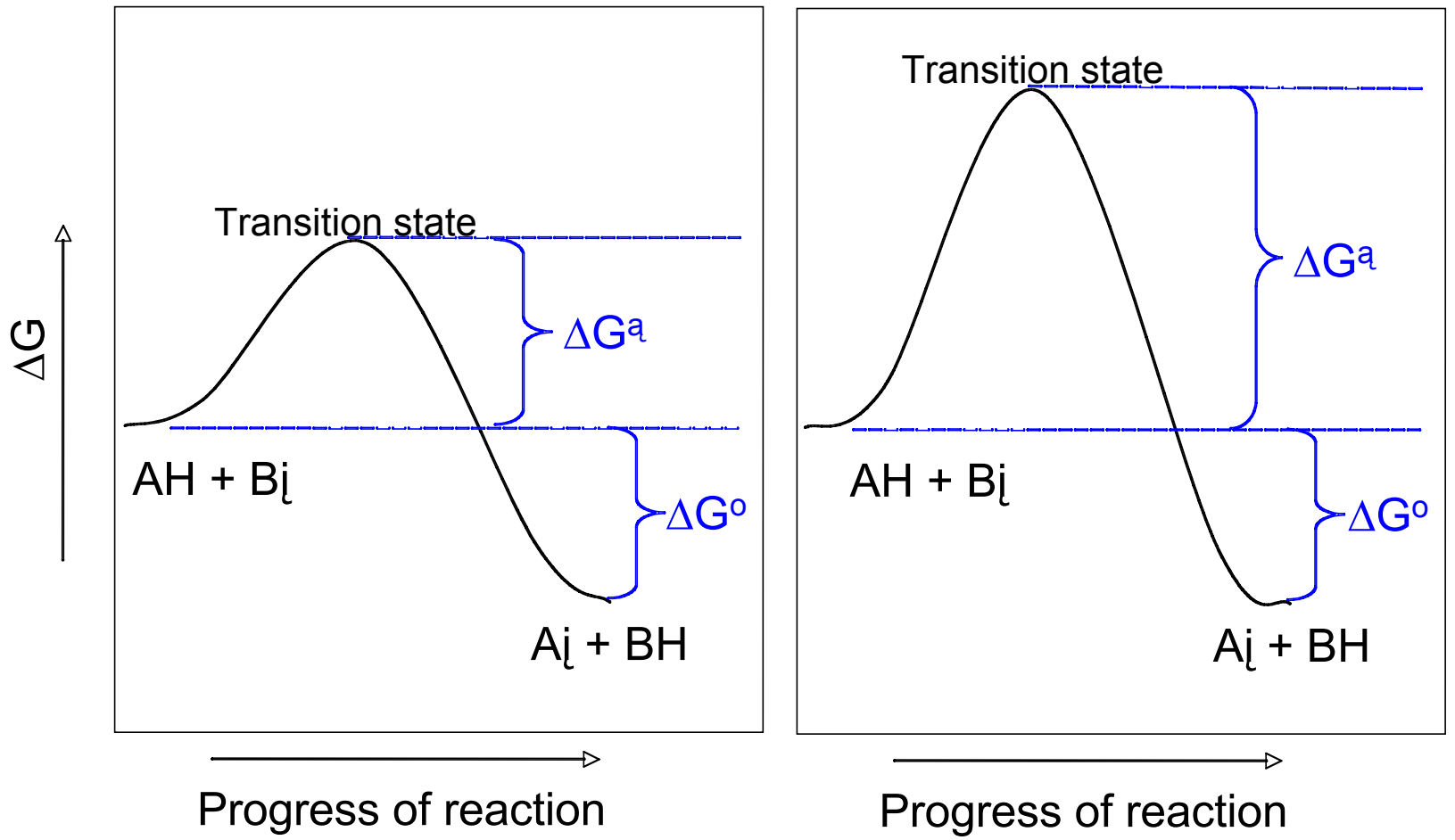
where *[Antioxidant]* is the instantaneous activity of the antioxidant

Thermodynamics tells you what could happen with or without the need to input energy.

⊗G^o must be negative for a reaction to occur spontaneously

But, ⊗G^o (or standard reduction potentials) does not tell you how fast anything happens- that is the subject of kinetics.

Which reaction will occur more rapidly?



$$\Delta G^{\ddagger}\ (energy\ of\ activation) = -RT\ \ln\ k\ /\ A$$

where A is the frequency factor for the reaction, R is the universal gas constant, and T is the temperature.

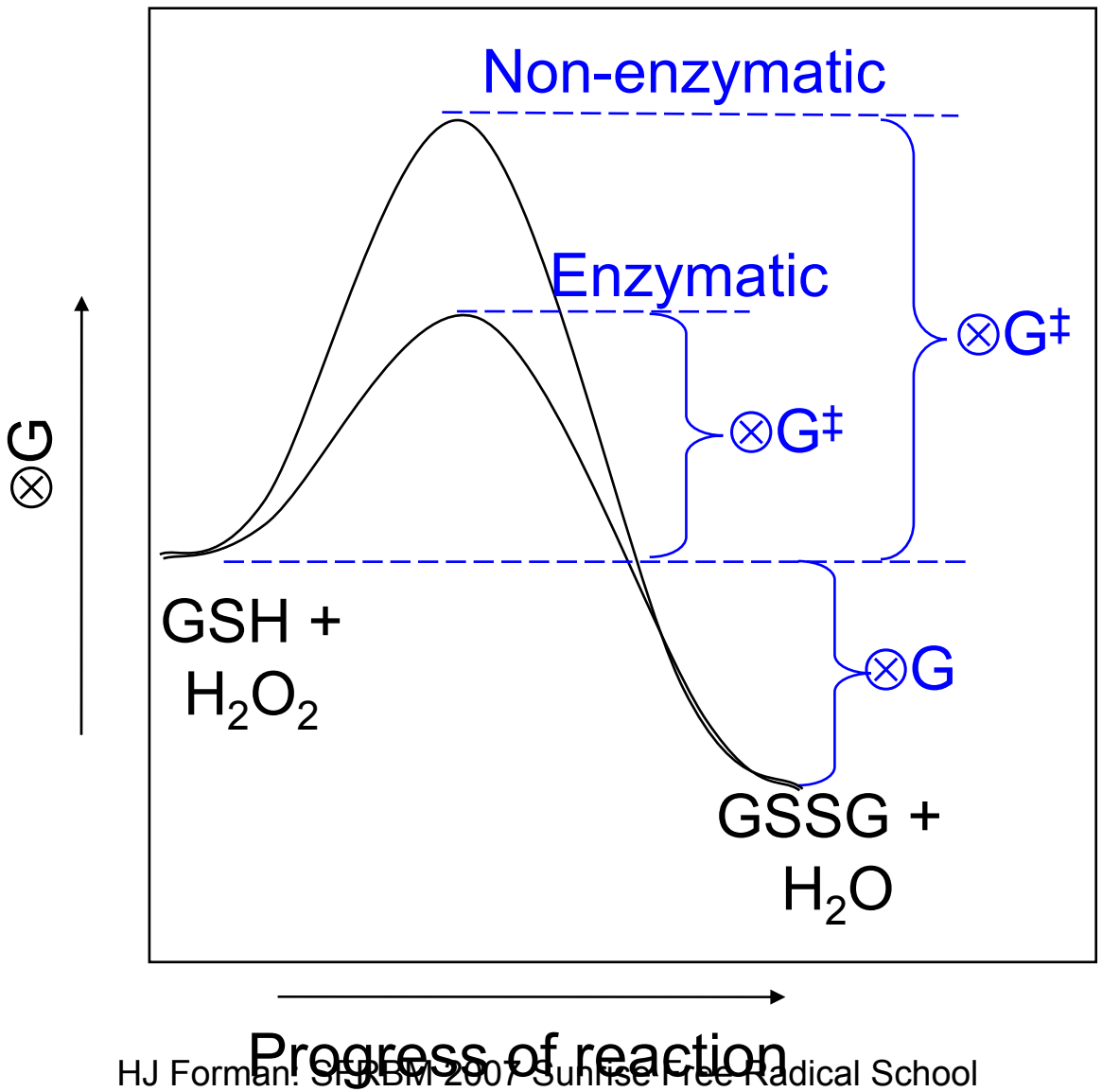
Qinghai-Tibet Railway: The highest point of the railway is 5,072 meters above sea level

But the Himalayas are the barrier (Mt. Everest rises to 8,848 meters)

⊗G is negative

Lucknow, India Elevation 128 meters

Enzymes accelerate reactions by decreasing ⊗G‡



An example is the reaction between glutathione (GSH) and H_2O_2

1- Kinetics and concentrations relation to bioavailability

$$Antioxidant + Oxidant \text{ É } Oxidized\ Antioxidant + Detoxified\ Oxidant$$

$$initial\ rate = k[Antioxidant][Oxidant]$$

So, will the [Antioxidant] and k both be high enough to prevent the oxidant from reacting with a biological target?

2- Will the antioxidant get to the right place?

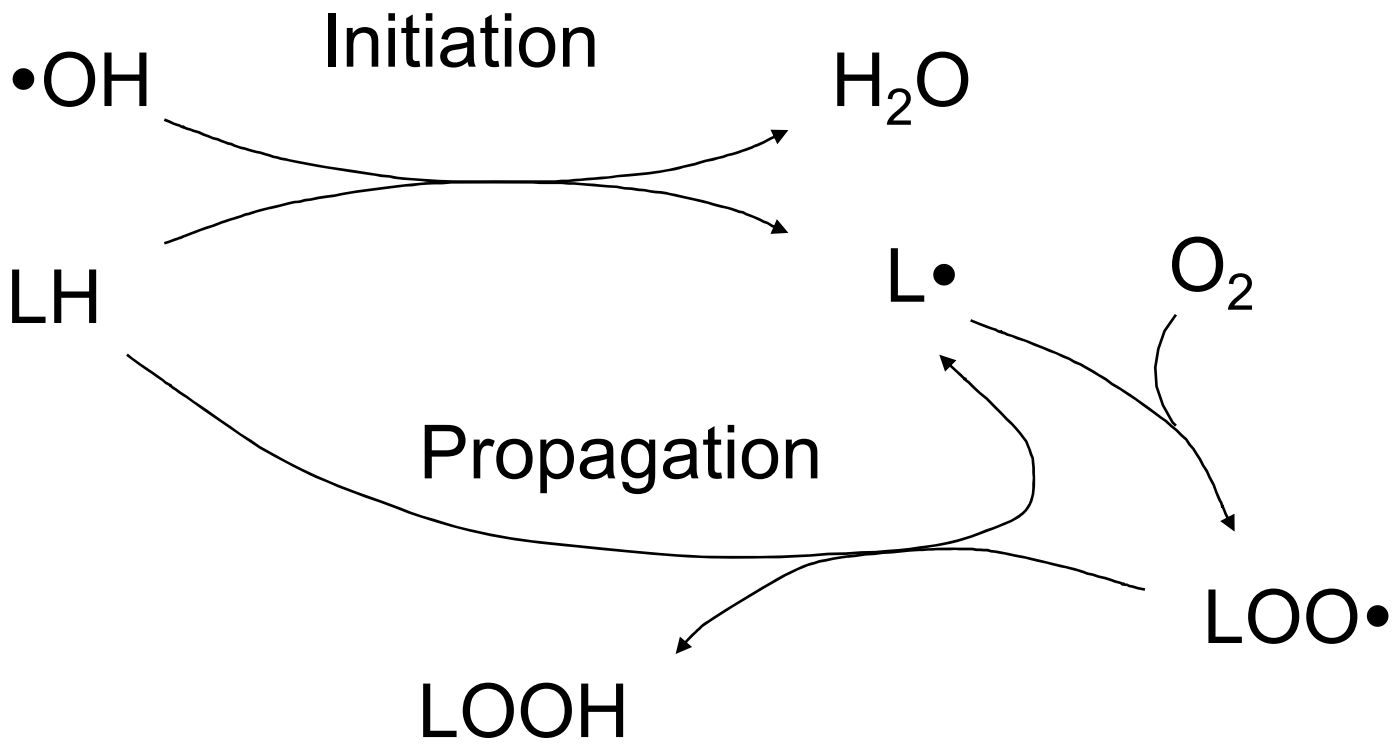
Factors influencing bioavailability

- Absorption through GI tract
- Metabolism in liver and other tissues
- Carrier molecules
  - e.g., for ⟨-tocopherol
  - LDL
- Solubility/partitioning into membranes

Examples of bioavailabilty issues

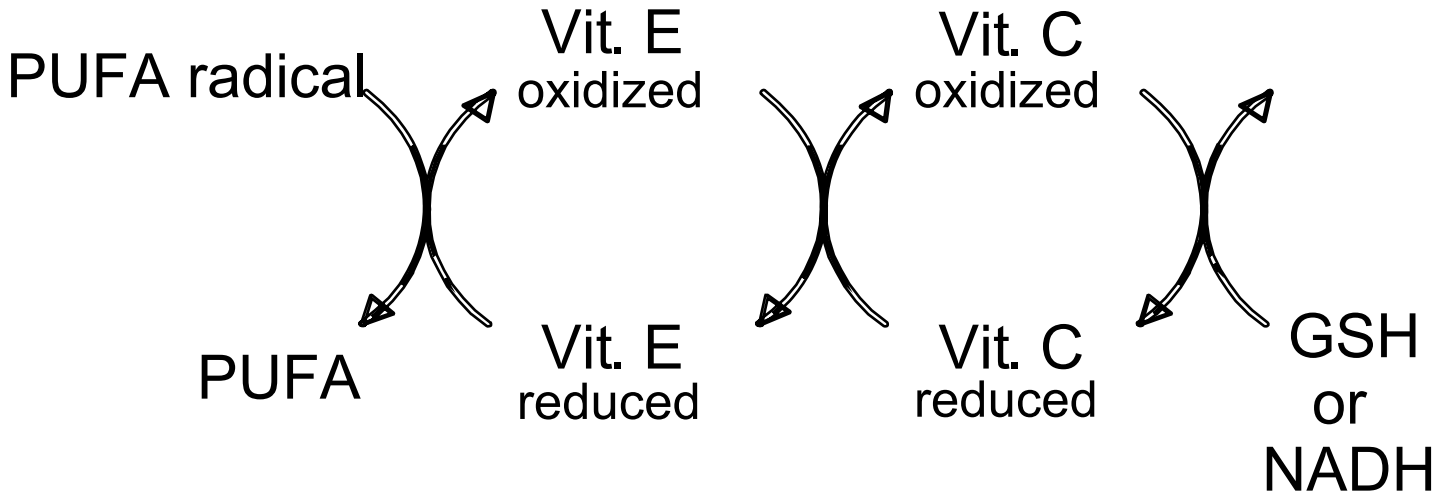
- Dietary ascorbate (vitamin C) approaches upper limit in plasma (1)
- Some studies of a tea polyphenol suggest effectiveness at < 1 ⌠M (the plasma peak) but most show need of > 10 ⌠M (2)
- Olive oil polyphenols reach at most 60 nM but are effective as scavengers only at > 50 ⌠M (3)

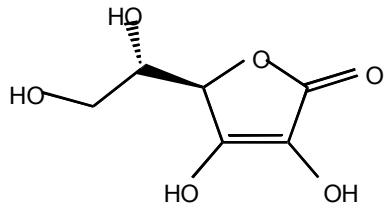
Lipid peroxidation
a major target for antioxidants



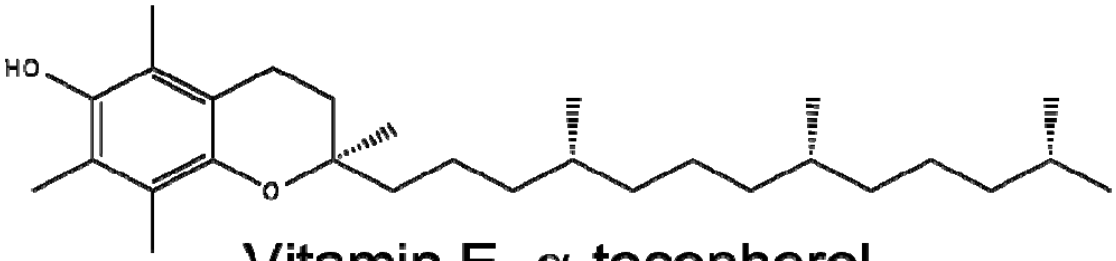
3- Potential interactions with co-antioxidants

Antioxidants in defense against lipid peroxidation

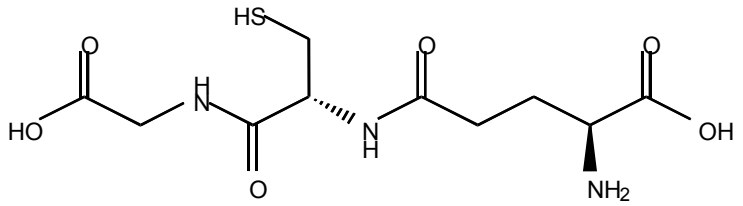




Vitamin C, ascorbic acid



Vitamin E, α-tocopherol



Glutathione, Ⓒ-glutamylcysteinylglycine

4- Specificity for scavenging ROS/RNS

Superoxide Dismutases

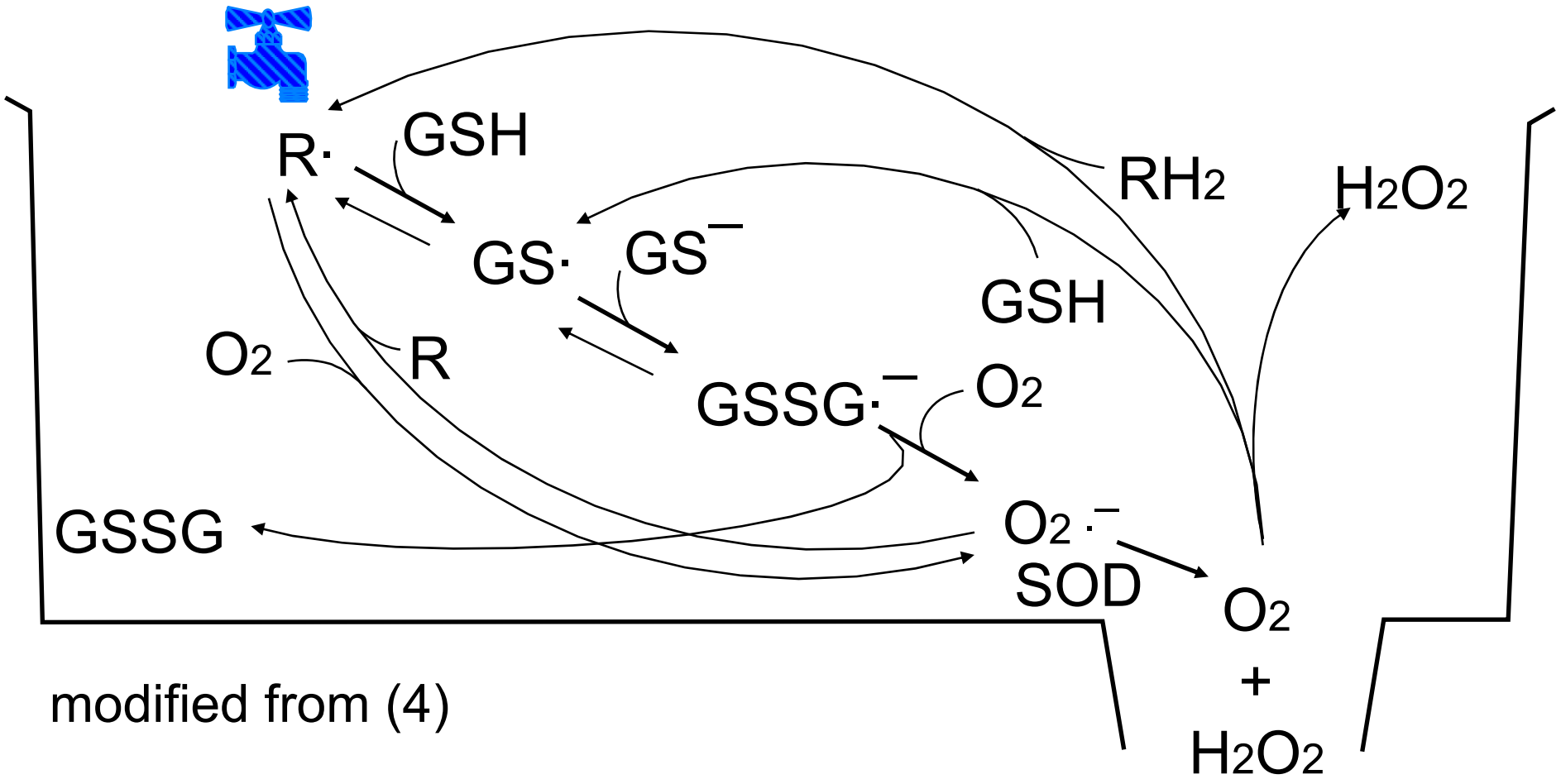
$$2O_2^{\cdot -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Metalloproteins with at least one transition metal (Cu, Mn, Fe)

Rate constants in the range of 10^9 M^{-1} s^{-1}

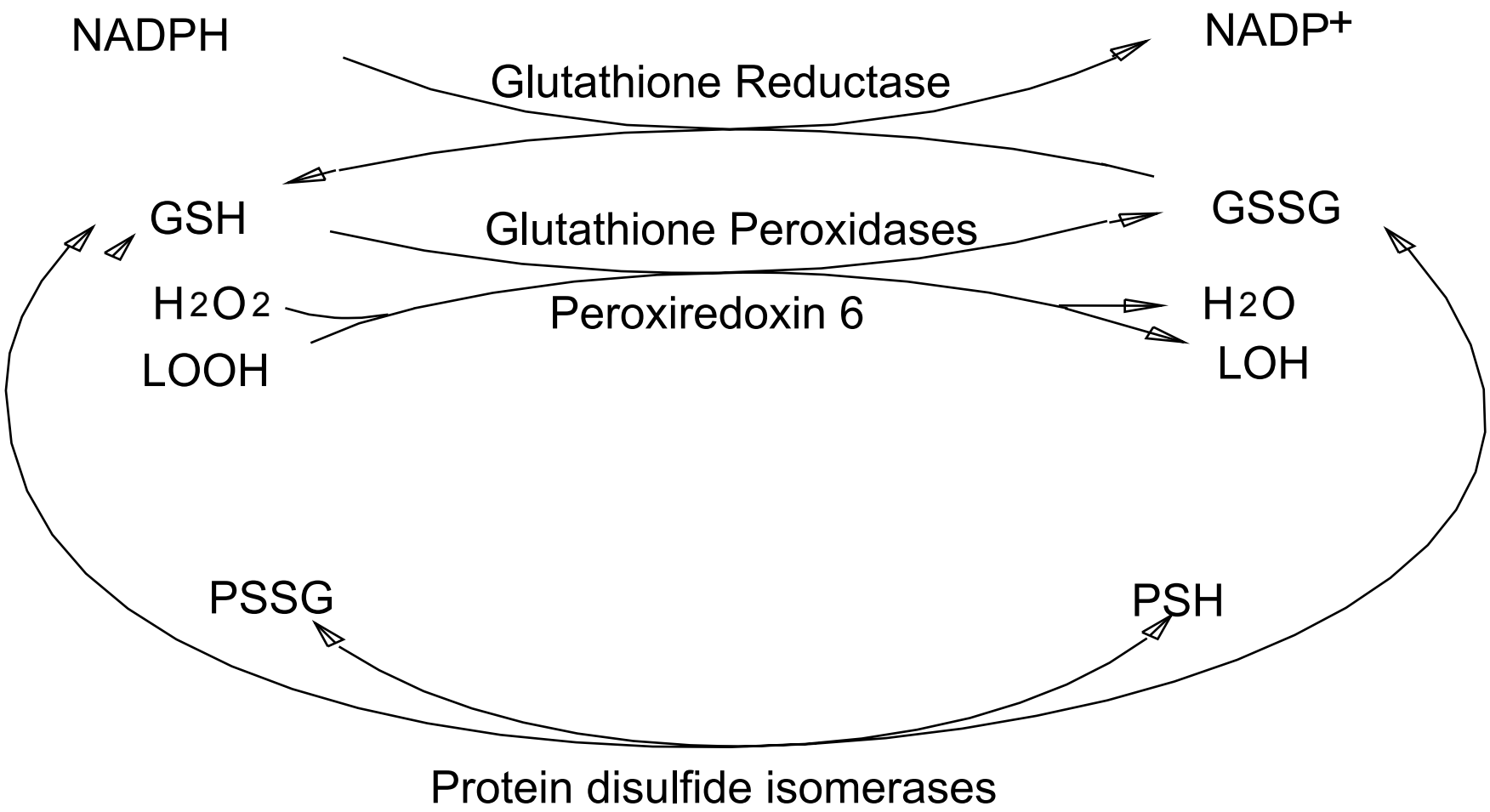
Found in cytosol, nucleus and extracellular compartments

The Radical Sink

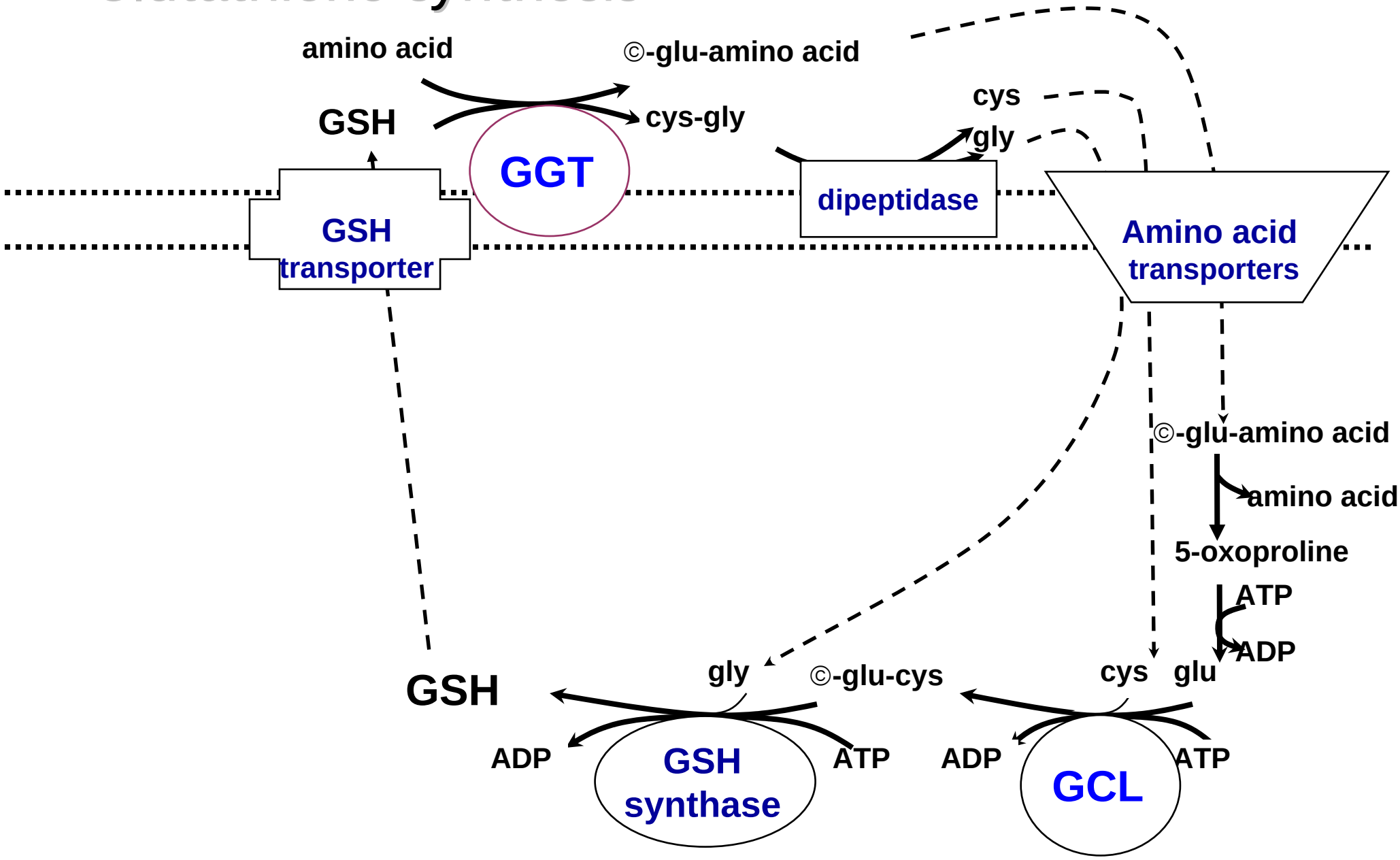


modified from (4)

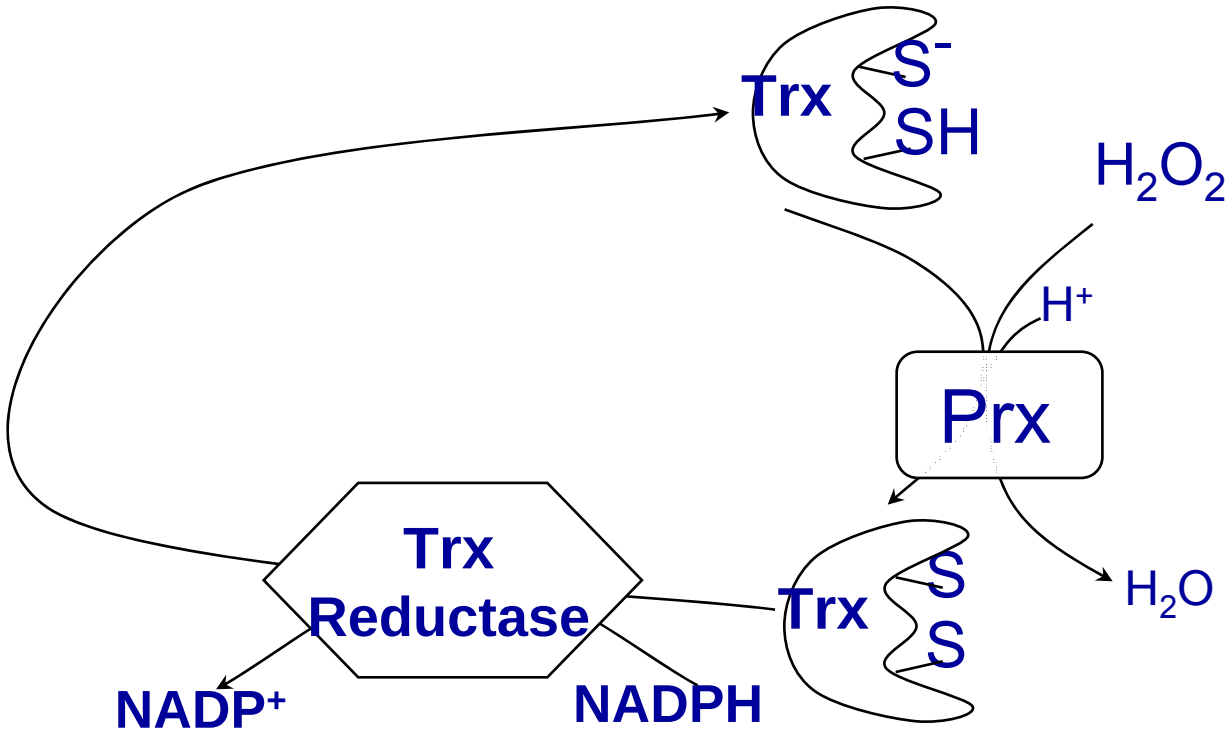
Glutathione as an enzyme substrate



Glutathione synthesis



Thioredoxin in elimination of H_2O_2

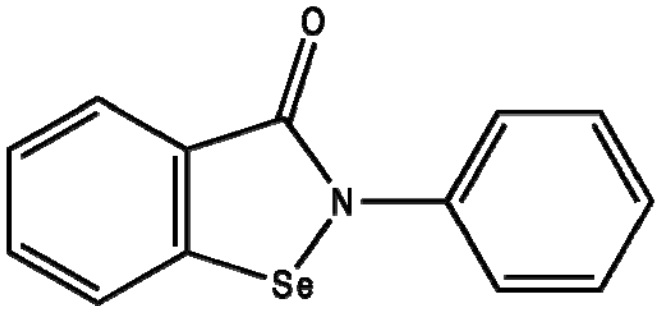


Non-enzymatic Trx oxidation is slow

Peroxynitrite is a substrate for GPx (5)

$$ONOO^- + 2GSH \xrightarrow{GPx} GSSG + H_2O + ONO^-$$

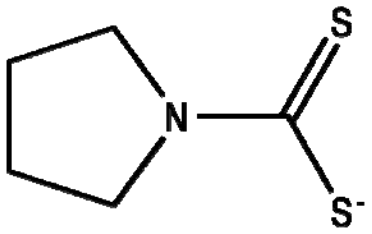
or Trx reductase with Ebselen or selenocystine



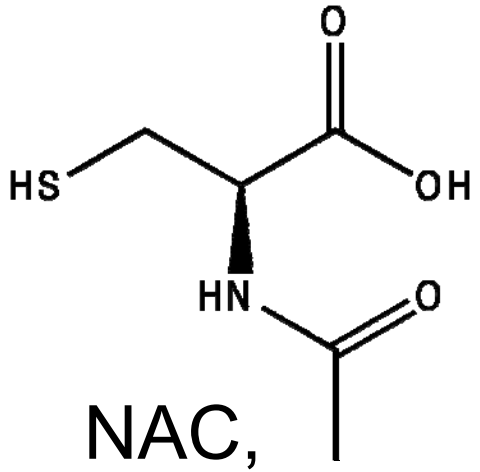
Ebselen

5- Can antioxidants function as pro-oxidants?

Thiols



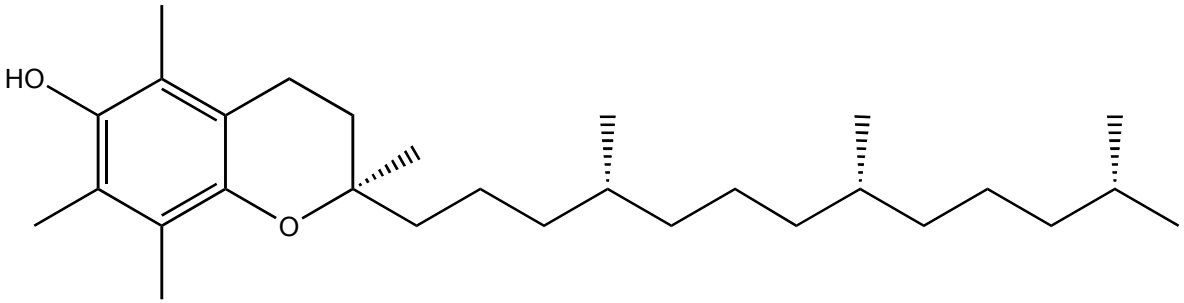
PDTC,
Pyrrolidinedithiocarbamate



NAC,
N-acetylcysteine

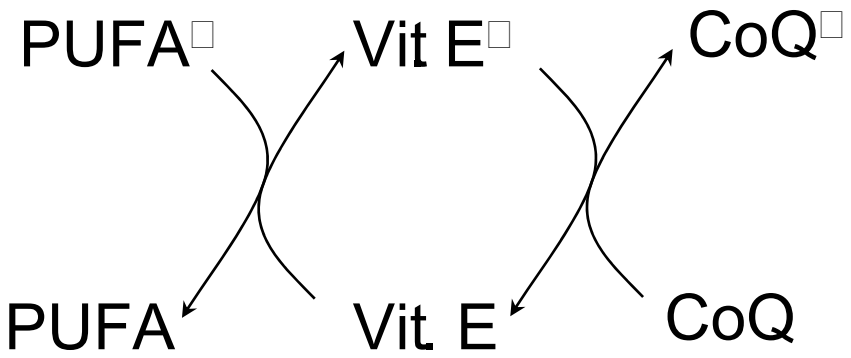
PDTC inhibits NF-| B DNA binding (6)
PDTC is a pro-oxidant copper chelator (7)

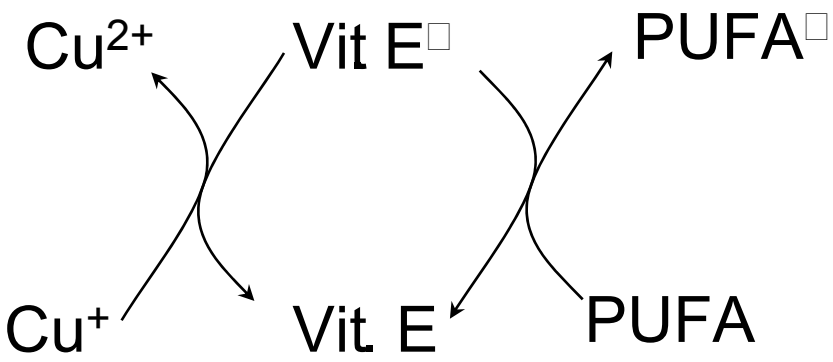
NF-| B activation by thiol oxidation (8)



⟨-tocopherol, vitamin E

In the absence of a co-antioxidant, ⟨-tocopherol can be a pro-oxidant (9)





6- Scavengers or cell signaling regulators?

The vitamin E debate **(**7/1/07 issue of Free Radic Biol Med)

Azzi (10) stated, that ⟨-tocopherol :

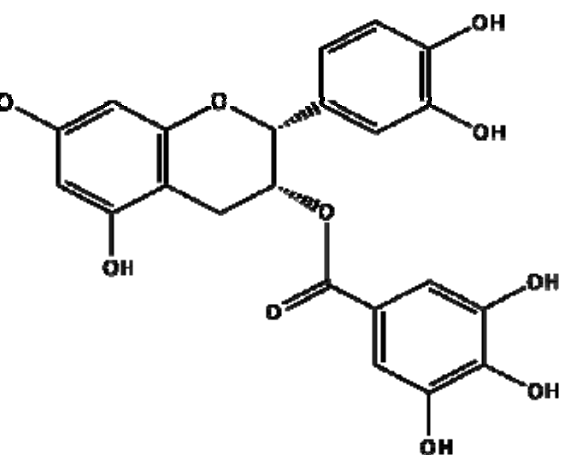
- has a natural function in cell signaling
- is the precursor of a more active form, ⟨-tocopheryl phosphate
- does not prevent oxidative damage *in vivo* or oxidative diseases

Traber (11) stated that ⟨- tocopherol:

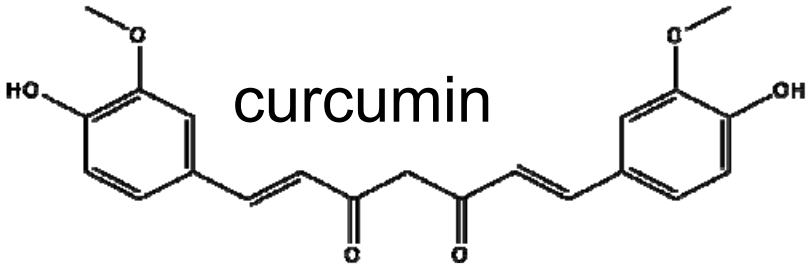
- participates only in signaling pathways involving oxidative stress
- protects PUFA and membrane changes affecting signaling
- has no direct signaling role

Commentary by Brigelius-Flohe and Davies, “Is vitamin E an antioxidant, a regulator of signal transduction and gene expression, or a 'junk' food?”

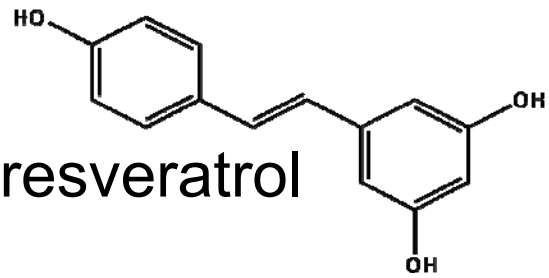
Polyphenols



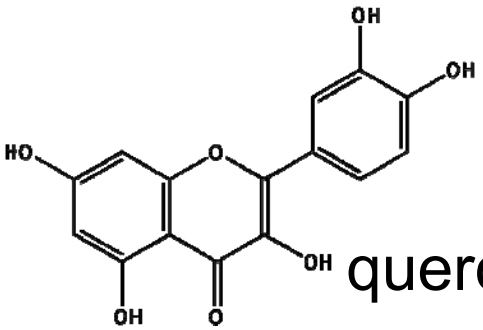
epigallocatechin gallate



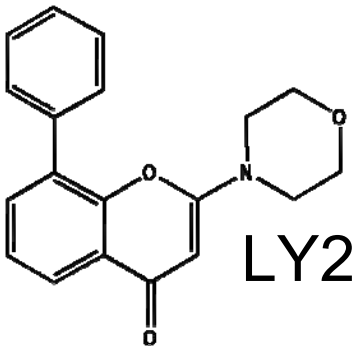
curcumin



resveratrol



quercetin



LY294002

Do we get enough of these “antioxidants” into tissues to be effective as scavengers?

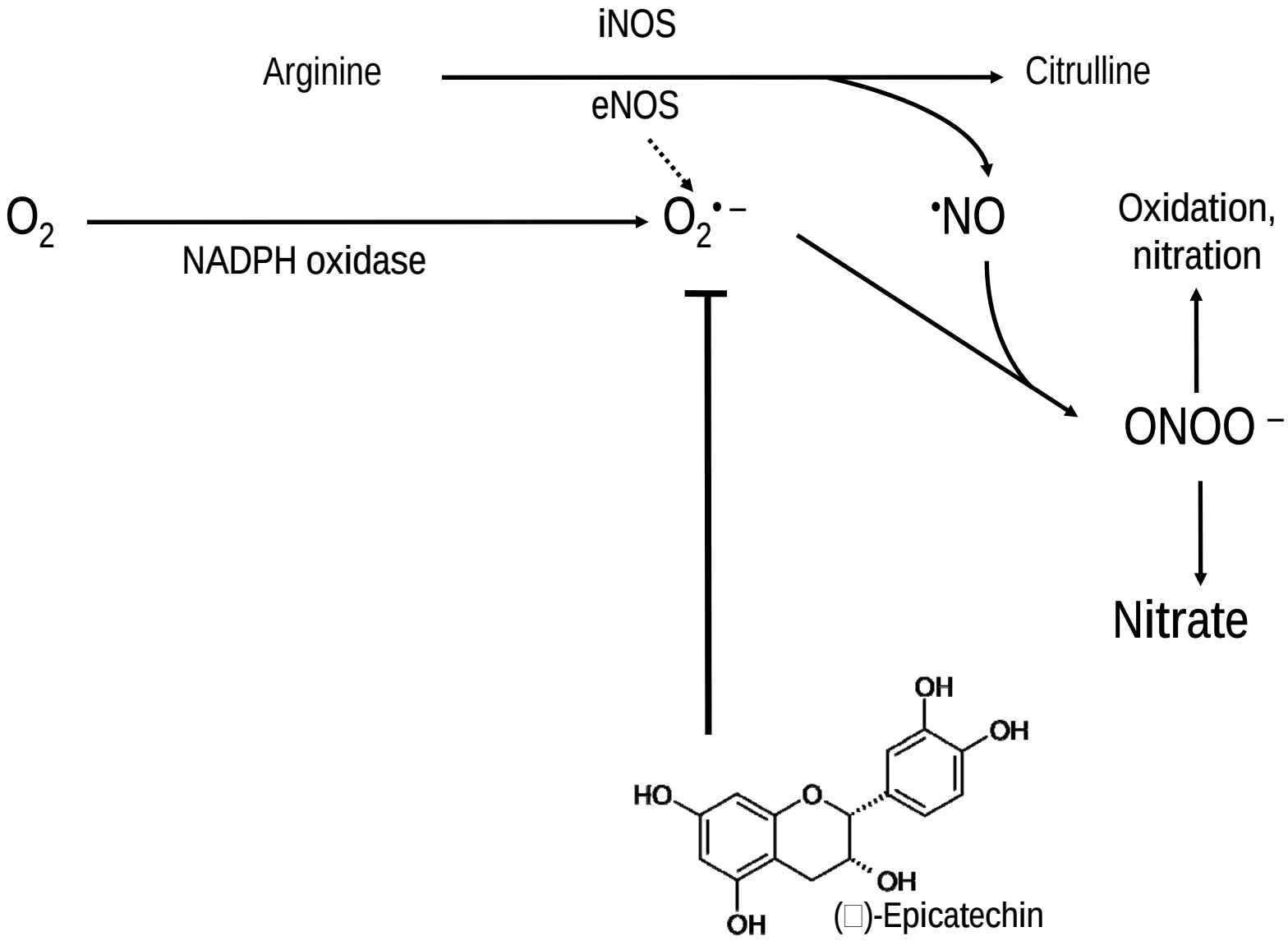
Do they act as antioxidants or have affects on signaling?

There can be up to 1 ⌠M in plasma from diet (13)

But, they can:

- act as pro-oxidants for EpRE signaling or caspase activation (14)
-modulators of protein kinases (15)

Indeed, LY294002, the most selective inhibitor of PI3 kinase, is an analog of quercetin (16)


iNOS
Arginine
Citrulline
eNOS
O_2
$O_2^{\bullet -}$
NADPH oxidase
•NO
Oxidation, nitration
$ONOO^-$
Nitrate
OH
OH
HO
O
OH
OH
(□)-Epicatechin

From (17)

Increases in Antioxidant Enzymes by Oxidant Stress another non-antioxidant function of “antioxidants”

MnSOD
CuZnSOD
catalase
GPx 1
Prdx 6
©-glutamyl transpeptidase
Glutamate cysteine ligase
NAD(P)H:quinone oxidoreductase 1
Heme oxygenase 1

The myth of the specific hydroxyl radical scavenger

- Almost all organic compounds react with •OH near diffusion limited rates.
- Thus, no compound has significantly more •OH scavenging activity than just about any other.

$$\frac{-d[^{\cdot}OH]}{dt} = (k_x[X] + k_a[all\ others])\, x\, [^{\cdot}OH]$$

So, you would have to drown it in!

References

1 Blanchard, Am J Clin Nutr 66:1165;1997
2 Lambert, J Nutr 133:32625;2003
3 Vissers, Eur J Clin Nutr 58:955;2004
4 Winterbourn, Free Radical Biol Med 14:85;1993
5 Sies, Free Radic Biol Med 28:1451;2000
6 Brennan, Biochem J 320:975;1996
7 Nobel, J Biol Chem 270:26202;1995
8 Das, Am J Physiol 269:L588;199
9 Thomas, Arterioscler Thromb Vasc Biol 16:687,199
10 Azzi, Free Radic Biol Med 43:16; 2007
11 Traber, Free Radic Biol Med 43:4; 2007
12 Brigelius-Flohe, Free Radic Biol Med 43:2; 2007
13 Scalbert, J Nutrition 130:2073S;2000
14 Owuor, Biochem Pharmacol 64:765;2002
15 Williams, Free Radic Biol Med 36:838;2004
16 Vlahos, J Biol Chem 269:5241;1994
17 Steffen Biochem Biophys Res Commun 359:828;2007