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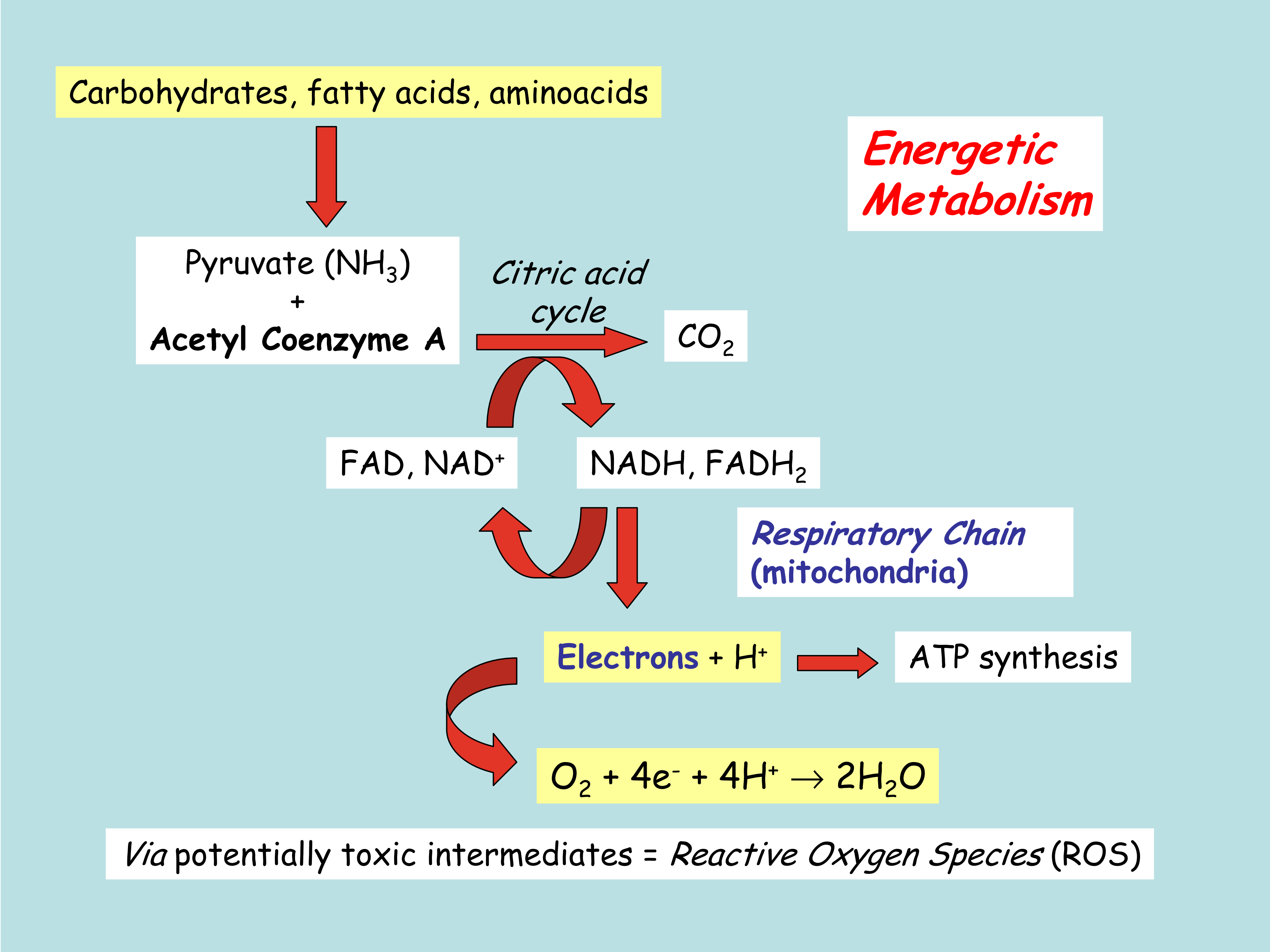
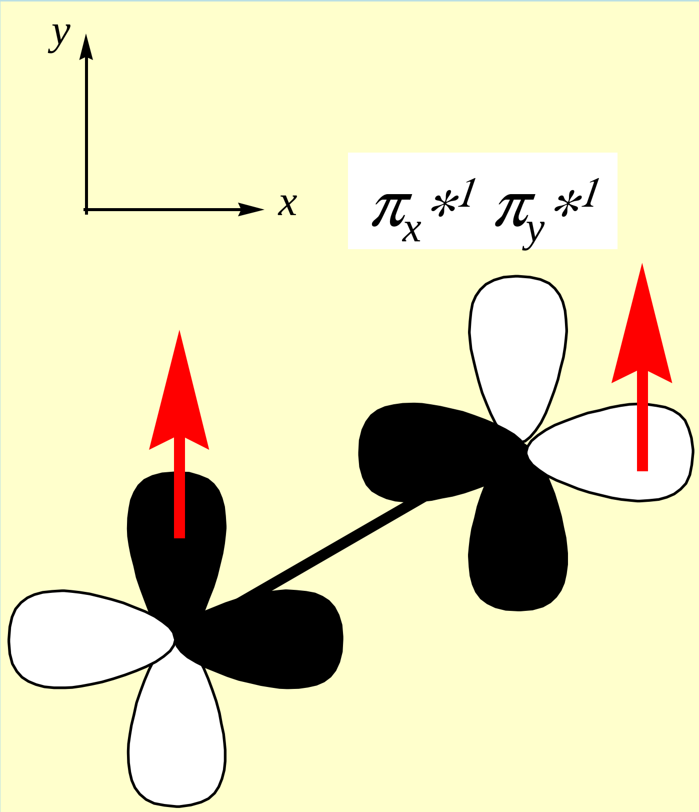
UNIVERSITÉ D'AVIGNON

Antioxidant Activity of Dietary Polyphenols

The Dangers of Aerobic Life:
Reactive Oxygen Species (ROS) & Oxidative Stress


Carbohydrates, fatty acids, aminoacids
Energetic Metabolism
Pyruvate (NH_3) + Acetyl Coenzyme A
Citric acid cycle
CO_2
FAD, NAD^+
NADH, $FADH_2$
Respiratory Chain (mitochondria)
Electrons + H^+
ATP synthesis
$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$
Via potentially toxic intermediates = *Reactive Oxygen Species* (ROS)

Dioxygen & ROS



Dioxygen is a biradical (S = 1)

O_2 (C = 1 M) + e^- → $O_2^{\bullet-}$, E^0 = -0.16 V

$O_2^{\bullet-}$ + $2H^+$ + e^- → H_2O_2, E^0 = 0.89 V

$2O_2^{\bullet-}$ + $2H^+$ → H_2O_2 + O_2, *fast*

H_2O_2 + H^+ + e^- → H_2O + $HO^\bullet$, E^0 = 0.38 V

$HO^\bullet$ + H^+ + e^- → H_2O, E^0 = 2.31 V

The most potent chemical oxidant (after $F^\bullet$)

ROS & antimicrobial defense (*inflammation*)

Enzymes in leucocytes produce ROS
for oxidative degradation of viruses, bacteria etc…

NADPH oxidase, a sophisticated metalloprotein complex

$NADPH + 2O_2 \rightarrow NADP^+ + 2O_2^{\bullet -} + H^+$

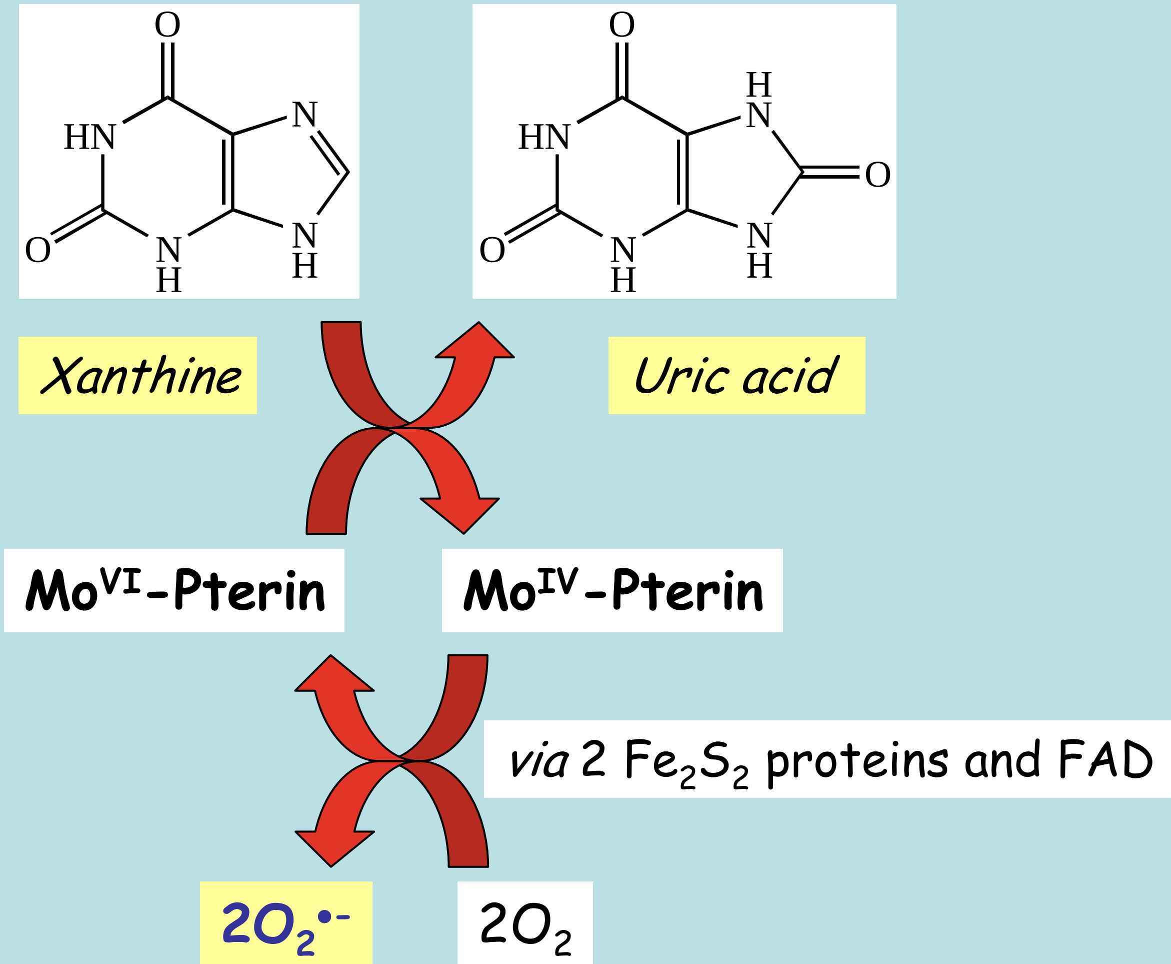
NO synthase $\Rightarrow$ **NO** $\rightarrow$ $O{=}N{-}O{-}O^-$ *peroxynitrite* $\xrightarrow{H^+,\ -NO_2}$ $HO^\bullet$

Myeloperoxidase (heme enzyme)

$Cl^- + H_2O_2 \rightarrow ClO^- + H_2O$

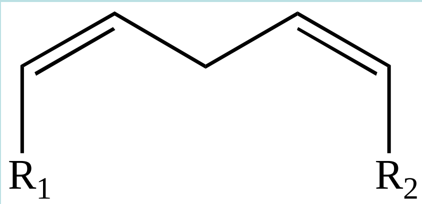
$ClO^- + H_2O_2 \rightarrow Cl^- + H_2O + {}^1O_2$

ROS & purine metabolism: xanthine oxidase (liver)



ROS & PUFA oxidation

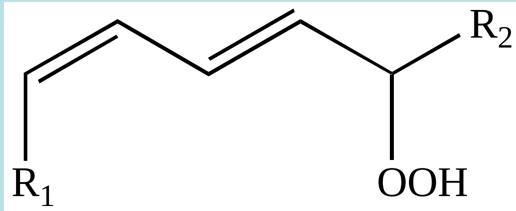
PUFAs (free form, in TAG, phospholipids, cholesteryl esters)
are among the most dioxygen-sensitive biomolecules



Polyunsaturated fatty acid (PUFA) = LH

O_2, initiator

Via **LOO•**



PUFA hydroperoxide = LOOH

<u>Initiator</u> = transition metal ions & metalloenzymes (*e.g.*, lipoxygenases), radiations

LOOH, LOO• → **LO•** → Electrophilic aldehydes & epoxides

→ *Chemical modifications of proteins & DNA*

PUFA oxidation takes place:

In plants

In response to various stresses (drought, chill, heat, salts, heavy metal ions), upon aging

In foods

During industrial treatments (heating, irradiation), storage, cooking

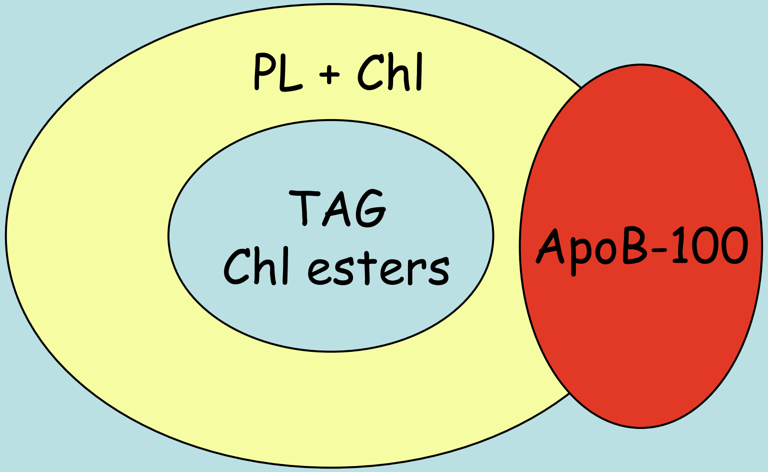
In humans

In the GI tract: oxidation of dietary lipids initiated by dietary pro-oxidant species such as heme iron

In cell membranes & internalized low-density lipoproteins:
- Following inflammation & cell injury: microbial infection, CVD, Alzheimer
- In various pathological conditions: obesity, hypertension, diabete…
- As a consequence of intoxication, smoking, aging & even physical exercise…

Low-Density Lipoproteins (LDL)

Transport of cholesterol in blood from liver to tissues
Density (vs. water): 1,006 - 1,063
Diameter: 15 - 25 nm
Composition: lipids = 78 %, protein (ApoB-100) = 22 %



TAG: triacylglycrols
Chl: cholesterol
PL: phospholipids

Minimally modified LDL (in artery wall)
oxidized lipids (dietary origin?), intact apoB

ROS-producing enzymes

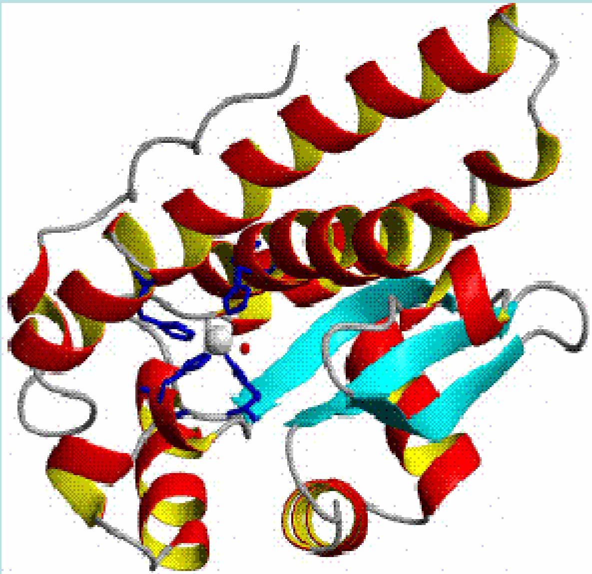
Highly oxidized LDL (ox-LDL)
Extensive lipid oxidation
modified apoB (Lys - lipid aldehyde linkages)

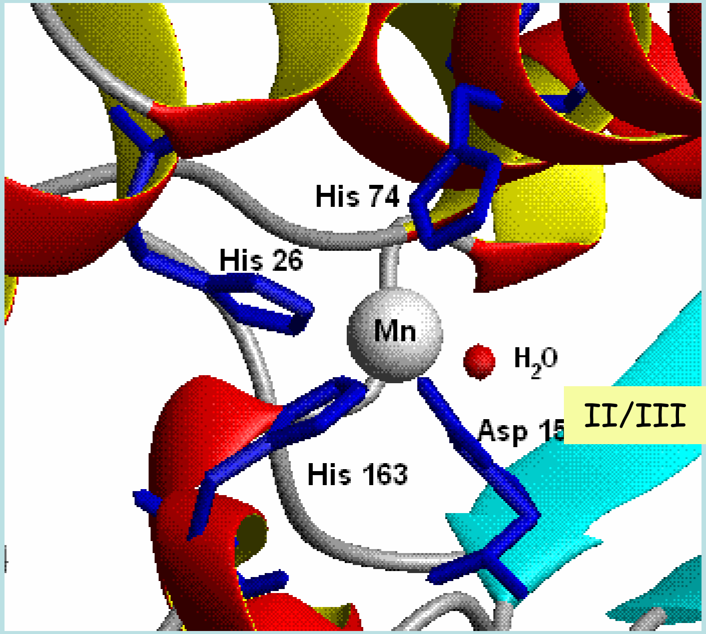
Accumulation in leucocytes (macrophages)

Atherosclerotic plaque
Cardiovascular diseases

Fighting ROS in excess: superoxide dismutase (SOD)

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





Mn human mitochondrial (Mn-SOD): $k_{cat} \approx 10^8\ M^{-1}\ s^{-1}$

CuZn-SOD (cytosol): $k_{cat} \approx 10^9\ M^{-1}\ s^{-1}$ (diffusion-controlled process)

Catalase (heme enzyme): $2H_2O_2 \rightarrow 2H_2O + O_2$

Naturel defense against the toxicity of lipid hydroperoxides: the glutathione peroxidases (GPx)

Isoforms GPx 1-4 are highly expressed in the gastro-intestinal mucosa

$ROOH + 2GSH \rightarrow ROH + GS\text{-}SG + H_2O$ (SelenoCys in the catalytic site)

GPx1 , GPx2: cytosolic enzymes, GPx1 also found in mitochondria, specificity for H_2O_2 and PUFA-OOH

GPx3: extracellular enzyme, reduction of H_2O_2, PUFA-OOH and PL-OOH

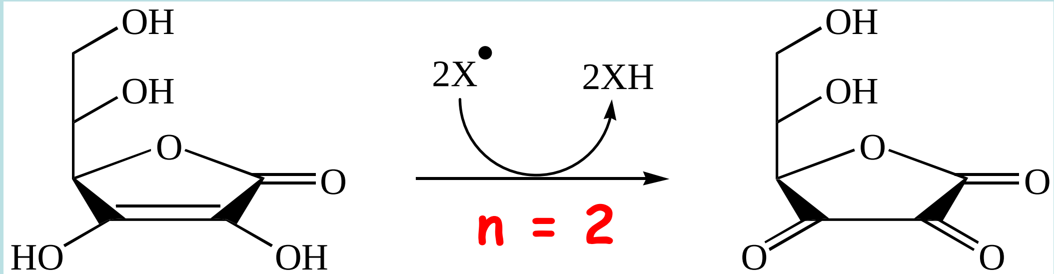
GPx4: cytosolic, mitochondrial and nuclear forms, reduction of PL-OOH, Chl-OOH and Thymine-OOH

F.-F. Chu *et al.*, *Free Rad. Biol. Med.*, 2004, *36*, 1481-1495

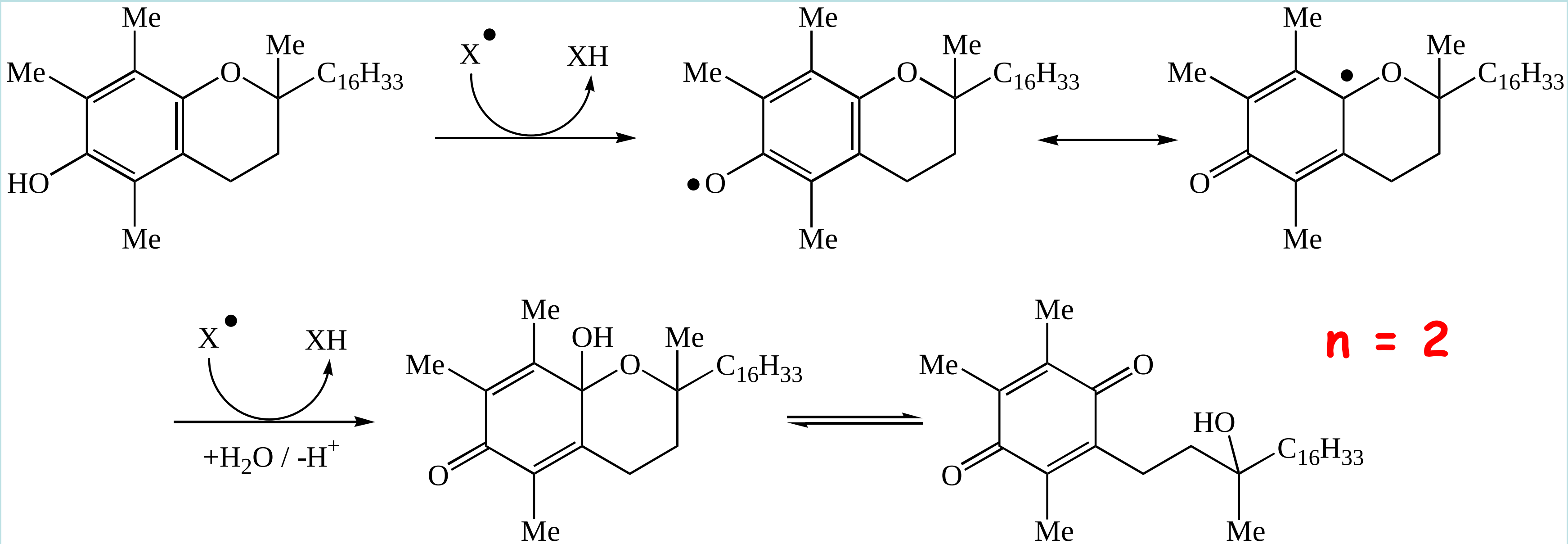
Important endogenous nonenzymatic antioxidants

n = number of one-electron oxidant (ROS) reduced by antioxidant molecule

L-Ascorbic acid
(vitamin C, *in blood*)



α-Tocopherol (vitamin E, *in blood lipoproteins, membranes*)



Glutathione (*in blood, cells*)

$2GSH + 2X^{\bullet} \rightarrow GS\text{-}SG + 2XH$ **n = 1**

Oxidative Stress = excessive ROS production overwhelming the capacity of antioxidant enzymes

Oxidative degradation of the host's biomolecules (lipids, proteins, DNA)

Chronic exposure to oxidative stress

- Genetic deficiency in antioxidant defenses
- Smoking, exposure to radiations and industrial pollutants
- Imbalanced diet (rich in fats and iron?) etc…

Increased risk to develop cardiovascular diseases, cancers, neurodegenerative diseases

Epidemiological studies suggest that diets rich in plant antioxidants could help

Dietary Polyphenols as Antioxidants

Most abundant dietary antioxidants = Polyphenols

UK-focused database

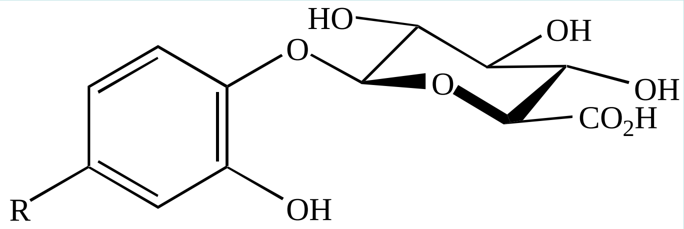
Clifford, M. & Brown, J.E. (2006)
In *Flavonoids: Chemistry, Biochemistry & Applications* (eds O. Andersen & K. Markham) pp. 319-370, CRC Press, Boca Raton.

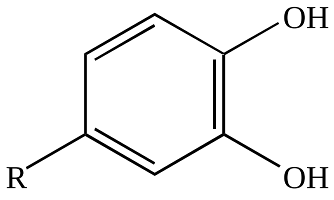
Mean dietary polyphenol intake = 450 – 600 mg / day (calculated as aglycones), i.e. millimole range

Main polyphenol providers = coffee and black tea

Bioavailability of Dietary Polyphenols

Extensive conjugation in the intestinal and hepatic cells

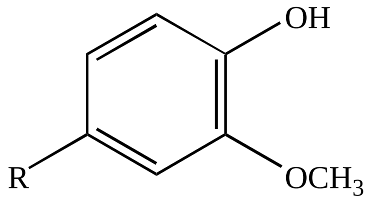




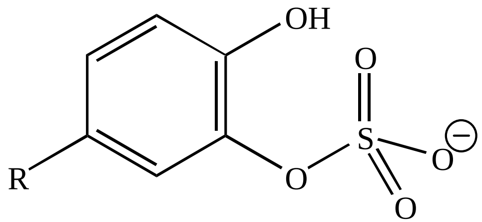
Native form
(potent antioxidant)

Conjugation

enzymes



Conjugated form
(weak antioxidant)



Plasma concentrations < 1 µM << glutathione or ascorbate concentrations

After intestinal absorption, the potential health effects of polyphenols are likely *not* dominated by their antioxidant activity

Potential Health Value of Polyphenols
(*preventive nutrition*)

Mechanisms of Action

Antioxidant
- ROS scavenging
- Binding of transition metal ions
- Regeneration of endogenous antioxidants

→ In food & gastric lipid emulsions

Polyphenol-protein interactions
- Inhibition of kinases
- Regulation of gene expression

etc…

→ Major cell effects
Relevance in the prevention of degenerative diseases

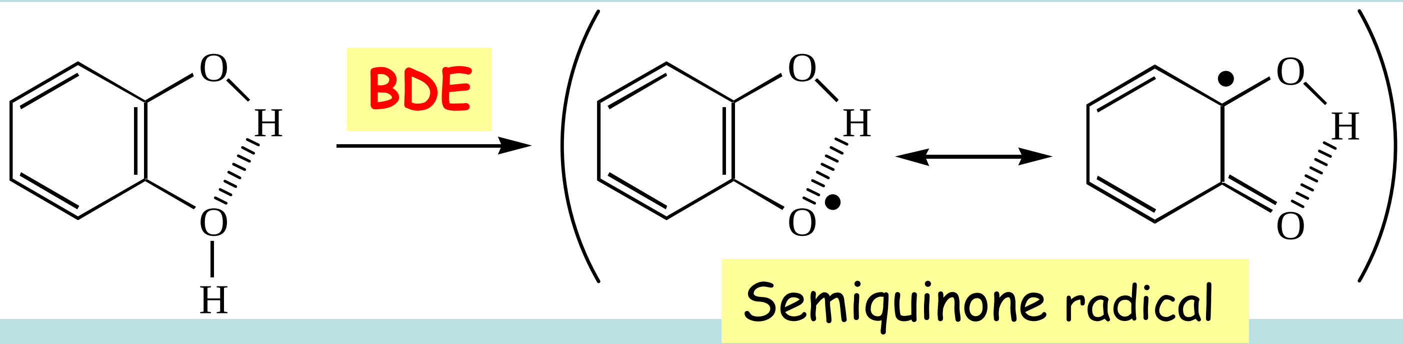
Both
- Inhibition of ROS-producing enzymes
- Cell-directed antioxidant activity (after deconjugation)
- Up-regulation of the expression of antioxidant enzymes

Polyphenols: chemical basis of their antioxidant activity

Structural unit critical to the antioxidant efficiency: the catechol (1,2-dihydroxybenzene) nucleus

compound	BDE(O-H) a) kcal mol^{-1}
phenol	88.0
1,2-dihydroxy benzene (*catechol*)	**81.2**
1,3-dihydroxy benzene	88 - 91

a) Bond Dissociation Energy, Gas phase, standard conditions, 298 K

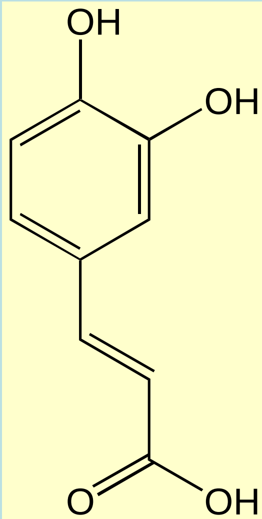


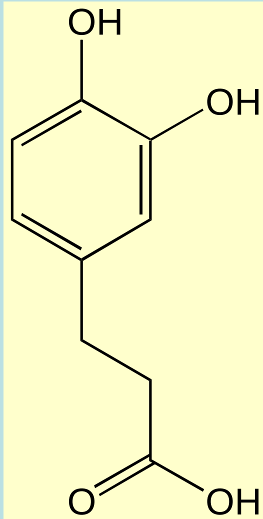
A simple assessment of the intrinsic antioxidant efficiency: the reduction of the DPPH radical

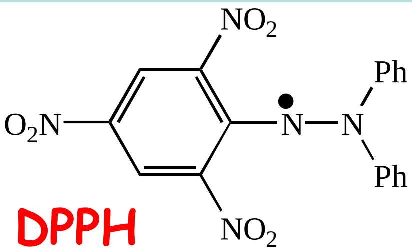
$$ArOH + DPPH^{\bullet} \xrightarrow{k_1} ArO^{\bullet} + DPPH\text{-}H$$

$$ArOH + \mathbf{n}DPPH^{\bullet} \rightarrow P_{Ox} + \mathbf{n}DPPH\text{-}H$$

P_{Ox} : inert oxidation products





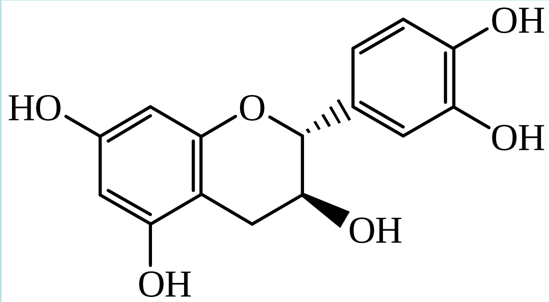


DPPH

	Caffeic Acid	Dihydrocaffeic Acid
k_1 (M^{-1} s^{-1})	1090	470
n	2.47	3.43

M. Roche, C. Dufour, N. Mora, O. Dangles
Org. Biomol. Chem., 2005, *3*, 423.

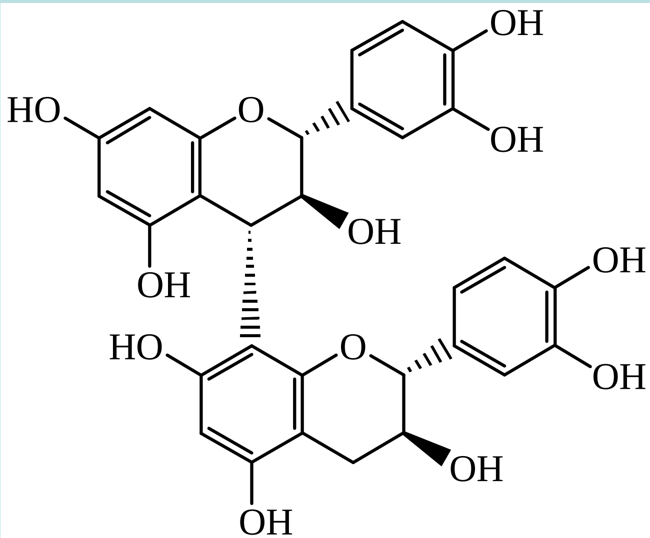
Which one is the best antioxidant?



Catechin

$k_1 = 1100\ M^{-1}\ s^{-1}$

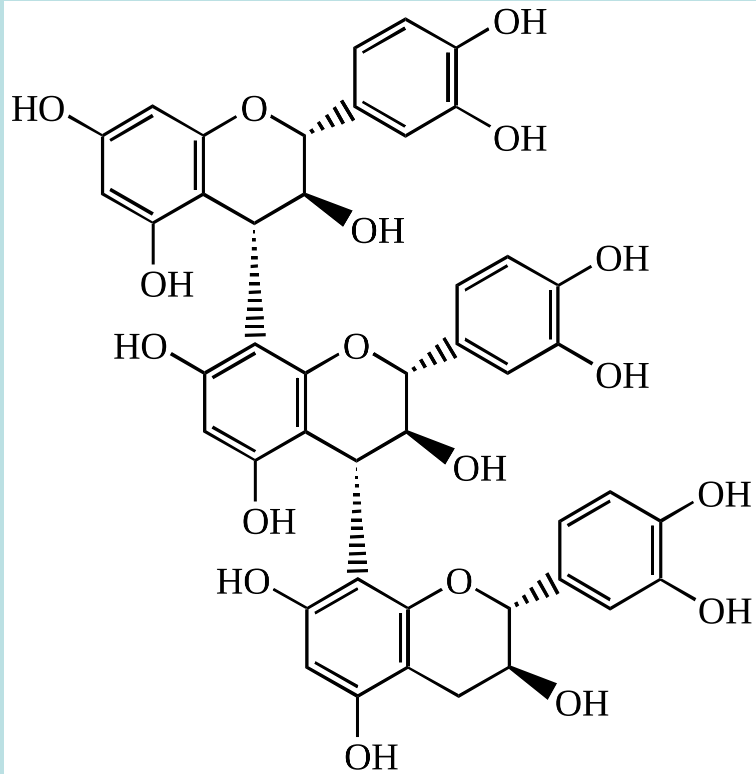
$n = 3.7$



Procyanidin B3

$k_1 = 1000\ \&\ 200\ M^{-1}\ s^{-1}$

$n = 6.5$ (*vs.* $2 \times 3.7 = 7.4$)



Procyanidin C2

(1 external more reactive sub-unit)

$k_1 = 1400\ \&\ 150\ M^{-1}\ s^{-1}$

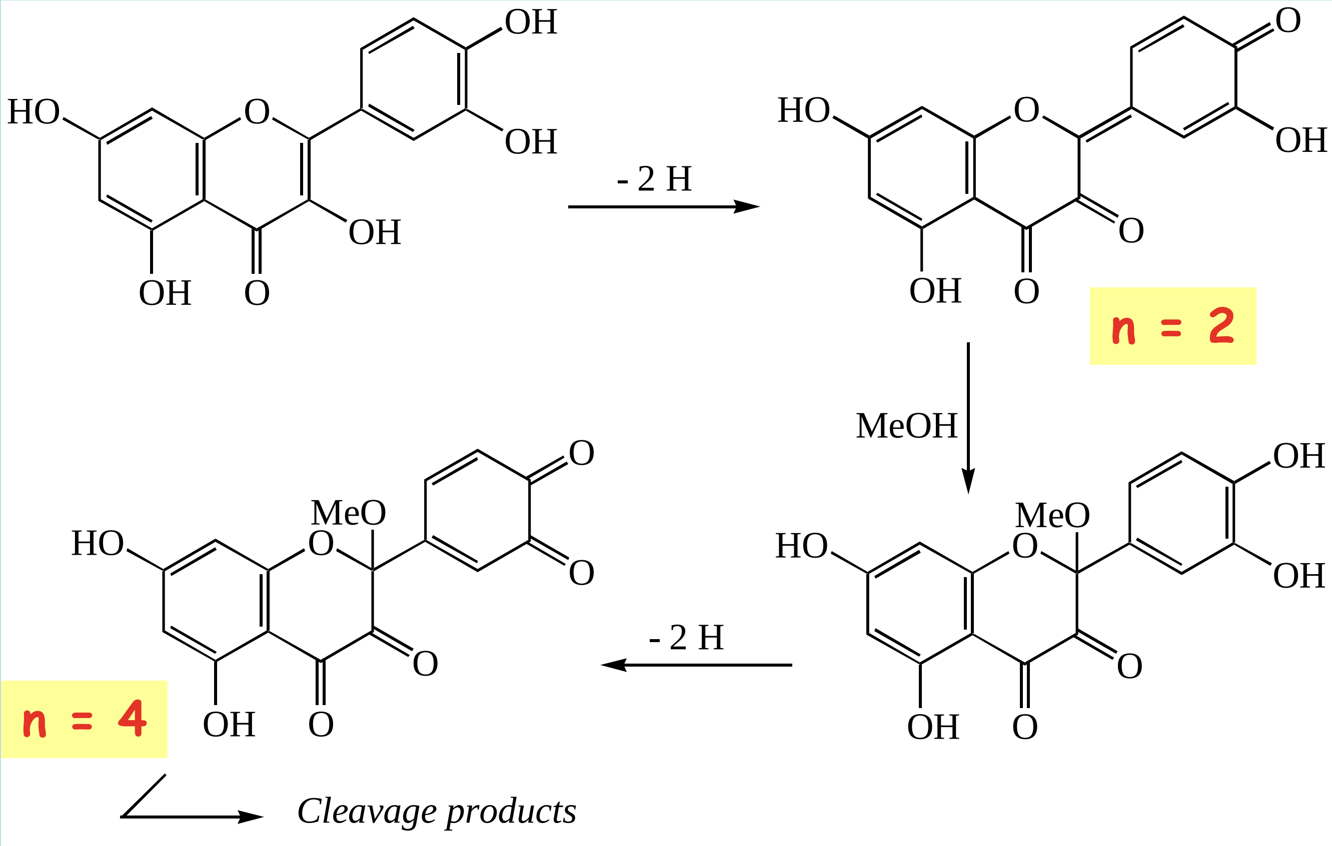
$n = 9.4$ (*vs.* $3 \times 3.7 = 11.1$)

P. Goupy, C. Dufour, M. Loonis, O. Dangles, *J. Agric. Food Chem.*, 2003, *51*, 615.

Quercetin

$k_1 = 2100\ M^{-1}\ s^{-1}$

$n = 4.9$



Inhibition of Xanthine Oxidase by Flavonoids

$$\text{Xanthine} + 2O_2 \rightarrow \text{Uric acid} + 2O_2^{\bullet -}$$

Flavonoid	IC_{50} / μM (*uric acid formation*)	IC_{50} / μM (*superoxide production*)
Quercetin	2.38 (± 0.13)	0.33 (± 0.03)
Catechin	> 100	0.48 (± 0.02)

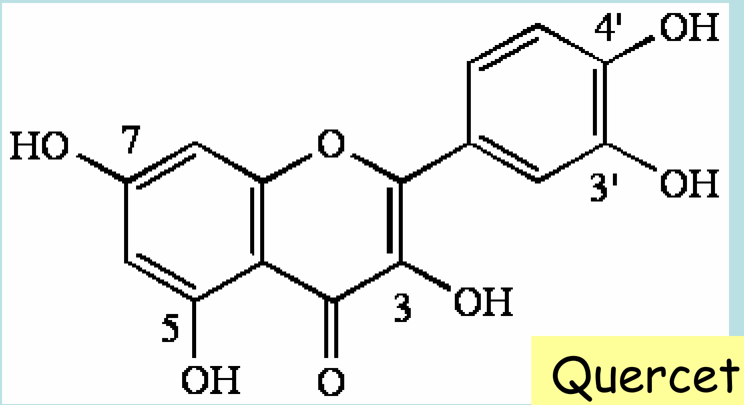
Quercetin (not catechin) binds the enzyme in competition with xanthine, thus quenching the superoxide source

Both are good superoxide scavengers

P. Cos *et al.*, *J. Nat. Prod.*, 1998, *61*, 71
C. M. Lin *et al.*, *BBRC*, 2002, *294*, 167

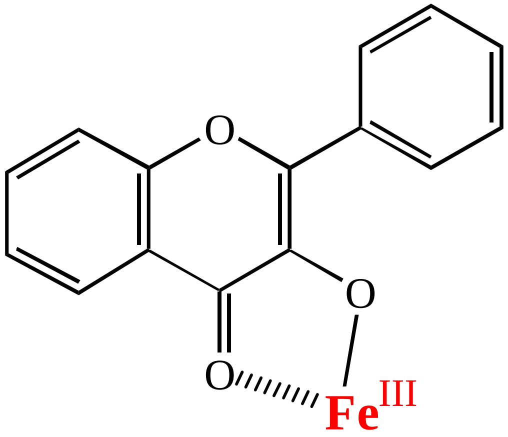
Inhibition of Xanthine Oxydase by Quercetin and its Glucuronides

Flavonol	Quercetin (Q)	Q3'-GlcU	Q4'-GlcU	Q7-GlcU	Q3-GlcU
K_d / µM	0.25	1.4	0.25	> 100	> 100



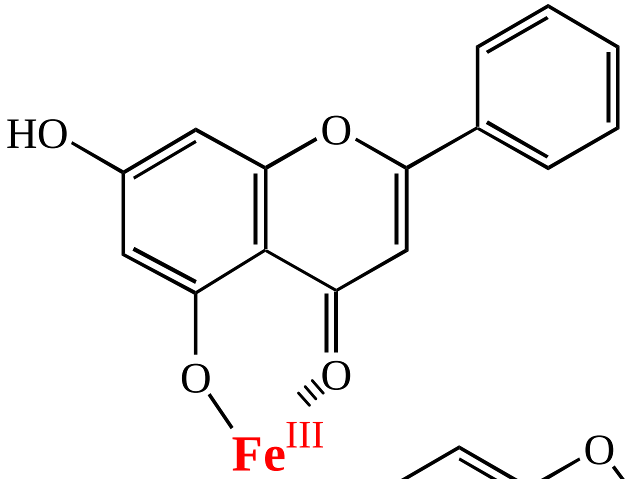
Quercetin

A.J. Day *et al.*, *Free Radic. Biol. Med.*, 2000, *29*, 1234.

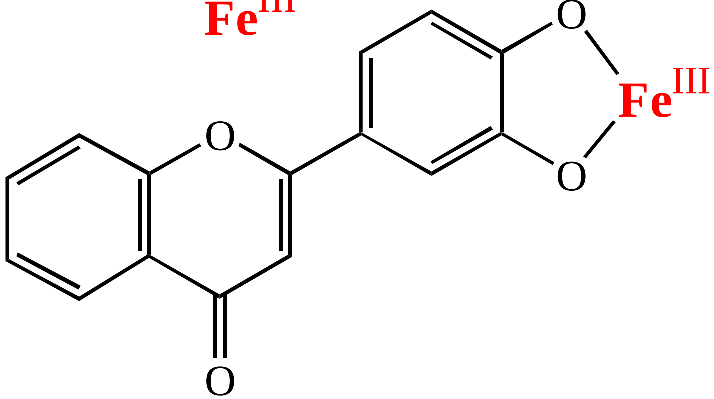


pK_a	10.0
$\log\beta$	13.3

Engelman *et al.*, JAFC, 2005, *53*, 2953



pK_a	7.9, 11.4
$\log\beta$	11.4



pK_a	8.4, 13.4
$\log\beta$	20.9

β: overall stability constant of the metal complex

Anti- vs. pro-oxidant effects

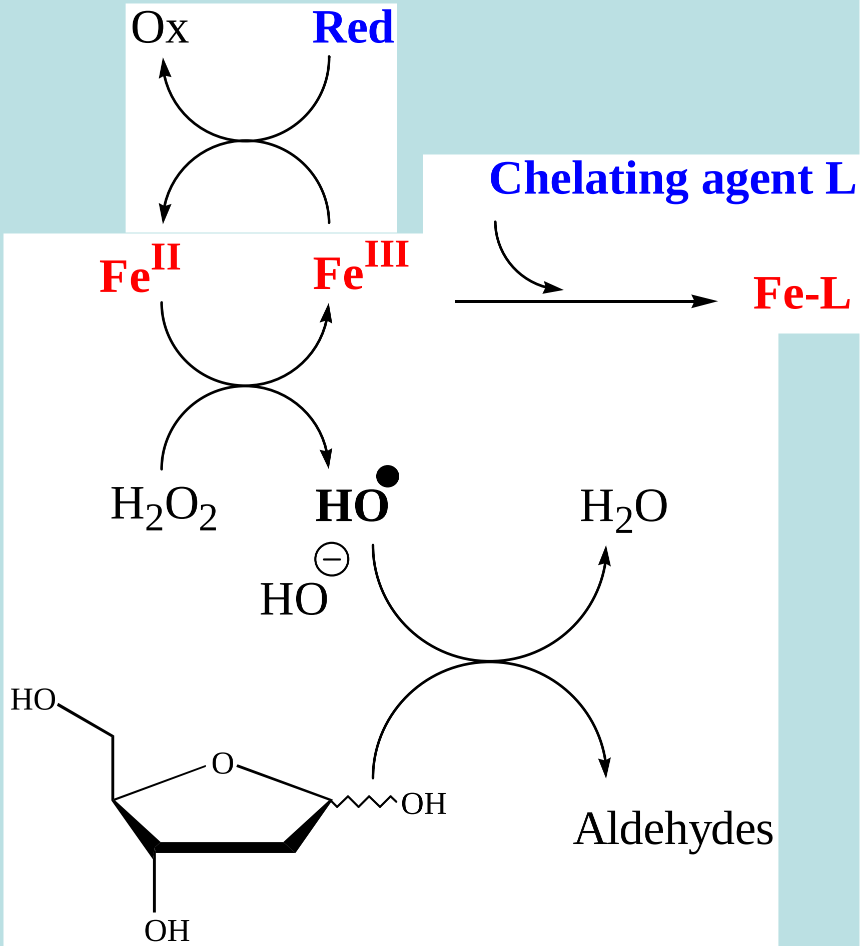
Deoxy-D-Ribose + H_2O_2 + $FeCl_3$
in phosphate buffer (pH 5.8 or 7.4), 37°C

I - Ascorbate + quercetin
Red = ascorbate, L = quercetin

Inert Fe-quercetin complex
Antioxidant activity

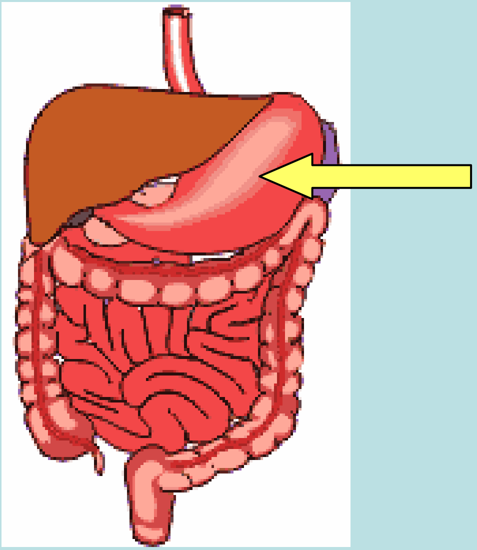
II - EDTA + quercetin
Red = quercetin, L = EDTA

Redox cycling of redox-active Fe^{II}-EDTA complex
Pro-oxydant activity



JF Moran *et al.*, *Free Radic. Biol. Med.*, 1997, *22*, 861

Chemical modelling of the antioxidant activity in the gastric compartment



Dietary polyunsaturated lipids (LH)
(triacylglycerols, phospholipids etc…)

Dietary Iron
Ex.: (met)myoglobin

O_2, H^+

LH + **O_2** → **LOOH** (hydroperoxides) → **L=O** (carbonyl compounds)
Via highly reactive lipid radicals **LOO•** & **LO•**

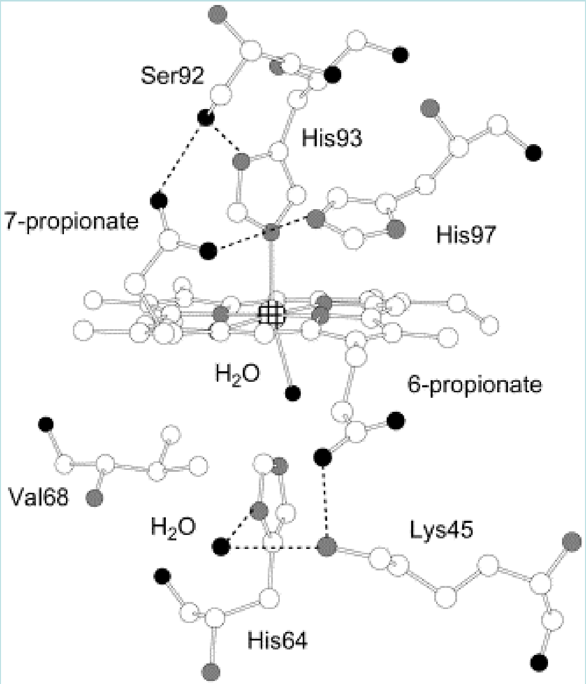
A possible antioxidant action for polyphenols regardless of their bioavailability

Activation of metmyoglobin by H_2O_2

$MbFe^{III}$
Metmyoglobin

$\xrightarrow{H_2O_2}$

$MbFe^{IV}=O$
Ferrylmyoglobin



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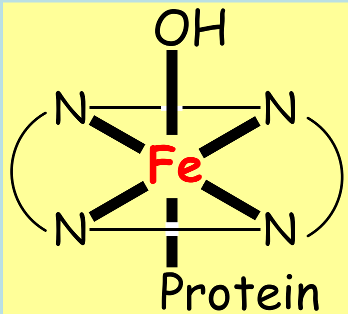
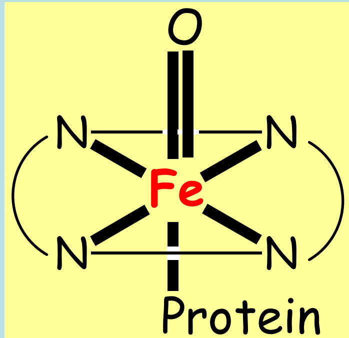
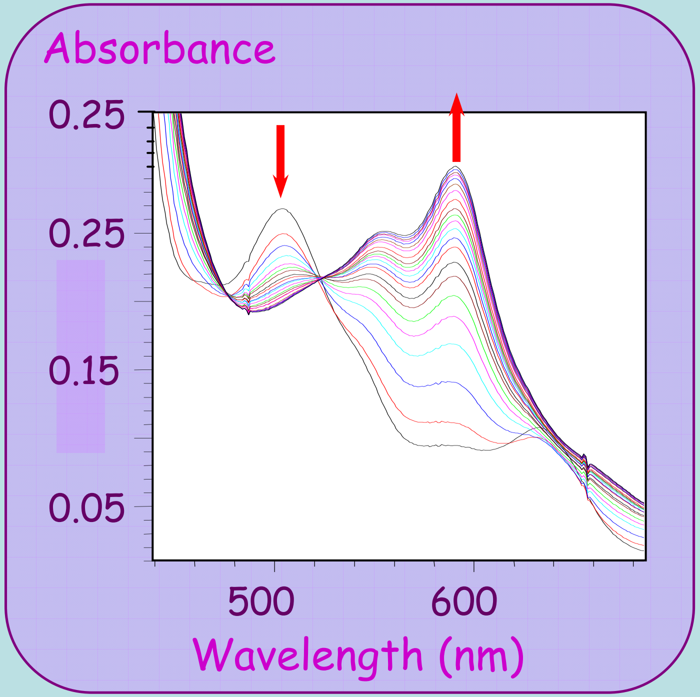




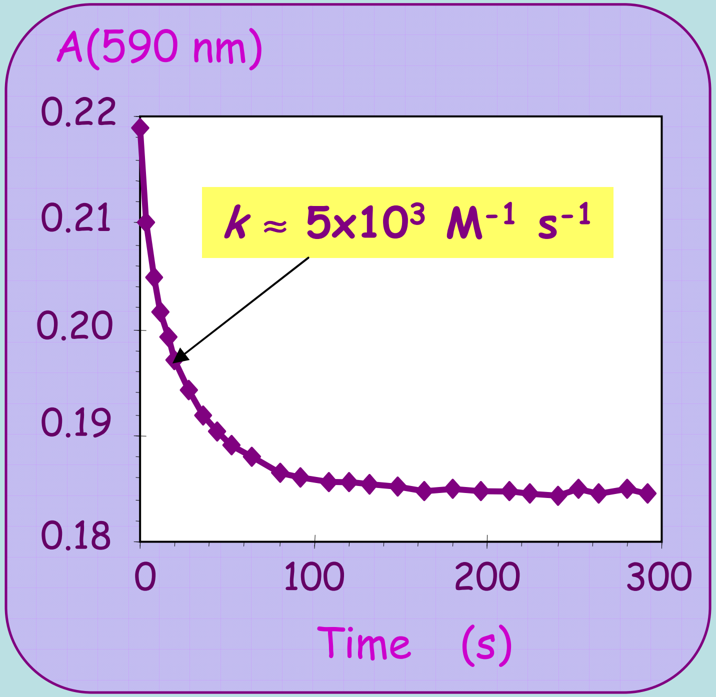
Hematin (cofactor) + Globin (protein) = Metmyoglobin

$MbFe^{III} + H_2O_2 \rightarrow MbFe^{IV}{=}O + H_2O$

Reduction of $MbFe^{IV}{=}O$ by quercetin (1 equiv.)



pH 5.8
37°C
2 mM
Tween 20

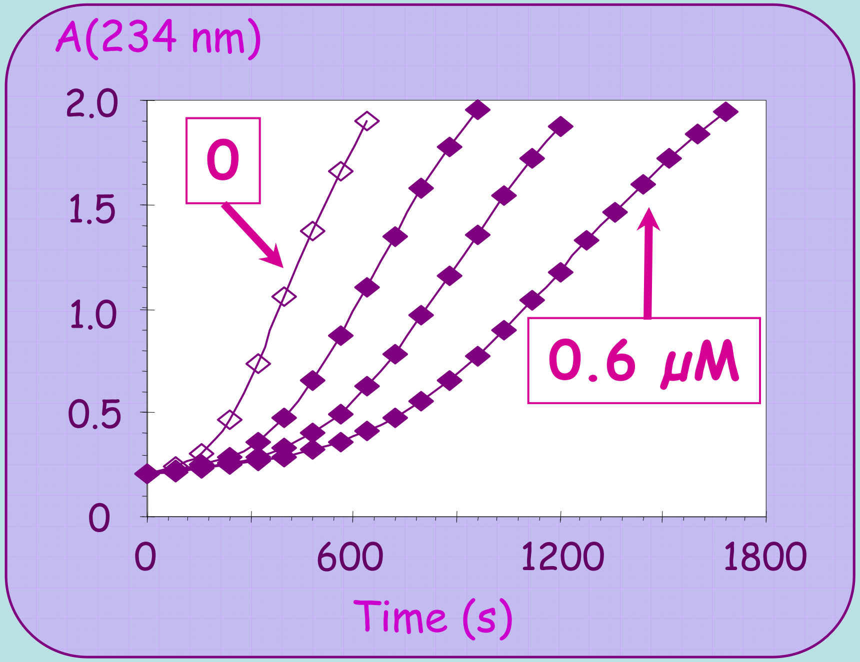


No reaction between $MbFe^{IV}{=}O$ and α-tocopherol

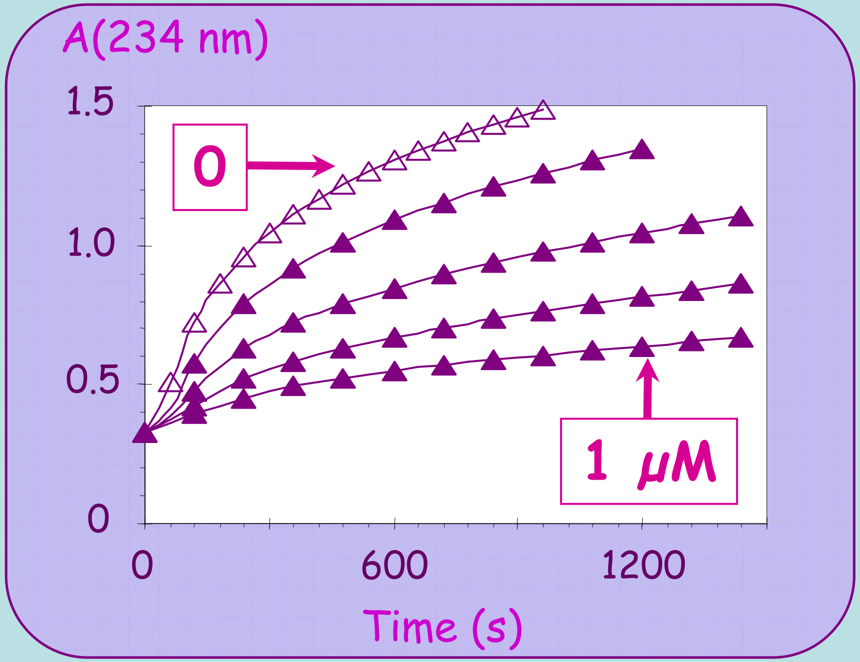
Inhibition of heme iron-induced lipid peroxidation

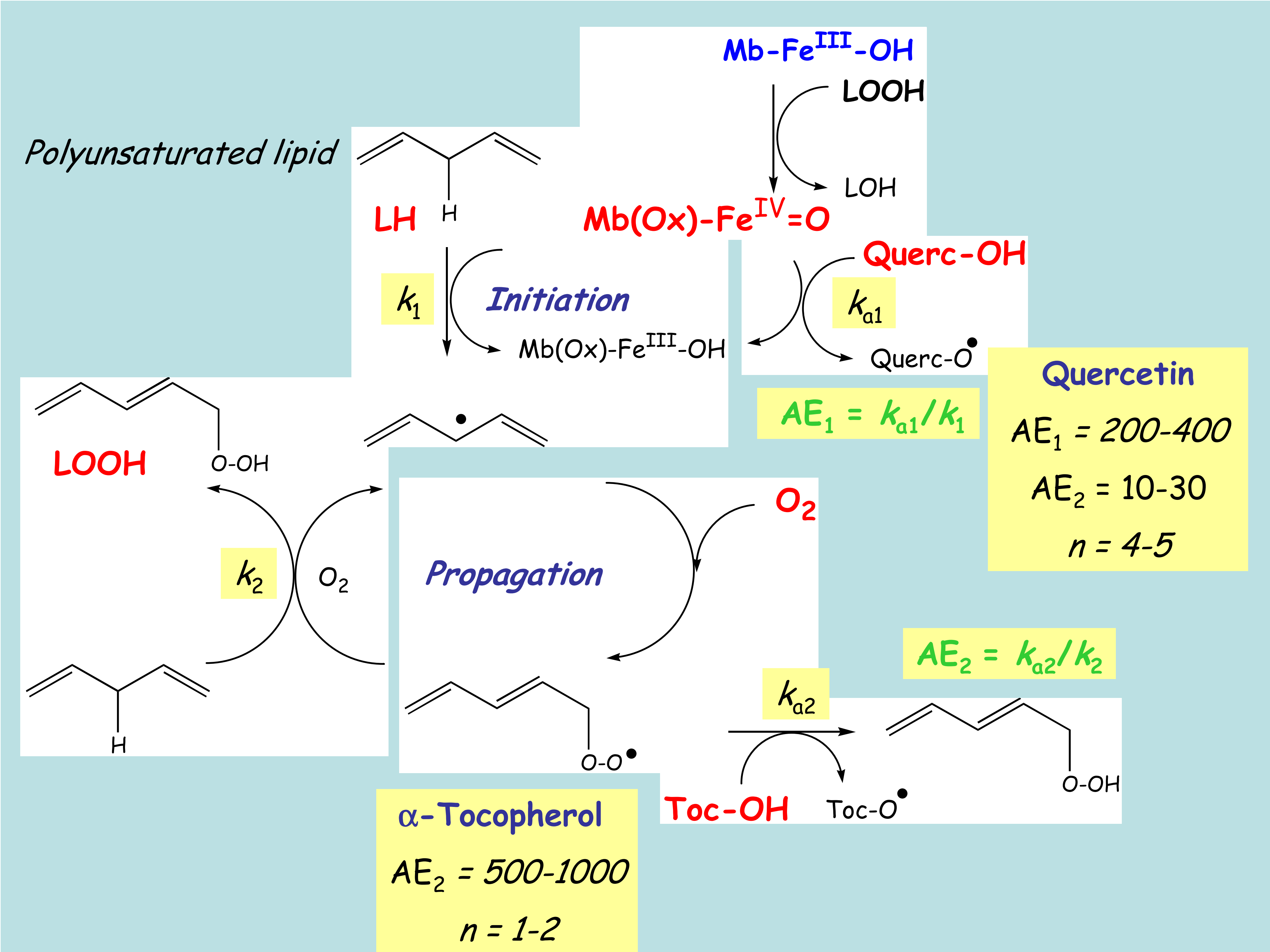
Linoleic acid (0.7 mM) + Quercetin (0-1 μM) + $MbFe^{III}$ (100 nM)
(37°C, pH 5.8, 2 mM Tween 20)

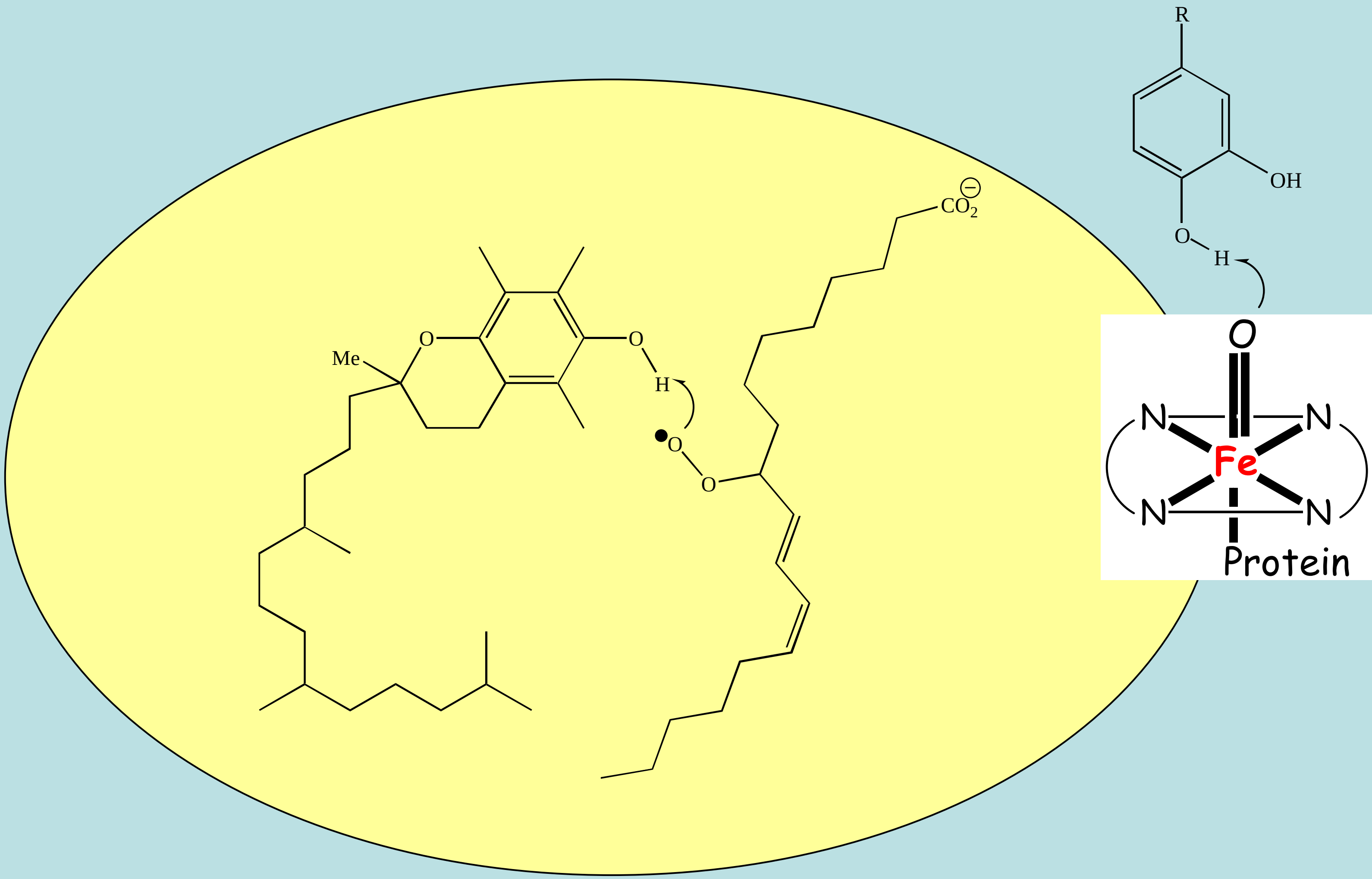
Metmyoglobin-induced peroxidation



Hematin-induced peroxidation



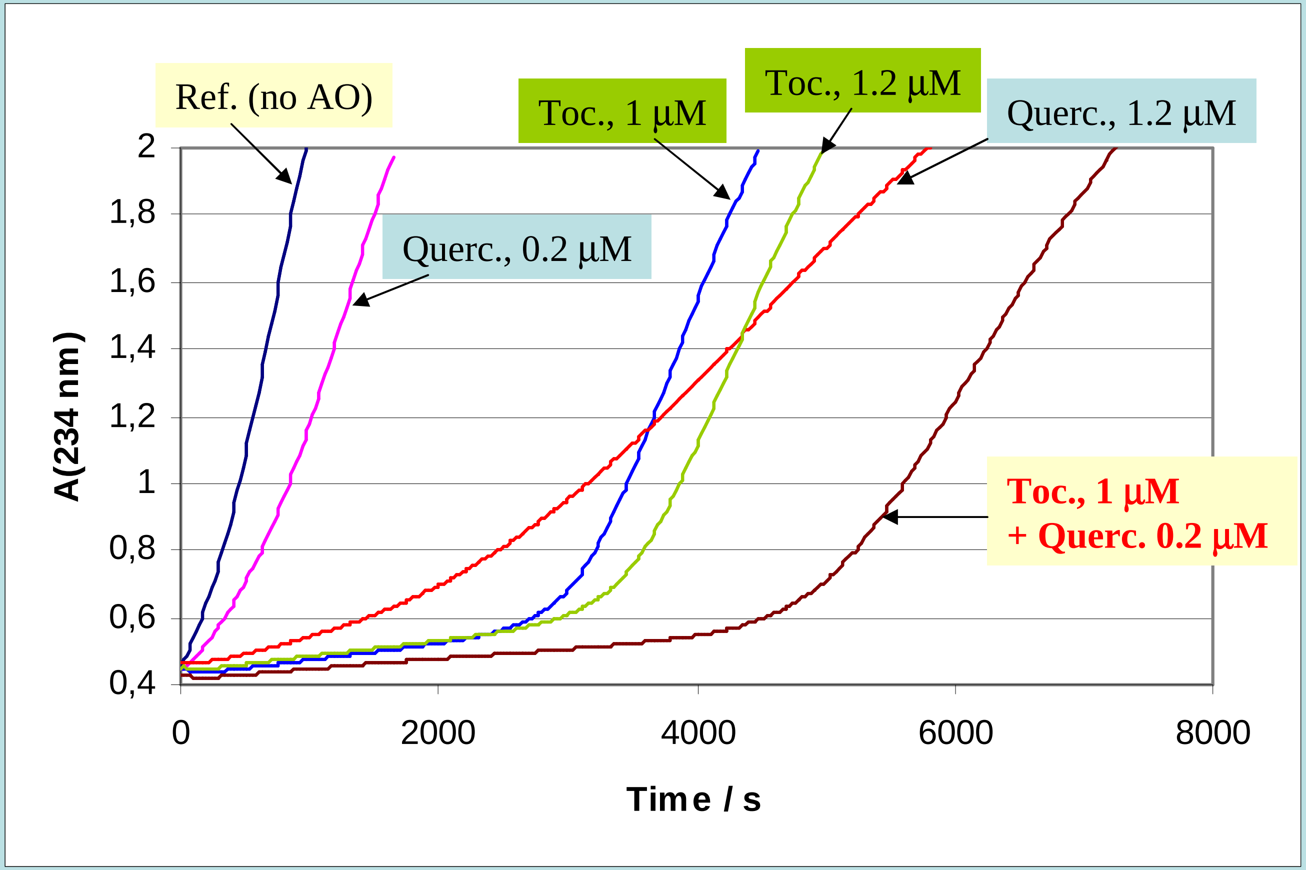

Polyunsaturated lipid
LH
H
Mb-Fe^III-OH
LOOH
LOH
Mb(Ox)-Fe^IV=O
k1
Initiation
Mb(Ox)-Fe^III-OH
Querc-OH
ka1
Querc-O•
AE1 = ka1/k1
Quercetin
AE1 = 200-400
AE2 = 10-30
n = 4-5
LOOH
O-OH
k2
O2
Propagation
O2
H
O-O•
ka2
AE2 = ka2/k2
Toc-OH
Toc-O•
O-OH
α-Tocopherol
AE2 = 500-1000
n = 1-2



Polyphenols: inhibition of initiation (*aqueous phase*)
Lipophilic antioxidants: inhibition of propagation (*lipid phase*)

E. Vulcain, P. Goupy, C. Caris-Veyrat, O. Dangles, *Free Rad. Res.*, 2005, *39*, 547-563.
P. Goupy, E. Vulcain, C. Caris-Veyrat, O. Dangles, *Free Radic. Biol. Med.*, 2007, *43*, 933-946.

Strong antioxidant synergism between quercetin and α-tocopherol



Linoleic acid (0.7 mM) + $MbFe^{III}$ (100 nM)
+ quercetin and/or α-tocopherol
37°C, pH 5.8, 2 mM Tween 20

IC_{50} = antioxidant conc. that doubles the period needed to accumulate a fixed LOOH conc.

Antioxidant	IC_{50} (μM)	Antioxidant	IC_{50} (μM)
α-tocopherol	0.28 (± 0.02)	**rutin**	0.63
quercetin	0.33 (± 0.01)	**caffeic acid**	0.49
β-carotene	1.52 (± 0.13)	**chlorogenic acid**	0.51
apigenin	3.33 (± 0.66)	**oenin**	0.27

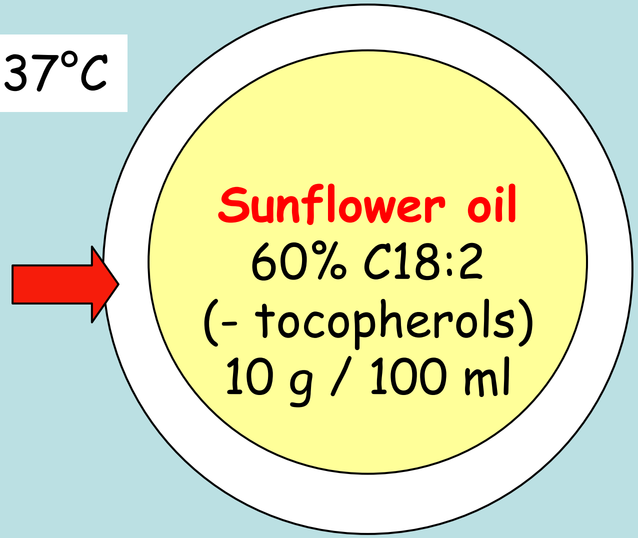
N.B.: rutin = quercetin-3-β-D-Glc-1,6-α-L-Rha, oenin = malvidin-3-β-D-Glc

Common dietary polyphenols are active in the sub-micromolar range

Refining the model by using oil-in-water emulsions

Acetate or phosphate buffer, pH 4-5.8, 37°C

Interface = bovin serum albumin (BSA)
or egg lecithin (phospholipids)

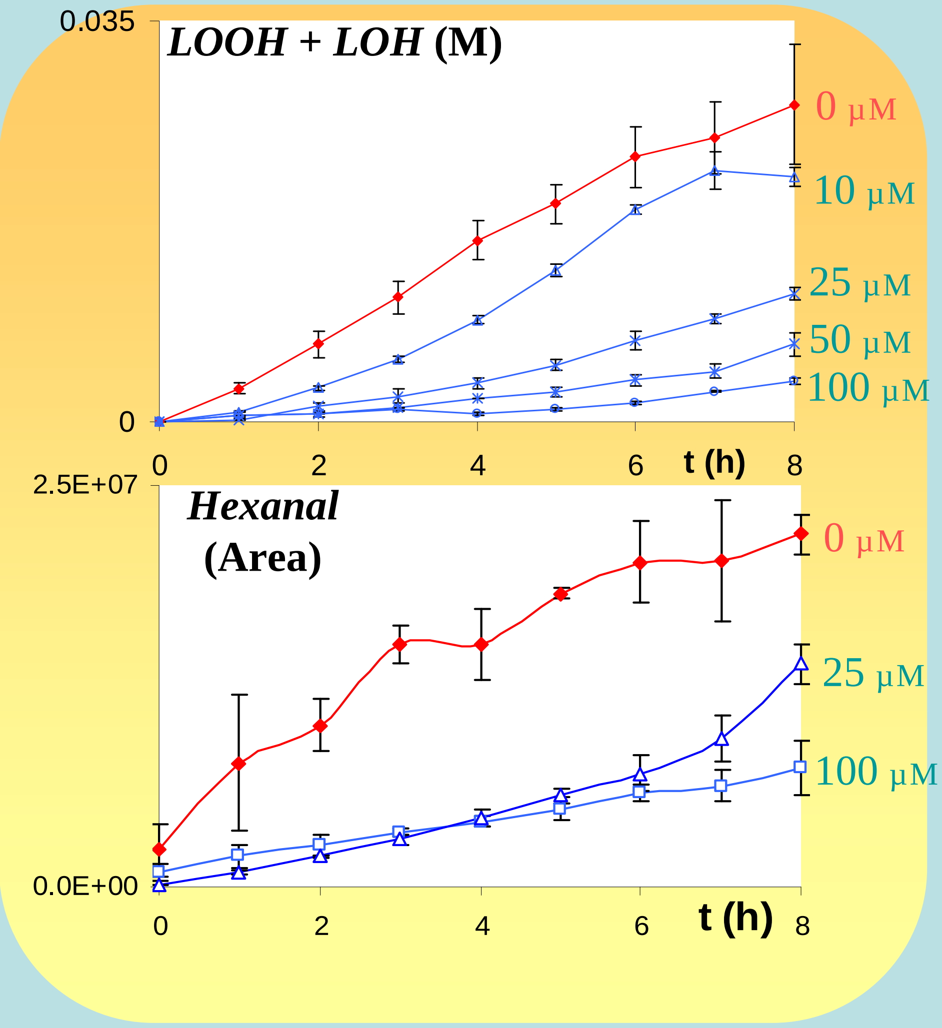


Metmyoglobin (20 μM)

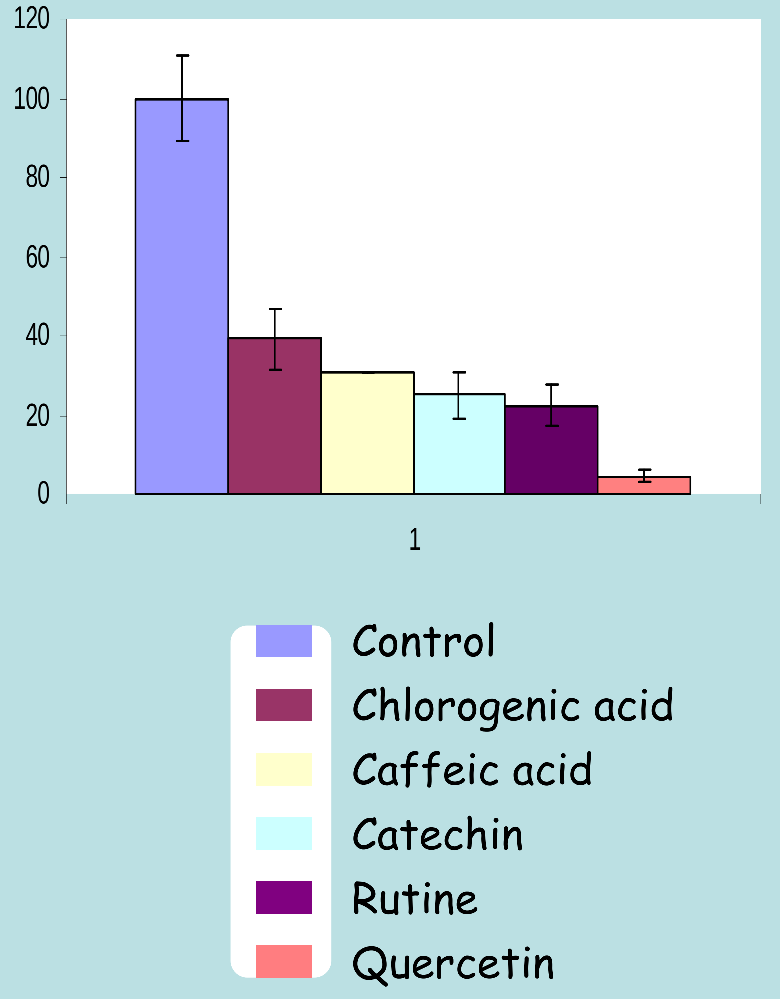
Inhibition of lipid oxidation by dietary antioxidants

Emulsifier = BSA

Antioxidant = quercetin, pH 5.8



t = 4 h, antioxidant conc. = 100 µM

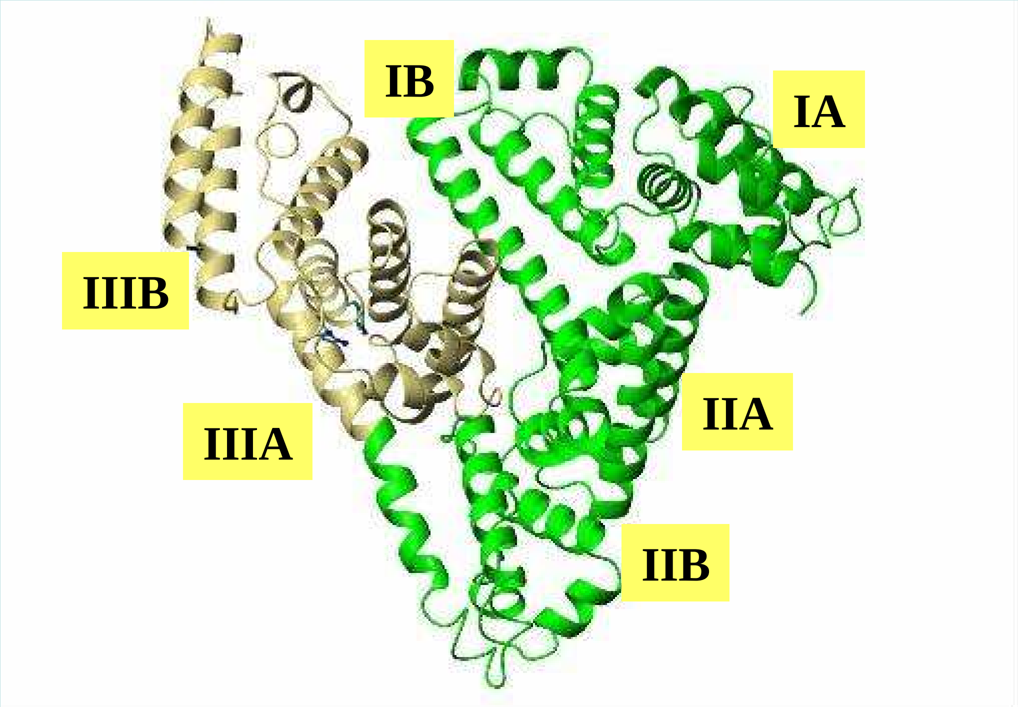


Does polyphenol-protein binding
(another typical property of polyphenols)
necessarily abolish
the antioxidant activity?

Human Serum Albumin

MM = 66500 g/mol

35-45 g/l of blood, *ca.* 0.6 mM

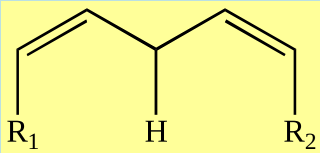


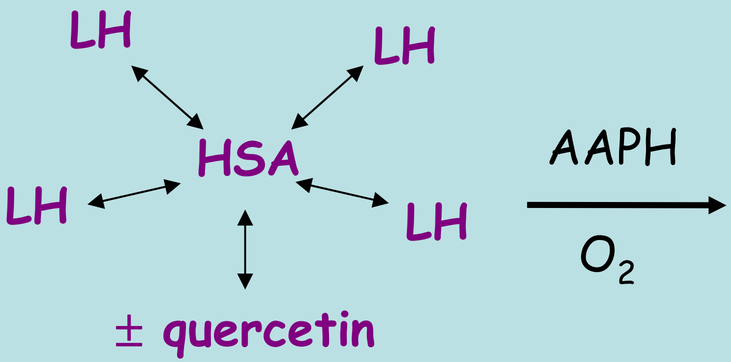
Key-role in the transport of:

- Free fatty acids
- A wide range of xenobiotics

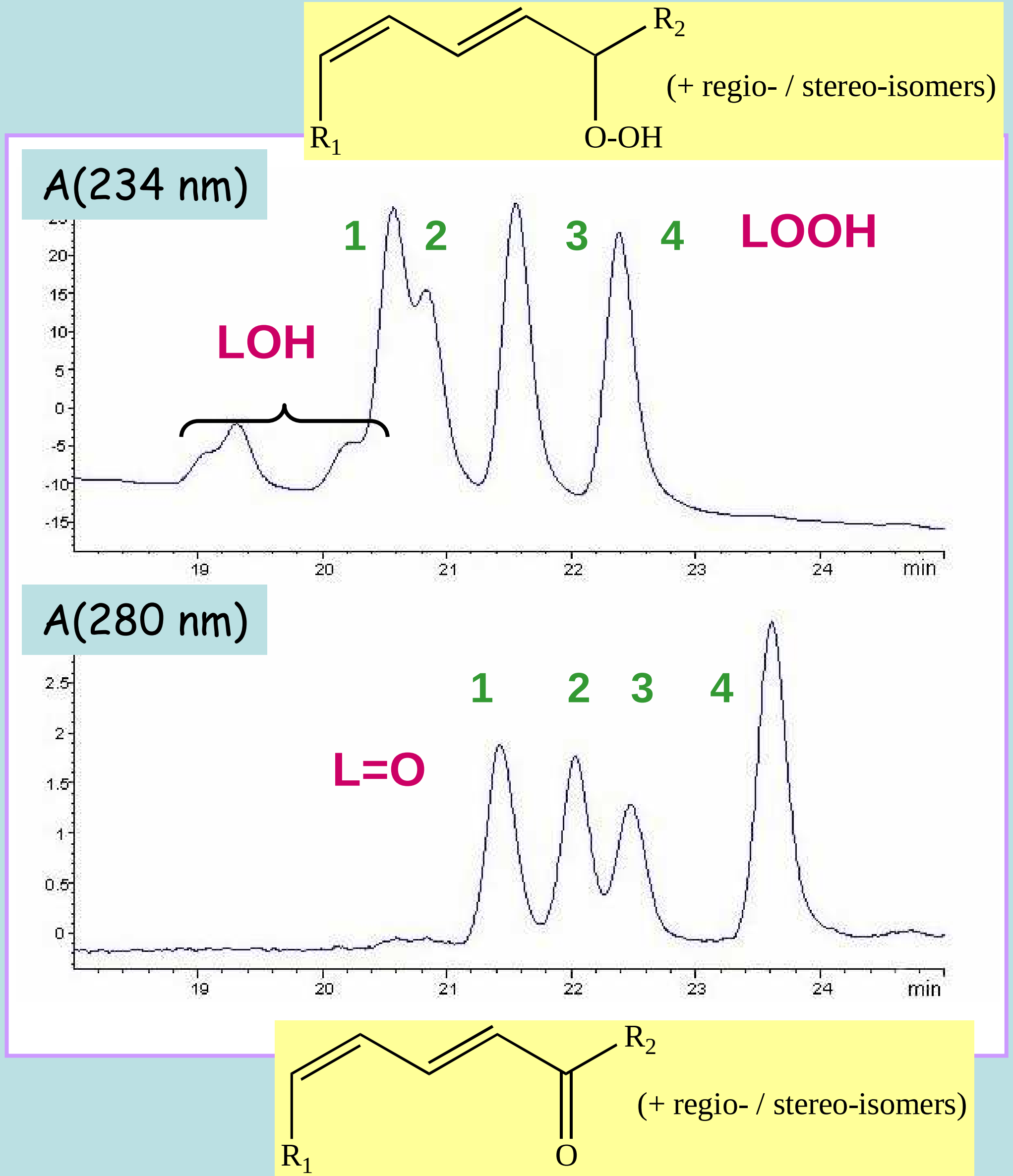
e.g., vitamins, metal ions, drugs, **dietary flavonoids**

Oxidation of HSA-bound linoleic acid and inhibition by quercetin



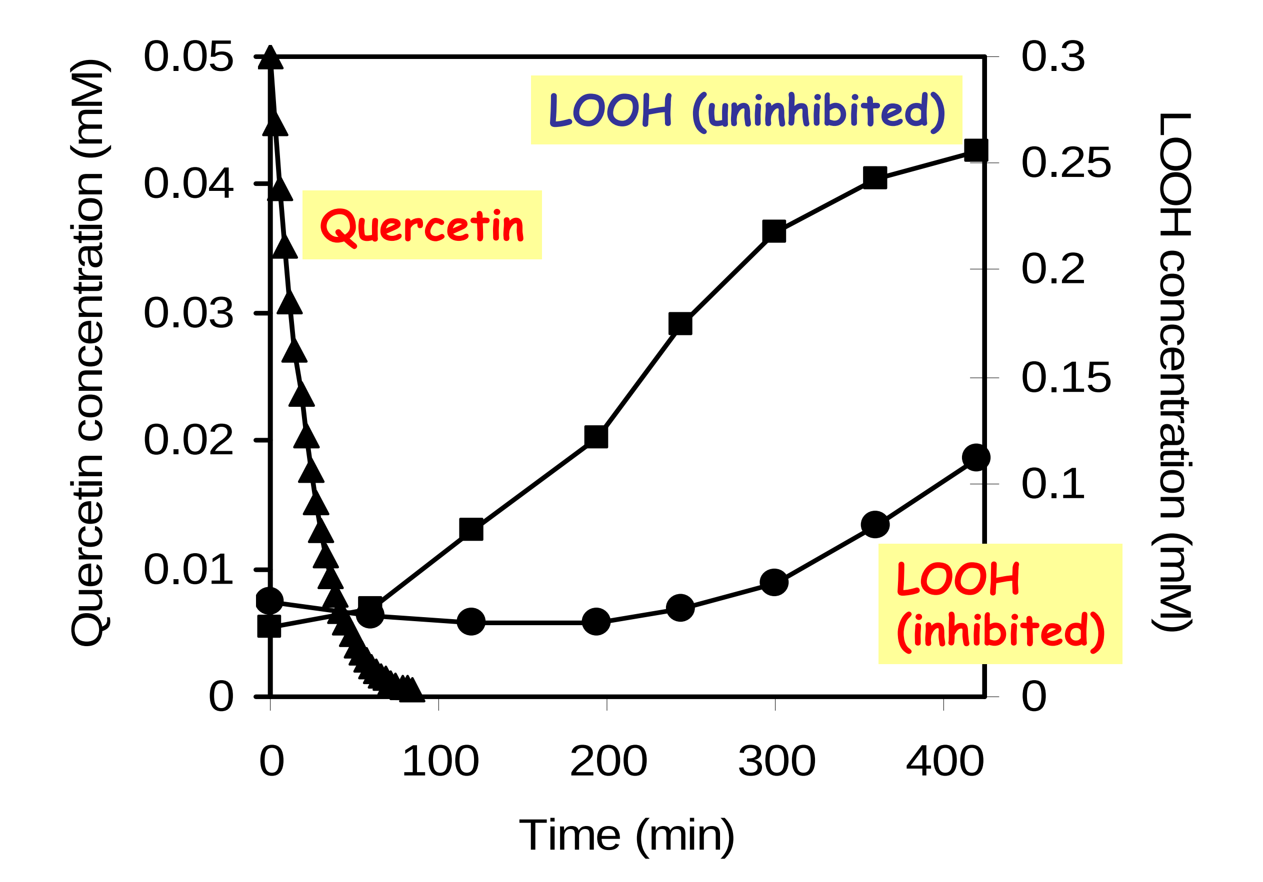


HSA: 0.5 mM
Linoleic acid (LH): 2 mM
Quercetin: 50 µM

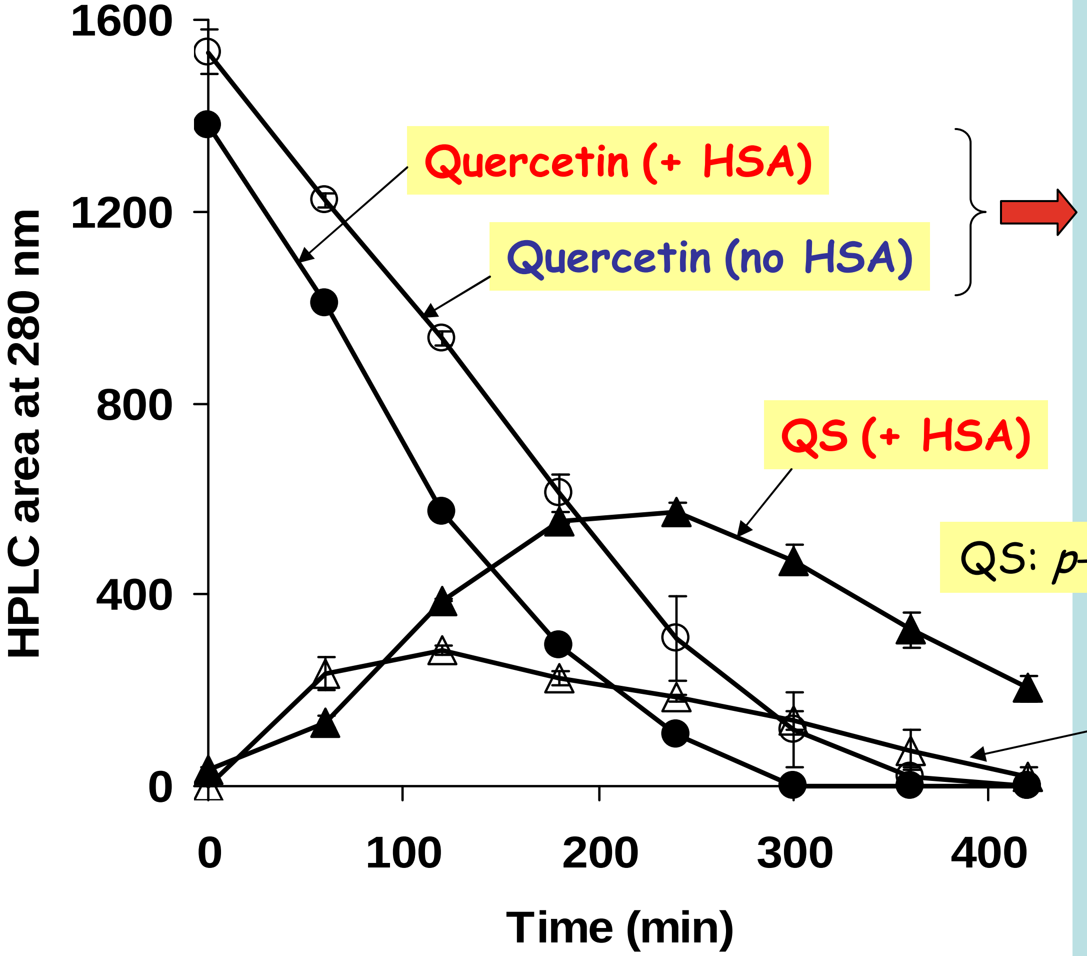


AAPH: hydrophilic diazo initiator

Oxidation of HSA-bound linoleic acid



Strong residual antioxidant activity of some quercetin oxidation products



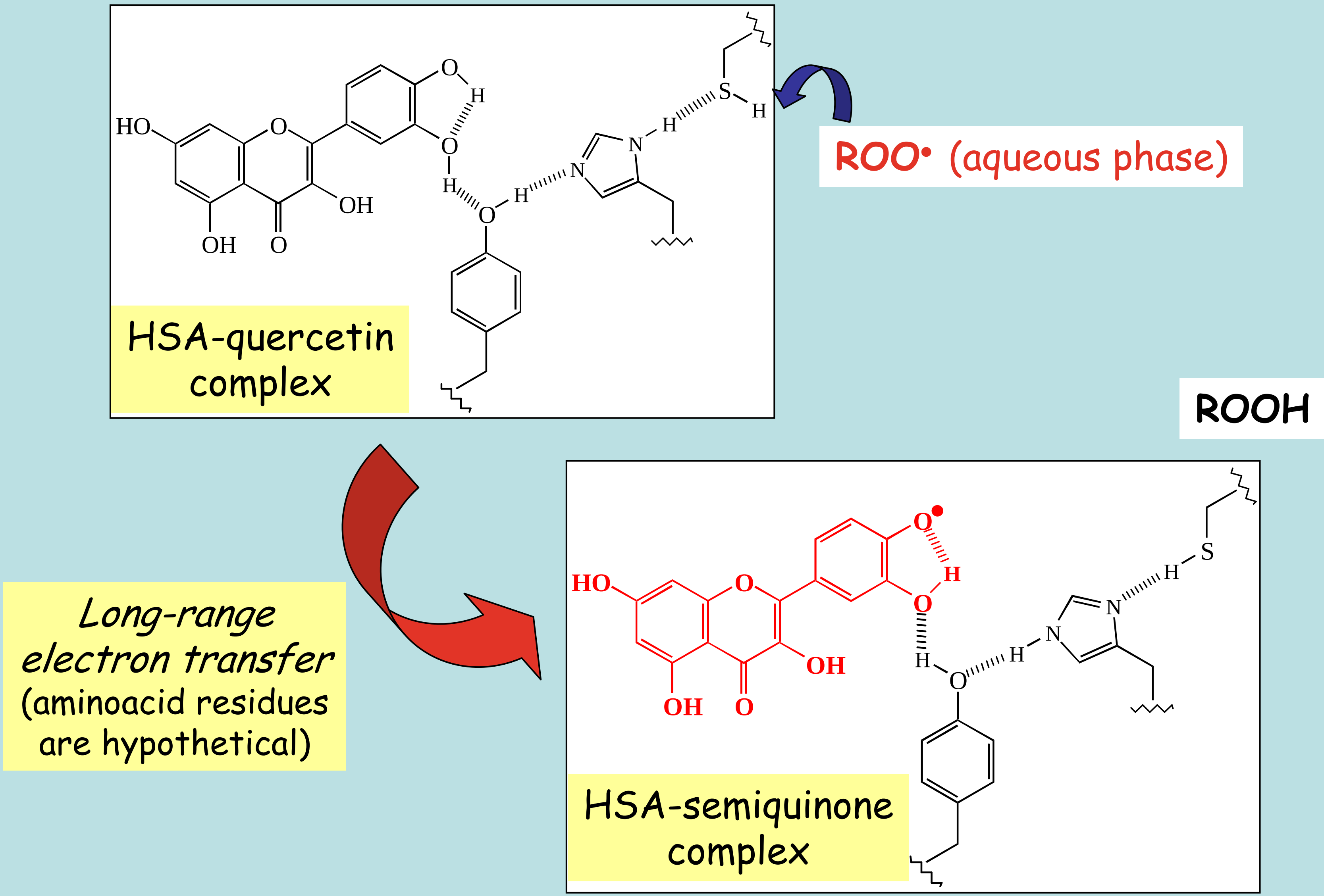
Binding to HSA does not protect quercetin from oxidation!!

QS: *p*-quinonemethide-water adduct

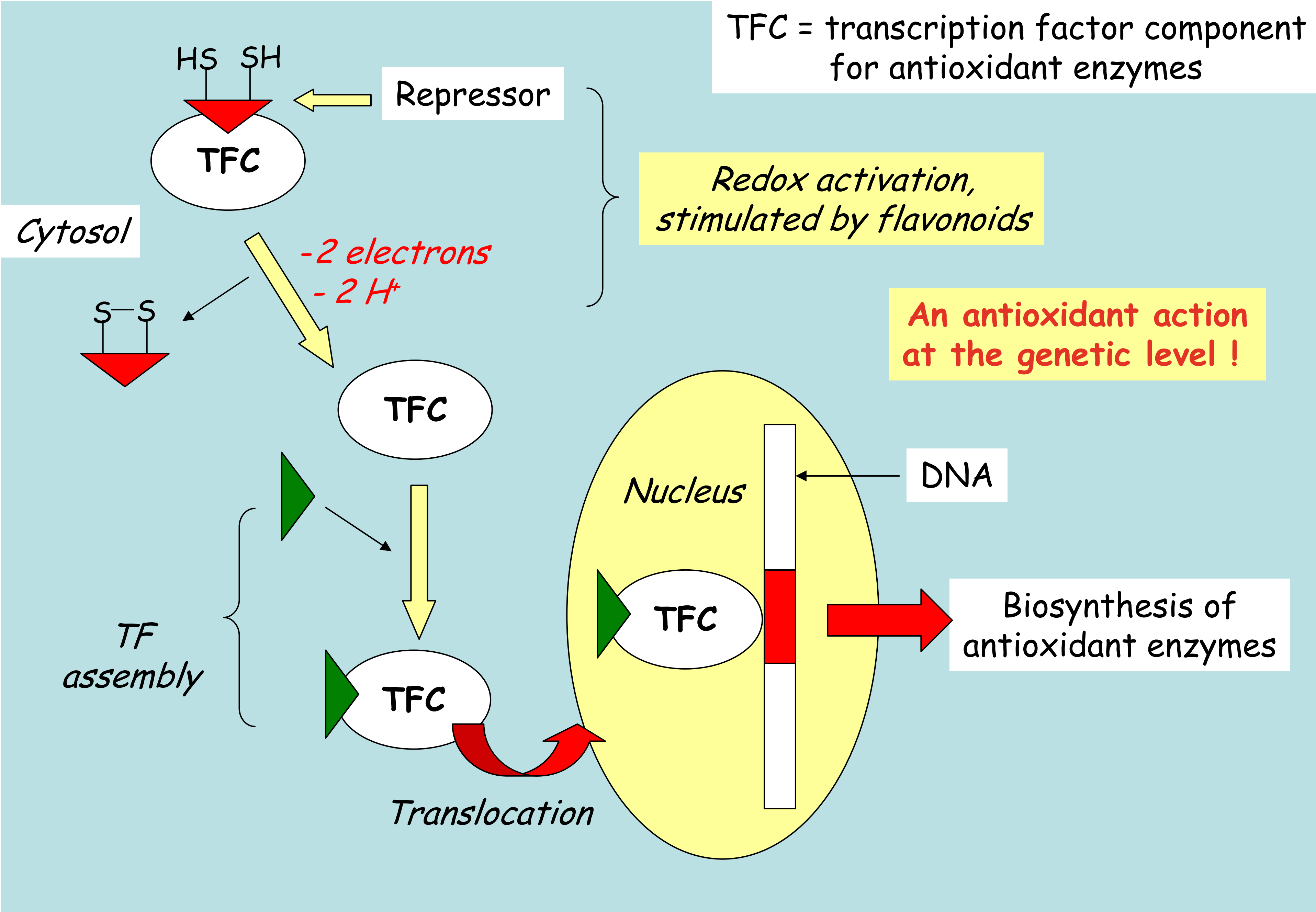
30 mM AAPH, 500 µM HSA, 400 µM quercetin

C. Dufour, M. Loonis, O. Dangles
Free Radic. Biol. Med., 2007, *43*, 241-252.

Binding to HSA maintains the antioxidant activity of quercetin and its susceptibility to oxidation



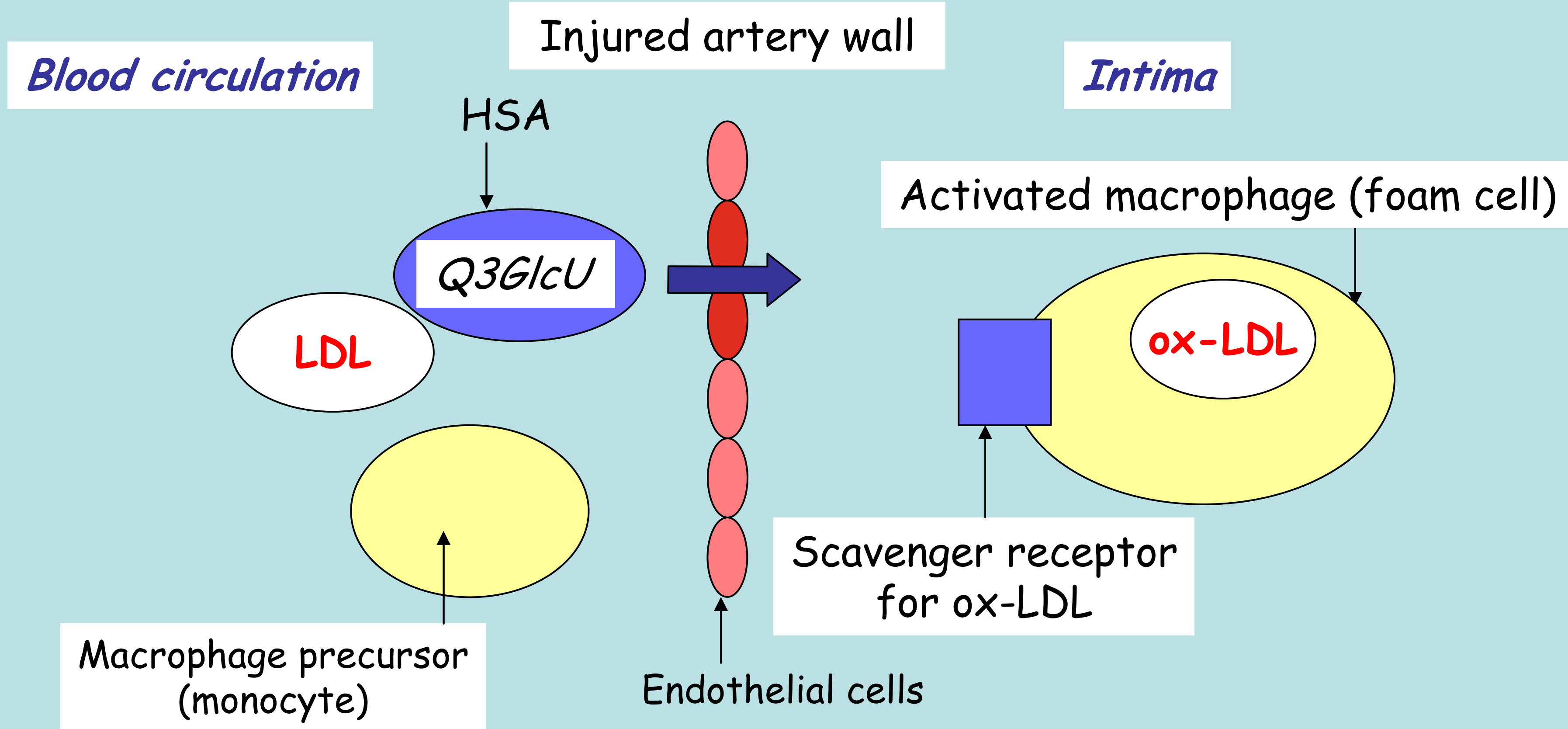
Final perspectives:
toward the elucidation of
specific antioxidant effects in cells



J. Ø. Moskaug *et al.*, *Mechanisms of Ageing and Development*, 2004, *125*, 315–324
P. Talalay *et al.*, *Adv. Enzyme Regul.*, 2003, *43*, 121-134

Specific protective effects of quercetin in atherosclerotic arteries

(Y. Kawai et al., *J. Biol. Chem.*, 2008, *283*, 9424-9434)



<u>Foam-cell specific effects of quercetin (Q)</u>

- Selective accumulation of Q3GlcU (main circulating metabolite)
- Partial deglucuronidation & methylation
- Inhibition of the expression of the scavenger receptor by 3′/4′MeQ
- Inhibition of LDL oxidation by Q & Q3GlcU

Dietary polyphenols as Antioxidants

Intrinsically potent ROS scavengers *if* catechol groups are present, high stoichiometries, antioxidant activity modulated (*not necessarily abolished*) by binding to proteins

Potent inhibitors of lipid peroxidation in food emulsions:
Antioxidant action of native polyphenols in the gastric compartment?

Specific antioxidant activity in cells:
- Activity of the circulating metabolites
+ specific (more antioxidant) cell metabolites (*e.g.*, aglycones)
- Inhibition of ROS-producing enzymes
- Up-regulation of the expression of antioxidant enzymes
- Antioxidant activity at inflammatory/atherosclerotic sites

Acknowledgements

UMR 408 University of Avignon - INRA

Emmanuelle VULCAIN, Bénédicte LORRAIN (PhD students)
Claire DUFOUR (INRA Researcher)
Pascale GOUPY & Michèle LOONIS (Technical staff)

Thank you for your attention !