Centrum pro výzkum toxických látek v prostředí

BIOMARKERS AND TOXICITY MECHANISMS

06 – Mechanisms Oxidative stress

Luděk Bláha, PřF MU, RECETOX
www.recetox.cz

Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.

EVROPSKÁ UNIE esf MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY OP Vzdělávání pro konkurenceschopnost

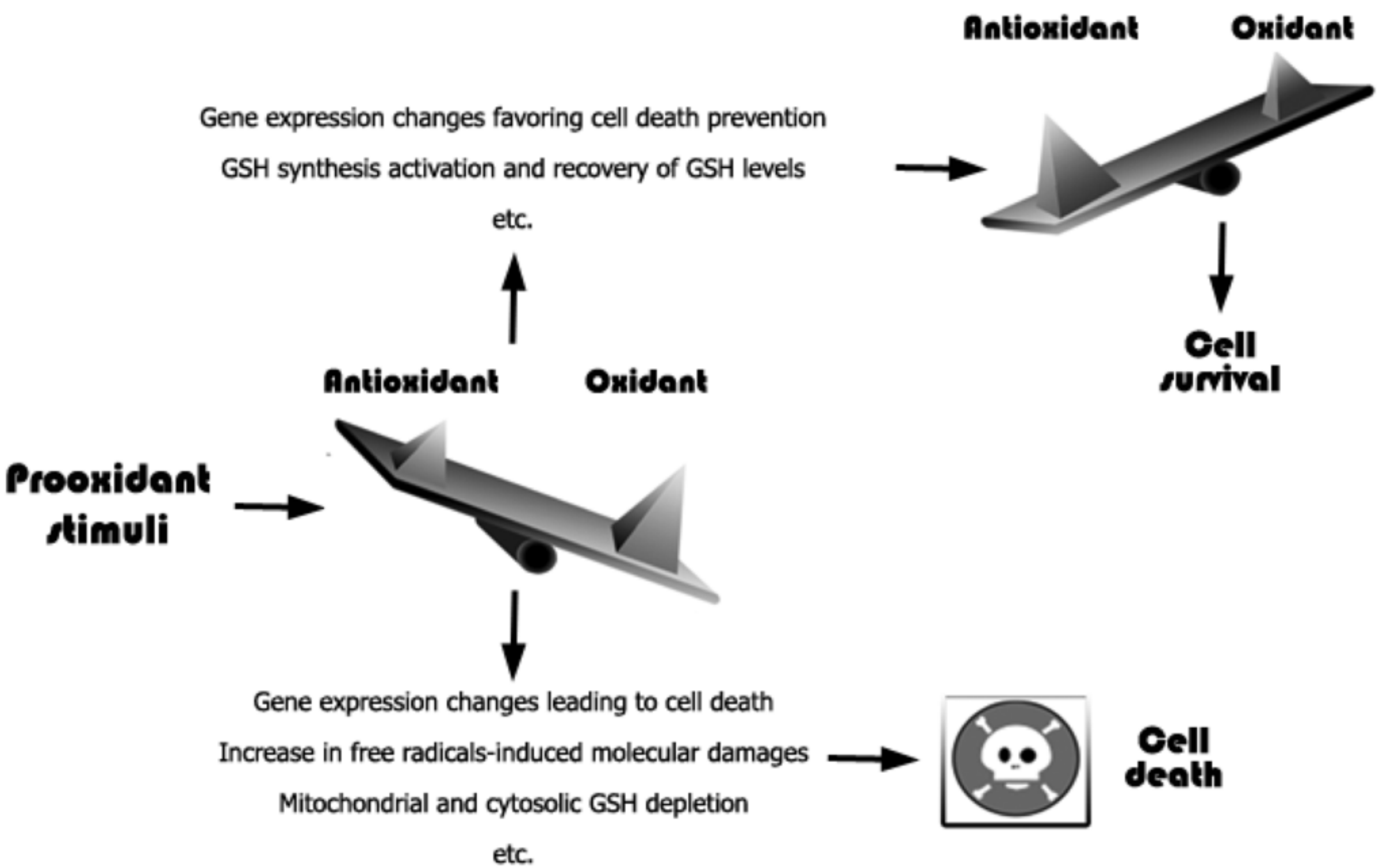
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Importance of redox (oxido-reduction) homeostasis

Traditional view – "too much oxidants" is bad

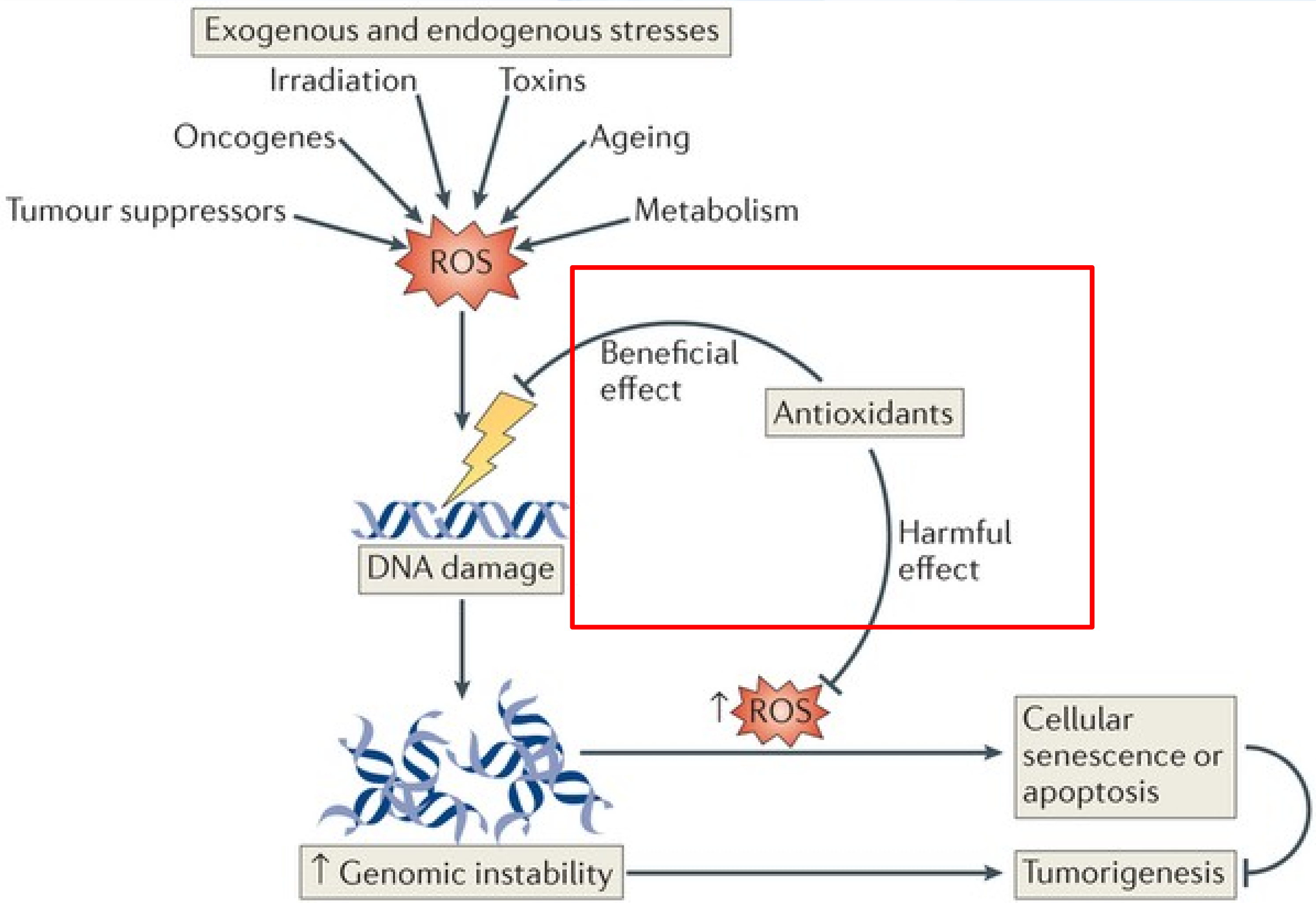
Prooxidants (Oxidative stress) → damage to macromolecules → death

Figure 1. Cellular redox balance control regulatory pathways determining cell viability.



Importance of redox (oxido-reduction) homeostasis

Modified view (2014) – "too much of anything is bad"



http://www.nature.com/nrm/journal/v15/n6/full/nrm3801.html

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Importance of redox (oxido-reduction) homeostasis

- Redox homeostasis
  - natural homeostatic levels of prooxidants and antioxidants
  - keeping cell metabolism and signalling balanced

- Disruptions of homeostasis
  - → **depletion of oxygen**
    - Change in metabolism, acidosis in tissues, signalling (e.g. TUMORS)
    - Less studied – new field – REDOX SIGNALLING
  - → **overproduction of prooxidants = oxidative stress**
    - GENERAL MECHANISM OF TOXICITY AND A

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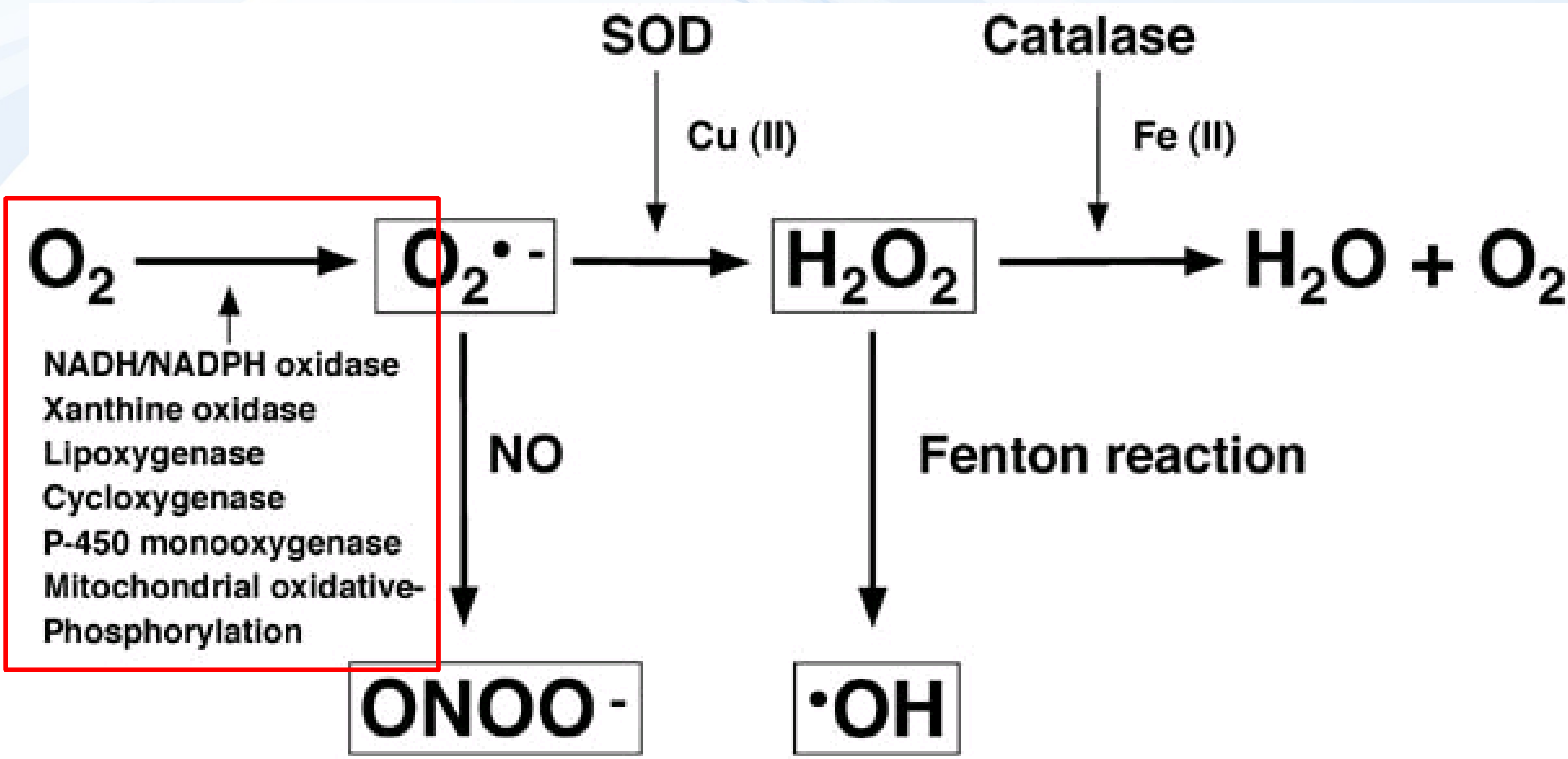
Pro oxidants

- **Oxygen (O2)**
  - principal molecule in living organisms
    - terminal acceptor of electones
  - highly reactive molecule
    - formation of reactive derivatives → ROS → toxicity

- **Other reactive molecules and ROS sources**
  - (details follow)
    - production in mitochondria (byproducts of metabolism)
    - redox-cycling (quinones of xenobiotics)
    - Fenton-reaction (metals)
    - oxidations mediated via MFOs (CYPs)
    - depletion of antioxidants (reactive molecules)

Key Reactive Oxygen Species (ROS)



SOD = Superoxide dismutase

Reduction of molecular oxygen to superoxide radical

$$O2 + e^- \rightarrow \boxed{^{\circ}O2^-}$$

Dismutation of superoxide radical

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

Transition metal catalyzed reaction (Fenton reaction)

$$\boxed{^{\circ}O_2^-} + Me^{n+} \rightarrow Me^{(n-1)+} + O_2$$

$$Me^{(n-1)+} + \boxed{H_2O_2} \rightarrow Me^{n+} + OH^- + \boxed{^{\circ}OH}$$

Haber-Weiss reaction

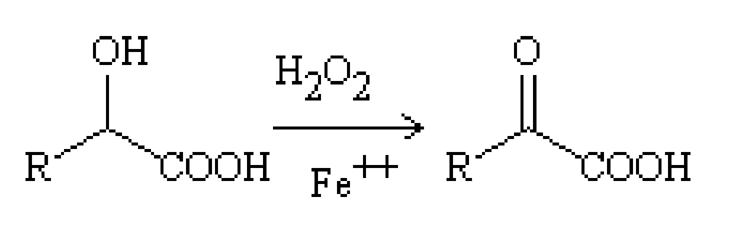
$$\boxed{^{\circ}O2^-} + \boxed{H_2O_2} \rightarrow O_2 + OH^- + \boxed{^{\circ}OH}$$

Me = metal (e.g. Fe^{3+}/Fe^{2+})

$^{\circ}O_2^-$ = superoxide radical (superoxide anion)
$^{\circ}OH$ = hydroxyl radical
OH^- = hydroxyl anion
H_2O_2 = hydrogen peroxide

TERMS, NAMES, REACTIONS

Fenton reaction
(from organic chemistry classes)



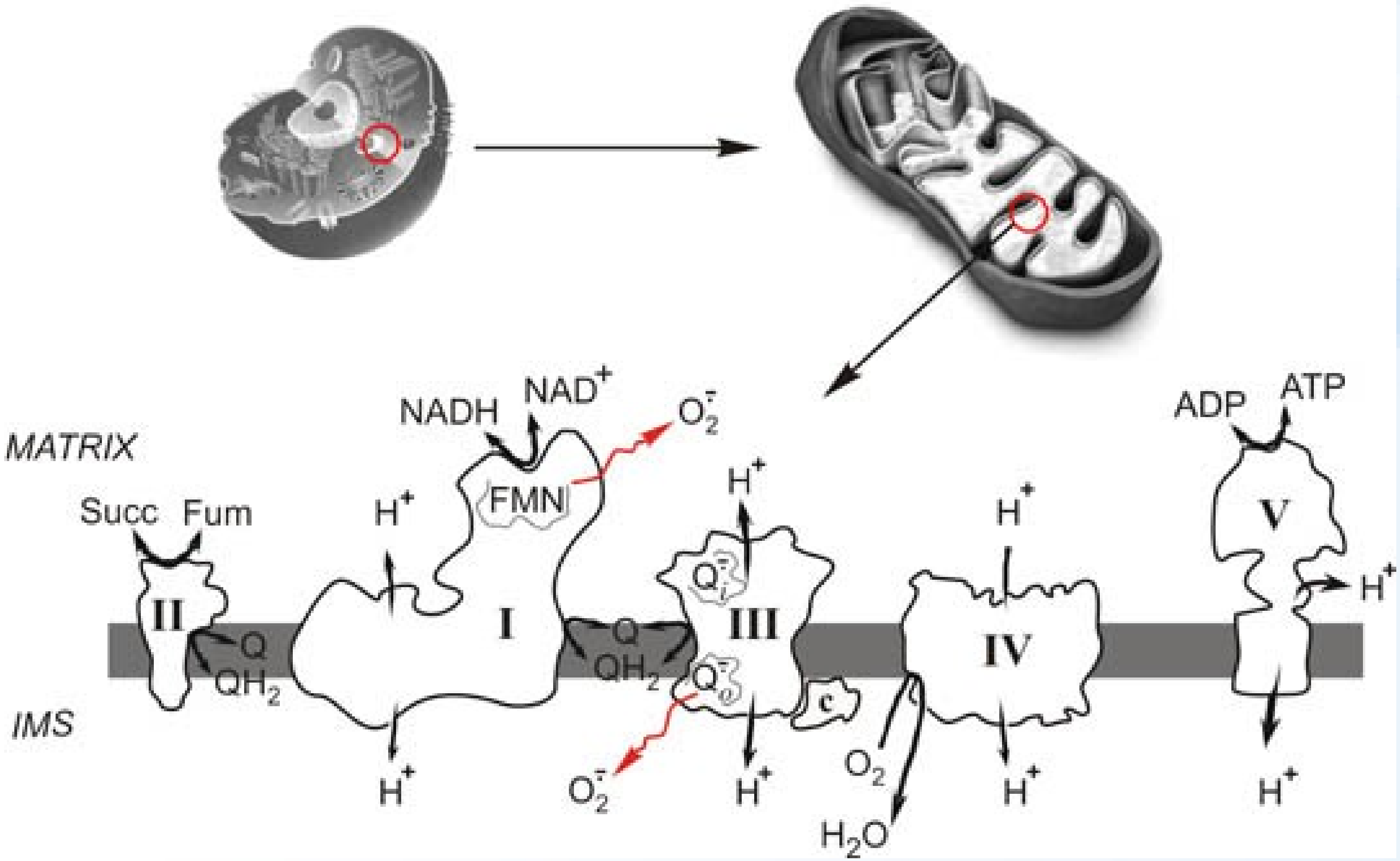
Fe3+/2+
But also **OTHER METALS (!)**

Reactivity of ROS (short rate → instability = reactivity)

ROS	Antioxidant	Rate constant, $M^{-1} \cdot sec^{-1}$
Superoxide anion of oxygen	carnosine	$5.0 \cdot 10^{-5}$
	carnosine	$0.8 \cdot 10^{-5}$
	ascorbate	$2.7 \cdot 10^{-5}$
	α-tocopherol	$2.0 \cdot 10^{-5}$
Singlet oxygen	carnosine	$3 \cdot 10^{-7}$
	imidazole	$2 \cdot 10^{-7}$
	ergothioneine	$2 \cdot 10^{-7}$
	NaN_3	$44 \cdot 10^{-7}$
Hydroxyl radical	carnosine	$(5\text{-}8) \cdot 10^{-9}$
		$9 \cdot 10^{-9}$

Sources or ROS

Mitochondria
Superoxide production in oxidative respiration



Redox cycling compounds and ROS production

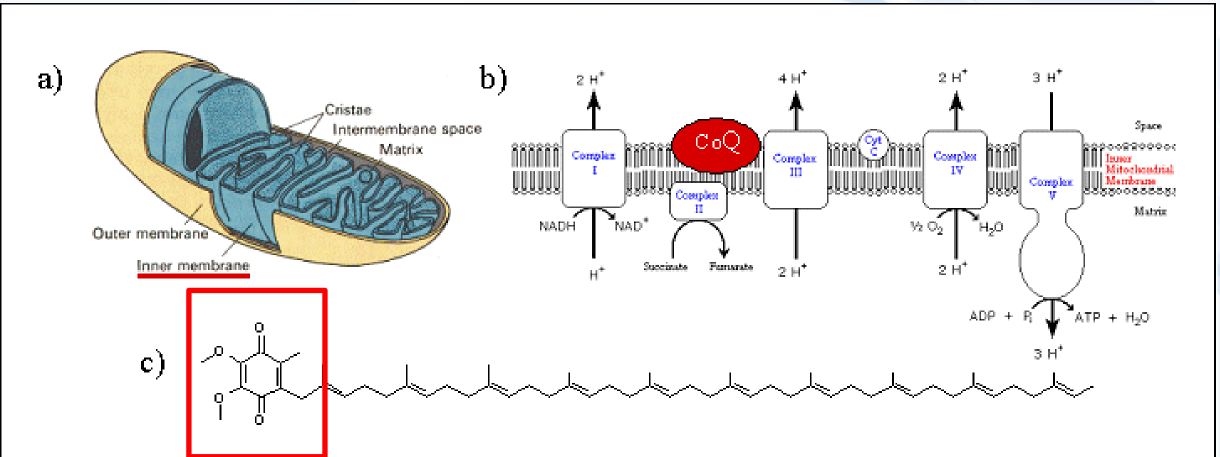
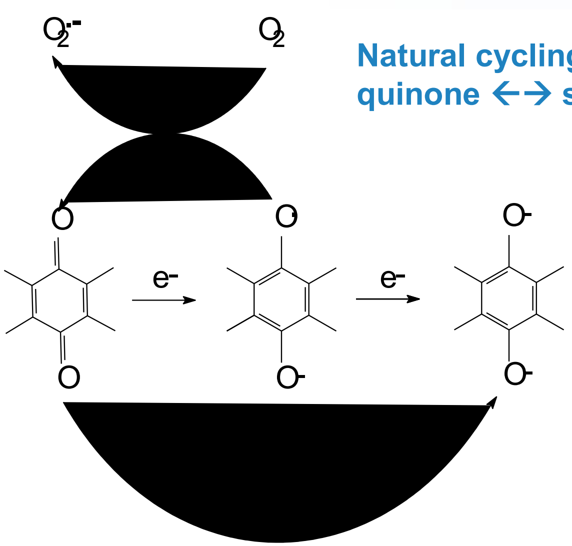


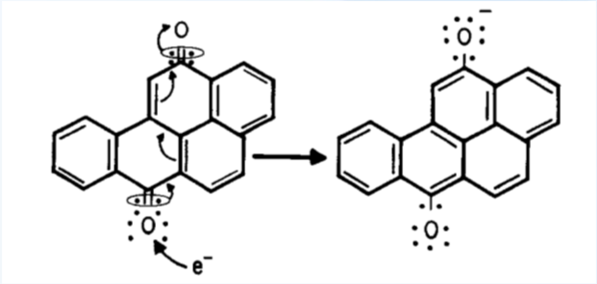
Figure 1. CoQ_{10} is an essential component of the electron transport chain within the mitochondria.[2,3]

Toxicity =
Interference with
“xeno”quinones
and similar compounds

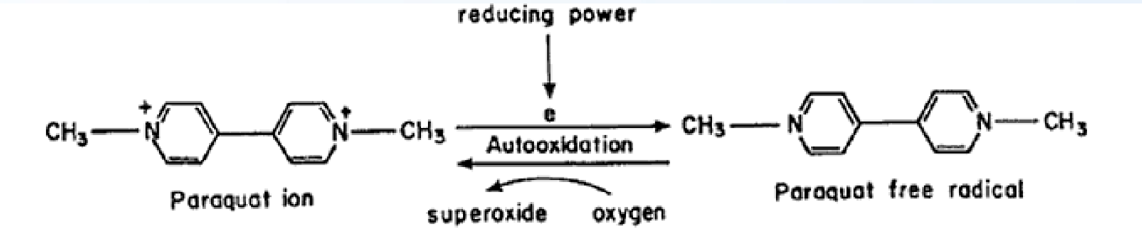


Natural cycling
quinone ←→ semiquinone

Example 1 – BaP quinone



Example 2 – Paraquate pesticide



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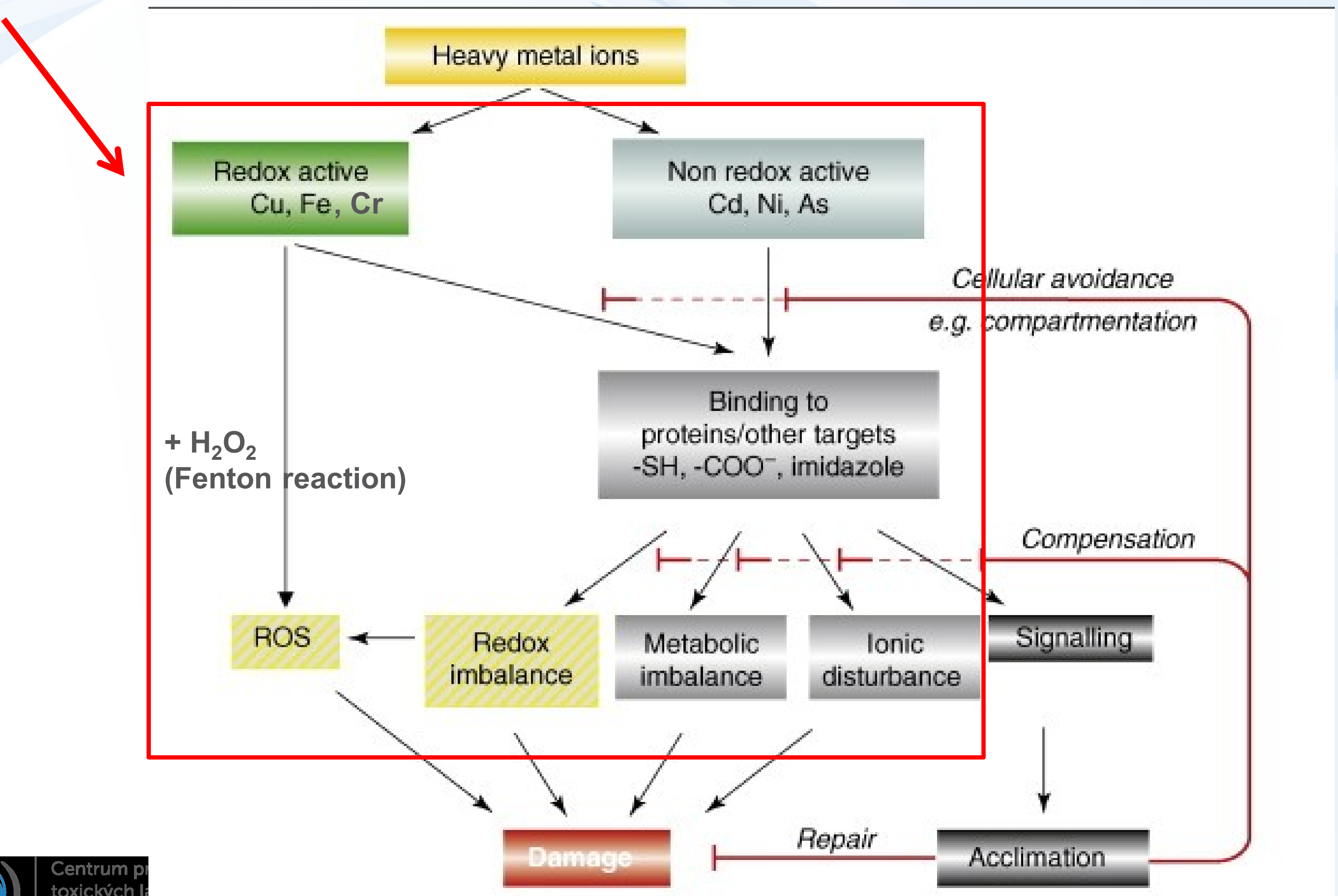
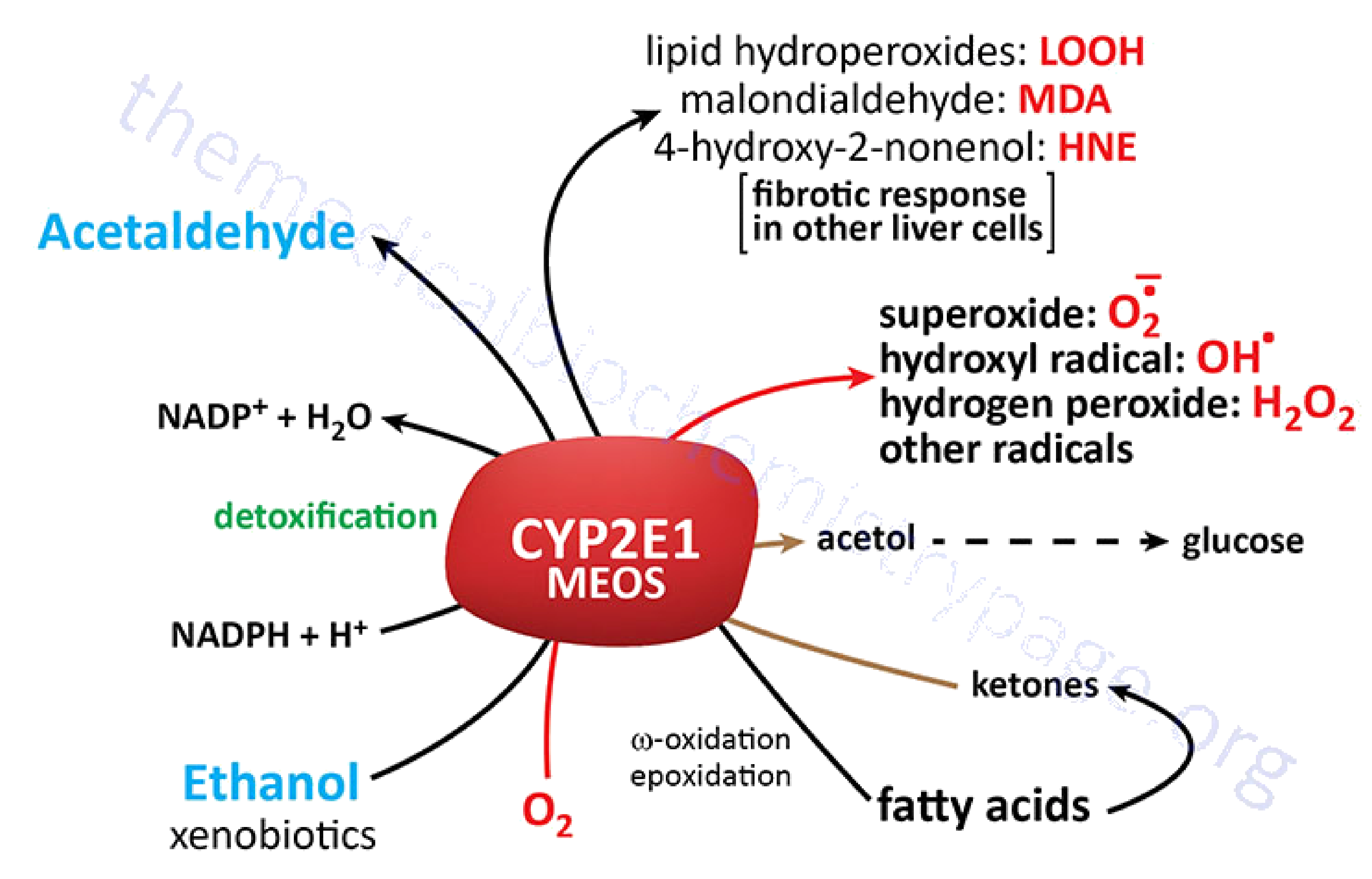
Metals and impacts on redox homeostasis

(* direct ROS production / * binding to proteins)



CYP450 as ROS source

(example CYP2E1, MEOS – microsomal ethanol oxidising system)



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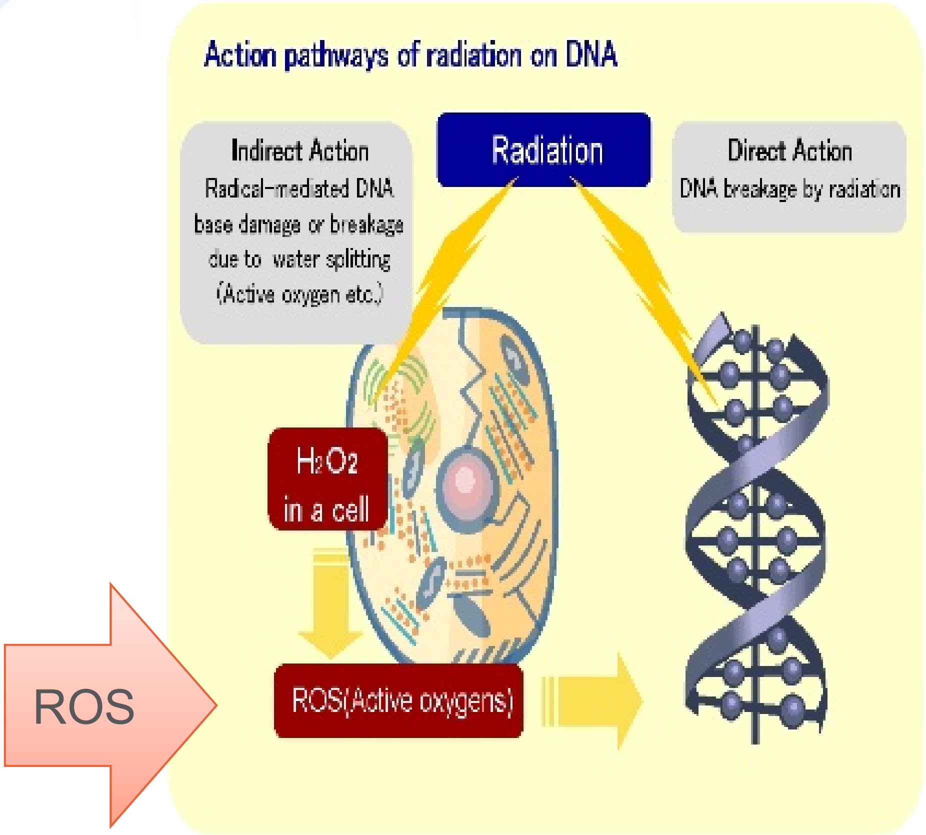
Irradiation as a source of ROS and oxidative damage
(reminder – check lectures on toxicity towards DNA)

Mechanism of Radiation action



- ✓The action pathway of radiation to the human body can visualized in two ways: one is direct action and the other one is an indirect action.
- ✓<u>The direct action</u> is DNA breakage. DNA has essential information to make body. The damaged DNA would cause apoptosis (cell death) and mutation of cells and increase a risk of diseases.
- ✓<u>The indirect action</u> is generation of radical oxygen in the human body.
- ✓We are influenced by radiation not only through environment exposure but also through breathing air and eating food.
- ✓The DNA base damage mediated by radical oxygen would disturb normal cell growth and cause a functional decline of the body.

Protection against ROS-induced damage

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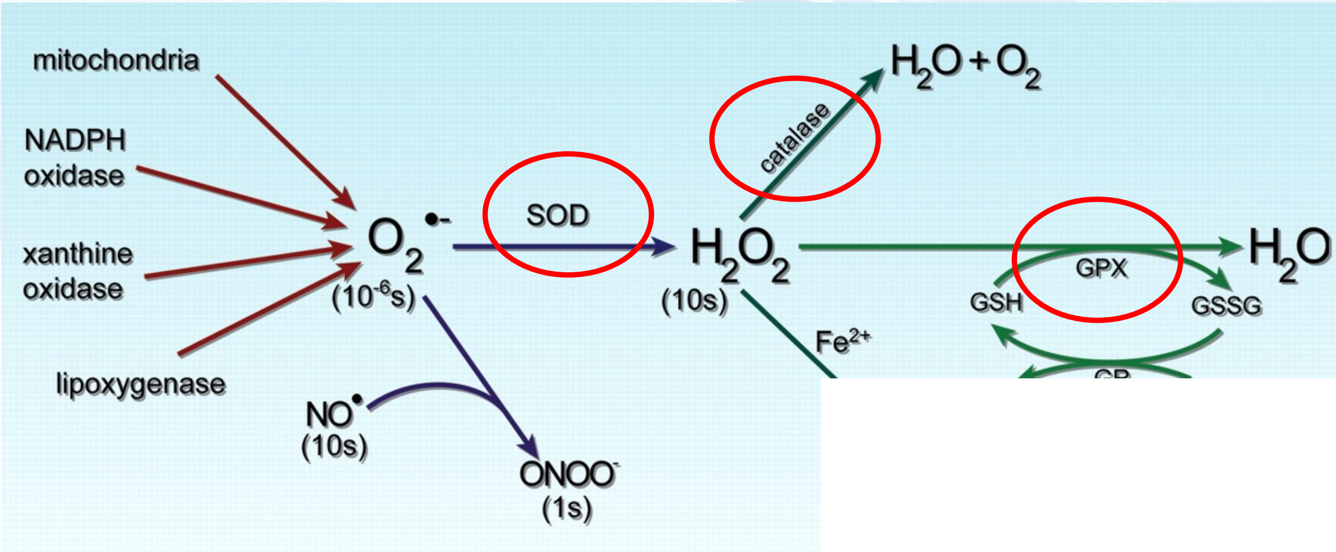
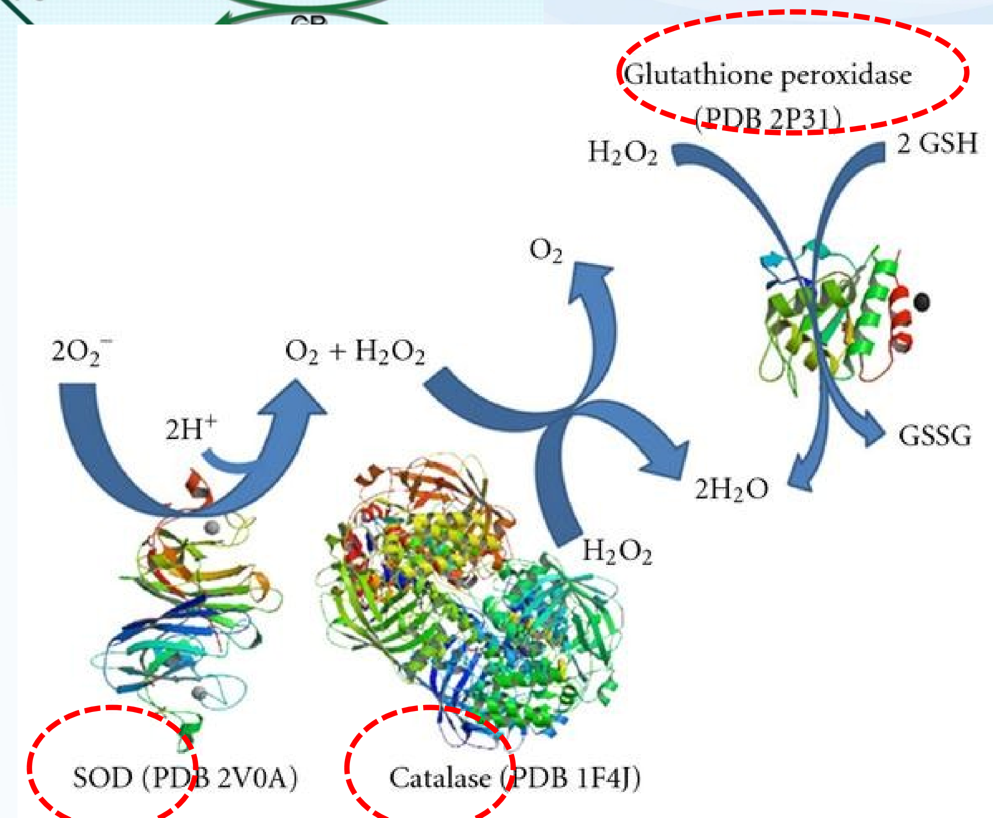
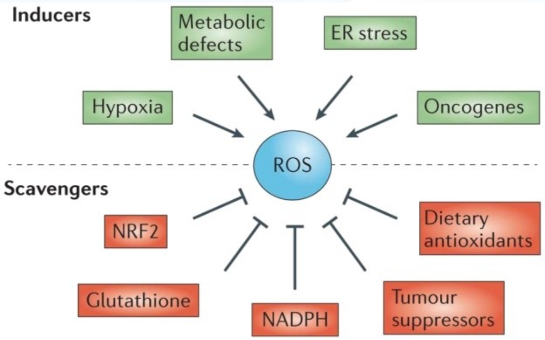
Antioxidant responses 1 - **enzymatic**



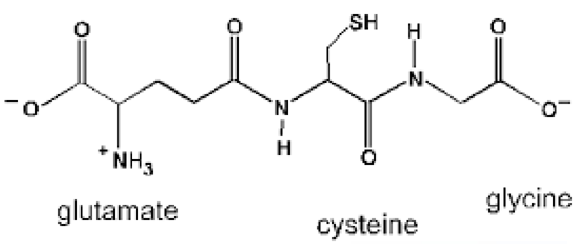
Highlighted enzymes
discussed further
within the „biomarker“ part

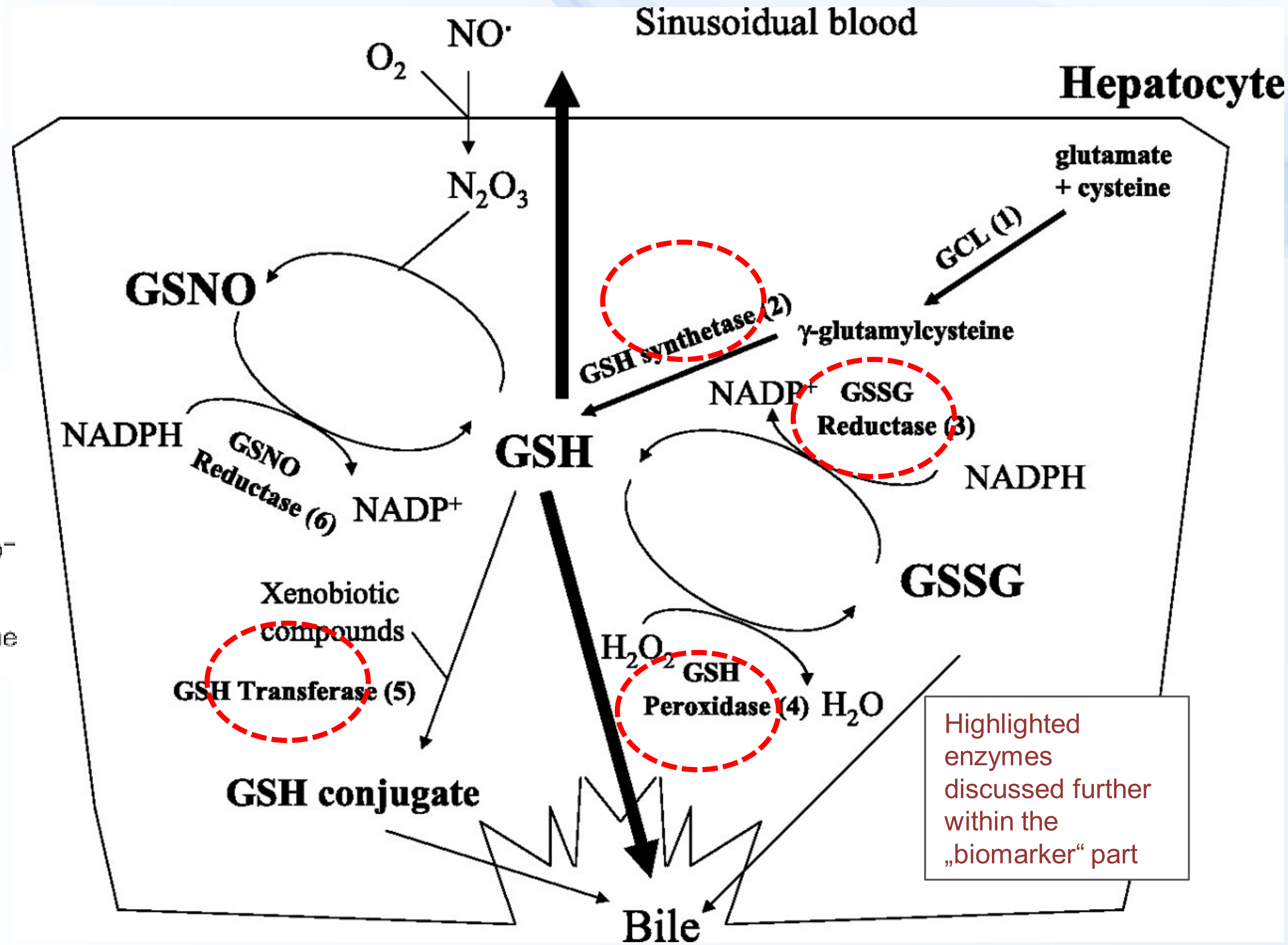


Antioxidant responses 2 – small molecules: GSH



glutathione (GSH)





Highlighted enzymes discussed further within the „biomarker“ part

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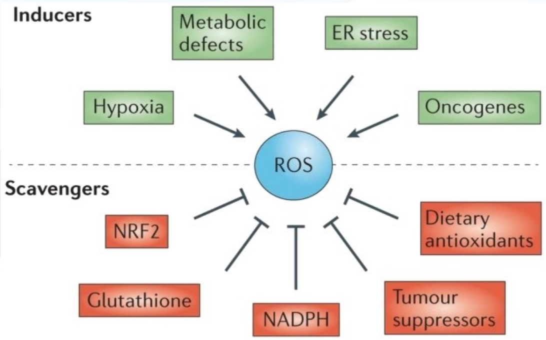
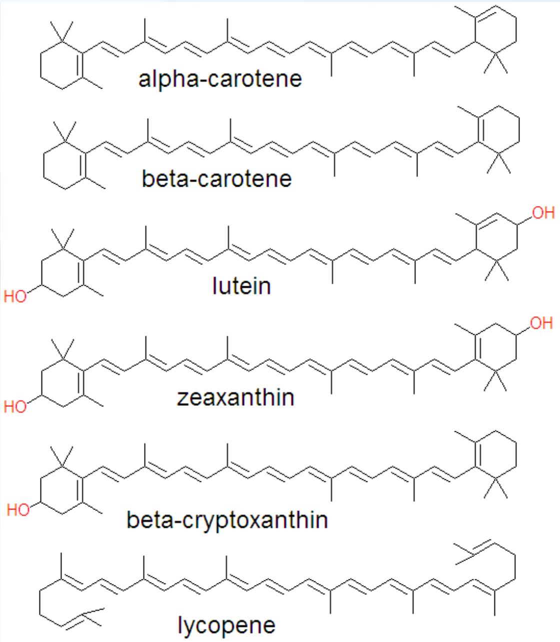
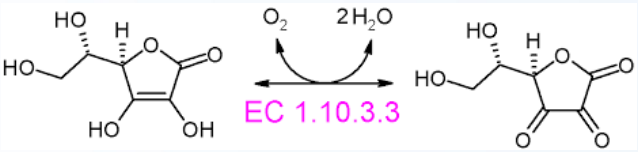
Antioxidant responses 2 – small molecules: dietary antioxidants



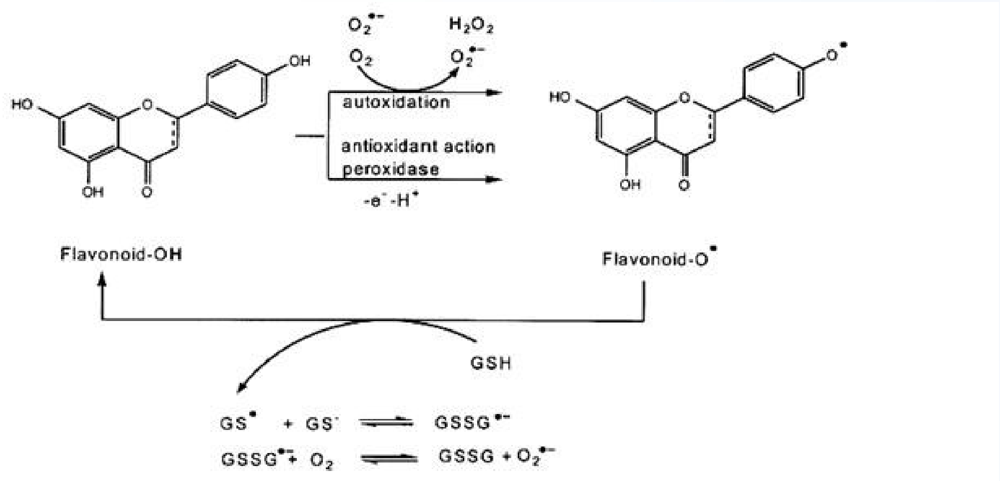
Carotenoids



Ascorbate



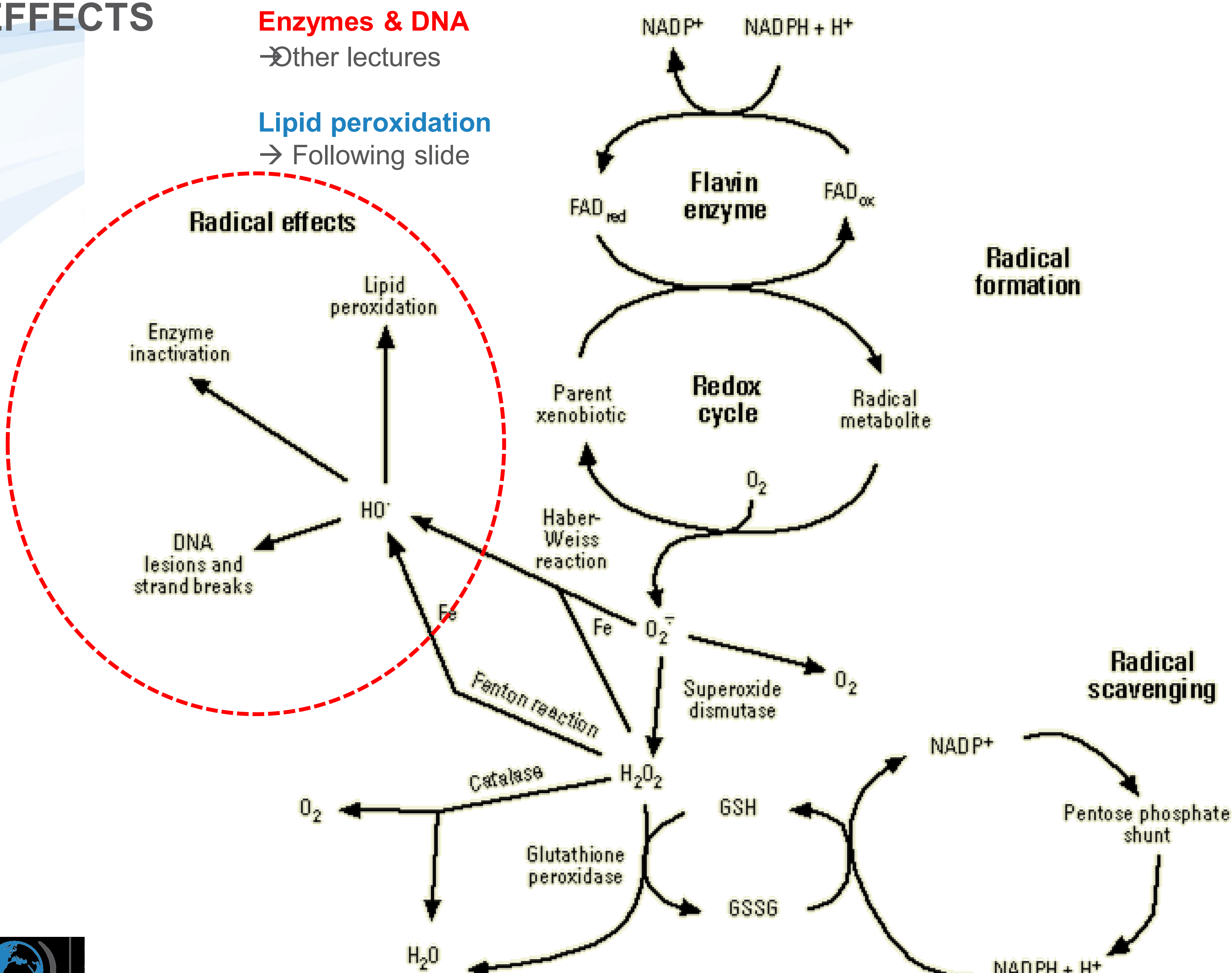
Flavonoids



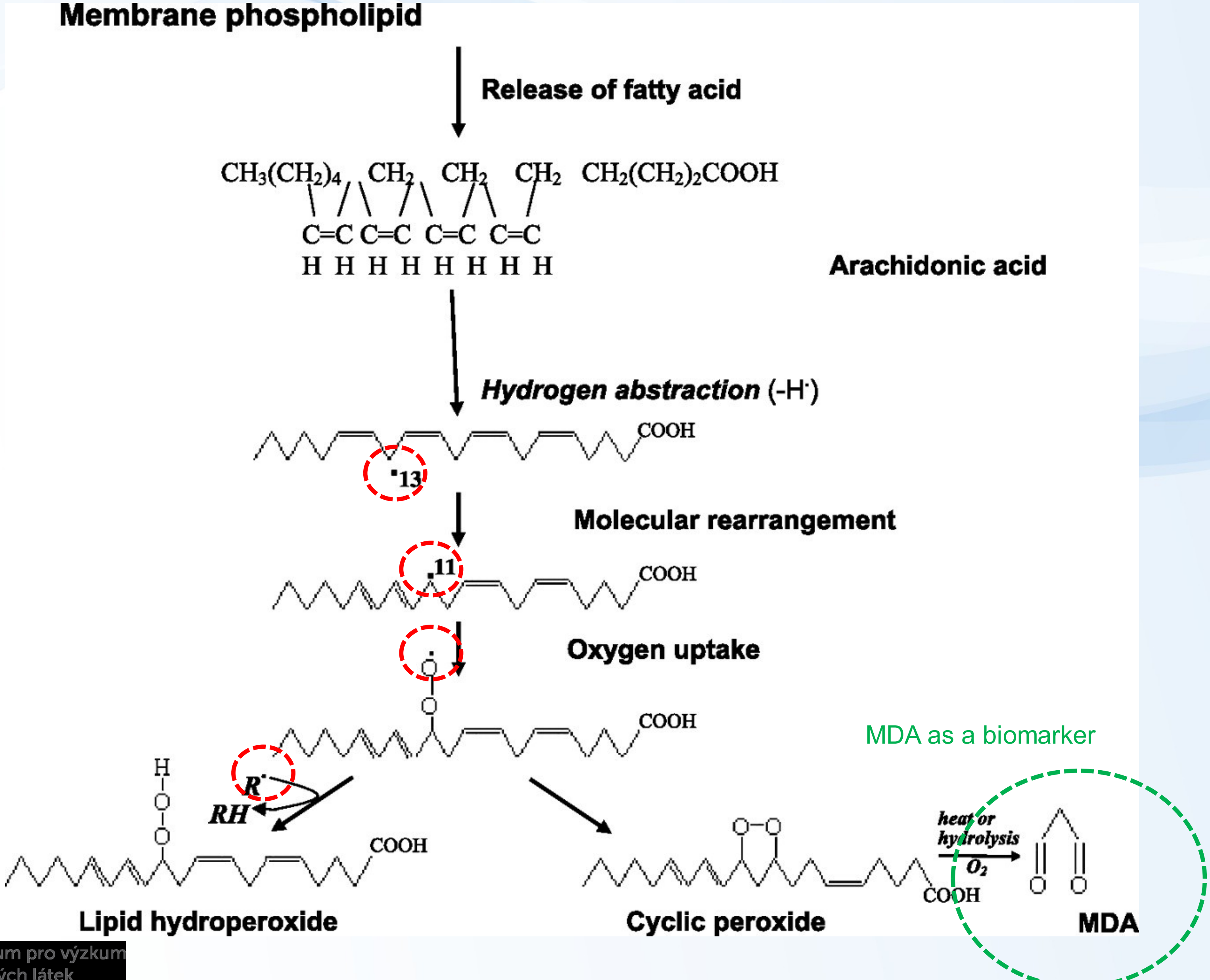
EFFECTS

Enzymes & DNA
→ Other lectures

Lipid peroxidation
→ Following slide



Lipid peroxidation = radical reaction → fast propagation



Biomarkers of oxidative damage
(will be discussed later)

BIOMARKER	AVAILABILITY	FREQUENTLY USED ASSAYS
Lipid Peroxidation		
F_2-isoprostanes	Plasma, urine	GC/MS, HPLC-MS/MS
Oxidized low-density lipoprotein (oxLDL)	Plasma, serum	ELISA
Malondialdehyde (MDA)	Plasma, serum, saliva, urine, exhaled breath condensate	Colorimetry, spectrophotometry, HPLC + fluorescence, GC/MS
Protein Oxidation		
Protein carbonyls	Plasma, serum	ELISA
DNA Oxidation		
8-hydroxy-2-deoxyguanosine (8-OHdG)	Plasma, serum, urine	HPLC-EC, HPLC-MS/MS*, GC/MS, Comet assay*

Health effects of oxidative stress … multiple

e.g. acute coronary syndrome (ACS) → myocardial infarction

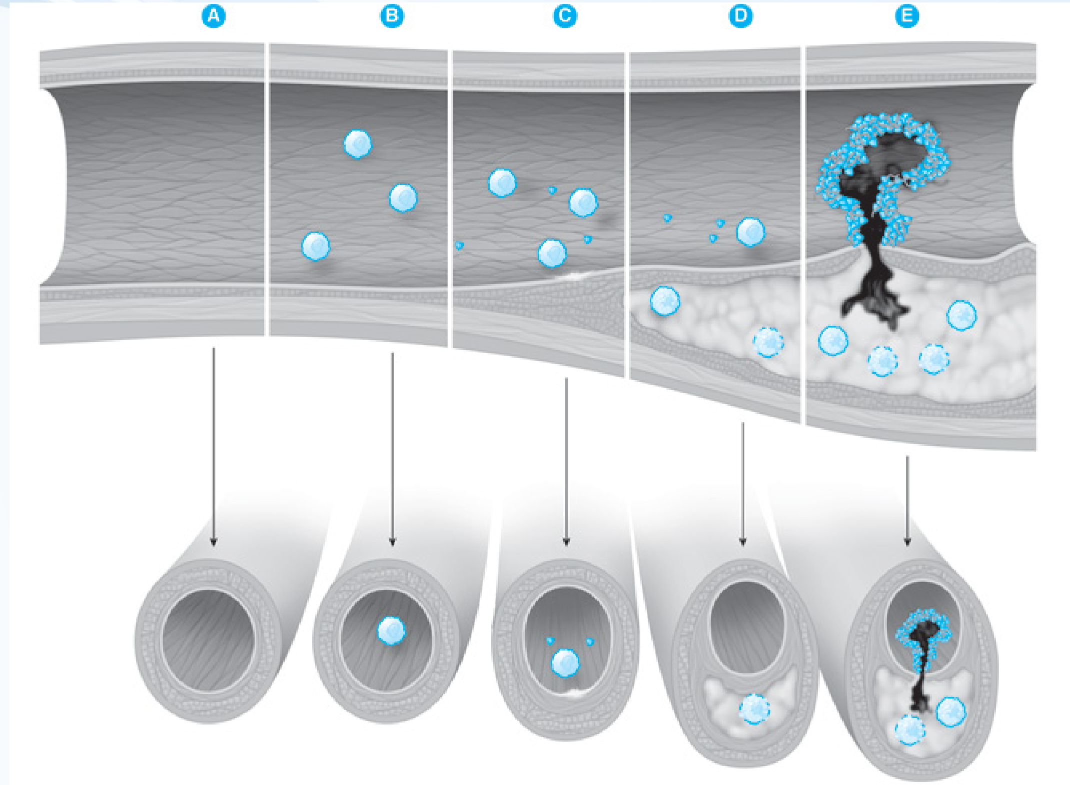


Figure 24-7. Pathogenesis of acutecoronary syndromes. A. A normal coronary artery has an intact endothelium surrounded by smooth muscle cells. **B.** Endothelial cell activation or injury recruits monocytes and T lymphocytes to the site of injury, leading to development of a fatty streak. **C.** Continued oxidative stress within a fatty streak leads to development of an atherosclerotic plaque. **D.** Macrophage apoptosis and continued cholesterol deposition cause further plaque organization, and may induce the expression of additional inflammatory proteins and matrix metalloproteinases. At this stage, the cap of the fibroatheroma remains intact. **E.** Continued inflammation within an atherosclerotic plaque leads to thinning of the fibrous cap and, eventually, to plaque erosion or rupture. Exposure of plaque constituents to the bloodstream activates platelets and the coagulation cascade, with resulting coronary artery occlusion.

Credit: Figure 24-7: Adapted with permission from Libby P. Current concepts of the pathogenesis of acute coronary syndromes. <i>Circulation</i> 2001;104:365–372.

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