



Centrum pro výzkum  
toxických látek  
v prostředí

# BIOMARKERS AND TOXICITY MECHANISMS

## 06 – Mechanisms

### Oxidative stress

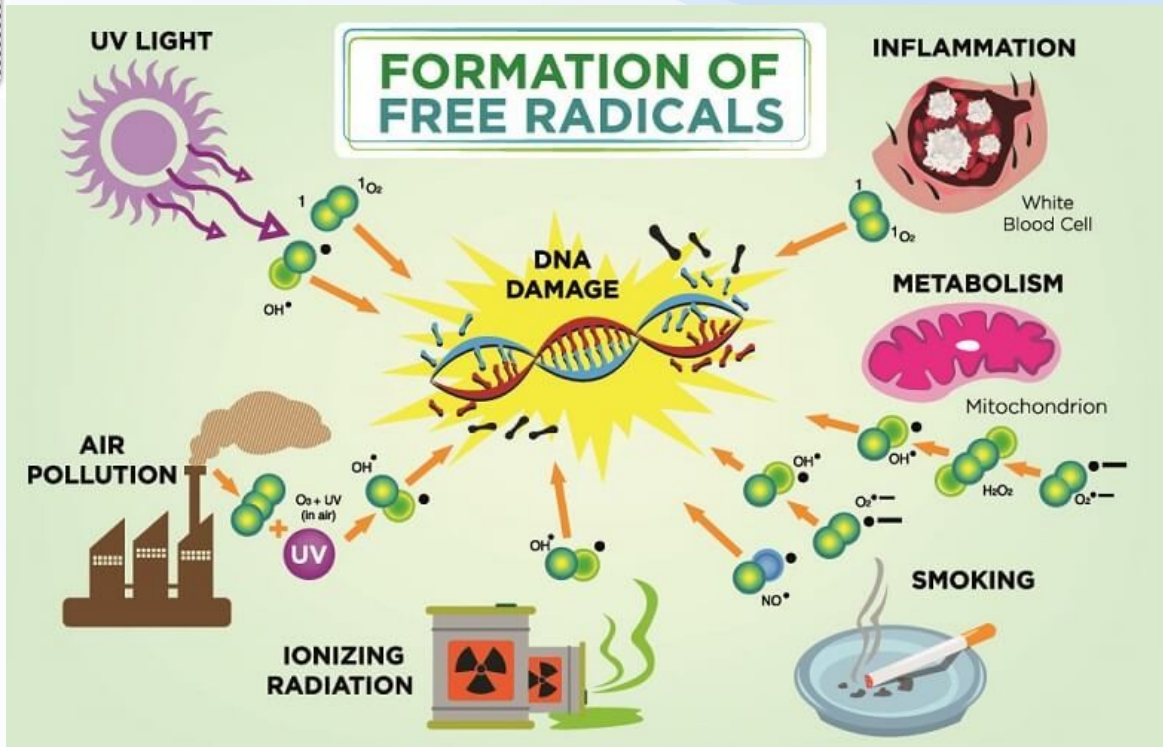
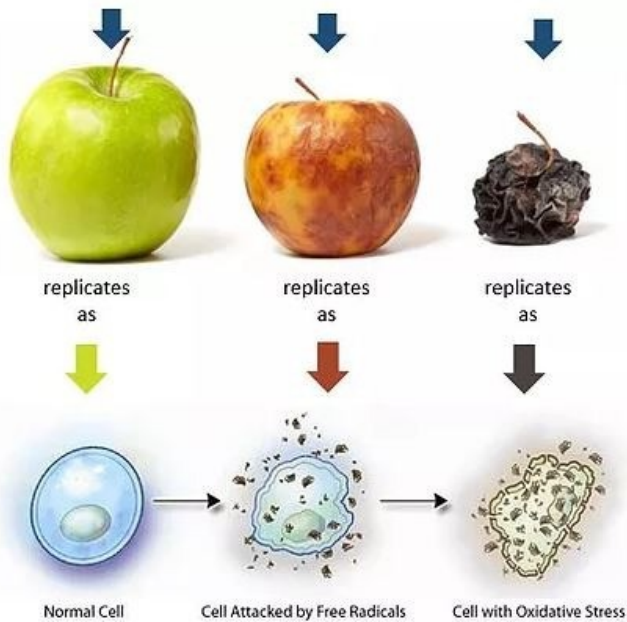
Luděk Bláha, PŘF MU, RECETOX  
[www.recetox.cz](http://www.recetox.cz)

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



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

# Stages of oxidative stress



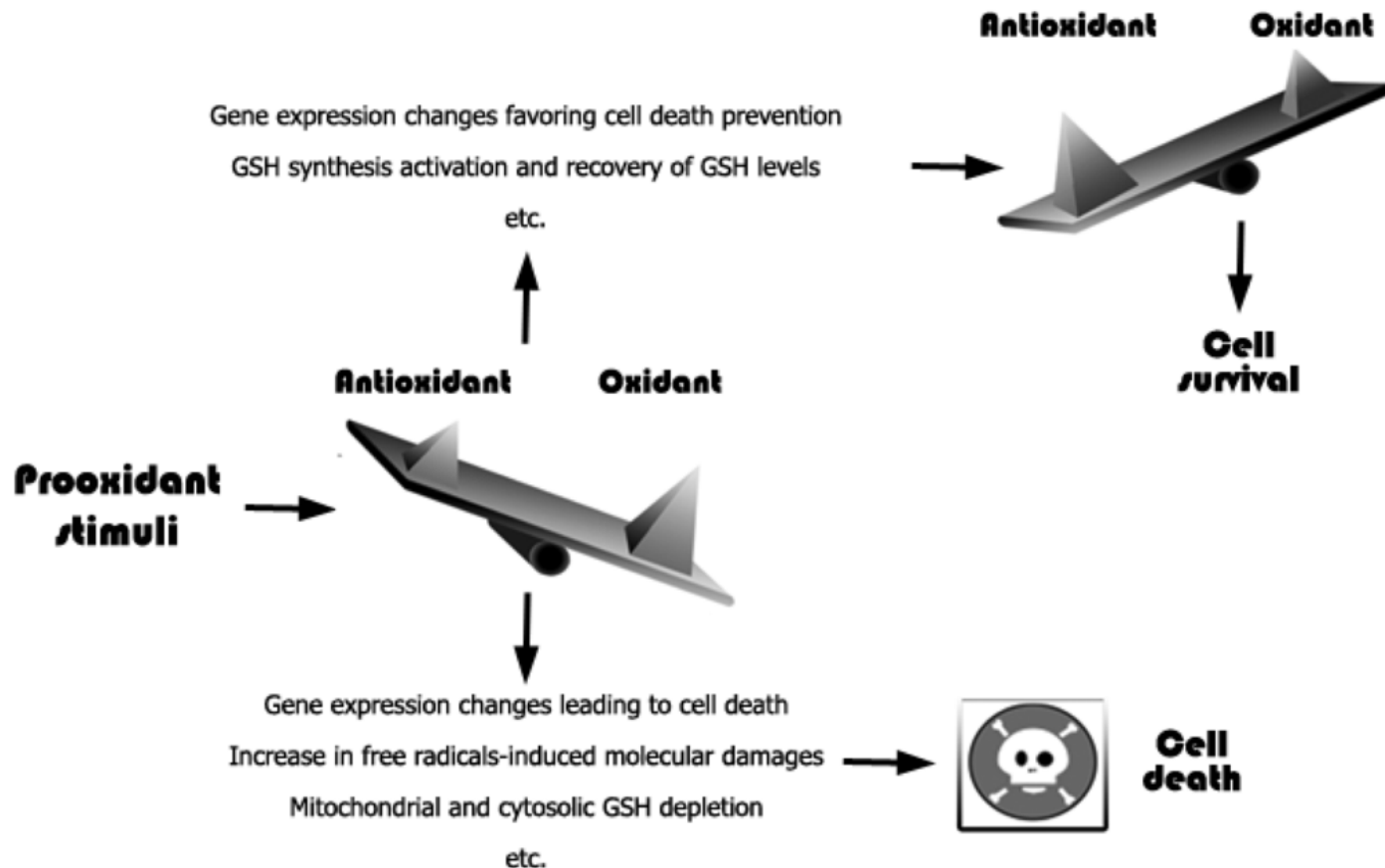
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# Importance of redox (oxido-reduction) homeostasis

## Traditional view – “too much oxidants” is bad

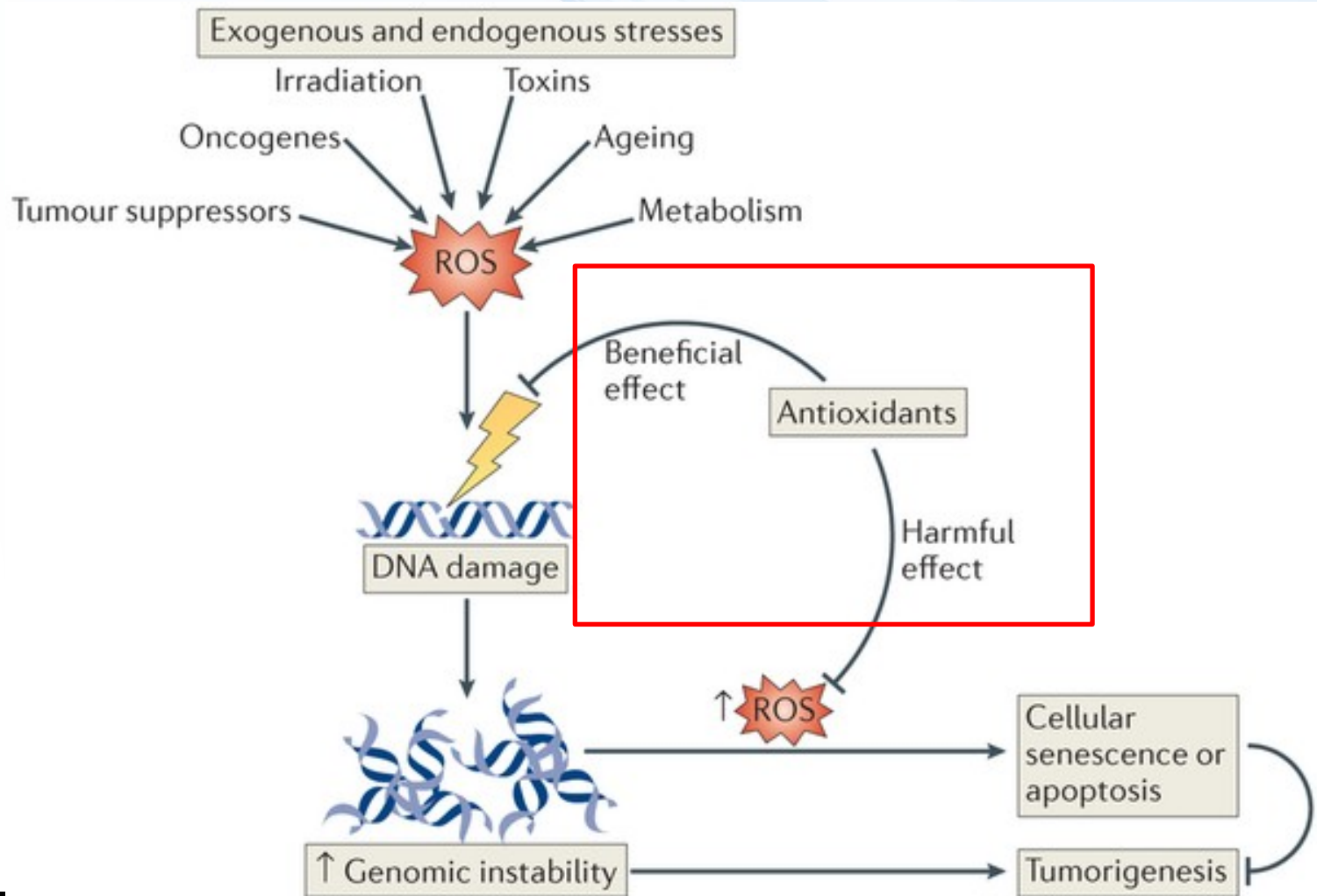
Prooxidants (Oxidative stress) → damage to macromolecules → death

**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>



# 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 AGING

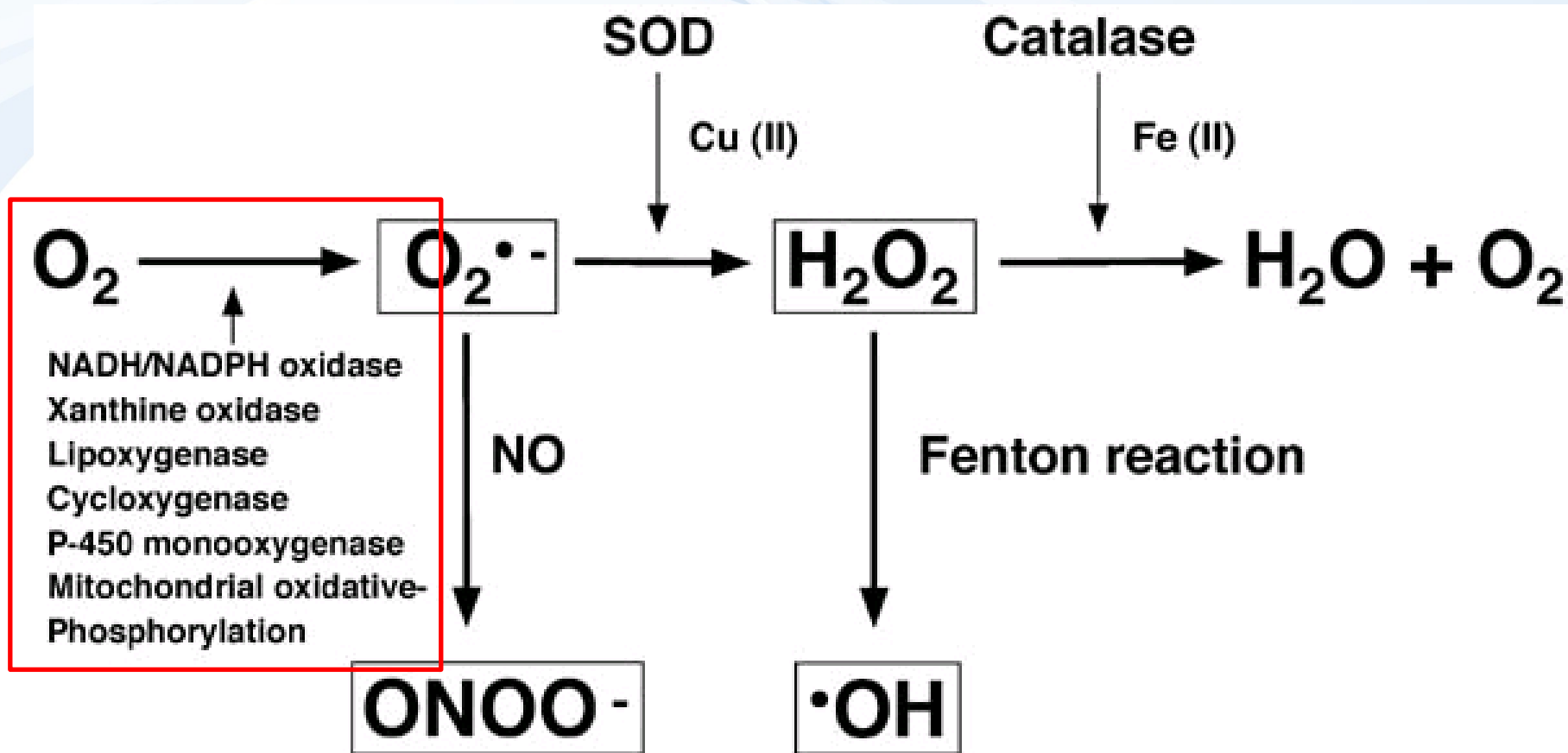


# Pro oxidants

- **Oxygen (O<sub>2</sub>)**
  - principal molecule in living organisms
    - terminal acceptor of electrons
  - 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)

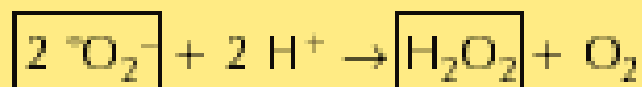


*SOD = Superoxide dismutase*

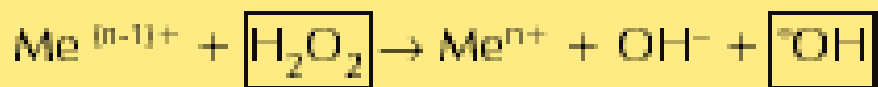
Reduction of molecular oxygen to superoxide radical



Dismutation of superoxide radical



Transition metal catalyzed reaction  
(Fenton reaction)



Haber-Weiss reaction



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

$\text{O}_2^{\cdot -}$  = superoxide radical (superoxide anion)

$\text{OH}^{\cdot}$  = hydroxyl radical

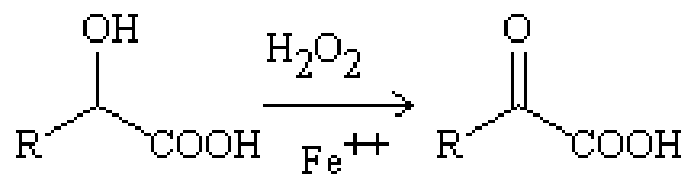
$\text{OH}^-$  = hydroxyl anion

$\text{H}_2\text{O}_2$  = hydrogen peroxide

## TERMS, NAMES, REACTIONS

### Fenton reaction

(from organic chemistry classes)



$\text{Fe}^{3+}/\text{Fe}^{2+}$

But also **OTHER METALS (!)**



# Reactivity of ROS (short rate $\rightarrow$ 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	$2.7 \cdot 10^{-5}$
	$\alpha$ -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	$(5-8) \cdot 10^{-9}$
		$9 \cdot 10^{-9}$

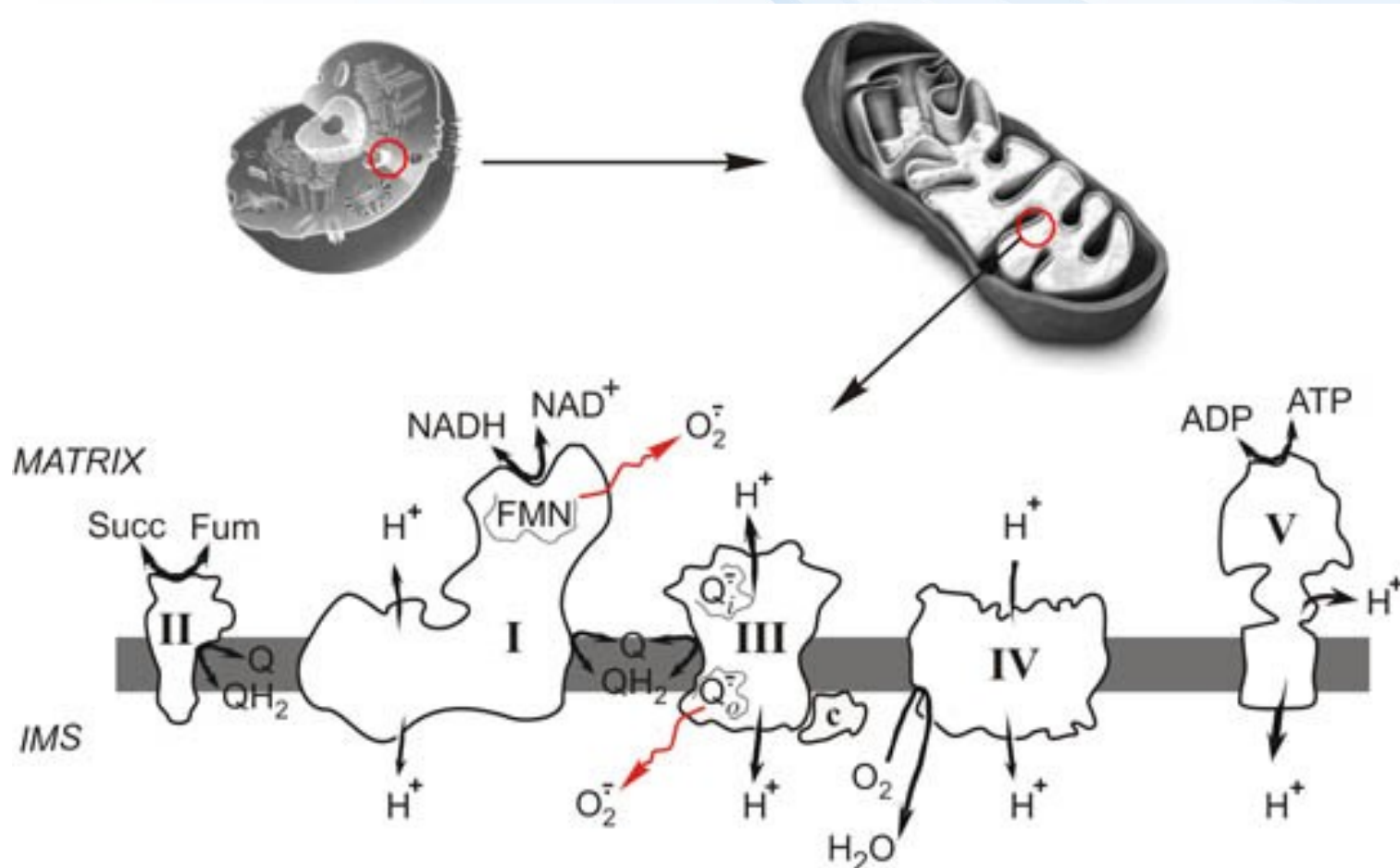


# Sources of ROS



# Mitochondria (= metabolism!)

## Superoxide production in oxidative respiration



# Redox cycling compounds and ROS production

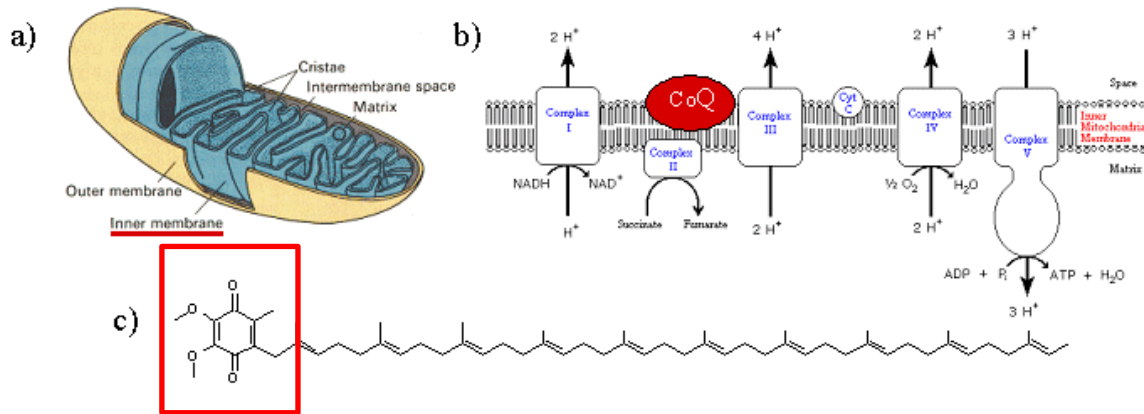
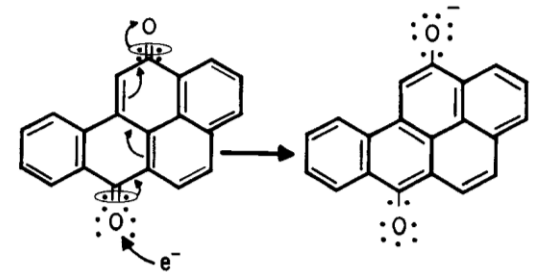


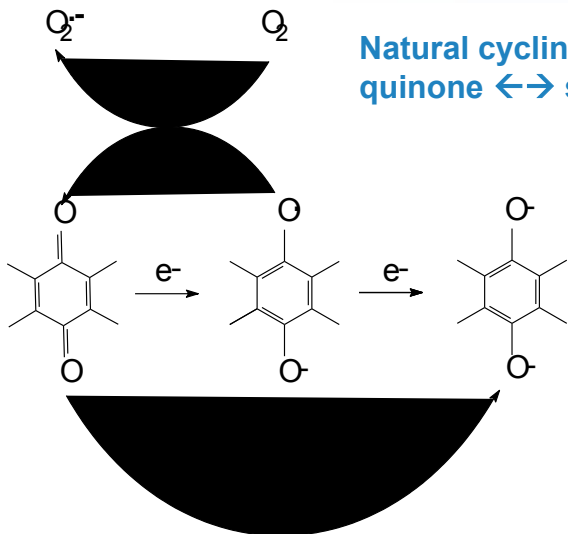
Figure 1. CoQ<sub>10</sub> is an essential component of the electron transport chain within the mitochondria.<sup>2,3</sup>

Toxicity =  
Interference with  
“xeno” quinones  
and similar compounds

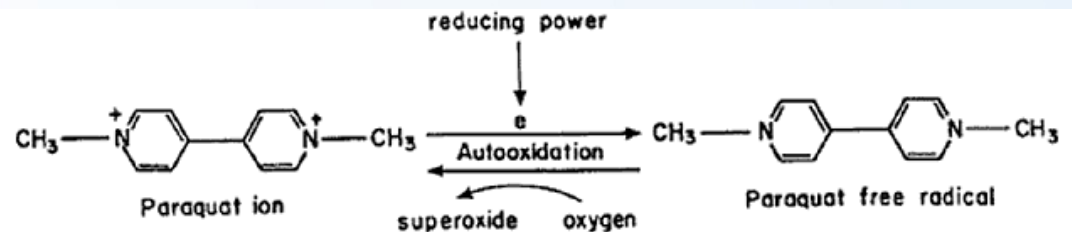
## Example 1 – BaP quinone



Natural cycling  
quinone ↔ semiquinone

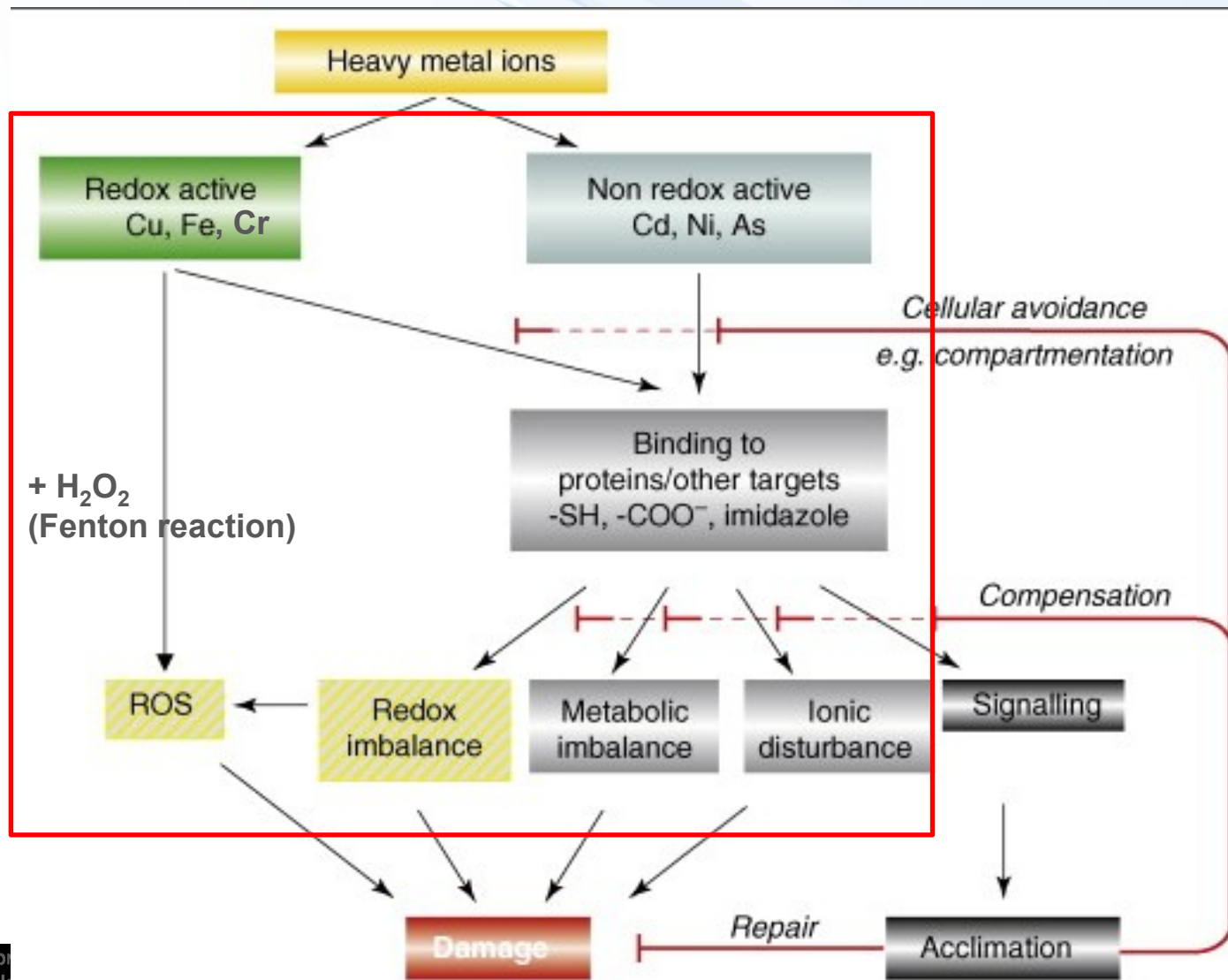


## Example 2 – Paraquat pesticide



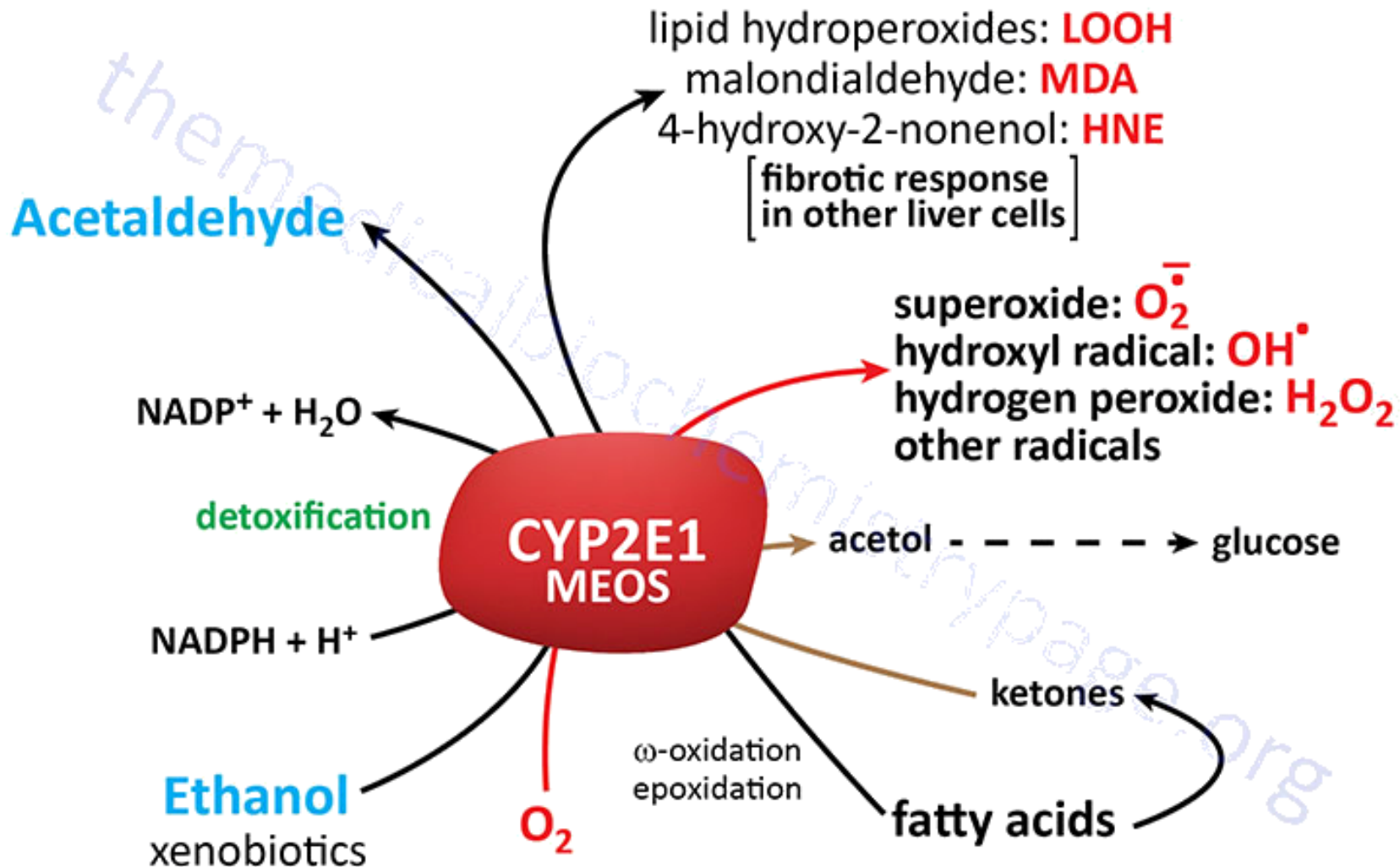
# Metals and impacts on redox homeostasis

(\* direct ROS production / \* binding to proteins)



# CYP450 as ROS source

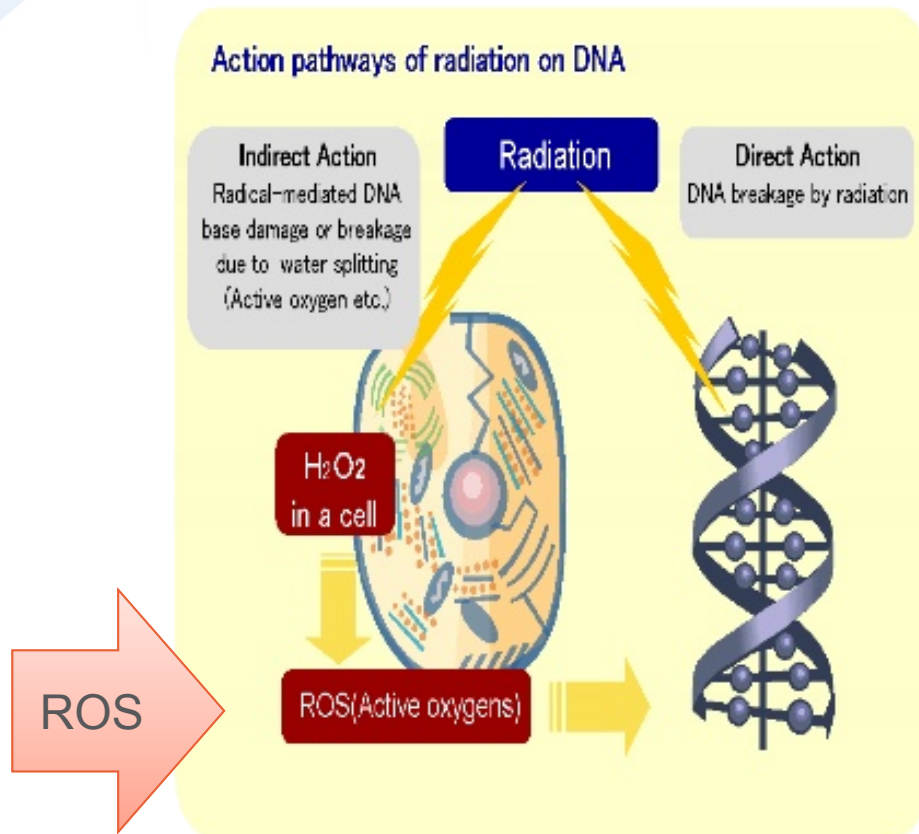
(example CYP2E1, MEOS – microsomal ethanol oxidising system)



# 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 be visualized in two ways: **one is direct action and the other one is an indirect action.**

✓ The direct action is **DNA breakage**. DNA has essential information to make the body. The damaged DNA would cause **apoptosis (cell death) and mutation of cells and increase a risk of diseases.**

✓ The indirect action 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.**

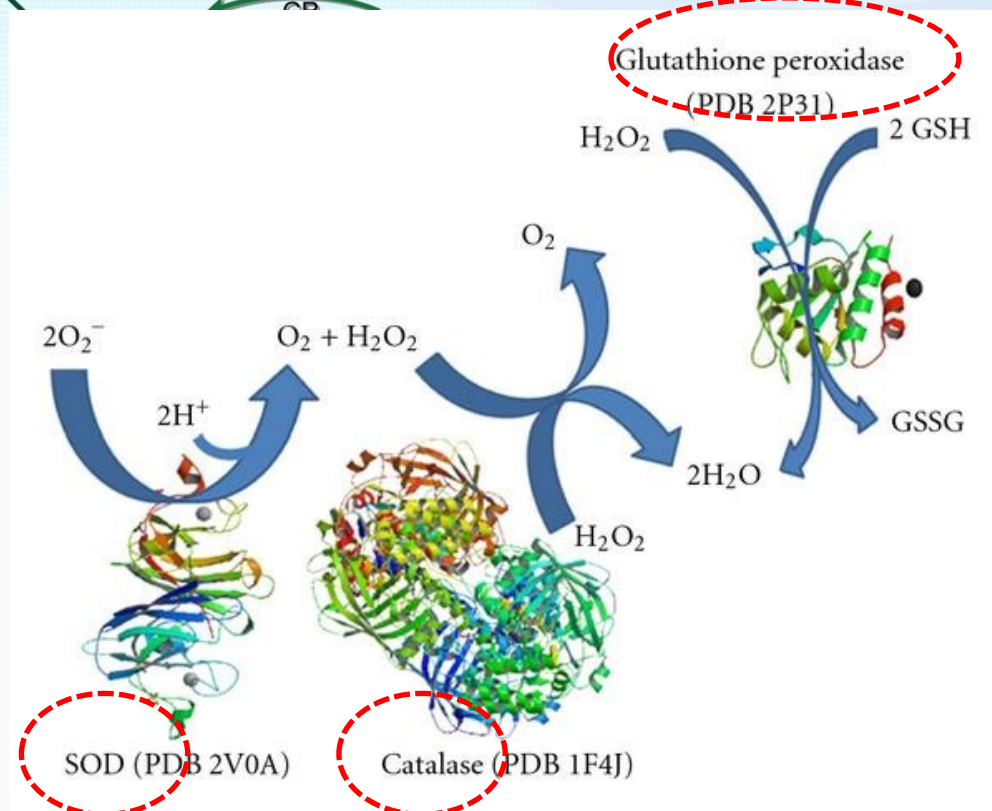
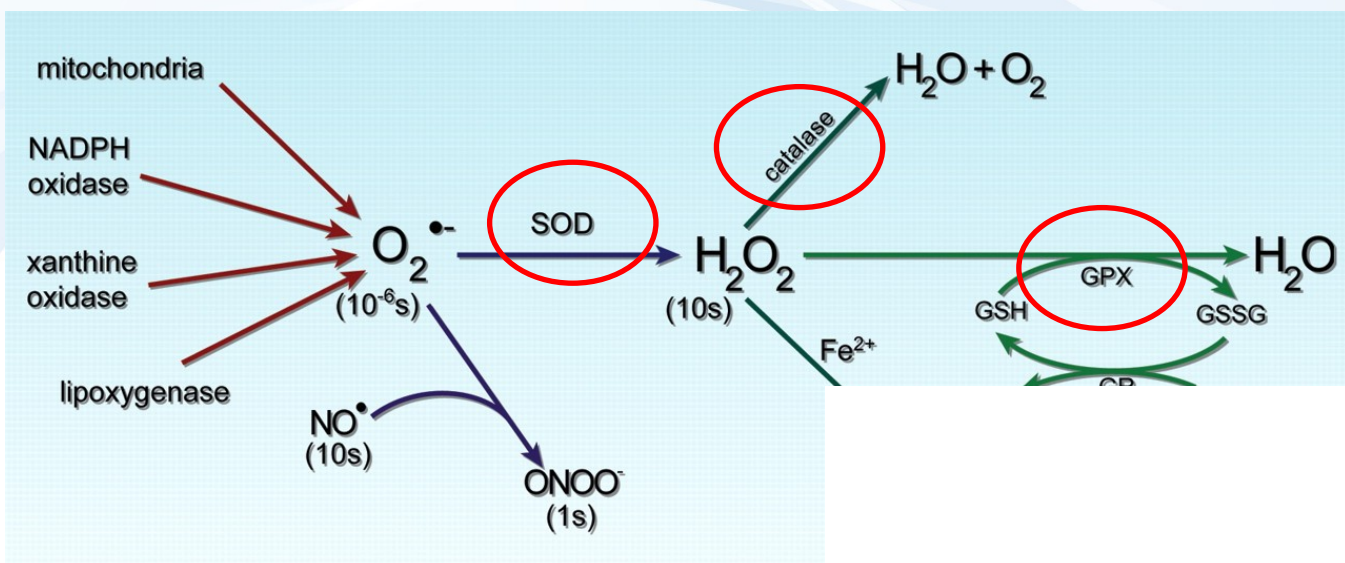


# Protection against ROS-induced damage



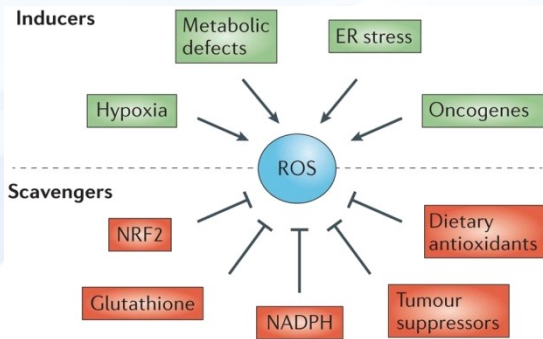


# Antioxidant responses 1 - enzymatic

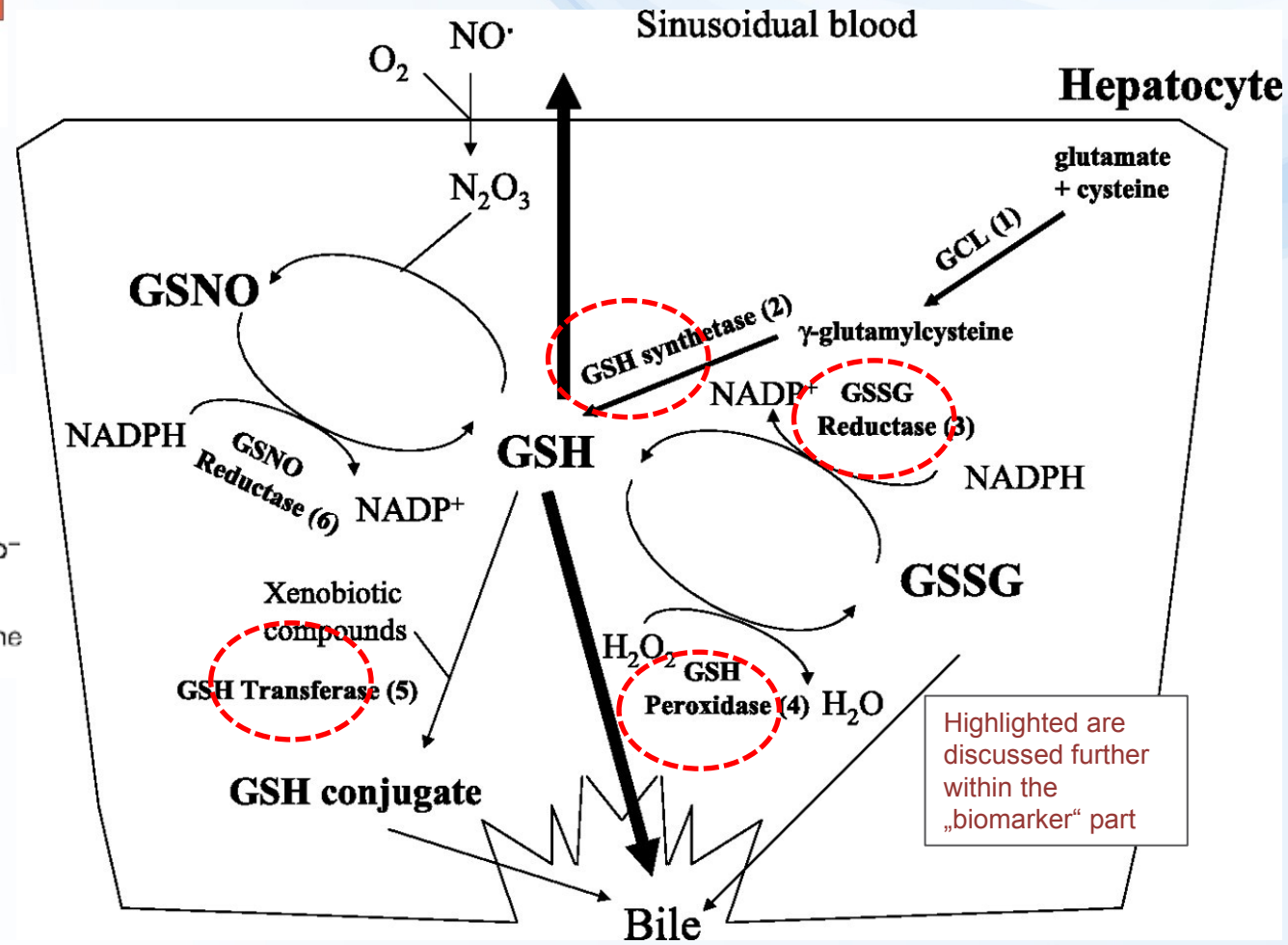
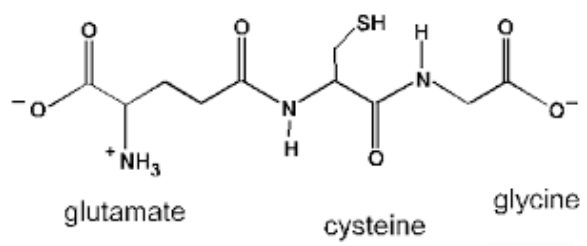


Highlighted enzymes discussed further within the „biomarker“ part

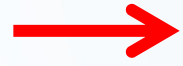
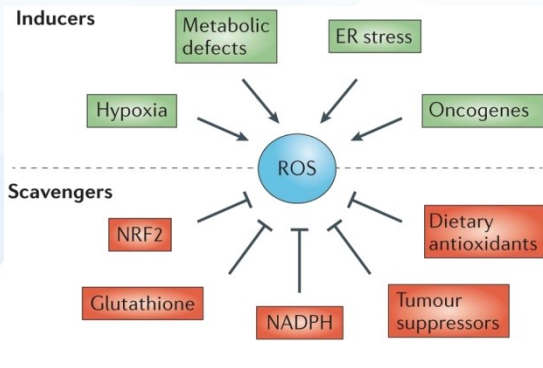
# Antioxidant responses 2 – small molecules: **GSH**



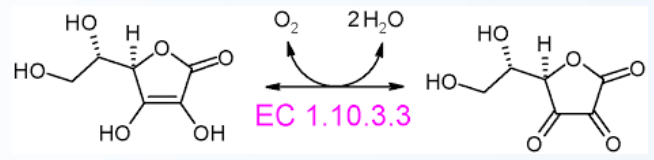
glutathione (GSH)



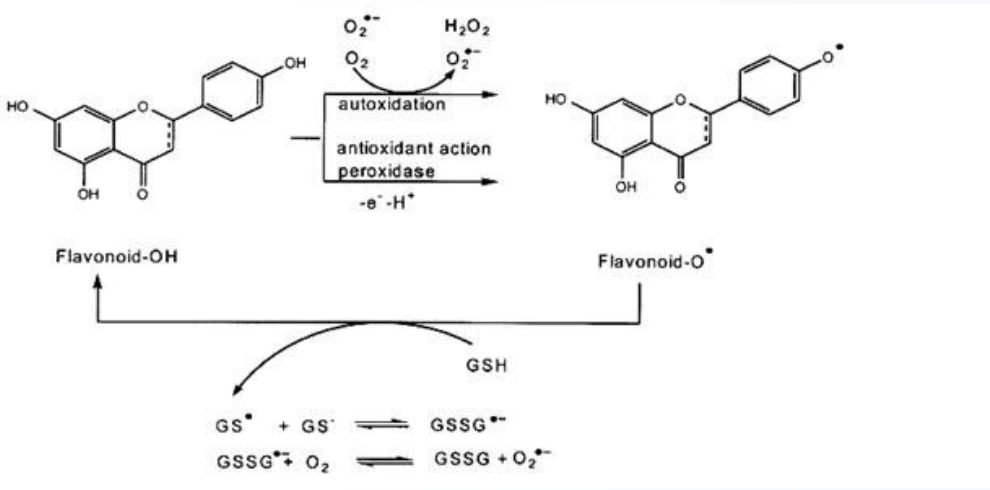
# Antioxidant responses 2 – small molecules: **dietary antioxidants**



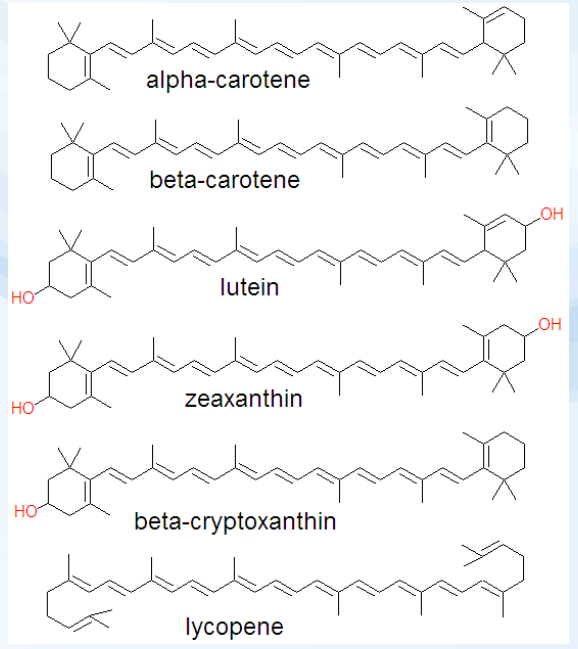
## Ascorbate



## Flavonoids



## Carotenoids



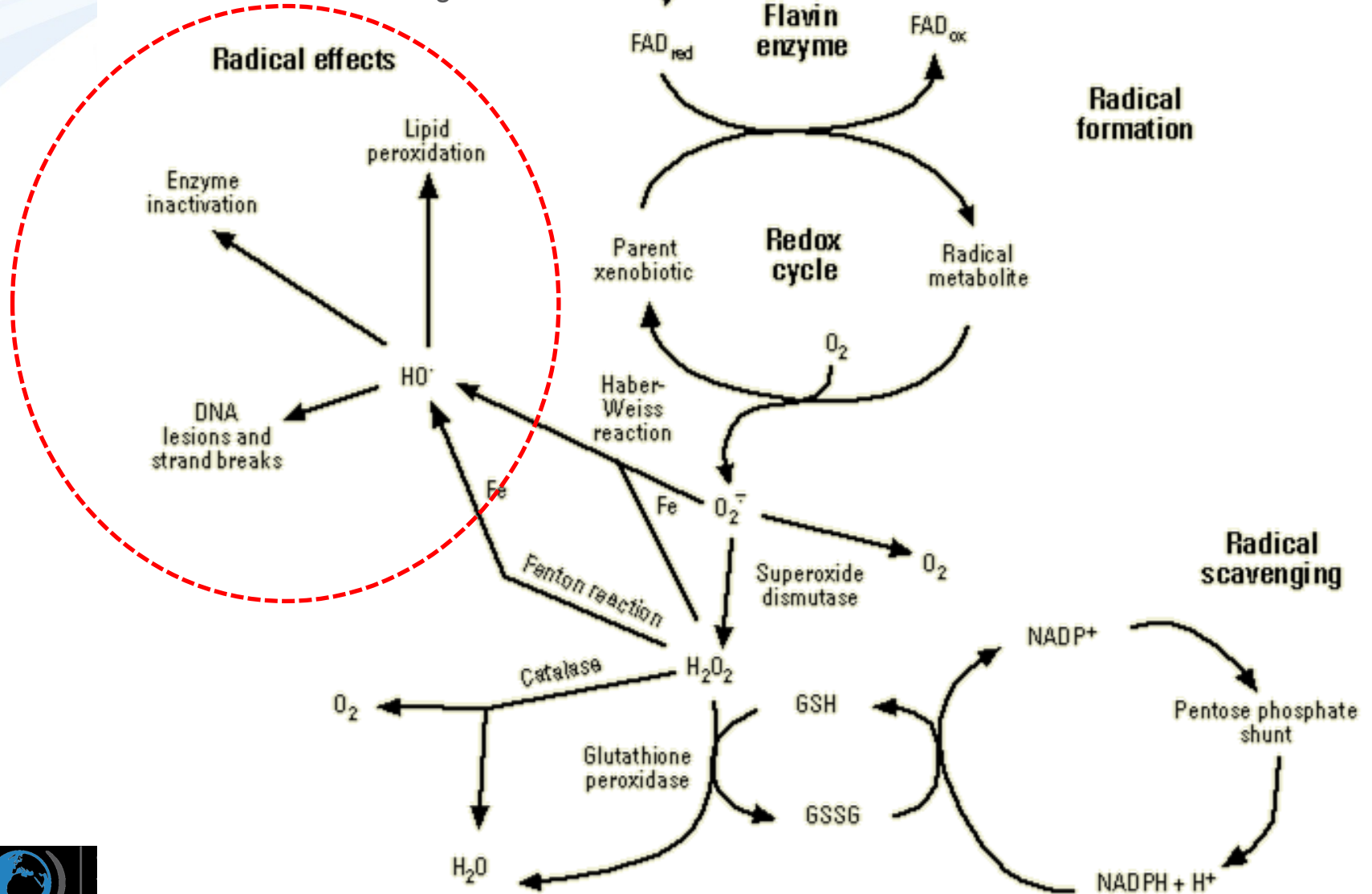
# EFFECTS

## Enzymes & DNA

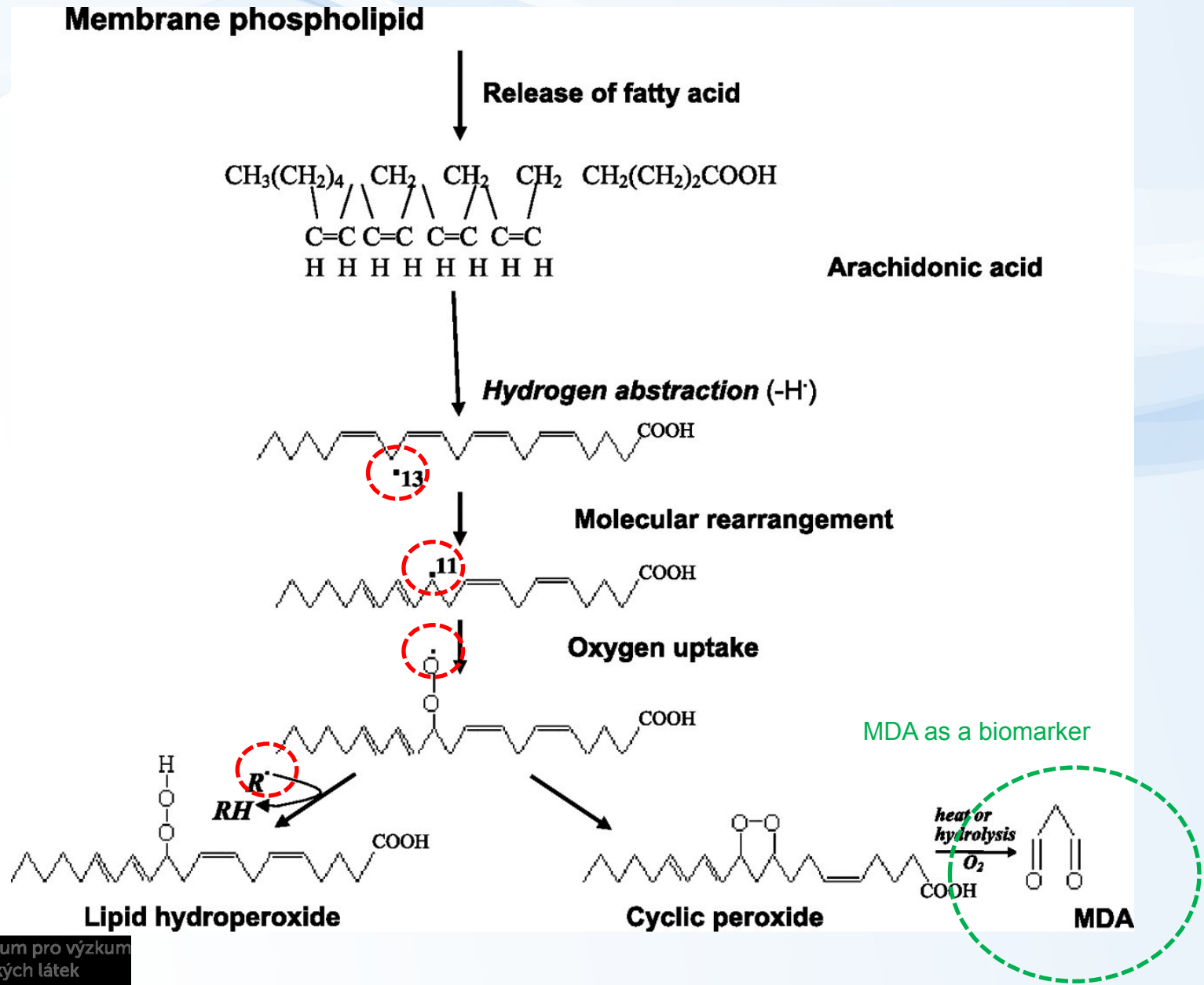
→ Other lectures

## Lipid peroxidation

→ Following slide



# Lipid peroxidation = radical reaction → fast propagation



## Biomarkers of oxidative damage

(will be discussed later)

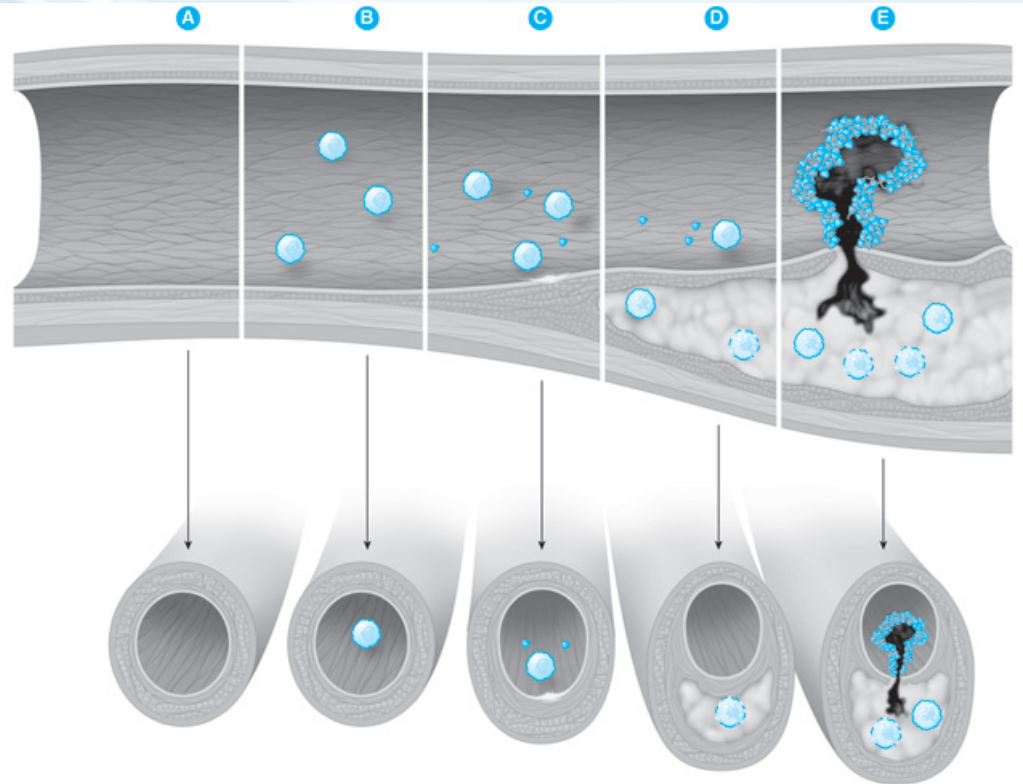
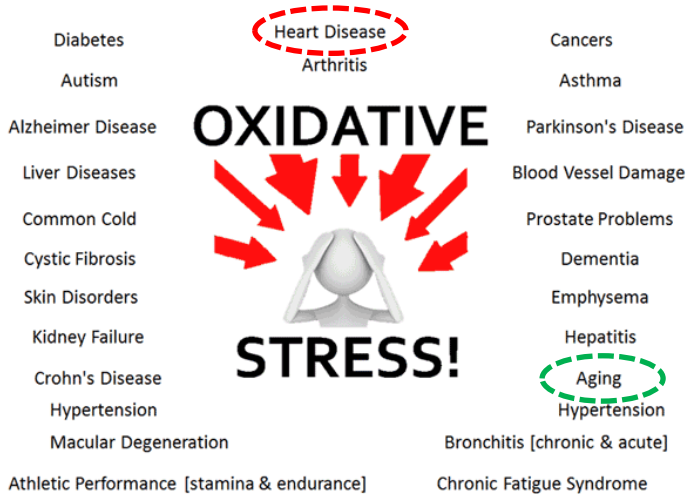
BIOMARKER	AVAILABILITY	FREQUENTLY USED ASSAYS
<b>Lipid Peroxidation</b>		
F <sub>2</sub> -isoprostanes	Plasma, urine	GC/MS, HPLC-MS/MS
Oxidized low-density lipoprotein (oxLDL)	Plasma, serum	ELISA
Malondialdehyde (MDA)	Plasma, serum, saliva, urine, exhaled breath condensate	Colorimetry, spectrophotometry, HPLC + fluorescence, GC/MS
<b>Protein Oxidation</b>		
Protein carbonyls	Plasma, serum	ELISA
<b>DNA Oxidation</b>		
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

## Diseases Related to Oxidative Stress



**Figure 24-7. Pathogenesis of acute coronary 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. *Circulation* 2001;104:365–372.