Intracelulární enzymatické zdroje volných radikálů

NADPH Oxidázy
Myeloperoxidáza

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Biological Sources of Oxidants/Radicals

$$O_2 \xrightarrow{e^-} O_2^{\bullet -} \xrightarrow{e^-} H_2O_2\ (O_2^{\,2-}) \xrightarrow{e^-} {}^{\bullet}OH \xrightarrow{e^-} H_2O$$

Diatomic oxygen — **Superoxide** — **Hydrogen Peroxide** — **Hydroxyl Radical**

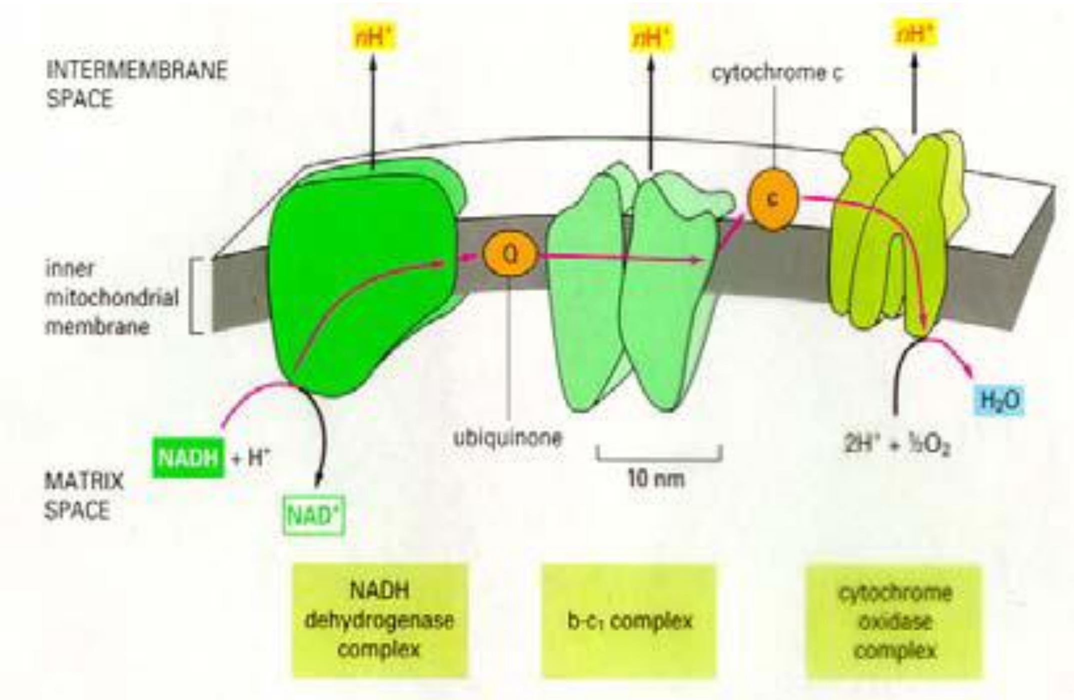
- **Leukocyte NADPH oxidase**
- **Non-Phagocytic NADPH oxidases (Nox family)**
- **Xanthine Oxidase**
- **Cytochrome P450 enzymes**
- **Uncoupled NO synthase**

Superoxide

- **Mitochondria - electron respiratory chain leak**
- **Cyclooxygenase**
- **Lipoxygenase**
- **Heme oxygenase**

ROS generally

• Mitochondria - electron respiratory chain leak



Complex I, II, III,IV

– Function is to reduce O_2 to H_2O

• Complex I (NADH-Ubiquinone reductase complex),
• Complex II (succinate dehydrogenase complex).
• Ubiquinone, also known as coenzyme Q, accepts electrons from both complexes and is sequentially reduced, one electron at a time, to ubisemiquinone and ubiquinol
• Complex III (ubiquinol-cytochrome c reductase)
• Complex IV (cytochrome c oxidase)

• Mitochondria - electron respiratory chain leak

Cytochrome oxidase is estimated to account for 90-95% of the total oxygen uptake in most cells

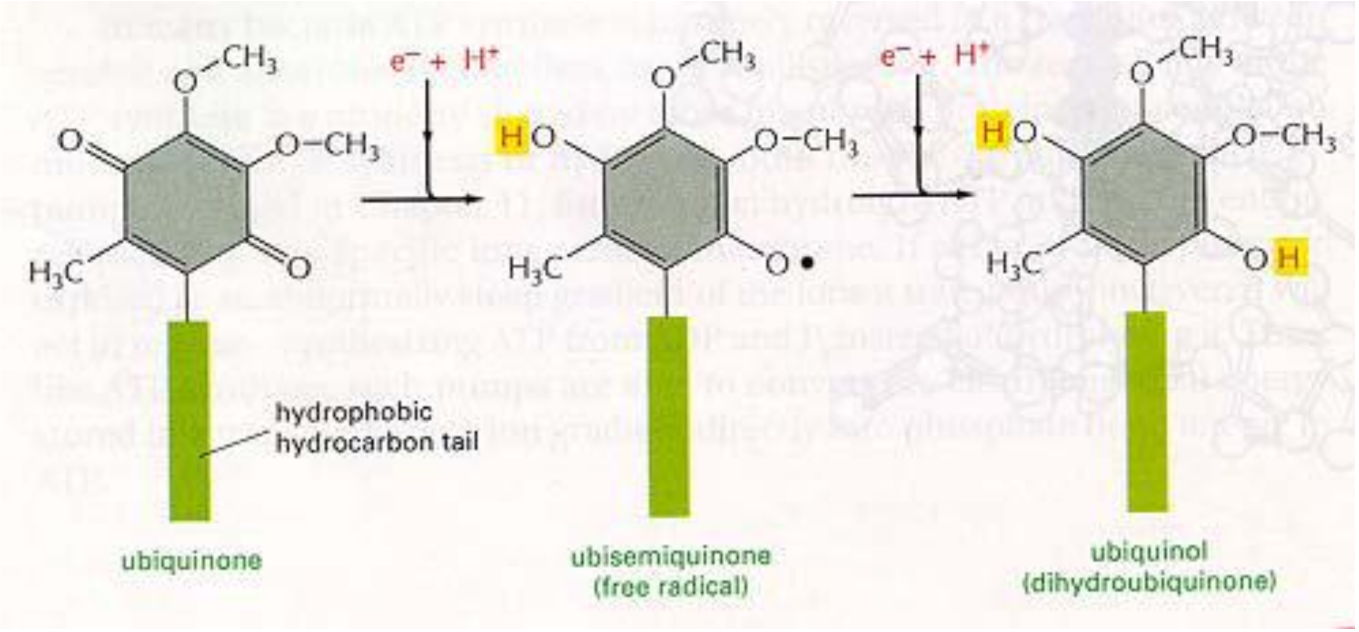
- What happens to other 5-10%

- formation of free radicals

Which complex is responsible for free radical leak?

- This electron is thought to come from the one-electron reduction of ubiquinone.

- Instead of accepting another electron and proton to form ubiquinol, ubisemiquinone may leak its unpaired electron to O_2, forming $O_2^{\bullet -}$.



Xanthine Oxidase/Dehydrogenase

Flavoprotein enzyme containing iron and molybdenum that promotes the oxidation especially of hypoxanthine and xanthine to uric acid and of many aldehydes to acids

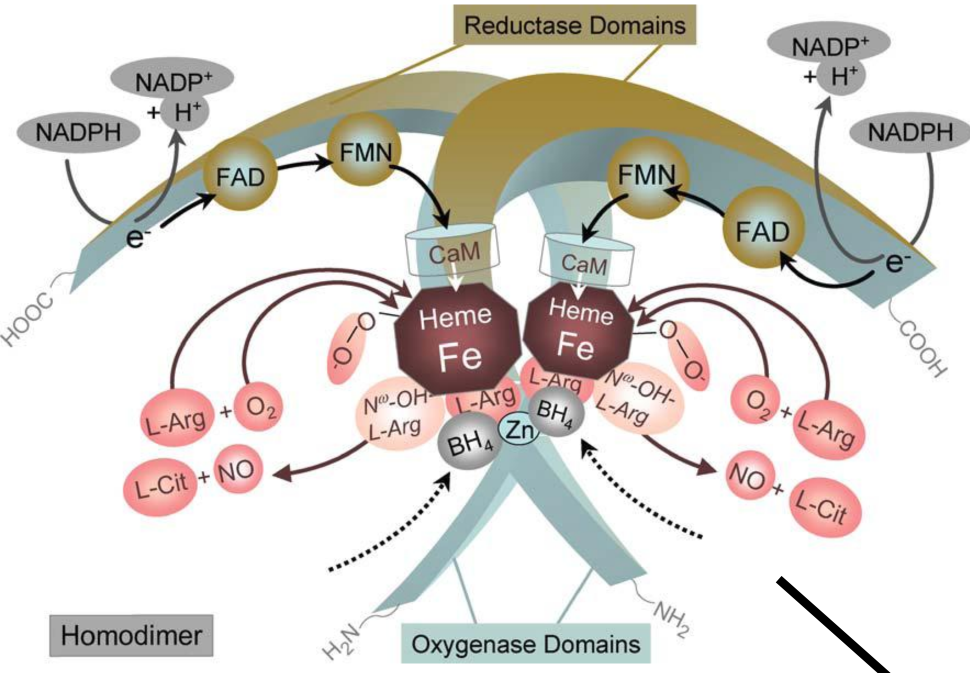
Xanthine $\xrightarrow{\textit{Xanthine dehydrogenase (XD)}}$ **NADH + Uric Acid**

Hypoxia
Cytokines → **XD** → **XO** ↑

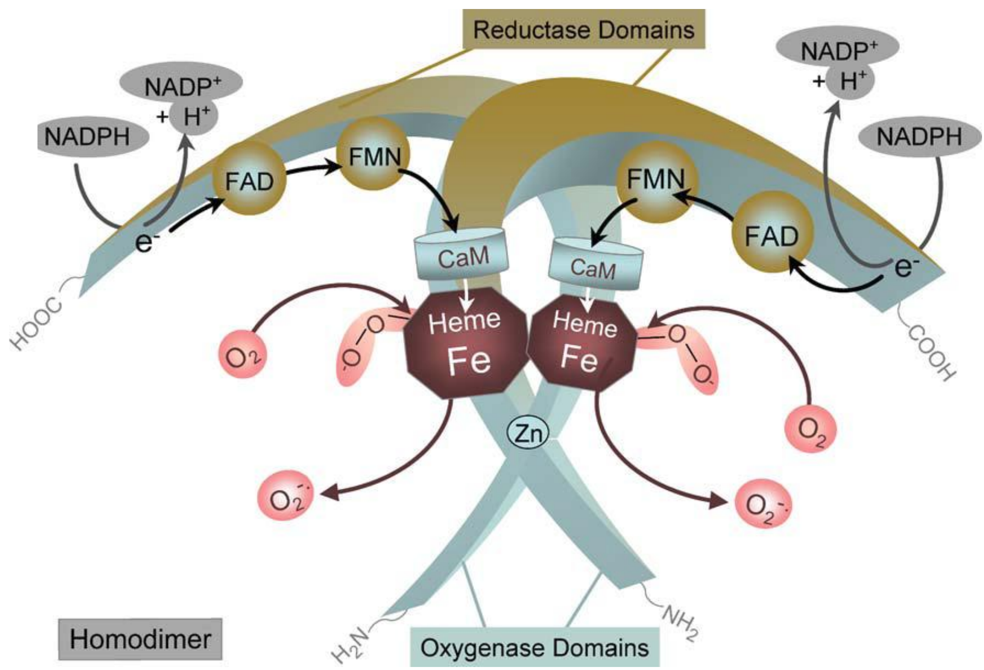
Hypoxanthine $\xrightarrow{\textit{Xanthine oxidase (XO)}}$ $O_2^{\cdot -}$ **+ Uric Acid**

Xanthine $\xrightarrow{\textit{Xanthine oxidase (XO)}}$ $O_2^{\cdot -}$ **+ Uric Acid**

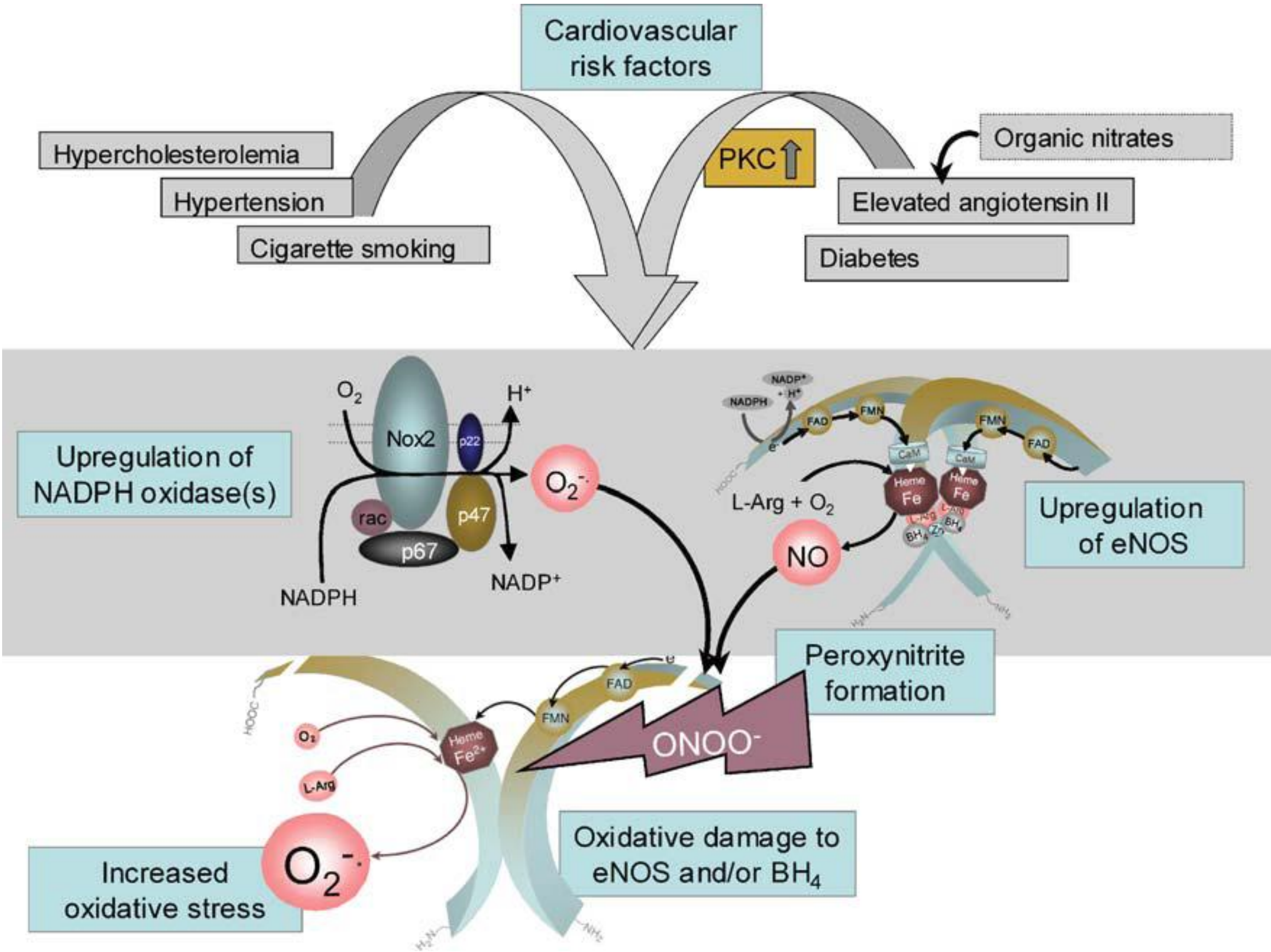
Uncoupling of NO synthase



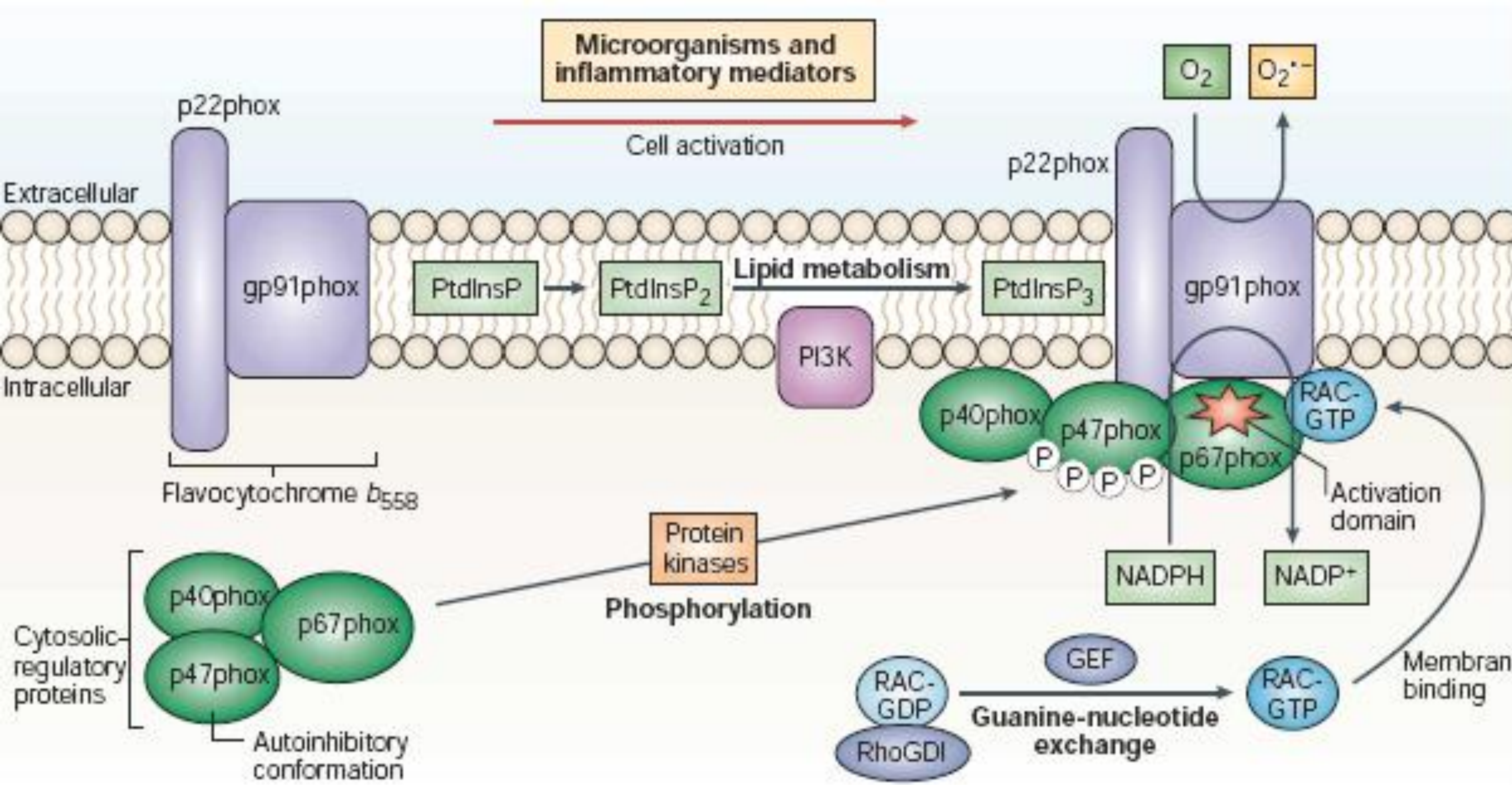
Oxidative stress



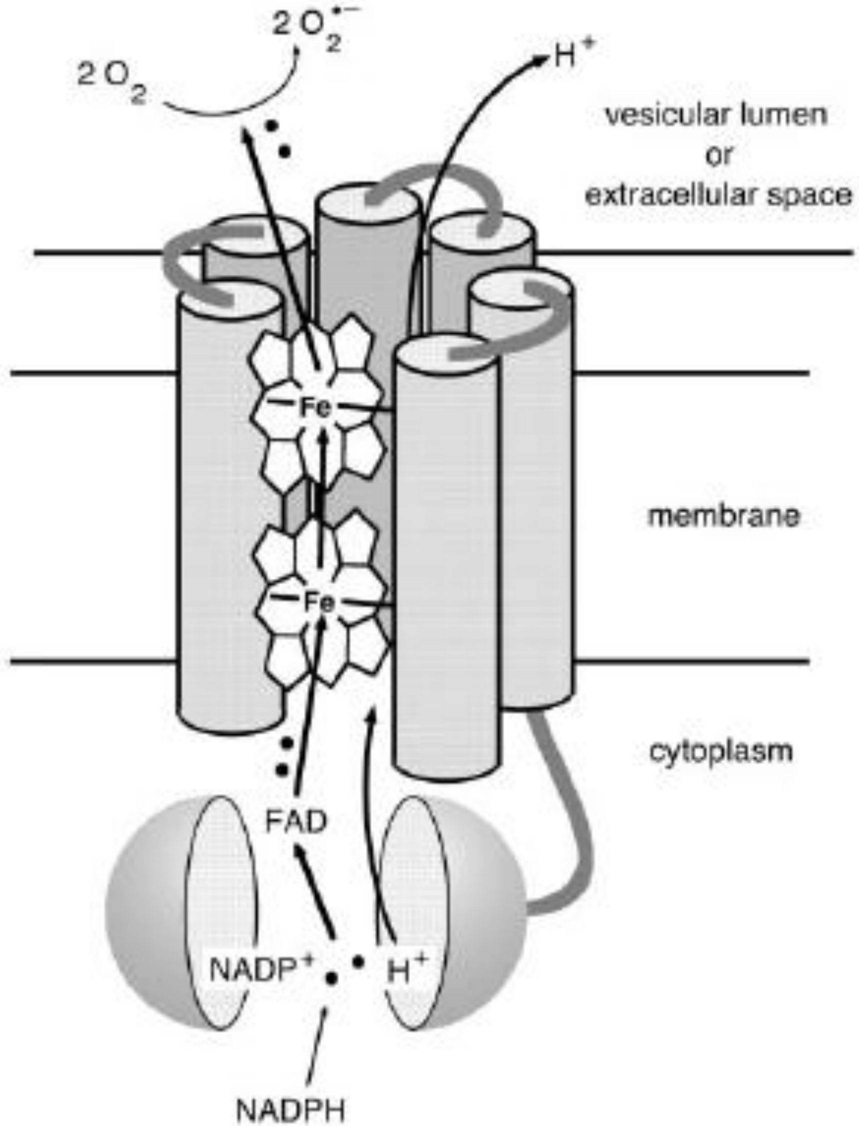
Pathological conditions leading to eNOS uncoupling



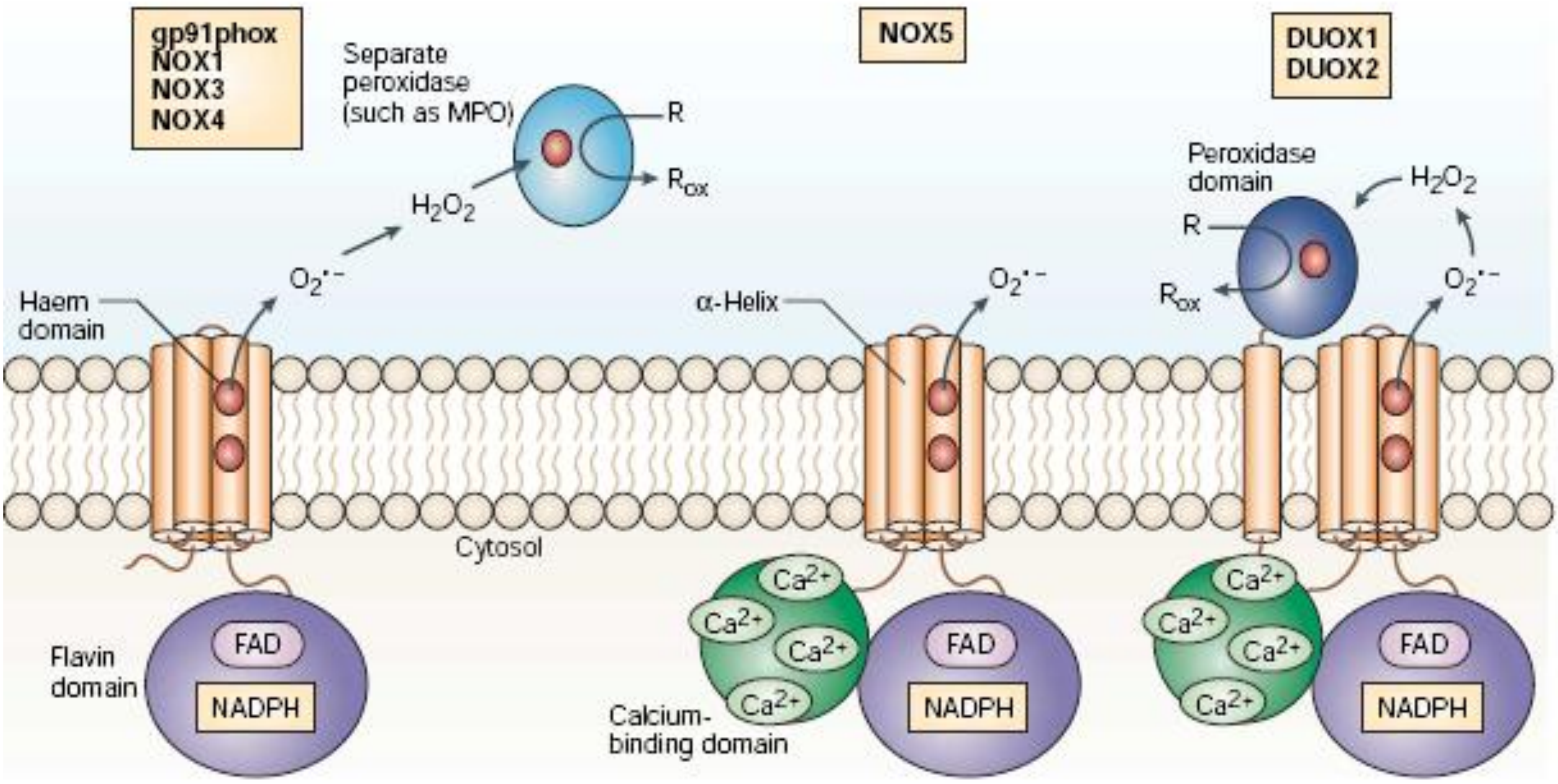
Struktura a aktivace fagocytární NADPH oxidázy (NOX2)



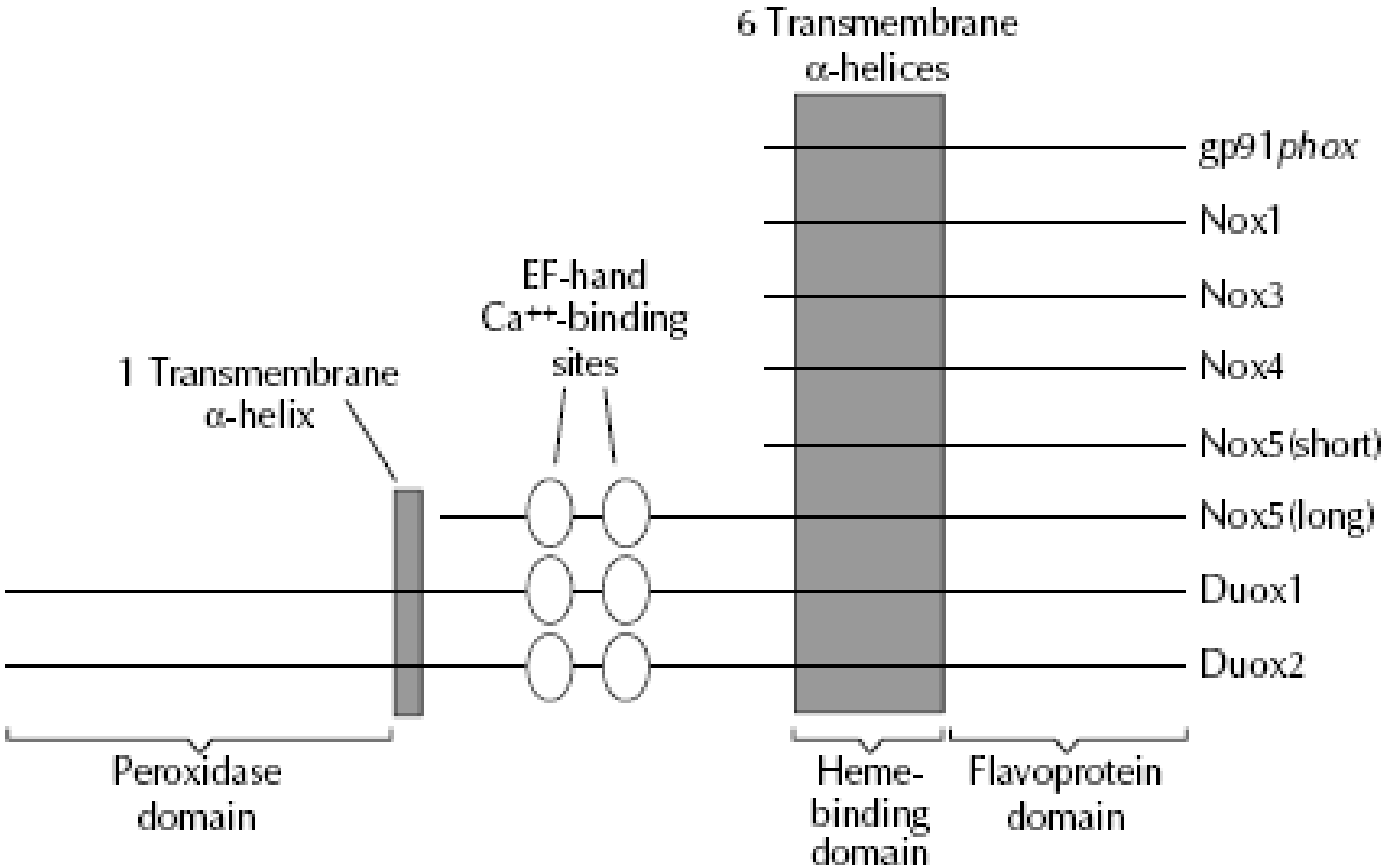
Transport elektronů NADPH oxidázou



Homology NADPH oxidáz a jejich struktura



Homology NADPH oxidáz a jejich struktura



Přehled homologů NADPH oxidáz

Table 1 | **Human NOX/DUOX enzymes**

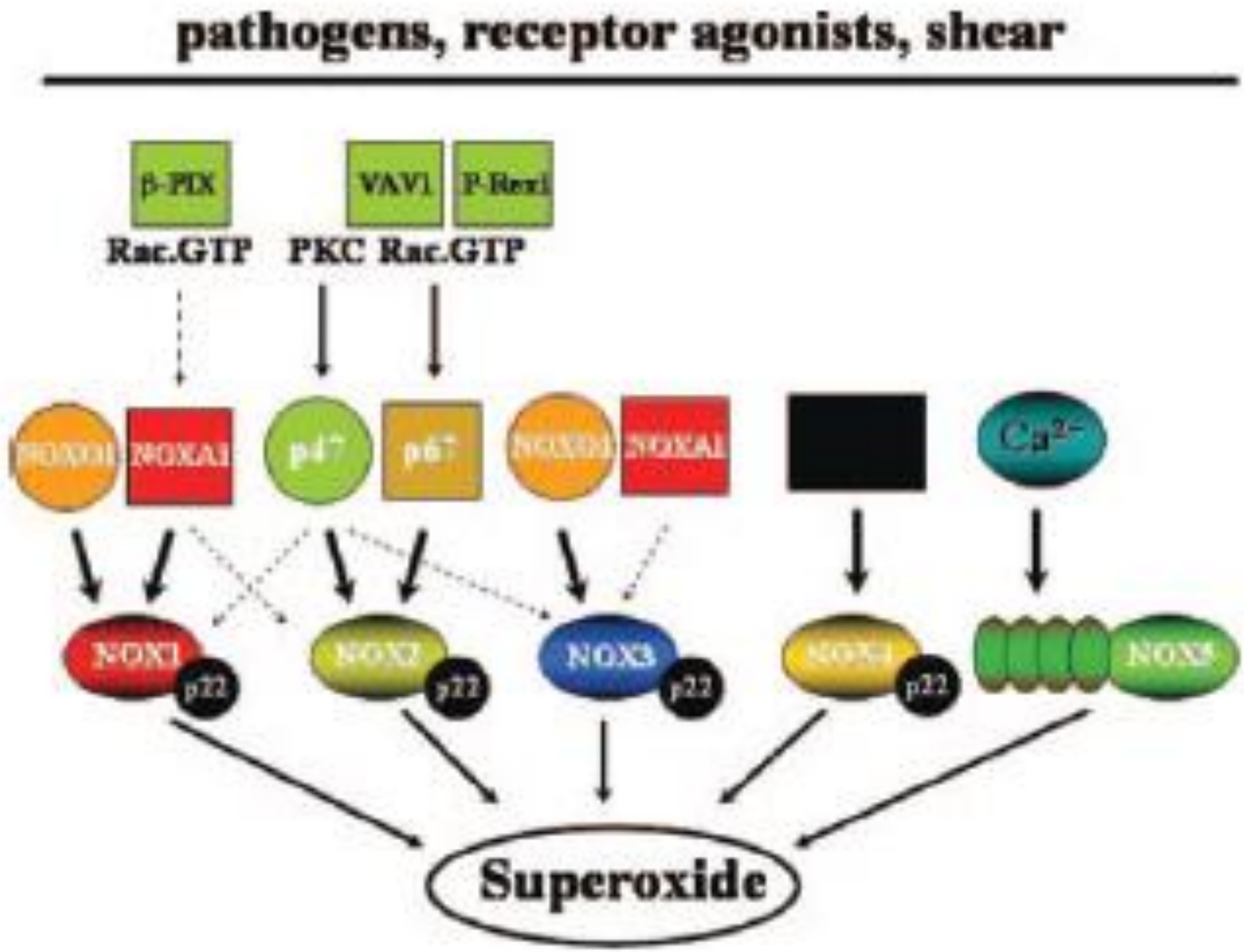
Enzyme	Highest level of expression	Known regulatory factors	References
gp91phox (NOX2)	Phagocytes	p47phox, p67phox, p40phox and RAC1/RAC2	14
NOX1	Inducible: colon and vascular smooth muscle	NOXO1, NOXA1 and p22phox	3,15,20,21
NOX3	Fetal kidney	N.D.	4,68
NOX4	Kidney, osteoclasts, ovary and eye; widespread	N.D.	6,68
NOX5	Spleen, sperm, mammary glands and cerebrum	Calcium	11,68
DUOX1	Thyroid, cerebellum and lungs	Calcium	4,69
DUOX2	Thyroid, colon, pancreatic islets and prostate	Calcium	13,69

DUOX, dual oxidase; N.D., not determined; NOX, NAPDH oxidase; NOXA1, NOX activator 1; NOXO1, NOX organizer 1.

Prokázaná existence řady orthologních NADPH oxidáz u myší, krys, Drosophil, Caenorhabditis elegans, a Diclostelium.

NADPH oxidázy také objeveny u kvasinek a rostlin

Aktivace NADPH oxidáz a jejich podjednotky



Předpokládané funkce NADPH oxidáz

- Obranná funkce
(fagocyty, střevní, plicní, ledvinný epitel, keratinocyty)

- Signální transdukce
(mitogení stimulace, apoptóza, senescence)

- Metabolismus látek
(biochemické reakce spojené se syntézou thyroidních hormonů a přestavbou kostí)

-**Regulace krevního tlaku v cévním sytému**

- Snímání koncentrace kyslíku v kůře ledvin

Myeloperoxidase

- Heme peroxidase ~ 150 kD

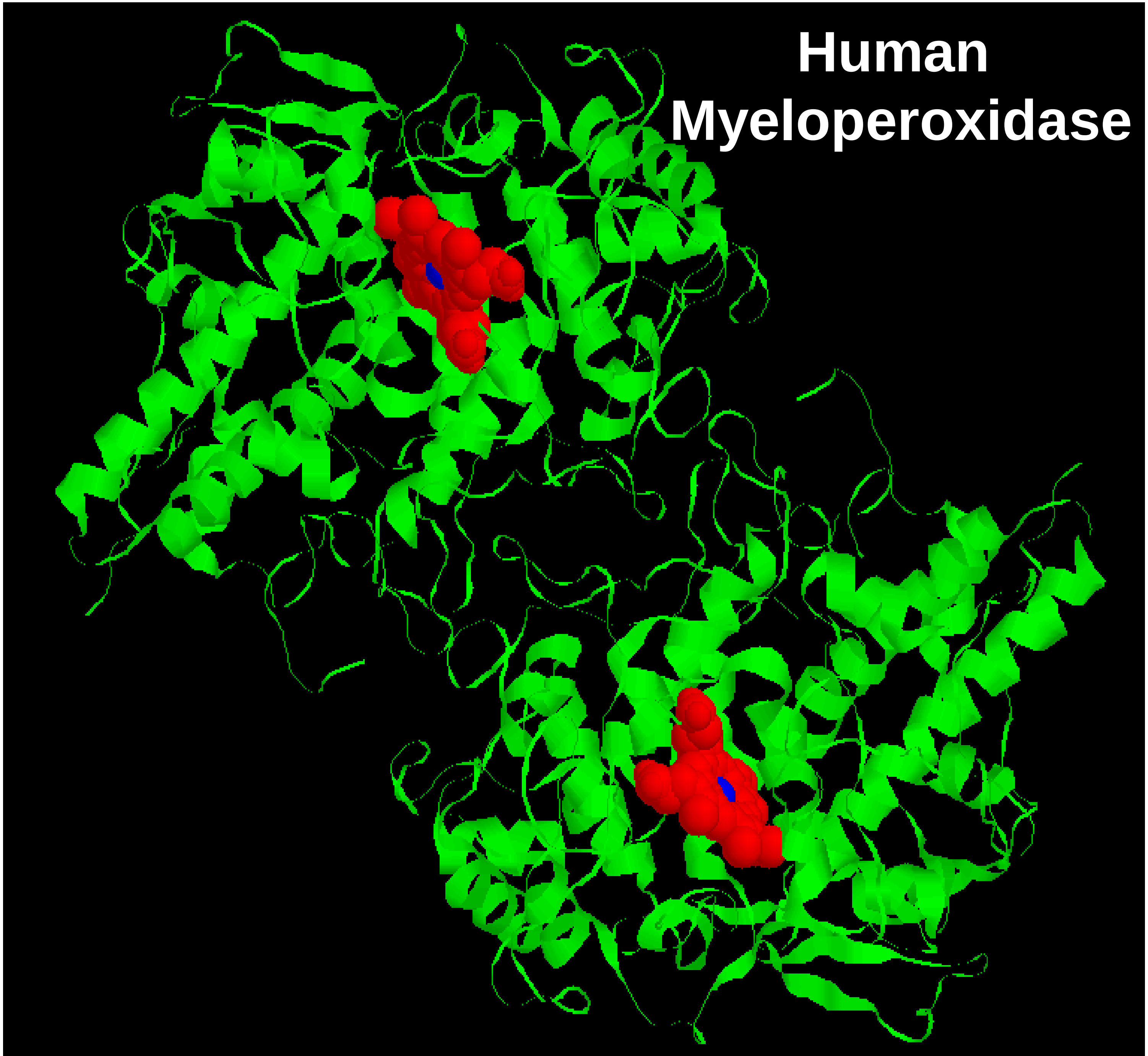
- Pair of protomers - α (heavy) subunit and β (light) subunit

- α subunit - two hemes and mannose-reach carbohydrate

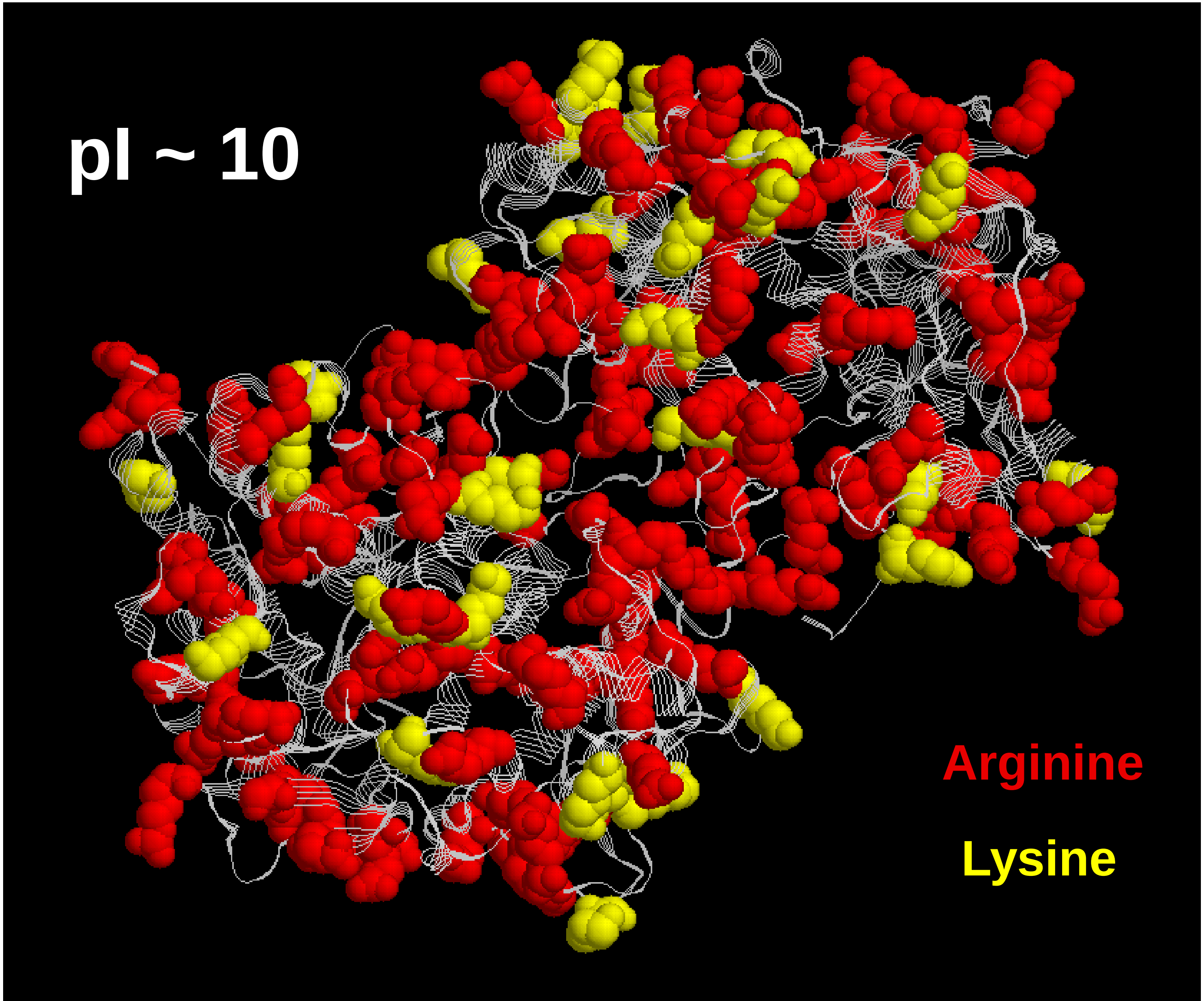
- Single gene located on chromosome 17

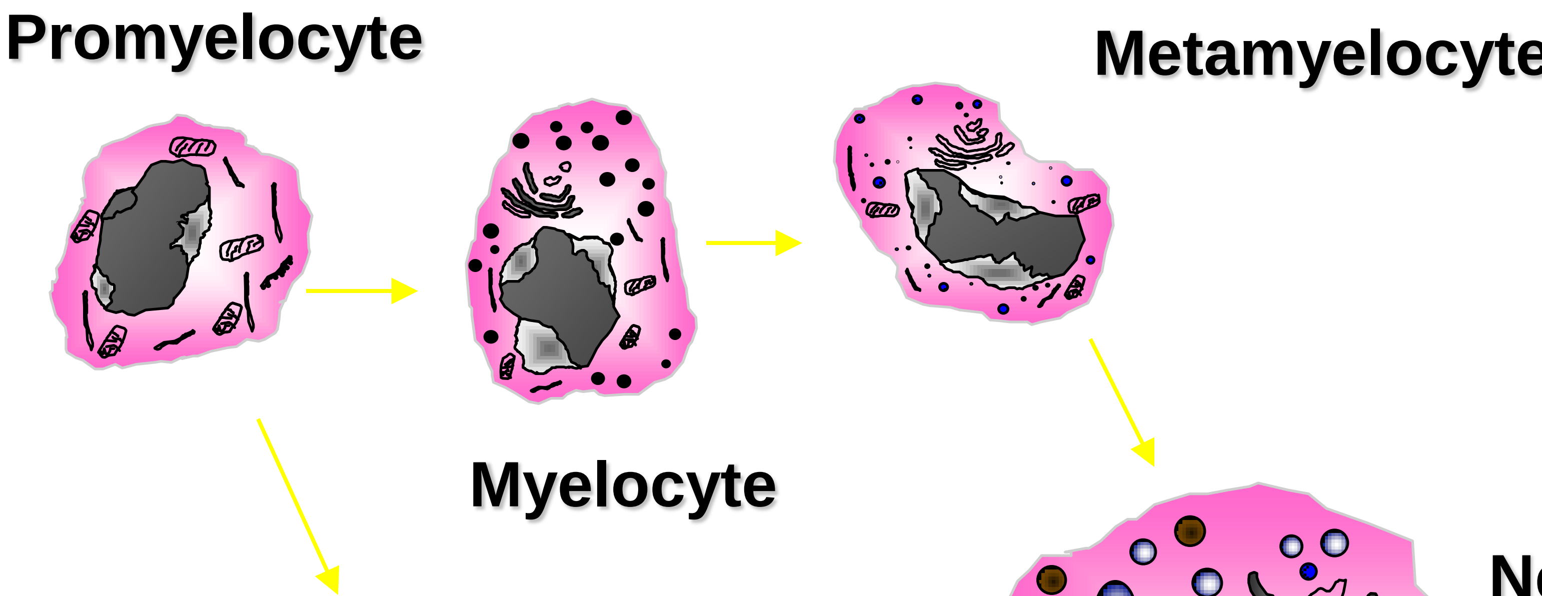
- MPO is up to 5% of total neutrophil proteins

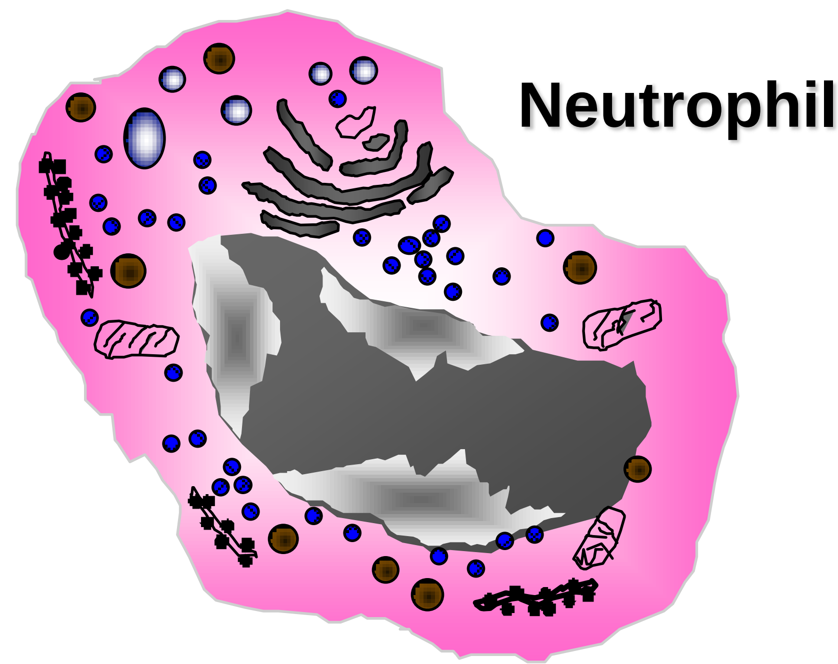
- High quantities of MPO are released and accumulated at the site of acute inflammation


Human
Myeloperoxidase

MPO is a Highly Cationic Protein







- Blood Monocytes

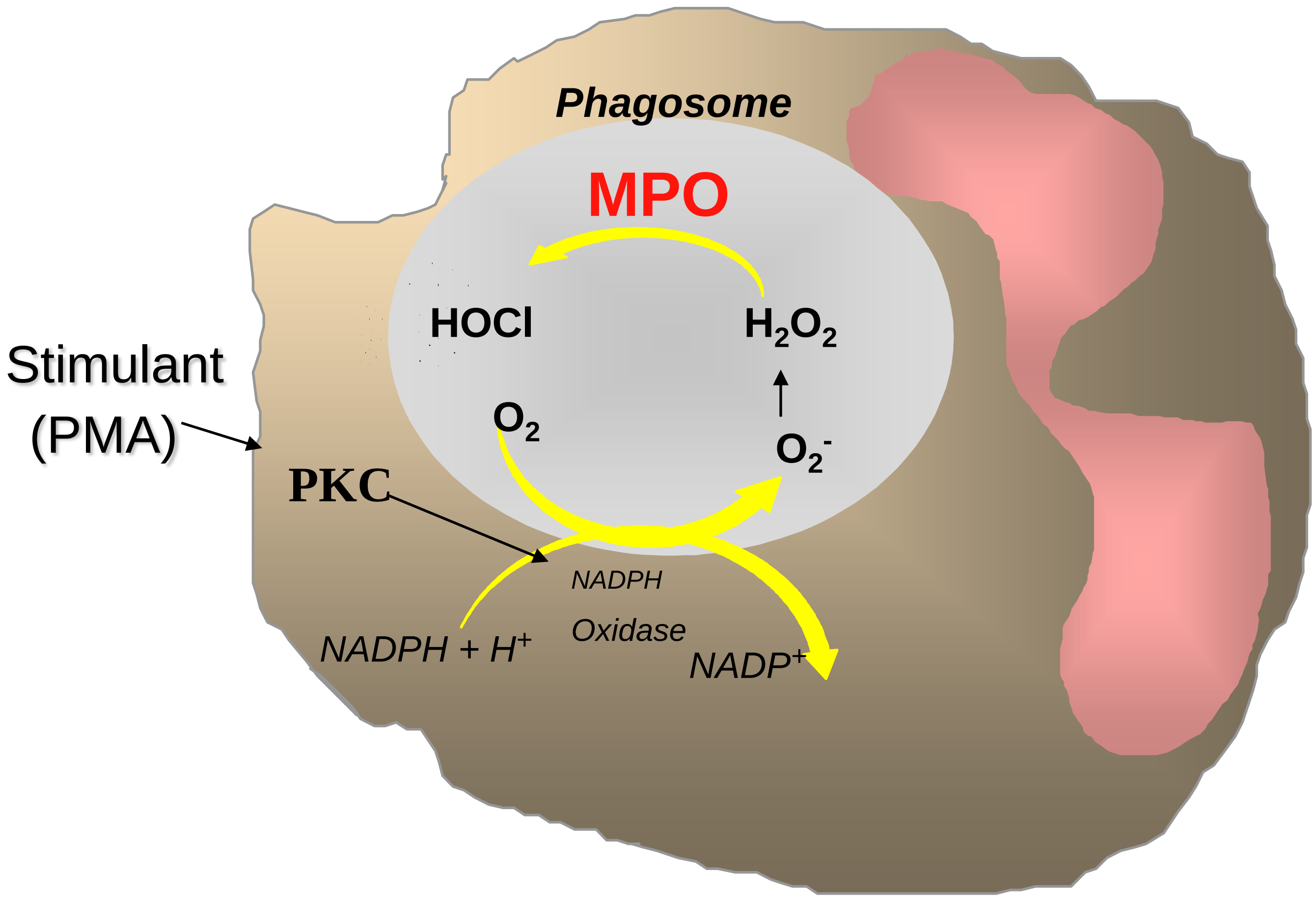
Tissue macrophages

- Kupffer cells

- Alveolar macrophages

- Microglia …

Oxidative Burst of Neutrophil



Phagocytes Utilize Myeloperoxidase to Form Bleach

$$\mathrm{MPO} \xrightarrow{H_2O_2} \mathrm{MPO\text{-}I} \xrightarrow{Cl^-} \mathrm{HOCl}$$

- Host Defense
- Tissue Injury

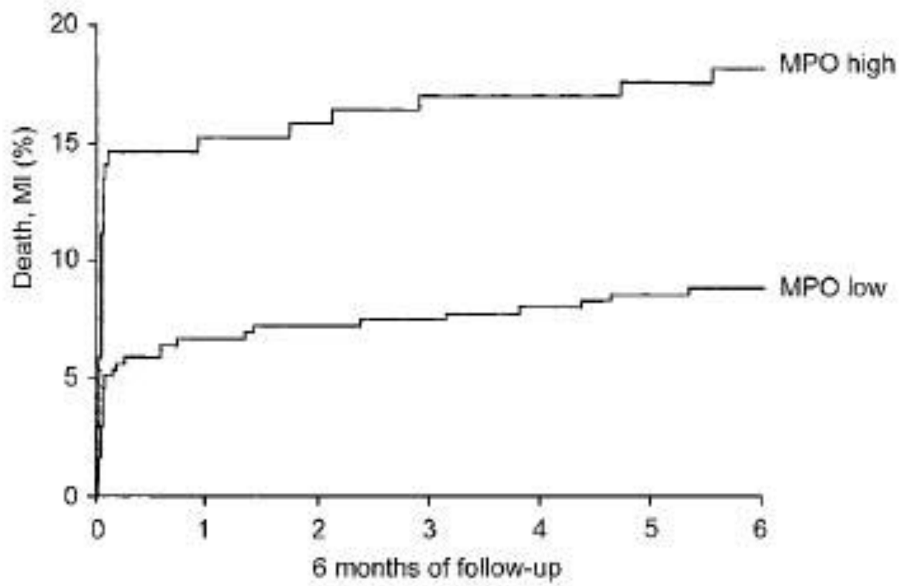
Chronic inflammation

MPO is an important factor in the pathophysiology of various disorders connected with chronic inflammation

- cardiovascular diseases
- renal diseases
- asthma
- obstructive pulmonary disease
- ….

Myeloperoxidase & Vascular diseases

Baldus, et al. (2003). “Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes” **Circulation** 108:1440-1445



Brennan, M. L. et al. (2003). "Prognostic value of myeloperoxidase in patients with chest pain." **N Engl J Med** 349(17): 1595-604.

Potential mechanisms of MPO mediated alterations of physiological functions

- Posttranslational modifications of proteins
- Modulation of intracellular H_2O_2 pool
- Modulation of availability of biologically active lipids
- Catabolism of NO

Myeloperoxidase-catalyzed Protein Oxidation

- Dityrosine Protein Cross-links

Tyr• + Tyr• ---> Tyr-Tyr

- 3-Chlorotyrosine

HOCl + Tyr ---> Cl-Tyr

- 3-Nitrotyrosine

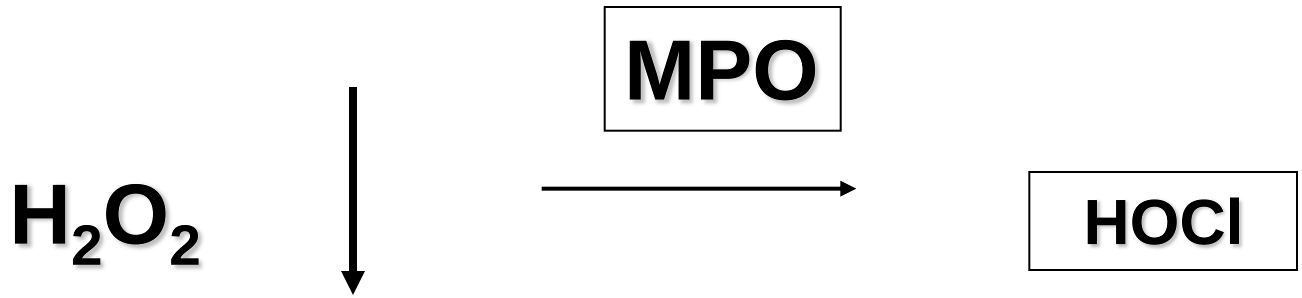
$\bullet NO_2$ + Tyr• ---> NO_2-Tyr

Myeloperoxidase-catalyzed Protein Activation/Inactivation

- Deactivation of proteins
  - Phagocytic NADPH oxidase
  - Chemotactic factors
  - Alpha1-proteinase inhibitor
  - Proteases (Matrix metalloproteinase 7)

- Activation of proteins
  - Proteases (collagenase, gelatinase)
  - MAP kinases
  - Tumor suppressor proteins

Modulation of Intracellular H_20_2 Pool



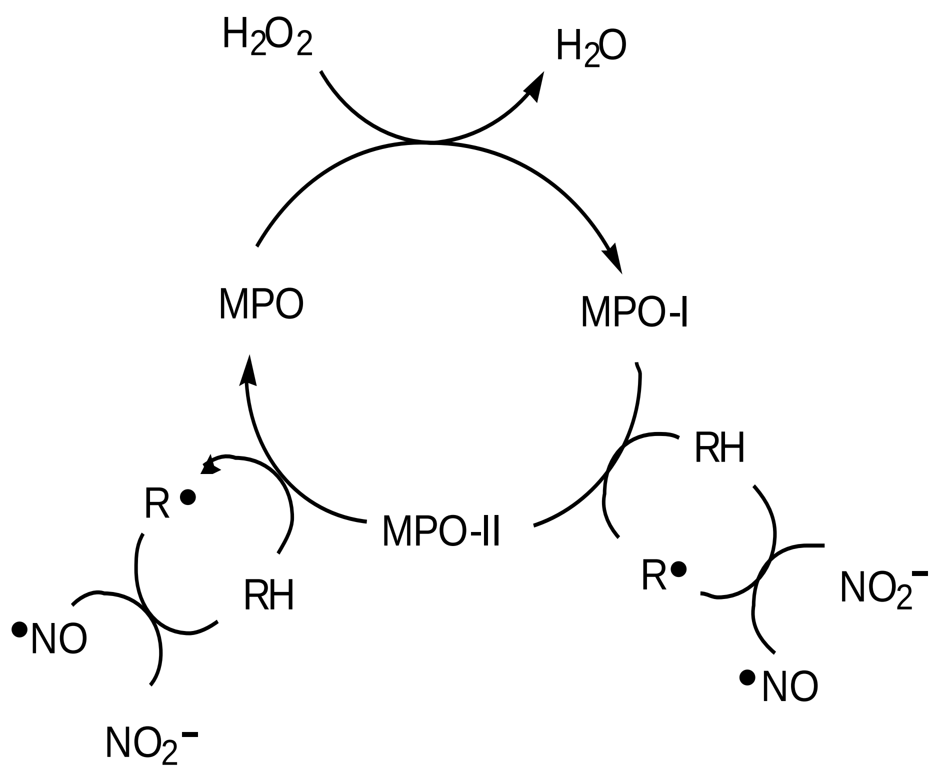
- Redox sensitive transcriptional factors

(gene expression)

- Enzymes with redox sensitive catalytic centers

(direct control of enzyme activity)

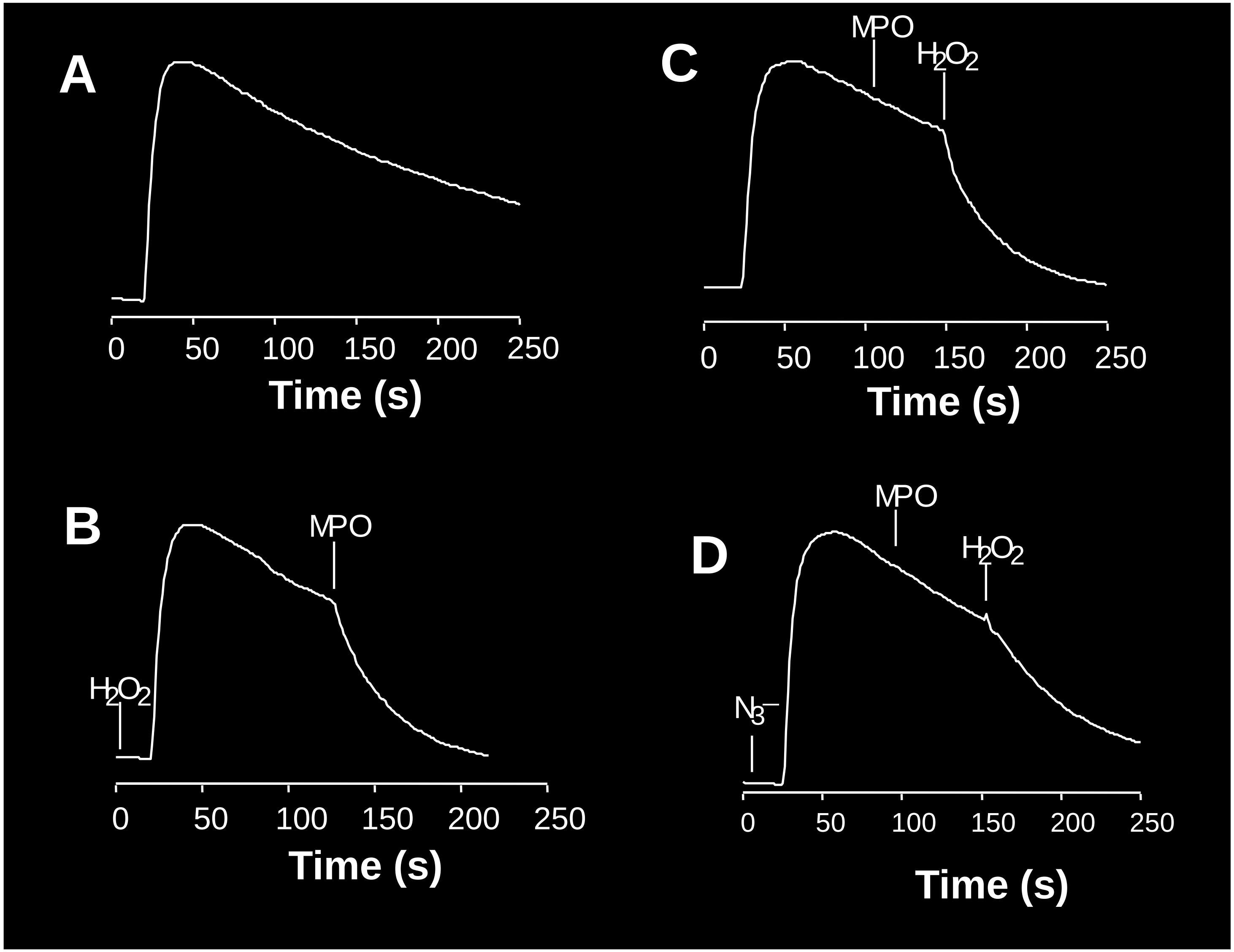
Radical-Mediated NO Consumption by MPO



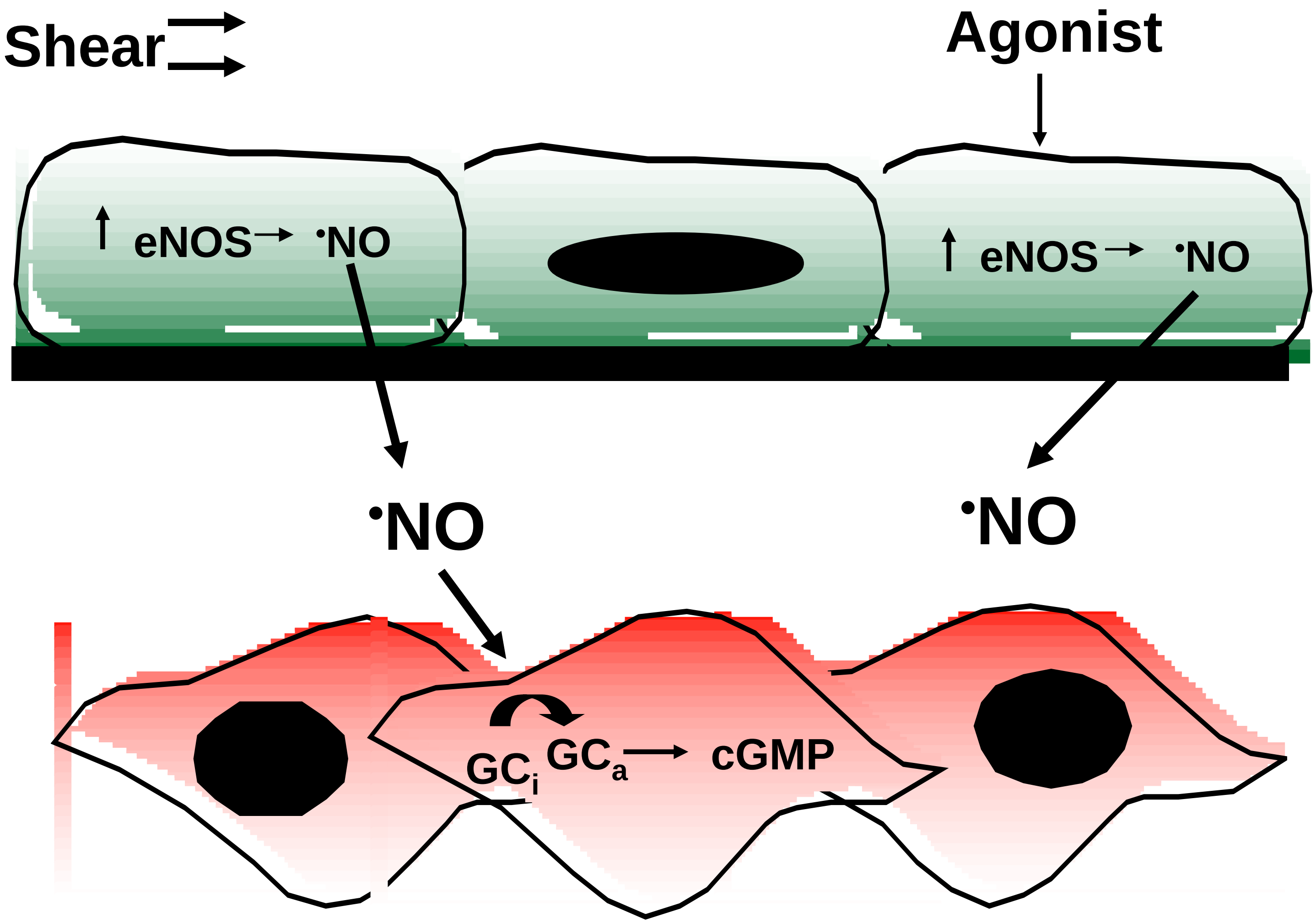
MPO is a catalytic sink for NO

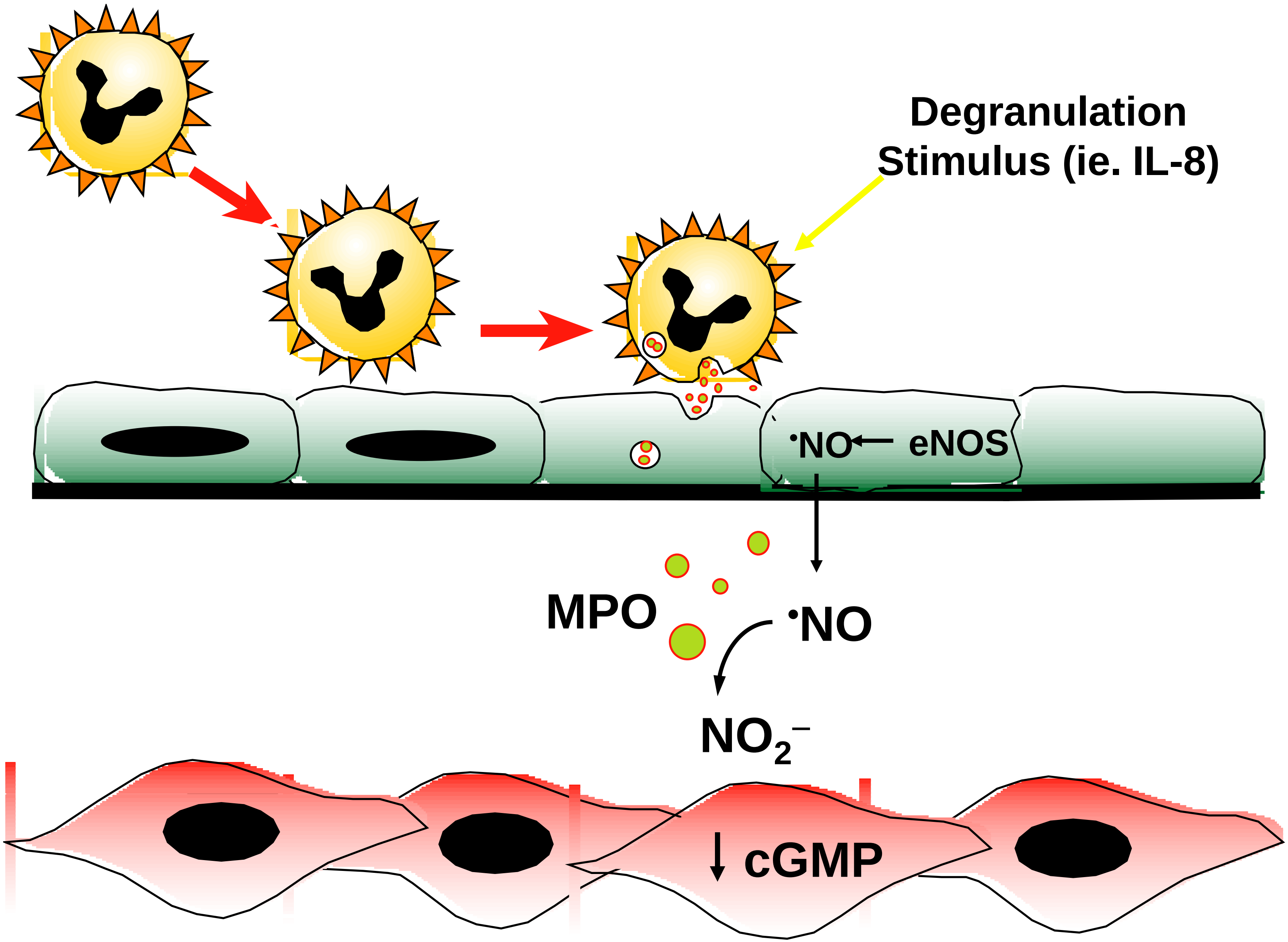
Eiserich et al. (2002) Science

Activated Heme Peroxidases Rapidly Consume •NO



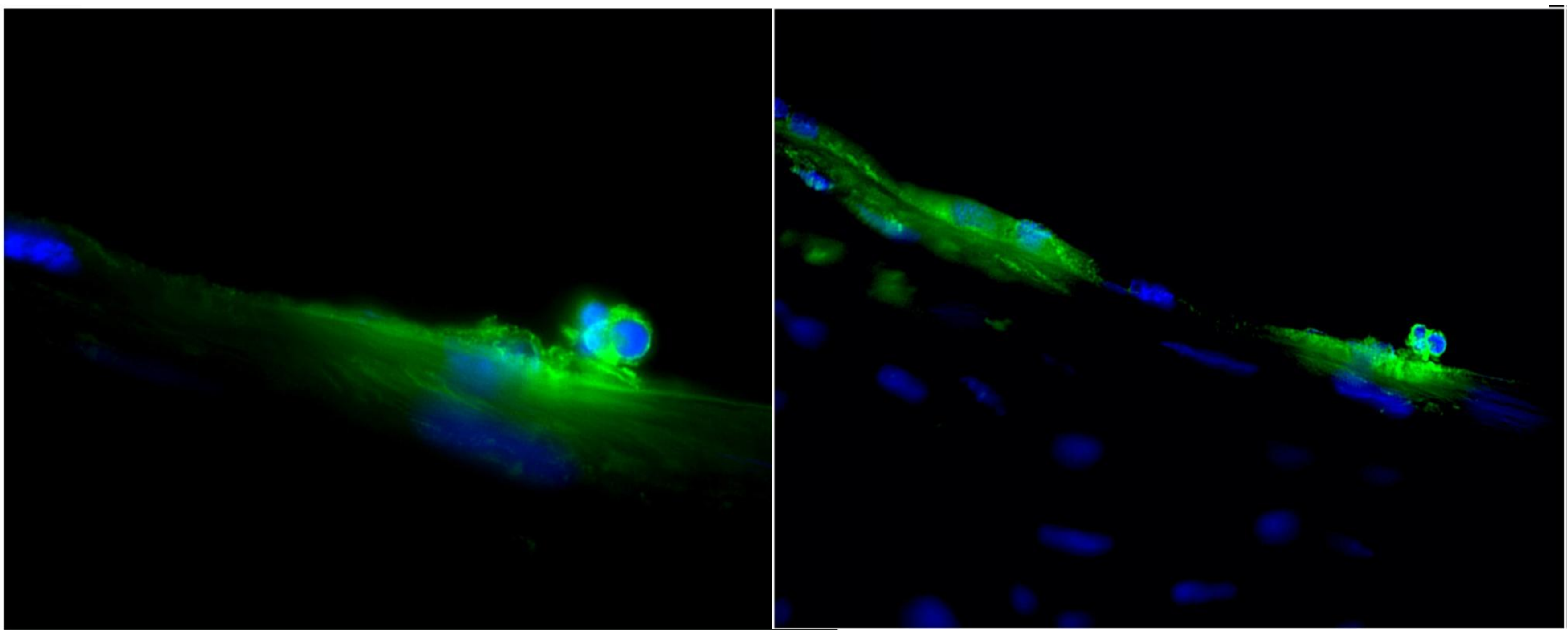
Nitric Oxide-Dependent Signaling in the Vasculature





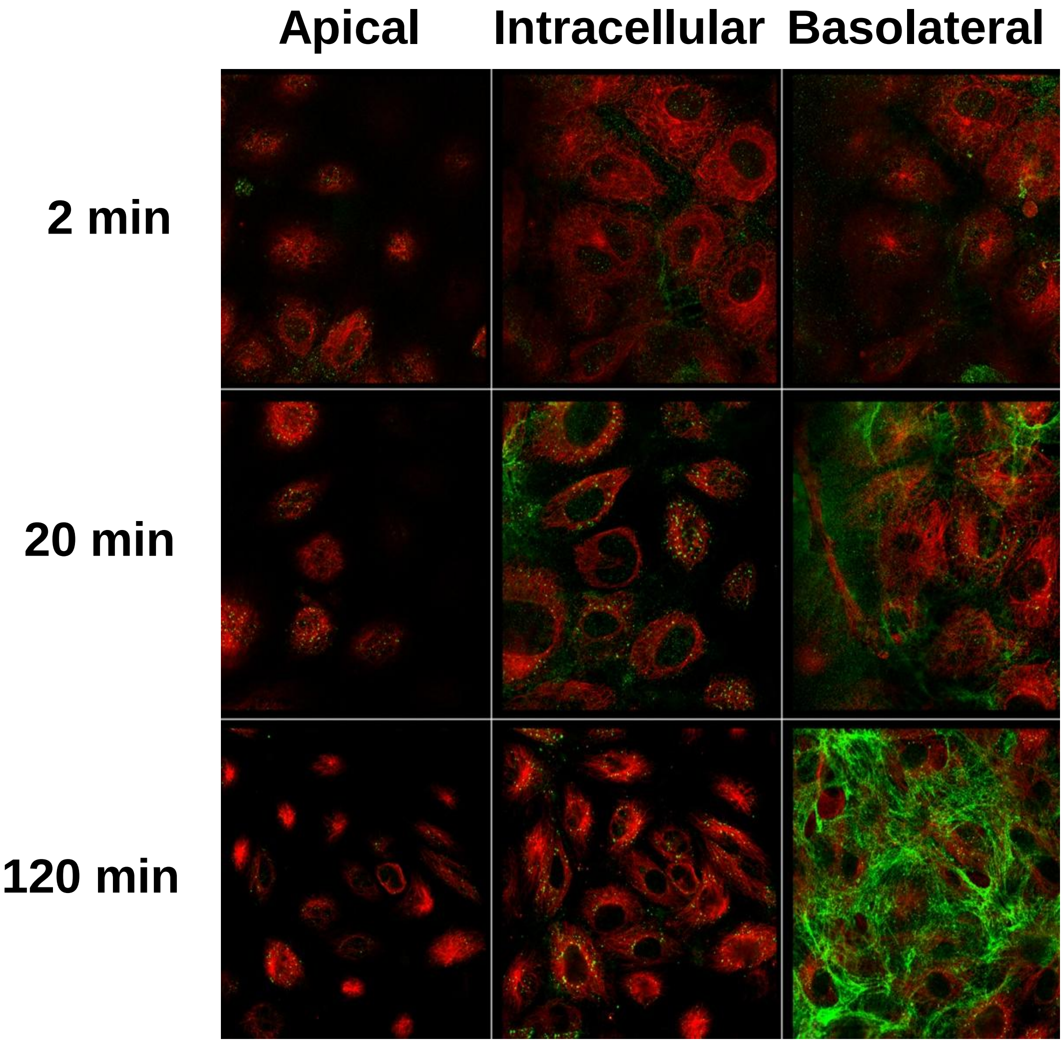
Degranulation
Stimulus (ie. IL-8)
•NO
eNOS
MPO
•NO
NO2–
↓ cGMP

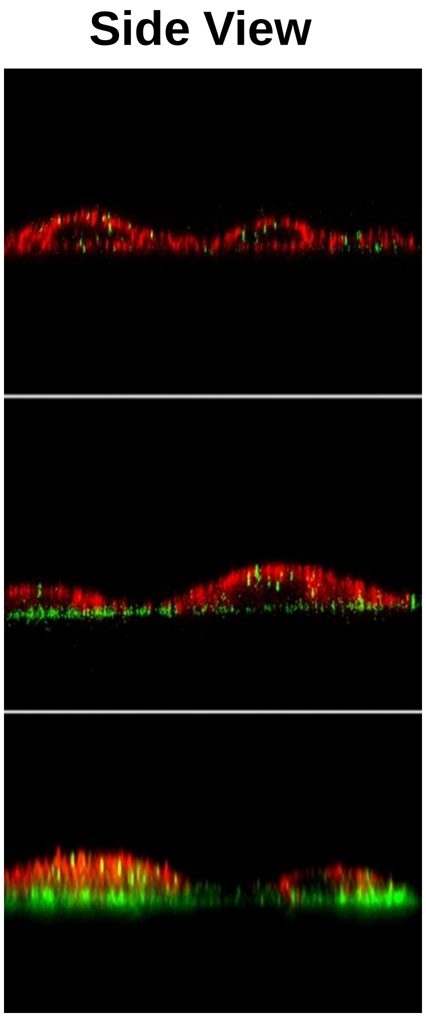
PMN Degranulation Results in Intimal Myeloperoxidase Localization



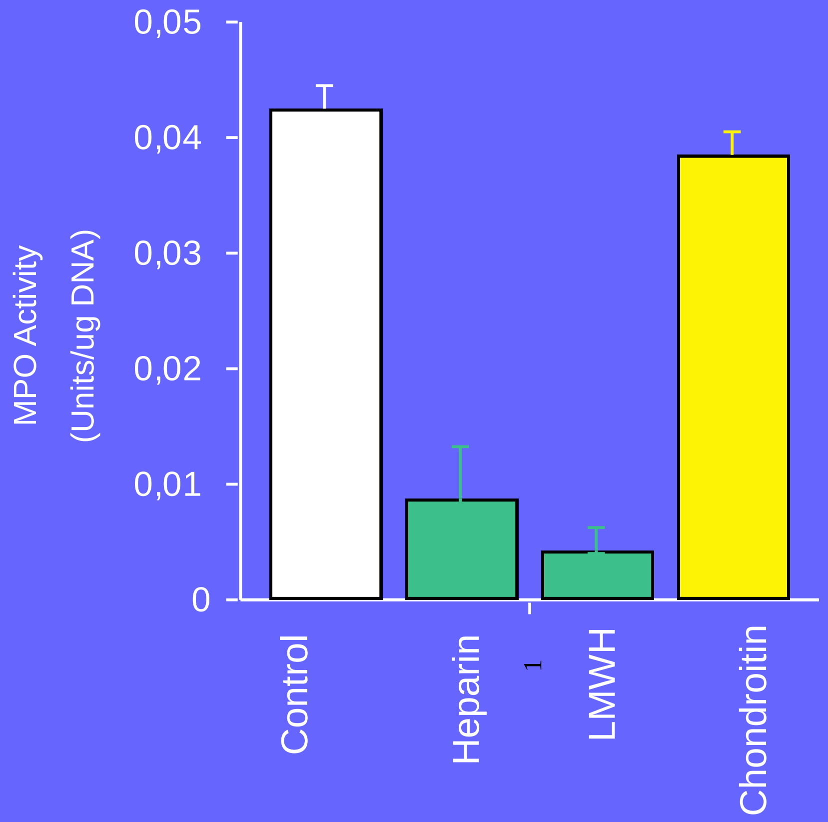
100x 50x

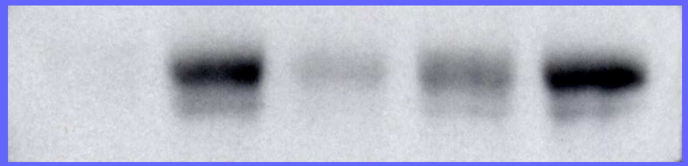
Endothelial Transcytosis of MPO



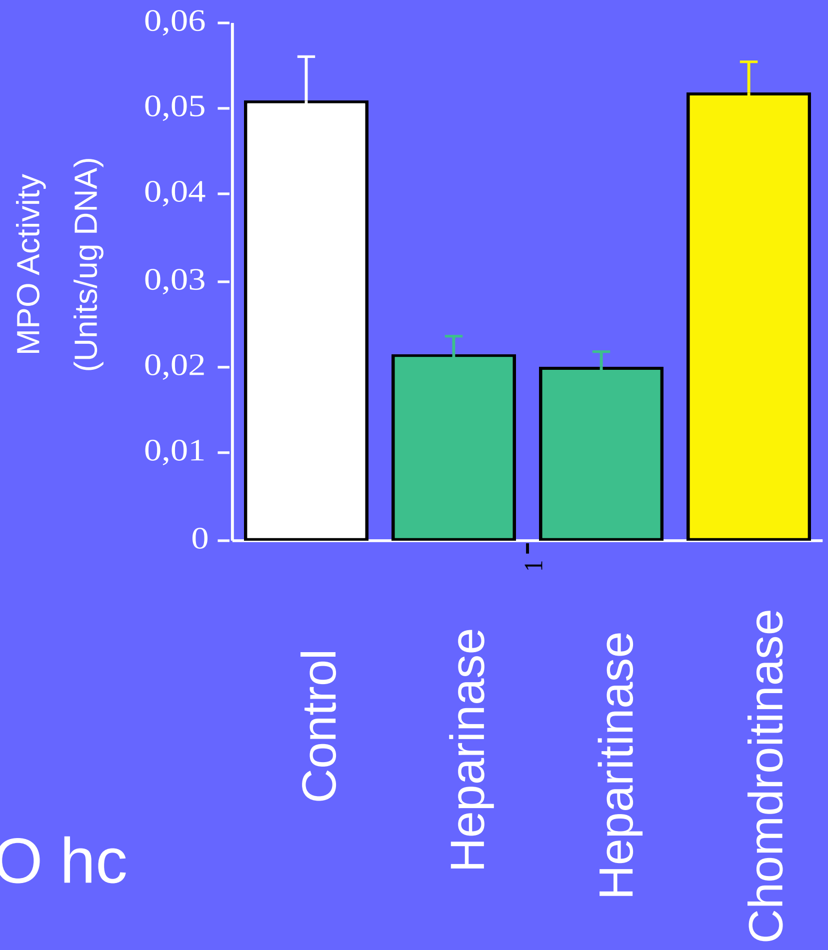


Binding of MPO is dependent on Heparin GAGs on cell surface

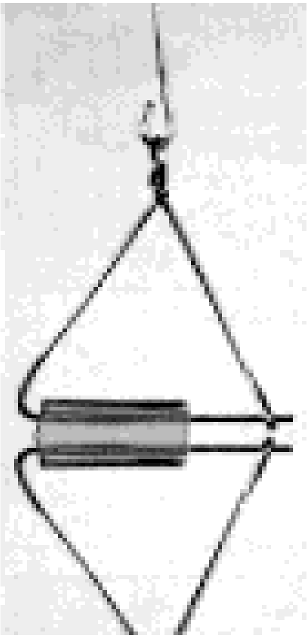




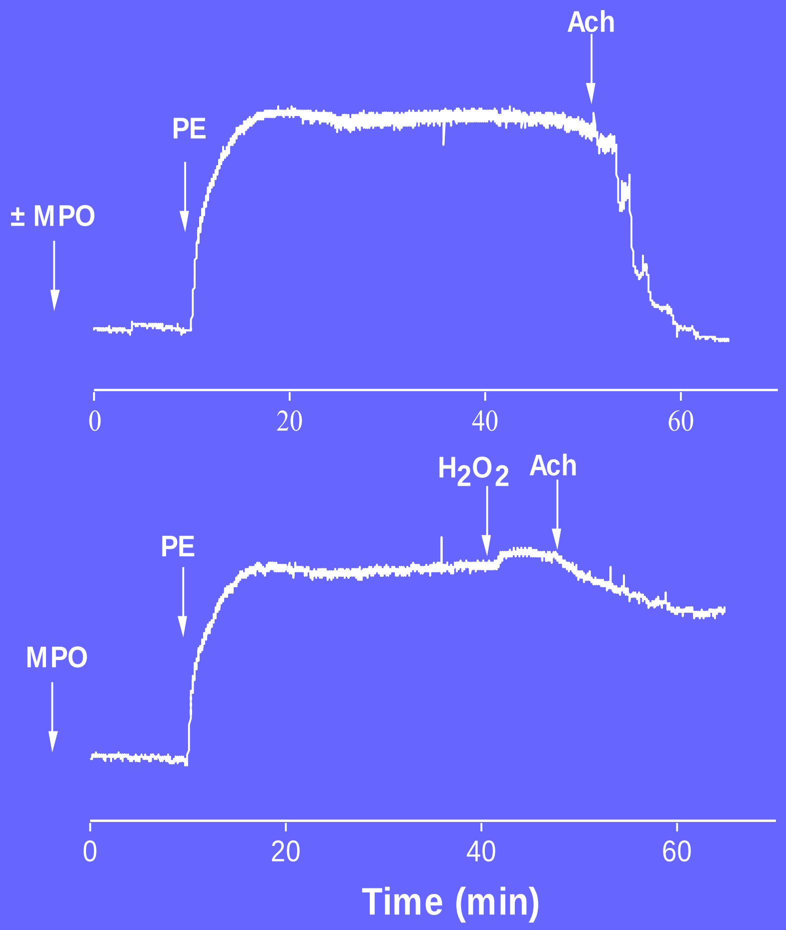
MPO hc



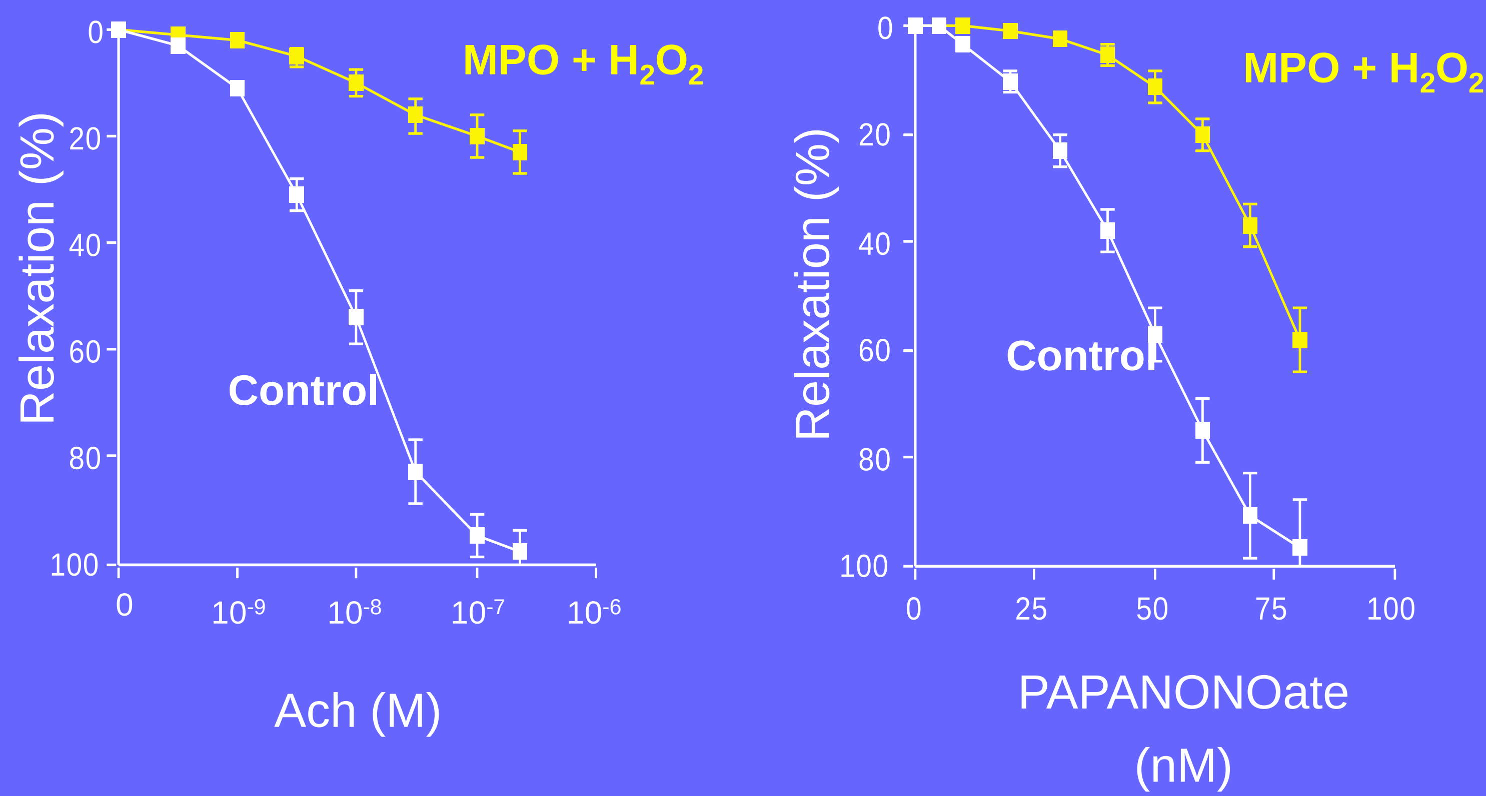
Organ Bath for Isometric Tension Measurements



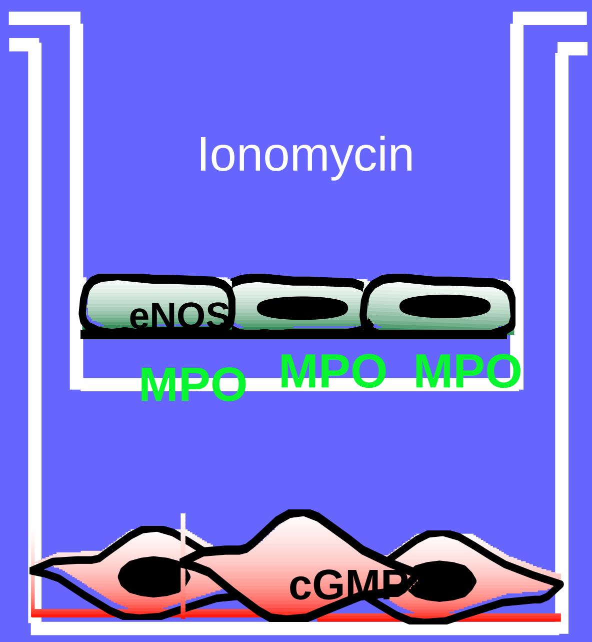
H_2O_2-Activated MPO Inhibits Aortic Relaxation in Response to Acetylcholine

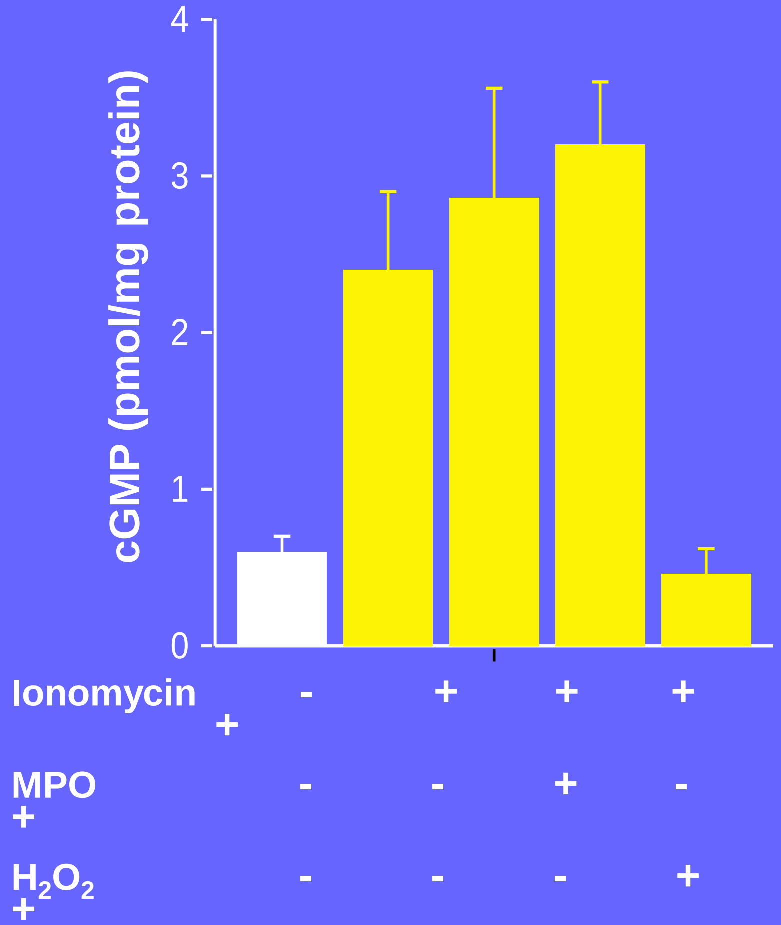


MPO impairs relaxation of rat aortic rings

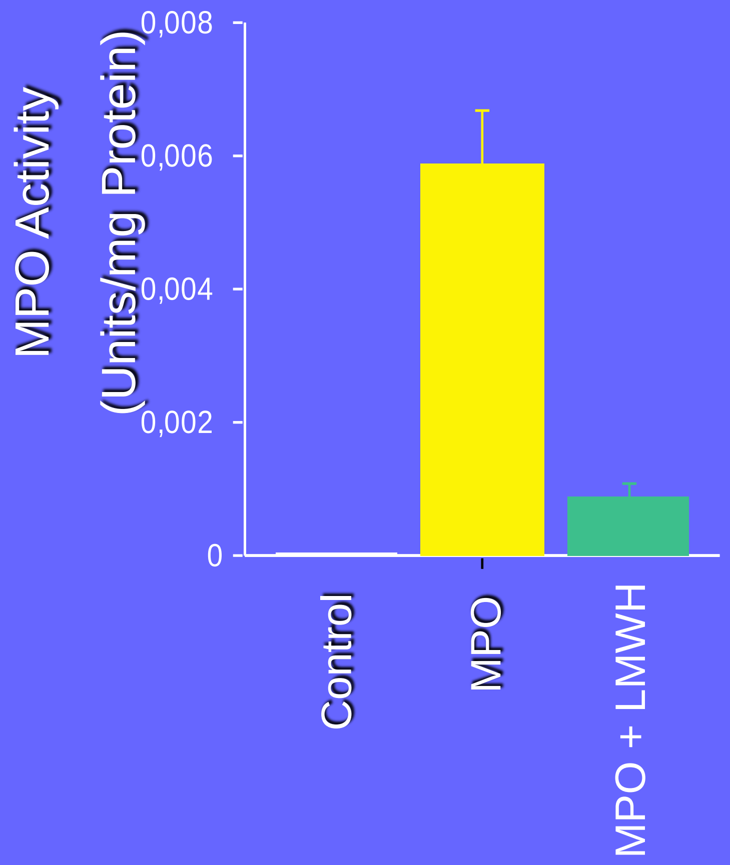


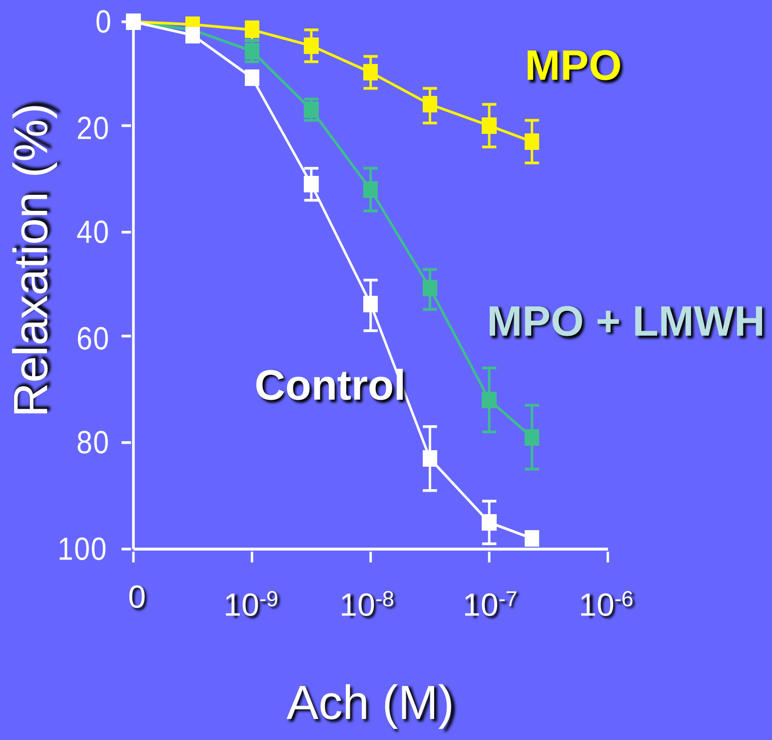
MPO attenuates cGMP levels in cocultures of EC-SMC





Heparin blocks MPO uptake into vascular tissue and restores vessel relaxation





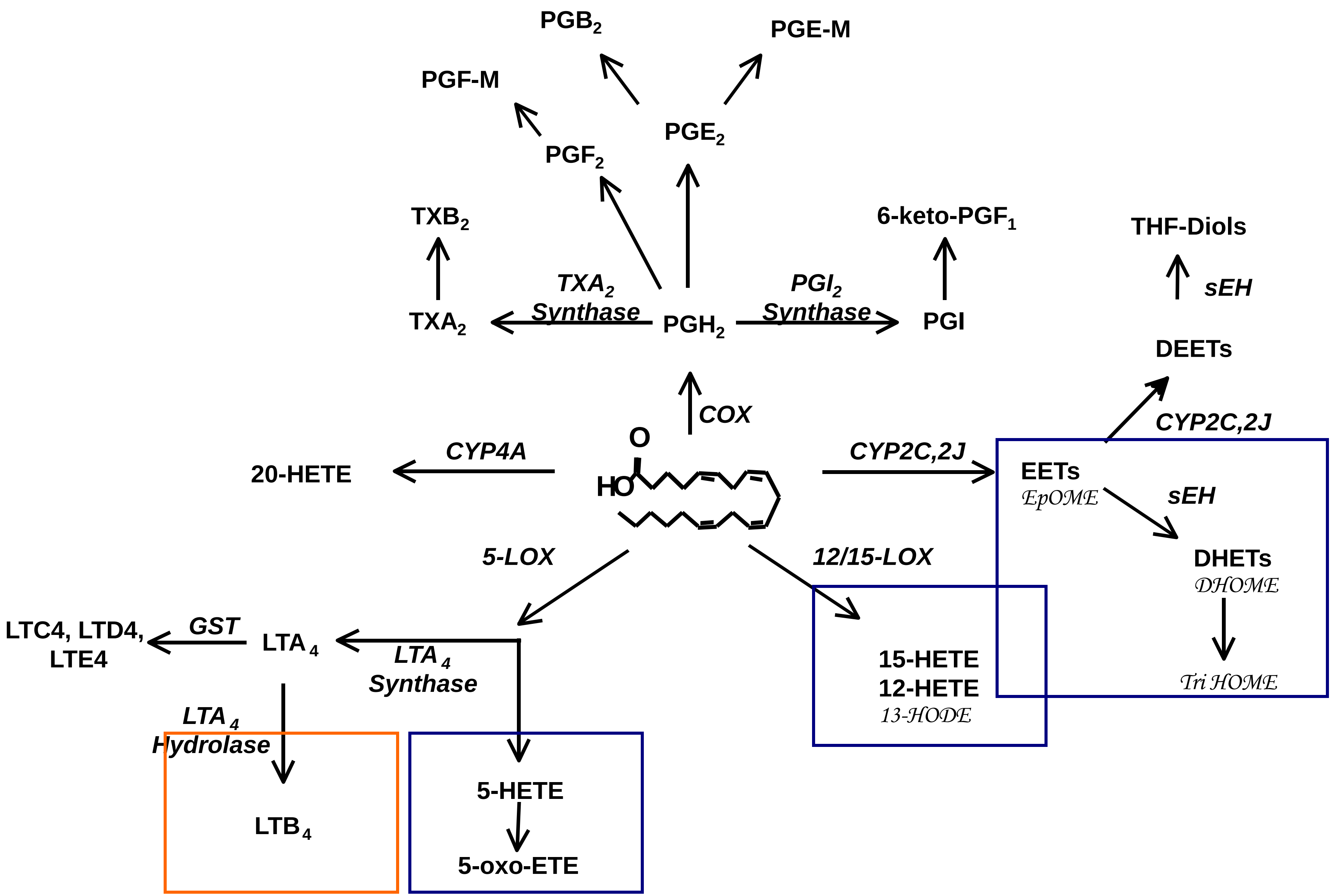
Baldus, Eiserich et al. (2001) JCI

MPO controls progress of inflammatory process by modification of bioavailability of lipid metabolites

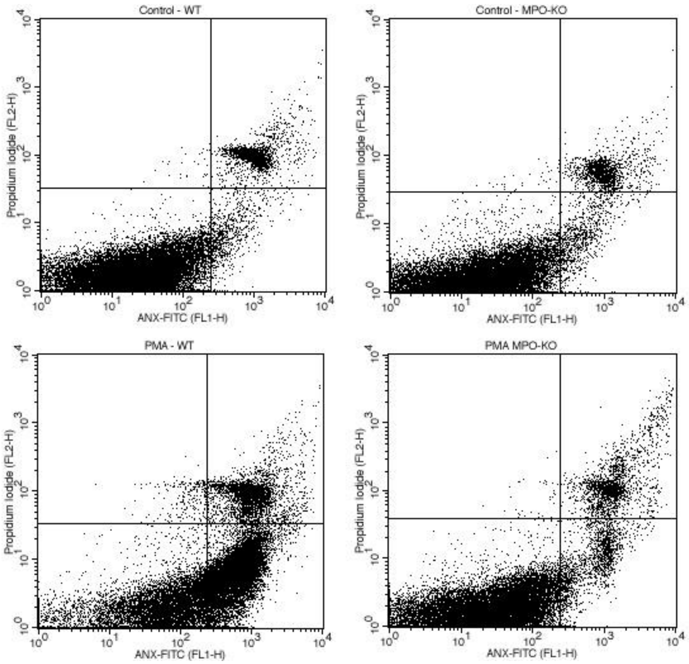
↑ MPO increases levels of **anti-inflammatory** biologically active lipid metabolites (EpOME)

↓ MPO decreased levels of **pro-inflammatory** biologically active lipid metabolites (LTB)

Target metabolites of the *Linoleic* and Arachidonic Acid Cascade



Myeloperoxidase deficiency delay onset of neutrophil granulocyte apoptosis



Anexin V and PI staining