Intracelulární enzymatické zdroje volných radikálů

NADPH Oxidázy
Myeloperoxidáza

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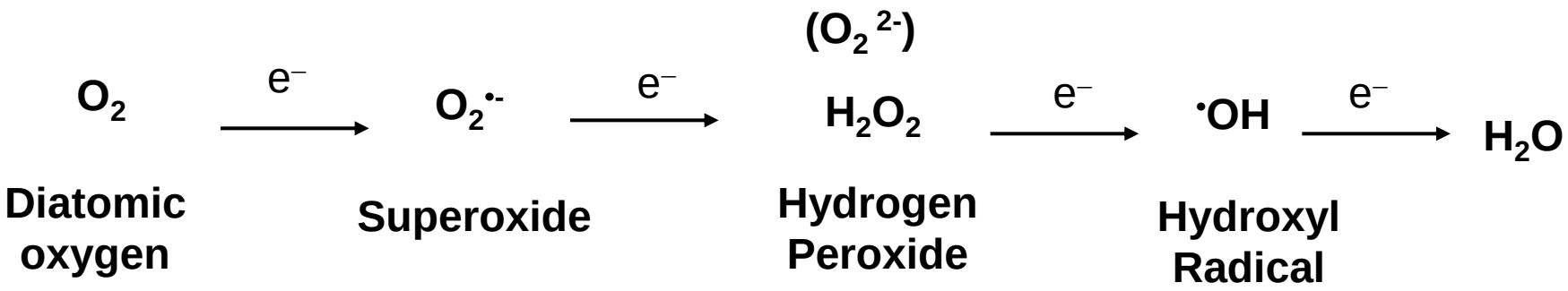
Biological Sources of Oxidants/Radicals



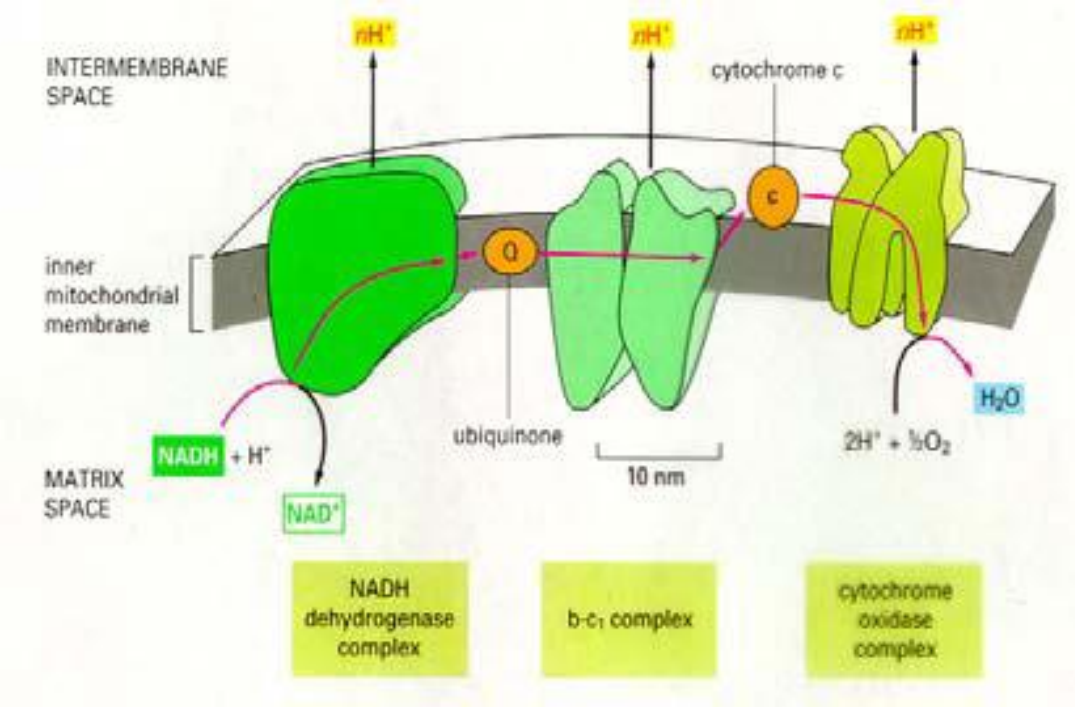
- Mitochondria - electron respiratory chain leak
- Cyclooxygenase
- Lipoxygenase
- Heme oxygenase

ROS generally

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

Superoxide

• Mitochondria - electron respiratory chain leak



Complex I, II, III,IV

– Function is to reduce O2 to H2O

• 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

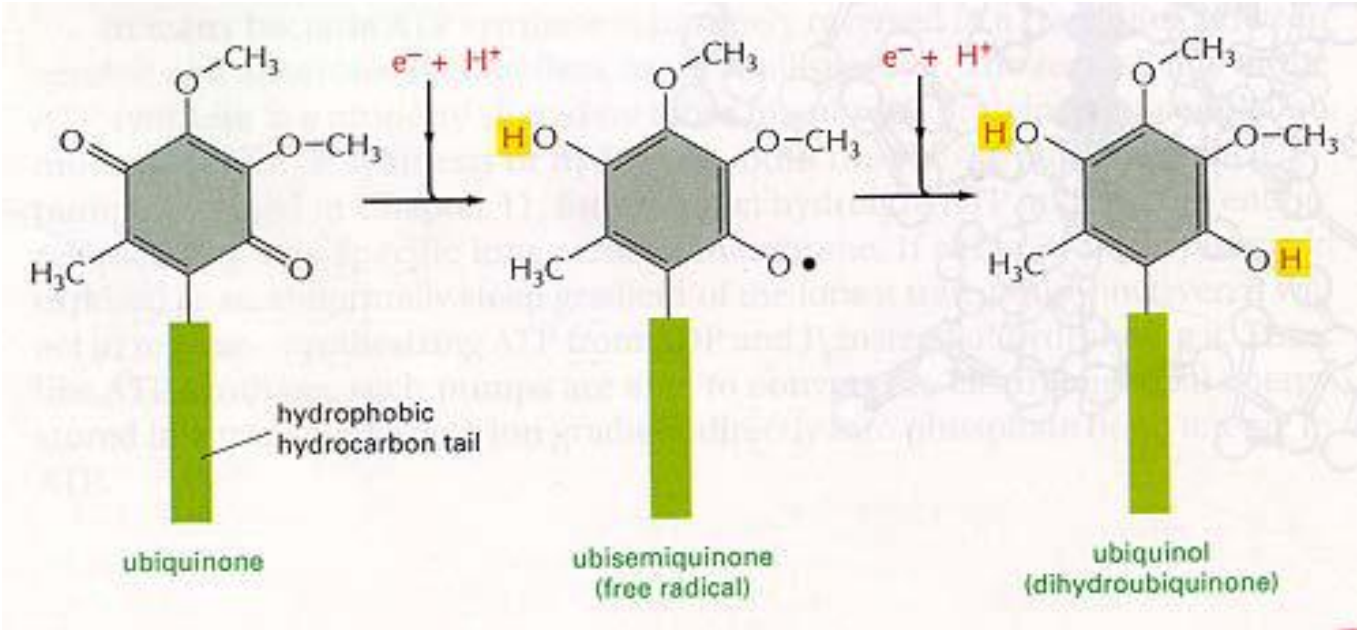
• What happens to other 1-5%

• ROS

Which complex is responsible for free radical leak?

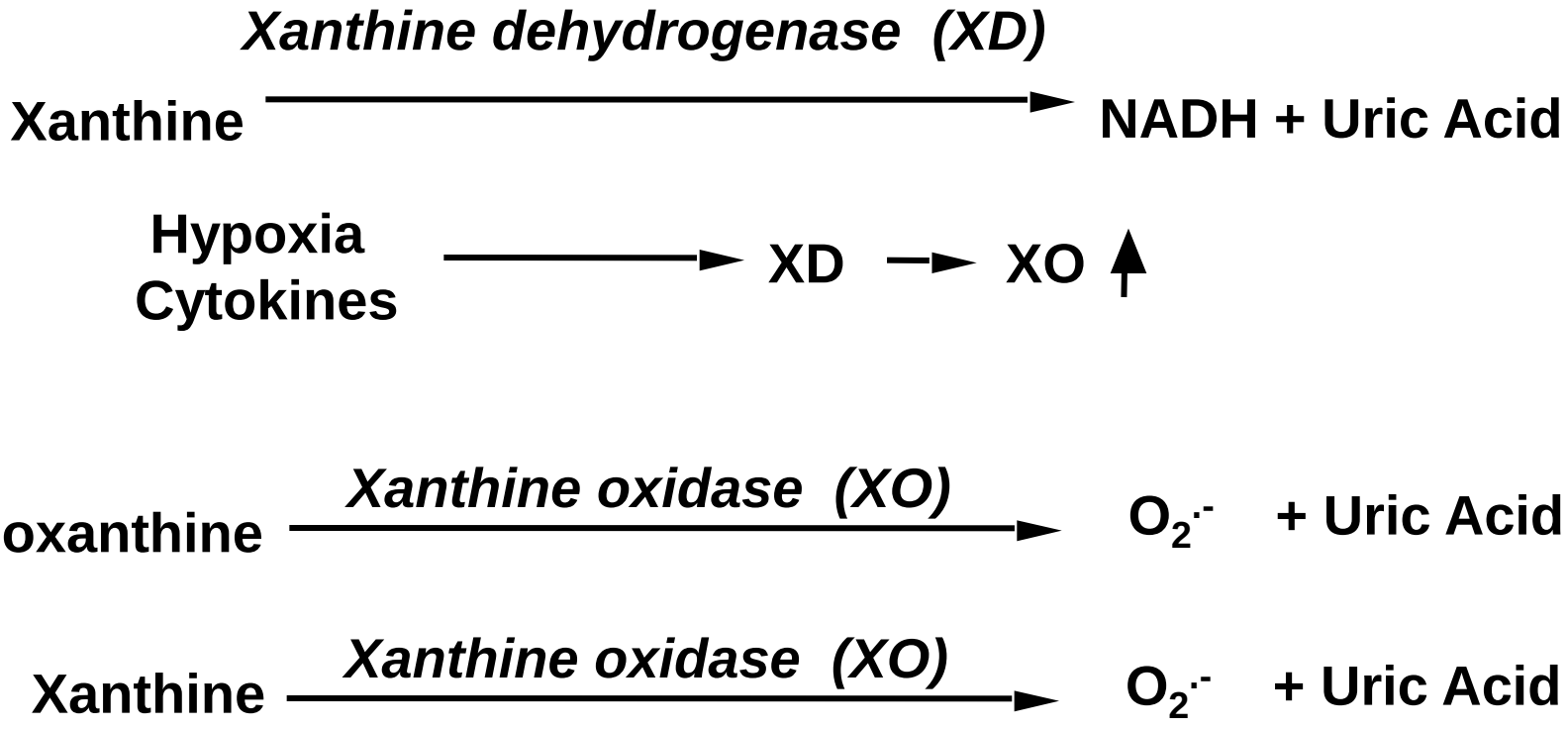
• This electron is thought to come from the one-electron reduction of ubiquinone, which generates the reactive intermediate ubisemiquinone formed by Complex III.

• Instead of accepting another electron and proton to form ubiquinol, ubisemiquinone may leak its unpaired electron to O2, forming O2•-.

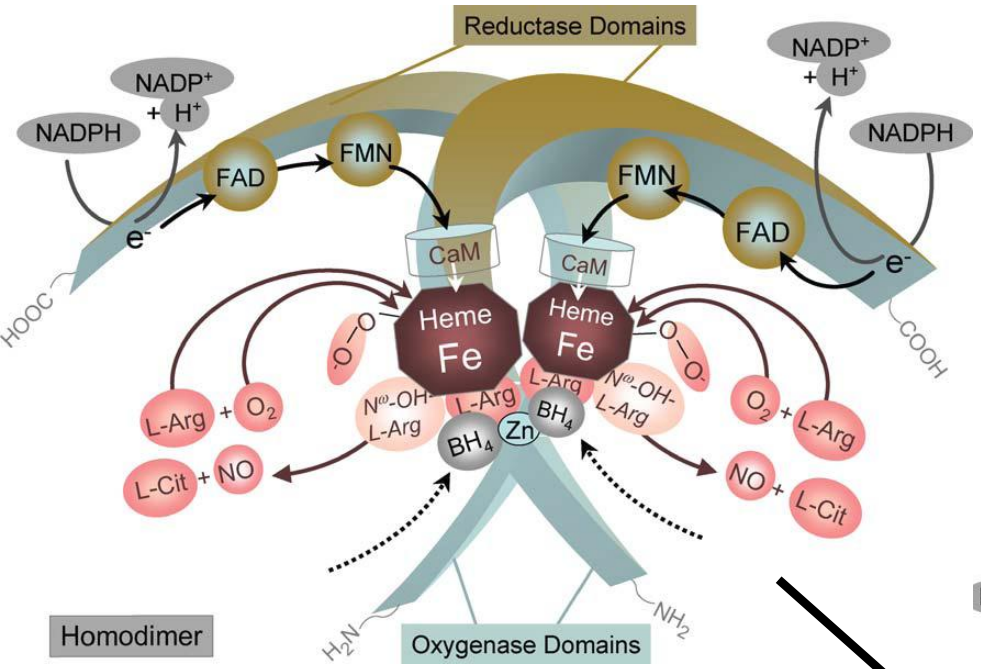


Xanthine Oxidase/Dehydrogenase

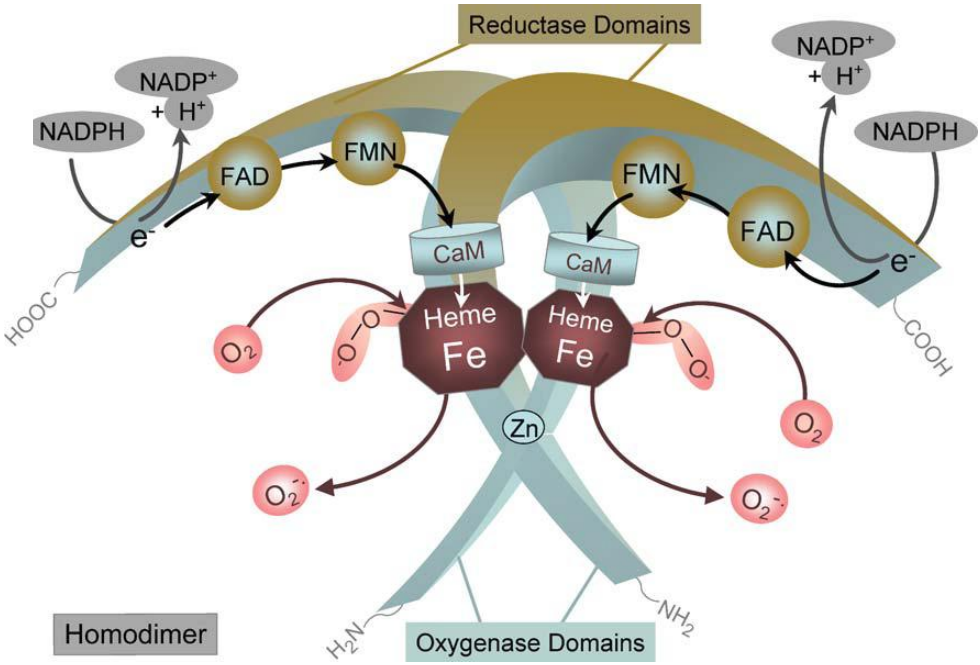
Flavoprotein enzyme containing iron and molybdenum that promotes the oxidation especially of hypoxanthine and xanthine to uric acid and of many aldehydes to acids



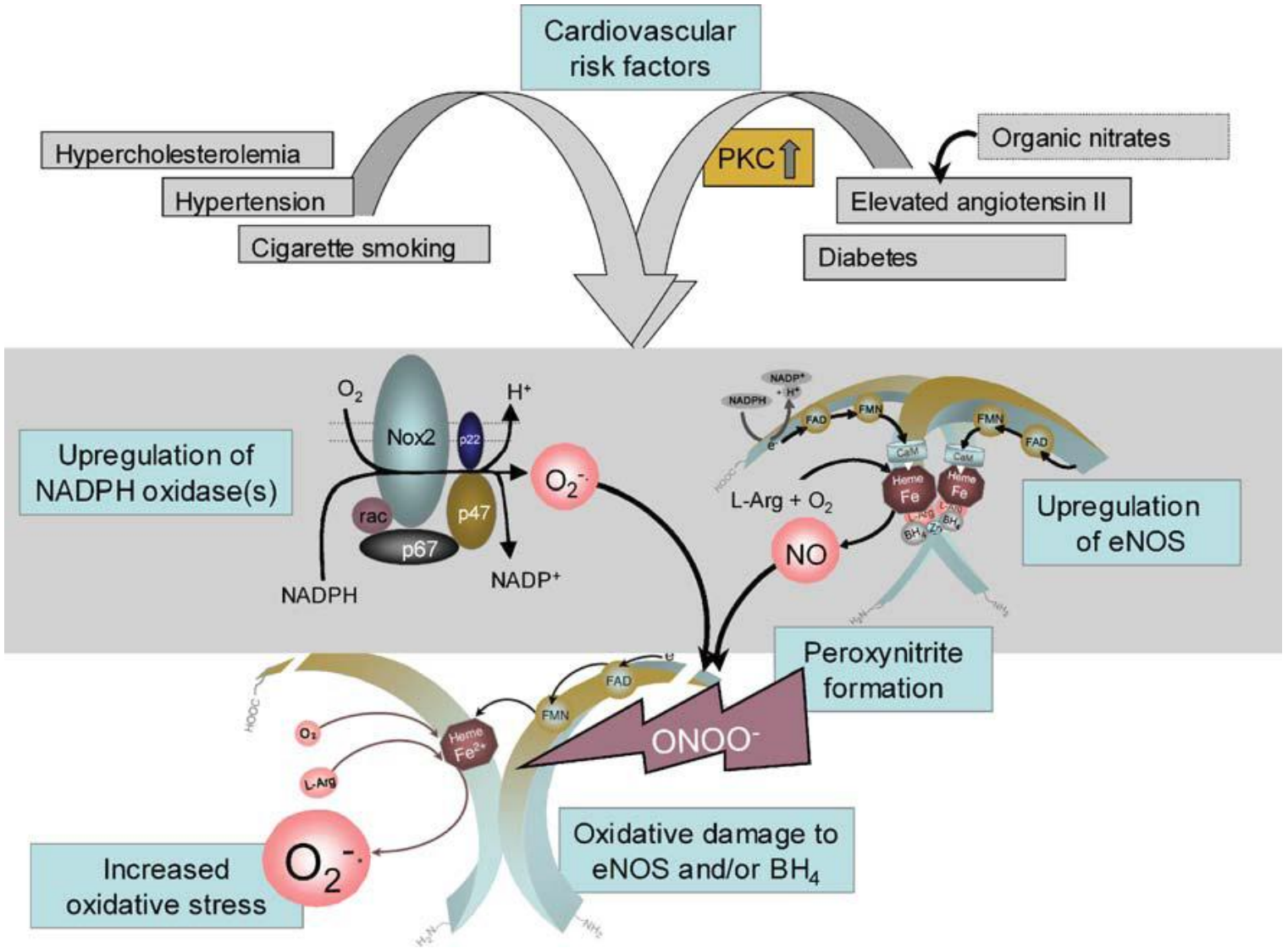
Uncoupling of NO synthase



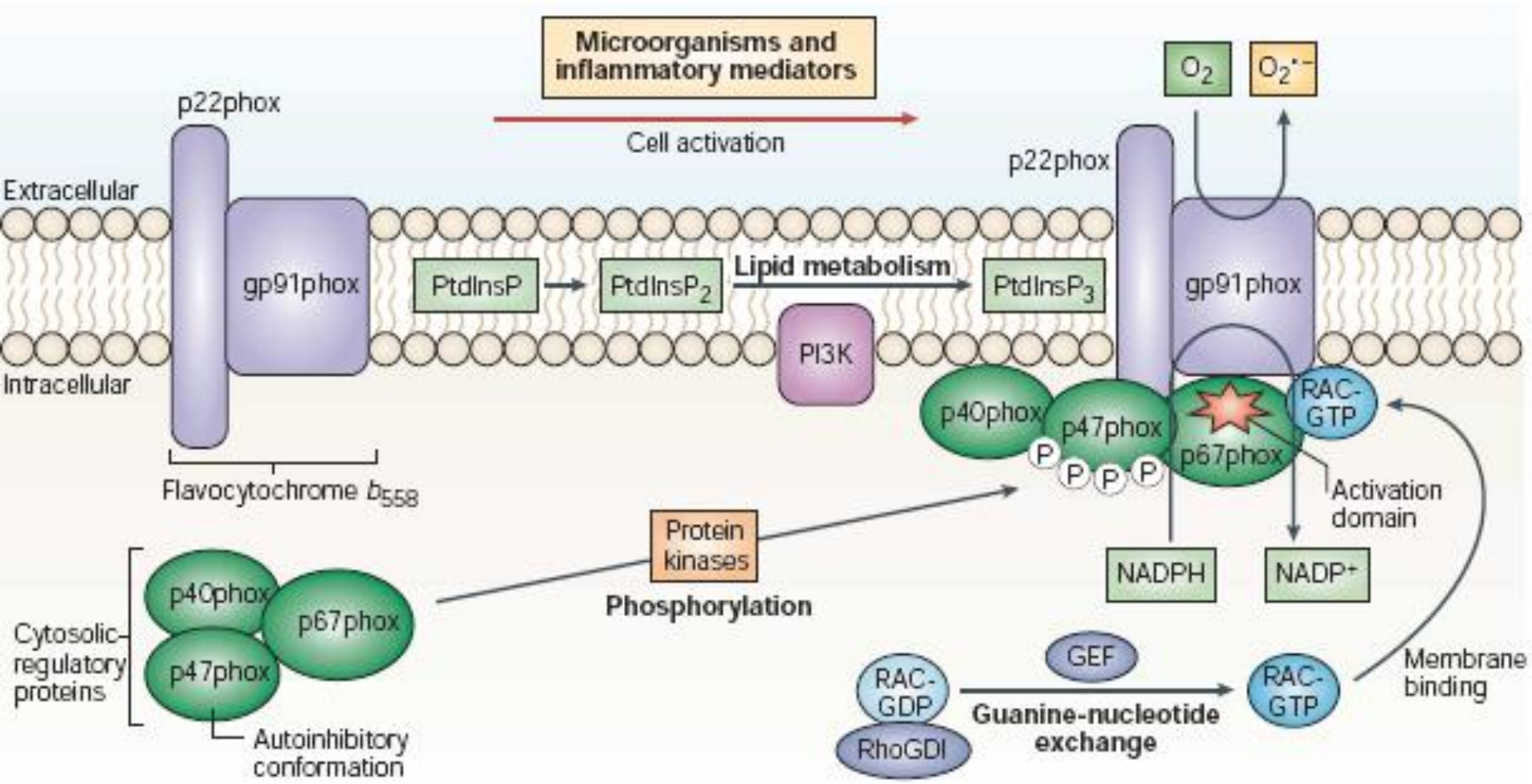
Oxidative stress



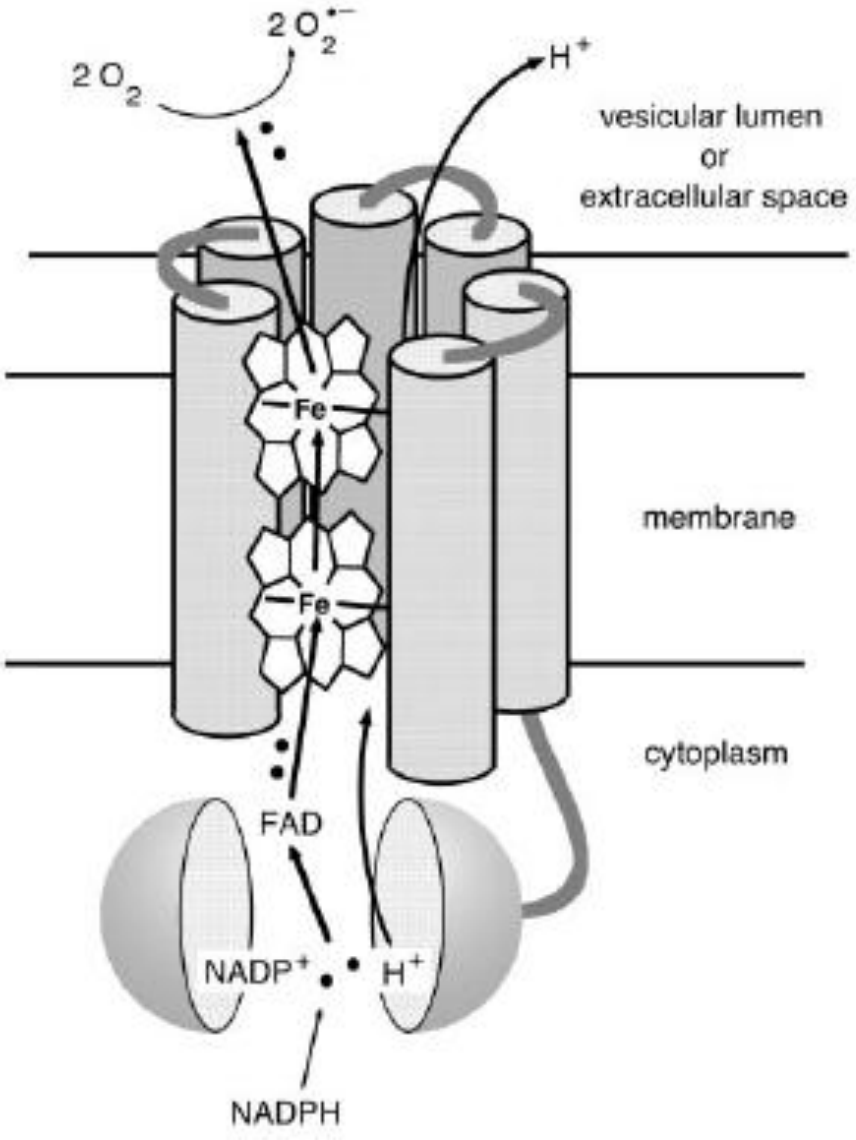
What leads to eNOS uncoupling



Struktura a aktivace fagocytární NADPH oxidázy (NOX2)



Transport elektronů NADPH oxidázou

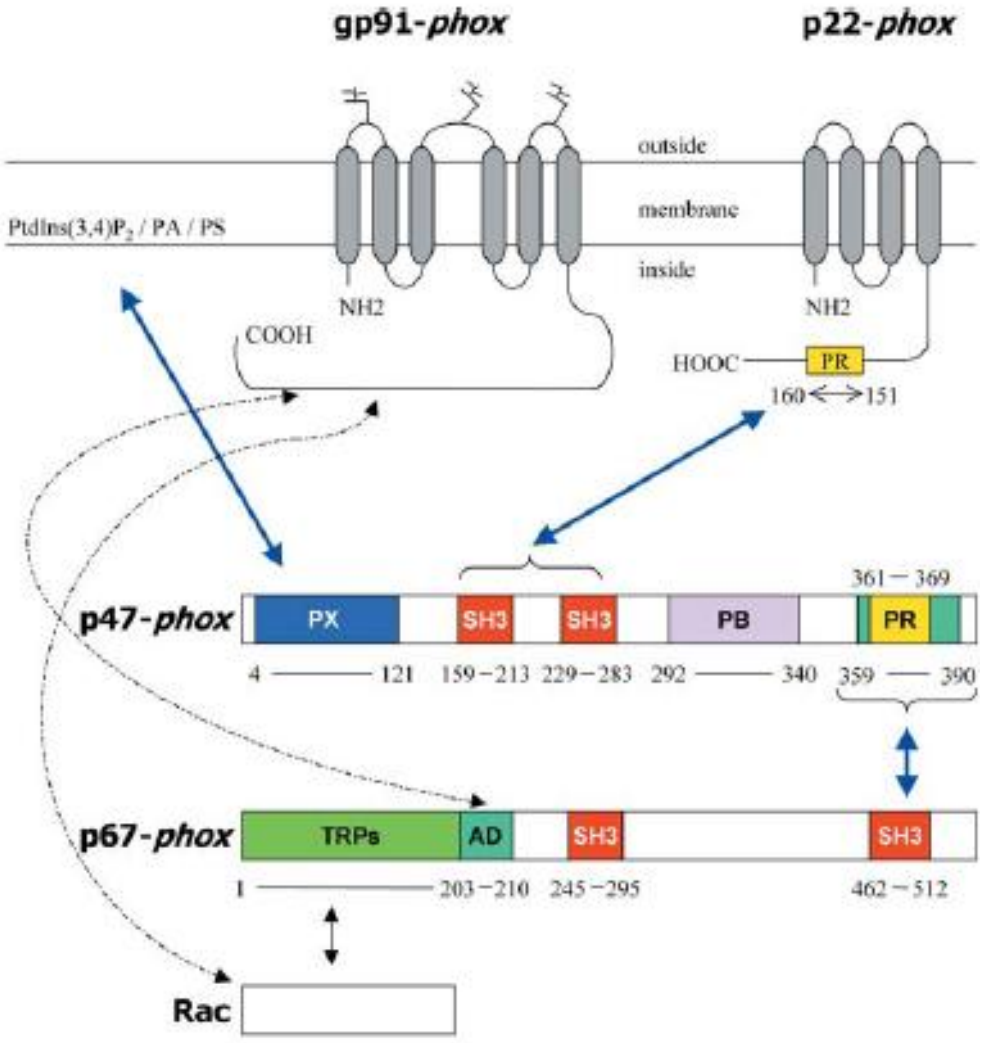


Charakteristika jednotlivých podjednotek NOX2 (fagocytární NADPH oxidázy)

Properties of the phagocyte respiratory burst oxidase (phox) components

	gp91^{phox}	p22^{phox}	p47^{phox}	p67^{phox}	p40^{phox}	Rac2
Gene and locus	*CYBB*; Xp21.1	*CYBA*; 16q24	*NCF-1*; 7q11.23	*NCF-2*; 1q25	*NCF-4*; 22q13.1	*Rac2*; 22q13.1
Amino acids	570	195	390	526	339	192
Molecular weight:						
Predicted	65,338 Da	20,959 Da	44,684 Da	59,735 Da	39,039 kDa	21,429 Da
By SDS-PAGE	~ 90 kDa	22 kDa	47 kDa	67 kDa	40 kDa	22 kDa
Glycosylation	Yes	No	No	No	No	No
pI	9.26	10.1	9.58	6.12	7.28	7.87
Phosphorylation	No	Minor	Yes	Minor	Yes	?
Location in PMN						
Resting	Specific granule and plasma membrane		Cytosol	Cytosol	Cytosol	Mainly cytosol
Stimulated	Plasma membrane and phagosome		Membrane	Membrane	Membrane	Membrane
Abundance pmol/10^6 cells (cytosol conc.)	1.0–2.0	1.0–2.0	6.0 (2750 nM)	1.0 (460 nM)	1.0 (460 nM)	2.6 (1200 nM)
Functional domains (see Fig. 2)	C-terminus binds cytosolic components. Haem, FAD and NADPH binding regions	C-terminal proline-rich region	Phosphorylation sites. PX domain, 2 SH3 domains, proline-rich region	Tetratricopeptide repeat, 2 SH3 domains, proline-rich domains	PX and SH3 domains, octicosapeptide repeat	GDP/GTP-binding; insert and effector regions, isoprenylation site

Vazebná místa jednotlivých podjednotek NOX2 (fagocytární NADPH oxidázy)



Assembly of the neutrophil–NOX complex

1 - phosphorylation of p47phox, releasing it from its autoinhibitory conformation.

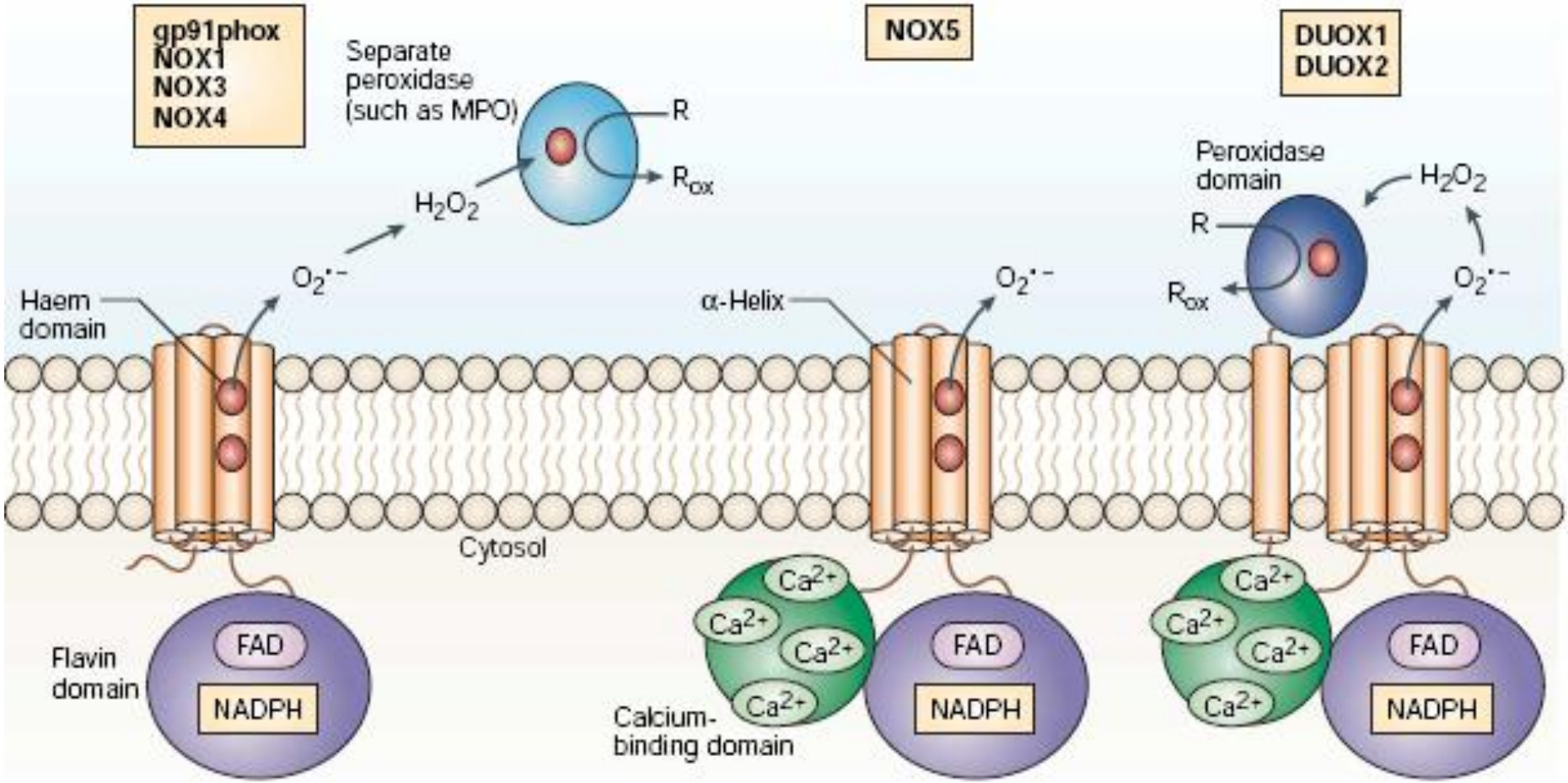
2- the p47phox SH3 domain binds the proline-rich region in p22phox

3- the PX domain of p47phox binds 3-phosphoinositides.

4- interaction of activated Rac2 with gp91phox is mediated by the N terminus of the p67phox subunit.

5- activation of the small GTPase Rac2 is associated with recruitment of p67phox, which associates with p47phox and with the cytochrome. Direct binding of Rac2 to the flavocytochrome has been implicated in the initial steps of the electron transfer reaction

Homology NADPH oxidáz a jejich struktura



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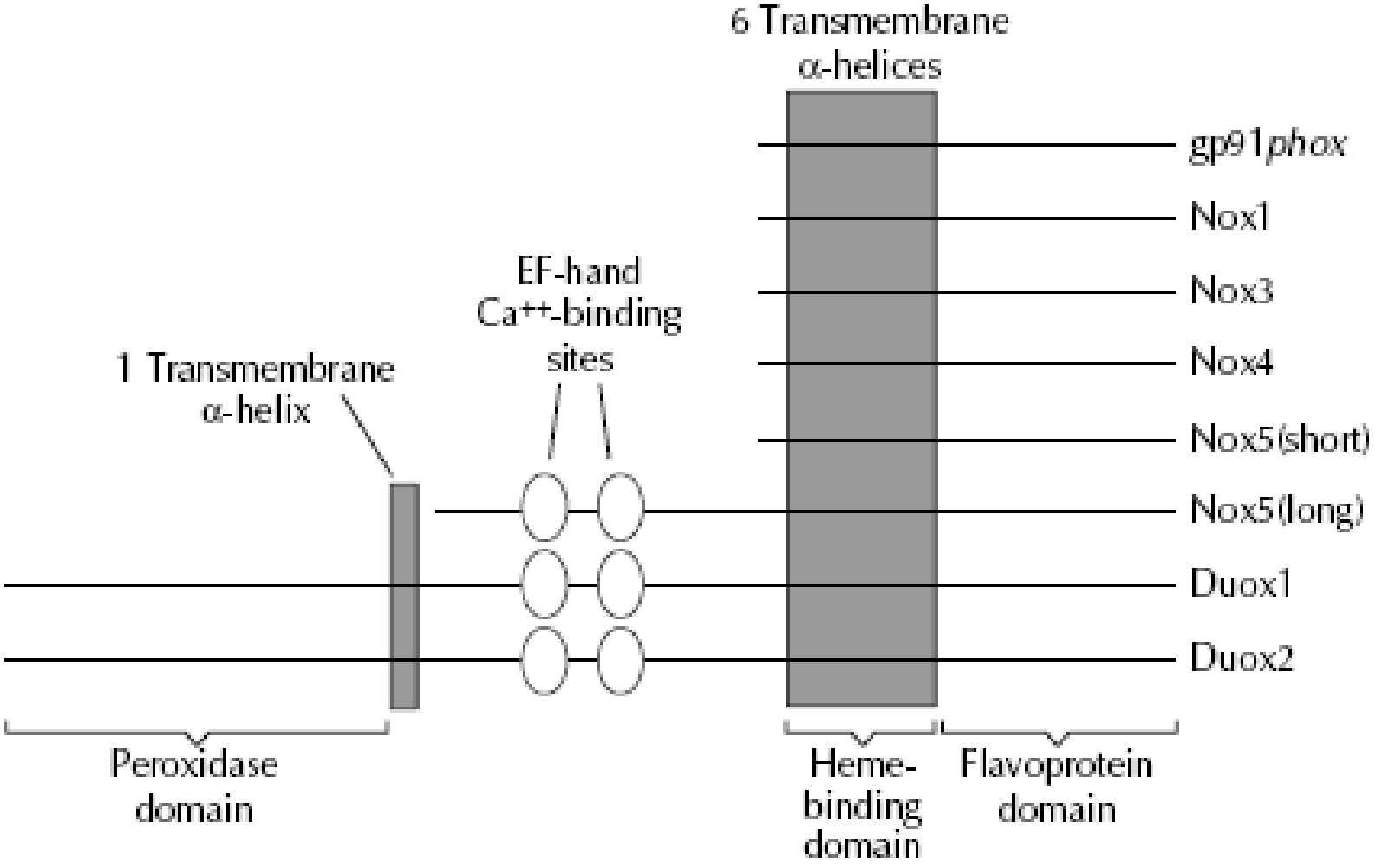


Přehled homologů NADPH oxidáz

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

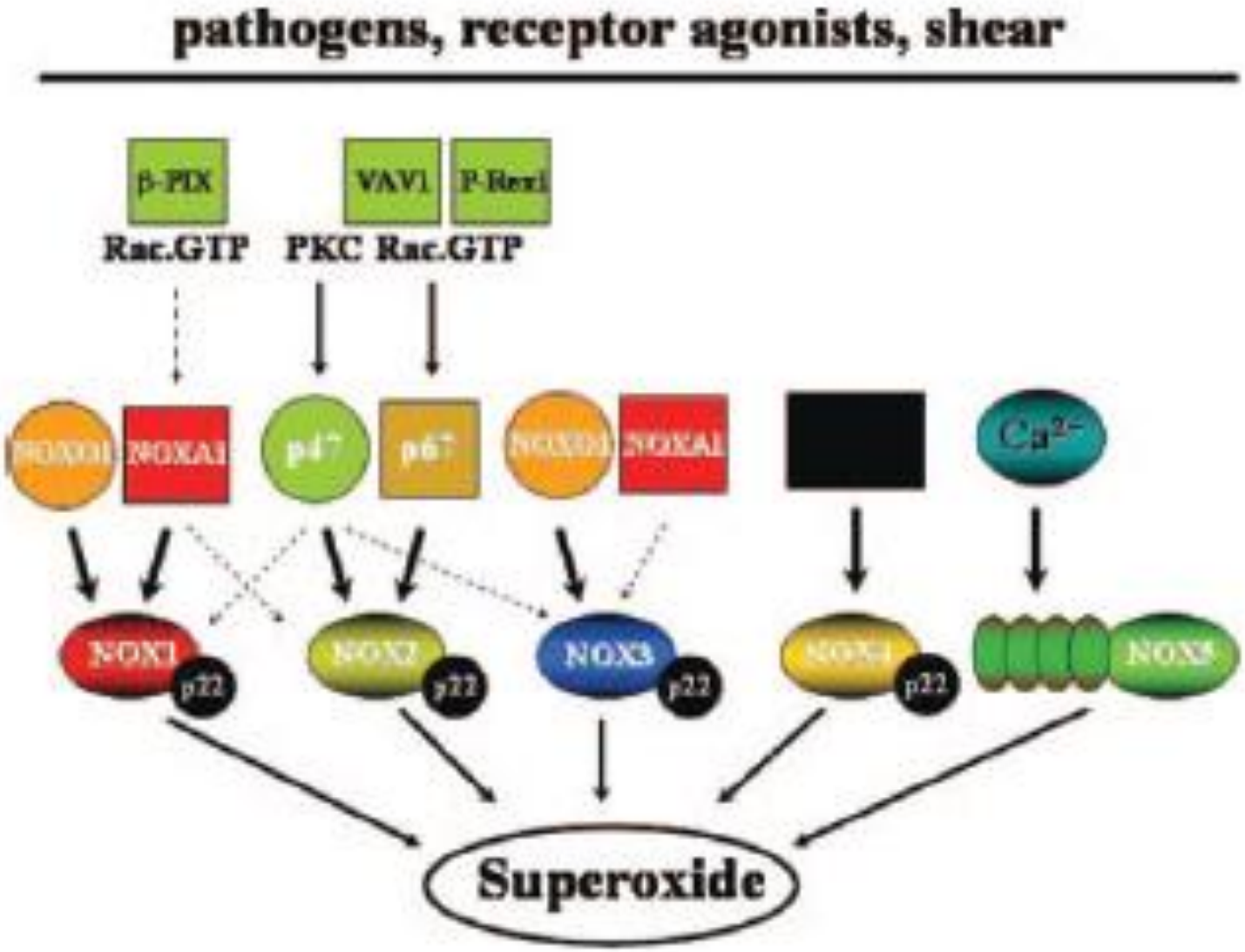
Enzyme	Highest level of expression	Known regulatory factors	References
gp91phox (NOX2)	Phagocytes	p47phox, p67phox, p40phox and RAC1/RAC2	14
NOX1	Inducible: colon and vascular smooth muscle	NOXO1, NOXA1 and p22phox	3,15,20,21
NOX3	Fetal kidney	N.D.	4,68
NOX4	Kidney, osteoclasts, ovary and eye; widespread	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.

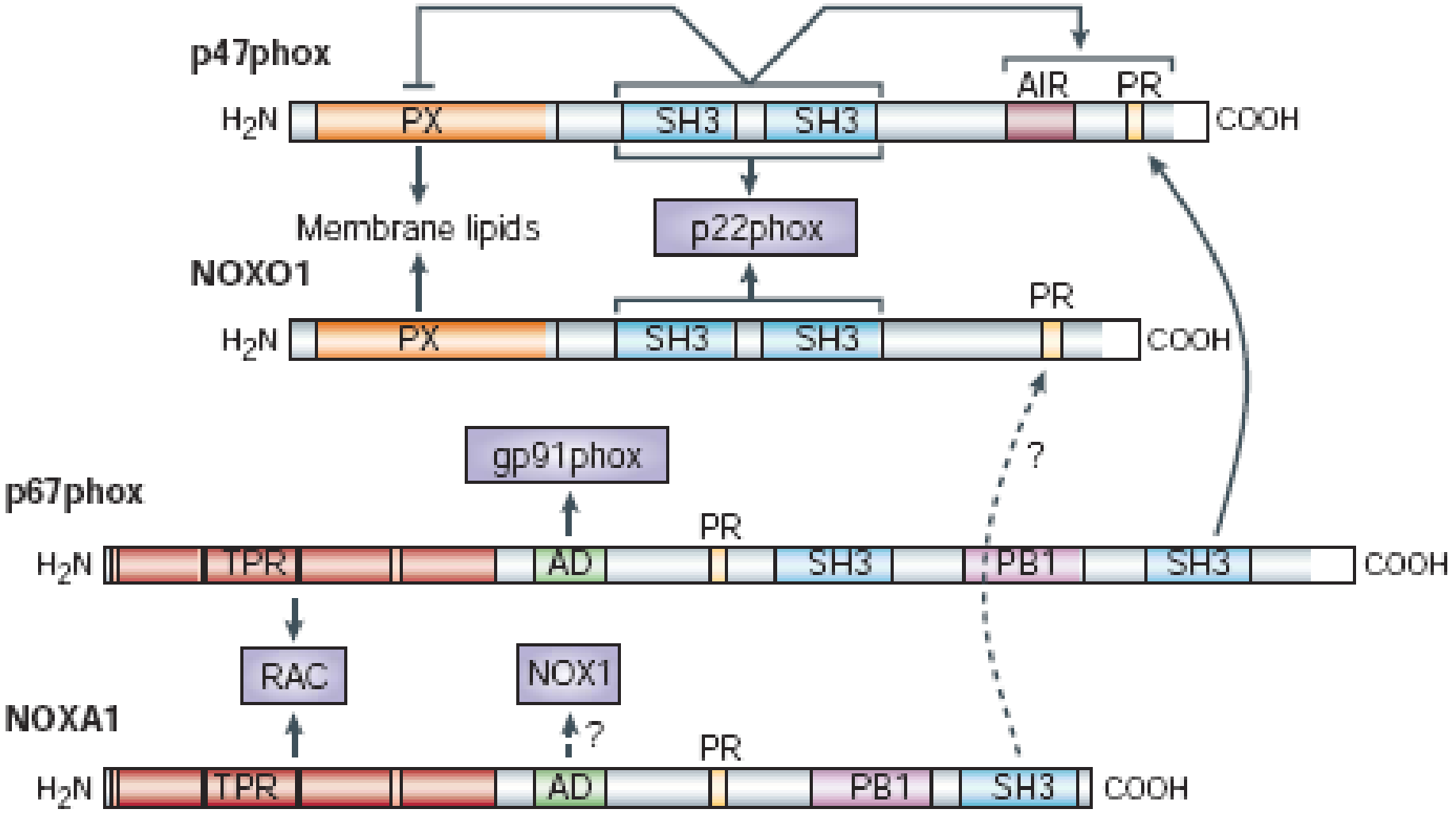
Prokázaná existence řady orthologních NADPH oxidáz u myší, krys, Drosophil, Caenorhabditis elegans, a Diclostelium.

NADPH oxidázy také objeveny u kvasinek a rostlin

Aktivace NADPH oxidáz a jejich podjednotky



Regulační podjednotky NOX1 aNOX3



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

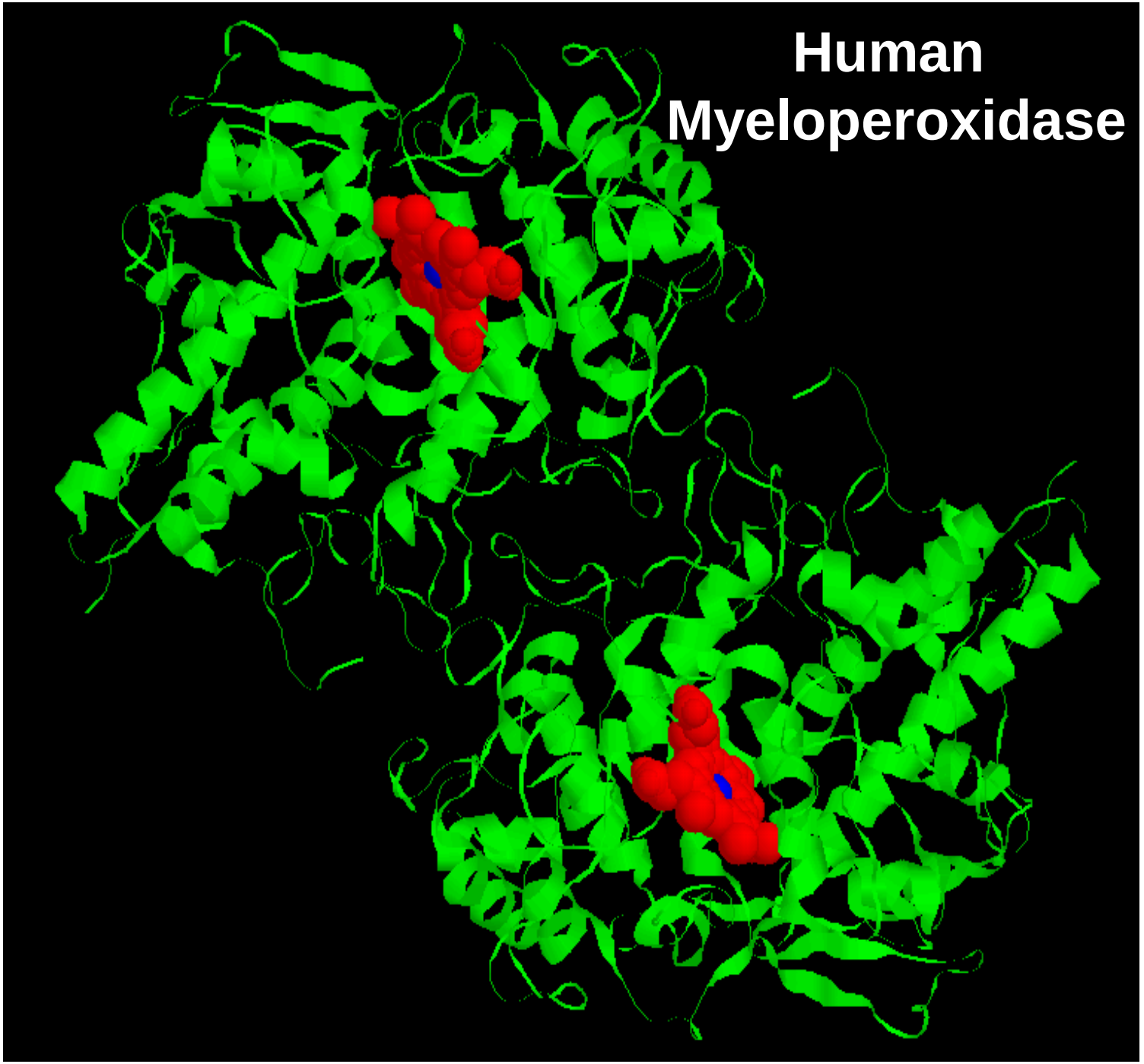
- Pair of protomers - α (heavy) subunit and β (light) subunit

- α subunit - two hemes and mannose-reach carbohydrate

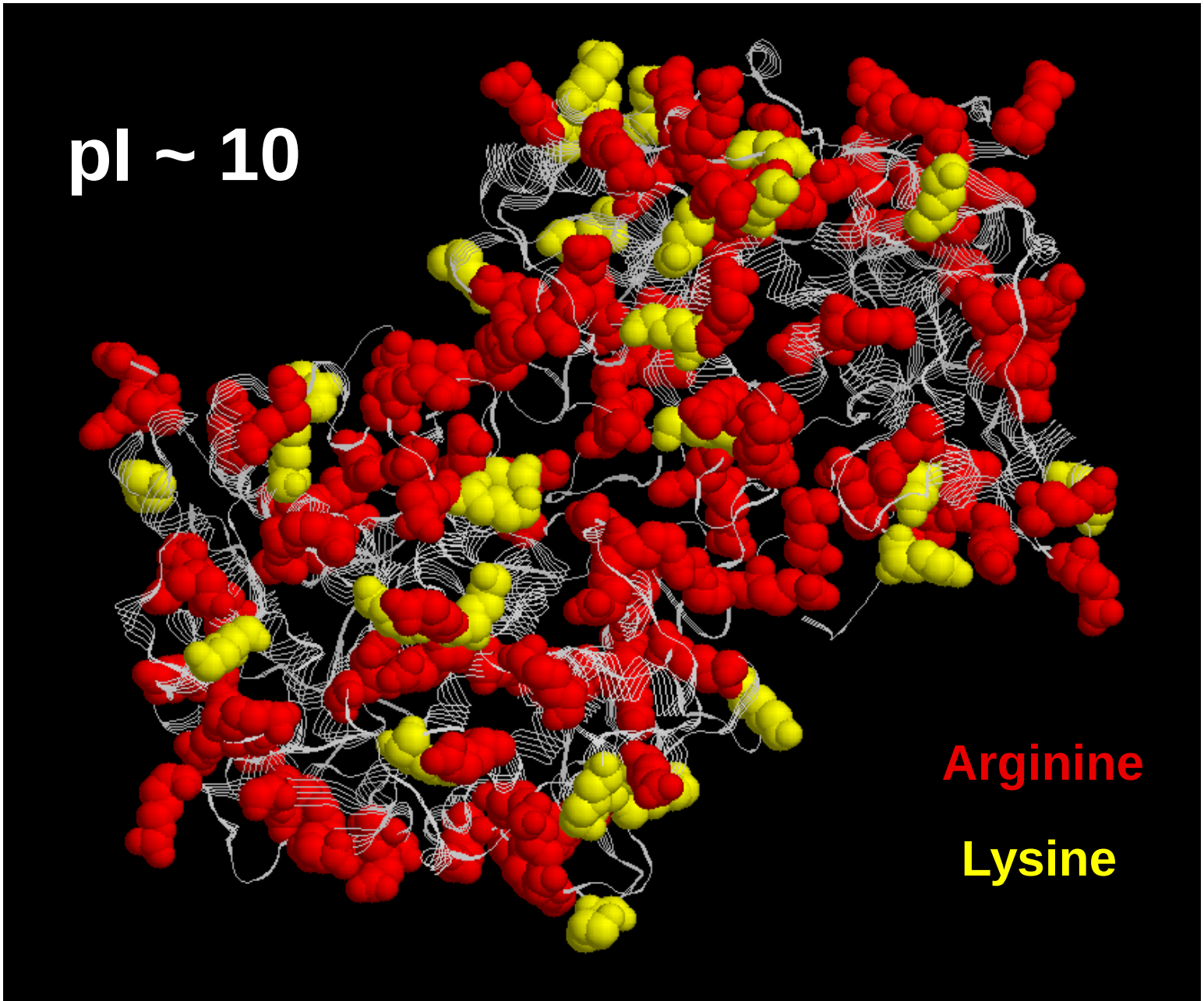
- Single gene located on chromosome 17

- MPO is up to 5% of total neutrophil proteins

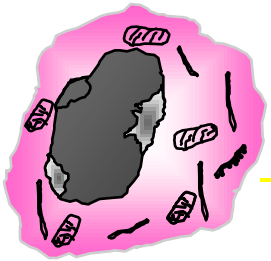
- High quantities of MPO are released and accumulated at the site of acute inflammation


Human
Myeloperoxidase

MPO is a Highly Cationic Protein



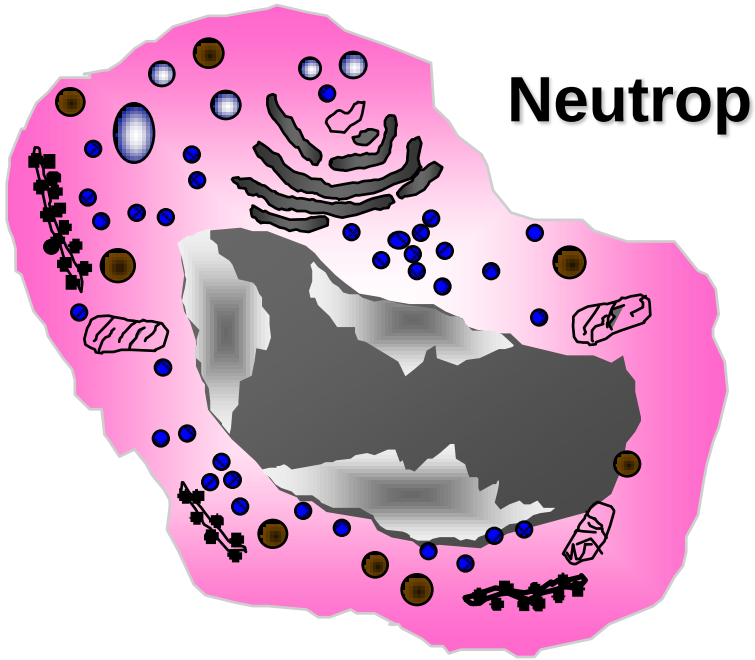
Promyelocyte



Myelocyte

Metamyelocyte

Neutrophil



- Blood Monocytes

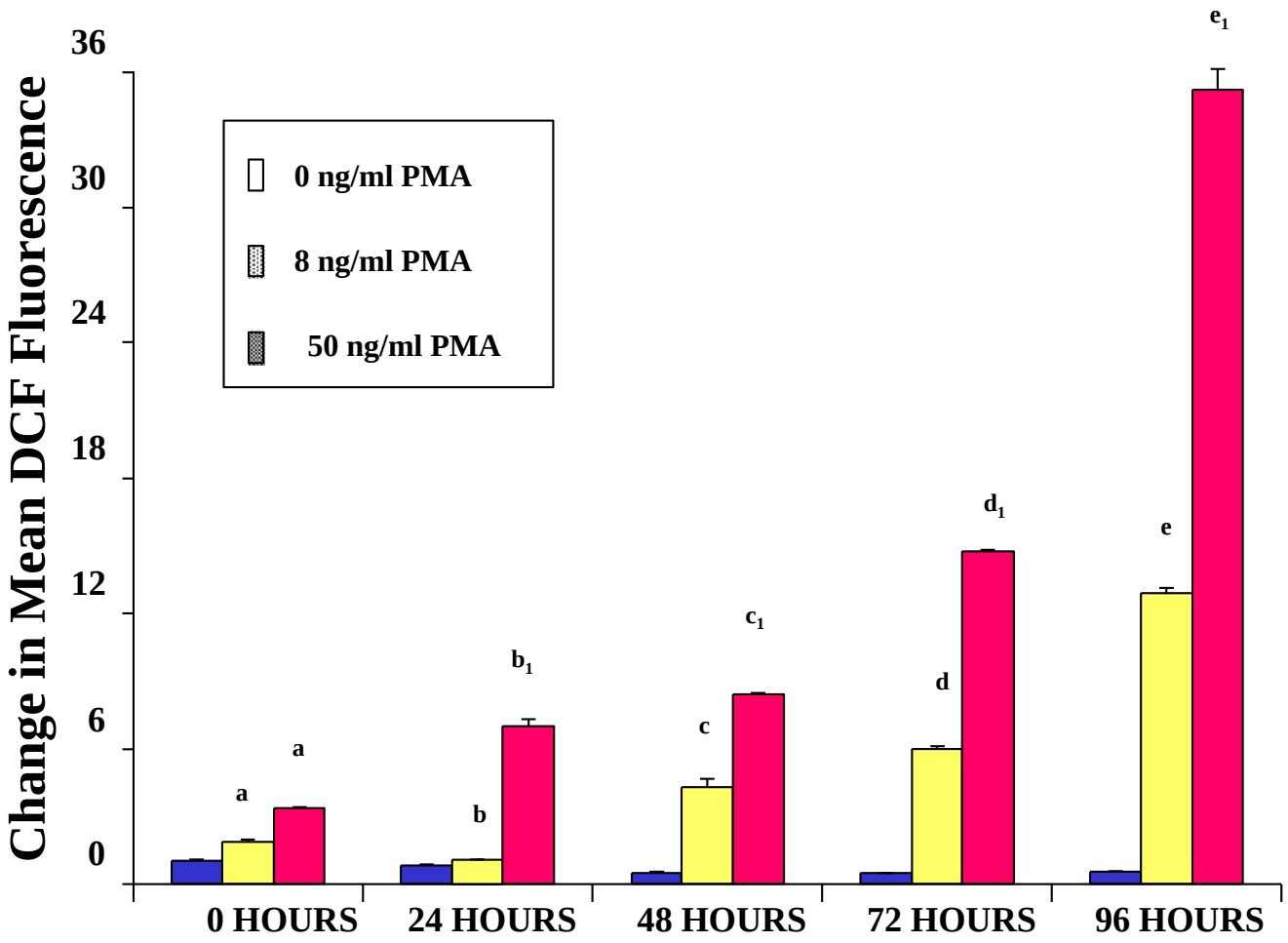
Tissue macrophages

- Kupffer cells

- Alveolar macrophages

- Microglia …

Oxidativní vzplanutí u buněk HL-60 během diferenciace



K.P. Narayanan et al. 1998

Control of myeloperoxidase expression

Allelic polymorphism at nucleotide -463 in the MPO promotor

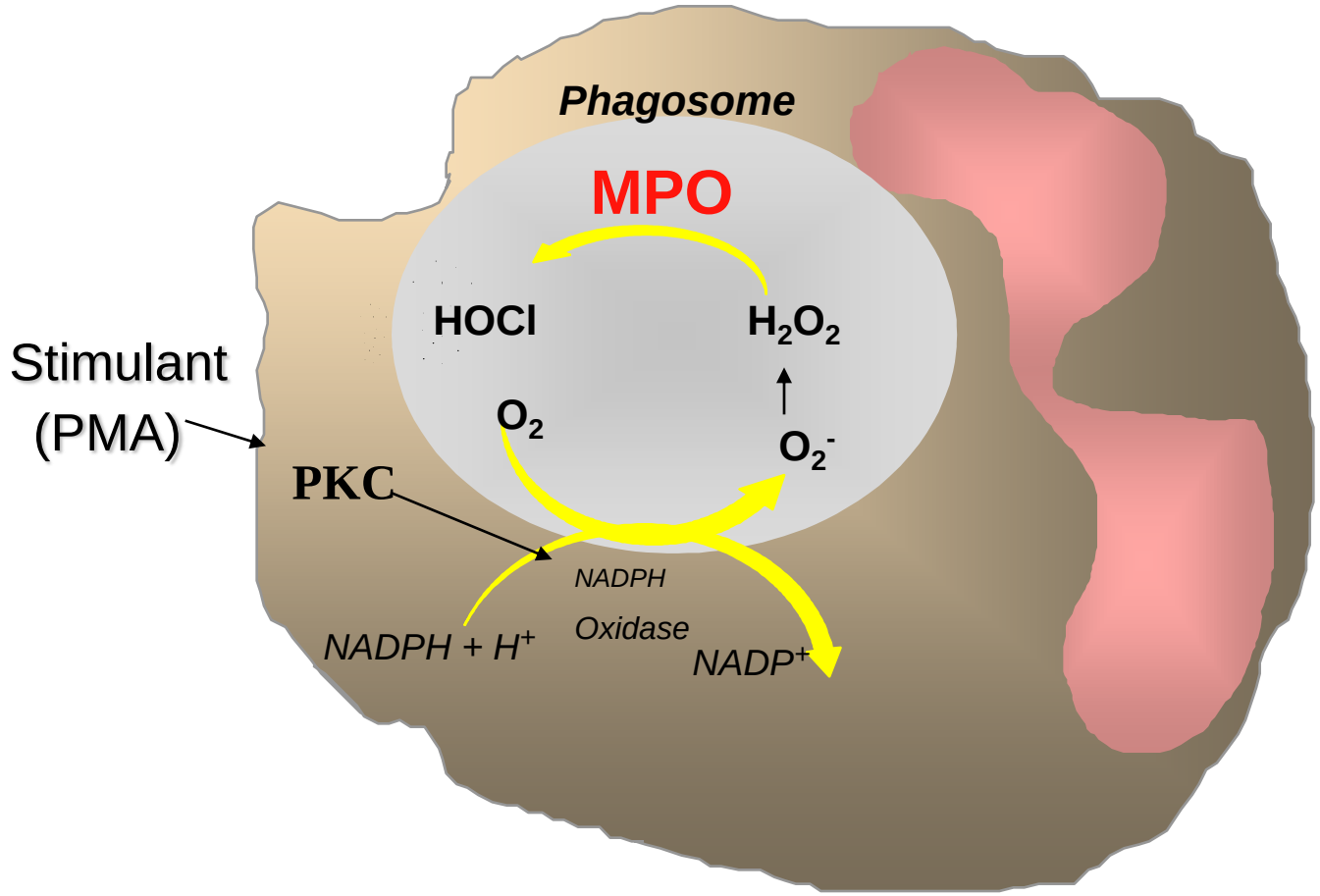
Allele -463G is connected with 25x higher transcription activity than -463A allele

In general population

GG: 61%, GA: 33% and AA: 6%

Total or partial MPO deficency - 1: 2000-4000

Oxidative Burst of Neutrophil



Phagocytes Utilize Myeloperoxidase to Form Bleach

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

- Host Defense
- Tissue Injury

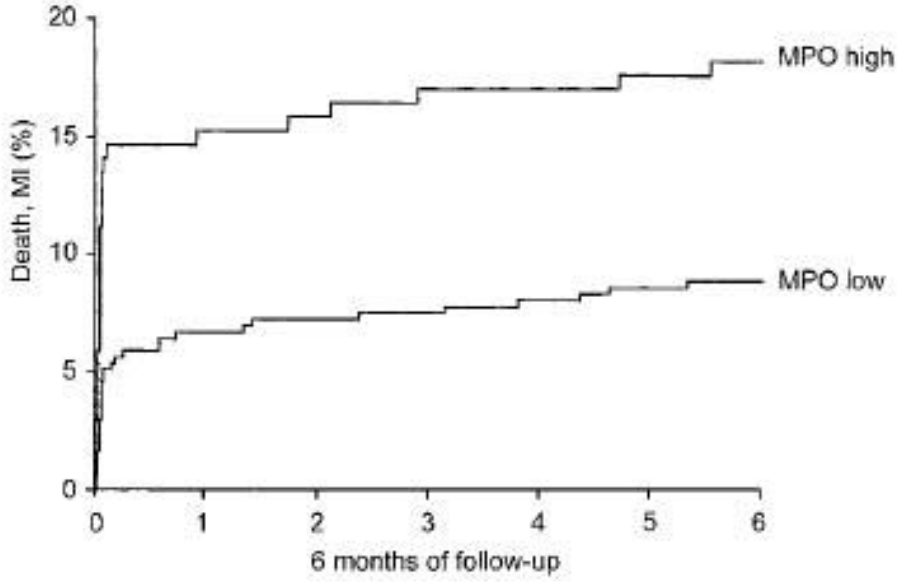
Chronic inflammation

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

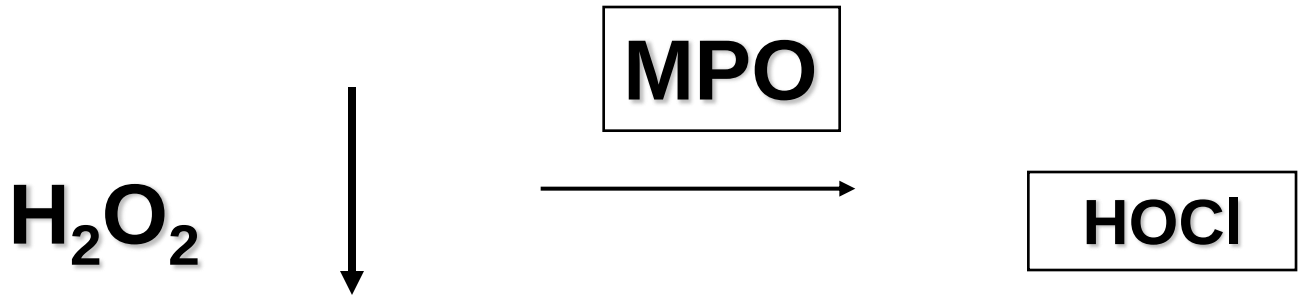
  $\bullet NO_2$ + Tyr• ---> NO_2-Tyr

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



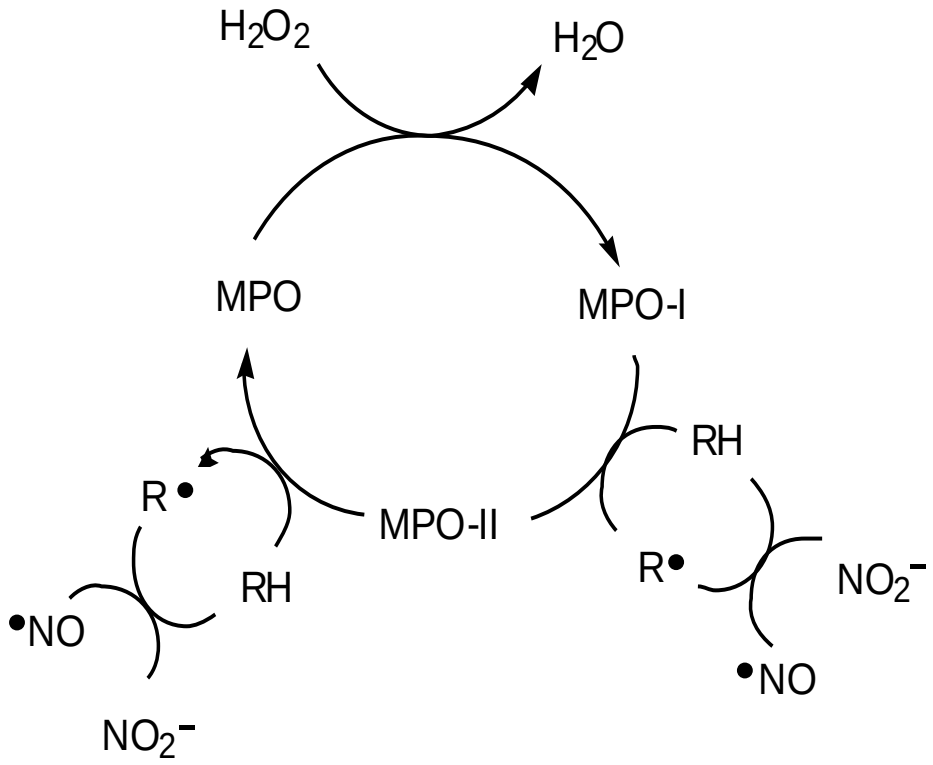
- Redox sensitive transcriptional factors

(gene expression)

- Enzymes with redox sensitive catalytic centers

(direct control of enzyme activity)

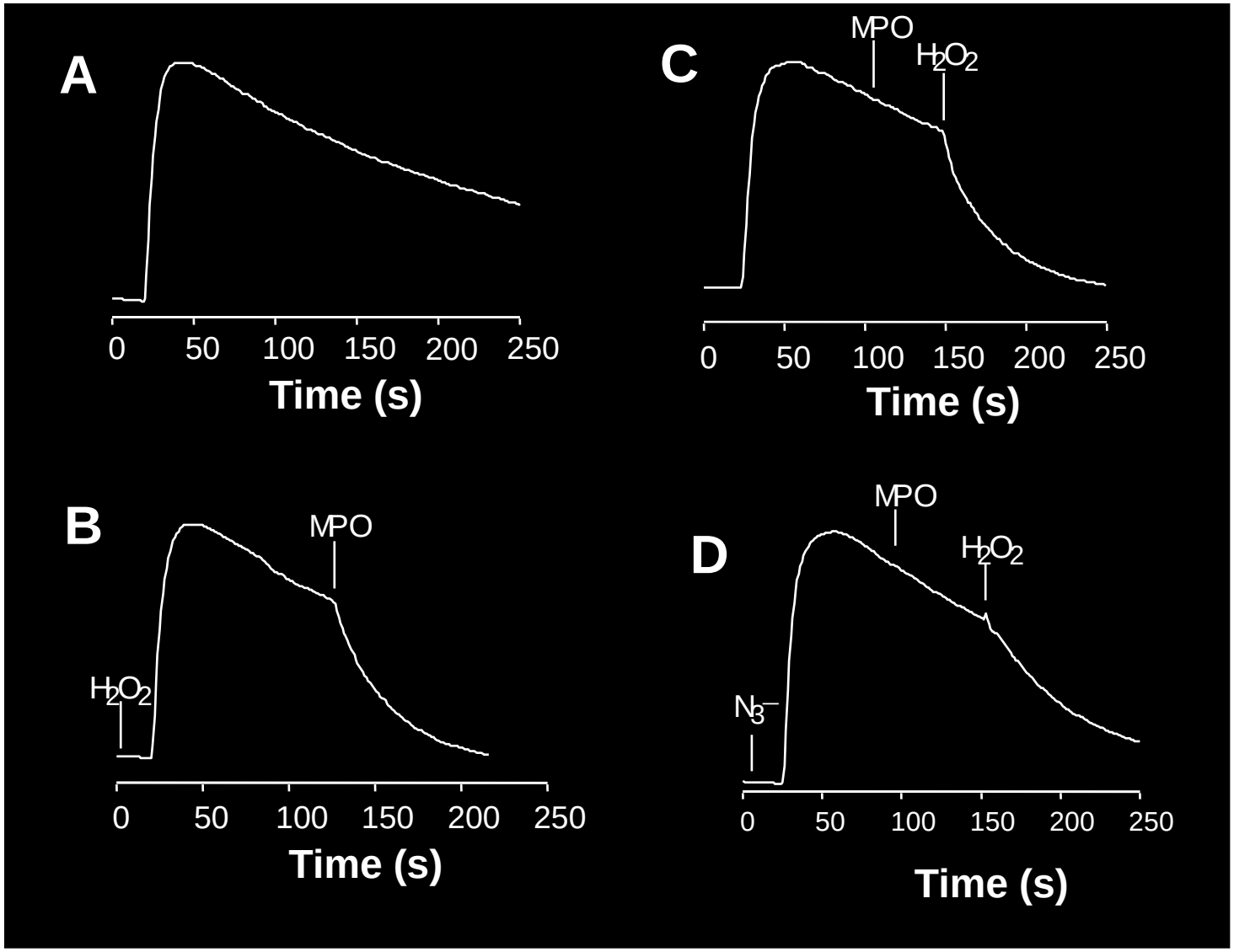
Radical-Mediated NO Consumption by MPO



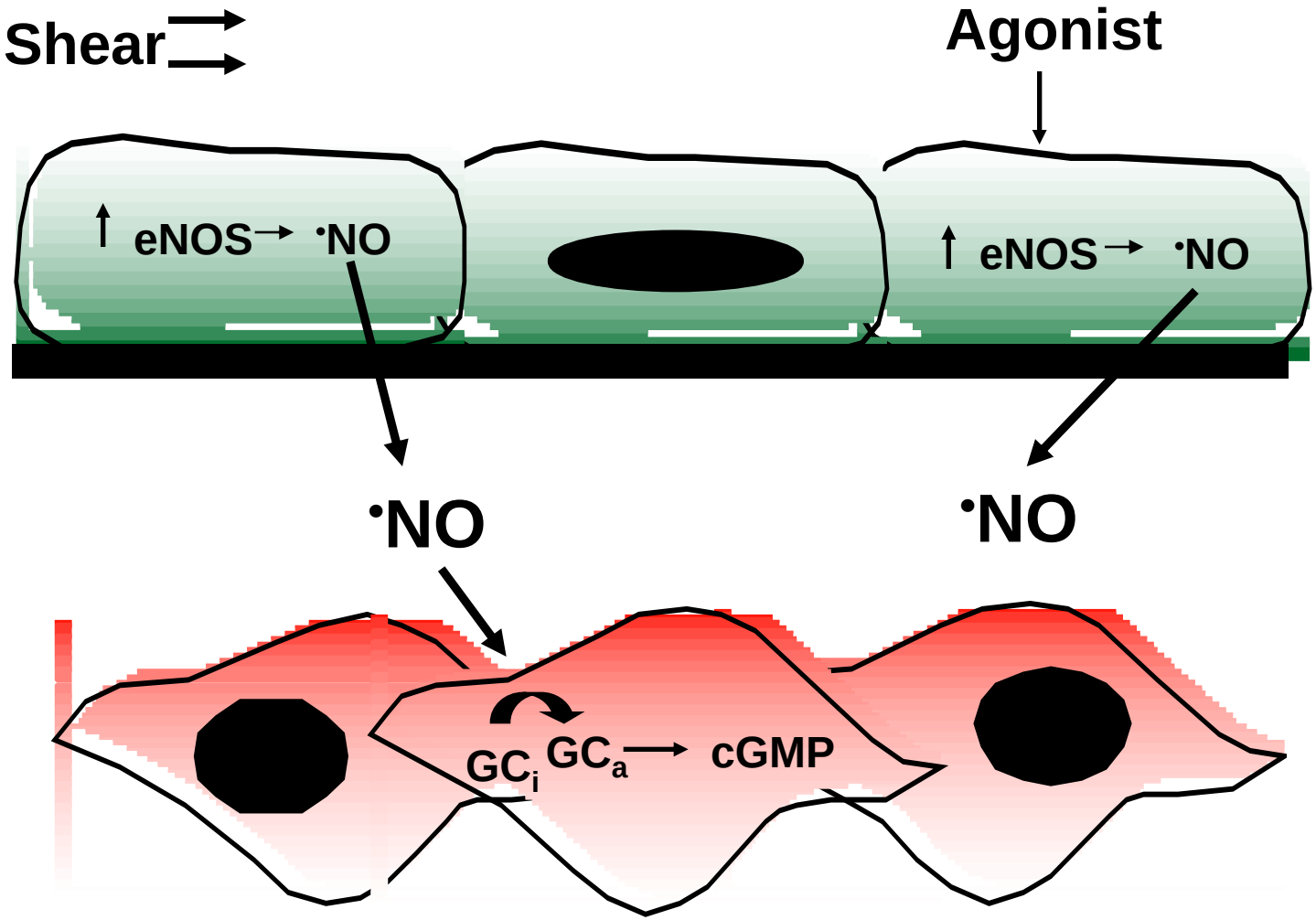
MPO is a catalytic sink for NO

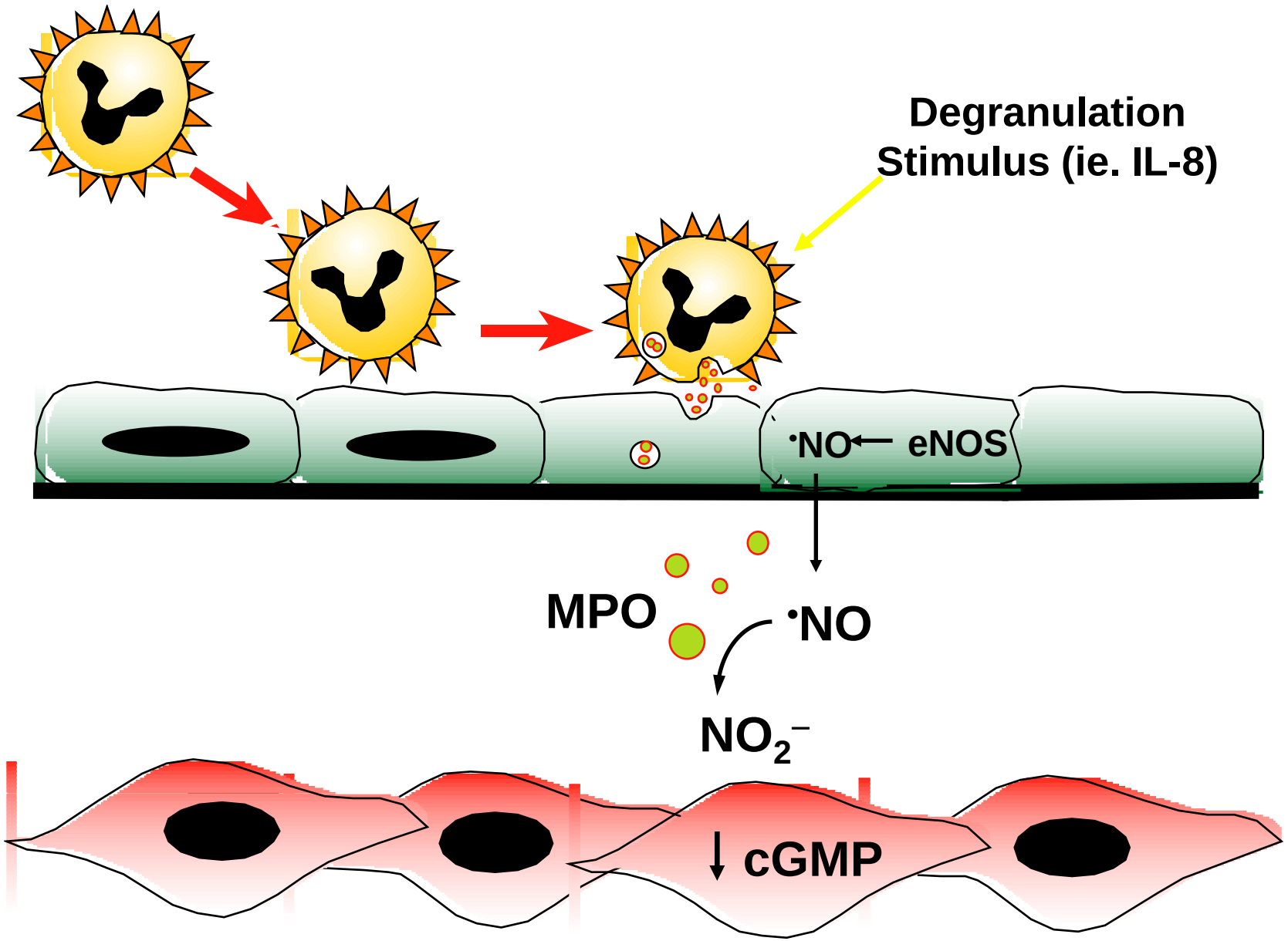
Eiserich et al. (2002) Science

Activated Heme Peroxidases Rapidly Consume •NO

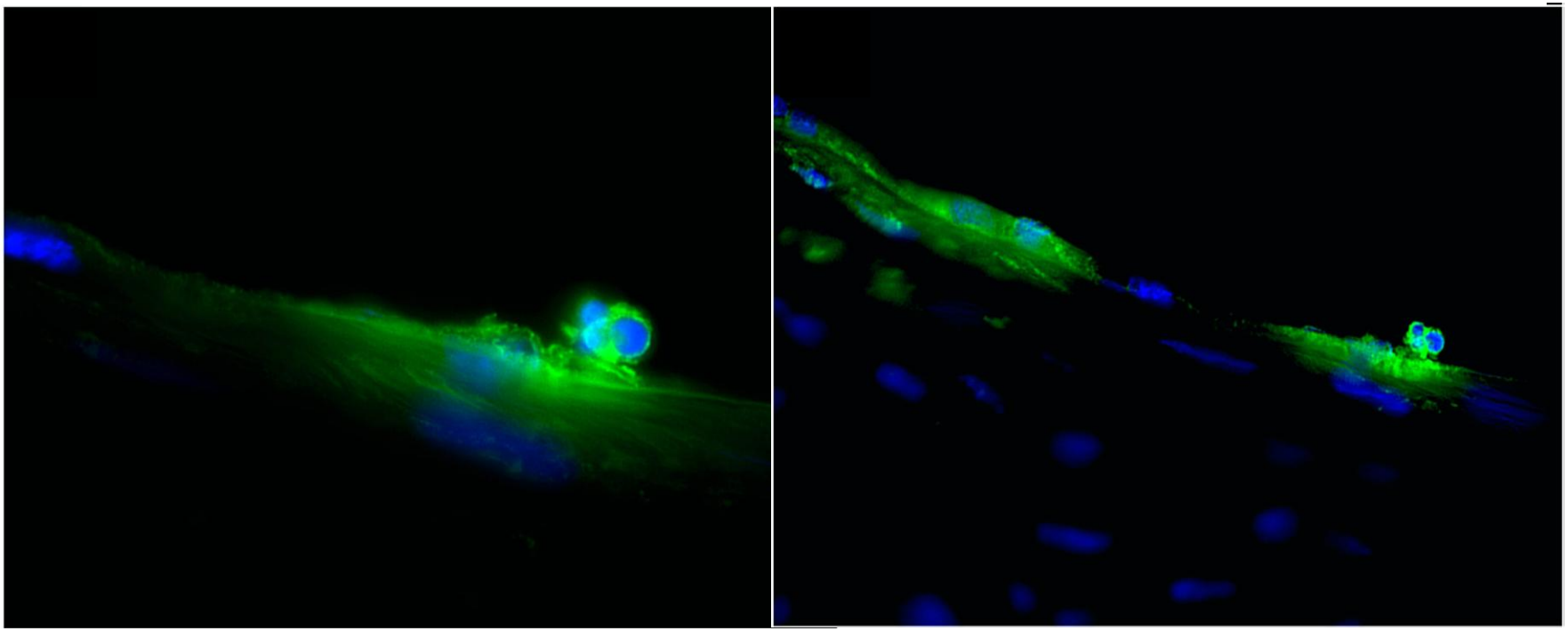


Nitric Oxide-Dependent Signaling in the Vasculature




Degranulation
Stimulus (ie. IL-8)
•NO ← eNOS
MPO
•NO
NO_2^-
↓ cGMP

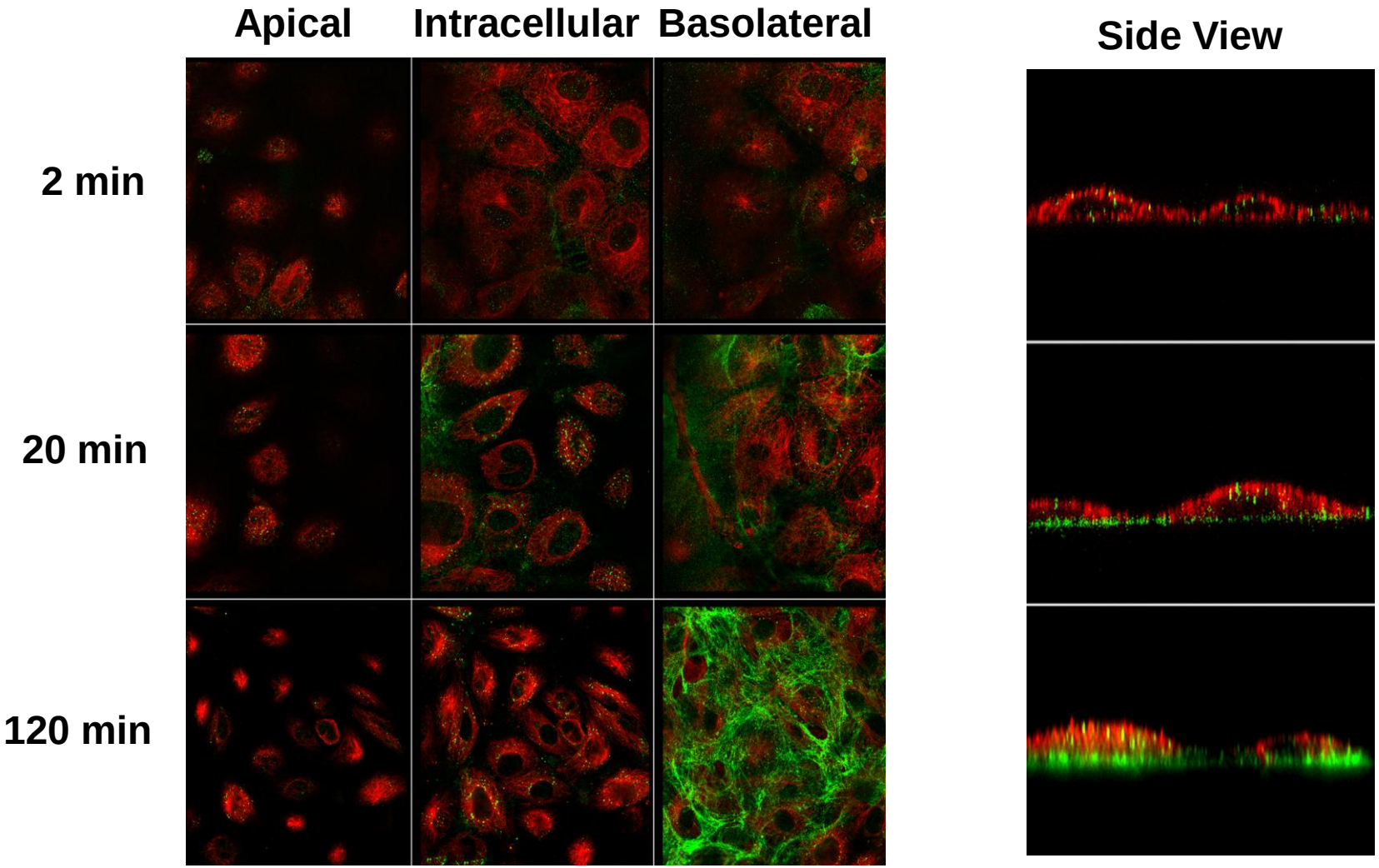
PMN Degranulation Results in Intimal Myeloperoxidase Localization



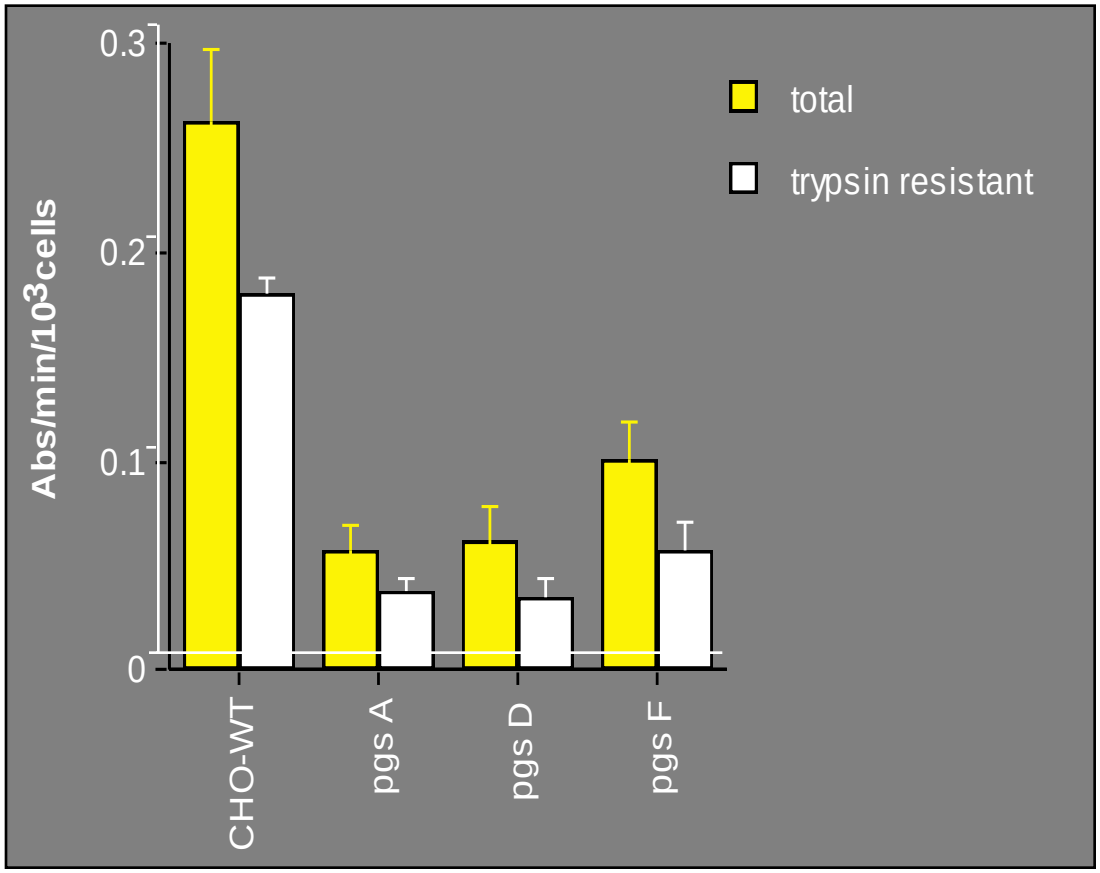
100x

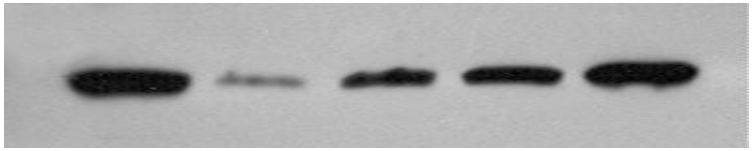
50x

Endothelial Transcytosis of MPO



Binding of MPO to GAG Mutants

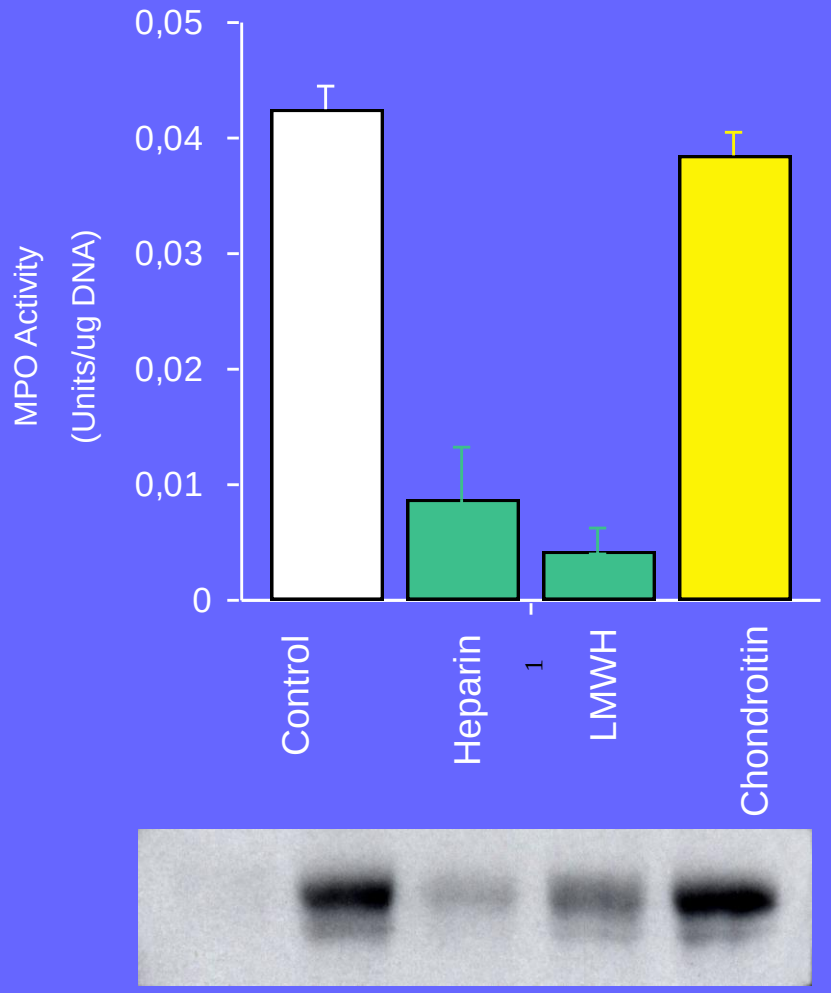




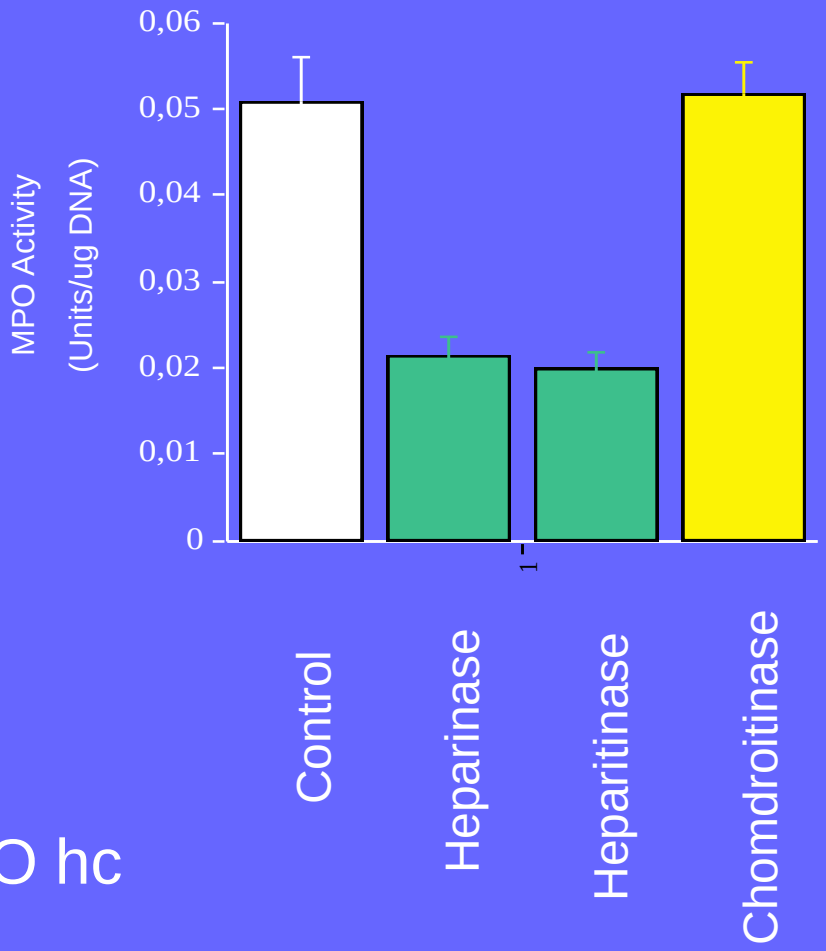
MPO
hc

CHO = Wild-type
pgsA = no GAGs
pgsD = no HS, 3X CS
pgsF = no SO_4-ation

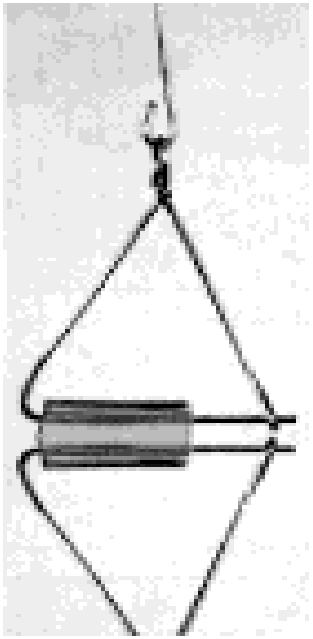
Binding of MPO is dependent on Heparin GAGs on cell surface



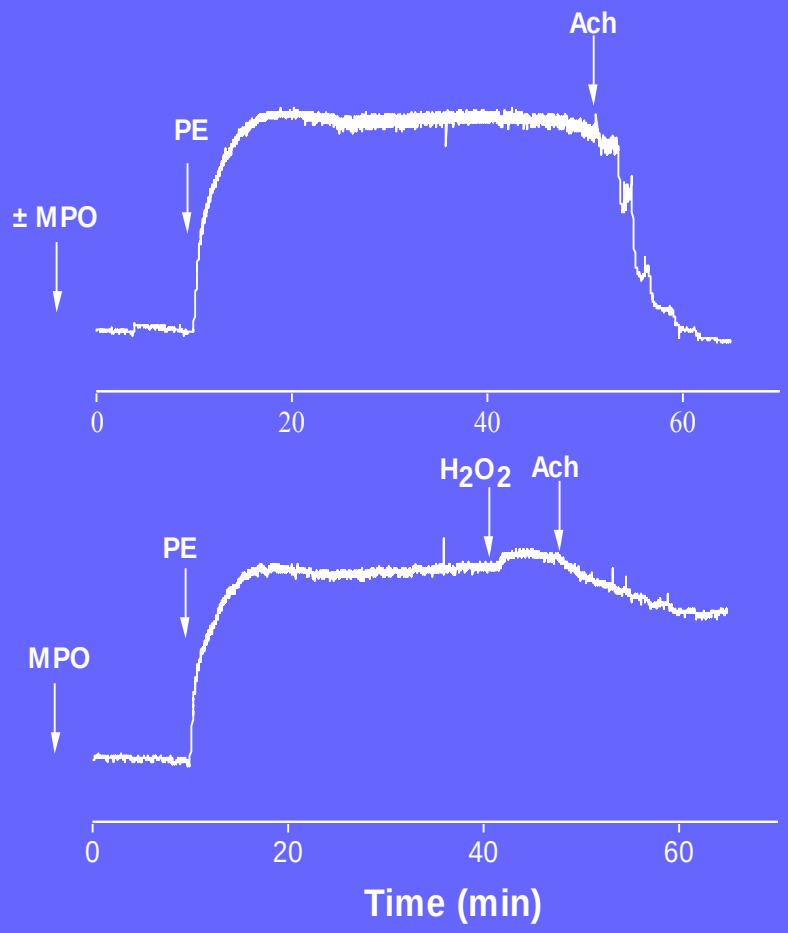
MPO hc



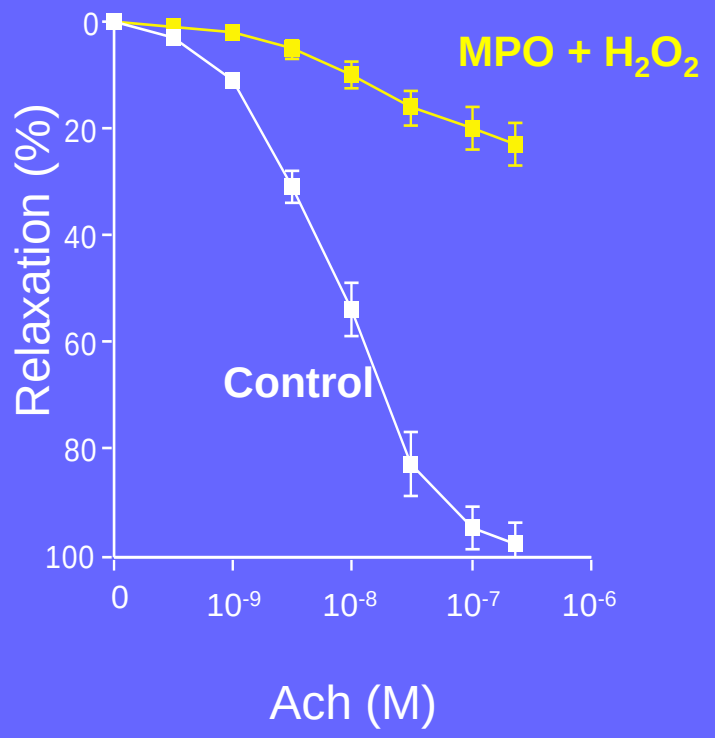
Organ Bath for Isometric Tension Measurements

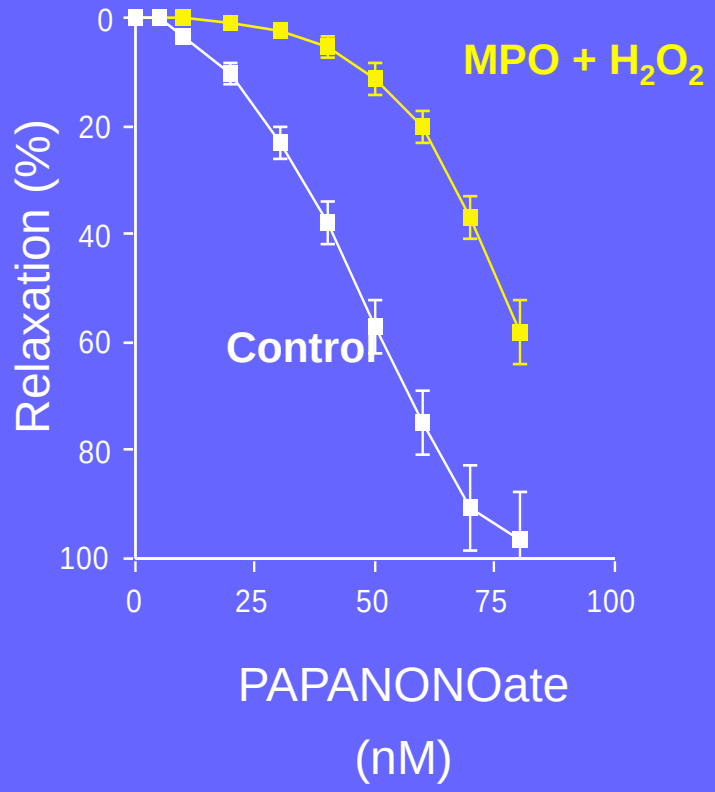


H_2O_2-Activated MPO Inhibits Aortic Relaxation in Response to Acetylcholine

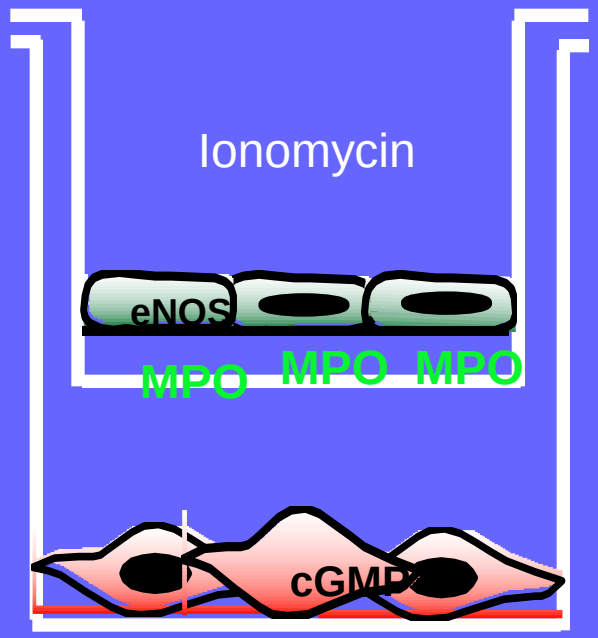


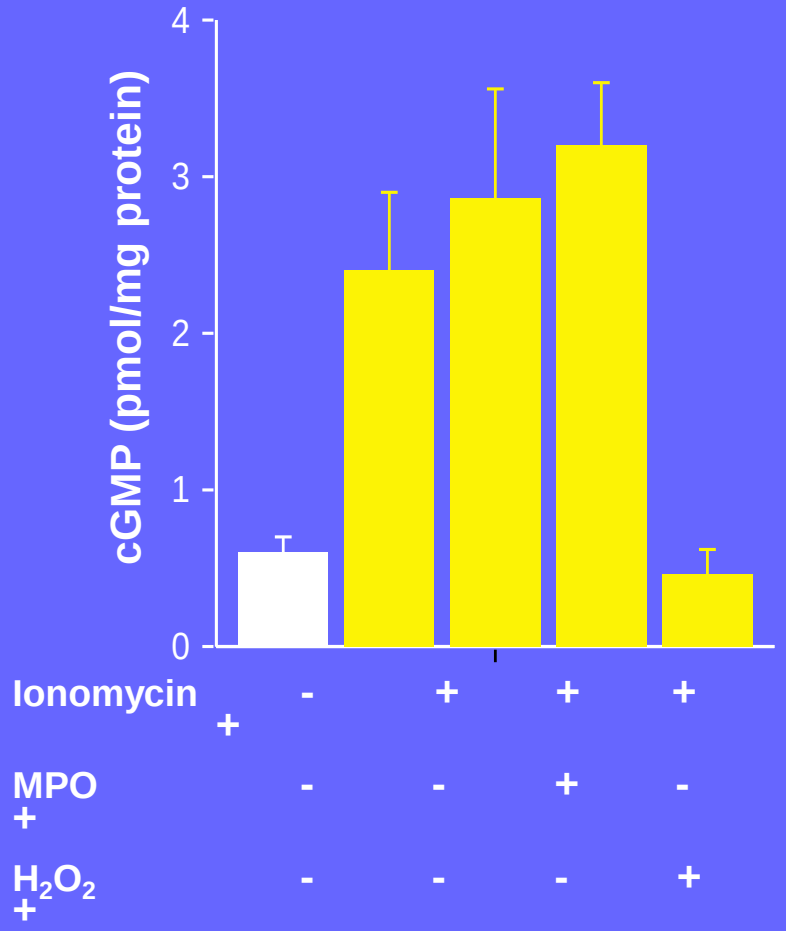
MPO impairs relaxation of rat aortic rings



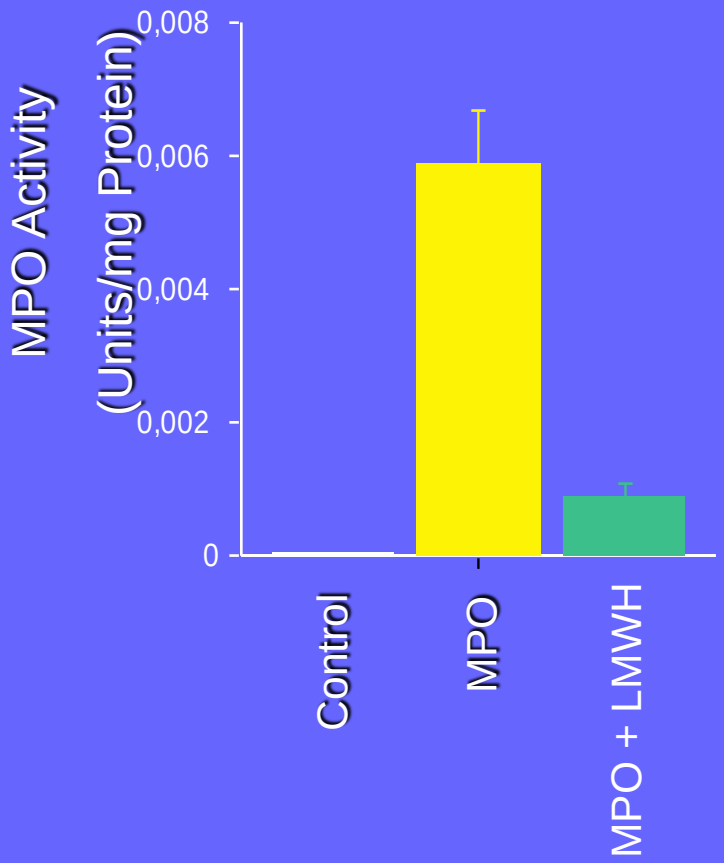


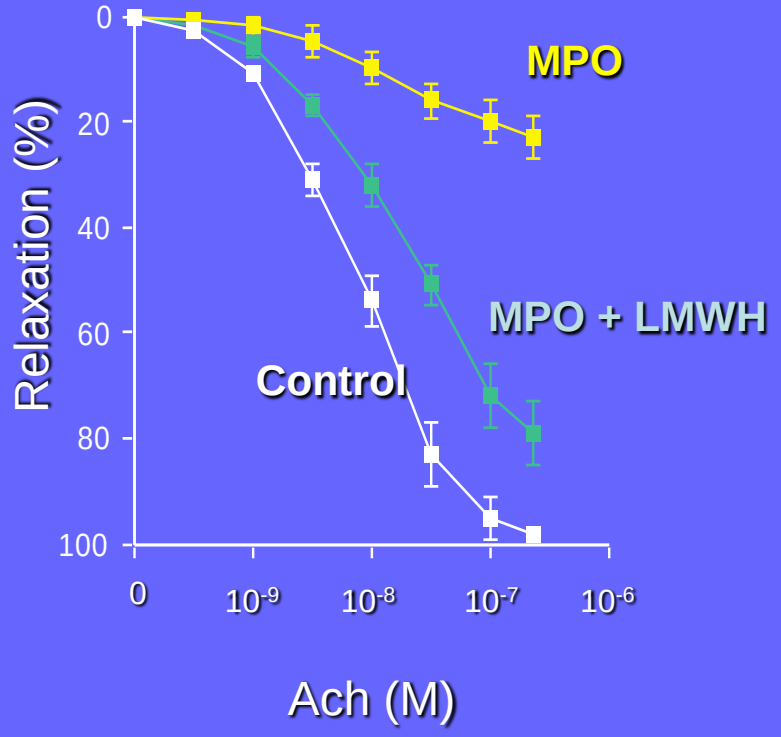
MPO attenuates cGMP levels in cocultures of EC-SMC





Heparin blocks MPO uptake into vascular tissue and restores vessel relaxation





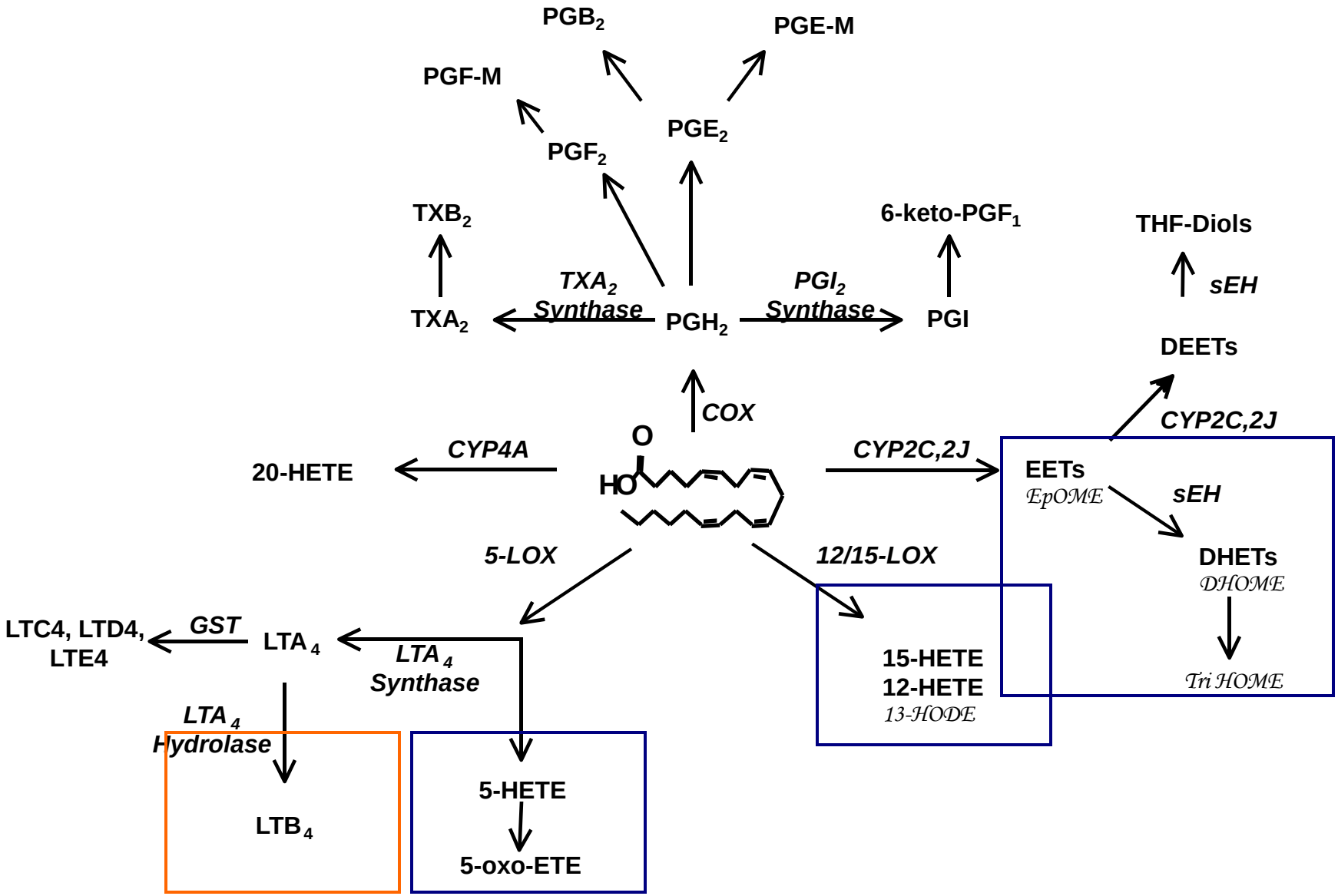
Baldus, Eiserich et al. (2001) JCI

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

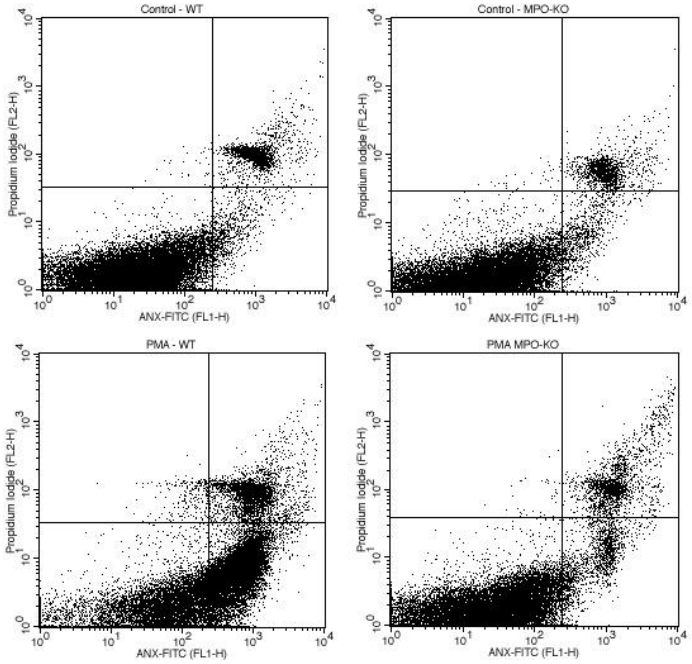
↑ MPO increases levels of **anti-inflammatory** biologically active lipid metabolites (EpOME)

↓ 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 apotosis



Anexin V and PI staining