

# RB biochemistry

## Redox Biology: Biochemistry

David A. Wink and Murali C. Krishna

National Institutes of Health  
National Cancer Institute  
Radiation Biology Branch  
Bldg. 10, Room B3-B69  
Bethesda, Maryland 20892

[wink@mail.nih.gov](mailto:wink@mail.nih.gov) or [murali@helix.nih.gov](mailto:murali@helix.nih.gov)

# Biochemistry Summary

## Biochemical Lecture

### I. Sources of the instigators

#### A. Exogenous sources of radicals

#### B. Endogenous

NADPH oxidase

NADPH P450 oxidoreductases

Xanthine oxidase

Mitochondria

Nitric oxide Synthase

Heme oxygenase

### II. Detoxification:

#### A. Enzymatic

SOD, CAT, GPx

DT-Diaphorase (2-e transfer)

#### B. Scavenging

GSH

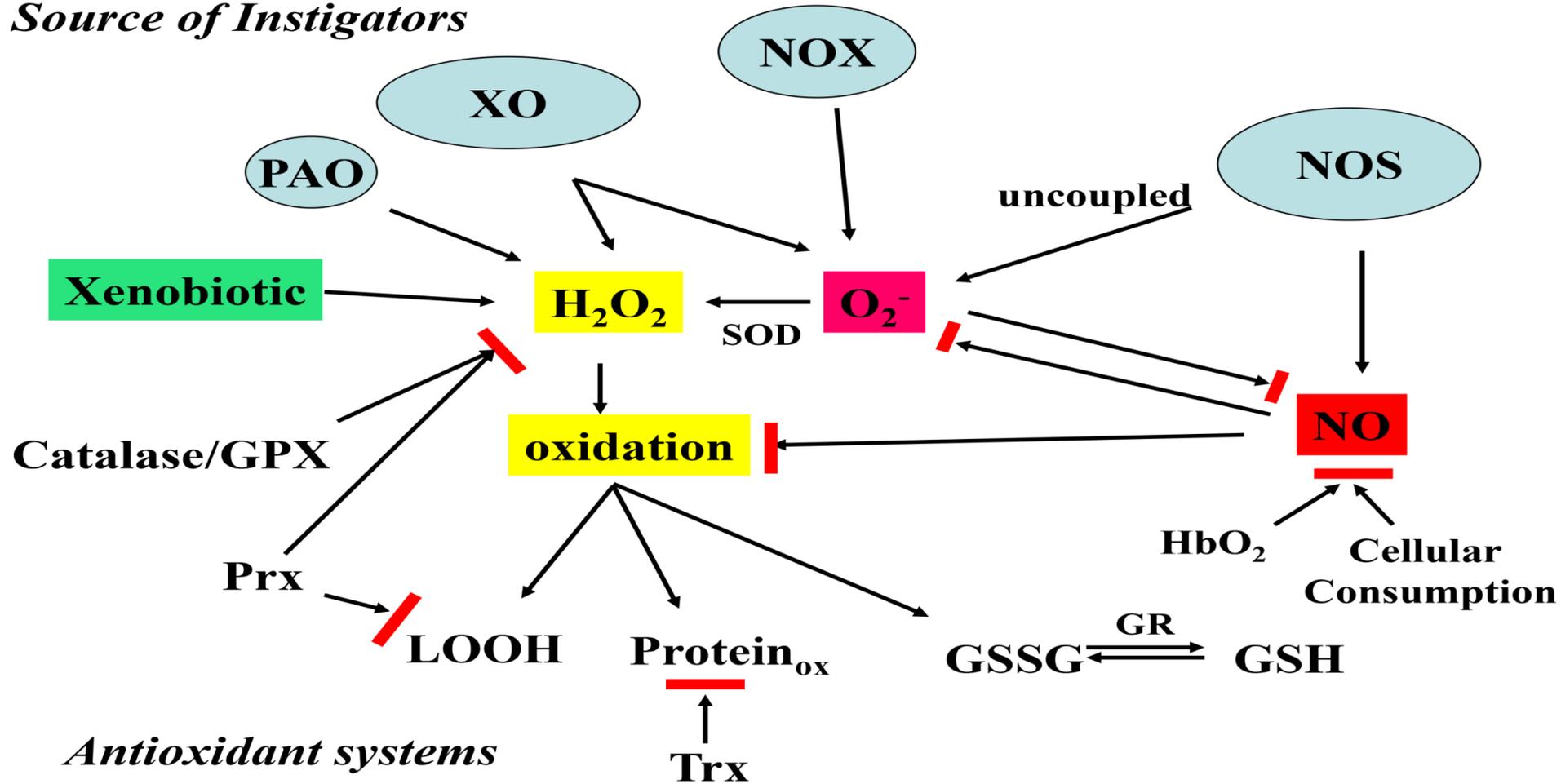
Ascorbate

Tochopherol

# Formation and Abatement of Oxidants

## Formation and Abatement of Oxidants in Redox Biology

### *Source of Instigators*



# Oxygen Reduction

## Reduction of oxygen to $\text{O}_2^-$ and $\text{H}_2\text{O}_2$

### Outer-sphere electron transfer

Thus, the determining factor is E and access



**Substance with more negative reduction potential than  $-0.33 \text{ V}$  can spontaneously reduce oxygen to superoxide.**

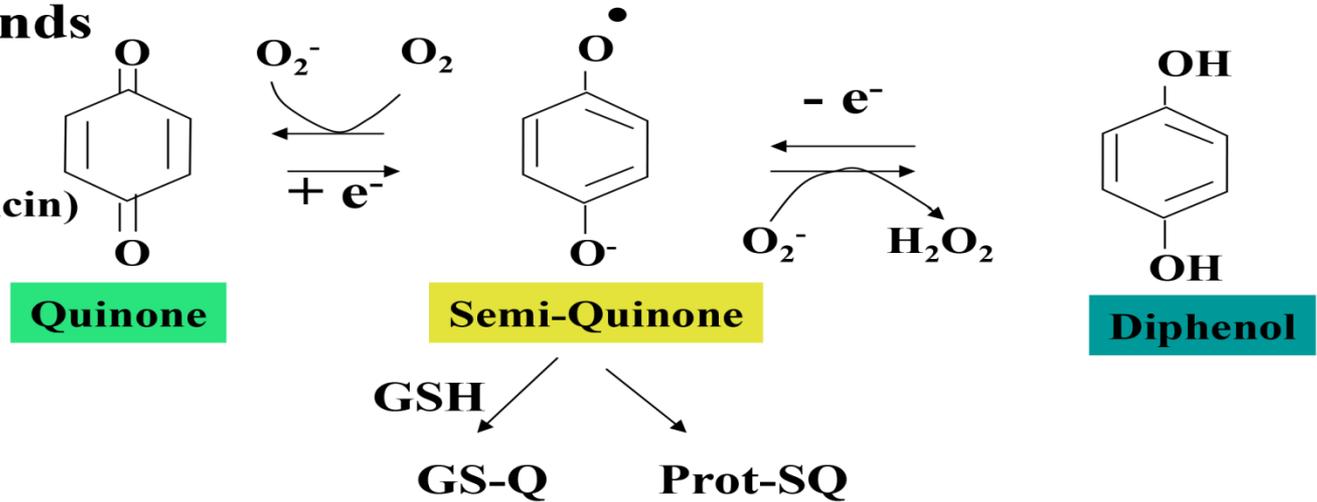


# Redox Cycling

## Redox Cycling to form Reactive Oxygen Species

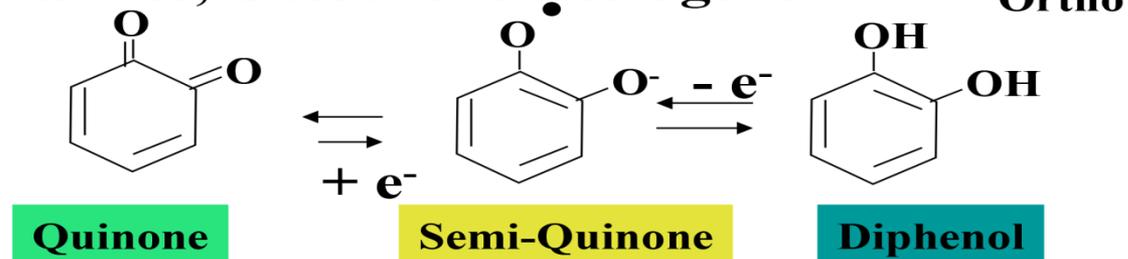
### Types of Compounds

Ubiquione (CoQ)  
Menidione  
Adraimycin (Doxorubicin)  
Tochoperol

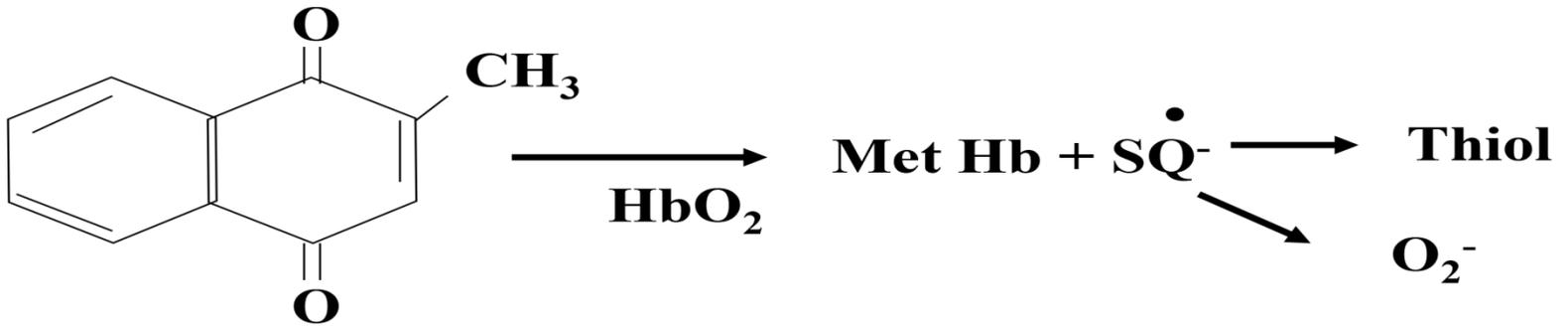


### Dopamine derivatives, Catecholic Estrogens

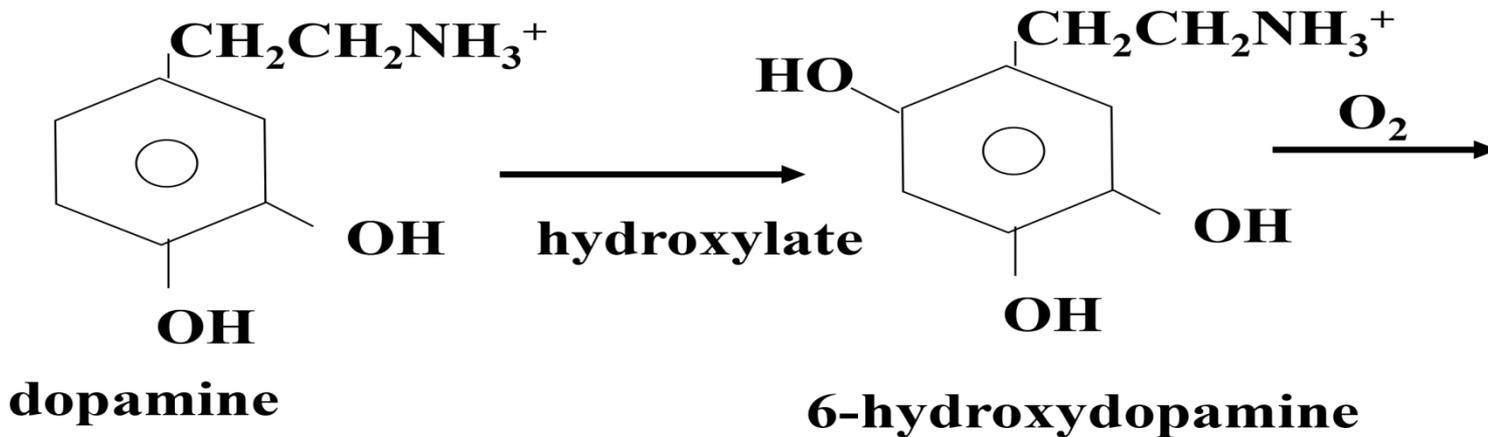
Adrenaline



# Menadione and Dopamine



**Menadione (Vitamin K<sub>3</sub>)**



**dopamine**

**6-hydroxydopamine**

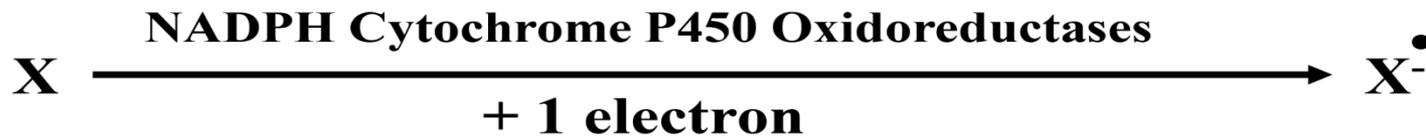




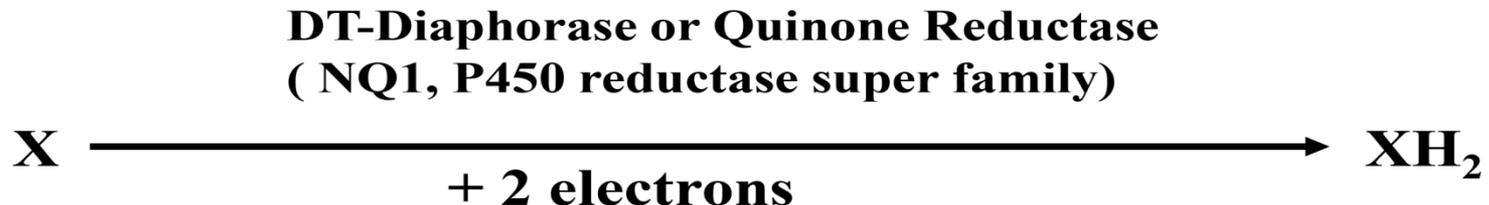
# Xenobiotic Metabolism

## Xenobiotic Metabolism

**Activation of Quinones, Paraquat etc.**



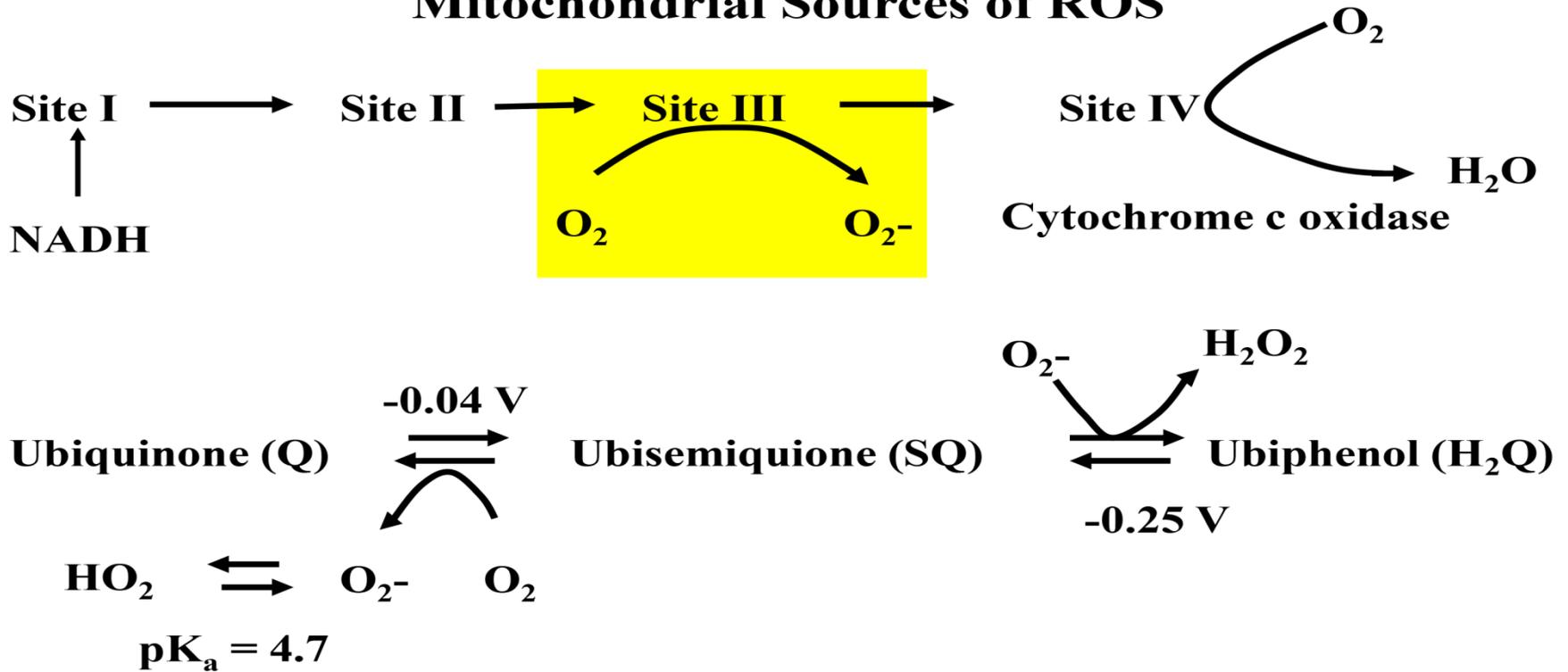
**Detoxification of Quinones, Paraquat etc.**



**Broccoli extracts induce DT-Diaphorase (NQ1) expression**

# Mitochondrial Sources of ROS

## Mitochondrial Sources of ROS



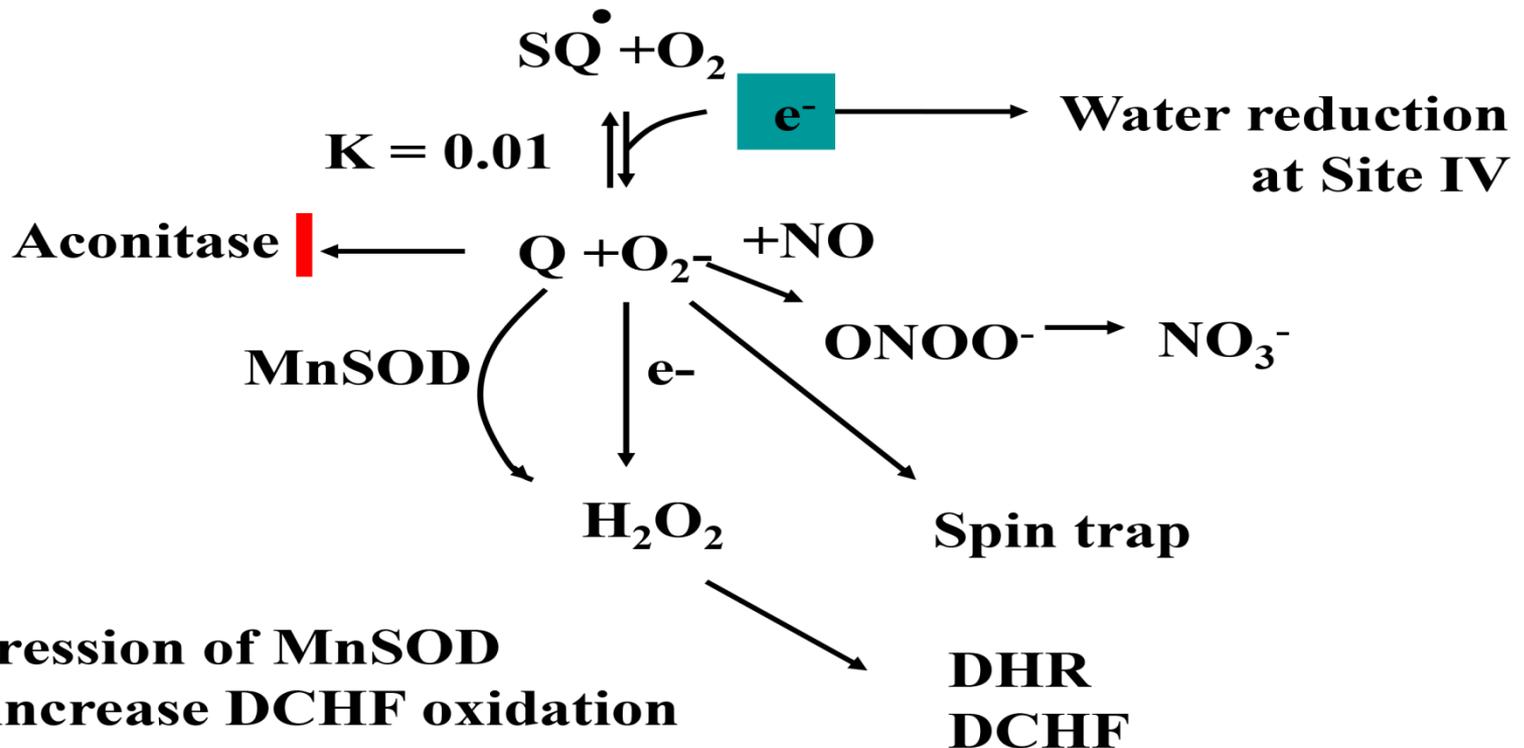
**Polarization/depolarization of the mitochondria can effect reduction where the proton gradient can be important to reduction of O<sub>2</sub>.**

# Energetics of ROS Formation

## Energetics of ROS formation from Site III

$E^\circ = -0.33$   
 $E^\circ = 0.04$   
 $\Delta E = -0.29$

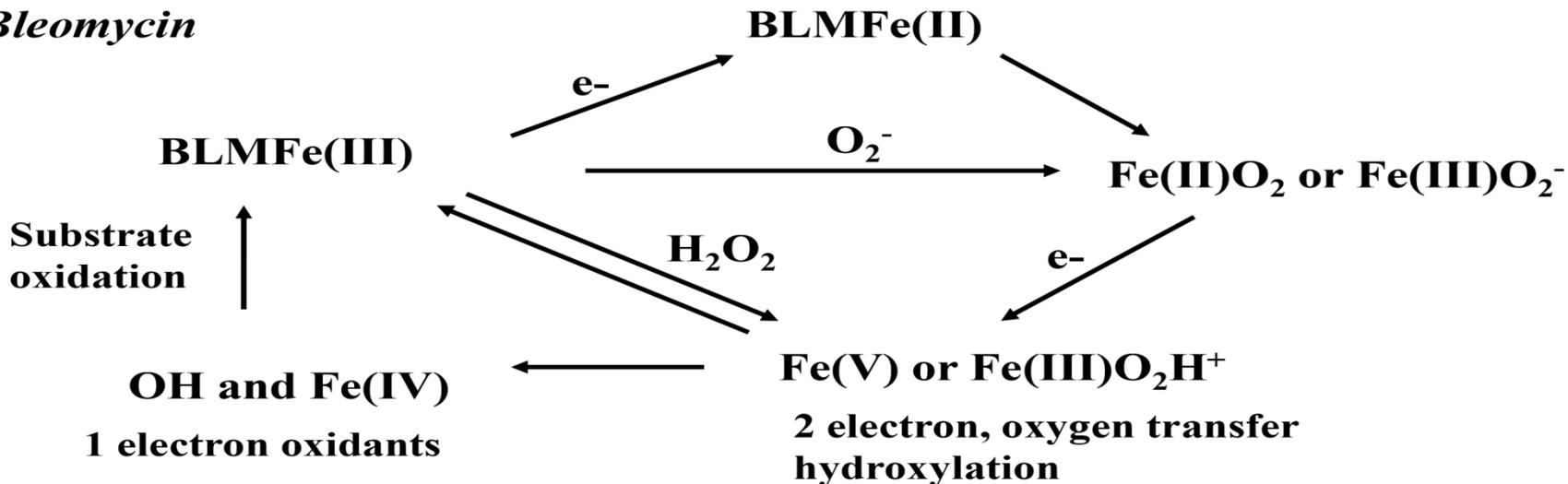
$\Delta G \sim 6 \text{ kcal}$   
 $K = 0.01$



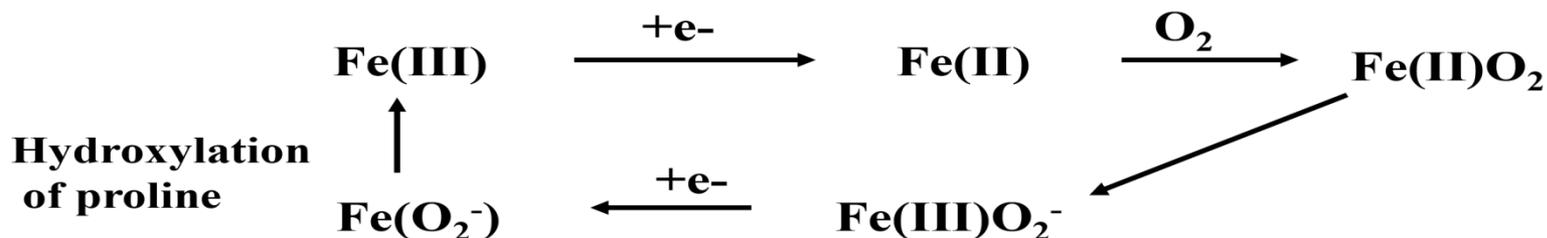
# Inner-Sphere Reduction Examples

## Examples of Inner-sphere reduction of Oxygen

### *Bleomycin*



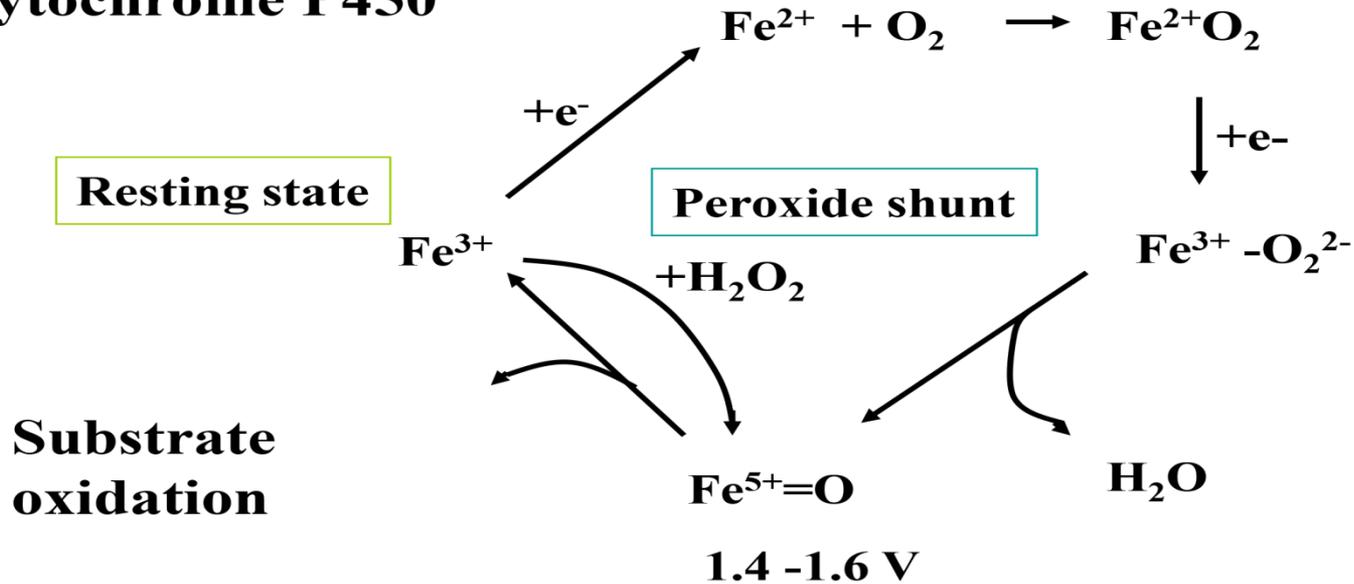
### *Proylyl Hydroxylase*



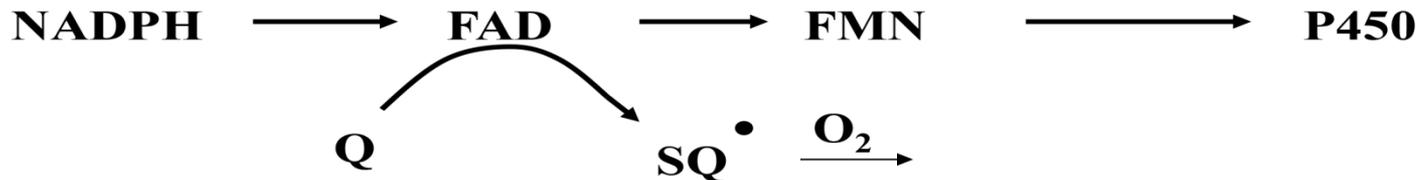
# P450 System

Different reduction mechanism of O<sub>2</sub> by P450 system

Cytochrome P450



P450 reductase



# **Enzymatic Generation Purpose**

## **Enzymatic generation of Reactive Oxygen Species**

### **Purpose:**

- **As an antimicrobial agent**
- **as a by product of metabolism**
- **Part of signal transduction mechanisms**

# Hydrogen Peroxide Formation Example

## Examples of Hydrogen peroxide formation

### Glucose Oxidase (Glc oxidase: Bacterial)



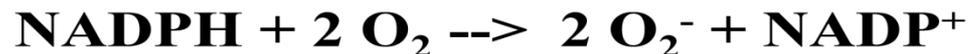
### Polyamine oxidase (PAO)



### Xanthine Oxidase (XO)



### NADPH oxidase (NOX)

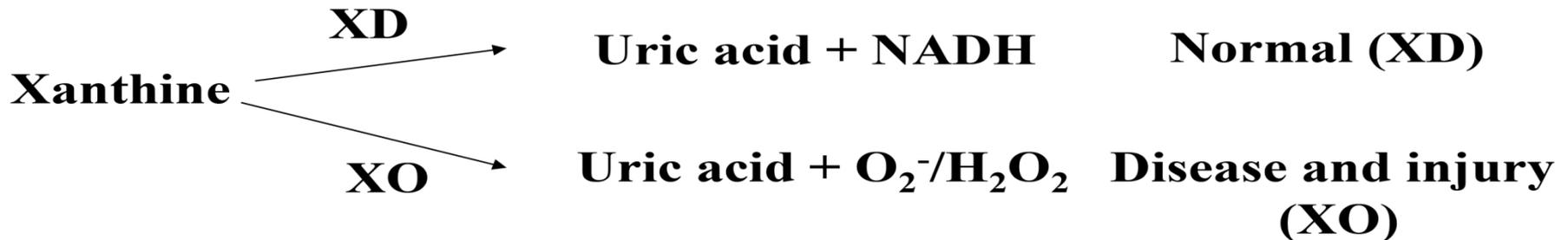


# Xanthine Oxidase

## Xanthine Oxidase

**Purpose:**

**purine metabolism  
secretion of milk drops  
Detoxification of Aldehydes  
Generation of ROS**



**XO**



**XD to XO by proteolysis or thiol oxidation**

# Xanthine Oxidase

## Xanthine Oxidase

Homodimer

Inhibited by allopurinol (abundant in goats milk)

HX or X

Urate

Mo

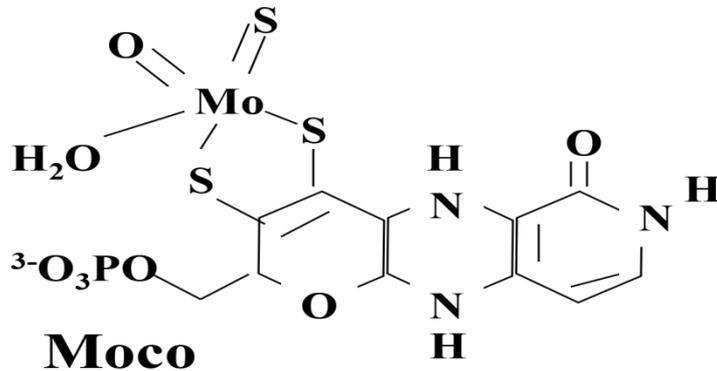
FAD

FADH<sub>2</sub>

Fe<sub>2</sub>S<sub>2</sub>

O<sub>2</sub>

O<sub>2</sub><sup>-</sup>/H<sub>2</sub>O<sub>2</sub>



Mouse utilizes XO more than humans

# NADPH Oxidase

## NADPH oxidase (NOX)

### Purpose:

**Phagocytic (Phox)**

**Membrane bound**

**gp91<sup>phox</sup>**

**p21<sup>phox</sup>**

**Cytosolic regulators**

**p47<sup>phox</sup>**

**p67<sup>phox</sup>**

**p40<sup>phox</sup>**

**Small GTPase/rac1/Rac2**

**Antimicrobial**

**Regulation of cell-surface signaling  
regulation of physiological function**

**NOX-1 colon VSMC, prostate**

**NOX-2 innate immune system**

**NOX-3`inner ear**

**NOX-4 kidney**

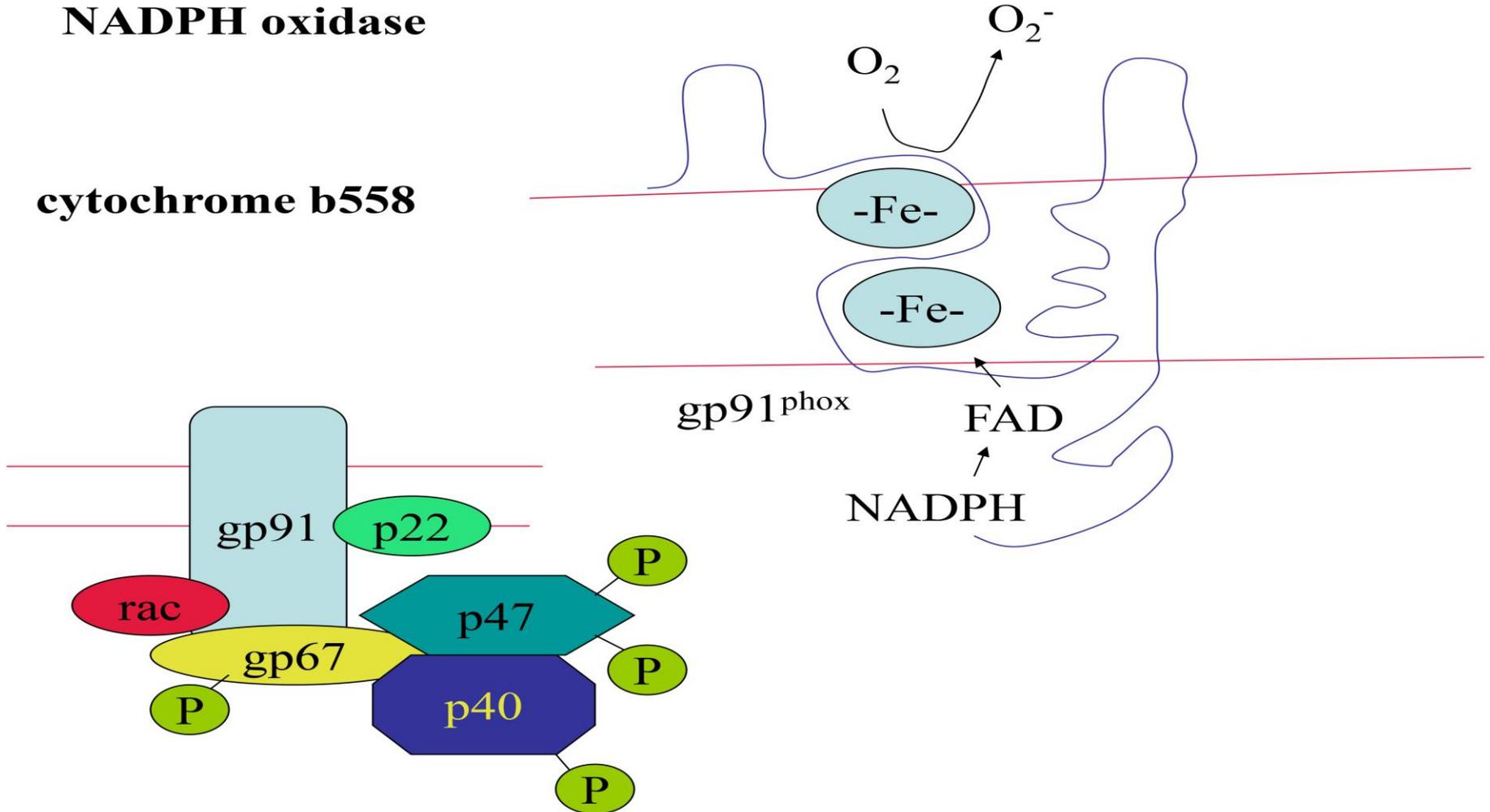
**NOX-5 spleen (human only)**

**Vascular NOX**

# NADPH Oxidase

NADPH oxidase

cytochrome b558



# Nitric Oxide Synthase

## Nitric Oxide Synthase

### Homodimer

**NOS-1 nNOS or neuronal NOS**  
**NOS-3 eNOS or endothelial NOS**

**Constitutive and calcium sensitive**

**NOS-2 iNOS or inducible NOS**

**Induced and calcium insensitive**

**P450 reductase domain**

**NADPH**

**Calmodulin binding**

**FMN**

**FAD**

**Heme oxidase domain**

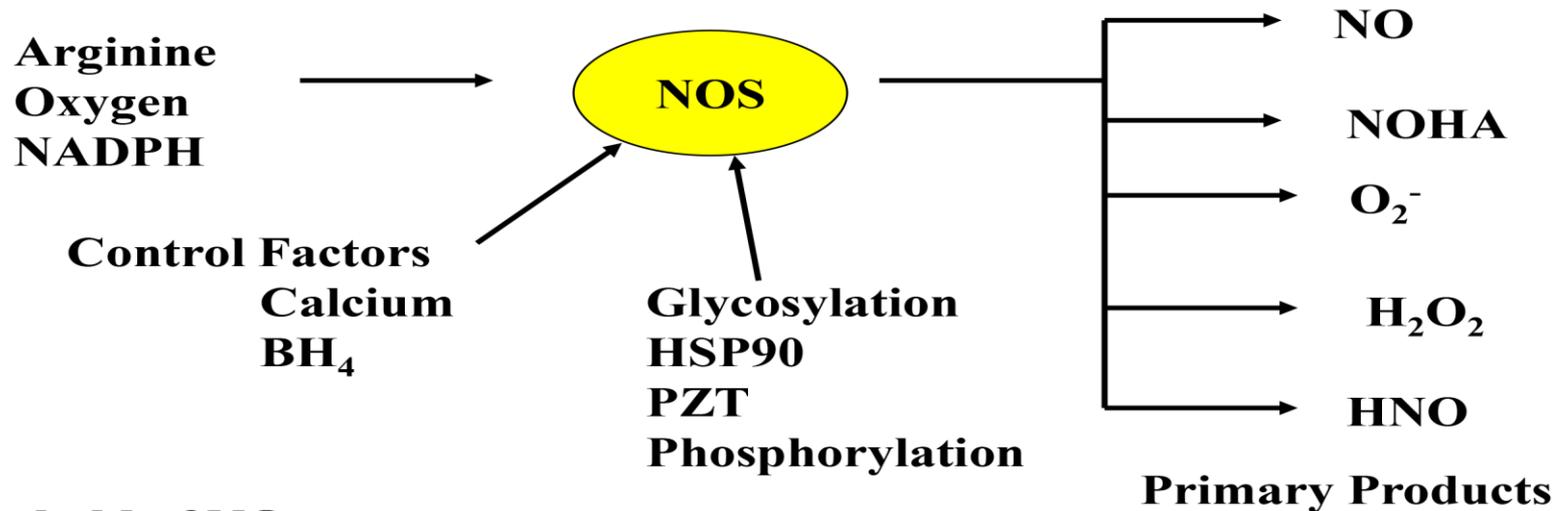
**O<sub>2</sub>**

**Arginine/*N*-hydroxyarginine (NOHA)**



# NOS Biochemistry

## NOS Biochemistry



$K_i^{\text{NO}}$       **iNOS >> nNOS or eNOS**

$K_m^{\text{O}_2}$       **eNOS, 25  $\mu\text{M}$ ; iNOS, 120  $\mu\text{M}$ ; nNOS, 400  $\mu\text{M}$**

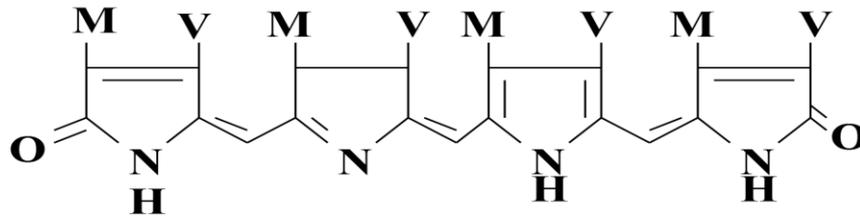
# Heme Oxygenase

**Heme Oxygenase**

**P450 superfamily**  
**Numerous factors induce HO-2**

**HO-1**  
**HO-2**

**Heme**  
↓ **HO**

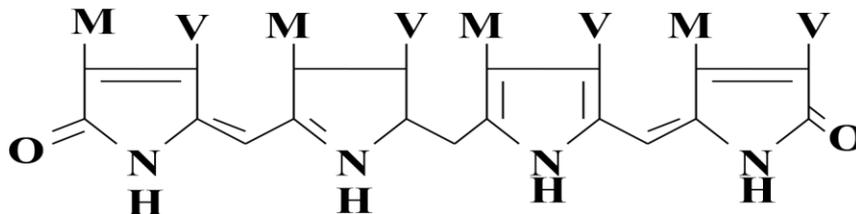


**Biliverdin**

+ **CO** → ?

**Guanylyl cyclase**  
**p38 activation**

↓ **NADPH**  
↘ **NADP<sup>+</sup>**



**Bilirubin**

→ **Antioxidant**

# Biochemistry of Prevention

## Biochemistry of Prevention of Oxidative or Nitrosative stress

### Different classes

#### *Preventive*

**Inhibition of production of instigators**

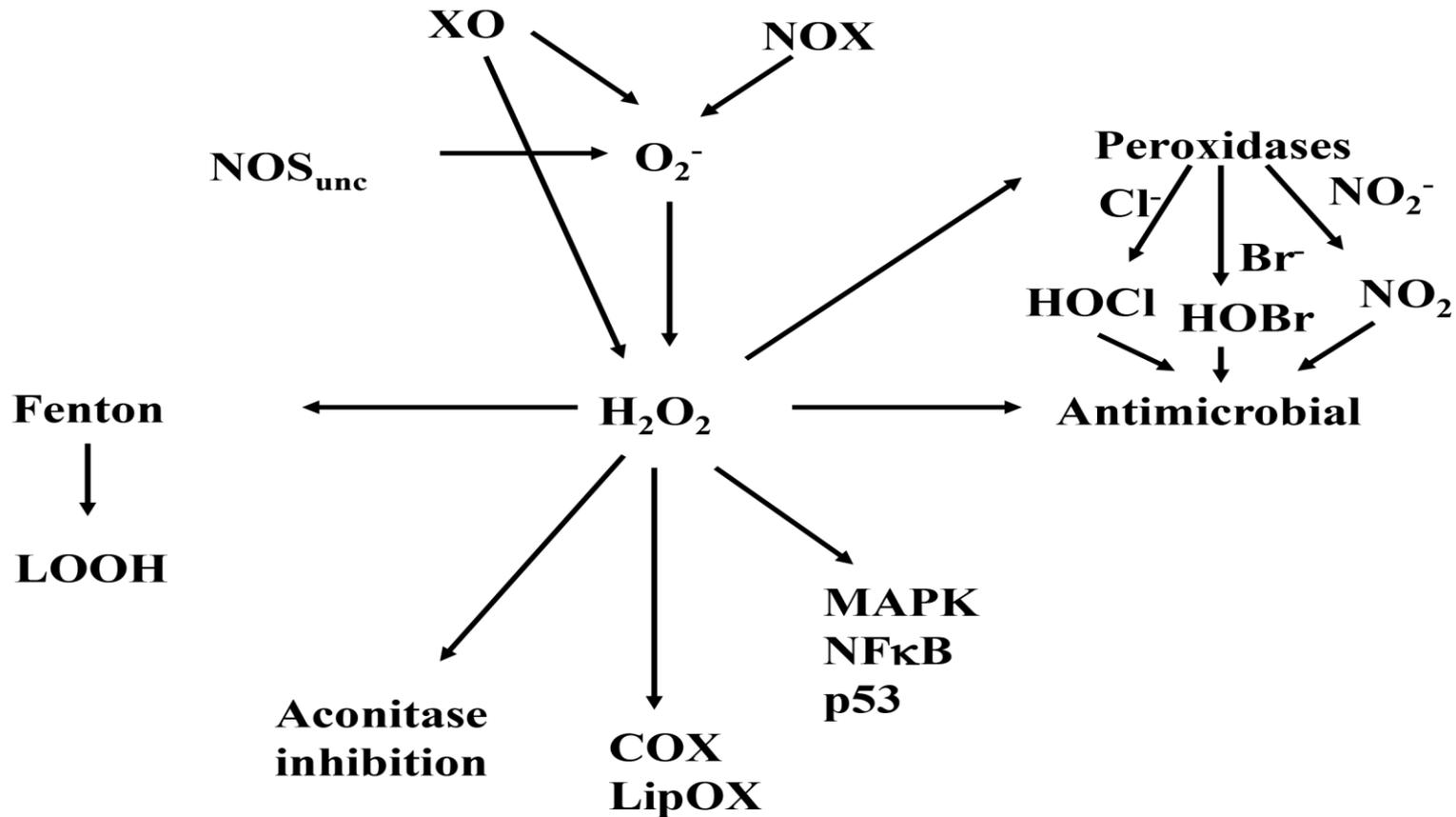
#### *Interception and Quenching*

**Scavenging of reactive intermediates formed**

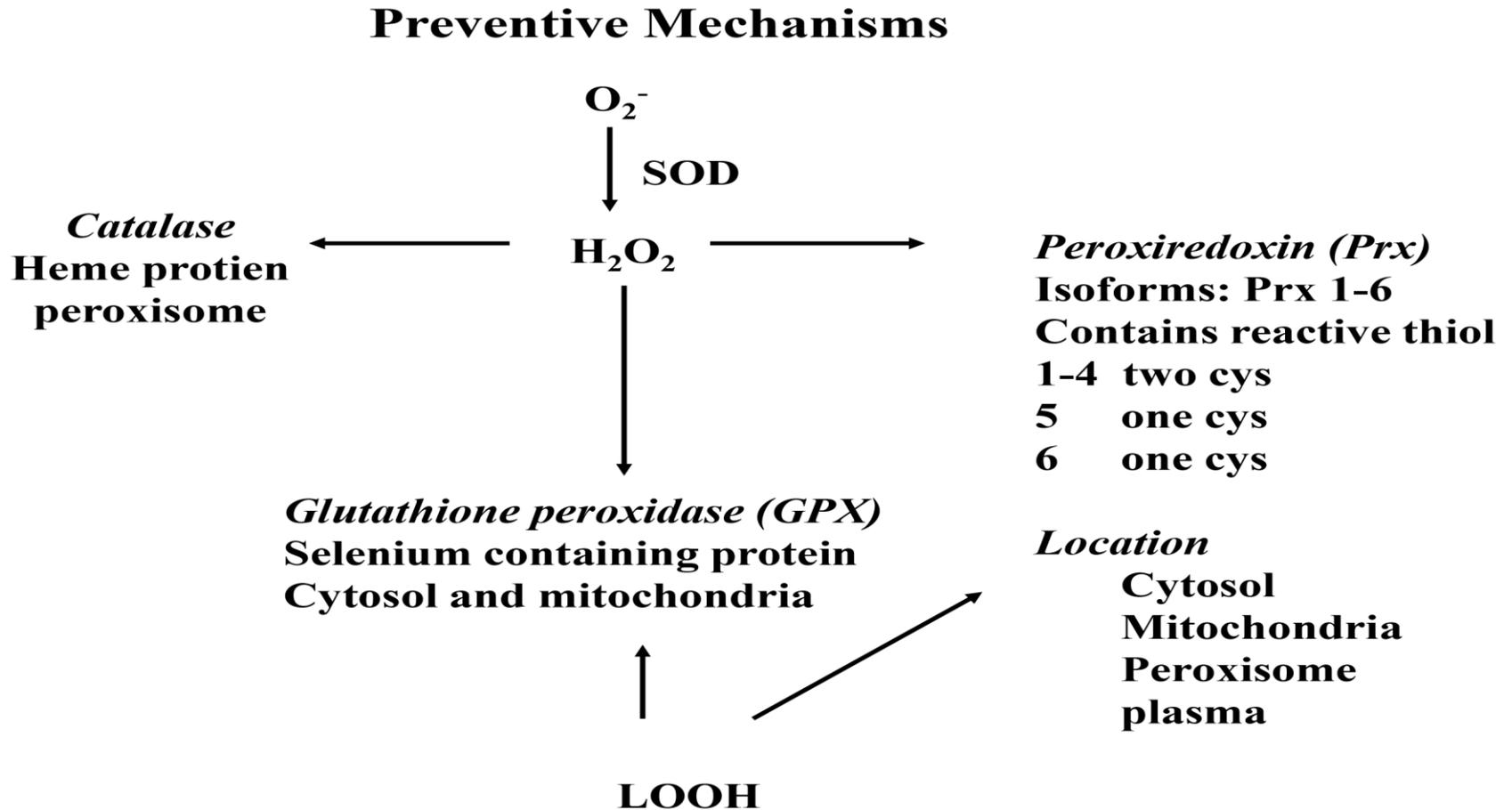
- 1) Want the product to be innocuous**
- 2) The product could be biologically recycled**
- 3) Prevent chain reactions from occurring (chain breaking)**

# Biochemistry Reactive Oxygen Species

## Biochemistry Reactive Oxygen Species



# Preventive Mechanisms



# Chemical Biology of Nitric Oxide

## Chemical Biology of Nitric Oxide

**Direct**

**Inhibition of Respiration**

**Guanylyl  
cyclase**

**NOS**

**NO**

**Radical scavenging  
Antioxidant properties**

**Inhibition of P450**

**Indirect**

**O<sub>2</sub><sup>-</sup>**

**ONOO<sup>-</sup>**

**+NO**

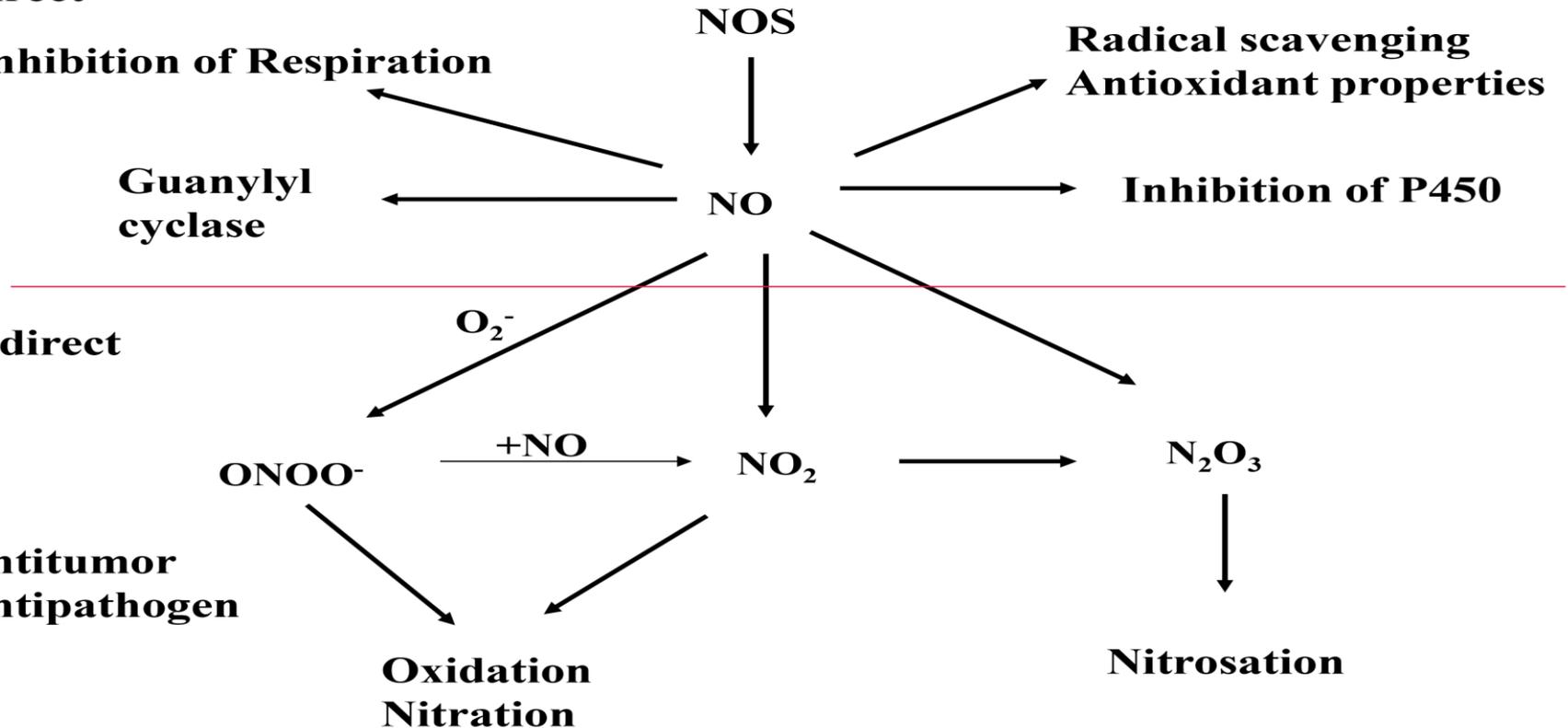
**NO<sub>2</sub>**

**N<sub>2</sub>O<sub>3</sub>**

**Antitumor  
Antipathogen**

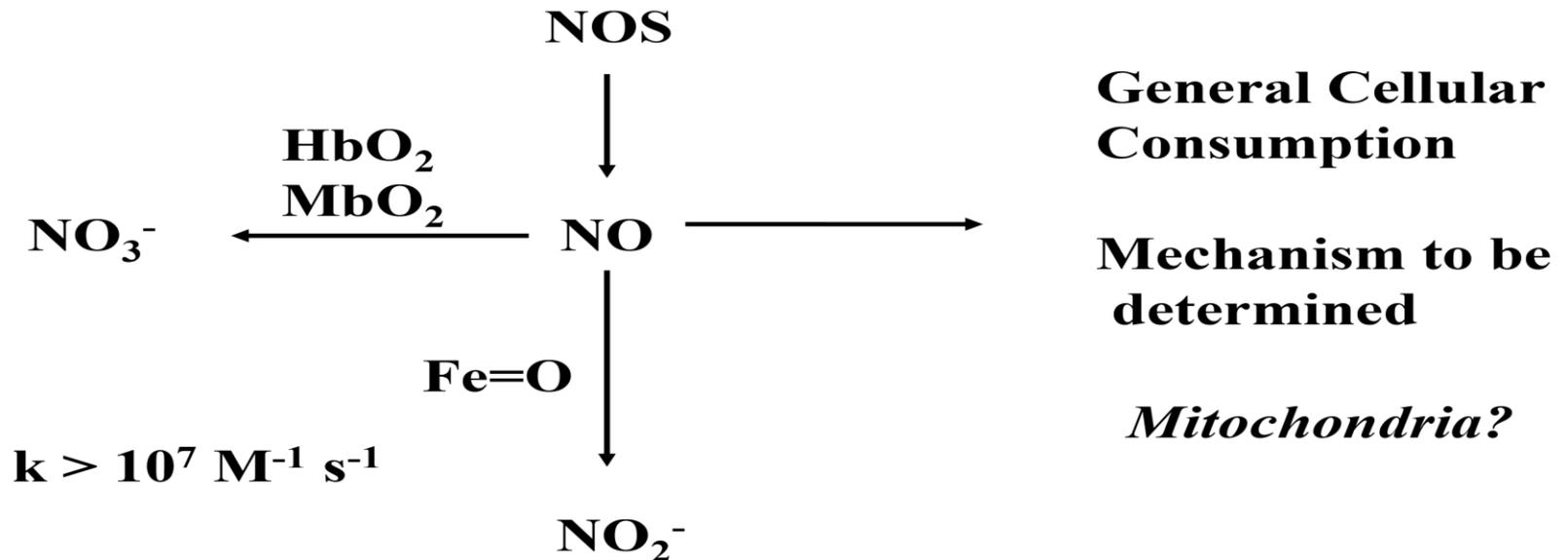
**Oxidation  
Nitration**

**Nitrosation**



# Scavenging of NO

**Scavenging of NO (Prevention of RNOS formation)**



**Critical to compartmentalizing NO effects**

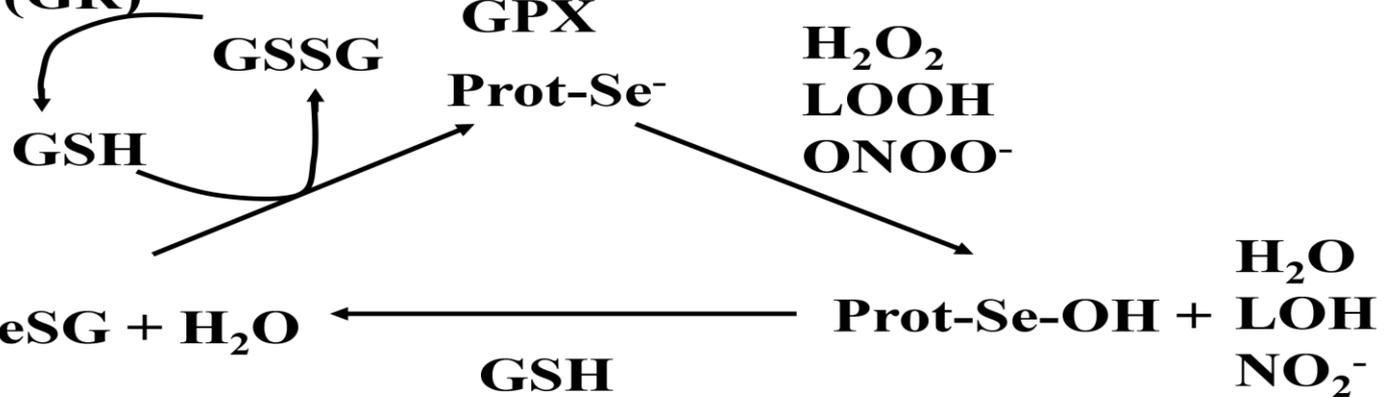
# Glutathione Peroxidase Biochemistry

## Biochemistry of Glutathione Peroxidase

**NADPH**



**Glutathione reductase (GR)**

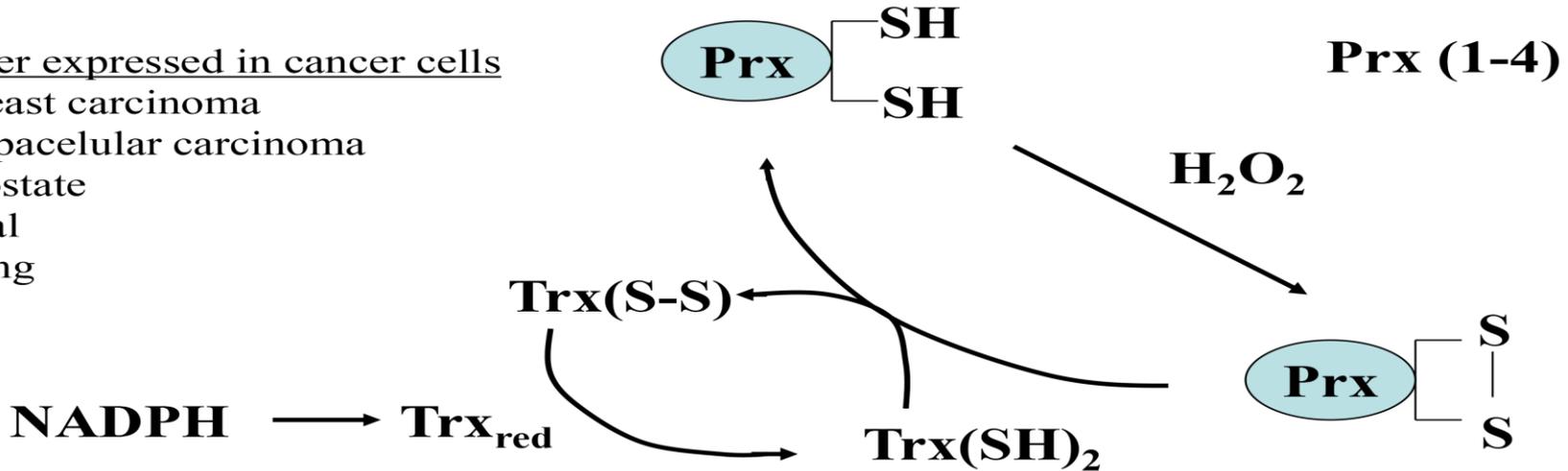


**Selenium compound Eblselen**

# Peroxioredoxin

Over expressed in cancer cells  
 Breast carcinoma  
 Hepacelular carcinoma  
 Prostate  
 Oral  
 Lung

## Peroxioredoxin (Prx)



**Trx = Thioredoxin (ER)**

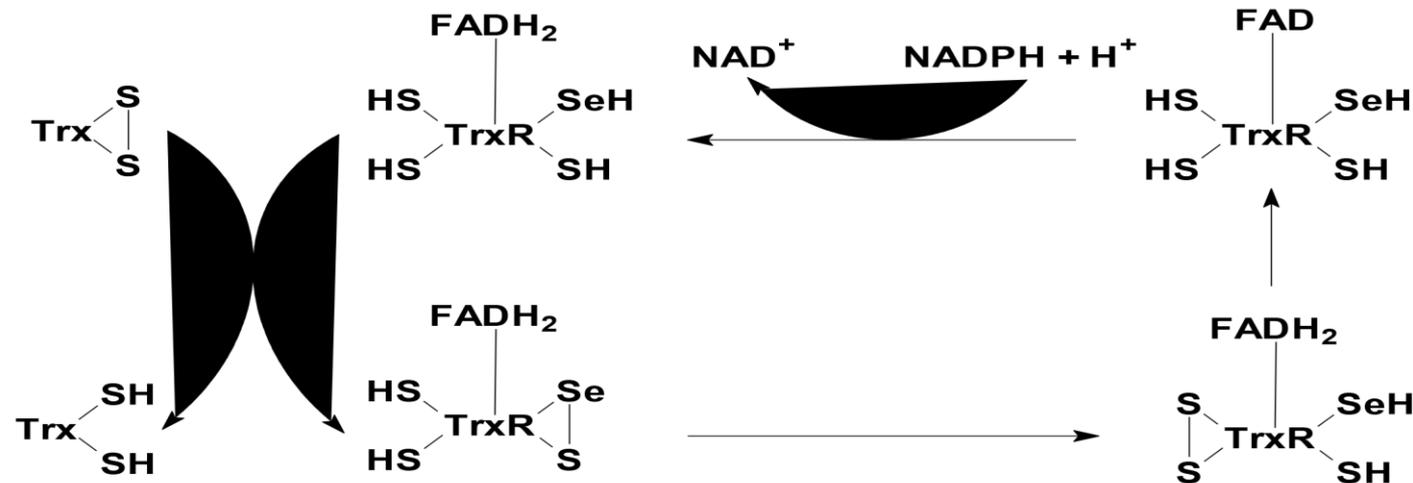
**Trx<sub>red</sub> = thioredoxin reductase (FAD)**



**Both GR and Trx<sub>red</sub> use *hydride transfer* to reduce the disulfides to sulfides**

# Seleno-Cysteine Reduction

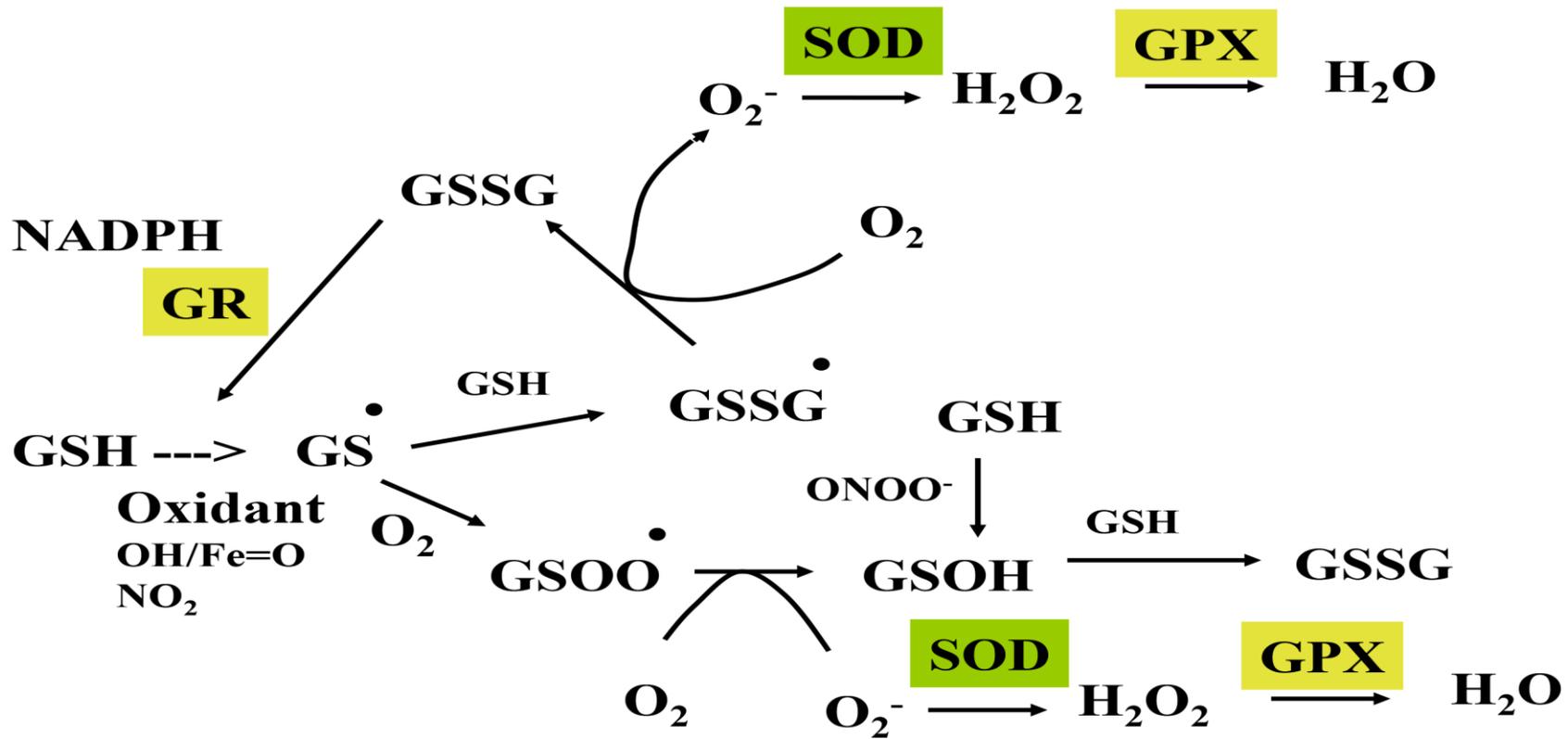
## Reduction of a seleno-cysteine



A selenylsulfide in a protein is reduced by a cysteine-exchange reaction and the resulting disulfide is then reduced by electron transfer. This example shows the reduction of thioredoxin (Trx) by thioredoxin reductase (TrxR).

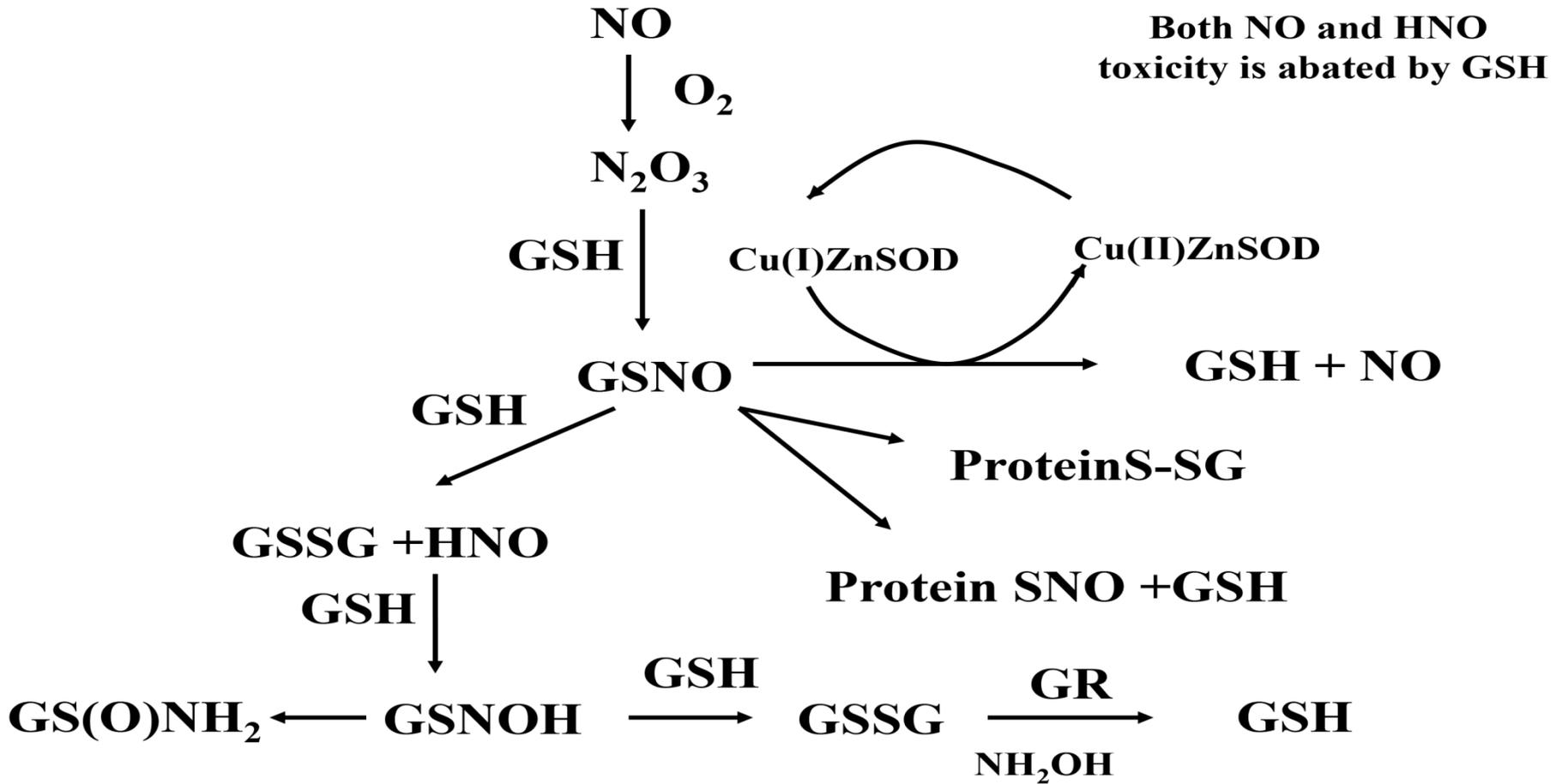
# Glutathione Metabolism

## Glutathione Metabolism



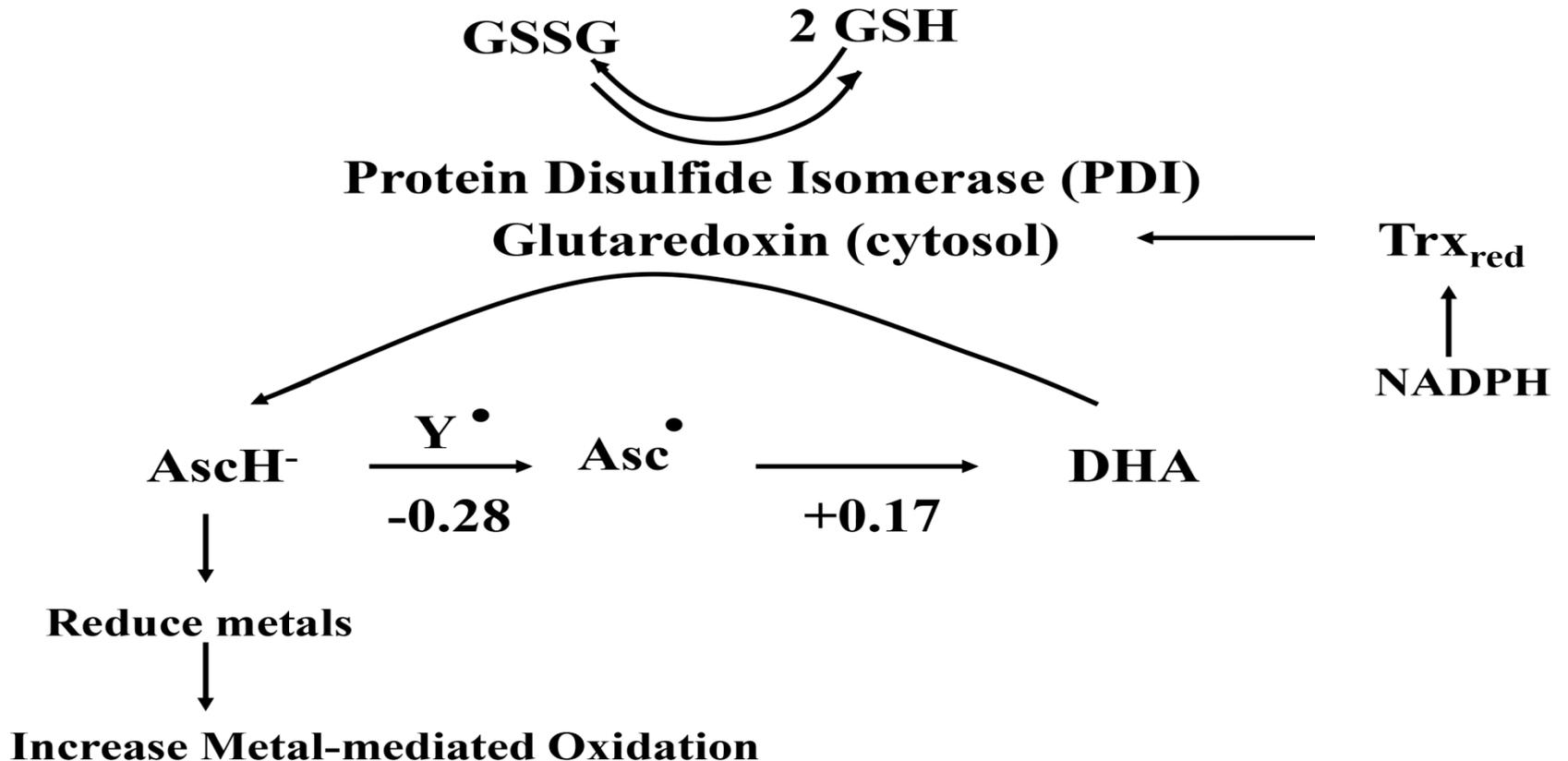
# Abatement of Nitrosative Stress

## Abatement of Nitrosative Stress



# Glutathione and Ascorbate Pools

## Glutathione and Ascorbate Pools Communicate



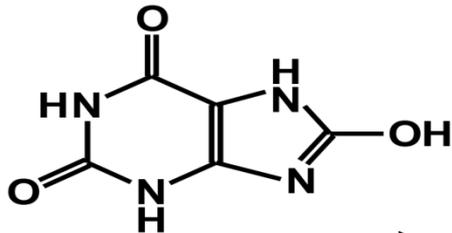
# Urate and Uric Acid

Urate and Uric Acid

Human Plasma 0.2-0.4 mM

Xanthine

XD or XO ↓



Uric acid

OH ↓

>10<sup>9</sup>

NO<sub>2</sub> ↓

10<sup>7</sup>

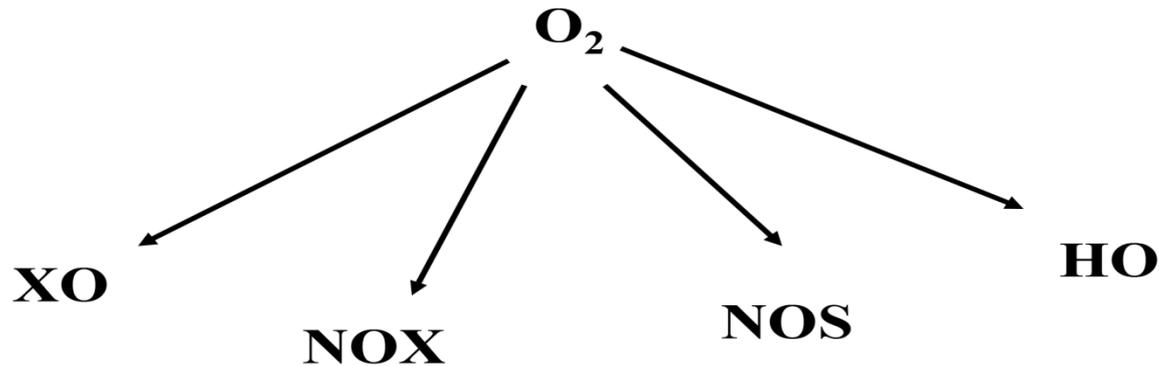
Urea and HOC-CHO  
Glyoxylic acid

↓  
Oxalic acid



# Oxygen and NADPH Essential

**All enzymes require Oxygen and NADPH**



**NADPH versus NADH**

**Metabolism of iron, oxygen and glucose will be critical factors in the cellular redox state**

**Now the adventure begins the biochemistry/chemistry in biology**