Redox chemistry by David Wink

Redox Biology: Chemistry

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Introduction to redox molecules

Introduction to Redox Molecules

Reactive Oxygen Species

H_2O_2 and O_2^- —metal→ **$OH^\bullet$**
Metalloxo species
Lipid radicals
Mediators of chemical stress

NO and Reactive Nitrogen Oxide Species

Aerobic conditions

NO → **NO_2**
N_2O_3
$ONOO^-$

Mediators of chemical stress

HNO —O_2→ **"$ONOO^-$"**

Carbon Monoxide

CO → **Ferrous-CO**

Identification of Chemical Species in Redox Biology

Classification of Chemical Species in Redox Biology

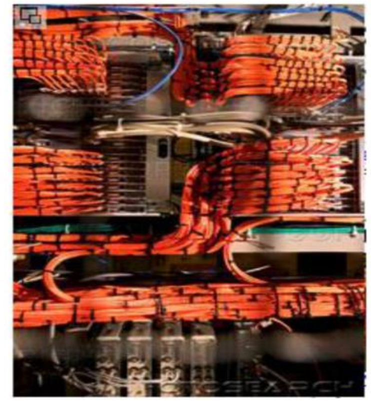
H_2O_2
↓
OH or $Fe^{4+}O$
↓
Lipid radicals
Carbonate radicals
↓
DNA oxidation
Oxidized lipids
↓
Oxidative stress

Instigators
↓
Primary Reactive Species
↓
Secondary Reactive Species
↓
Biochemical Product
↓
Biological Effects

NO
↓
NO_2
N_2O_3
$ONOO^-$
↓
Nitrosamines
S-nitrosothiols
Nitrotyrosine
↓
Nitrosative stress

Concepts in Redox Stress

Concepts in Redox Stress



Redox Chemistry

↓

Biological Redox Balance

Chemical Stress ↔ **Changes in Redox Homeostasis**

Disease

Different Mechanisms for the Chemistry of Oxidative Stress

Different Mechanisms for the Chemistry of Oxidative Stress

Chemical effect

where the biochemical reactions directly destroys the cellular and tissue structure.

lipid peroxidation
metal catalyzed DNA oxidation

Production of an oxidized product

that results in change in the cell or tissue function.

Cholesterol oxidation
DNA base oxidation and miss repair

Changes in biological redox balance

Ischemia reperfusion and recruitment of leukocytes
Shear stress in coronary circulation

The Chemistry Outline

The Chemistry Outline

I. Fundamental Concepts in electron transfer and oxidative chemistry

II. Chemistry of formation of reactive species

III. Chemistry of the reactive species

- **DNA**
- **Proteins**
- **Lipid chemistry**

IV. Chemistry of ROS and NO/RNOS: Chemical Biology of NO

Balancing NO and ROS

Basic Chemical Concepts in Electron Transfer Reactions

Basic Chemical Concepts in Electron Transfer Reactions

$\Delta G = \Delta H - T\Delta S = -nF\Delta E$ — **More Negative ΔG is the faster reaction**

$$E' = E^o + \frac{RT}{nF} \log \frac{(oxidized)}{(reduced)}$$

Nernst equation

$M + e \dashrightarrow M^-$

Half-cell potentials or standard reduction potentials

Ferricytochrome c + e^- ---> Ferrocytochrome	**+0.26**
O_2^- ---> O_2 + e^-	**+0.33**
Ferricytochrome c + O_2^- --> Ferocytochrome c + O_2	**+0.59**

Pecking Order of Redox Species and Reactions

Pecking Order of Redox Species and Reactions

Oxidants

Positive ΔE for the reduction means it's a good oxidant. If negative the product is a good reductant.

Reductants

Redox Couple (one-electron reductions)	E°'/mV
$HO^{\square}$, H^+/H_2O	+ 2310
$RO^{\square}$, H^+/ROH (aliphatic alkoxyl radical)	+ 1600
$ROO^{\square}$, $H^+/ROOH$ (alkyl peroxyl radical)	+ 1000
$GS^{\square}/GS^{\square}$ (glutathione)	+ 920
$PUFA^{\square}$, H^+/PUFA-H (*bis*-allylic-H)	+ 600
$TO^{\square}$, H^+/TOH (tocopherol)	**+ 480**
H_2O_2, H^+/H_2O, $HO^{\square}$	+ 320
$Asc^{\square\square}$, $H^+/AscH^-$ (Ascorbate)	**+ 282**
$CoQ^{\square-}$, $2H^+/CoQH_2$	+ 200
Fe(III) EDTA/Fe(II) EDTA	+ 120
$CoQ/CoQ^{\square-}$	- 36
$O_2/O_2^{\square\square}$	- 160
$Paraquat/Paraquat^{\square\square}$	- 448
Fe(III)DFO/Fe(II)DFO	- 450
$RSSR/RSSR^{\square\square}$ (GSH)	- 1500
$H_2O/e^{\square}_{aq}$	- 2870

What is Chemistry?

What is redox Chemistry? (basic concepts)

Electron transfer

Oxidation

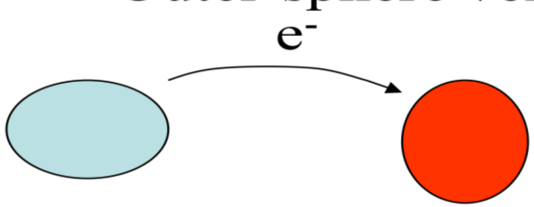
Substrate $\xrightarrow{-e^-}$ $Substrate^{+\bullet}$

Reduction

Substrate $\xrightarrow{+e^-}$ $Substrate^{-\bullet}$

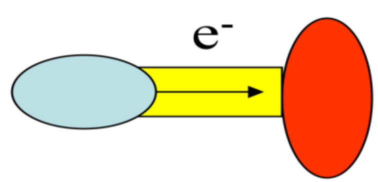
> “free radicals”:
> Substance with unpaired electrons

Outer-sphere versus inner-sphere electron transfer



Like Static electricity

Ex: Reduction of oxygen by Flavoproteins



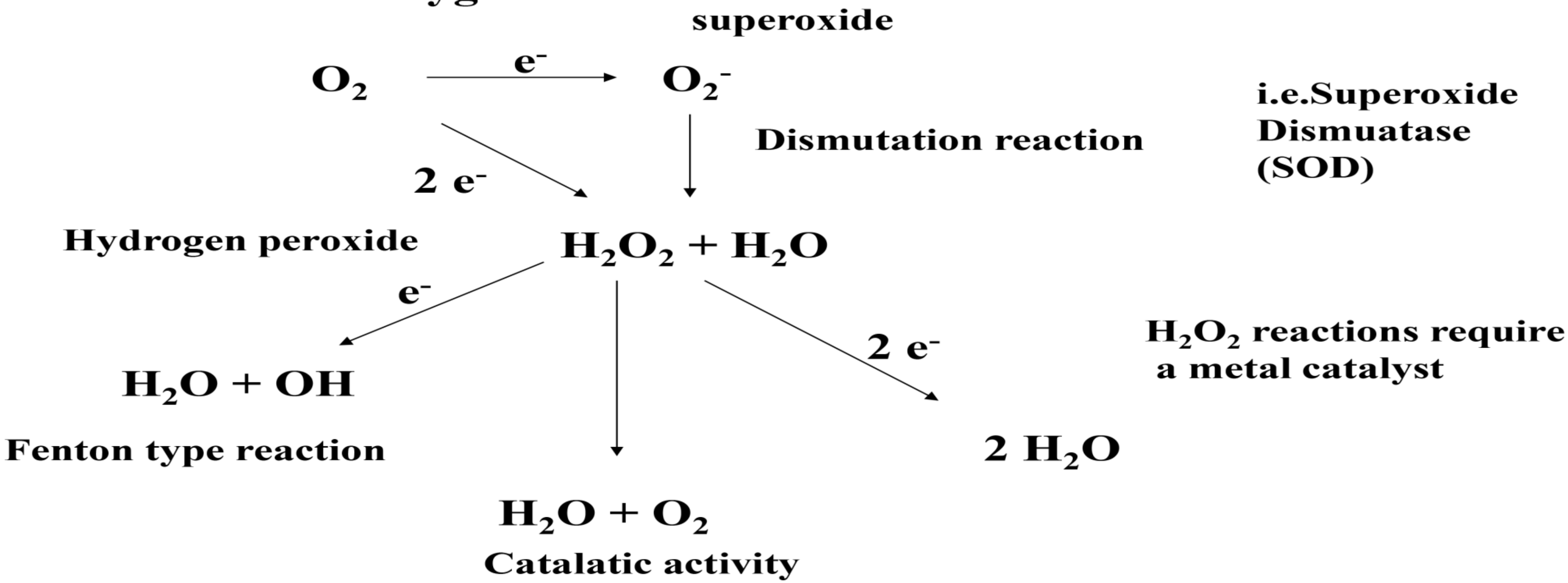
Through Covalent Bonds

Ex: Reduction of disulfide bonds

Examples of Outer-sphere electron transfer

Examples of Outer-sphere electron transfer

Reduction of Oxygen



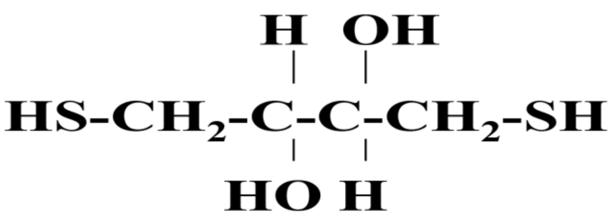
Examples of Inner-sphere electron transfer

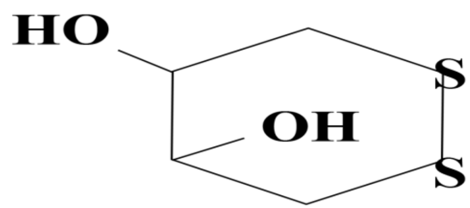
Examples of Inner-sphere electron transfer

Thiol reduction

RSSR + DTT_{red} ⟶ 2 RSH + DTT_{ox}

Lipoic acid **Thioredoxin**





Disulfide

Hydride Transfer

NADPH ⟶ $FADH_2$ **2 electron reducing agent**

Oxidation Mechanisms of Biological Membranes

Oxidation Mechanisms of Biological Molecules

One electron oxidation "free radical formation"

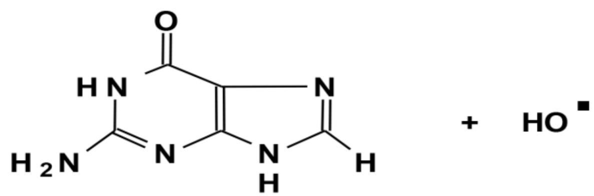
$OH^{\bullet} + RH \longrightarrow R^{\bullet} + H_2O$

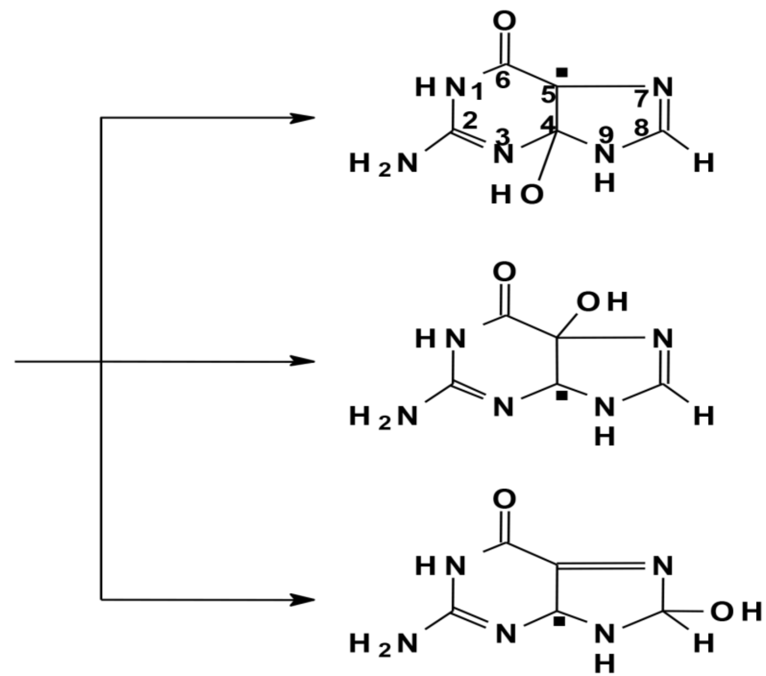
$OH^{\bullet} + \text{Ribose} \longrightarrow \text{Ribose}^{\bullet} + OH^-$

Hydroxylation

$OH^{\bullet} + RH \longrightarrow RH(OH)^{\bullet}$

Guanine





Oxygen addition or insertion

$M{=}O + \text{Nucleophile} \longrightarrow M + Nuc{=}O$

Special Chemical Terms for Nitrogen Oxide Related Chemistry

Special Chemical Terms for Nitrogen Oxide Related Chemistry

Nitrosylation

Substrate + NO --> Sub-NO (nitrosyl)

Metal + NO --> M-NO

Radicals + NO --> R-NO

Nitrosation

Nucleophile + "NO^+" ⟶ RNO

RSH + N_2O_3 --> RSNO **S-nitrosothiol**

Amine + N_2O_3 --> RNNO **Nitrosamine**

Nitration

Nucleophile + "NO_2^+" ⟶ RNO_2

Tyrosine + 2 NO_2 ⟶ Nitrotyrosine

The Chemical Reactions of hydrogen peroxide

The Chemical Reaction of H_2O_2

Fenton reaction generation of powerful oxidant

$$Fe^{2+} + H_2O_2 \longrightarrow OH^{\bullet} + Fe^{3+}\ H_2O \text{ or } Fe^{4+}O + H_2O$$

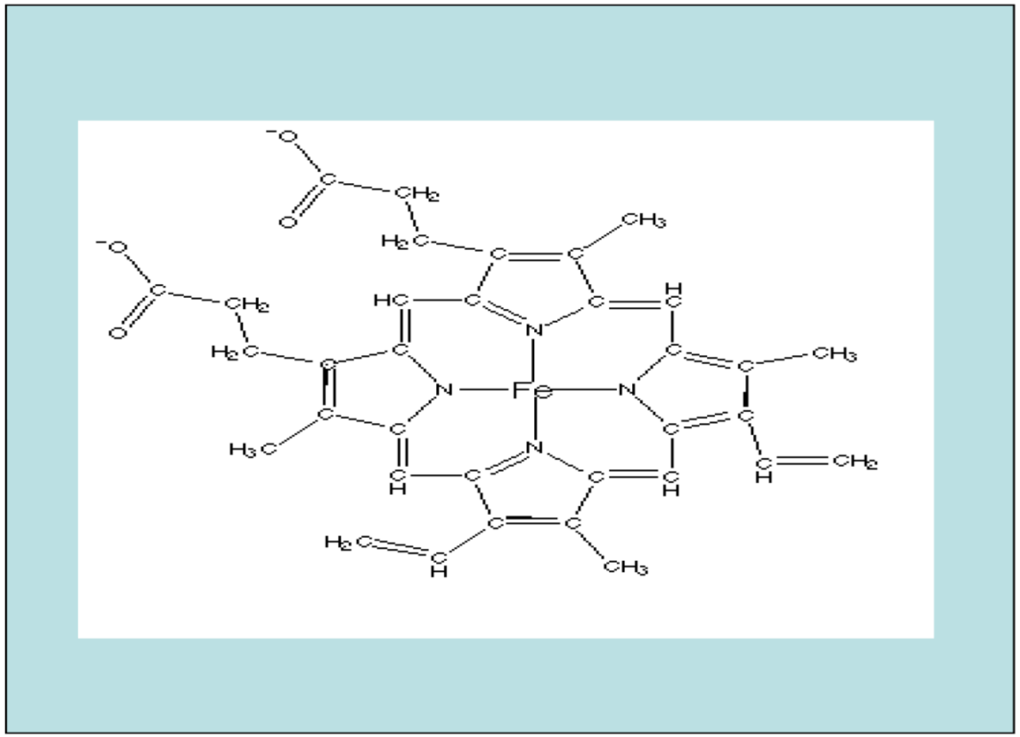
Ligand field will determine the oxidation potential of the species. Hard ligands (oxides and amine will tend to result in species with higher oxidation potential then those which are softer (ie ligand with aromaticity or sulfur). These changes in the ligand field can tailor make the redox chemistry of metal complexes.

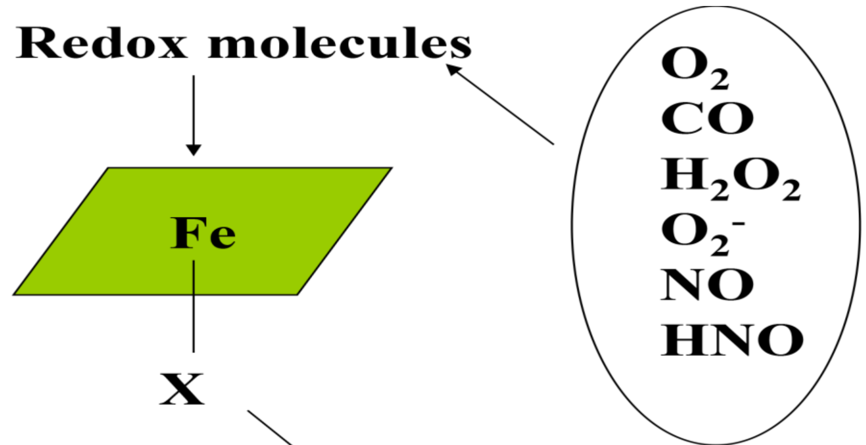
Hard ligands EDTA
Soft ligand Porphyrin ring and sulfides

Other redox active metals can reduce hydrogen peroxide to form oxidants. copper, nickel, cobalt, manganese, chromium, vanadium, and titanium.

Heme complexes

Heme Complexes

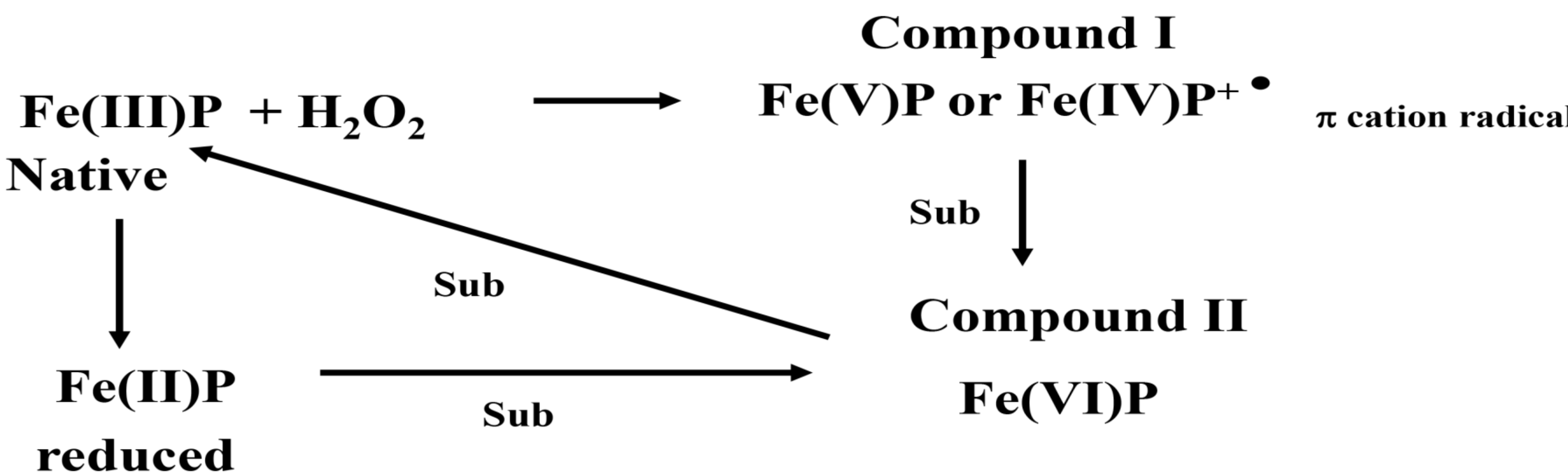




Histidine	**Hemoglobin** **myoglobin** **peroxidases**
Cystiene	**Cytochrome P450** **Nitric oxide synthase**
Tyrosine	**Catalase**

Peroxidase Chemistry or Peroxidatic Activity

Peroxidase Chemistry or Peroxidatic Activity



Substrate is H_2O_2 then it is catalatic activity (catalase)

If substrate is halogen then it is haloperoxidase (example myleoperoxidase)

Nitrite can be oxidized to NO_2 a powerful RNOS oxidant

Haber-Weiss cycle

Superoxide

$$O_2^{\bullet -} \xrightarrow{-e^-} O_2 + e^- \quad +0.3\ V\ pH = 7$$

$$O_2^{\bullet -} \xrightarrow{+e^-} H_2O_2 \quad +0.9\ V\ pH=7$$

Haber-Weiss Cycle

$$Fe^{2+} + H_2O_2 \longrightarrow Fe(III) + OH^{\bullet}$$

$$OH^{\bullet} + H_2O_2 \longrightarrow H_2O + H^+ + O_2^{\bullet -}$$

$$Fe^{3+} + O_2^{\bullet -} \rightleftarrows Fe^{2+} + O_2$$

$$O_2^{\bullet -} + Fe^{2+} \longrightarrow Fe^{3+} + H_2O_2$$

Dismutation of Superoxide

Dismutation of Superoxide

$$2\,O_2^{\bullet -} + 2\,H^+ \longrightarrow O_2 + H_2O_2$$ **(pH dependent)**

Superoxide dismutase: copper zinc(cytosolic)
Manganese (Mitochondria), Iron (bacterial)

$$O_2^{\bullet -} + Cu^{2+} \longrightarrow Cu^+ + O_2$$

$$O_2^{\bullet -} + Cu^+ \xrightarrow{2H^+} H_2O_2 + Cu^{2+}$$

SOD mimetics are chemical compounds will readily dismutate superoxide

Why nature wants to control superoxide

Aconitase inactivation
Prevent Haber Weiss cycling
Prevent NO scavenging and generation of RNOS

$$O_2^{\bullet -} + NO \longrightarrow ONOO^-$$

Reactivity of OH Radical and Metallo-oxo Species

Reactivity of OH Radical and Metallo-oxo Species

2.3 V $\quad OH^{\bullet} + CO_3^{2-} \longrightarrow \dot{C}O_3^{-} + OH^{-}$ $\quad$ 1.4 V

$OH^{\bullet} + NO_2^{-} \longrightarrow NO_2^{\bullet} + OH^{-}$ $\quad$ 1.0 V

$OH^{\bullet} + RH \longrightarrow R^{\bullet} + H_2O$

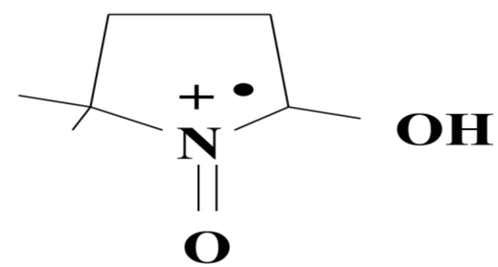
Free radical

Detection

Spin trapping

$\dot{O}H + DMPO \longrightarrow DMPO(OH)^{\bullet}$

Unstable (τ =nsec) $\quad$ Stable adduct (τ =min, EPR)



Trapping

Benzoic acid
Salicylic acid $\longrightarrow$ Hydroxylated products (HPLC)

DNA Oxidation Chemistry by Fenton-derive Oxidants

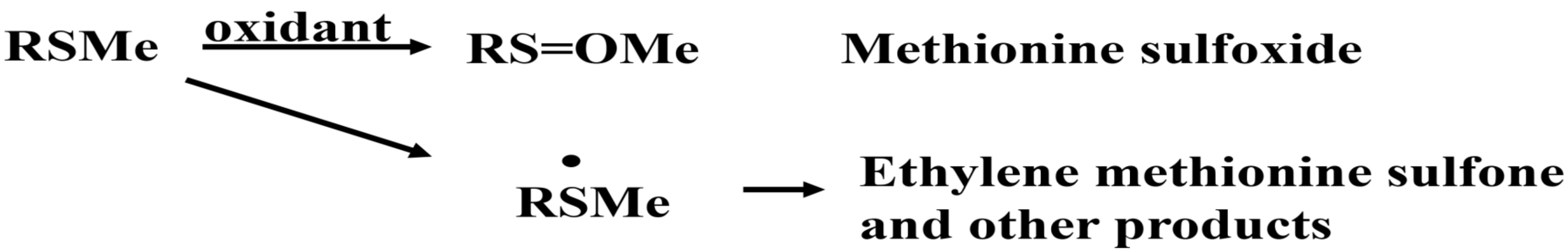
DNA Oxidation Chemistry by Fenton-derived Oxidants

Oxidation of Ribose	→	Strand breaks
Oxidation of Bases	→	Base Oxidation Cross reaction Deamination

Some base modification have been used as dosimeters of oxidative damage

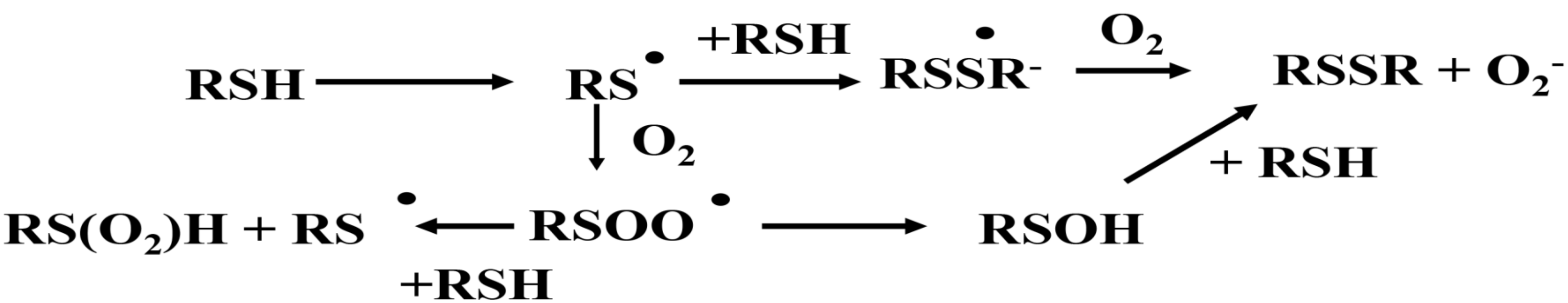
Sulfur Oxidation

Methionine



Cysteine

Intracellular antioxidant (Glutathione or GSH)



Lipid peroxidation

Lipid peroxidation

Initiation LH $\xrightarrow{\text{OH or M=O}}$ L•

Propagation

L• + O_2 ⟶ LOO•

LOO• + LH ⟶ L• + LOOH Hydroperoxide

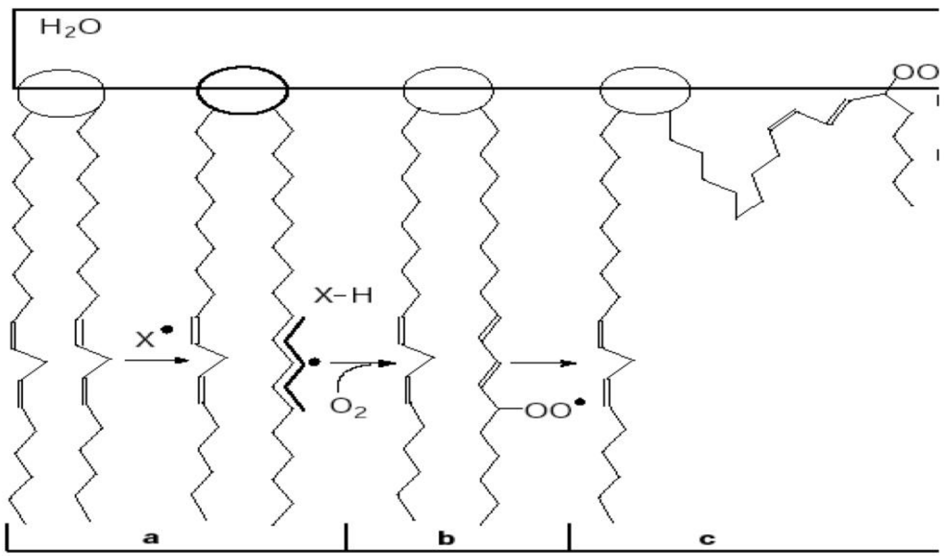
LOOH + metal ⟶ LO• Alkoxy radical
LOO• Peroxy radical

Commonly measured decomposition products

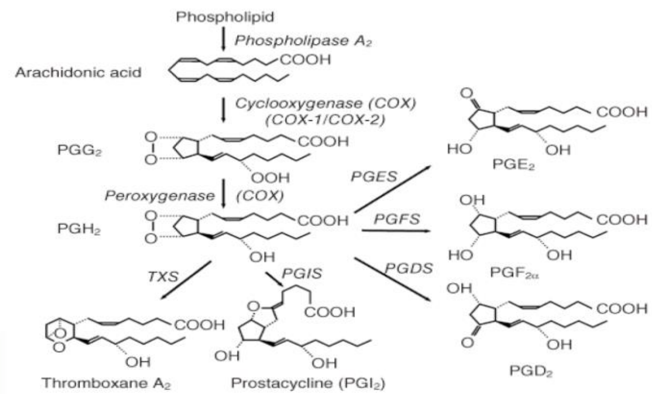
Alkanes
Malondialdehyde
4 hydroxynananol
8-isoprostanes

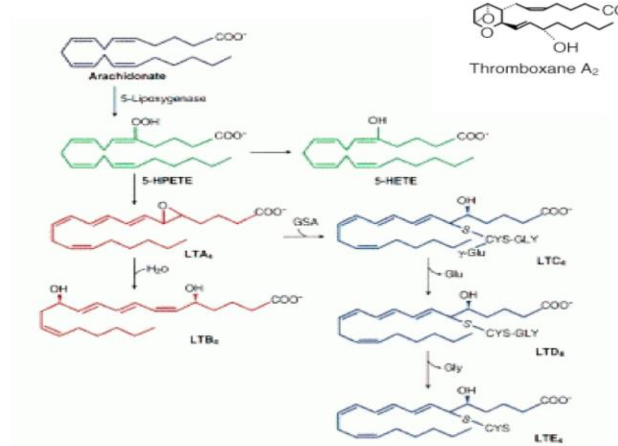
Lipid Peroxidation in Chemical Stress versus Lipid oxidation for regulatory Function

Lipid Peroxidation in Chemical Stress versus Lipid oxidation for regulatory Function



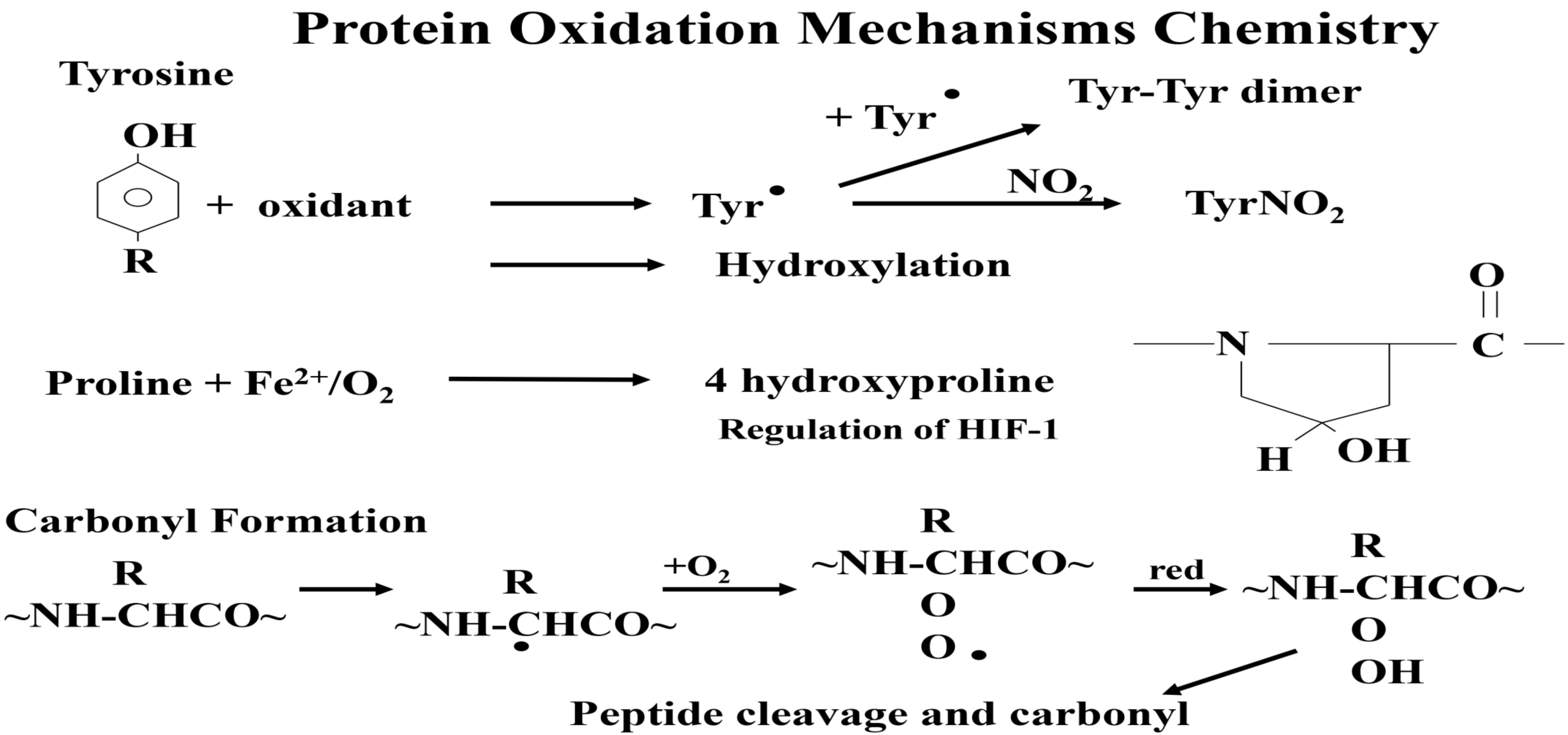
Destruction of the Membrane





Prostaglandin and leukotriene synthase

Protein Oxidation mechanism Chemistry



Sulfur Oxidation

Sulfur Oxidation

Methionine

$$RSMe \xrightarrow{\text{oxidant}} RS{=}OMe \quad \text{Methionine sulfoxide}$$

$$RSMe \longrightarrow RS^{\bullet}Me \longrightarrow \text{Ethylene methionine sulfone and other products}$$

Cysteine

Intracellular antioxidant (Glutathione or GSH)

$$RSH \longrightarrow RS^{\bullet} \xrightarrow{+RSH} RSSR^{\bullet -} \xrightarrow{O_2} RSSR + O_2^{-}$$

$$RS^{\bullet} \xrightarrow{O_2} RSOO^{\bullet}$$

$$RSOO^{\bullet} \xrightarrow{+RSH} RS(O_2)H + RS^{\bullet}$$

$$RSOO^{\bullet} \longrightarrow RSOH \xrightarrow{+\,RSH} RSSR + O_2^{-}$$

Nitric Oxide and Reactive Nitrogen Oxide Species

Nitric Oxide and Reactive Nitrogen Oxide Species

A stable free radical

$N{=}O^{\bullet}$

Redox Chemistry

$$\underset{\text{-0.8 V}}{} \quad NO^- \xleftarrow{\text{reduction}} NO \xrightarrow{\text{oxidation}} NO^+ \quad \text{-1.0 V}$$

NO⁻ nitroxyl → (H^+) → **HNO**; $pK_a = 11.5$

NO^+ Nitrosonium ion

Chemistry of Nitric Oxide formation of Nitrosyl complexes

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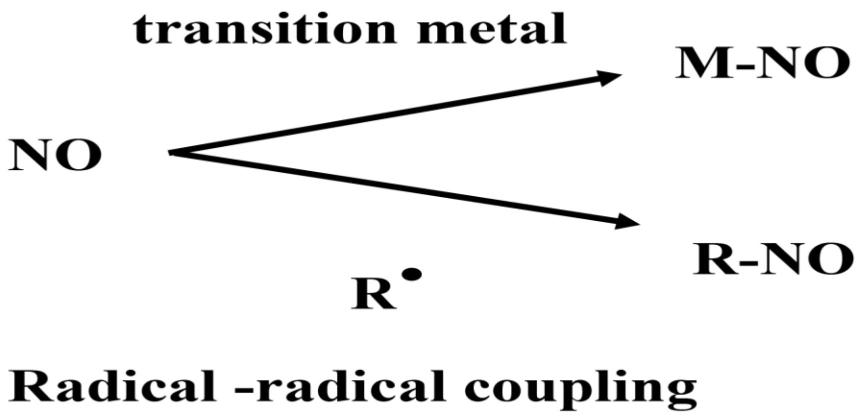


Metal nitrosyl complex
Guanylyl cyclase

Abatement of lipid peroxidation
Radiation induced DNA radicals

These are some of the fastest reactions of NO in biology

Autoxidation of NO

Autoxidation of NO

$$4\,NO + O_2 + 2\,H_2O \longrightarrow 4\,HNO_2$$

Gas Phase and lipid layers

Rate limiting step

$$2\,NO + O_2 \longrightarrow 2\,NO_2 \quad \textbf{Nitrogen dioxide}$$

$$NO_2 + NO \longrightarrow N_2O_3$$

$$N_2O_3 + H_2O \longrightarrow 2H^+ + NO_2^-$$

Aqueous

$$2\,NO + O_2 \xrightarrow{+\,2\,NO} N_2O_3$$

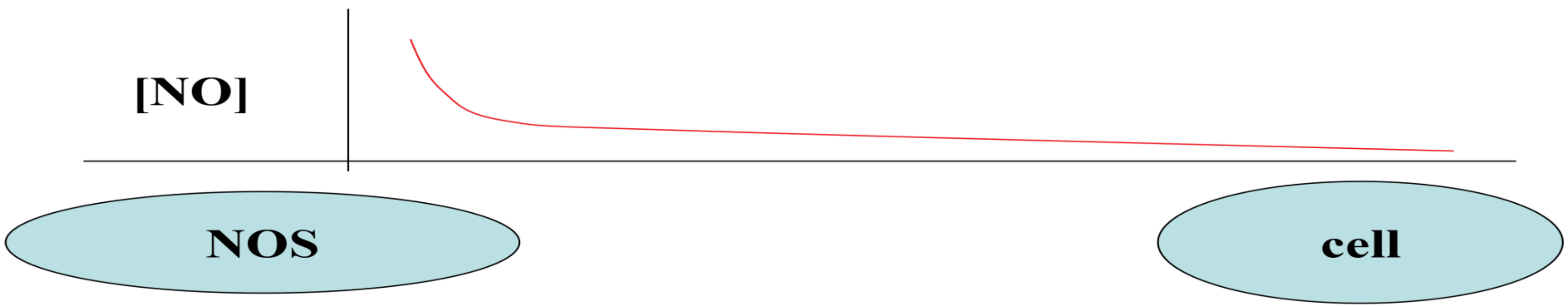
Unique kinetics of the NO/O2 reaction

Unique Kinetics of the NO/O_2 reaction answer some question as an agent on biology

$$-d[NO]/dt = d[NO_2^-]/dt = k[NO]^2[O_2]$$

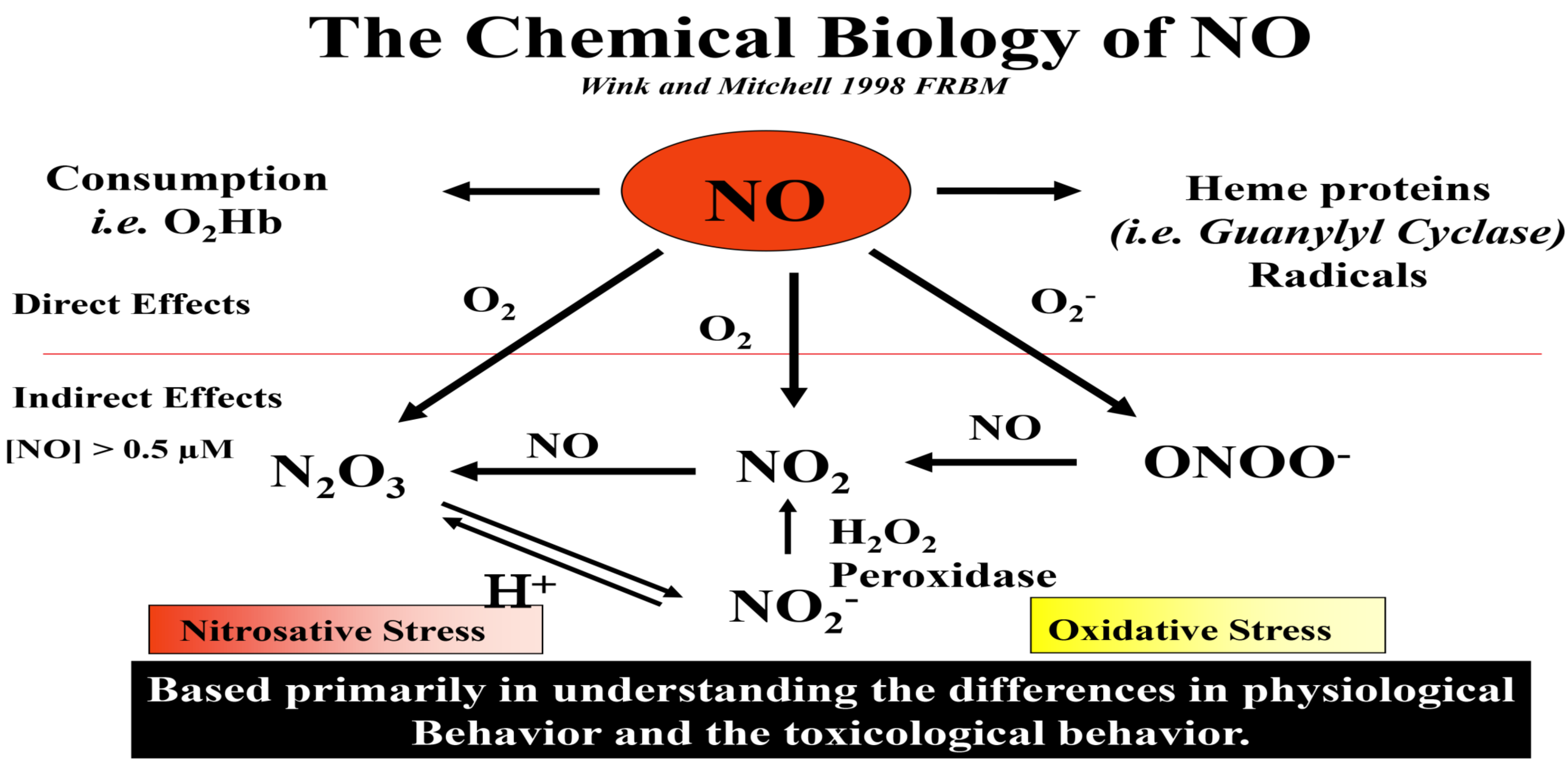
The lifetime of NO is porportional to the concetration of NO

NO conc.	**1 μM**	**100 μM**
Half-life	**800 sec**	**8 sec**



In vivo NO has life time < 1 sec at submicromolar conc.

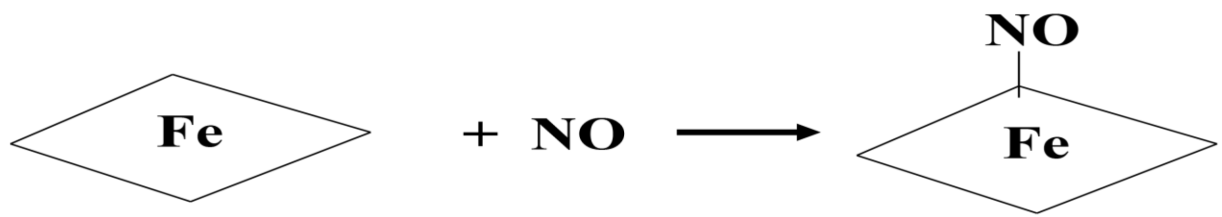
The Chemical Biology of NO



NO Direct Reactions

NO Direct Reactions

Nitrosylation



Soluble Guanylyl Cyclase
GTP --> cGMP

Dioxygen complex

$Hb(Fe^{2+})(O_2) + NO \longrightarrow Met(Hb)\ (Fe^{3+}) + NO_3^-$

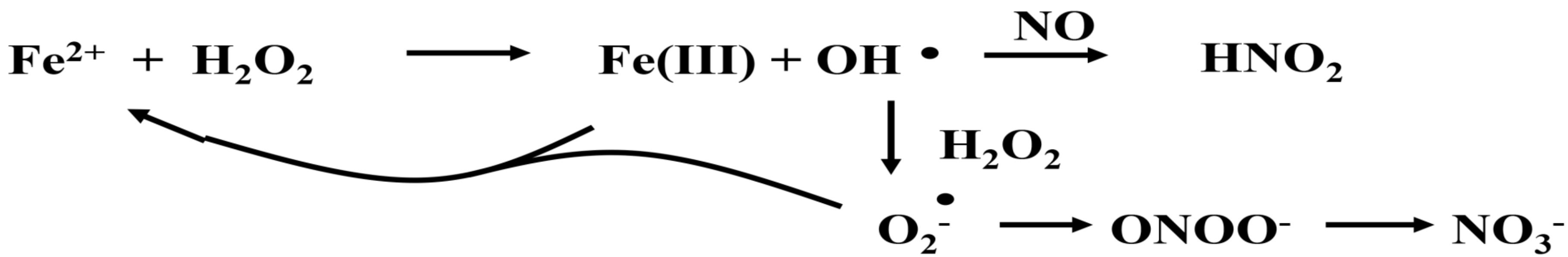
Metallo-Oxo

$P\ Fe{=}O\ \ +\ 2\ NO \longrightarrow PFe\ +\ NO_2-$

Antioxidant properties of NO

Antioxidant properties of NO

Haber Wiess



Lipid Peroxidation

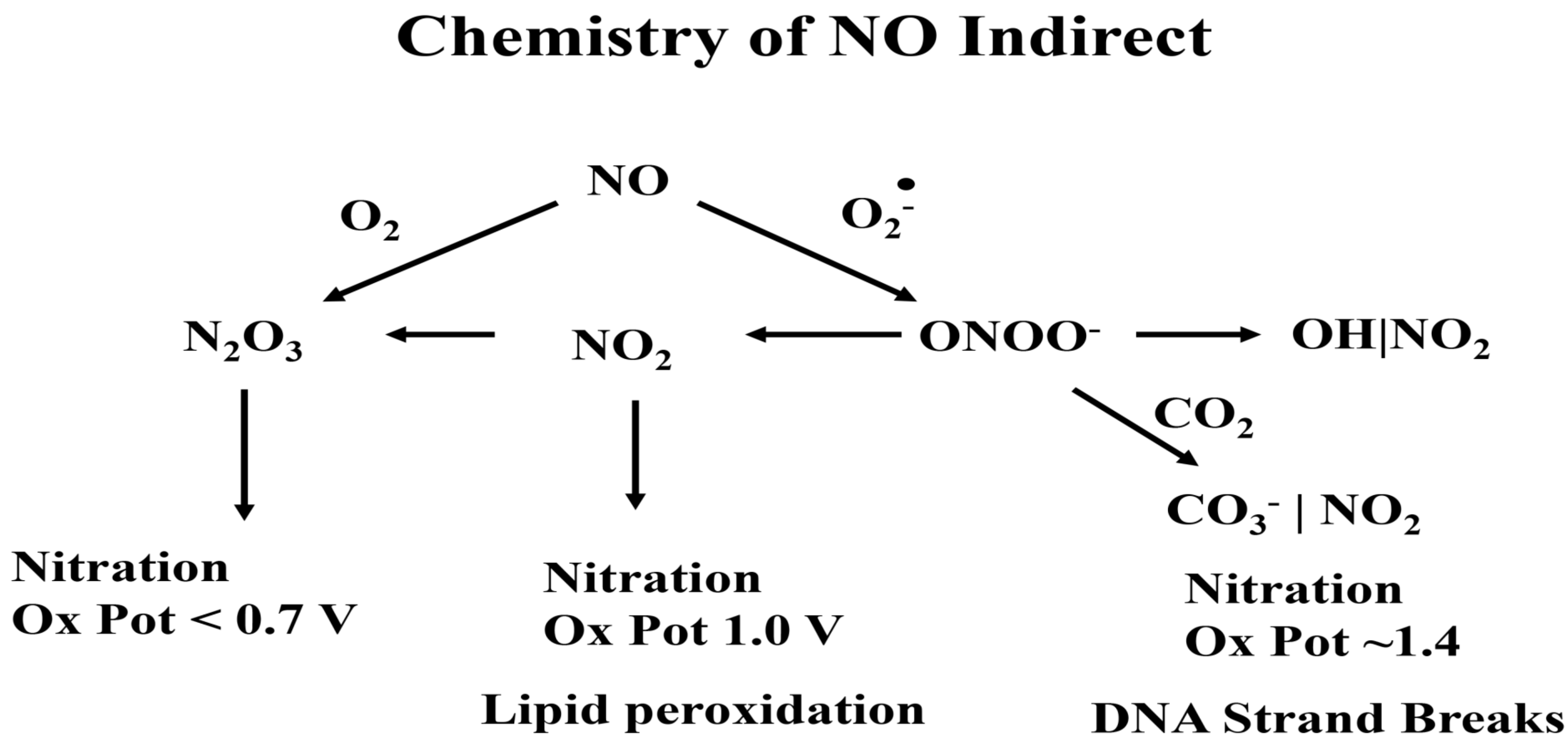
$$LH \xrightarrow{OH \text{ or } M=O} L^{\bullet}$$

$$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet} \xrightarrow{NO} LOONO$$

$$LOO^{\bullet} + LH \longrightarrow L^{\bullet} + LOOH$$

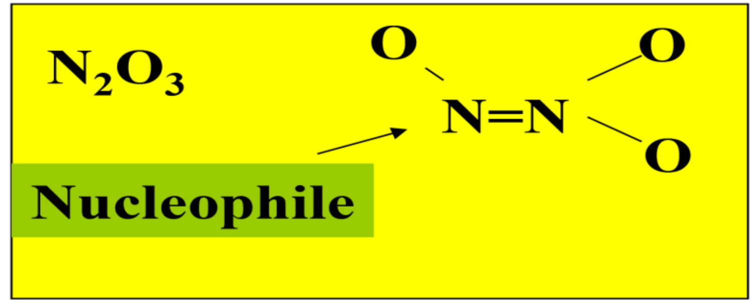
$$LOOH + \text{metal} \longrightarrow LO^{\bullet},\ LOO^{\bullet} \xrightarrow{NO} LONO,\ LOONO$$

Chemistry of NO indirect



Nitrosation Chemistry

Nitrosation Chemistry



S-Nitrosothiol Formation

$$RSH + N_2O_3 \longrightarrow RSNO + NO_2^-$$

Nitrosamine Formation

$$R_2NH + N_2O_3 \longrightarrow R_2NNO + HNO_2$$

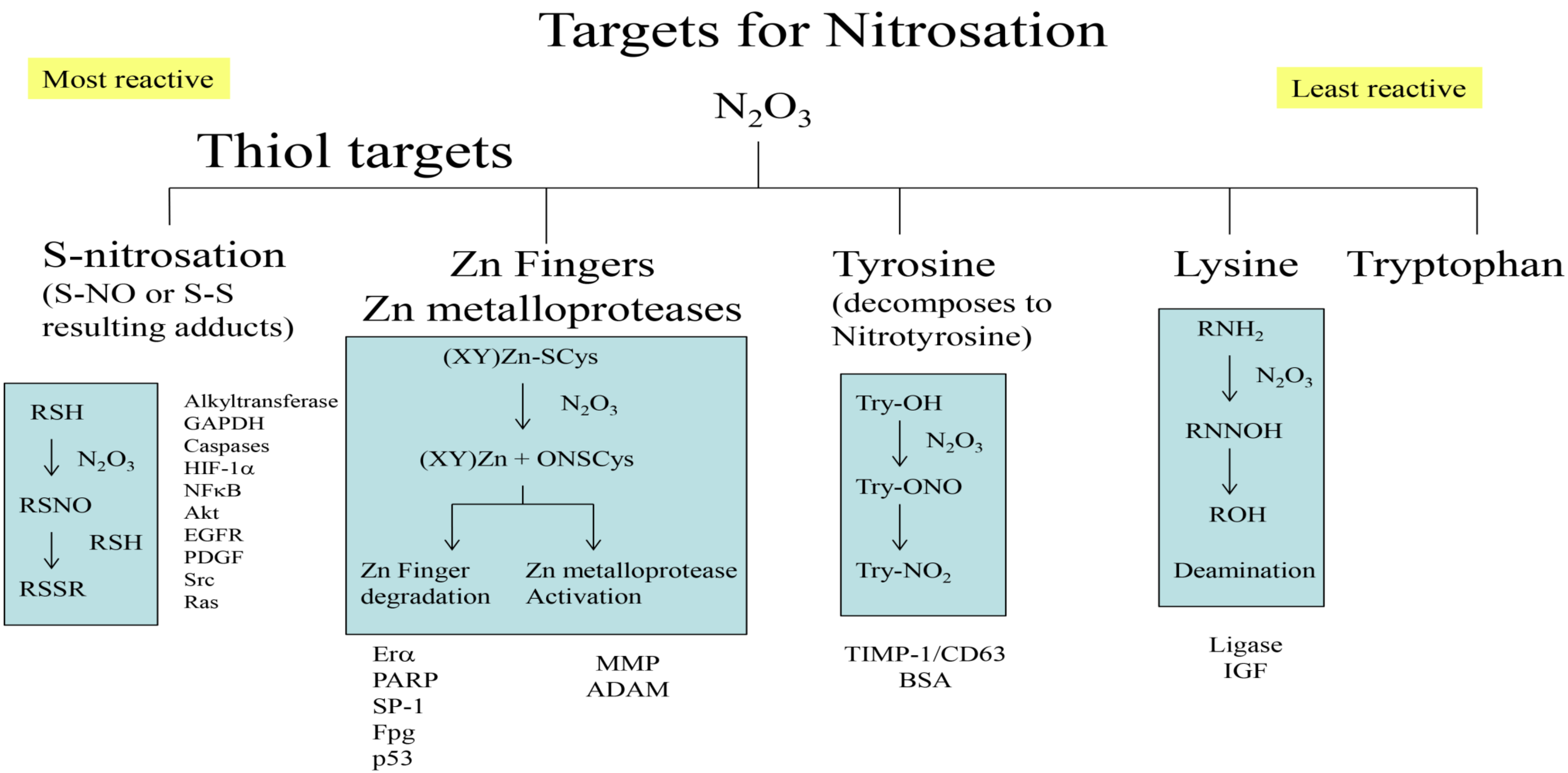
Secondary nitrosamine

$$RNH_2 + N_2O_3 \longrightarrow RHNNO + HNO_2$$

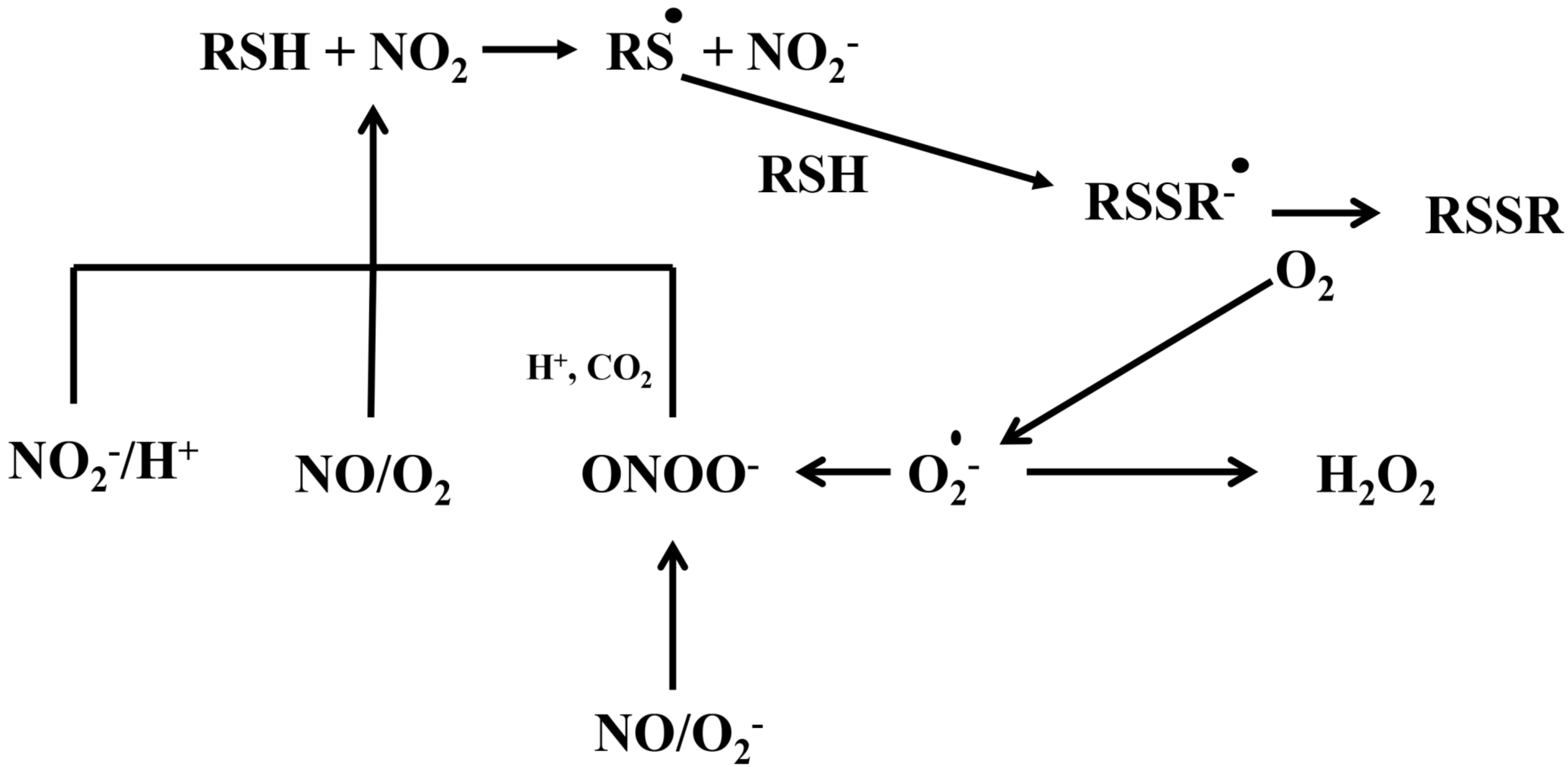
↓ Deamination

$$RNNOH \longrightarrow ROH$$

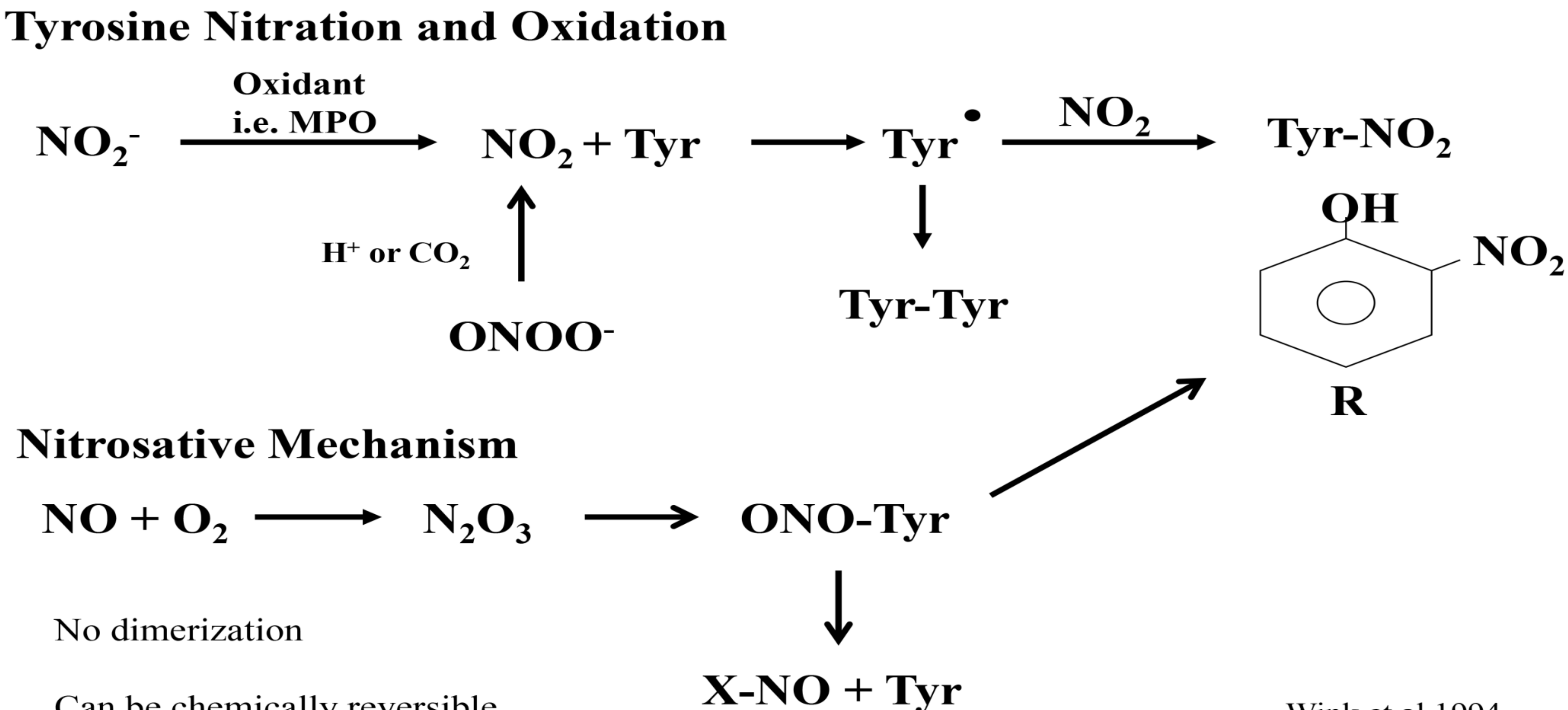
Targets for Nitrosation



Thiol oxidation by NO_2



Tyrosine nitration

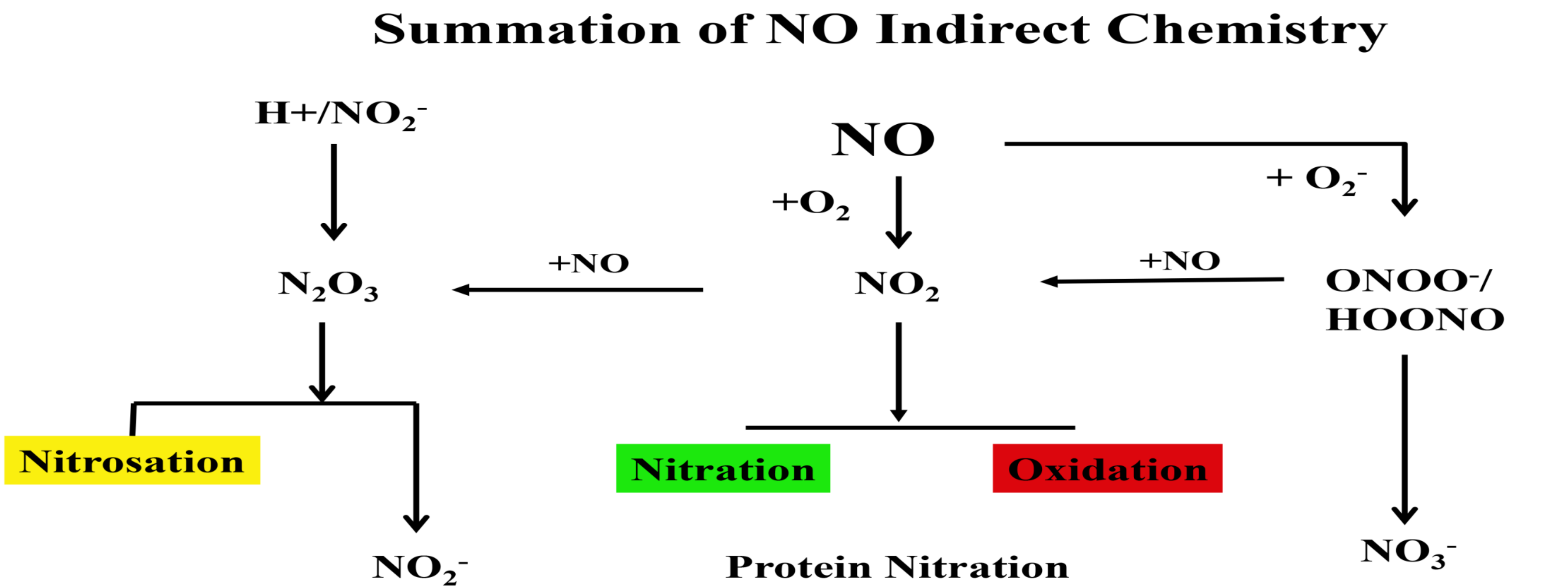


No dimerization

Can be chemically reversible

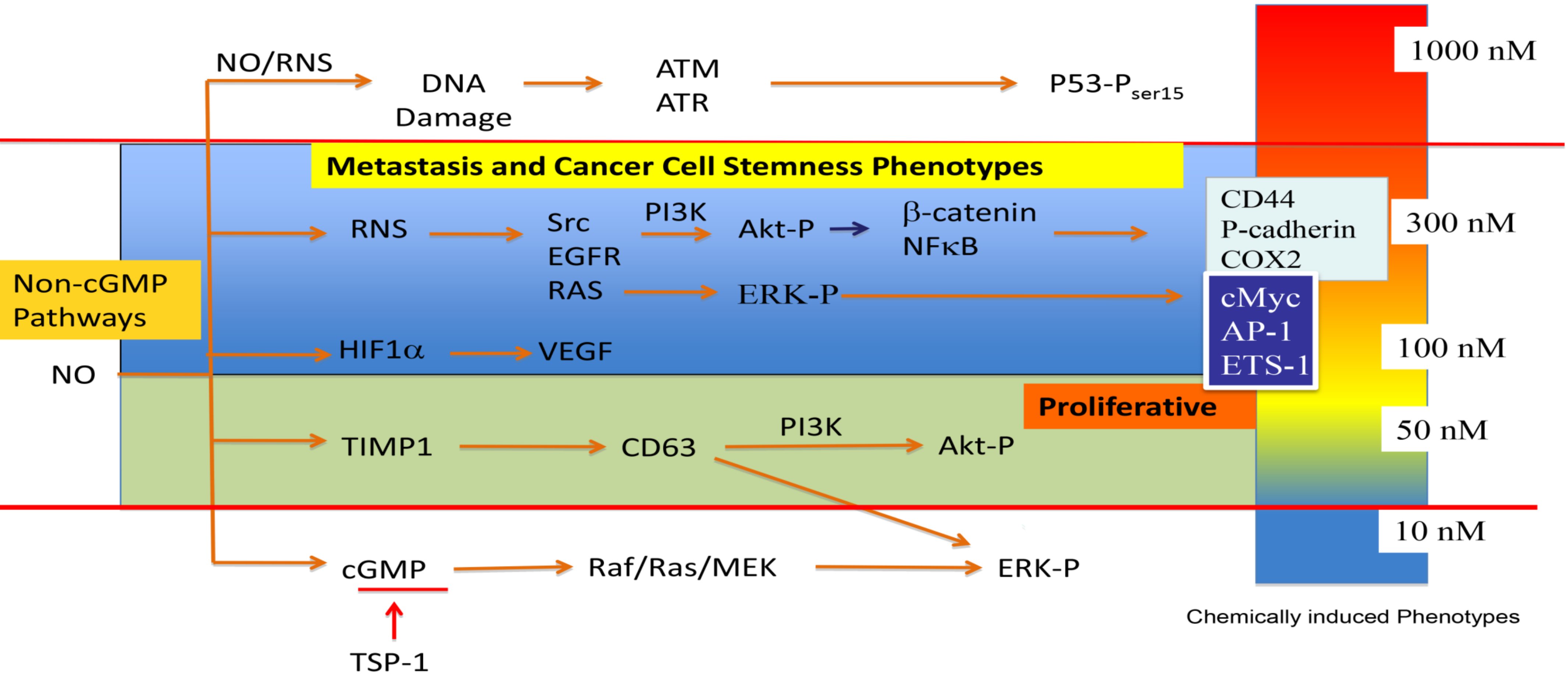
Wink et al 1994
Simon et al 1996
Ridnour et al. 2012

Summation of NO indirect chemistry



Levels of NO

Levels of NO that Effect Key Cancer-related Pathways



Cancer related pathways