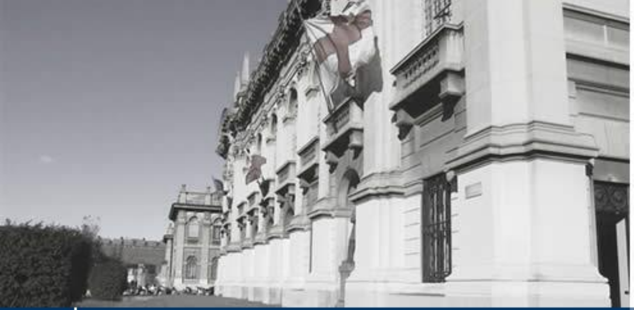


POLITECNICO DI MILANO

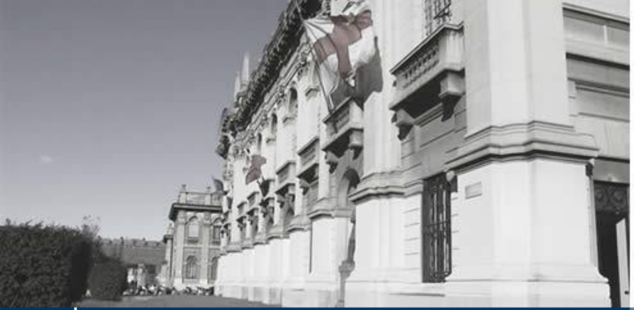
Department CMIC
Lecture 4 – FR4

Free-Radicals: Chemistry and Biology

Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”

http://iscamap.chem.polimi.it/citterio/education/free-radical-chemistry/

Content



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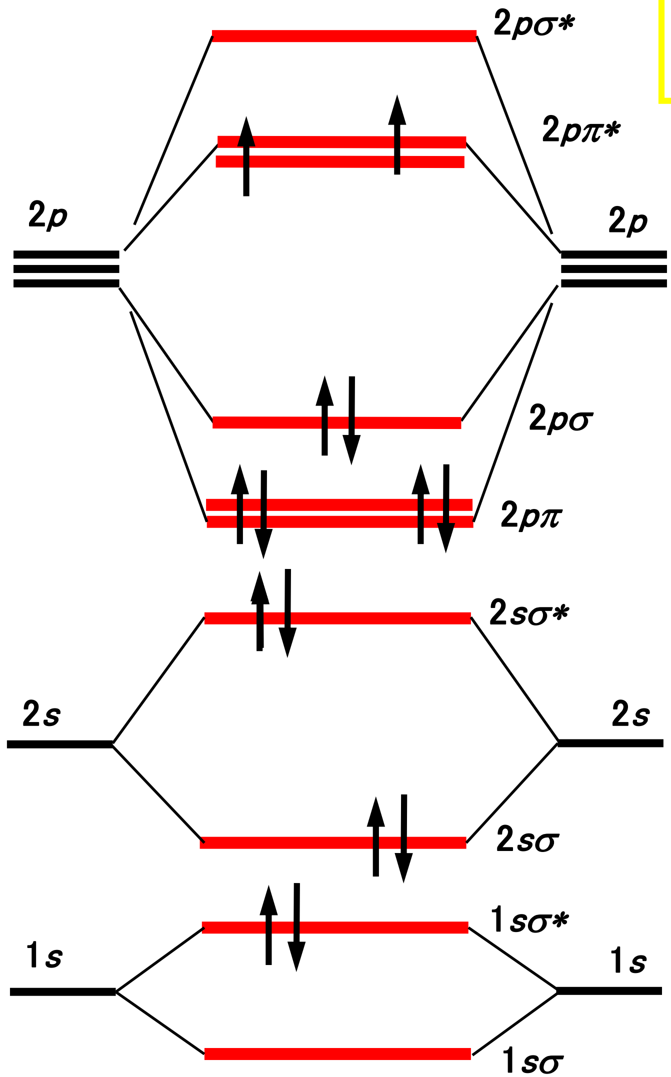
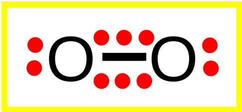
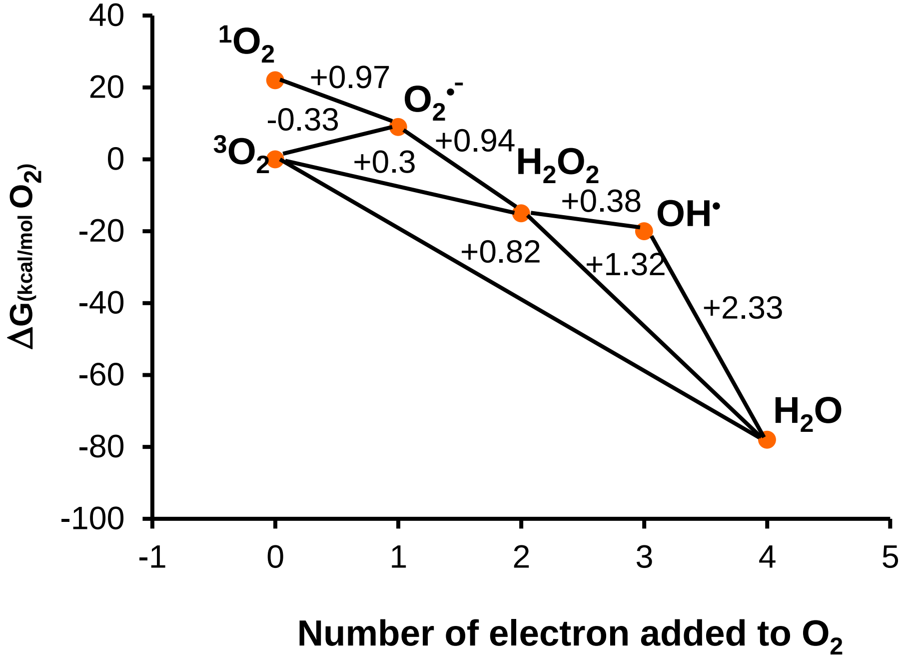
Active Oxygen Species

Prof. Attilio Citterio
Dipartimento CMIC "Giulio Natta"

Oxygen Molecule and Free Energy Diagram of Oxygen Species



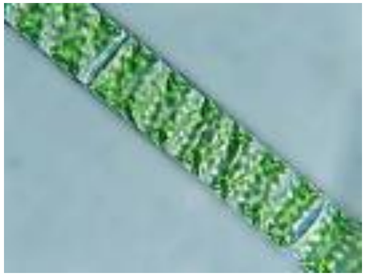




Diatomic Oxygen Generation

The more efficient oxygen generators are plant and plant-like AUTOTROPHIC organisms via "photosynthesis" :

$$n\, \mathbf{H_2O} + \mathbf{n\, CO_2} + \mathbf{h\nu} \rightarrow \mathbf{n\, O_2} + \mathbf{[CH_2O]_n}$$



green algae

O_2 oxygen atoms arise from water and those of sugar from CO_2. Complex reaction catalyzed by chlorophyll (green pigment based on magnesium-porphyrin).

Oxygen appears in earth's atmosphere ~ 2.5 billion years ago, following the activity of green algae, and eukaryotic cells (appeared 1.5 billion years ago) raised the level to actual concentrations with some fluctuations.

Industrially, oxygen is obtained by fractional distillation of air (10 Mt/year) or by water electrolysis. Some oxoacid salts decompose to oxygen :

$2KClO_3$ (400°) $\rightarrow 2KCl + 3O_2$

$2KMnO_4 \rightarrow K_2MnO_4 + MnO_2 + O_2$

Kinetics of Diatomic Oxygen Reactions

- Direct reactions of dioxygen tend to be slow because ground state dioxygen is a triplet and most reactants are singlets.
- Triplet-to-singlet spin conversions are forbidden by quantum mechanics and hence are slow.
- A collision between two molecules occurs much more rapidly than a spin flip and so cannot be concerted.
- Instead, the number of unpaired electrons remains the same before and after each elementary step of a chemical reaction, and spin flips must be thought of as kinetically separate steps.
- For these reasons, we know that it is impossible for a spin forbidden reaction to go in one concerted step.

$$\underbrace{{}^{3}O_2\ (\uparrow\uparrow)}_{\text{triplet}} + \underbrace{{}^{1}X\ (\uparrow\downarrow)}_{\text{singlet}} \nrightarrow {}^{1}XO_2\ (\uparrow\downarrow)$$

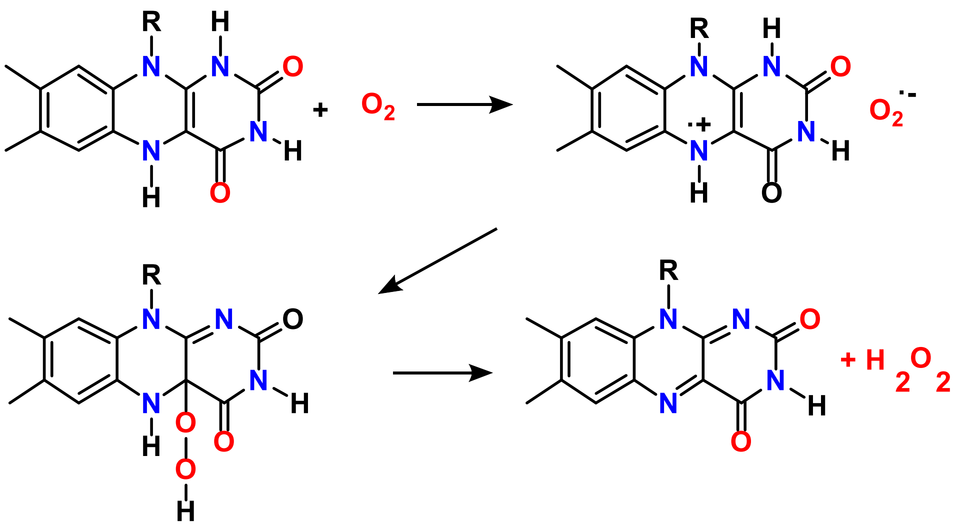
A Direct Reaction of O_2 in Which Each Step is Spin Allowed:

$$^3O_2\ (\uparrow\uparrow) + {}^1X\ (\uparrow\downarrow) \rightarrow {}^2O_2^-\ (\uparrow) + {}^2X^+\ (\uparrow)$$

$$^2O_2^-\ (\uparrow) + {}^2X^+\ (\uparrow) \rightarrow {}^2O_2^-\ (\uparrow) + {}^2X^+\ (\downarrow)$$

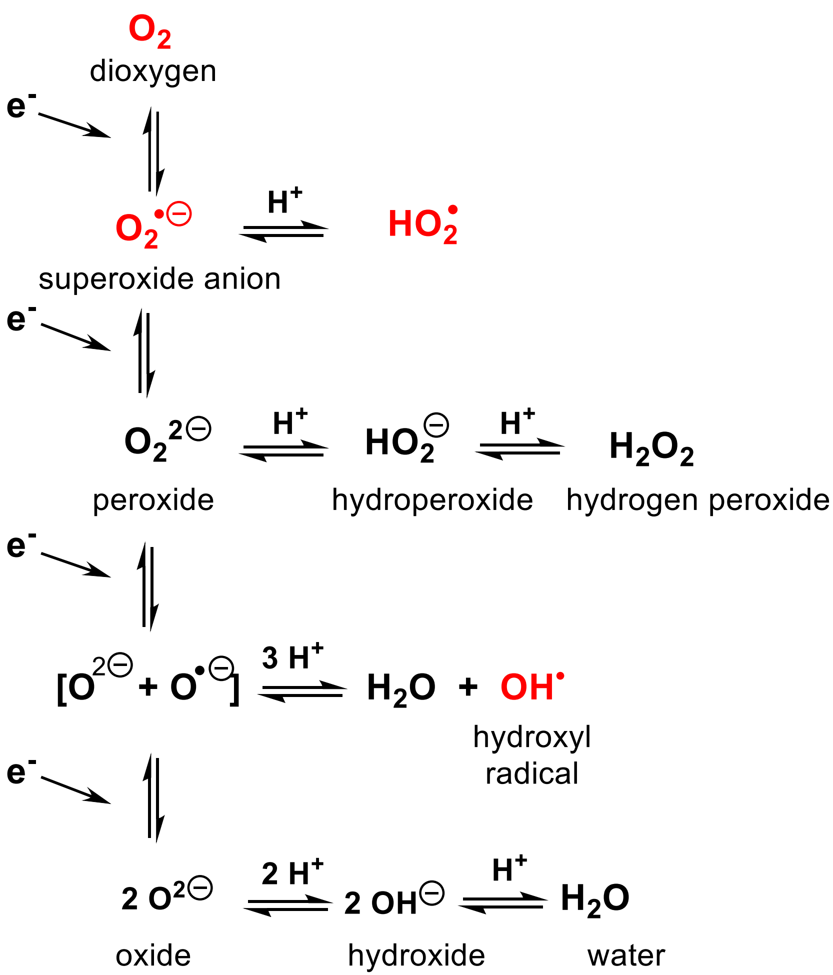
$$^2O_2^-\ (\uparrow) + {}^2X^+\ (\downarrow) \rightarrow {}^1XO_2\ (\uparrow\downarrow)$$

Reaction of dioxygen with reduced Flavin



This type of reaction is very unusual because most substrates are not good enough reducing agents to make superoxide.

Reactive Oxygen Species (ROS)



- ROS are a minor product of the oxidative respiratory chain (~1-2%), mostly in the form of superoxide.
- Excess production of ROS may result from iron overload and inflammation or immune responses.

Kaim w. and Schwederski B. “Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life.” J. Wiley and Sons, 1994, New York

"Persistence" of Reactive Species in Water

Reactive Species	Half-life
Hydrogen peroxide Organic hydroperoxides Hypohalous acids	~ minutes
Peroxyl radicals Nitric oxide	~ seconds
Peroxynitrite	~ milliseconds
Superoxide anion Singlet oxygen Alkoxyl radicals	~ microsecond
Hydroxyl radical	~ nanosecond

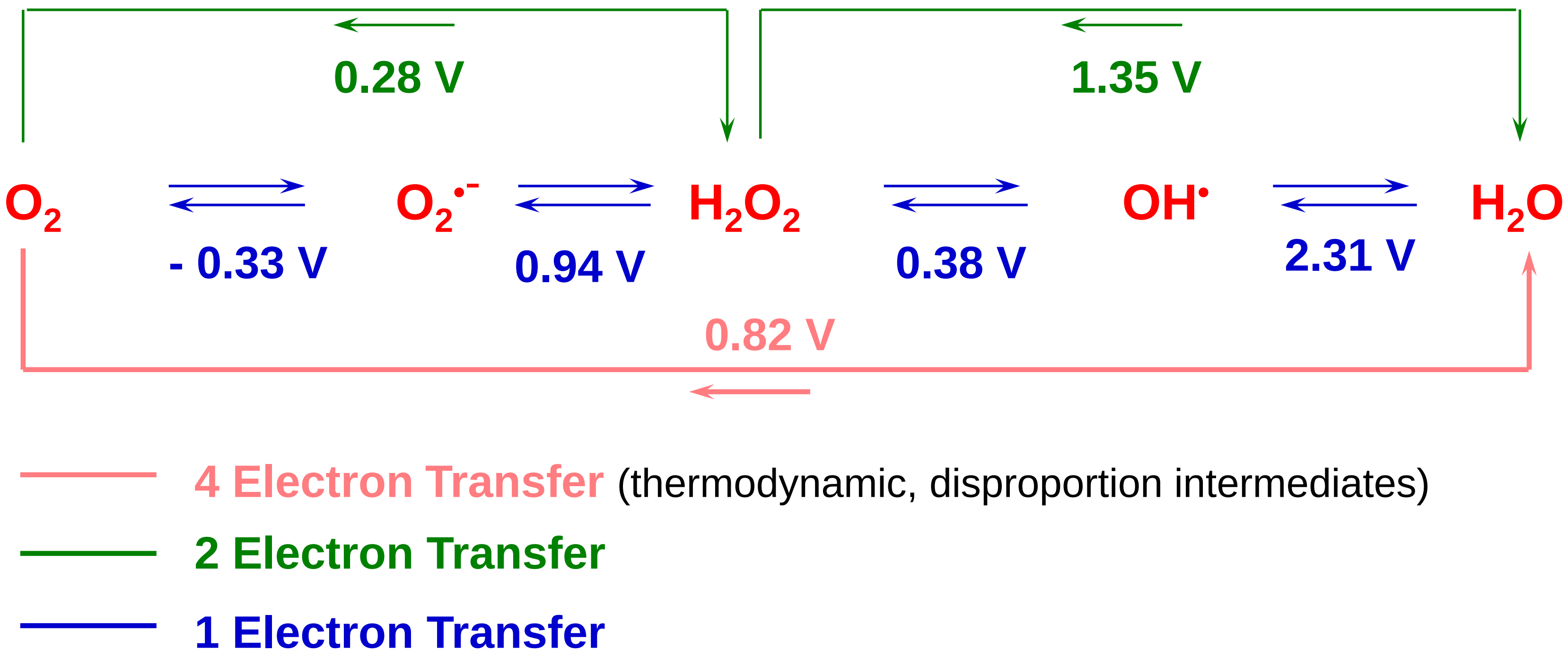
Standard Reduction Potential for Oxygen Species in Water (pH = 7, 25°C)

Reaction	E°, V vs. NHE
$O_2 + e^- \rightarrow O_2^{\bullet -}$	- 0.33[a]
$O_2^{\bullet -} + e^- + 2\ H^+ \rightarrow H_2O_2$	+ 0.94
$H_2O_2 + e^- + H^+ \rightarrow H_2O + {}^{\bullet}OH$	+ 0.38
${}^{\bullet}OH + e^- + H^+ \rightarrow H_2O$	+ 2.31
$O_2 + 2\ e^- + 2\ H^+ \rightarrow H_2O_2$	+ 0.281[a]
$H_2O_2 + 2\ e^- + 2\ H^+ \rightarrow 2\ H_2O$	+ 1.349
$O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O$	+ 0.815[a]

[a]The standard state used here is unit pressure. If unit activity is used for the standard state of O_2, the redox potential for reactions of that species must be adjusted by +0.17 V.

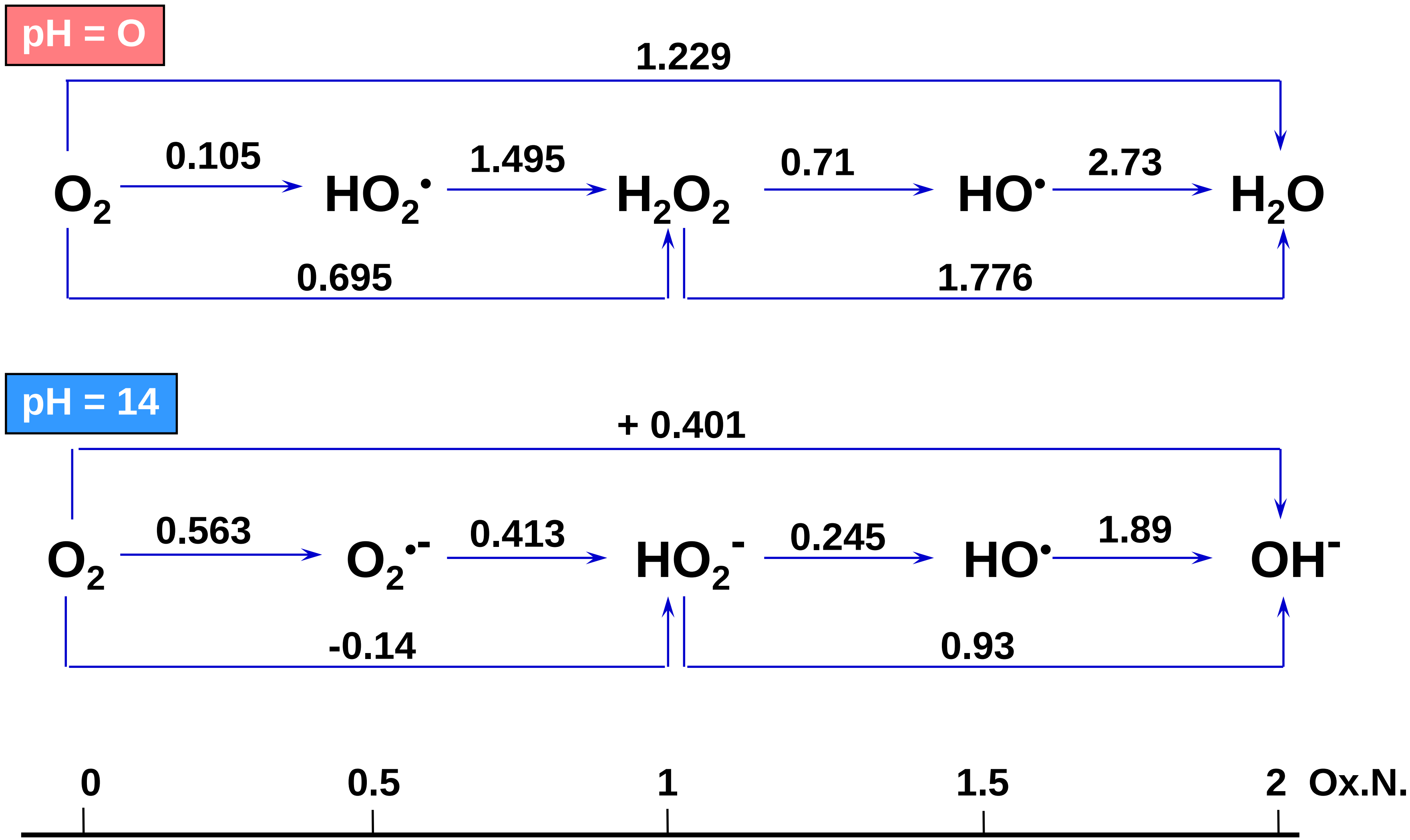
Redox Properties of O_2/H_2O System at pH = 7

Ox.N.	**0**	**(-1)**	**-1**	**(-2)**	**-2**
Name	**Oxygen**	**Superoxide ion**	**Hydrogen peroxide**	**Hydroxyl radical**	**water**

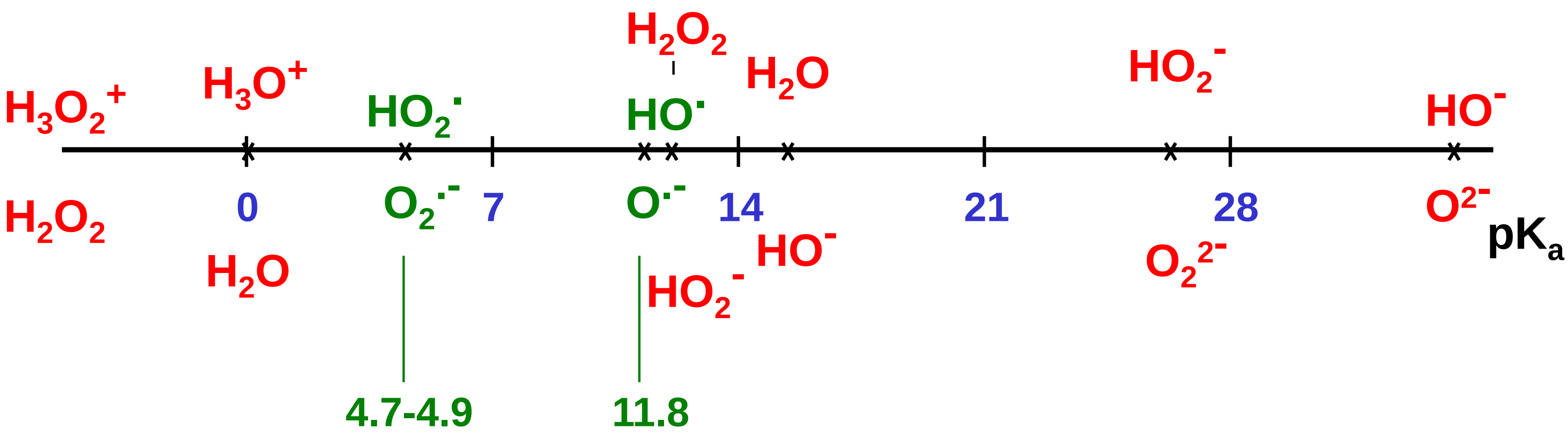


Halliwell B, Gutteridge JMC, Free radicals in biology and medicine. New York: Oxford University Press; 2003.

Redox Potential of Main Oxygen Derived Species in Acid and Basic Media

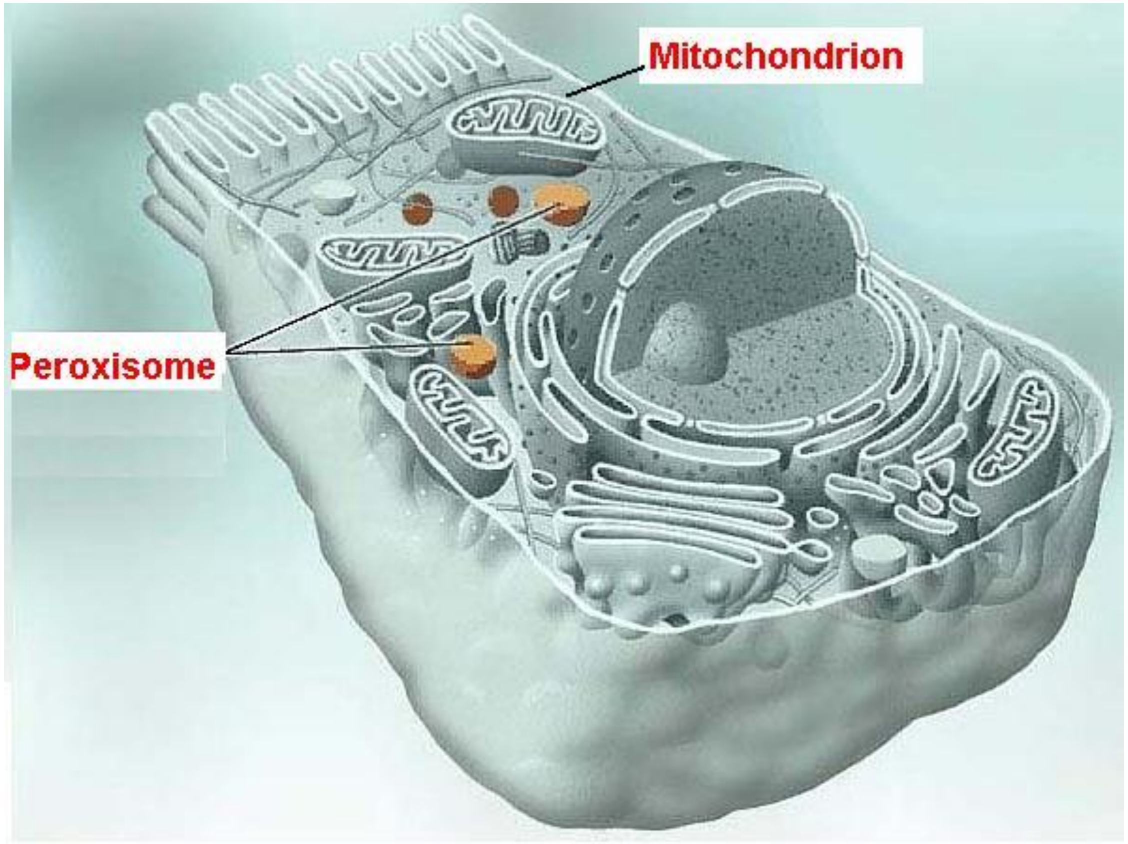


Acid-Base Properties of Oxygen Species



- Radical species are more acid than the corresponding hydro derivatives
- $H_3O_2^+$, H_2O_2 and HO_2^- are more acid than derivatives H_3O^+, H_2O and OH^-
- $HO_2^{\bullet}$ e $O_2^{\bullet -}$ are more weaker bases than $OH^{\bullet}$ and $O^{\bullet -}$.

Biological Role of Diatomic Oxygen

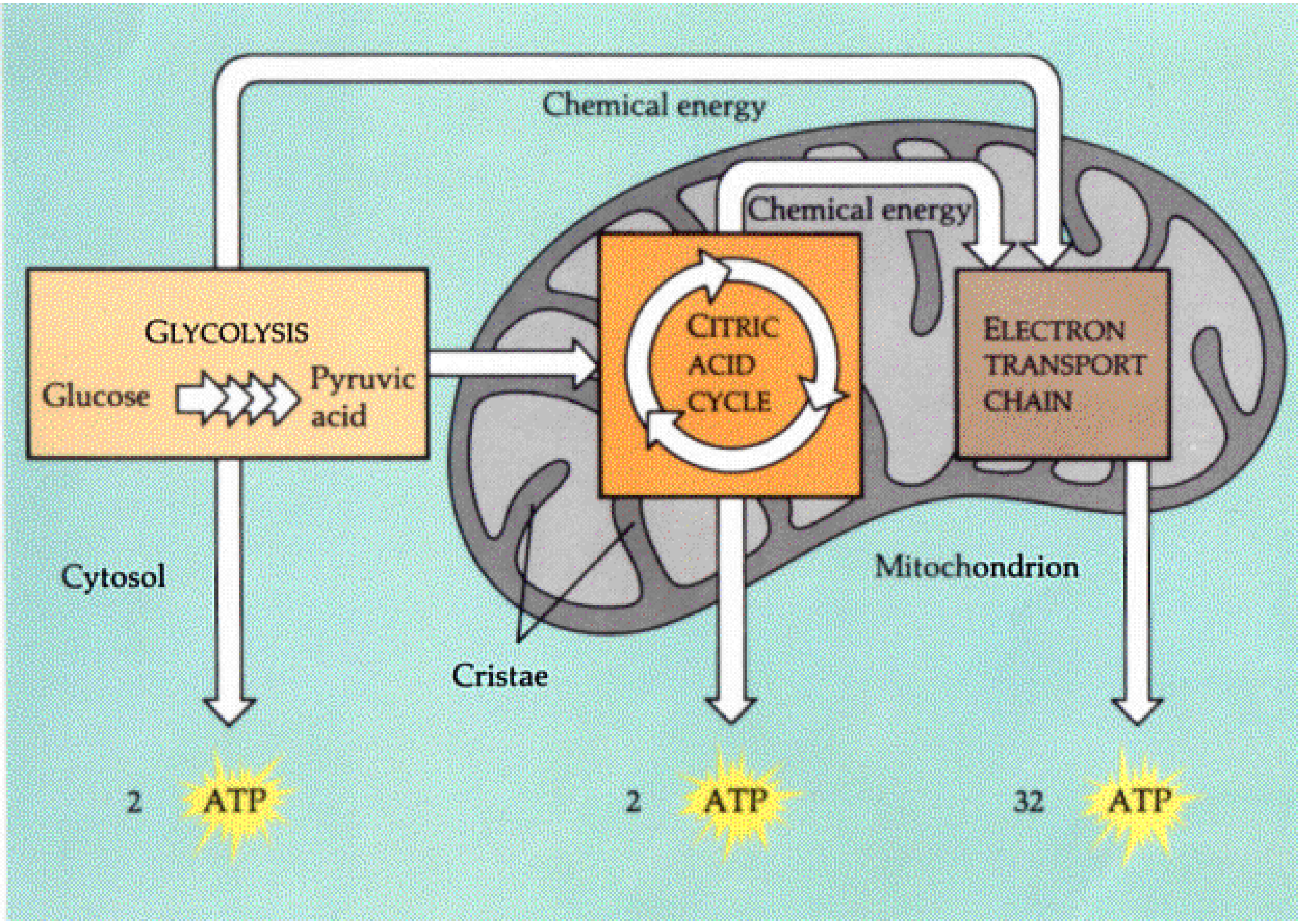


- Carbohydrate metabolism for ATP Production (Mitochondria)
- Degradation of metabolic by-products (Peroxisomes)

Normal Oxygen Metabolism

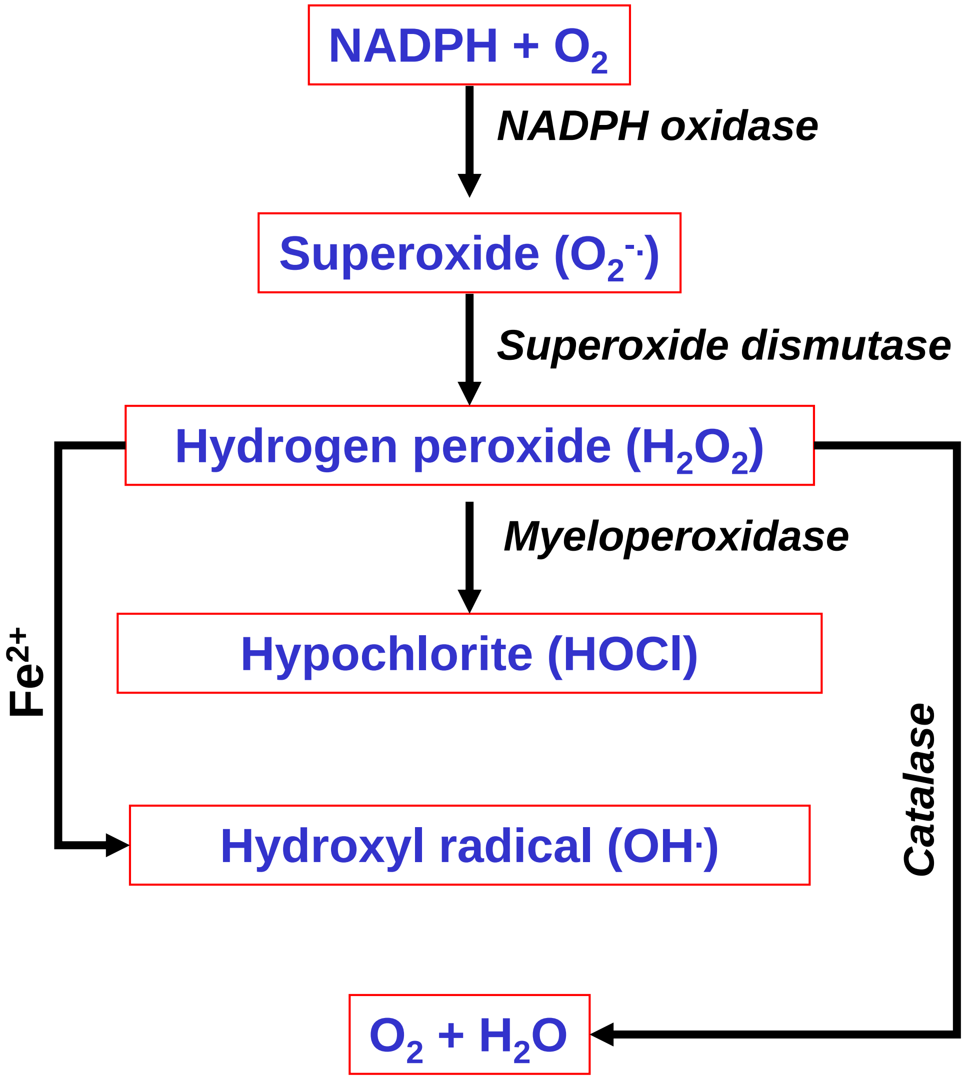
- Human cellular consumption
  - 20×10^6 O_2/m -cell surface per second
  - 95% Used in metabolic processes
- Semireduced oxygen species produced
  - 2~5% (H_2O_2 $O_2^{\bullet -}$ $^{\bullet}OH$)
- Normal steady-state levels of semireduced Oxygen species
  - H_2O_2 ~ 10 nM
  - $O_2^{\bullet -}$ ~ 0.1 nM
  - $^{\bullet}OH$ ~ 1 pM to 1 fM
- Oxidized protein
  - ~ 1 oxidized protein produced for each 100 molecules of oxygen consumed
- Oxidized DNA and RNA
  - ~ 1 oxidized nucleic acid produced for each 200 molecules of oxygen

Cellular Respiration



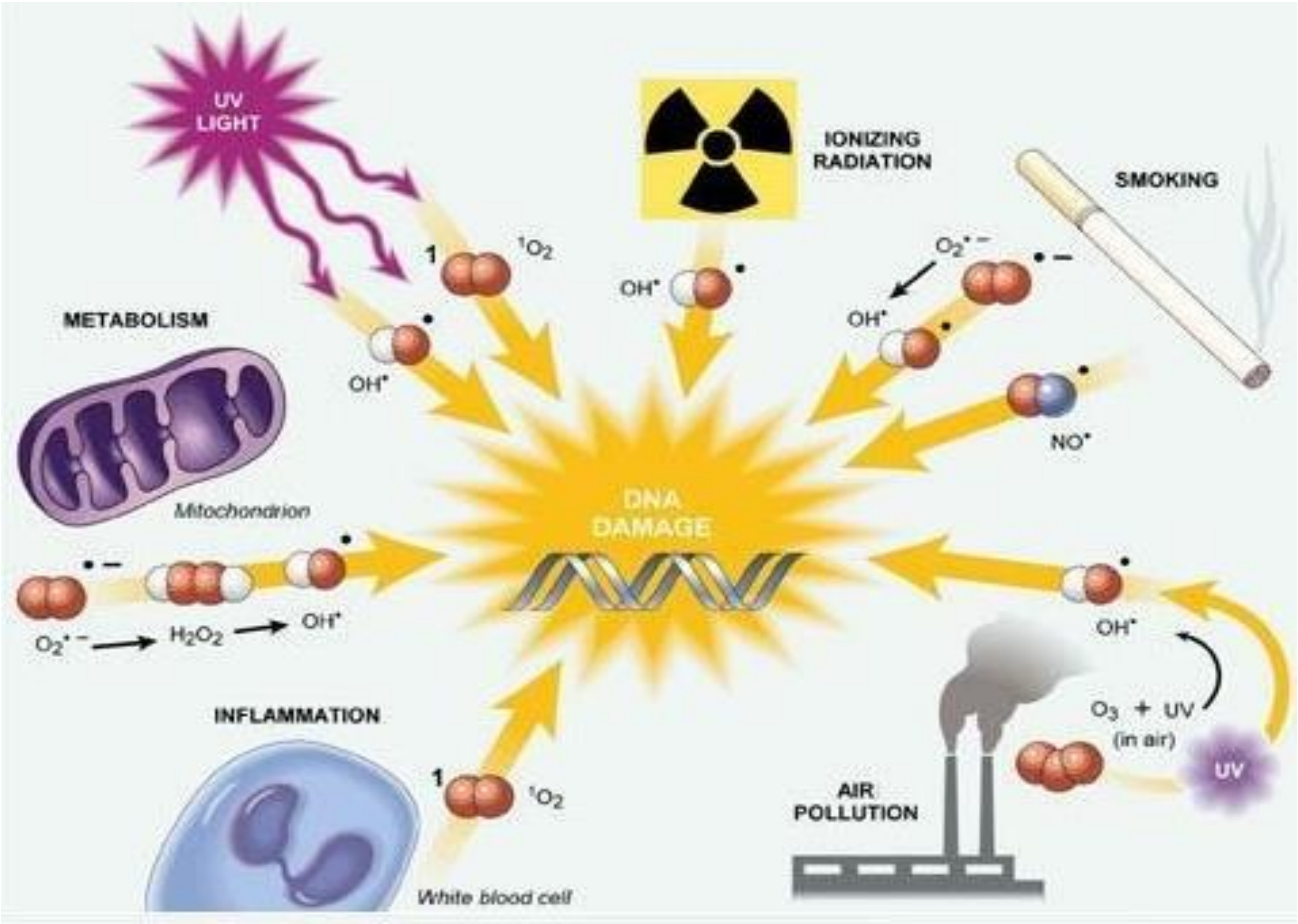
Production of Reactive Oxygen Species

Reactive Oxygen Species (ROS)



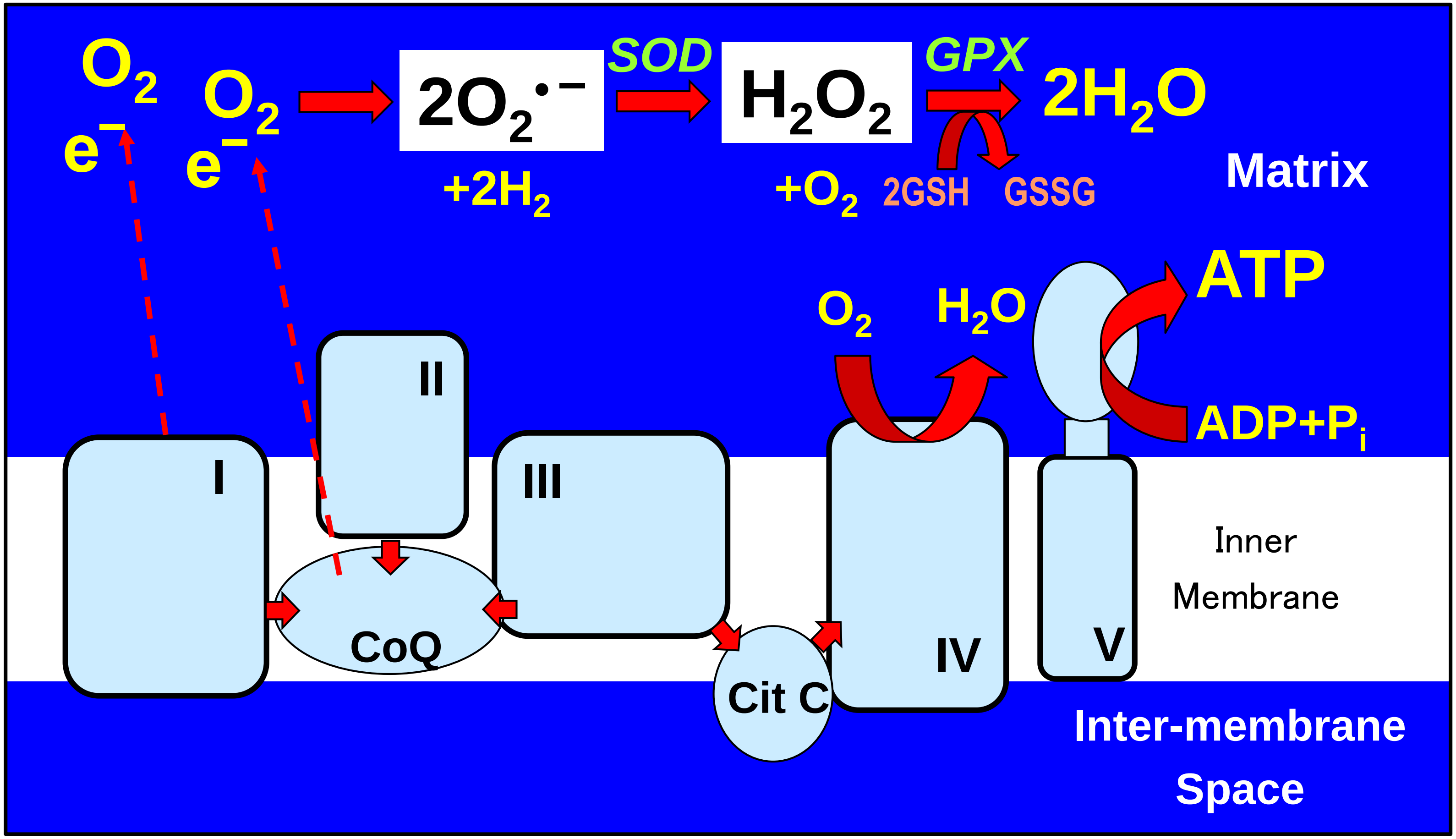
- In the respiratory chain 1-2 % of daily oxygen consumption goes to superoxide anion ($O_2^{\bullet -}$) generation
- Adult person produces 200-400 mmol $O_2^{\bullet -}$ / day

Generation of Reactive Oxygen Species



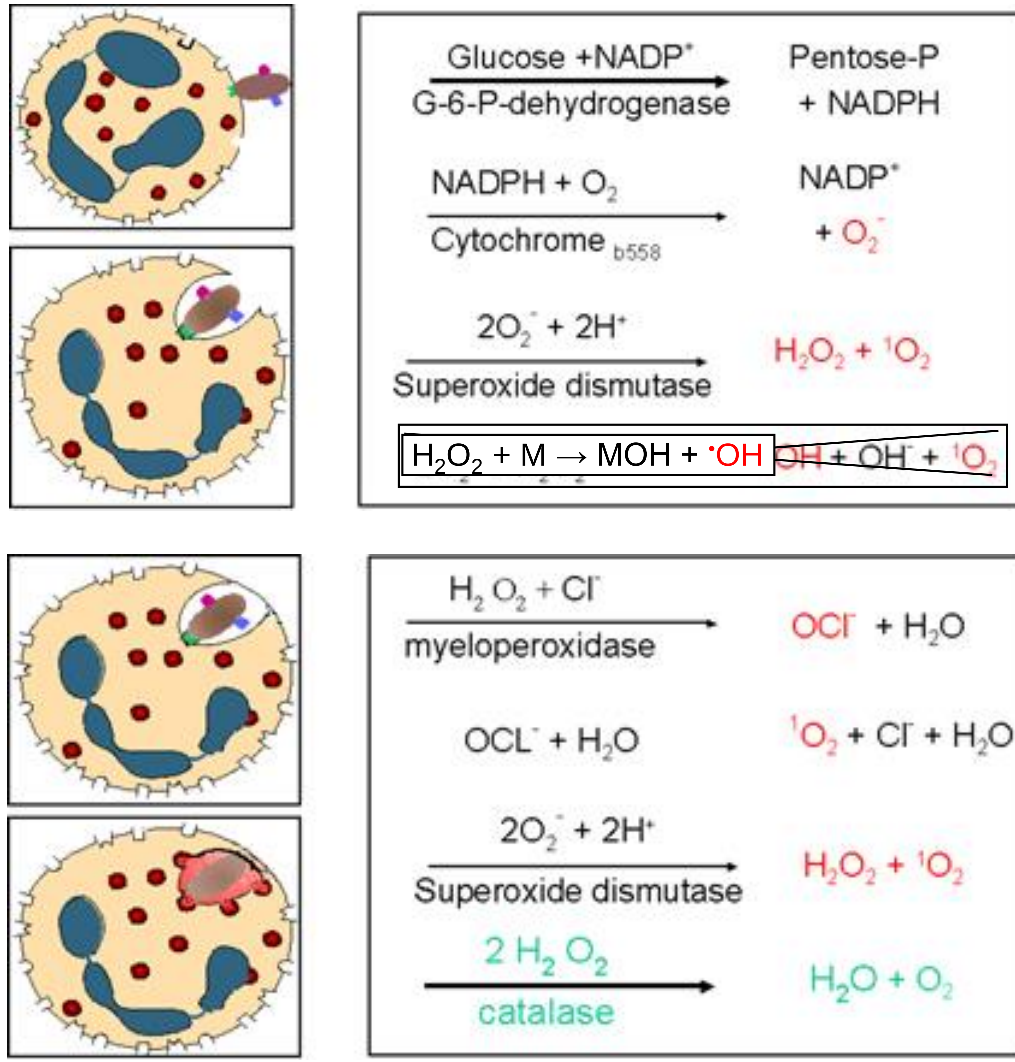
Elstner, E.F. 1982. Oxygen activation and oxygen toxicity. Ann. Rev. Plant Physiol. 33:73–96.

ROS Production and Detoxification

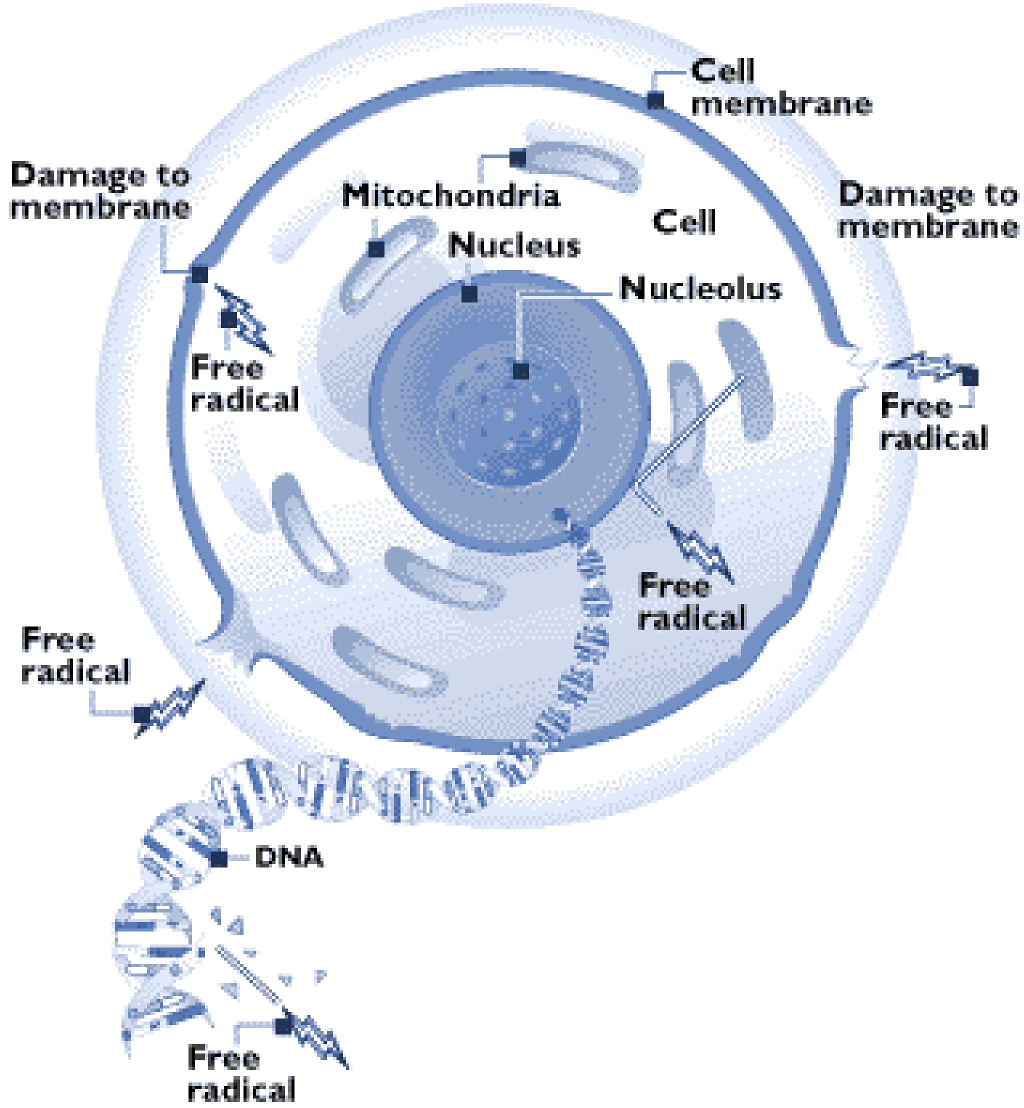


Respiratory Burst –
Generation of ROS in Immune Defenses

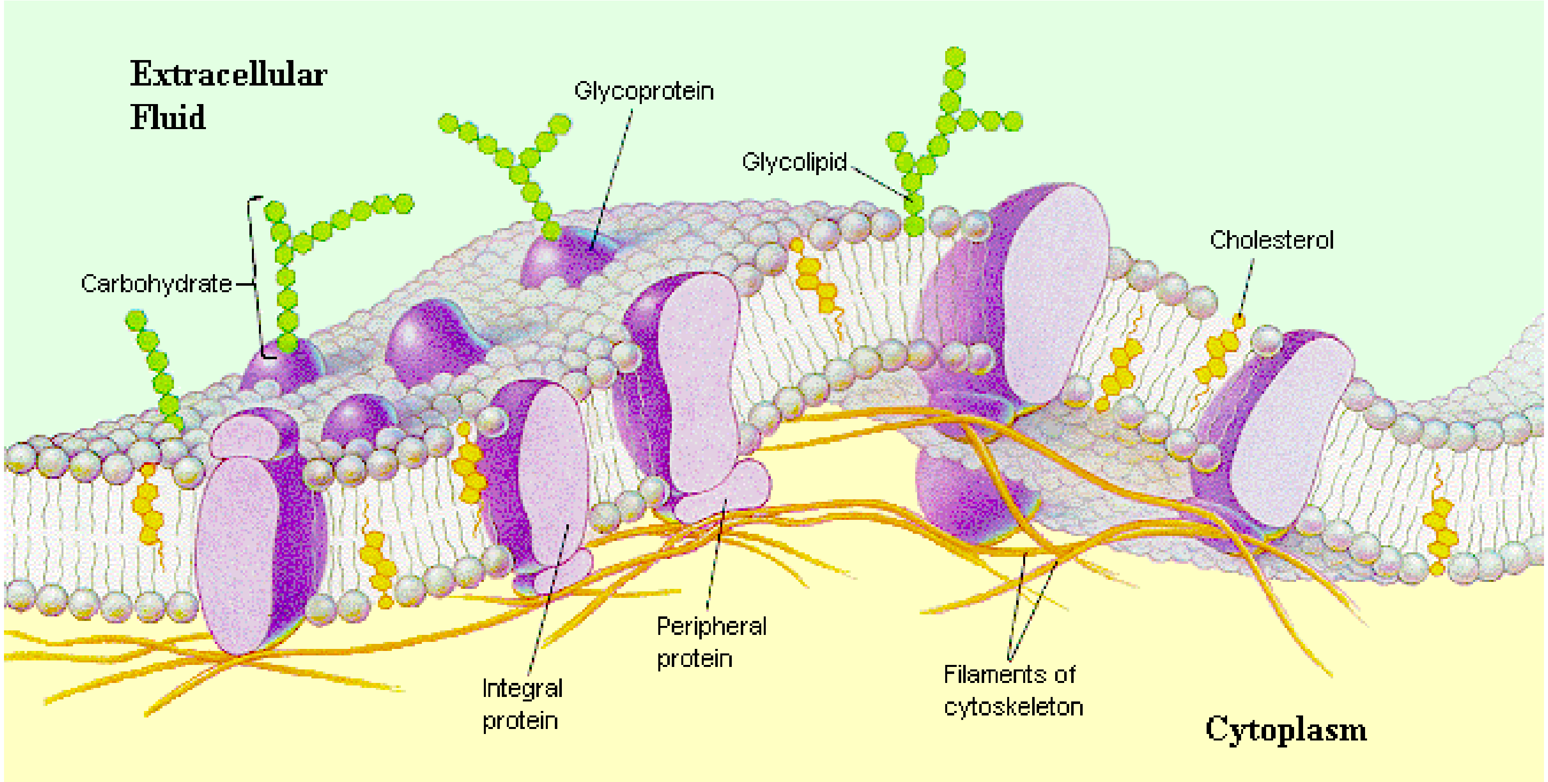
- Occurs in macrophages during phagocytosis
- Abrupt rise in oxygen consumption
- Increased glucose consumption
- HMP Shunt (Pentose phosphate pathway)
- Large amounts of reactive oxygen intermediates
- Enzyme – **NADPH Oxidase**



Oxidative Damages

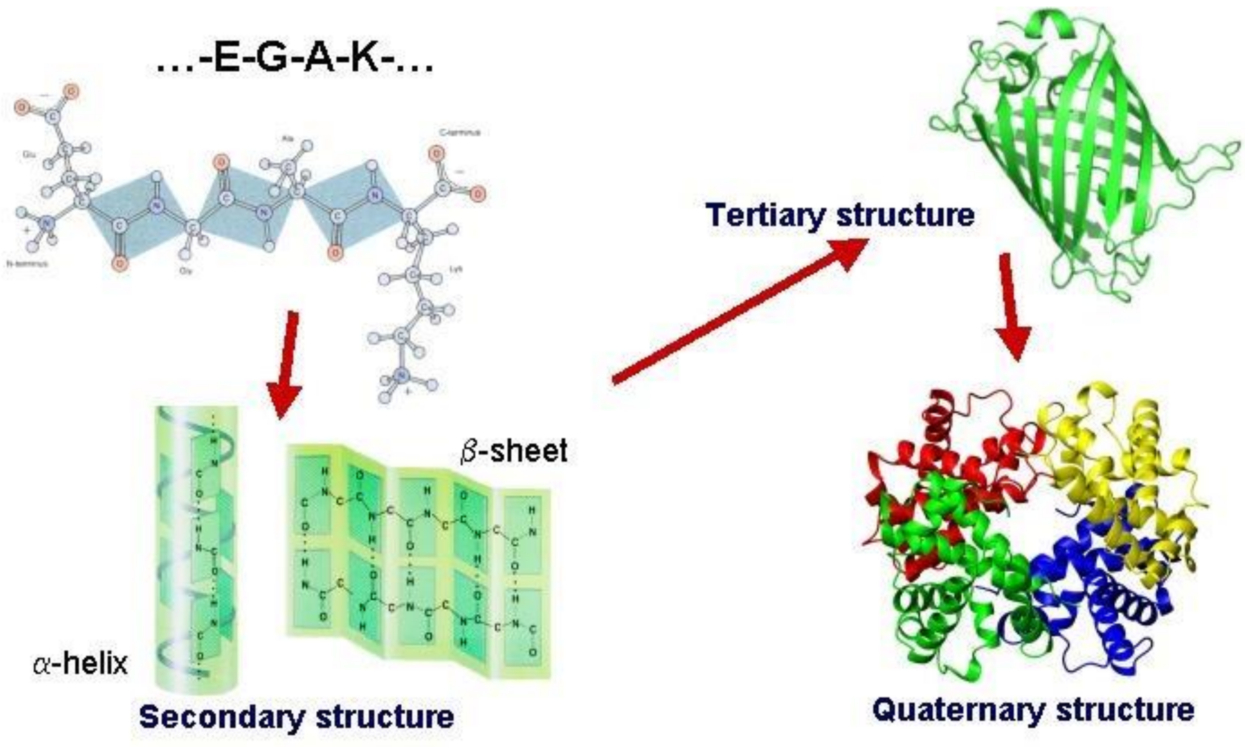


Oxidative Damage to Lipids



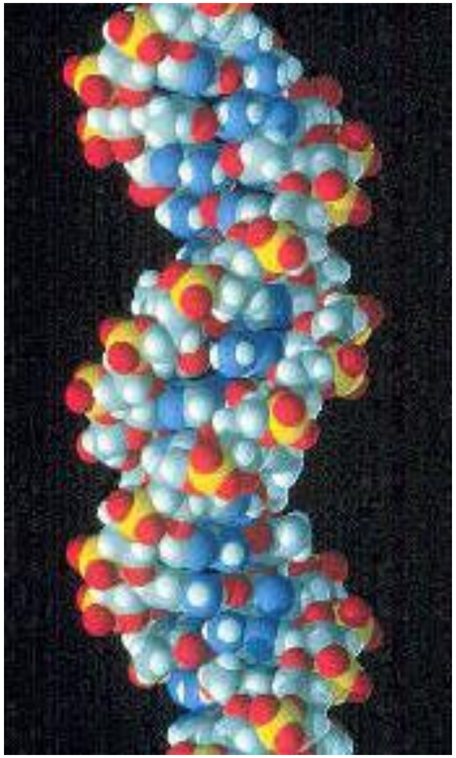
- **Increase membrane rigidity**
- **Reduce activity of membrane-bound enzyme**
- **Alter activity of membrane receptors**
- **Alter cell permeability**

Oxidative Damage to Proteins

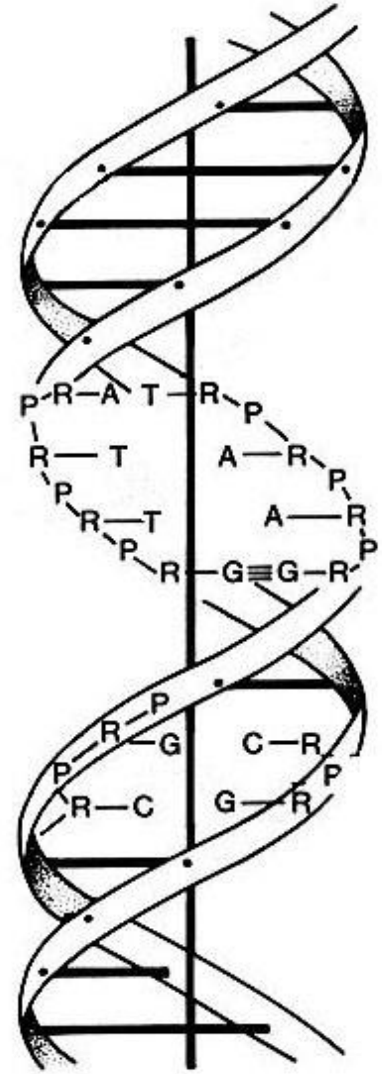


- Site-specific amino acid modifications
- Fragmentation of peptide chain
- Aggregation of cross-linked reaction products
- Increased susceptibility to proteolysis
- Degradation of enzymes

Oxidative Damage to DNA



- Mutation
- Single strand breakage
- Nucleotide degradation
- Cross-linking to protein



Free radical biology and medicine: it‘s a gas, man! William A. Pryor, Kendall N. Houk, Christopher S. Foote, John M. Fukuto, Louis J. Ignarro, Giuseppe L. Squadrito, Kelvin J. A. Davies; *Am J Physiol Regul integr Comp Physiol* **2006**, *291*: R491-R511,

Higher Levels of Oxidative Stress (H_2O_2)

- 9 – 14 µmol $H_2O_2/10^7$ cells: permanent growth arrest

  → loss of divisional competence, but no cell death arrested cells still exclude trypan blue, maintain membrane ionic gradients, utilize oxygen, make ATP good cellular models for certain cell ageing processes

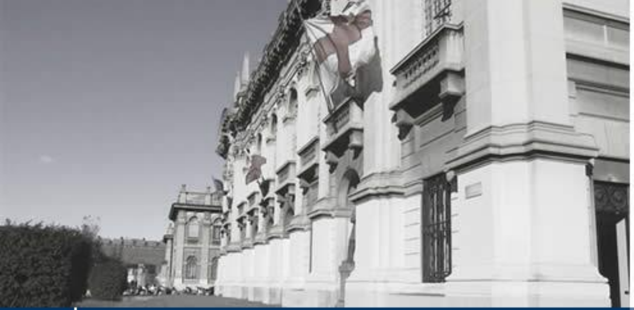
- 15 – 30 µmol $H_2O_2/10^7$ cells: apoptotic pathway

  → loss of mitochondrial transmembrane potential, release of cytochrome C to cytoplasm, loss of bcl-2, down-regulation and degradation of mitochondrial encoded m-RNA, r-RNA, DNA, diminished transcription of the mitochondrial genome

- 150 – 300 µmol $H_2O_2/10^7$ cells: necrosis

  → membrane integrity breaks down oxidation induced necrosis may play a role in: heart attacks, strokes, macular degeneration, necrotic cells cause inflammatory responses in surrounding tissues

  → secondary inflammation (rheumatoid arthritis, lupus?)



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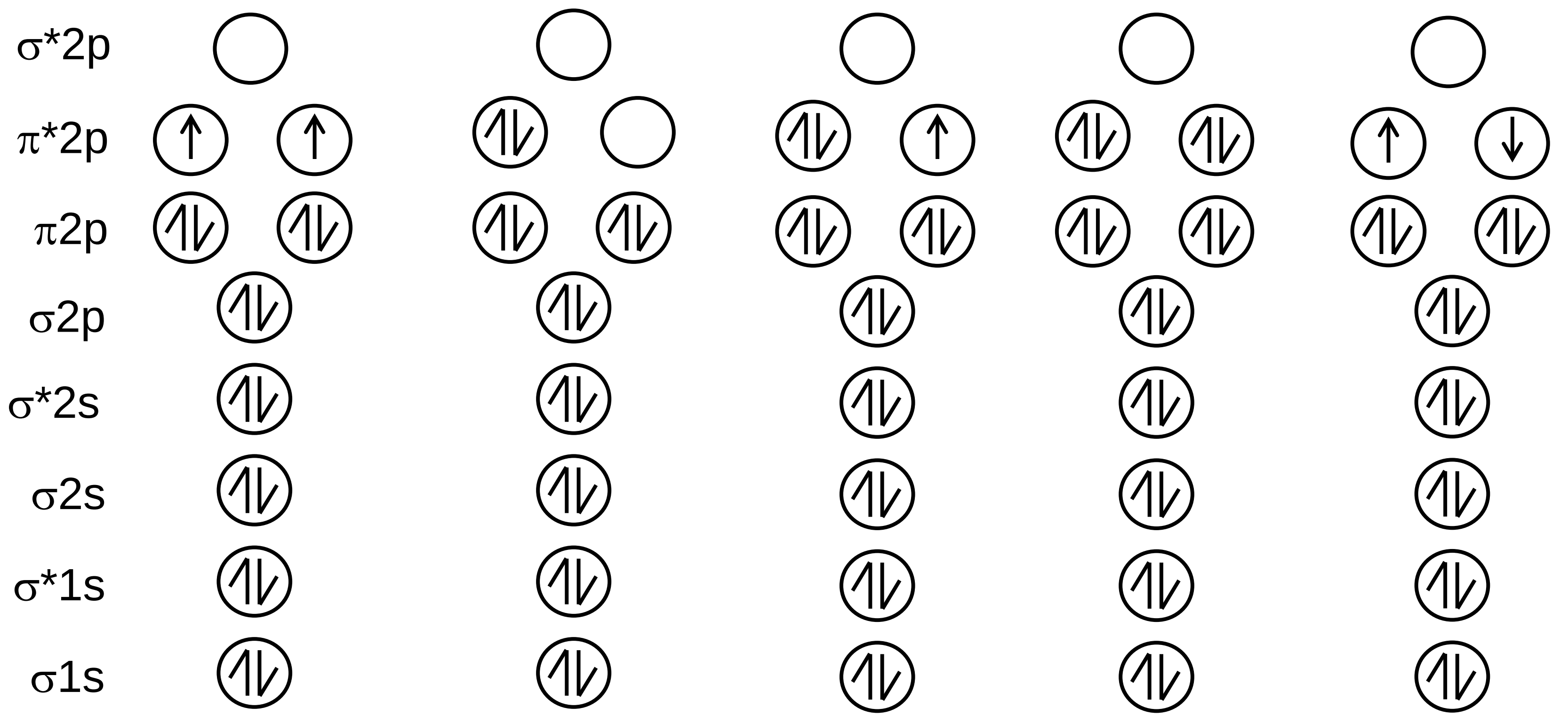
Active Oxygen Species:
O_2, $O_2^{\bullet-\cdot}$, $HO_2^{\bullet}$, $^1O_2$, H_2O_2, $HO^{\bullet}$

Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”

Electronic Structure of the Main Neutral and Anionic O_2 Species



Ground-state O_2	Singlet O_2	Superoxide	Peroxide ion	Singlet O_2
$(^3\Sigma_g O_2)$	$(^1\Delta_g O_2)$	$(O_2^{\bullet -})$	(O_2^{2-})	$(^1\Sigma_g^+ O_2)$
Paramagnetic		Paramagnetic		Paramagnetic

Prooxidants

Free Radicals:

- Any species capable of independent existence that contains one or more unpaired electrons
- A molecule with an unpaired electron in an outer valence shell

$R_3C^{\bullet}$	Carbon-centered
$R_3N^{\bullet}$	Nitrogen-centered
$R\text{-}O^{\bullet}$	Oxygen-centered
$R\text{-}S^{\bullet}$	Sulfur-centered

Non-Radicals:

- Species that have strong oxidizing potential
- Species that favor the formation of strong oxidants (e.g., transition metals)

H_2O_2	Hydrogen peroxide
HOCl	Hypochlorous acid
O_3	Ozone
$^1O_2$	Singlet oxygen
$ONOO^-$	Peroxynitrite
Me^{n+}	Transition metals

Free Radical Autoxidation:
The Main Source of Oxygen-Centered Radicals

Initiation: $X_2 \rightarrow 2\,X^{\bullet}$

$X^{\bullet}\,(\downarrow) + RH \rightarrow XH + R^{\bullet}\,(\downarrow)$

Propagation: $R^{\bullet}\,(\downarrow) + O_2\,(\uparrow\uparrow) \rightarrow ROO^{\bullet}\,(\uparrow)$

$ROO^{\bullet}\,(\uparrow) + RH \rightarrow ROOH + R^{\bullet}\,(\uparrow)$

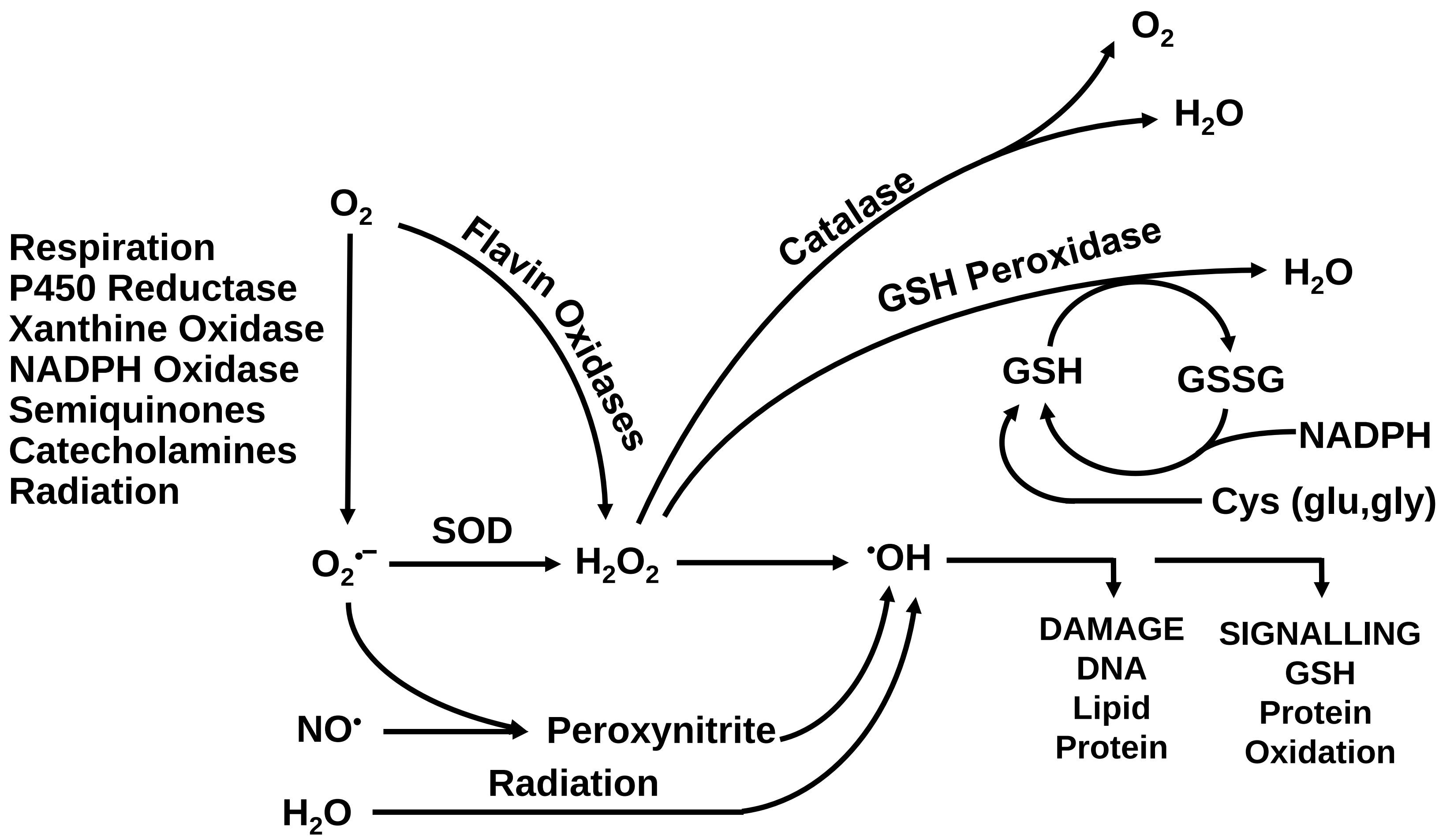
Termination: $R^{\bullet} + ROO^{\bullet} \rightarrow ROOR$

$2\,ROO^{\bullet} \rightarrow ROOOOR \rightarrow O_2 + ROOR$

(plus other oxidized products such as ROOH, ROH, RC(O)R, RC(O)H)

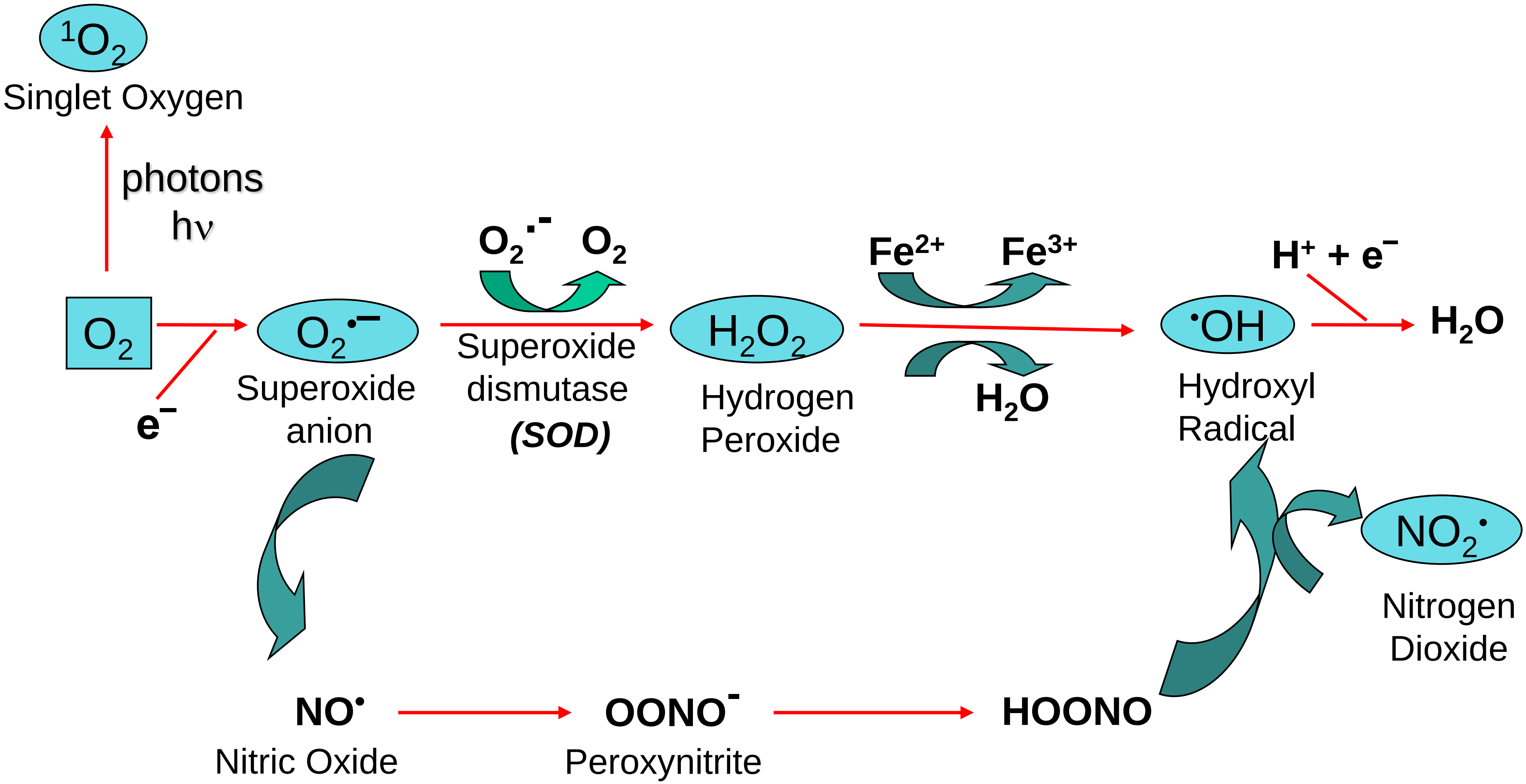
- Much more common than expected (inhibition is essential!!).
- Very small traces of redox metal ions and peroxide can initiate.
- Hydroperoxides are secondary sources of alkoxy radicals by reduction.

Generation and Fate of ROS in Biological Systems – An Overview



Cell Mol.Life Sci. **2000**, 57, 1287-1305

Interconversion of Reactive Oxygen Species in Biological Systems



Metabolic Sources of Oxygen Radicals - 1

- **Ionizing radiation causes lysis of water producing $^{\bullet}OH$**

- **Reaction of transition metals with O_2 or H_2O_2**
  - **$M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + {}^{\bullet}OH + OH^{-}$**
  - metabolically important metal ions include Cu^{+}, Co^{2+}, Ni^{2+}, Fe^{2+}

- **Production of $^{\bullet}NO$ (nitric oxide – endothelium-derived relaxation factor) by hydroxylation of arginine**
  - **$^{\bullet}NO$ reacts with $O_2^{\bullet -}$, forming peroxynitrite, which can decay to $^{\bullet}OH$**

Metabolic Sources of Oxygen Radicals - 2

- **Respiratory burst of macrophages**
  - the cytotoxic action of macrophages is due to production of halogen, oxygen and other radicals

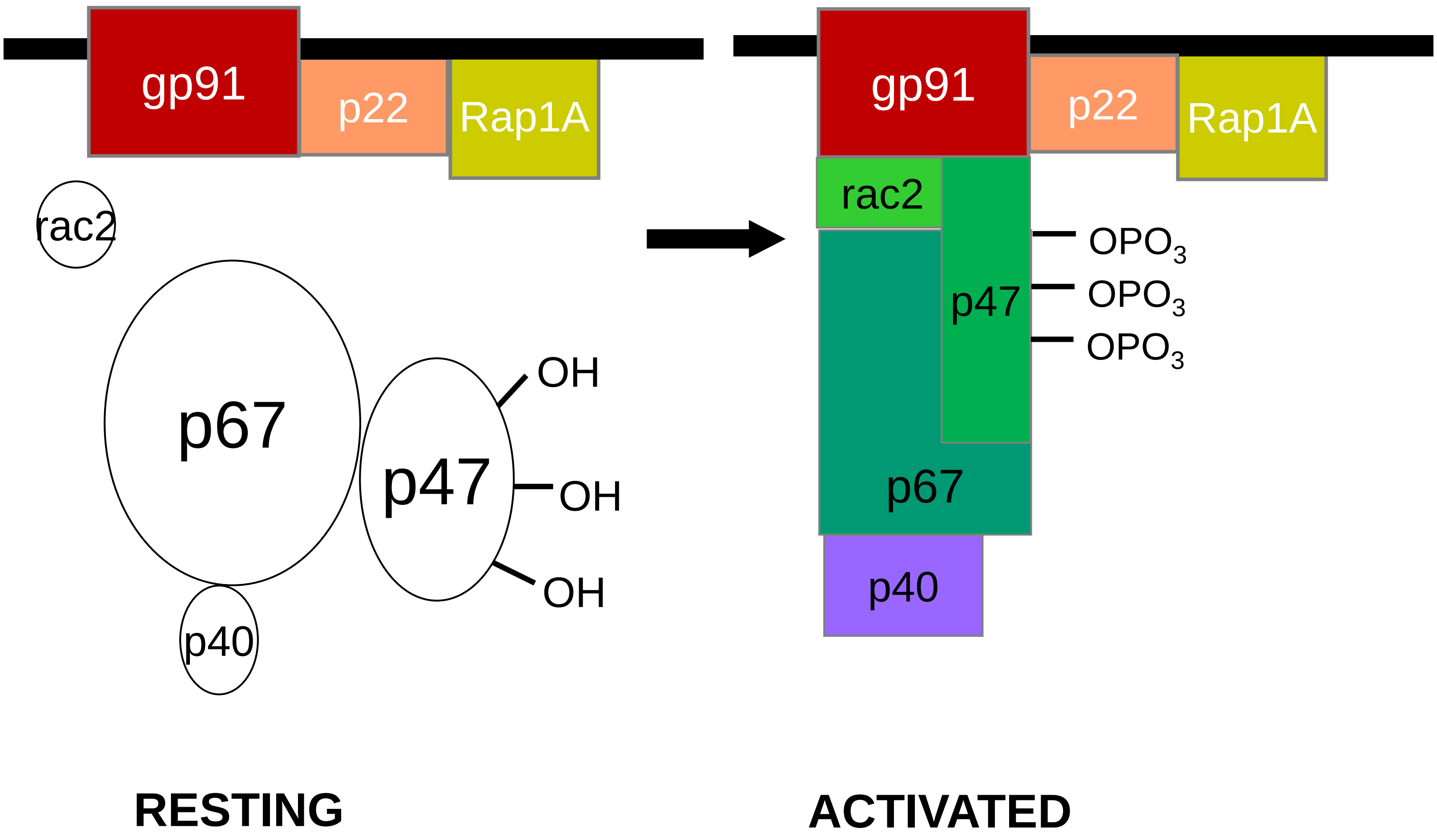
The respiratory burst of activated macrophages is increased utilisation of glucose to permit reduction of $NADP^+$ to NADPH, and increased utilisation of oxygen to oxidise NADPH.

The respiratory burst oxidase (NADPH oxidase) is a flavoprotein that reduces O_2 to $O_2^{\bullet -}$

$$\mathbf{NADPH + 2\ O_2 \rightarrow NADP^+ + 2\ O_2^{\bullet -} + 2H^+}$$

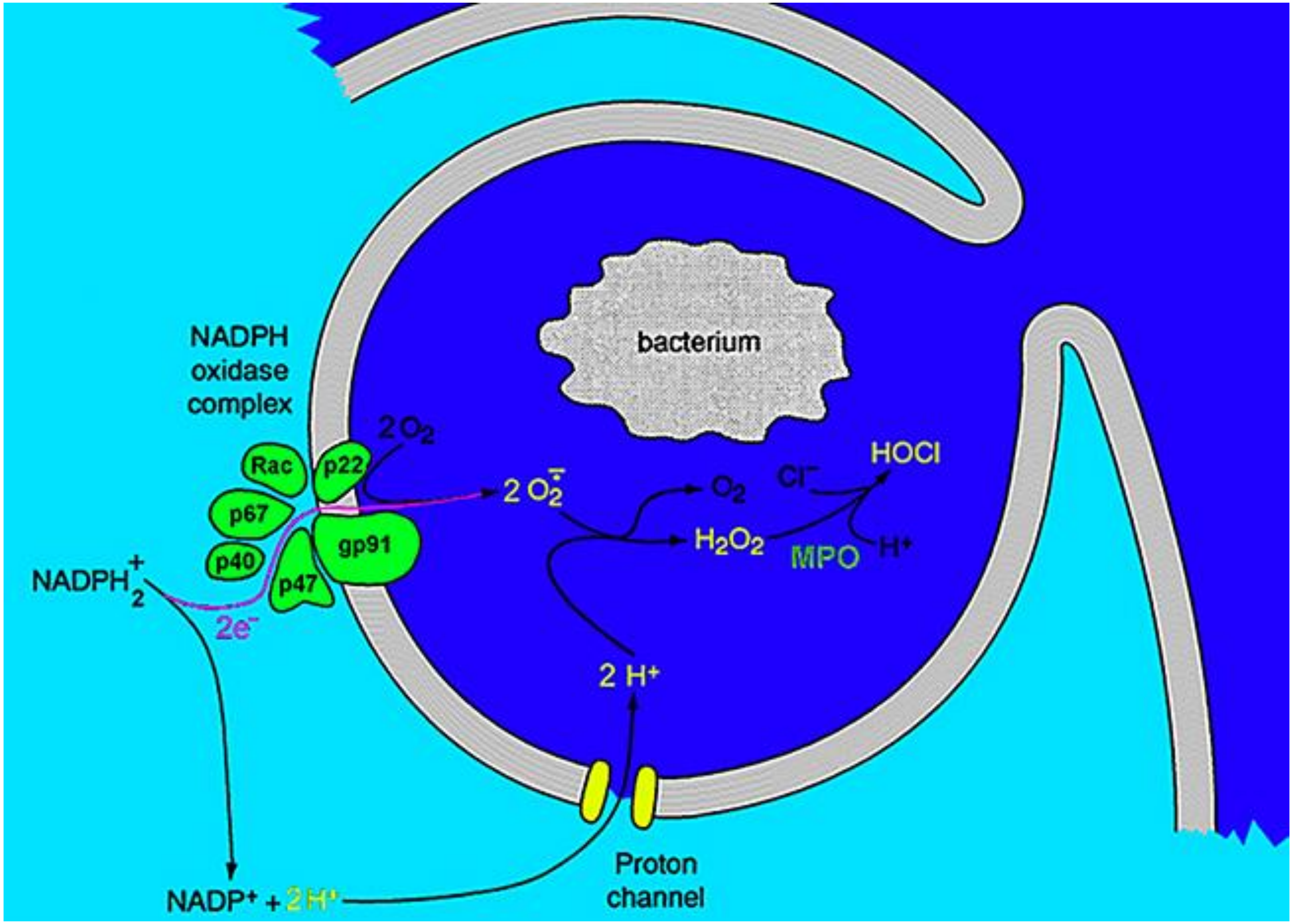
Originally described in 1973 by Babior.

NADPH Oxidase



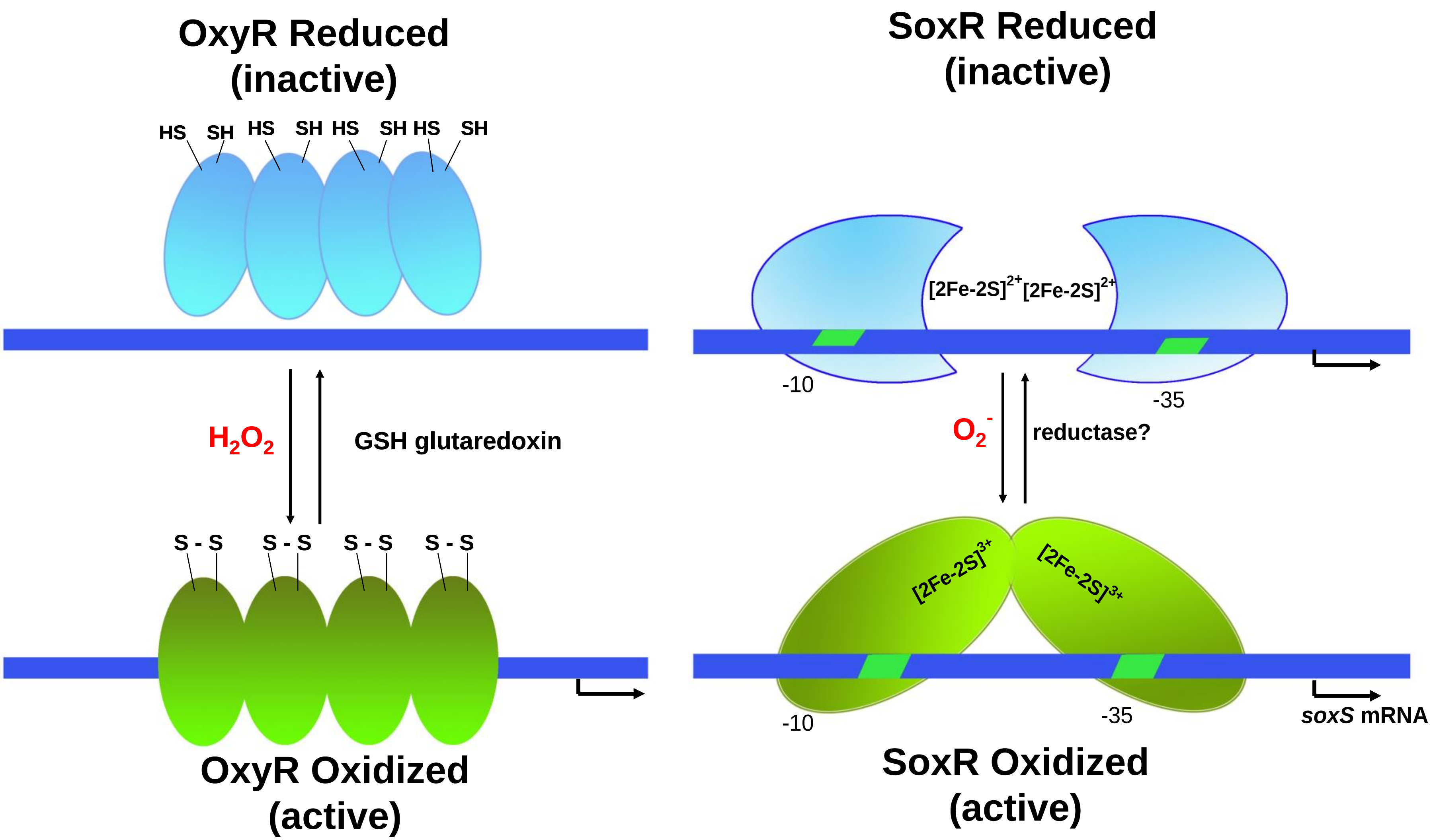
Ref-Babior,B.M;Blood **1999**, *93*,1464-1476.

Formation of Phagocytic Vesicle



http://www.bioscience.org/2003/v8/s/1191/fig8.jpg

OxyR and SoxR Transcriptional Regulators Protect Bacteria from the Lethal Effects of ROS



Metabolic Sources of Oxygen Radicals - 3

- **Reoxidation of reduced flavins in the respiratory chain**

$$X\text{-}H_2 + O_2 \rightarrow X + H_2O_2$$

$$XH_2 + \text{flavin} \rightarrow X + \text{flavin-}H_2$$

$$\text{flavin-}H_2 + O_2 \rightarrow \text{flavin} + H_2O_2$$

- **Reoxidation of reduced flavins in mixed function oxidases – the metabolism of foreign compounds**
- **Fully reduced flavin-H_2 reacts with oxygen to form flavin semiquinone radical and superoxide**

$$\text{flavin-}H_2 + O_2 \rightarrow \text{flavin-}H^{\bullet} + {}^{\bullet}O_2$$

- **Flavin semiquinone and superoxide react to form flavin hydroperoxide**

$$\text{flavin-}H^{\bullet} + O_2^{\bullet -} \rightarrow \text{flavin-HOOH}$$

- **Flavin hydroperoxide breaks down to flavin semiquinone and perhydroxyl**

$$\text{flavin-HOOH} \rightarrow \text{flavin-}H^{\bullet} + {}^{\bullet}O_2H$$

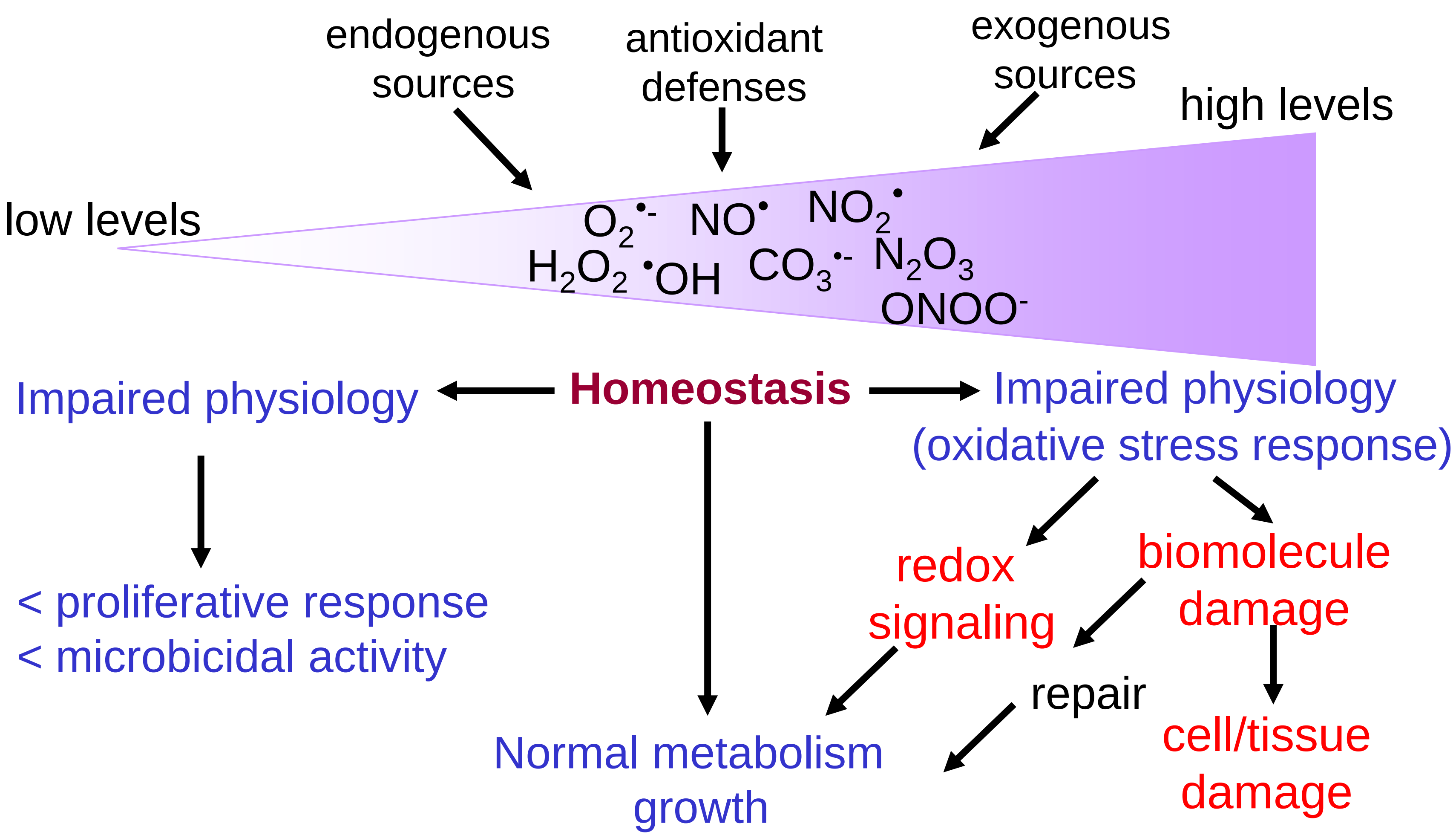
- **Perhydroxyl decays to superoxide plus a proton**

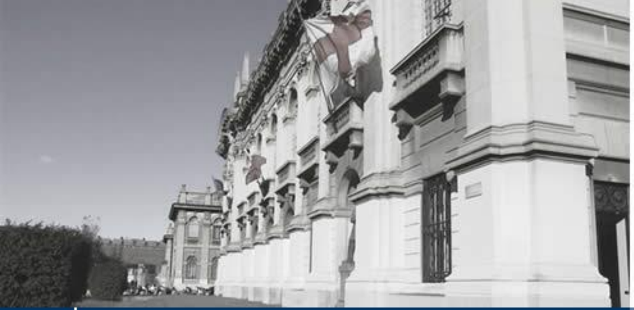
$${}^{\bullet}O_2H \rightarrow H^+ + O_2^{\bullet -}$$

- **In the presence of H^+, flavin semiquinone and superoxide react to yield hydrogen peroxide and oxidised flavin**

$$\text{flavin-}H^{\bullet} + H^+ + O_2^{\bullet -} \rightarrow \text{flavin} + H_2O_2$$

Biomarkers of oxidative damage & early biomarkers





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Active Oxygen Species: Chemistry

Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”

Hydroxyl Radicals

Hydroxyl radical $HO^{\bullet}$ is a:

- strong oxidant

  (A.E. = 1.83 eV;   E° = 2.85 V)

- quite reactive and unselective agent towards:
  - reducing species
  - organic C-H bonds
  - unsaturated bonds
  - transition metal complexes

Reactivity of Hydroxyl Radical ($HO^•$)

Hydrogen abstraction reactions

$$R\text{-}H + HO^• \longrightarrow R^• + HO\text{-}H$$

Compound	log k ($M^{-1}s^{-1}$)	ΔH($kJ\cdot mol^{-1}$)
HOO-H	7.30	- 119
$HOOCCH_2$-H	7.15	
$HOCH_2$-H	8.70	
$RC(NH_2)(COOH)$-H	9.03	
$CH_3CH(OH)$-H	9.04	
RS-H	9.48	- 167

Addition Reaction

$$HO^• + \rangle C{=}C\langle \longrightarrow HO\text{-}\overset{|}{\underset{|}{C}}\text{-}\overset{|}{\underset{|}{C}}^•$$

Compound	log k ($M^{-1}s^{-1}$)
Benzene	9.54
Ethylene	9.61
Phenol	9.85
Hydroquinone	10.08

Electron transfer Reactions: $HO^• + Red \rightarrow HO^- + Ox$

Ion	Ce^{3+}	Mn^{2+}	Sn^{2+}	Tl^+	NO_2^-	N_3^-	CNS^-	Cl^-	Br^-
log k/ $M^{-1}s^{-1}$	8.34	8.2	9.3	9.88	9.55	9.81	9.11	8.6	10.56

SINGLET OXYGEN

Bradley, D.E., Min. D.B. 1992. Singlet oxygen oxidation of foods. Cat. Rev. Food Sci. Nutri. 31: 211-236.

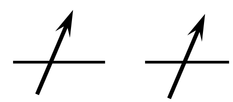
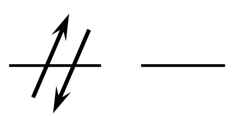
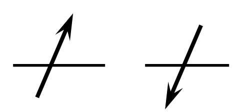
Molecular Oxygen Species (O_2)

Triplet Oxygen: colorless, odorless gas (low solubility: 31 ml/l at 20°C)

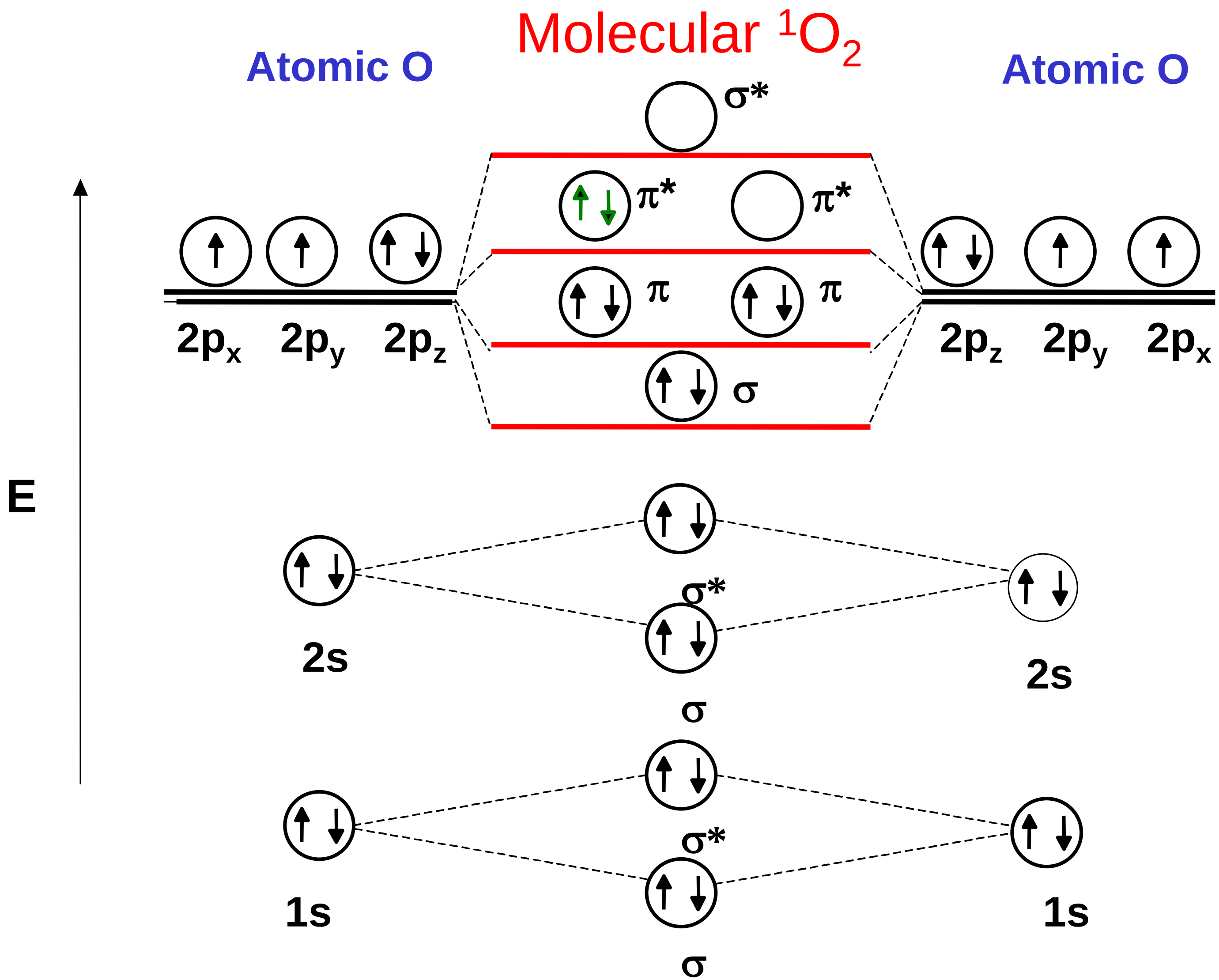
Electronegativity: 3.5 (the highest after F)

$T_{crit.}$: –118°C; b.p. = -183°C (see N_2: b.p. = -196°C)

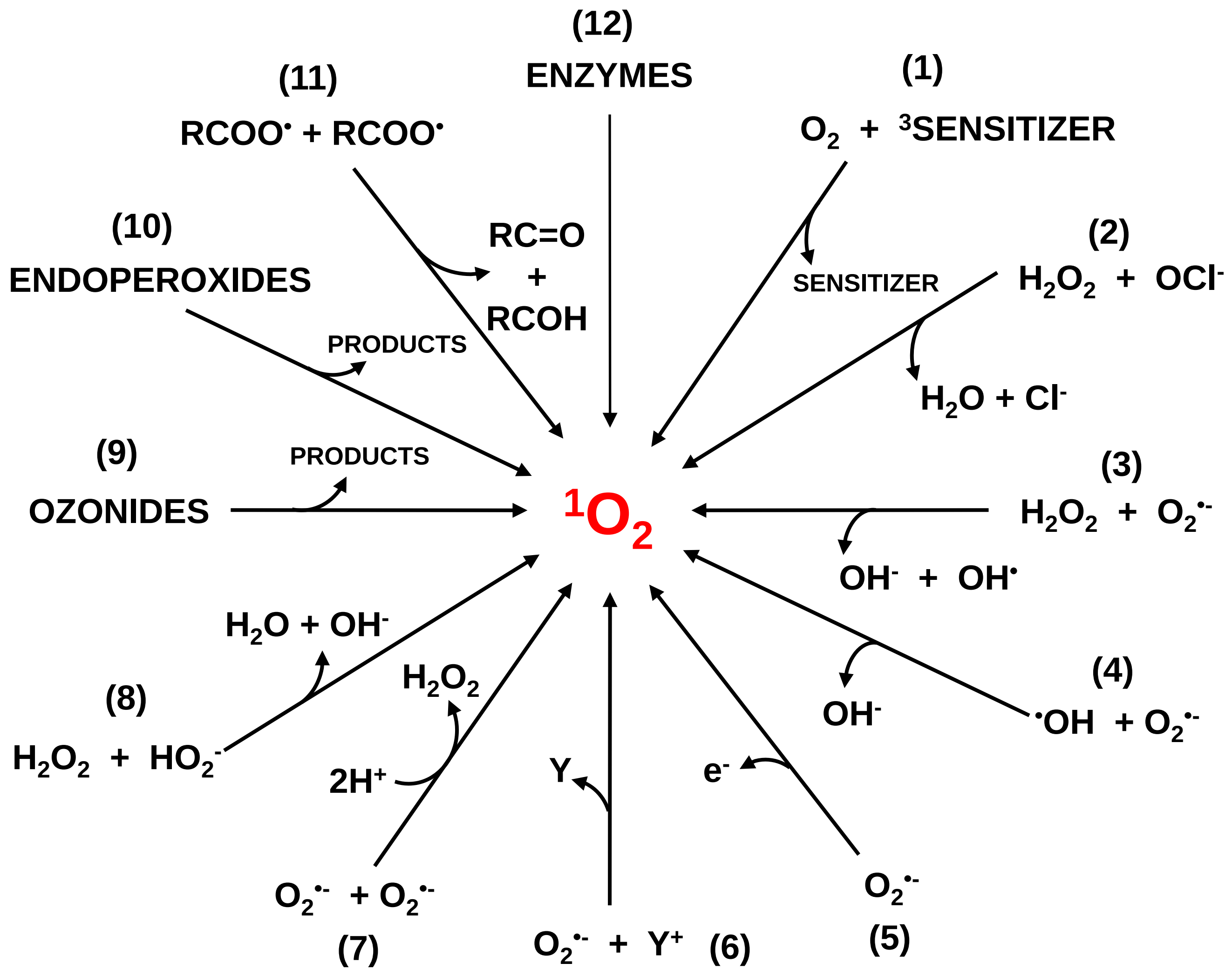
O_2 in ground state is a paramagnetic specie with two unpaired electrons (triplet). There are 2 excited forms :

Notation	*π Orbitals*	*El. State*	*lifetime*	*properties*
:O:::O: **triplet**		$^3\Sigma_g^-$	**persistent**	**paramagnetic oxidant**
:Ö=Ö: **singlet**		$^1\Delta_g$	**45-50 min**	**diamagnetic very reactive**
·Ö--Ö· **excited singlet**		$^1\Sigma_u$	**10^{-11} sec**	**excited diradical very reactive**

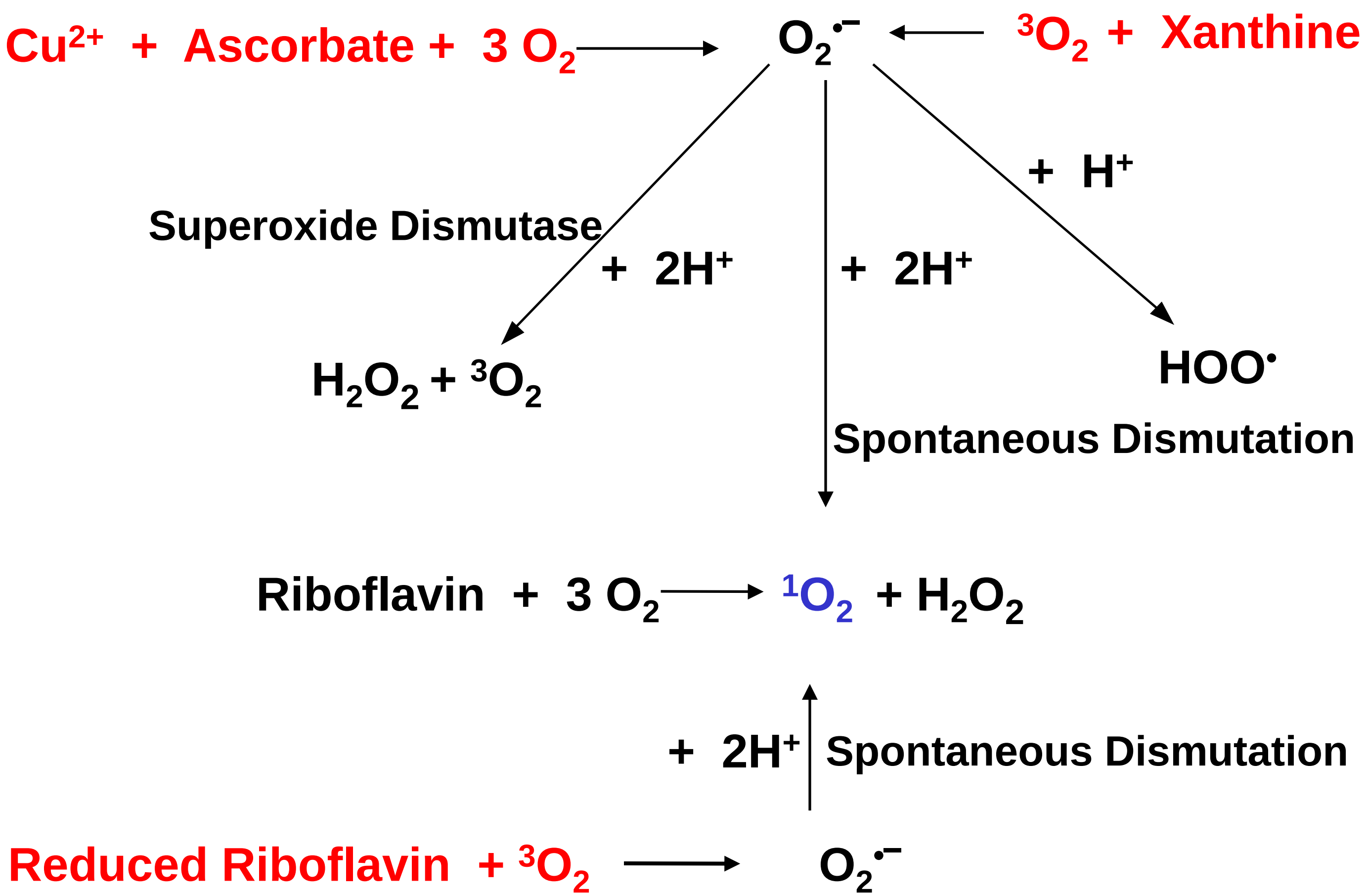
Molecular Orbital of Singlet Oxygen



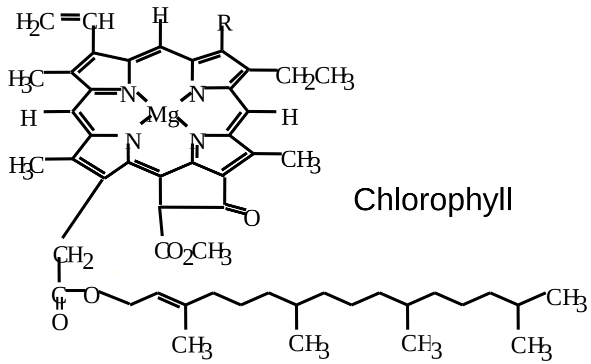
Production of $^1O_2$ by Photochemical, Chemical, and Biological Systems



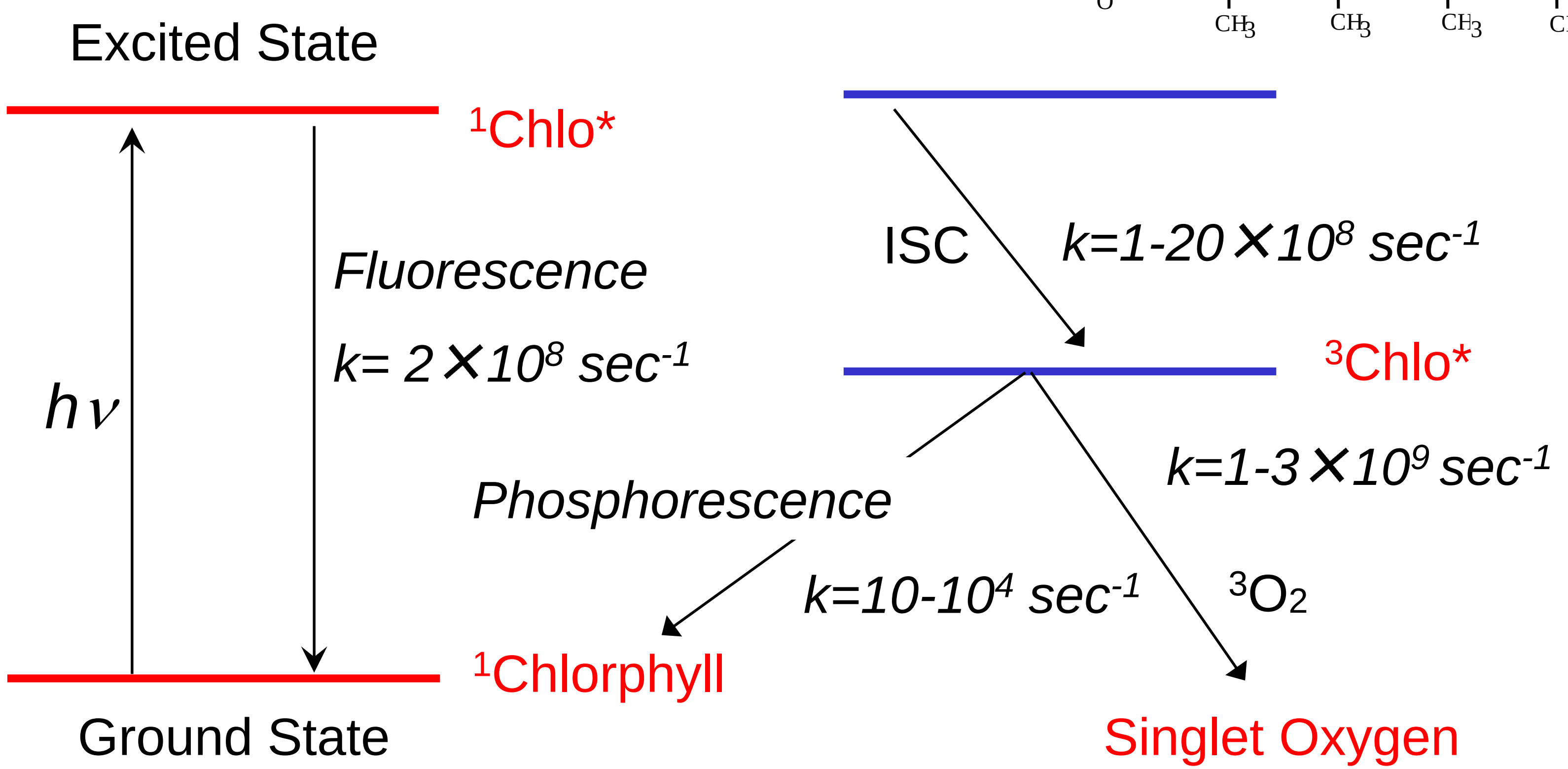
In Vivo Singlet Oxygen Formation Mechanisms



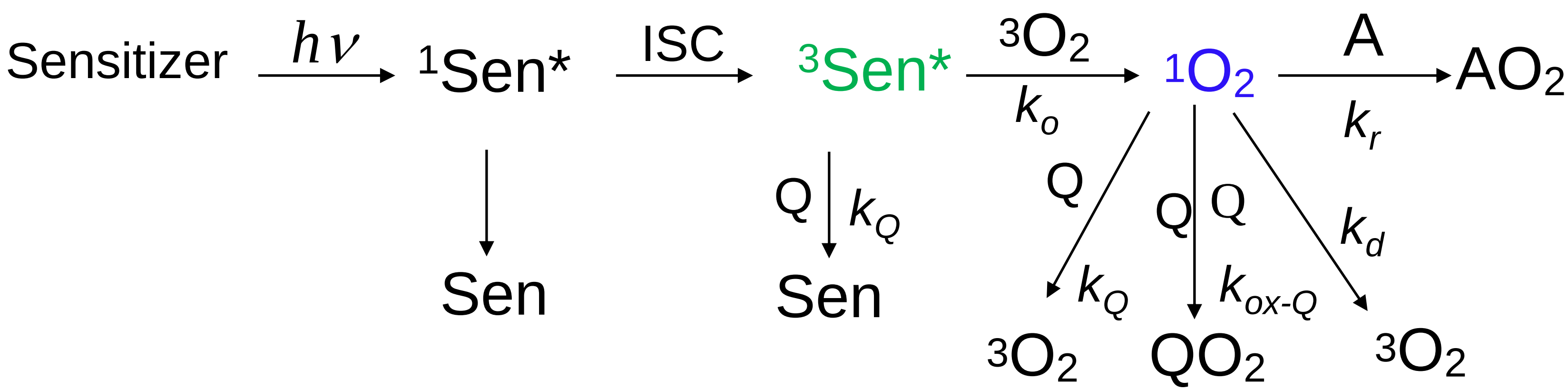
Singlet Oxygen Formation by Photosensitizer



Chlorophyll

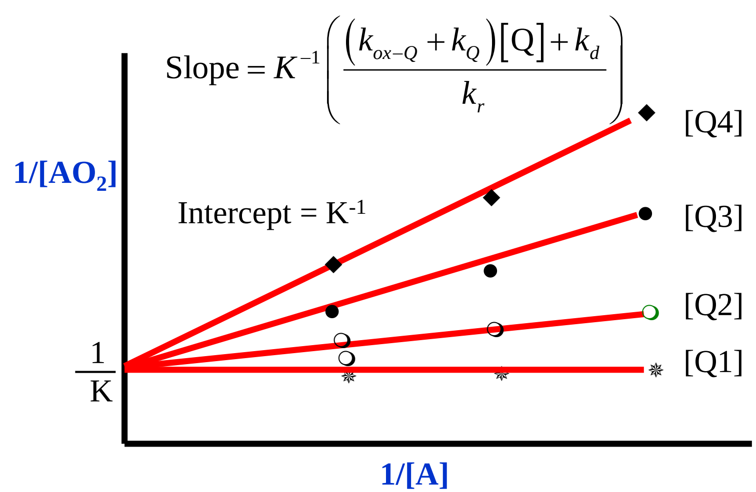


Chlorophyll Photosensitized Singlet Oxygen Soybean Oil Oxidation

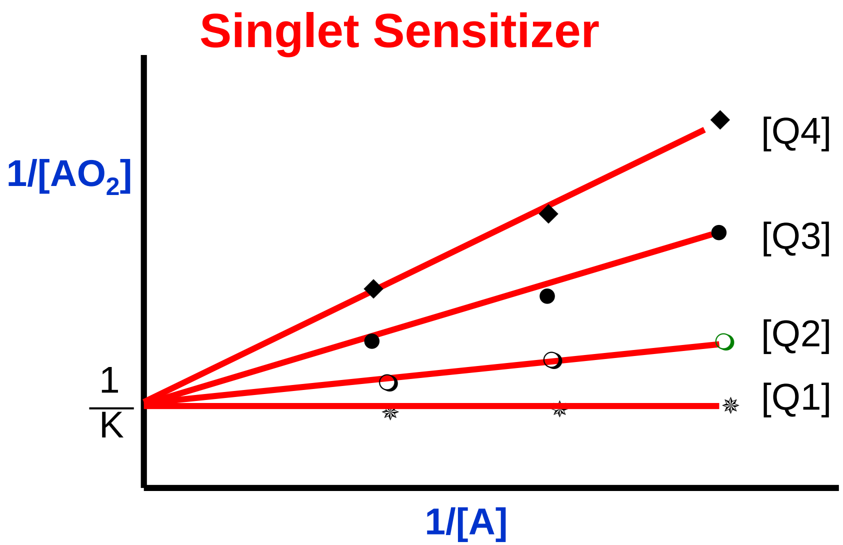


$$\frac{d[\mathrm{AO_2}]}{dt} = K\left(\frac{k_o[{}^3O_2]}{k_o[{}^3O_2]+k_Q[Q]}\right)\left(\frac{k_r[A]}{k_r[A]+\left(k_{ox-Q}+k_Q\right)[Q]+k_d}\right)$$

Plot of $[\mathrm{AO_2}]^{-1}$ vs. $[A]^{-1}$ at various [Q]

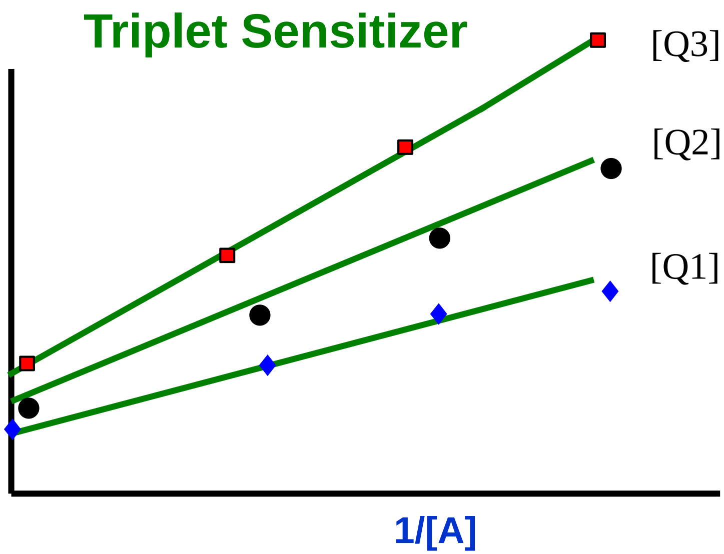


Sensitizer Quenching Plot



$$\text{Slope} = K^{-1}\left(\frac{\left(k_{ox-Q} + k_Q\right)[\mathrm{Q}] + k_d}{k_r}\right)$$

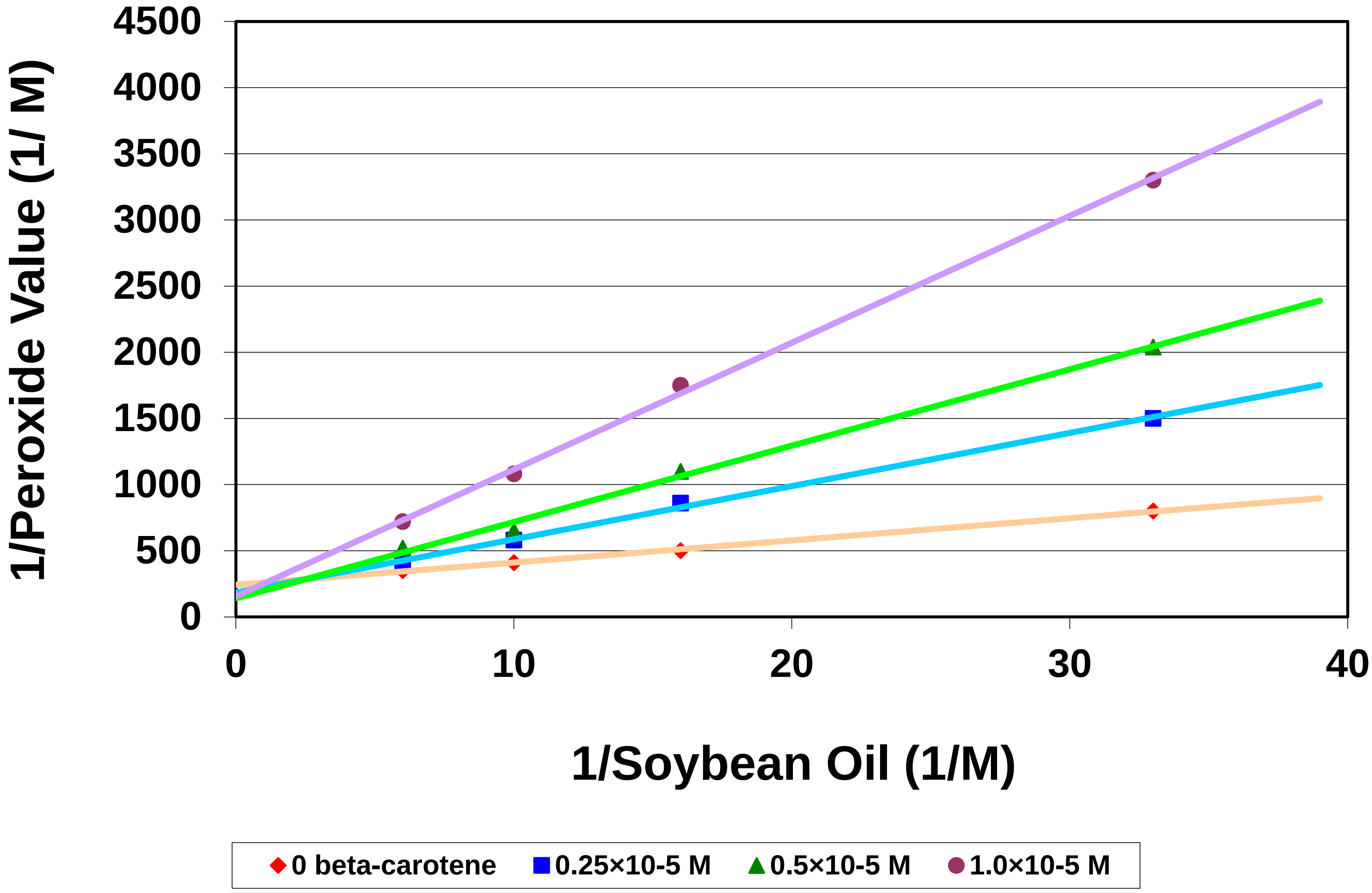
$$\text{Intercept} = K^{-1}$$



$$\text{Slope} = K^{-1}\{k_d\,(k_o\,[^3O_2] + k_Q[Q]) / k_o[^3O_2]k_r\}$$

$$\text{Intercept} = K^{-1}\{(k_o\,[^3O_2] + k_Q[Q]) / k_o[^3O_2]\}$$

Quenching Mechanism of β-Carotene



Singlet Oxygen Quenching Rate ($k_q + k_{ox\text{-}Q}$) of Carotenoids

Carotenoids	Number of Conjugated Double Bonds	Quenching Rate ($M^{-1}\cdot sec^{-1}$)
β-apo-8'-Carotenal	10	2.86×10^9
β-Carotene	11	4.60×10^9
Canthaxanthin	13	1.12×10^{10}

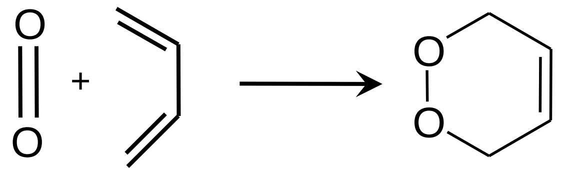
$$^1O_2 + {}^1\beta\text{-Carotene} \xrightarrow{\text{Energy Transfer}} {}^3O_2 + {}^3\beta\text{-Carotene}$$

$$^3\beta\text{-Carotene} \xrightarrow{\text{Radiationless Transfer}} {}^1\beta\text{-Carotene}$$

Typical Singlet Oxygen Quenchers

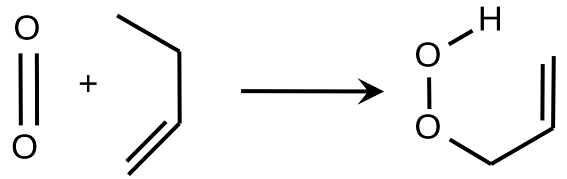
Quencher	Quenching Rate ($M^{-1}sec^{-1}$)
β-Carotene	4.60×10^9
Ascorbic acid	1.08×10^8
α-Tocopherol	2.70×10^7

1,4-Cycloaddition:



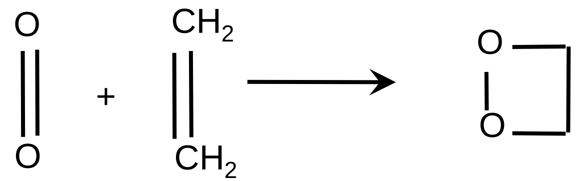
Endoperoxide

ENE Reaction :



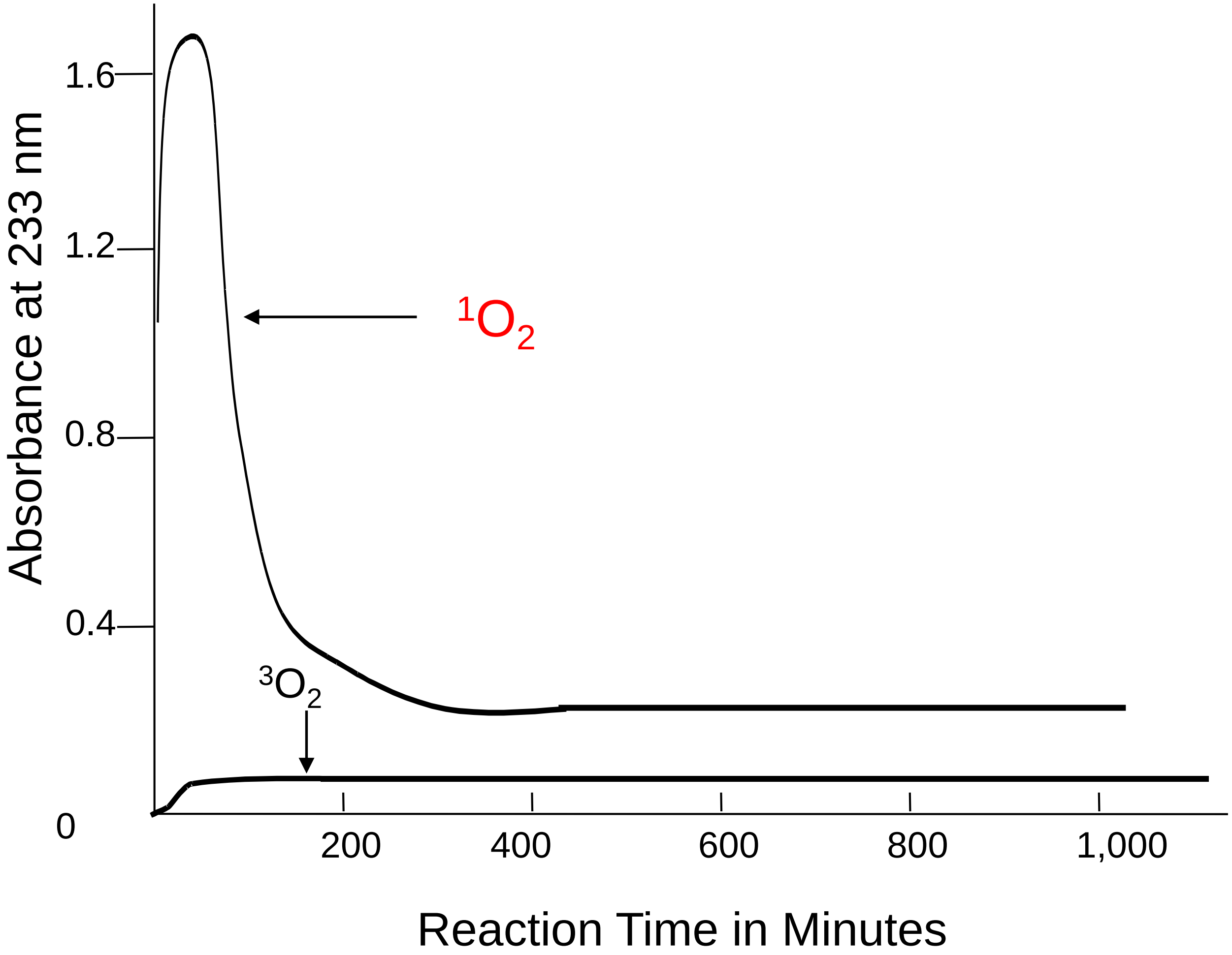
Allyl Hydroperoxide

1,2-Cycloaddition:

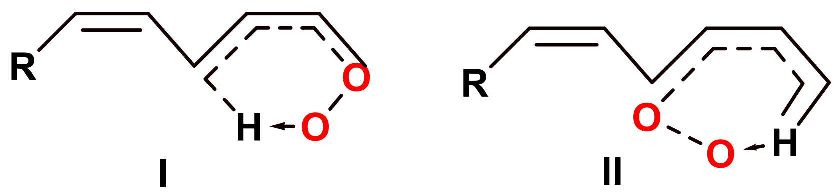


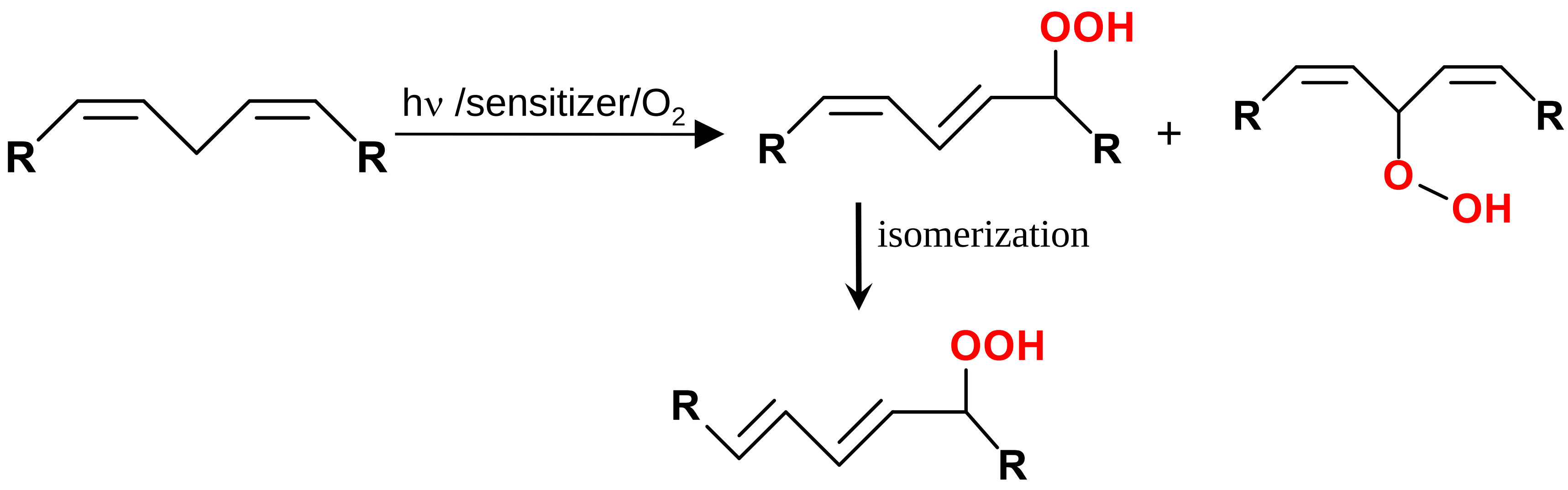
Dioxetane

$^1O_2$ and $^3O_2$ with Linoleic Acid



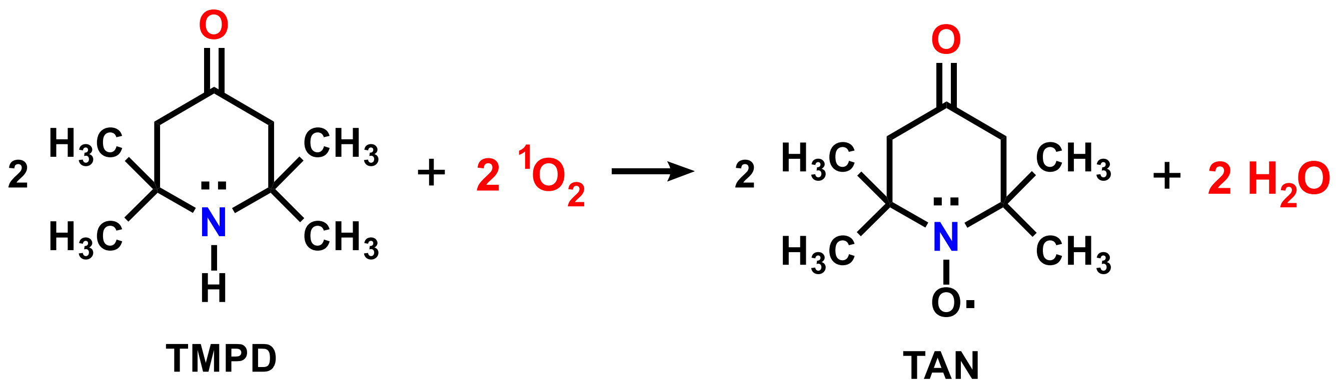
Reactions of Singlet Oxygen with Double Bonds





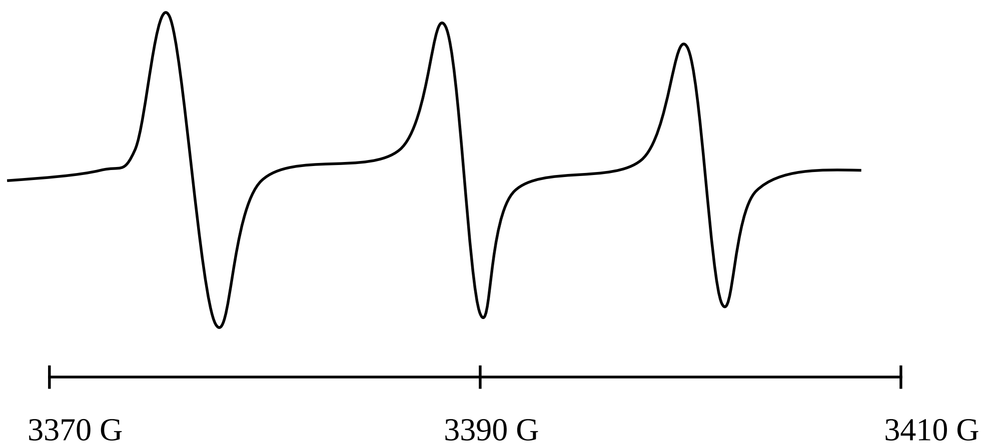
Conjugated and Nonconjugated Hydroperoxides via the 6-Centered Transition State

Singlet Oxygen Trapping



2,2,6,6−Tetramethyl−4−Piperidone−N−Oxyl

EPR Detection



SUPEROXIDE RADICAL ANION

Afanas'ev, I.B. 1985. Superoxide Ion: Chemistry and Biological Implications Volume 1. CRC Press, Boca Raton.

Beyer, W., Imlay, J., Fridovich, I. 1991. Superoxide Dismutases. Prog. Nucl. Acid Res. 40:221–253.

Bowler, C. and Van Montague, M. and Inzé, D. 1992. Superoxide dismutase and stress tolerance. Ann Rev. Plant Physiol. Plant Mol. Biol. 43:83–116.

Bowler C., Van Camp W. , Van Montagu M. and Inze D. 1994. Superoxide dismutase in plants. Critical Rev. Plant Sci. 13: 199–218

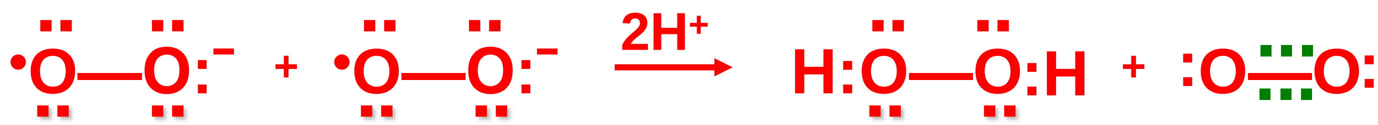
Davies, K.J.A. 1987. Protein damage and degradation by oxygen radicals. I General aspects. J. Biol. Chem. 162:9895–9901.

Superoxide Anion Radical

Two reactions are important for this radical:

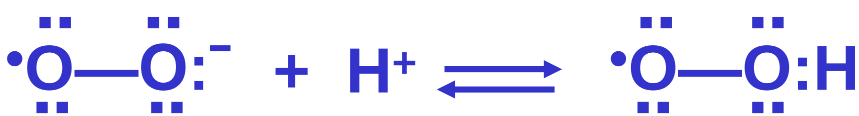
- Disproportionation / dismutation: reaction of superoxide anion with itself:

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



- Protonation of superoxide anion: formation of perhydroxyl radical

$$O_2^{\bullet -} + H^+ \rightleftarrows HO_2^{\bullet} \qquad pK_a = 4.8$$



$$HO_2^{\bullet} + HO_2^{\bullet} \rightarrow H_2O_2 + O_2 \qquad k = 8.3\times10^5\ M^{-1}\cdot s^{-1}$$

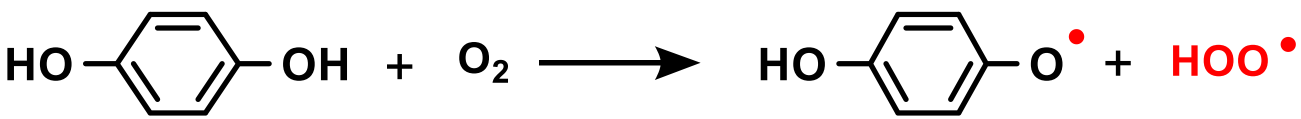
$$HO_2^{\bullet} + O_2^{\bullet -} \xrightarrow{H^+} H_2O_2 + O_2 \qquad k = 9.7\times10^7\ M^{-1}\cdot s^{-1}$$

Superoxide Anion Radical as Oxidant

The rare cases in which $O_2^{\bullet -}$ is observed to oxidize substrates at high rates occur only when proton transfer is simultaneous with electron transfer, resulting in formation of HO_2^- rather than O_2^{2-}.

$$X \cdots O_2^{\bullet -} \cdots H\text{-}Y \rightarrow X^+ + HO_2^- + Y^-$$

An example of a fast oxidation by superoxide in which such proton-coupled electron transfer to superoxide is likely to be occurring is the rapid oxidation of hydroquinones by superoxide.·



k = 1.7´10^{7} M^{-1}s^{-1}

Superoxide Anion Radical Activation by Metal Ions

- Alternatively, a metal ion may be oxidized by superoxide in an oxidative addition reaction to give a metal peroxo complex, where the peroxide is stabilized by coordination to the metal ion rather than by protonation, followed by peroxide dissociation, resulting in overall oxidation of the metal ion.

- In this case, the electron transfer to form a metal-bound peroxide can precede the protonation step because the metal ion stabilizes the O_2^{2-} ligand as it is formed.

$$M^{n+} + O_2^{-} \rightarrow M^{(n+1)+}(OO^{2-}) \xrightarrow{H^+} M^{(n+1)+} + H_2O_2$$

Nucleophilic Reactivity of SRA

1. **Enhancement by superoxide of hydrolysis of phosphatidylinositol (PIP) to inositol 1,4,5-tris-phosphate (IP_3) in rat aortic smooth cells:** **PIP $\Rightarrow$ ($O_2^{\bullet-}$) $\Rightarrow$ IP_3**

L Wu and J de Camplain, Hypertension, 1999

2. **Induction of apoptosis in mesangial cells by superoxide-dependent inhibition of phosphorylation of serine-threonine kinase Akt (protein kinase B) and activation of pro-apoptotic protein BAD**

Glucose $\rightarrow$ $O_2^{\bullet-}$ $\rightarrow$ Inhibition of Akt $\rightarrow$
BAD activation $\rightarrow$ apoptosis in mesangial cells

PS Kang et al., Am J Physiol. 2003

3. **The enhancement of expression of phosphorylated Akt after cerebral ischemia in SOD1 transgenic mice and a decrease in BAD activation due to decrease in superoxide formation.**

N Noshita, et al., Stroke 2003

SRA is a “Supernucleophile”

Not being a “super-oxidant”, superoxide is “super-nucleophile” with high reactivity in heterolytic reactions:

HYDROLYSIS OF ESTERS

$$O_2^{\bullet-} + RCOOR' \rightarrow RC(O)OO^{\bullet} + R'O^{-}$$

$$RC(O)OO^{\bullet} + O_2^{\bullet-} \rightarrow RC(O)OO^{-} + O_2$$

DEPROTONATION

$$O_2^{\bullet-} + ROH \rightarrow RO^{-} + HOO^{\bullet}$$

$$HOO^{\bullet} + O_2^{\bullet-} \rightarrow HOO^{-} + O_2$$

Sources of Superoxide Anion

Enzymatic reactions

- NADH oxidase
- NADPH-P450 reductase
- xanthine oxidase

Cellular sources

- leukocytes and macrophages
- mitochondrial electron transfer
- microsomal monooxygenase

Environmental factors

- ultraviolet light
- X-rays
- toxic chemicals
- aromatic hydroxylamines
- aromatic nitro compounds
- insecticides
- chemotherapeutic agents

Superoxide Radical Anion

OVERPRODUCTION OF SUPEROXIDE IS ONE OF MAJOR FACTORS OF MITOCHONDRIAL AGING

Notwithstanding its famous name, superoxide is a no "super-oxidant," but it can be a precursor of other reactive species.

1. THE FENTON REACTION

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

$$Fe^{2+} + H_2O_2 \Rightarrow Fe^{3+} + HO^{\bullet} + HO^{-}$$

2. DESTRUCTION OF ACONITASE

$$O_2^{\bullet -} + [2Fe^{2+}2Fe^{3+}\text{-}4S] + 2\,H^{+} \Rightarrow H_2O_2 + [Fe^{2+}3Fe^{3+}\text{-}4S]$$

$$[Fe^{2+}3Fe^{3+}\text{-}4S] \Rightarrow [3Fe^{3+}\text{-}4S] + Fe^{2+}$$

3. THE FORMATION OF PEROXYNITRITE

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

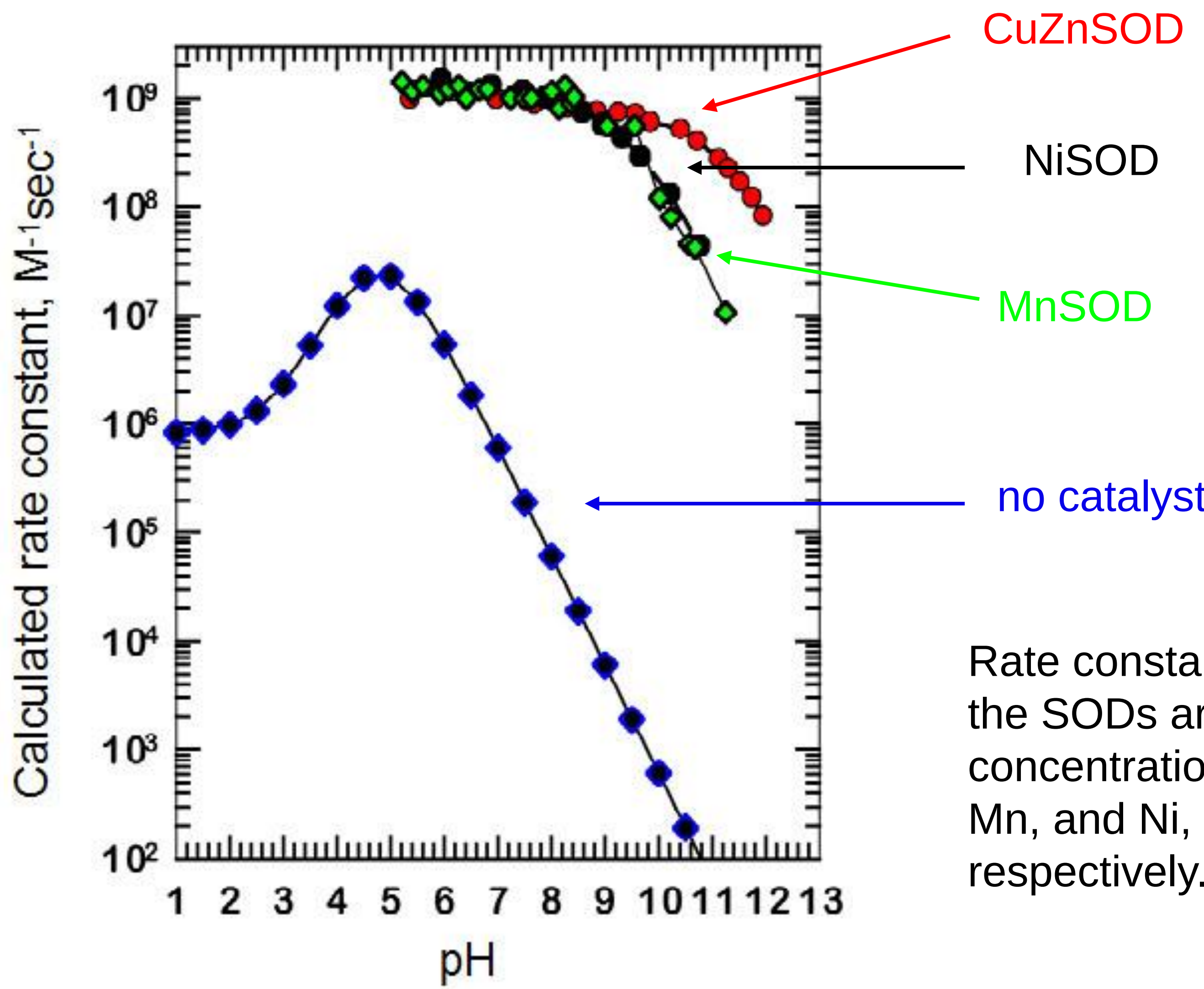
Superoxide Dismutase (SOD)

- The discovery of enzyme superoxide dismutase (SOD) by McCord and Fridovich in 1969 started a new era of research on the role of free radicals in biology and medicine.
- Now it has been found that SOD is ubiquitous in every aerobic organism from microbes to human. In animal cells, there are two kinds of SODs, a cellular SOD containing a CuZn active site and a mitochondria SOD containing a Mn active site. An extracellular CuZn-SOD (EC-SOD) is also found in mammalian extracellular fluids such as plasma, lymph, synovial fluid, cerebrospinal fluid and seminal plasma.
- Prokaryotic SODs are more diverse in active site composition consisting of CuZn, or Mn, or Fe, or Ni metal centers.

$$\mathbf{O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2}$$

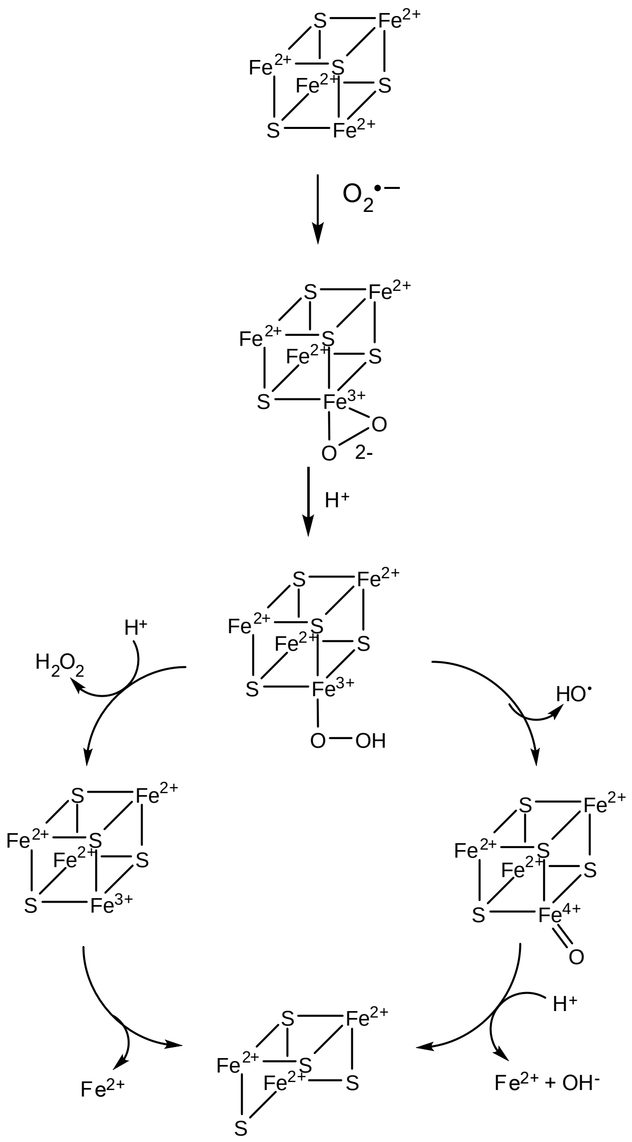
One SOD activity unit is defined as the amount of SOD that inhibits the rate of cytochrome C reduction by half at pH 7.8 and 25°C under specific conditions.

Rate Constants for Superoxide Dismutation



Rate constants for the SODs are per concentration Cu, Mn, and Ni, respectively.

Superoxide Reactivity Towards Fe_4S_4 Cluster



Hypothetical mechanism for reaction of $[Fe_4S_4]^{2+}$ cluster with superoxide. Individual charges have been assigned to iron atom in the figure for convenience in keeping track of redox changes, but it should be emphasized that electron–density in Fe–S clusters is known to be highly delocalized.

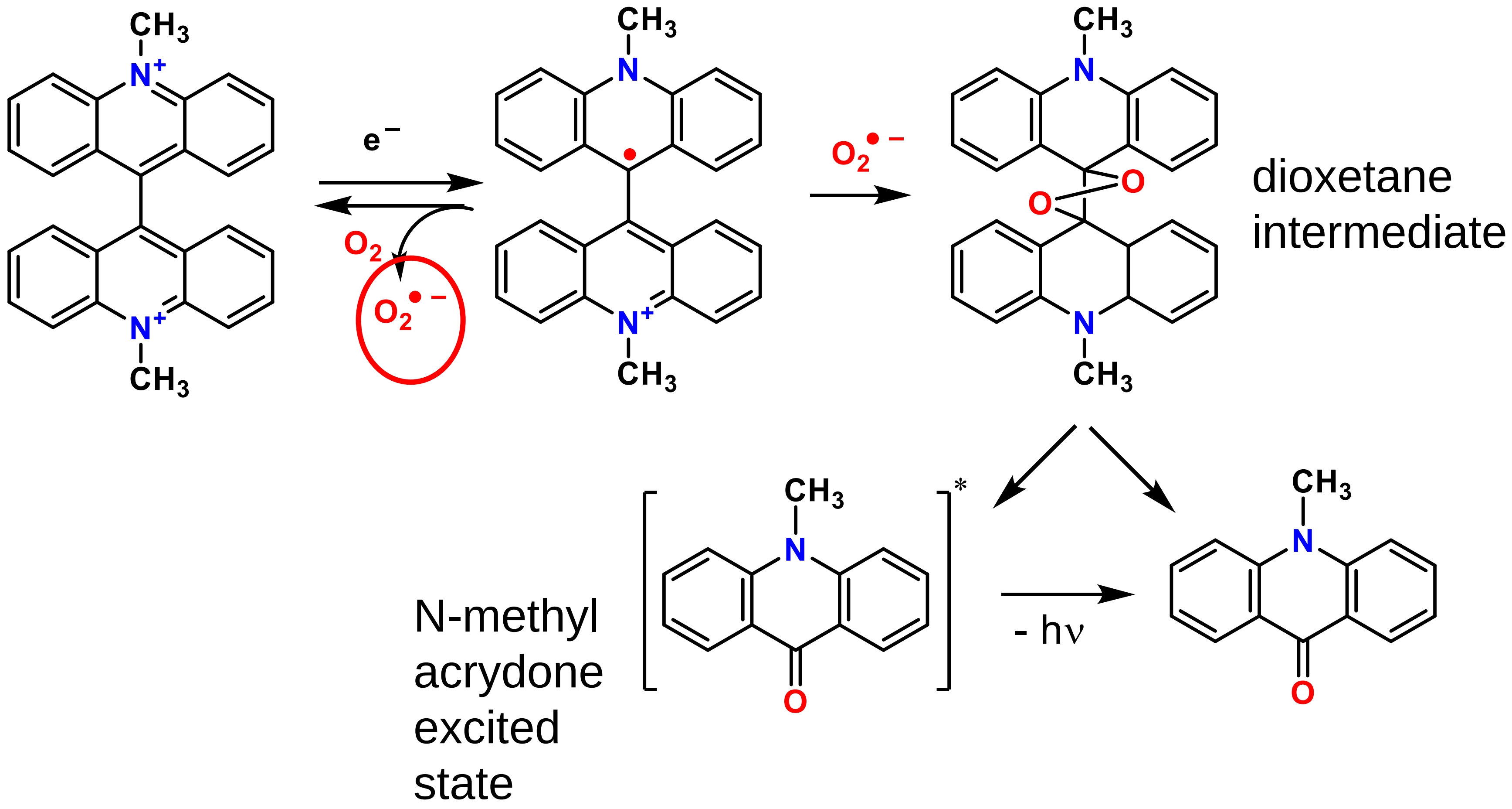
(a) Reaction of superoxide with the solvent–exposed iron center at one corner of the cube produces ferric peroxo intermediate, $[Fe_4S_4(O_2)]^+$, and (b) protonation of the ferric peroxo yield (c) a ferric hydroperoxide $[Fe_4S_4(OOH)]^{2+}$. Decomposition of the cluster might occur by one or two indicated pathways: (d) protonation and loss of hydrogen peroxide, forming an $[Fe_4S_4]^{3+}$ cluster which loses Fe^{2+} to give the $[Fe_3S_4]^+$ cluster, or (e) homolytic cleavage of the hydroperoxo ligand to give hydroxyl radical and a ferryl–containing cluster, $[Fe_4S_4(O)]^{2+}$, which could also give the $[Fe_3S_4]^+$ cluster upon protonation and loss of Fe^{3+} and hydroxide.

Problems with Direct $O_2^{\bullet -}$ Detection

1. $O_2^{\bullet -}$ has extremely short life-time (~ 1 ms).
2. It is present at very low steady-state concentration (~ 1 nM).
3. No EPR spectrum at room temperature.

Superoxide cannot be directly detected in biological samples.

Detection of $O_2^{\bullet -}$ by Lucigenin – a Chemiluminescent Probe

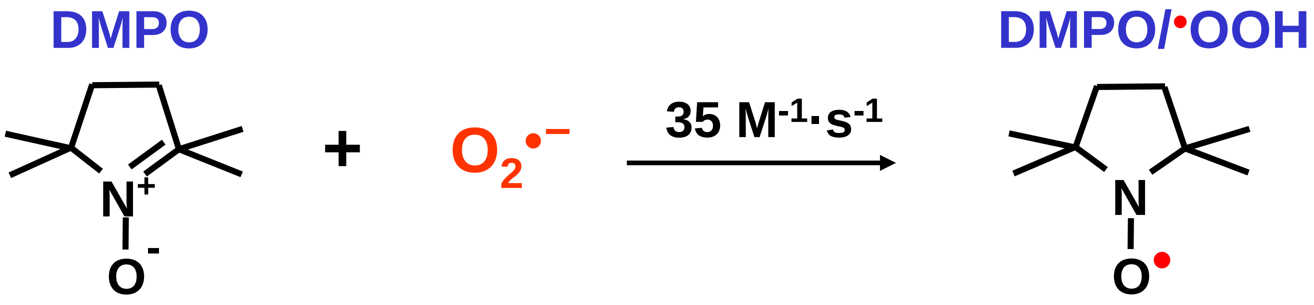


Detection of $O_2^{\bullet-}$ with EPR spectroscopy

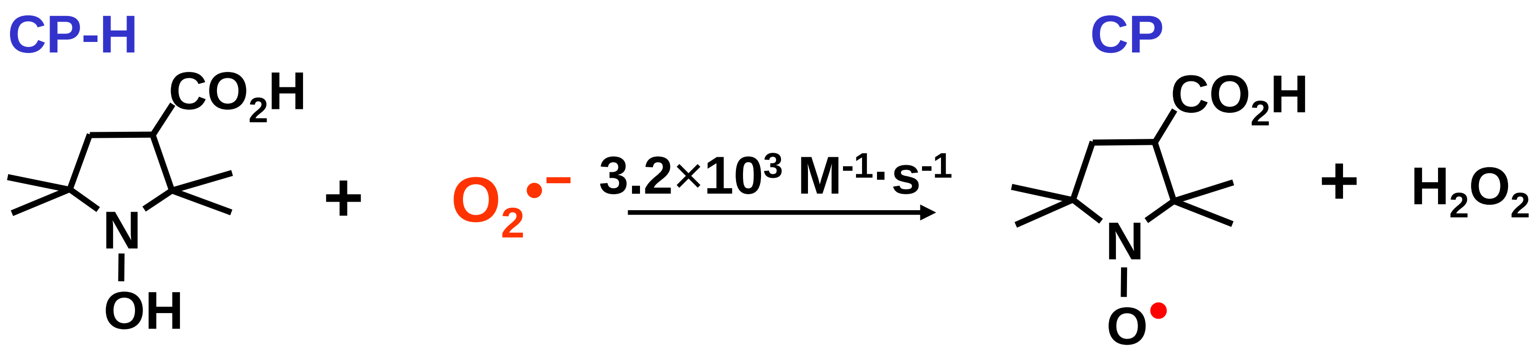
1. **Direct detection**

$$O_2 + 1\,e^- \rightarrow O_2^{\bullet-} \xrightarrow{SOD} H_2O_2$$

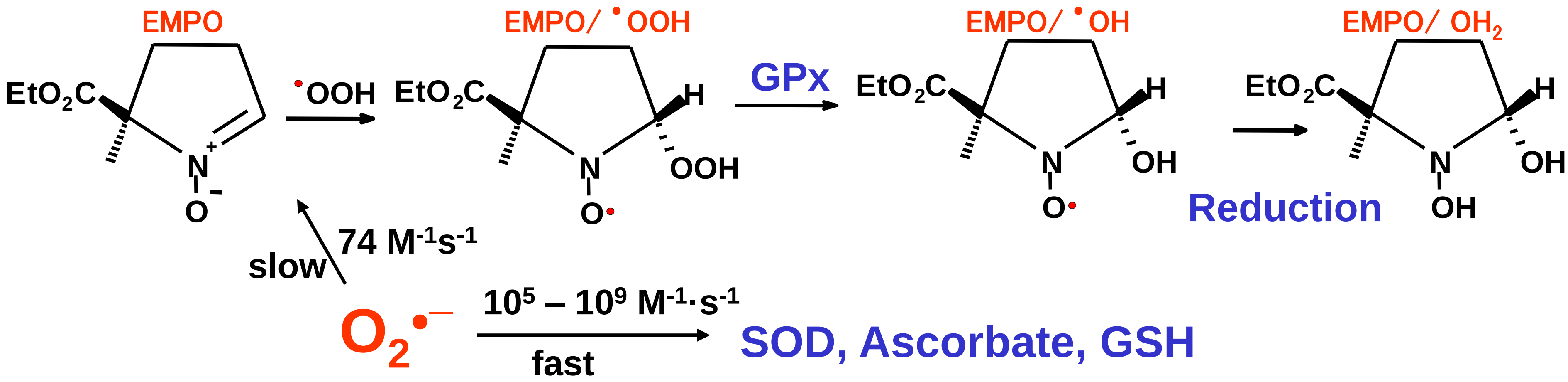
2. **Spin trapping (DMPO, EMPO, DEPMPO)**



3. **Spin probes (cyclic hydroxylamines)**



Problems with Spin Trapping of $O_2^{\bullet -}$



1. Slow kinetics of $O_2^{\bullet -}$ trapping and obstruction by antioxidants

EMPO + $^{\bullet}$OOH ⟶ EMPO/ $^{\bullet}$OOH (74 $M^{-1}\cdot s^{-1}$)

2. Decomposition to OH-radical adduct (GSH peroxidase)

EMPO/ $^{\bullet}$OOH ⟶ EMPO/ $^{\bullet}$OH

3. Reduction to EPR silent R_2NOH (ascorbate, metals, enzymes)

EMPO/ $^{\bullet}$OOH + Fe^{2+} ⟶ EMPO/OH_2 + Fe^{3+}

Spin trapping is limited by slow kinetics and biodegradation of the radical adducts.

POLITECNICO DI MILANO

Active Oxygen Species:
H_2O_2 and Peroxides

Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”

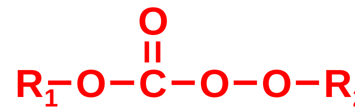
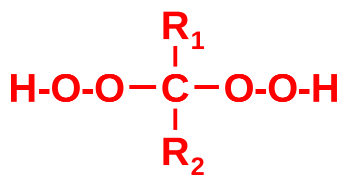
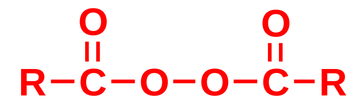
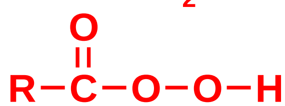
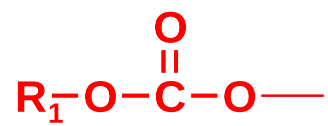
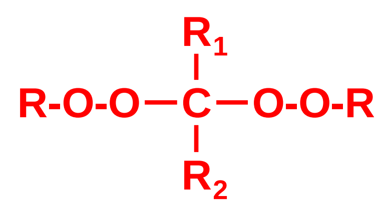
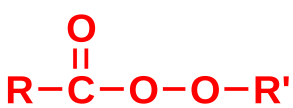
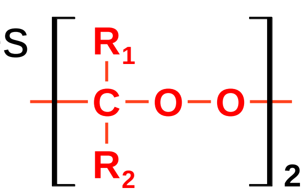
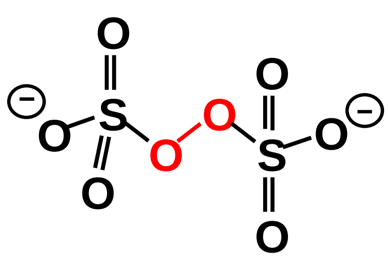
Hydrogen Peroxide Biochemistry

- **Hydrogen peroxide is not a free radical and *per se* is very little reactive at low concentration.**
- **Its reactivity in biological systems depends on two properties:**
  - **It can diffuse long distances crossing membranes**
  - **It reacts with transition metals by homolytic cleavage yielding the highly reactive hydroxyl radical**
- Hydrogen peroxide is the precursor of hydroxyl radicals and can be depleted into a hydroxyl ion and the high reactive hydroxyl radical ($^{\bullet}OH$), catalyzed by transition metals. The Fenton reaction describes the hydroxyl radical generation caused by iron ions

  1) $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$ 1') $Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2$
- **Hydroxyl radicals can be generated by UV-induced homolytic fission of the O-O bond in hydrogen peroxide**

  2) $H_2O_2 \rightarrow 2\ {}^{\bullet}OH$

Classes of Peroxides

Type of peroxide	*Structure*	*Type of peroxide*	*Structure*
Hydroperoxides	**R-O-O-H**	Peroxycarbonates	
Ketone peroxides		Diacylperoxides	
Peroxyacids		Peroxydicarbonates	
Dialkylperoxides	**R-O-O-R**	Peroxyketals	
Peroxyesters		Cyclic ketone peroxides	
Persulfates			

Abbreviations of Typical Peroxide

Code	*Chemical name**	*CAS nr.*
BPIC	Tert-butyl peroxy isopropylcarbonate (Trigonox BPIC)	2372-21-6
BPO	Dibenzoyl peroxide (Lucidol, Cadet)	94-36-0
BTMHP	Bis(3,5,5-trimethylhexanoyl) peroxide (Trigonox 36)	3851-87-4
CPDC	Dicetyl peroxydicarbonate (Perkadox 24)	26322-14-5
DCP	Dicumyl peroxide (Perkadox BC)	80-43-3
DTAP	Di-tert-amyl peroxide (Trigonox 201)	10508-09-5
DTBP	Di-tert-butyl peroxide (Trigonox B)	110-05-4
EHP	Bis(2-ethylhexyl) peroxydicarbonate (Trigonox EHP)	16111-62-9
LPO	Dilauroyl peroxide (Laurox)	105-74-8
MPDC	Dimyristyl peroxydicarbonate (Perkadox 26)	53220-22-7
TBCPDC	Bis(4-tert-butylcyclohexyl) peroxydicarbonate (Perkadox 16)	15520-11-3
TBHP	Tert-butyl hydroperoxide (Trigonox A)	75-91-2
TBPB	Tert-butyl peroxybenzoate (Trigonox C)	614-45-9
TBPEH	Tert-butyl peroxy-2-ethylhexanoate (Trigonox 21)	3006-82-4
TBPIB	Tert-butyl peroxyisobutanoate (Trigonox 41)	109-13-7
TBPP	Tert-butylperoxy pivalate (Trigonox 25)	927-07-1

Half-life Times of Various Organic Peroxides as Function of Temperature