”Redox cycling”

— *basic concepts of redox biology*

Elias Arnér, MD PhD
Division of Biochemistry
Medical Biochemistry and Biophysics
Karolinska Institutet
Stockholm, Sweden

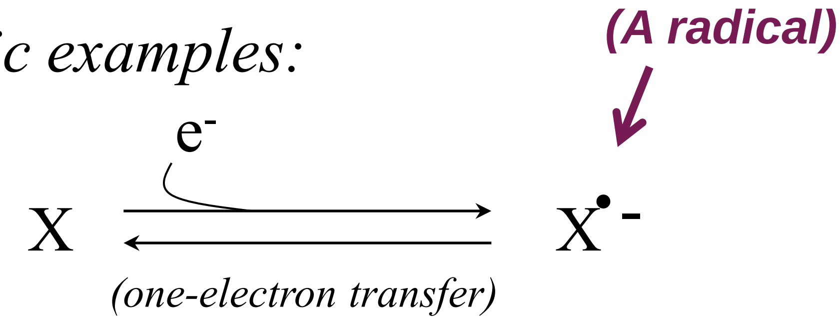
Elias.Arner@ki.se

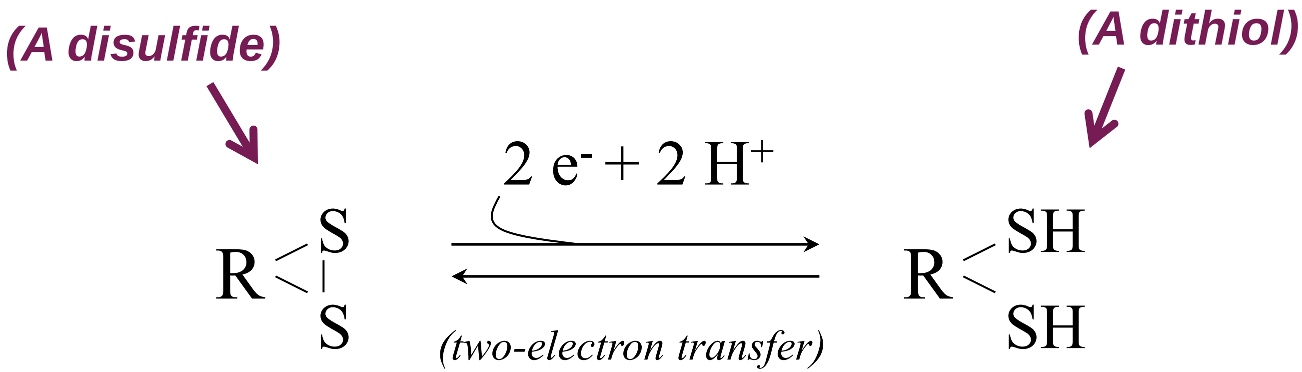
What is “redox”?

Redo**x**: “**Red**uction and **ox**idation”

→ usually involves reactions with transfer of one or more electrons between two compounds.

Two schematic examples:





Definition of redox reactions?

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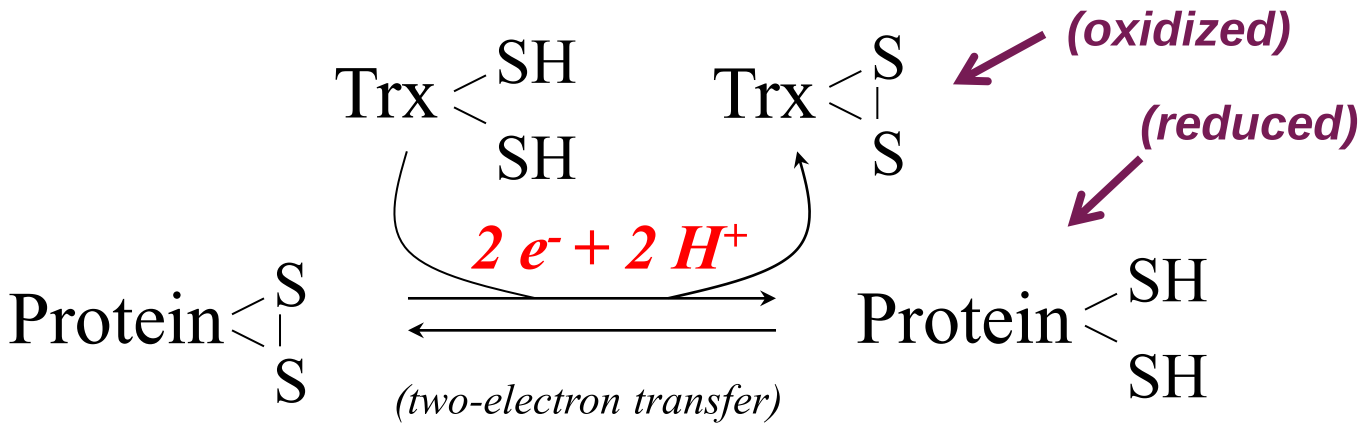
“Chemical reactions whereby the ***oxidation state*** of a molecule is either *increased (oxidation)* or *decreased (reduction)”*

→ almost always involve coupled transfers of electrons from one molecule to another, whereby the **loss of electrons gives oxidation** and the **uptake of electrons yields reduction**



The importance of the electrons

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- Note that electrons can never just “appear” and can thereby only be donated by electron donors, and they can never “disappear” and can thereby only be transferred to electron acceptors
- Compounds that easily donate electrons have a tendency to reduce other compounds and are therefore often called ***reductants***
- Compounds that easily take up electrons can often oxidize other compounds and can therefore be called ***oxidants***
- The oxidized and reduced forms of the same molecule are together called ***redox couple***
- Redox reactions are often propelled through chains of consecutive redox reactions, when several different redox active moieties are involved
- The chemical tendency to either donate or take up electrons by a molecule (or moiety) is reflected by its ***redox potential***
- The ***differences*** in redox potential between two molecules will determine the ***equilibrium*** between their oxidized and reduced forms

The redox potential

- The ***redox potential*** (or "reduction potential”, $\boldsymbol{E_h}$) is a measure (in volts) of the affinity of a molecule for electrons
- This value is usually compared to that for hydrogen, which is set arbitrarily at zero in order to give the ***standard redox potential*** ($\boldsymbol{E_0}$)
- Substances that are more strongly oxidizing than hydrogen have ***positive redox potentials*** (usually considered ***”oxidizing agents”***)
- Substances that are more reducing than hydrogen have ***negative redox potentials*** (***”reducing agents”***)
- The redox potential of a molecule becomes affected by ***concentration, pressure, pH and temperature***
- The redox potential can be defined or described using the ***Nernst equation***
- The probability of a redox reaction occurring can be indicated by the **Gibbs free energy** of that reaction

Redox reactions, rates and (non)equilibria

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Review Article

REDOX ENVIRONMENT OF THE CELL AS VIEWED THROUGH THE REDOX STATE OF THE GLUTATHIONE DISULFIDE/GLUTATHIONE COUPLE

Freya Q. Schafer and Garry R. Buettner

Free Radical Research Institute & ESR Facility, The University of Iowa, Iowa City, IA, USA

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Abstract—Redox state is a term used widely in the research field of free radicals and oxidative stress. Unfortunately, it is used as a general term referring to relative changes that are not well defined or quantitated. In this review we provide a definition for the redox environment of biological fluids, cell organelles, cells, or tissue. We illustrate how the reduction potential of various redox couples can be estimated with the Nernst equation and show how pH and the concentrations of the species comprising different redox couples influence the reduction potential. We discuss how the redox state of the glutathione disulfide-glutathione couple (GSSG/2GSH) can serve as an important indicator of redox environment. There are many redox couples in a cell that work together to maintain the redox environment; the GSSG/2GSH couple is the most abundant redox couple in a cell. Changes of the half-cell reduction potential (E_{hc}) of the GSSG/2GSH couple appear to correlate with the biological status of the cell: proliferation $E_{hc} \approx -240$ mV; differentiation $E_{hc} \approx -200$ mV; or apoptosis $E_{hc} \approx -170$ mV. These estimates can be used to more fully understand the redox biochemistry that results from oxidative stress. These are the first steps toward a new quantitative biology, which hopefully will provide a rationale and understanding of the cellular mechanisms associated with cell growth and development, signaling, and reductive or oxidative stress. — © 2001 Elsevier Science Inc.

Keywords—Glutathione, NADPH, Nernst equation, Reduction potentials, Free radicals

Nernst equation

Gas constant
(8.31 J K^{-1} mol^{-1})

Temperature (K)

Redox potential

$$E_{\text{red}} = E^{\ominus}_{\text{red}} - \frac{RT}{zF} \ln \frac{a_{\text{Red}}}{a_{\text{Ox}}}$$

Ratio of reduced vs. oxidized species at equilibrium

Standard redox potential

Number of electrons transfered in the reaction

Faraday constant
(9.65×10^4 C mol^{-1})

The Gibbs free energy is related to the redox potential: **ΔG = - *n*F □E**
(**□E** is the difference in redox potential between two reacting compounds)

Redox reactions, rates and (non)equilibria

Redox-pair 1	$Red_1 \rightarrow Ox_1 + e^-$
Redox-pair 2	$Ox_2 + e^- \rightarrow Red_2$
Redox-reaction	$Red_1 + Ox_2 \rightleftharpoons Ox_1 + Red_2$

$$aRed_1 + bOx_2 \leftrightharpoons cOx_1 + dRed_2$$

$$Q = \frac{[Ox_1]^c\,[Red_2]^d}{[Red_1]^a\,[Ox_2]^b}$$

$$\Delta E = \Delta E^\circ - \frac{RT}{nF}\ln Q$$ **Nernst Equation**

SCHAFER and BUETTNER (2001) FRBM, Vol. 30, pp. 1191–1212

The redox potential

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Molecule/Redox reaction	*Typical standard redox potential (E_0 in V)*
$NADP^+ + H^+ + 2\ e^- \rightarrow NADPH$	-0.320
$GSSG + 2\ H^+ + 2\ e^- \rightarrow 2\ GSH$	-0.240
$FAD + 2\ H^+ + 2\ e^- \rightarrow FADH_2$ (free)	-0.219
$FAD + 2\ H^+ + 2\ e^- \rightarrow FADH_2$ (bound to flavoproteins)	~0
$O_2 + 2\ H^+ + 2\ e^- \rightarrow H_2O_2$	+0.7
$O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O$	+1.23

more reducing ↑ ↓ *more oxidizing*

Note:

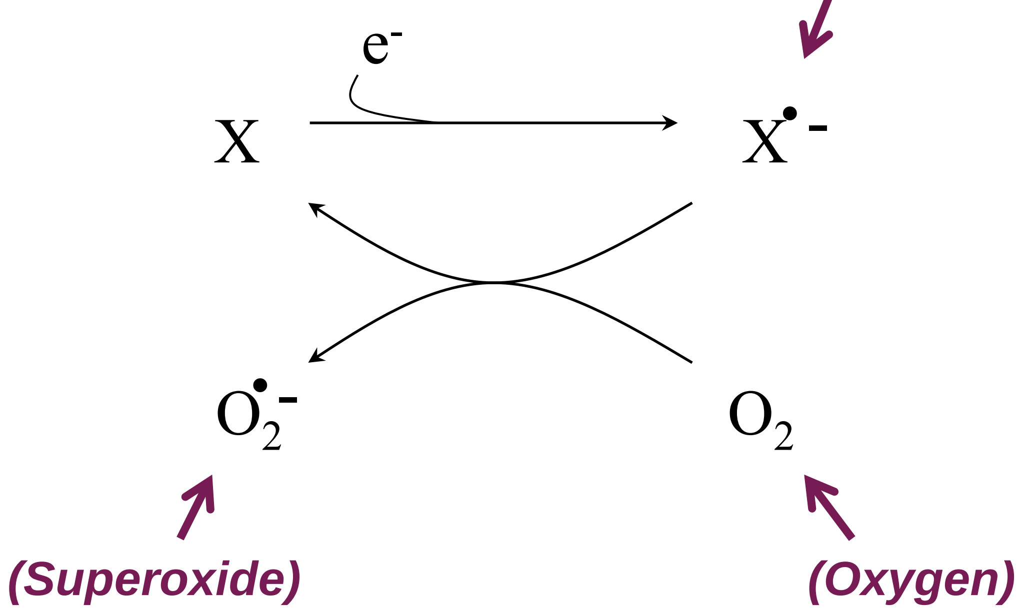
- In biological systems, redox couples are seldom (never?) in equilibrium!
- The concentration of reduced over oxidized forms are continuously affected by enzyme activities and fluctuating concentrations, due to metabolism
- *Example:*

[NADPH]:[NADP⁺] ≥ 100:1 while **[NADH]:[NAD⁺] ≈ 30:70**
(although NADPH and NADH have identical redox potentials)

What is “redox cycling”?

Redox cycling: Repetitively coupled reduction and oxidation reactions, often involving oxygen and reactive oxygen species

An example of one-electron transfer reactions with redox cycling:



What is "redox cycling"?

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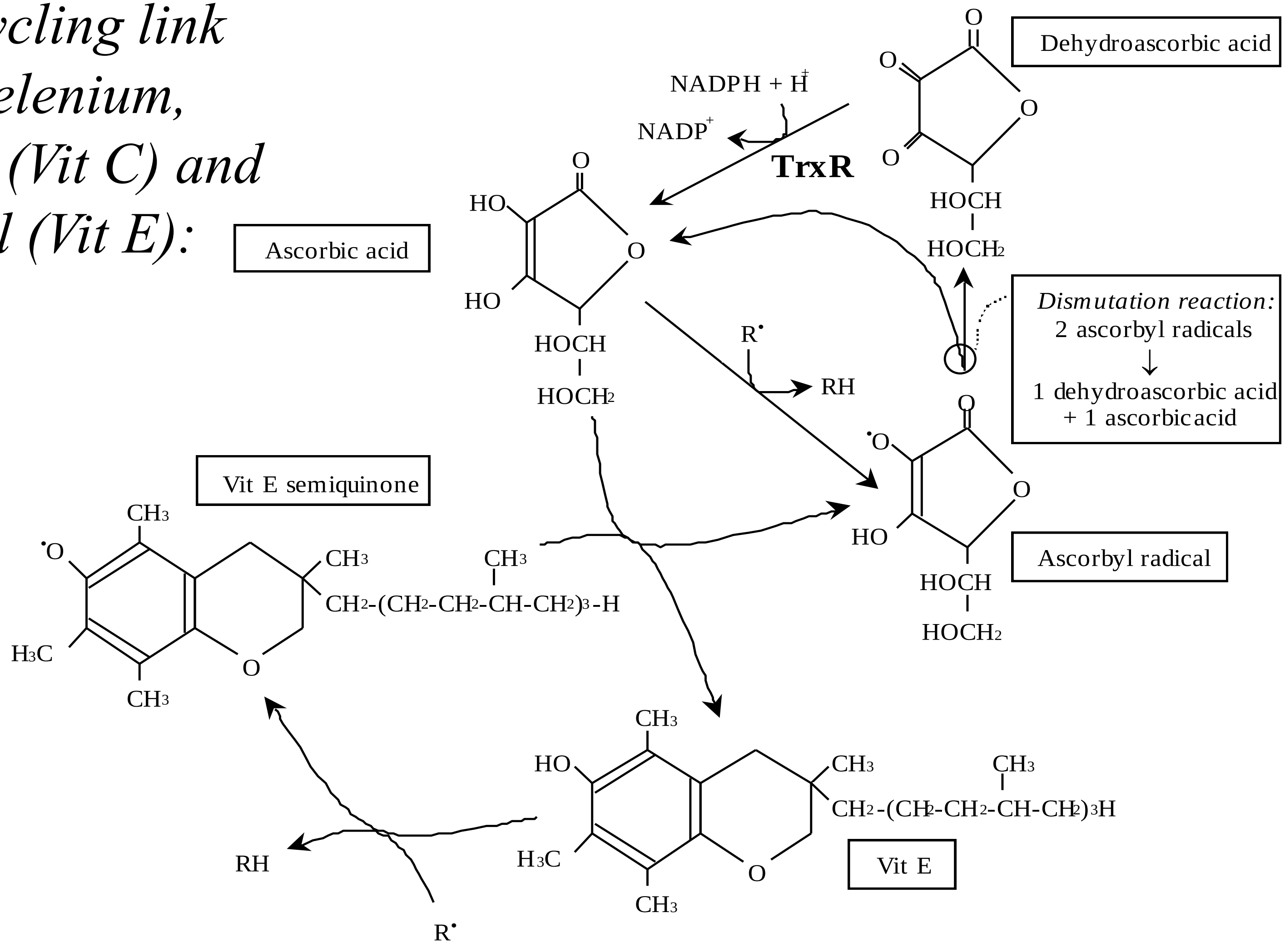
- ***All*** redox active compounds (i.e. compounds that can donate or accept electrons) could in theory participate in redox cycling
- The extent of redox cycling at a given moment in a given cellular compartment will depend upon the local conditions and the combination and state of the involved redox active molecules

Thioredoxin reductase, Vit C and Vit E

A redox cycling link between selenium, ascorbate (Vit C) and tocopherol (Vit E):



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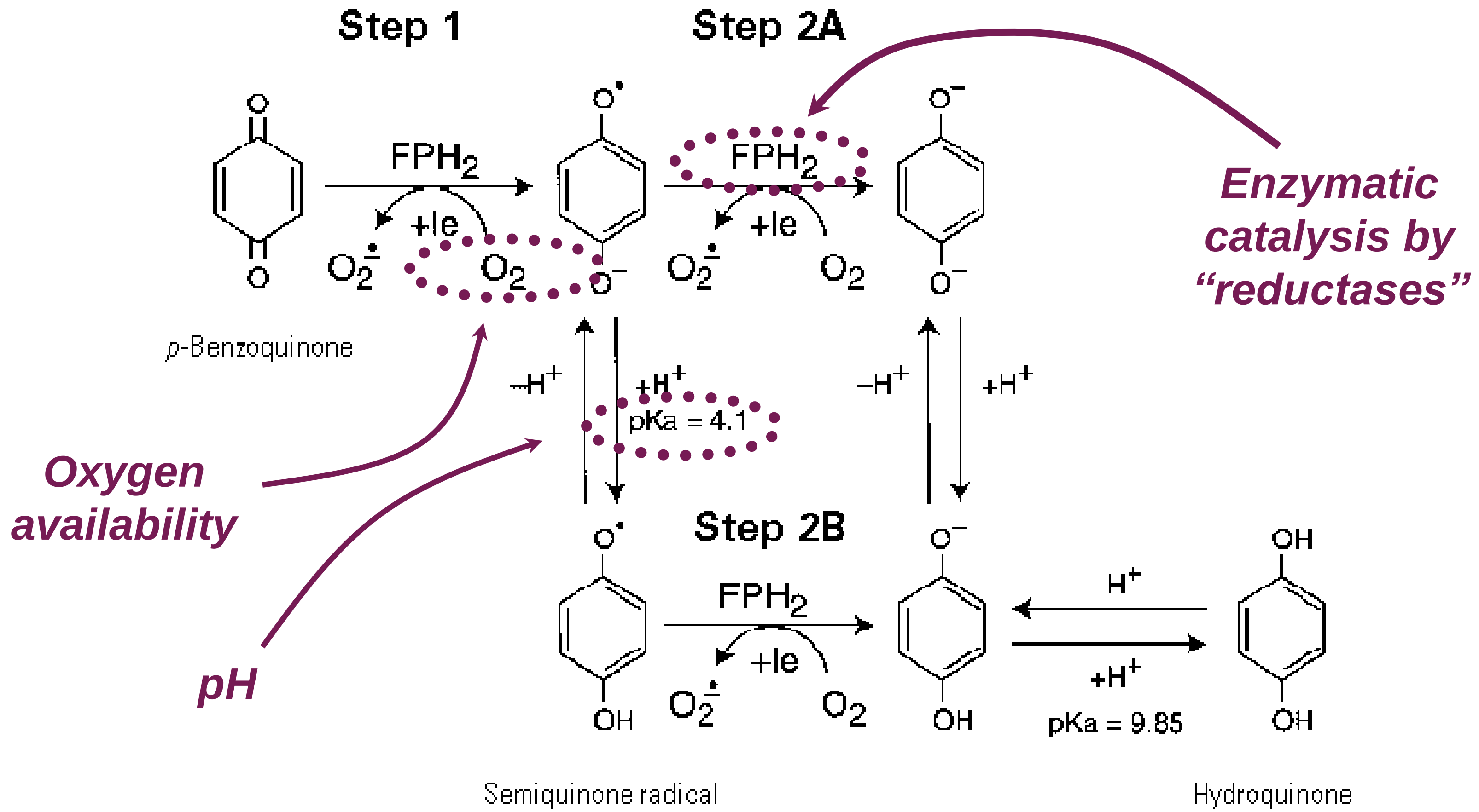
Redox cycling - examples with *p*-benzoquinone



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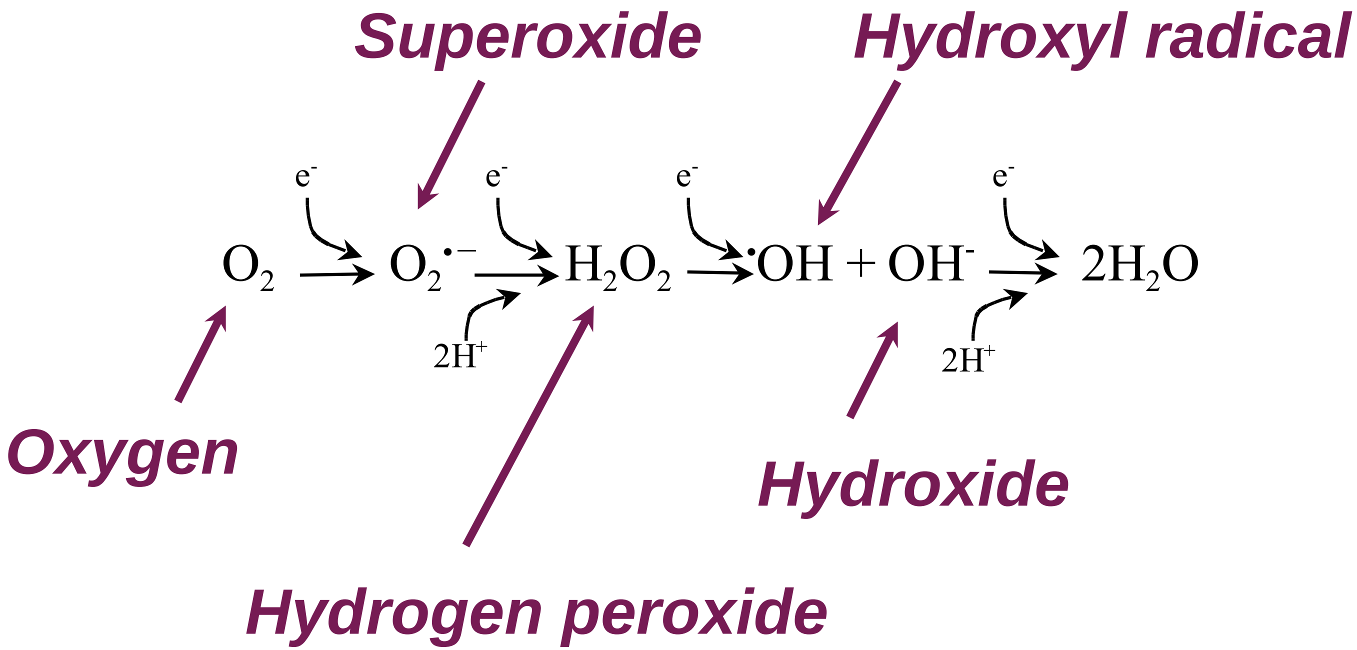
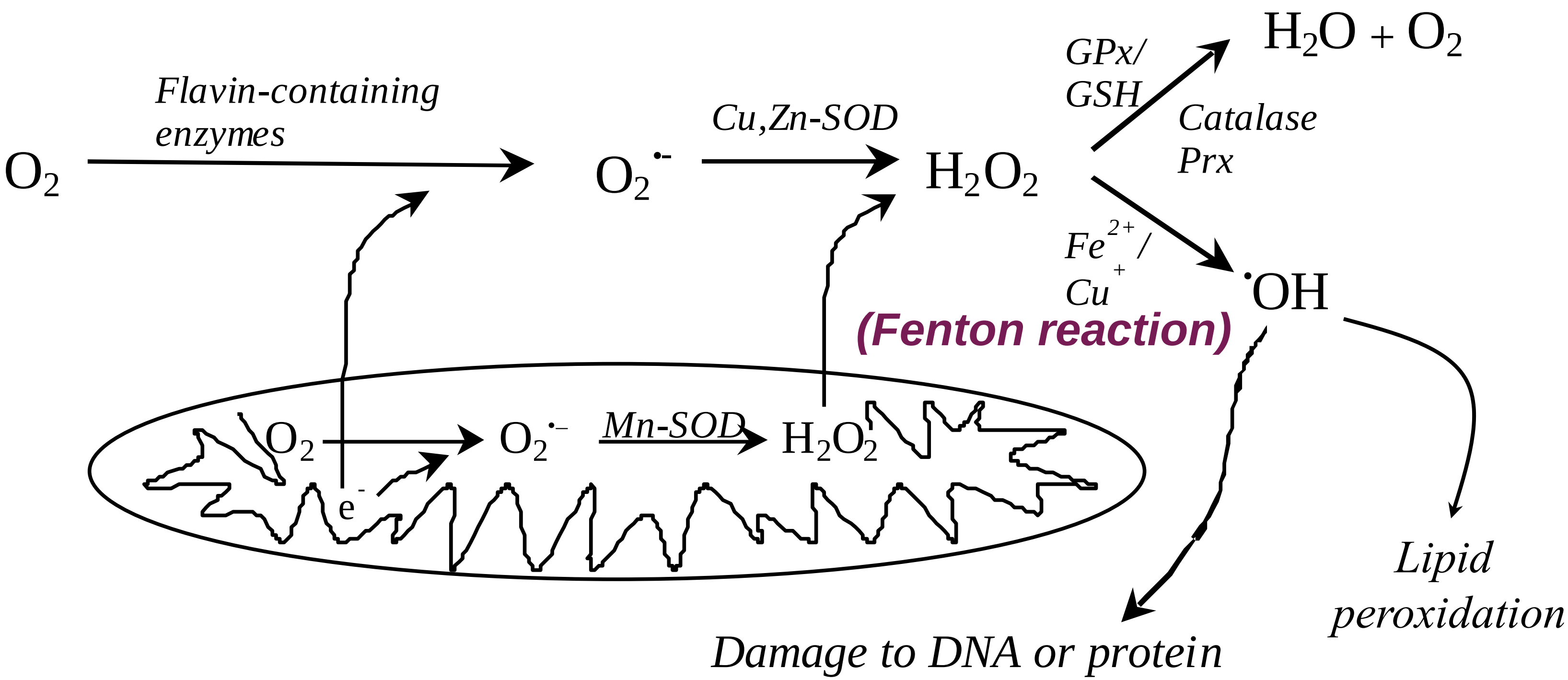
The importance of oxygen and of reactive oxygen species (ROS)

Reactive oxygen species (ROS)



Nordberg J. & Arnér E.S.J. (2001). *Free Rad. Biol. Med.*31,1287-1312.

Reactive oxygen species (ROS)
- a simple scheme of production

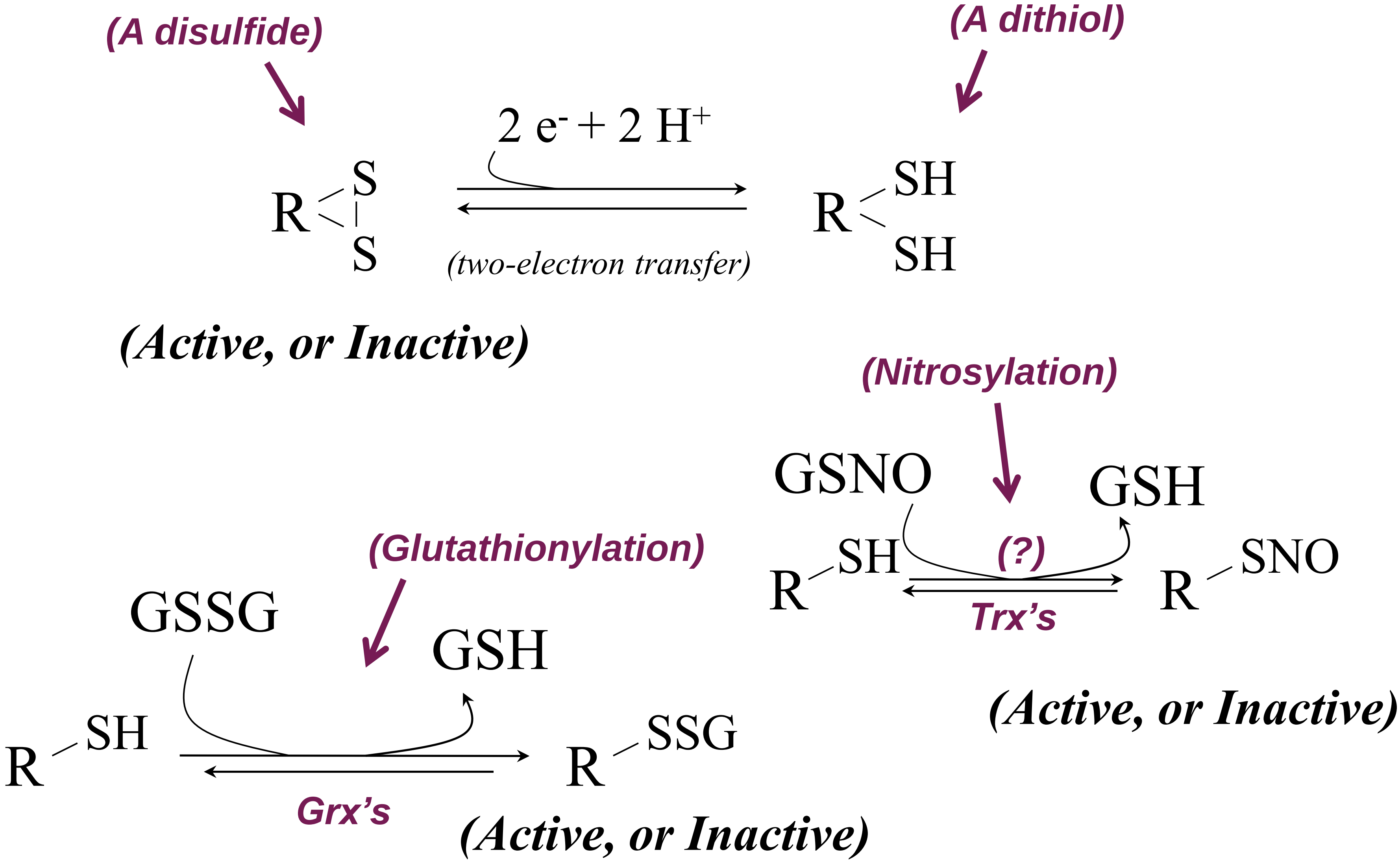


Nordberg J. & Arnér E.S.J. (2001). *Free Rad. Biol. Med.*31,1287-1312.

Redox regulation

— A process of regulated activation or inhibition through redox control

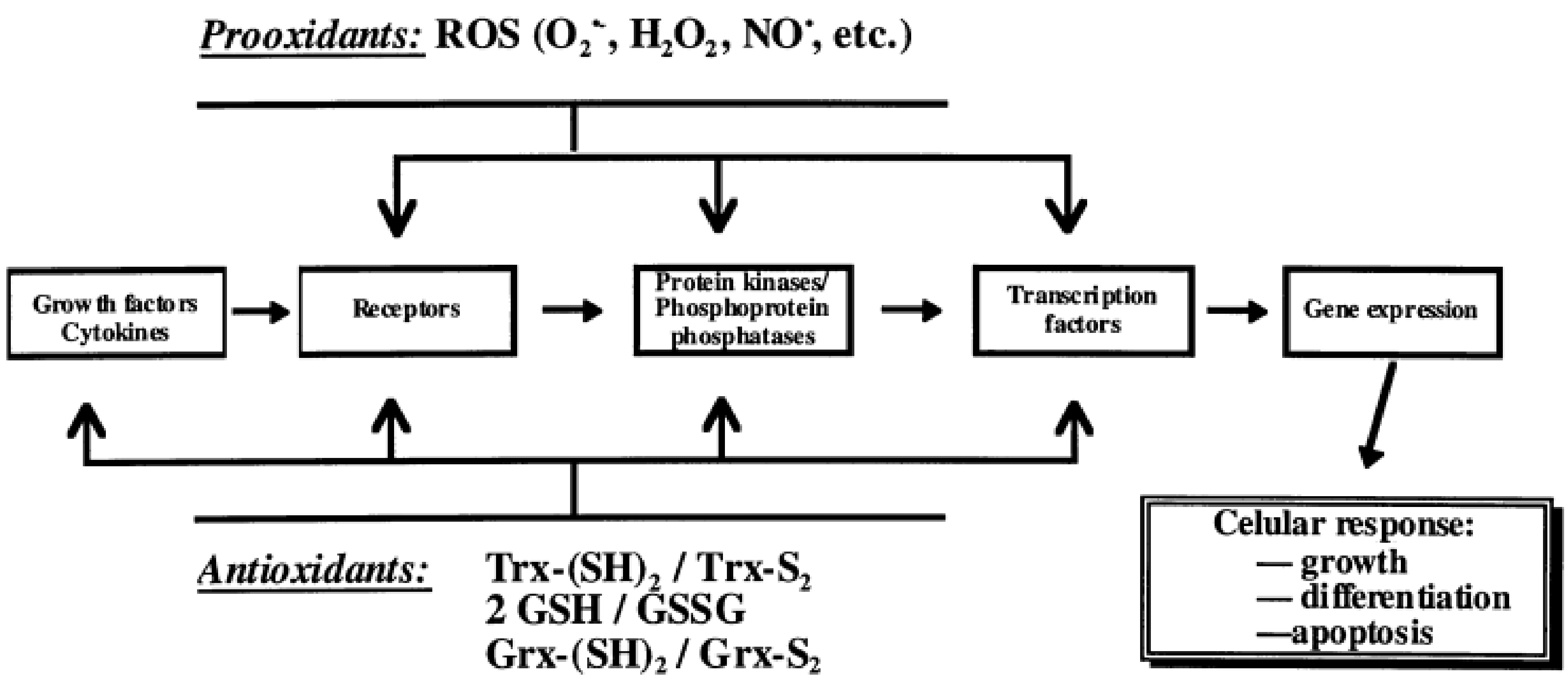
Redox regulation
- examples of reactions



Redox regulation
- a few examples of regulated proteins

- Oxidation of **Keap-1** releases and *activates* the transcription factor **Nrf2**, which induces transcription of a number of predominantly GSH-dependent antioxidant enzyme systems
- Several protein tyrosine phosphatases (**PTP's**) are transiently *inactivated* by oxidation (probably due to local NOX activity) as a step in protein phosphorylation cascades, thus *activating* protein phosphorylation cascades
- Many proteins have been identified to be reversibly **glutathionylated**, the significance of which is yet largely unknown
- **Nitrosylation** of caspase-3 may *inactivate* the caspase and prevent apoptosis (this nitrosylation can be removed by the thioredoxin system)

Many levels of redox regulation in cells
- there is much yet to discover



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Conclusions

- ✓ Redox reactions are reversible and most redox active compounds may have either pro- or antioxidant properties, acting as either “oxidant” or “reductant”, depending upon overall conditions

- ✓ The redox potential is *one* determinant for reactions occurring with a redox active compound, but enzyme metabolism yields non-equilibrium states of most, if not all, redox active compounds in biological systems

- ✓ Redox regulation is likely to be important for control of several cellular signaling pathways

- ✓ A thorough knowledge of the ***chemistry*** of redox reactions as well as the ***biological context*** will therefore be essential for the understanding of different processes in redox biology