„Oxidant stress" - Basic Concepts

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Founded 1829
University of London

Basic Concepts: recommended resource

http://www.sfrbm.org/sections/education/frs-presentations

Defining oxidant stress

"*Redox imbalance leading to over-abundance of oxidants*"

- is this too simplistic?

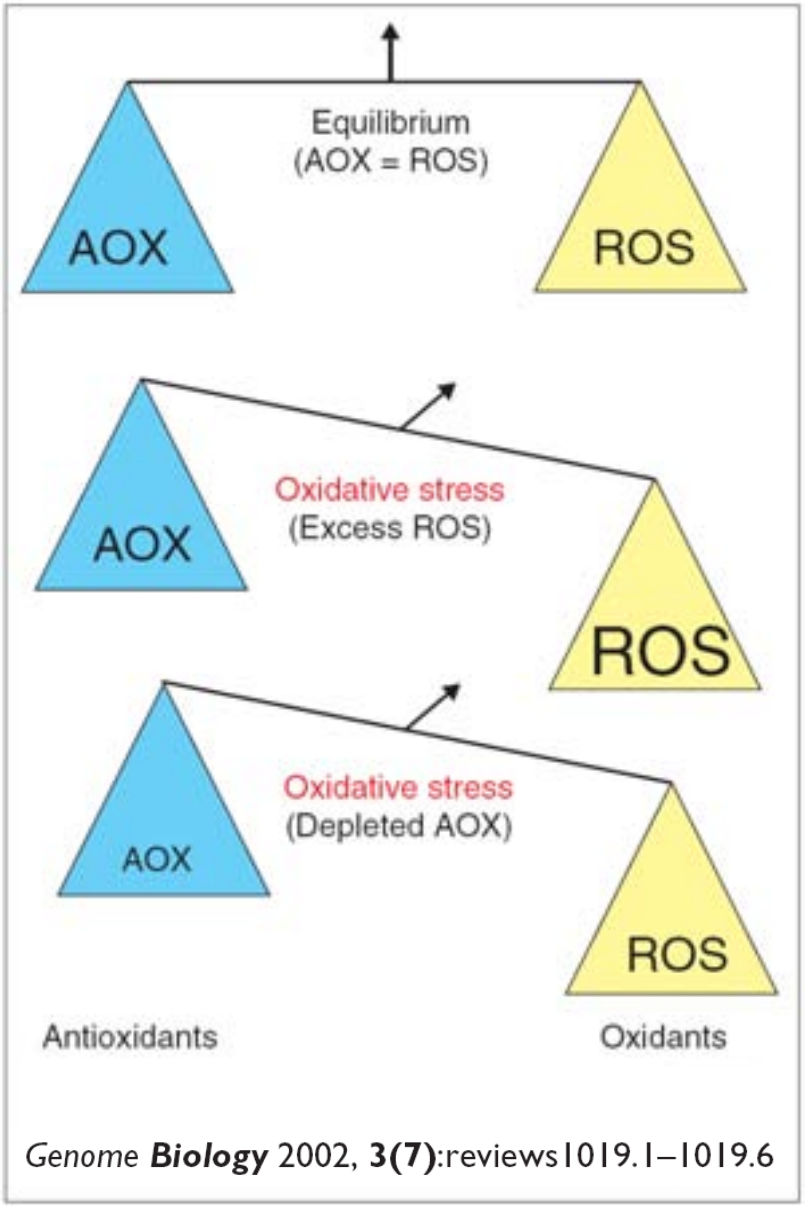
no universally agreed definition

issues with semantics

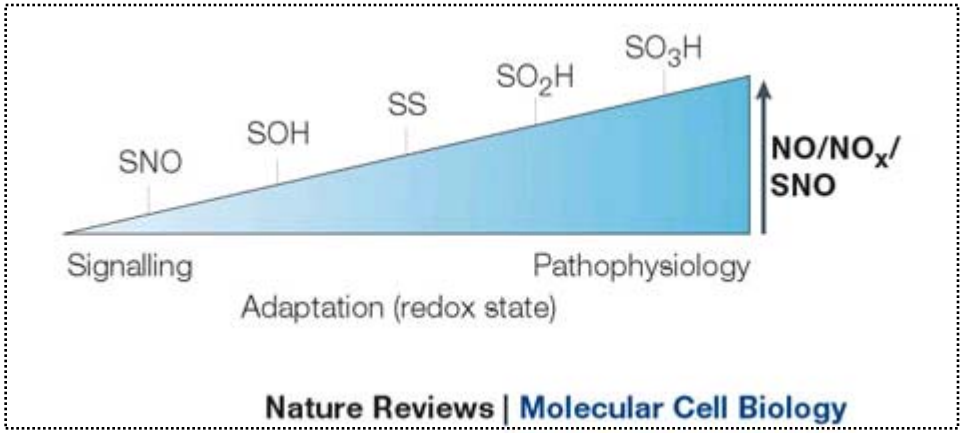
Is term "stress" always appropriate?


Oxidant stress
Damage
dysfunction and disease
Signalling
regulatory or adaptive response

Common illustrations of oxidant stress



Genome ***Biology*** 2002, **3(7)**:reviews1019.1–1019.6

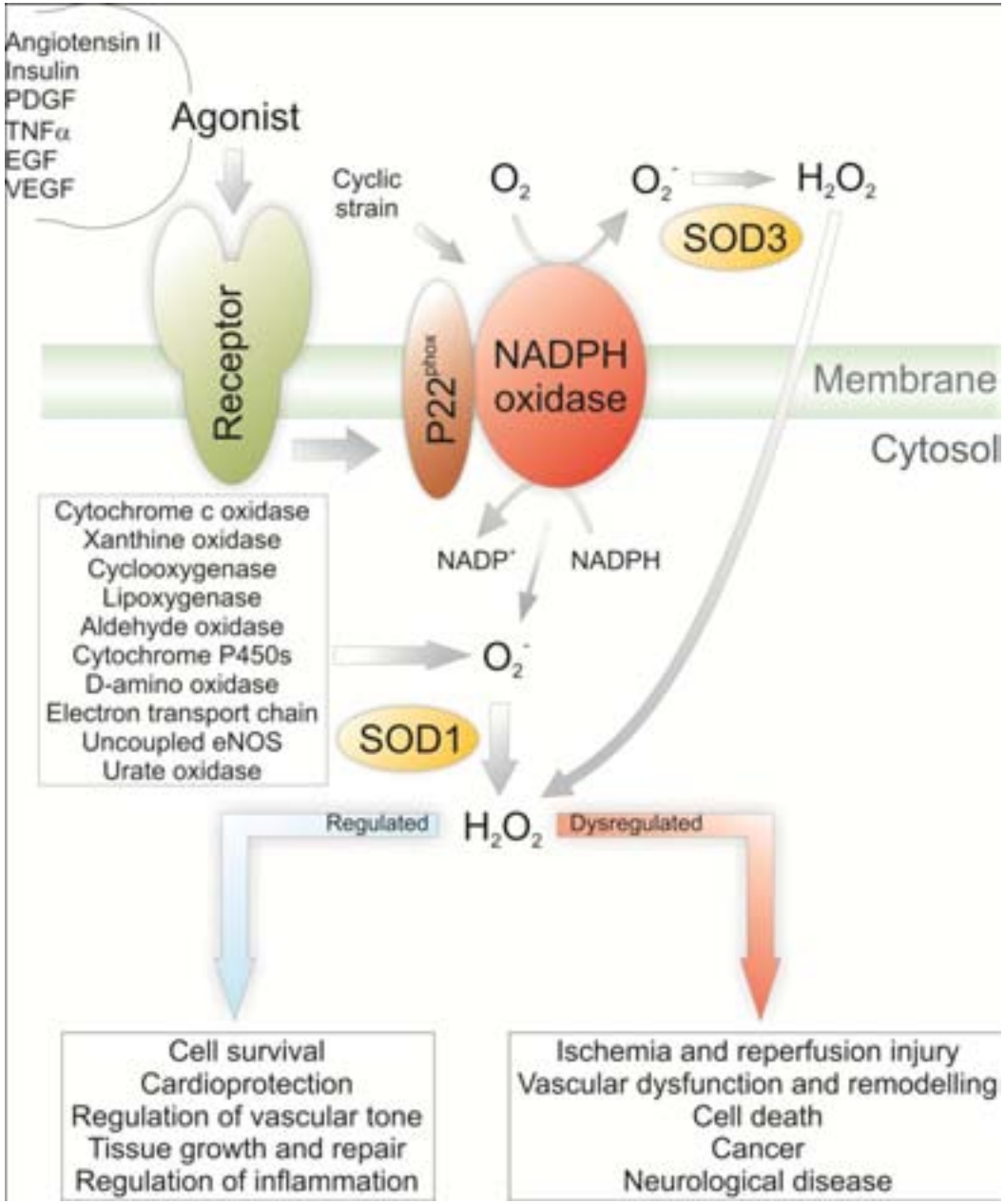


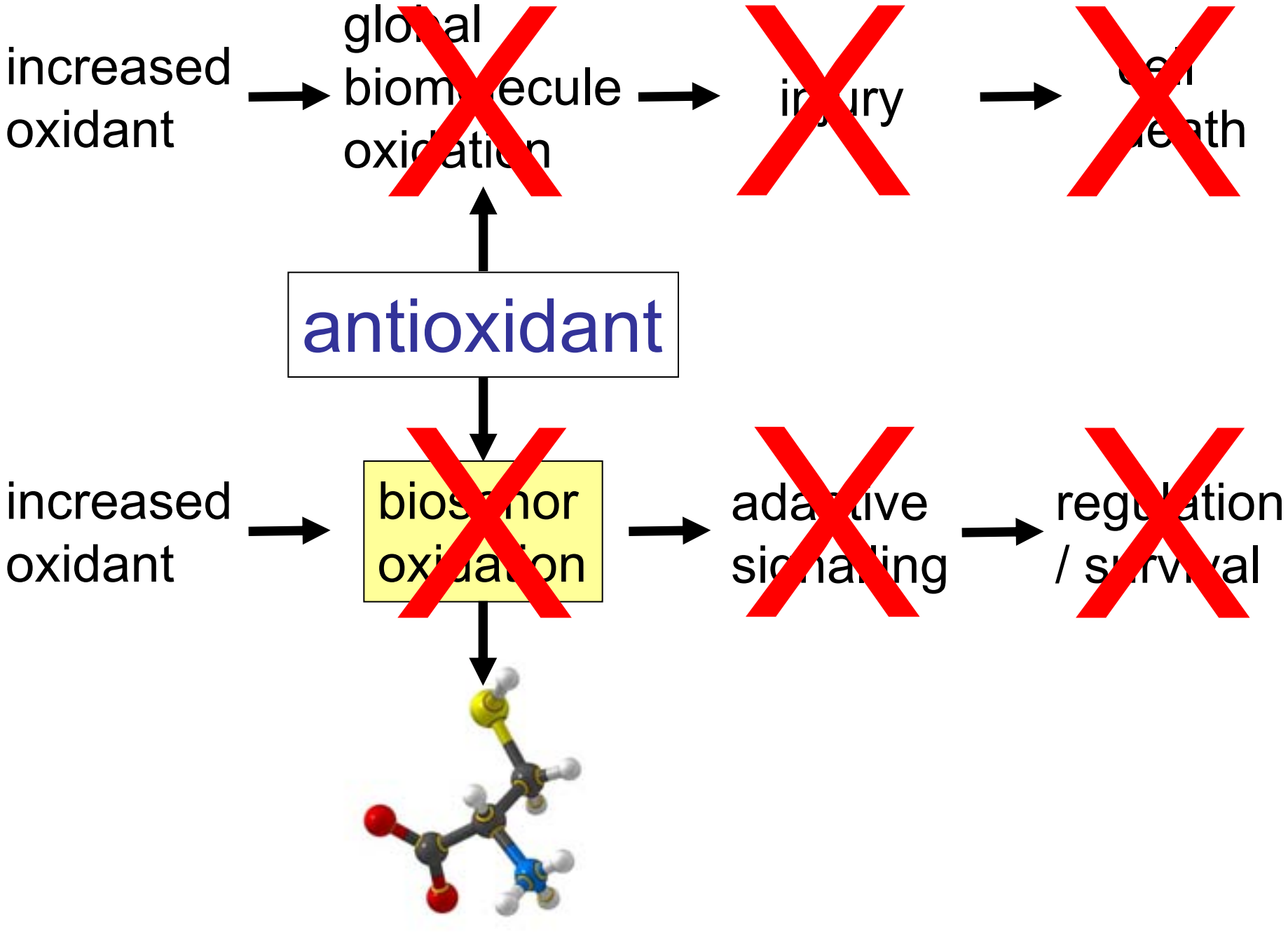
Easy but inadequate concept?

Not all redox components are in equilibrium

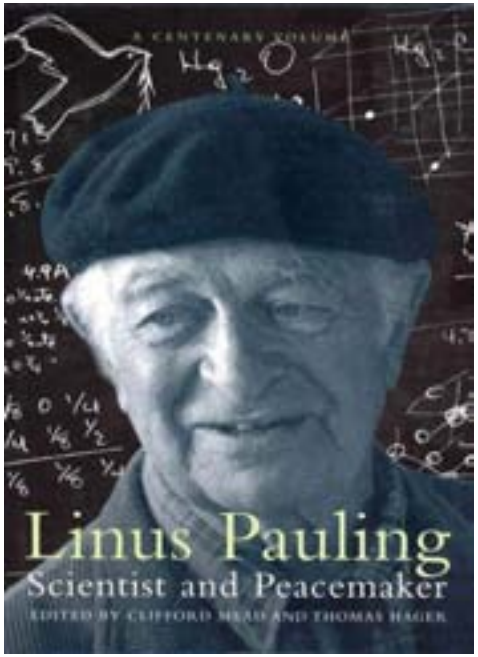
- chemically
- spatially
- Temporally

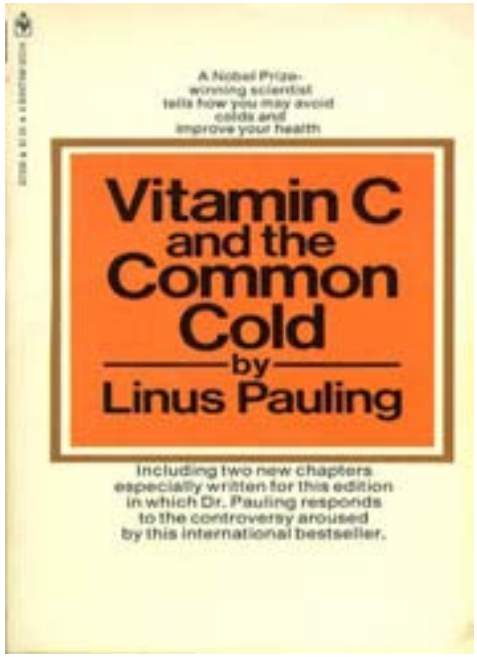
Require some consideration of chemistry


Angiotensin II
Insulin
PDGF
TNFα
EGF
VEGF
Agonist
Cyclic strain
O_2
O_2^-
H_2O_2
SOD3
Receptor
$P22^{phox}$
NADPH oxidase
Membrane
Cytosol
Cytochrome c oxidase
Xanthine oxidase
Cyclooxygenase
Lipoxygenase
Aldehyde oxidase
Cytochrome P450s
D-amino oxidase
Electron transport chain
Uncoupled eNOS
Urate oxidase
$NADP^+$
NADPH
O_2^-
SOD1
Regulated
H_2O_2
Dysregulated
Cell survival
Cardioprotection
Regulation of vascular tone
Tissue growth and repair
Regulation of inflammation
Ischemia and reperfusion injury
Vascular dysfunction and remodelling
Cell death
Cancer
Neurological disease


increased oxidant
global biomolecule oxidation
injury
cell death
antioxidant
increased oxidant
biosensor oxidation
adaptive signaling
regulation / survival

Why do many people think oxidants are simply “bad” and antioxidants “good”?





In 1991 the Linus Pauling Institute recommended daily doses of

6g to 18g vitamin C

400 to 1600 IU vitamin E

25000 IU of vitamin A,

plus other supplements

Evidence?

Meta-Analysis: High-Dosage Vitamin E Supplementation May Increase All-Cause Mortality

Edgar R. Miller III, MD, PhD; Roberto Pastor-Barriuso, PhD; Darshan Dalal, MD, MPH; Rudolph A. Riemersma, PhD, FRCPE; Lawrence J. Appel, MD, MPH; and Eliseo Guallar, MD, DrPH

Background: Experimental models and observational studies suggest that vitamin E supplementation may prevent cardiovascular disease and cancer. However, several trials of high-dosage vitamin E supplementation showed non–statistically significant increases in total mortality.

Purpose: To perform a meta-analysis of the dose–response relationship between vitamin E supplementation and total mortality by using data from randomized, controlled trials.

Patients: 135 967 participants in 19 clinical trials. Of these trials, 9 tested vitamin E alone and 10 tested vitamin E combined with other vitamins or minerals. The dosages of vitamin E ranged from 16.5 to 2000 IU/d (median, 400 IU/d).

Data Sources: PubMed search from 1966 through August 2004, complemented by a search of the Cochrane Clinical Trials Database and review of citations of published reviews and meta-analyses. No language restrictions were applied.

Data Extraction: 3 investigators independently abstracted study reports. The investigators of the original publications were contacted if required information was not available.

Data Synthesis: 9 of 11 trials testing high-dosage vitamin E (≥400 IU/d) showed increased risk (risk difference > 0) for all-cause mortality in comparisons of vitamin E versus control. The pooled all-cause mortality risk difference in high-dosage vitamin E trials was 39 per 10 000 persons (95% CI, 3 to 74 per 10 000 persons; $P = 0.035$). For low-dosage vitamin E trials, the risk difference was −16 per 10 000 persons (CI, −41 to 10 per 10 000 persons; $P > 0.2$). A dose–response analysis showed a statistically significant relationship between vitamin E dosage and all-cause mortality, with increased risk of dosages greater than 150 IU/d.

Limitations: High-dosage (≥400 IU/d) trials were often small and were performed in patients with chronic diseases. The generalizability of the findings to healthy adults is uncertain. Precise estimation of the threshold at which risk increases is difficult.

Conclusion: High-dosage (≥400 IU/d) vitamin E supplements may increase all-cause mortality and should be avoided.

Ann Intern Med. 2005;142:37-46. www.annals.org
For author affiliations, see end of text.

Re-evaluated 19 vitamin E antioxidant trials between1994 and 2004

136,000 patients in North America, Europe and China

Death rate increased: 1 in 20 chance of dying earlier if 200IU Vitamin E

25% Americans use vitamin E supplements, with >60% taking >400IU day

How many die early?

Vitamin supplementation trials - smoking

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study

29,000 male smokers randomly assigned to of beta carotene, vitamin E, both, or placebo

After 6 years lung cancer incidence was 16 percent higher in supplement group

The all-cause death rate was also 8% higher

Trial stopped early

The Beta-Carotene and Retinol Efficacy Trial (CARET)

18,000 smokers, former smokers, or workers exposed to asbestos

Randomly assigned to beta-carotene and vitamin A (or placebo)

Four years follow up lung cancer incidence was 28 percent higher in supplement group

All-cause death rate was also 17% higher

Trial stopped early

Vitamin trials in other diseases – pre-eclampsia

Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial

L Poston, A L Briley, P T Seed, F J Kelly, A H Shennan, for the Vitamins in Pre-eclampsia (VIP) Trial Consortium*

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Vitamins C and E and the Risks of Preeclampsia and Perinatal Complications

Alice R. Rumbold, Ph.D., Caroline A. Crowther, F.R.A.N.Z.C.O.G., Ross R. Haslam, F.R.A.C.P., Gustaaf A. Dekker, F.R.A.N.Z.C.O.G., and Jeffrey S. Robinson, F.R.A.N.Z.C.O.G., for the ACTS Study Group*

No benefit from supplementation

- indications of harm?

Molecular basis of oxidant stress (damage)

oxidant + target biomolecule

target biomolecule oxidation = damage

Molecular basis of oxidant stress (signalling)

oxidant + target biomolecule

target biomolecule oxidation = signalling

How can we differentiate damage from signalling?

Defining oxidation and reduction

These are reactions where electrons are transferred from one species to another

Oxidation is the loss of electrons from a species
- the agent causing the loss of electrons is an oxidant

Reduction is the gain of electrons
- the agent donating the electrons is a reductant

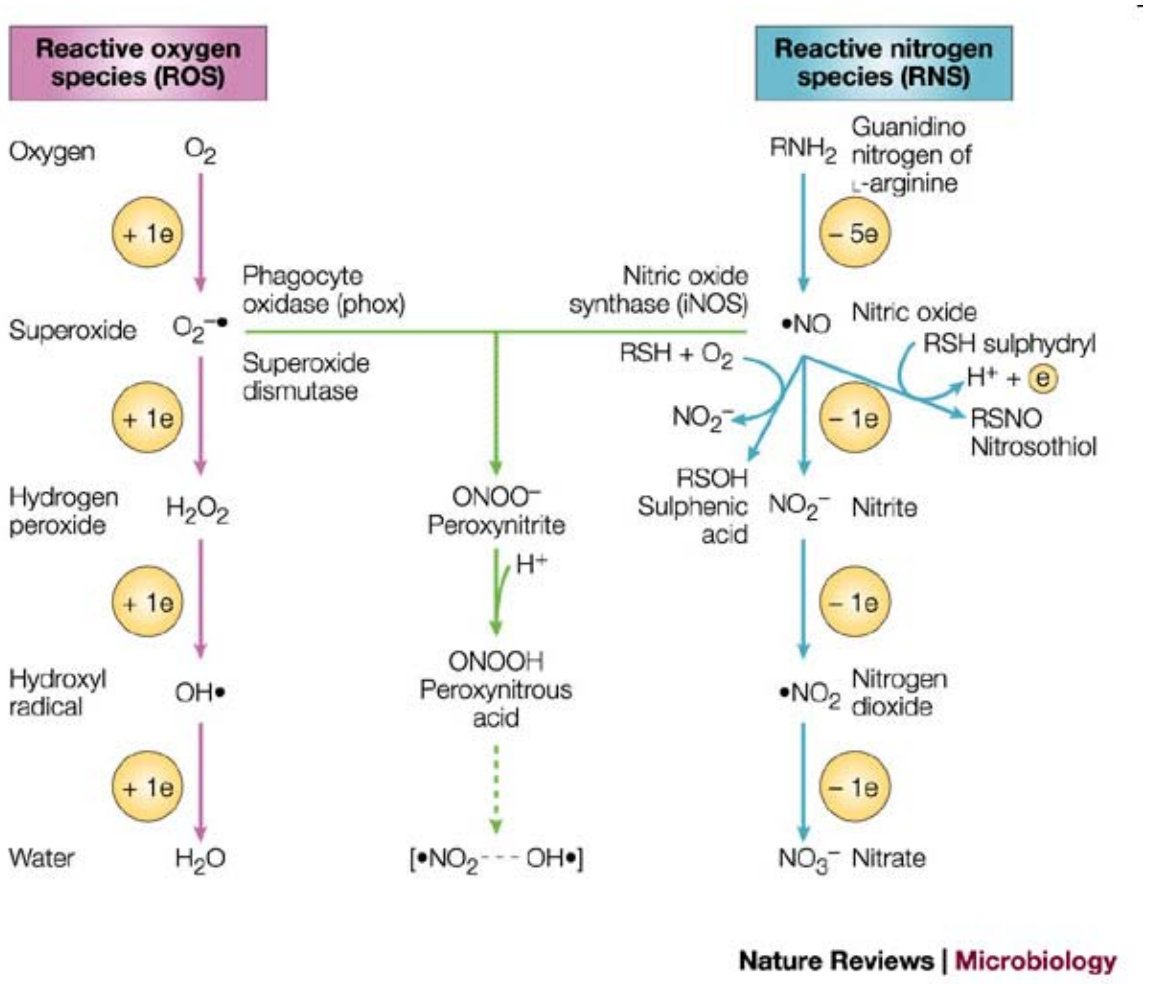
Oxidation and reduction in terms of hydrogen transfer

- oxidation is loss of hydrogen

- reduction is gain of hydrogen

e.g. $2GSH + H_2O_2 \rightarrow G\text{-}S\text{-}S\text{-}G + 2H_2O$

Major biological oxidants



Nature Reviews Microbiology 2, 820-832 (2004)

Reactions of oxidants with biological targets

Oxidants can target virtually all biological molecules

•DNA, RNA, cholesterol, lipids, carbohydrates, proteins and antioxidants

Extent of target oxidation depends on many factors

•concentration of oxidant and target
•rate constant for reaction of oxidant with target
•location of target versus oxidant
•occurrence of secondary damaging events
•occurrence of transfer reactions
•repair and scavenging reactions

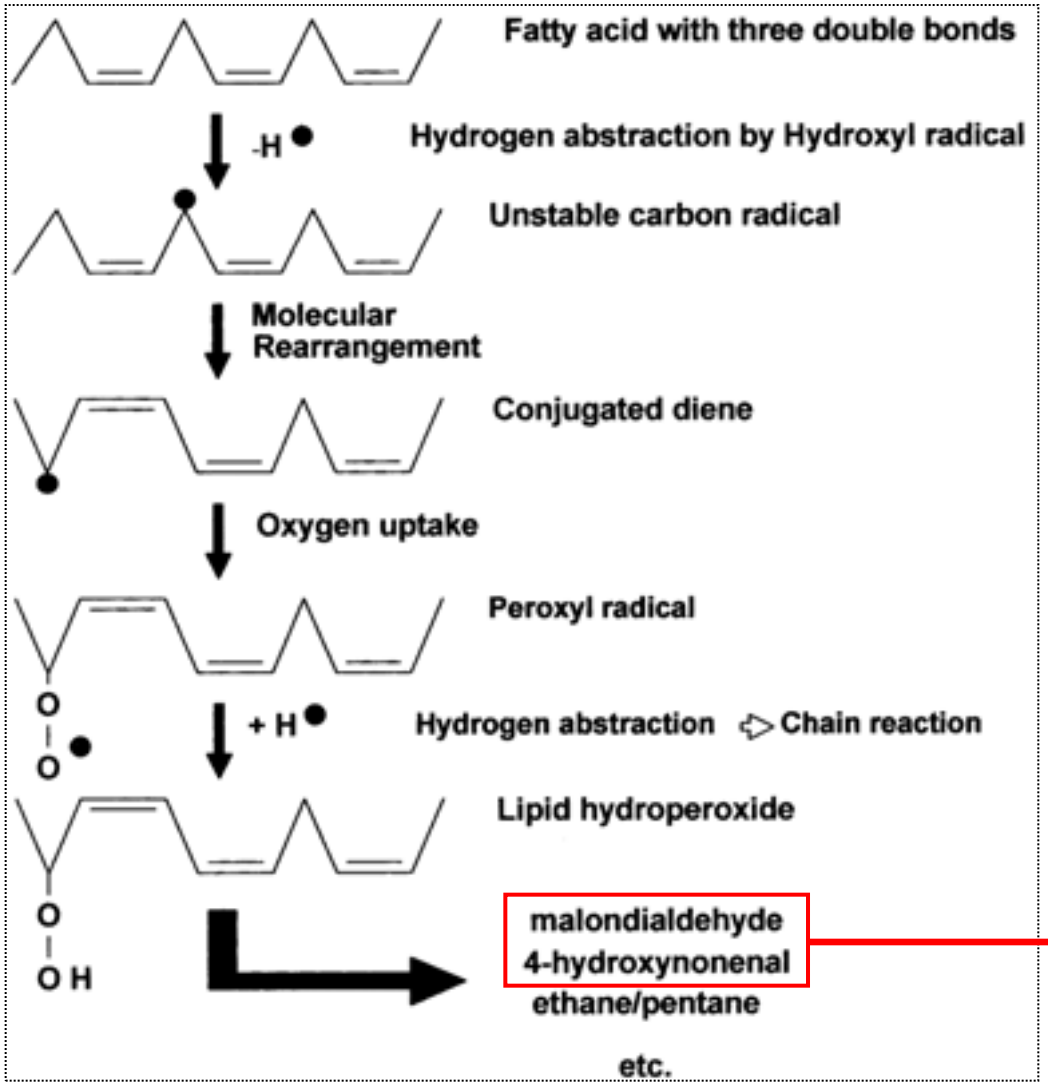
target oxidation = signalling or damage?

Biochem. J., 1997, 324, 1-18; Biochim. Biophys. Acta, 2001, 1504, 196-219

Biological oxidant species – reaction rates

Hydroxyl radical (•OH)	10^{-9}
Alcoxyl radical (RO•)	10^{-6}
Singlet oxygen (1O2)	10^{-5}
Peroxynitrite anion (ONOO-)	0.05 – 1.0
Peroxyl radical (ROO•)	7
Nitric oxide (•NO)	1 - 10
Semiquinone radical	minutes/hours
Hydrogen peroxide (H_2O_2)	Spontaneous / hours / days (accelerated by enzymes)
Superoxide anion (O2•-)	Spontaneous / hours / days (by SOD accelerated to 10^{-6})
Hypochlorous acid (HOCl)	depends on substrate

Lipid peroxidation



damaged membranes

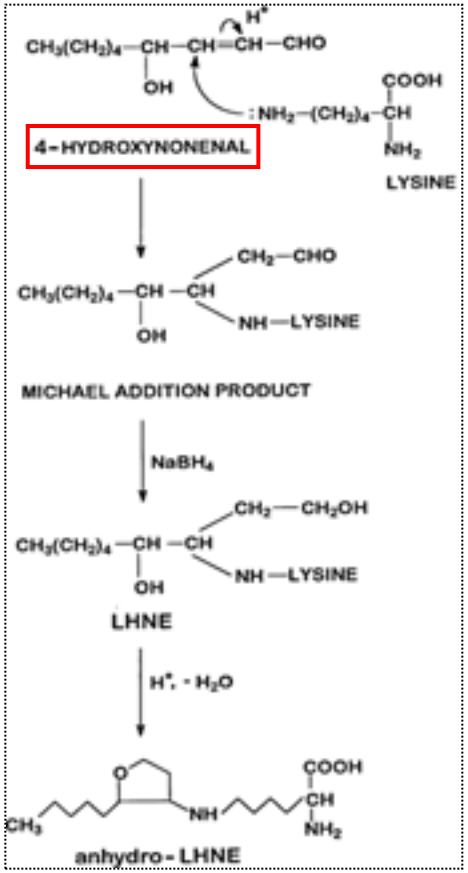
= compromised cell

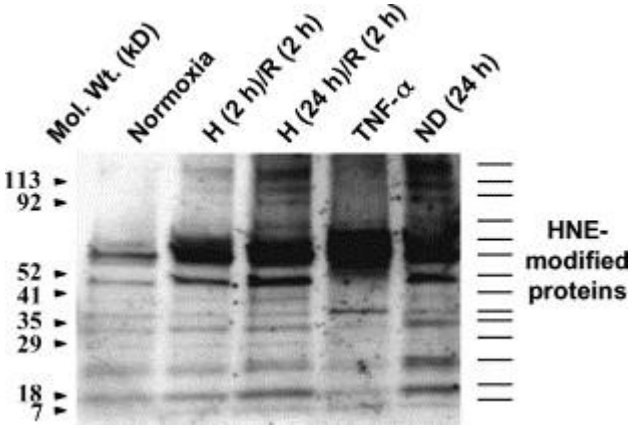
Secondary Oxidants
protein adducts
(injury, signaling)

DNA adduct

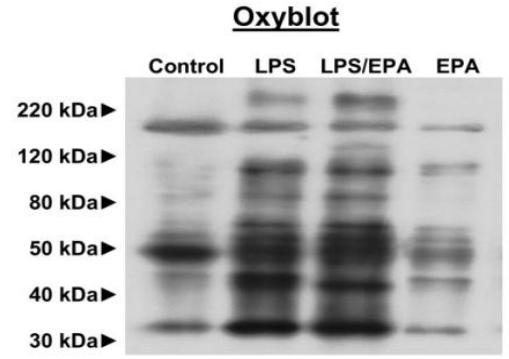
Young and McEneny Biochemical Society Transactions (2001) 29, (358–362)

Other reactive oxidized lipids that adduct proteins

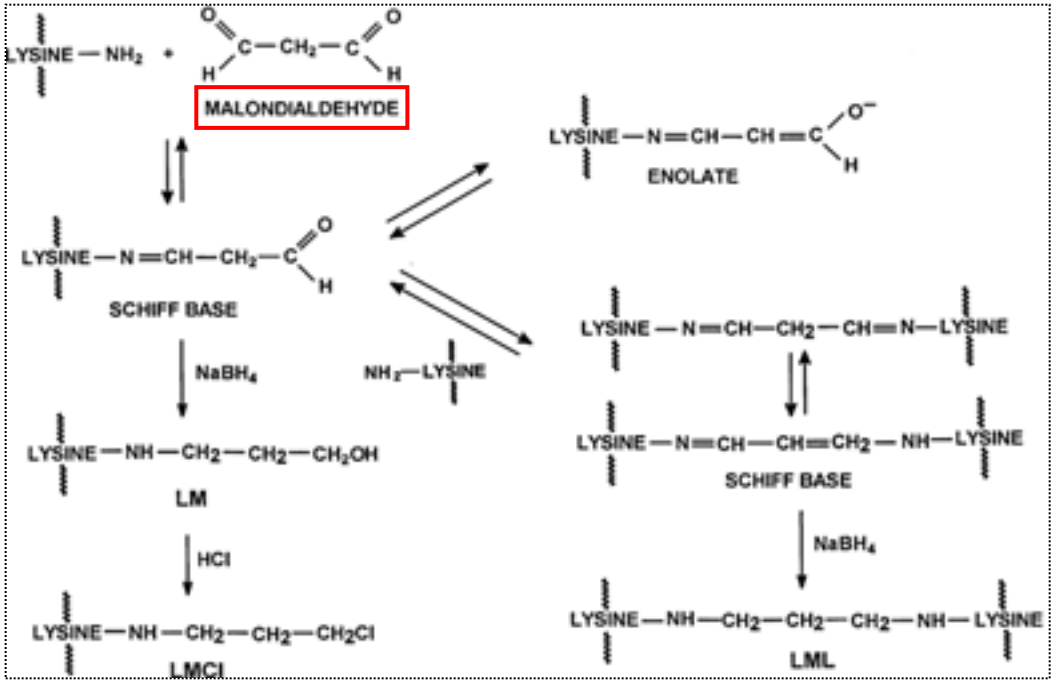




Martin et al, Vasc Pharm (2005)
IB: anti protein-HNE`



Supinski et al, Crit Care (2010)



Requena et al, Biochem. J. (1997)

DNA Oxidation

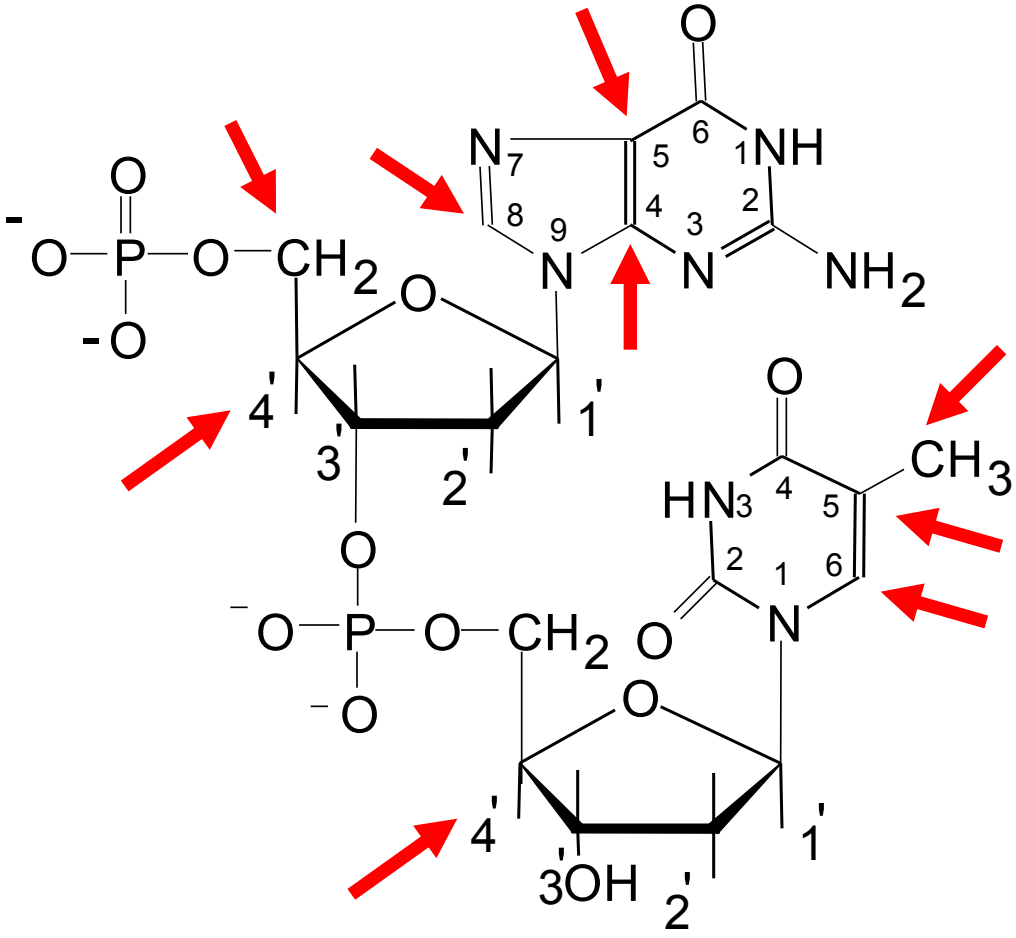
hundreds of different DNA oxidation states

sites of oxidative attack shown (→)

bases and sugars susceptible

strand-breaking oxidations

mutagenic



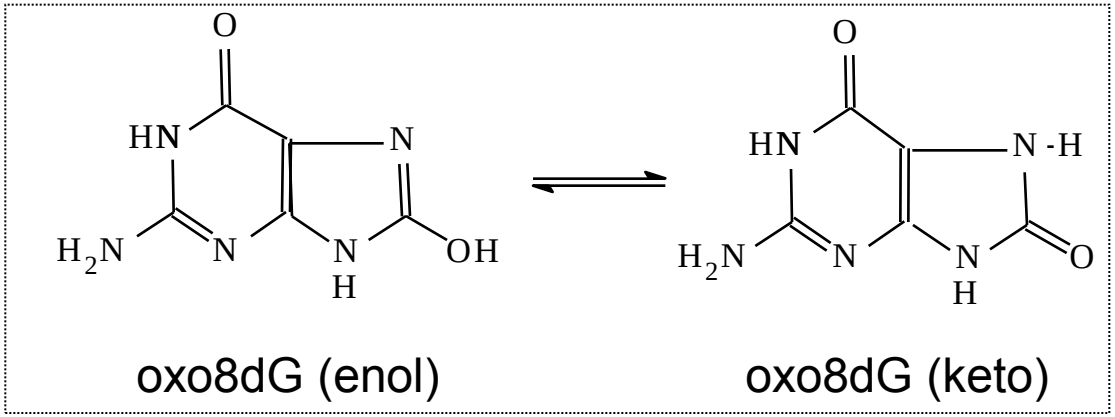
DNA Oxidation – examples

8-Oxo-deoxyguanosine (oxo8dG)

8-Oxo-deoxyadenosine

5-Hydroxy-2-deoxycytidine (5-HMdU)

Thymidine glycol



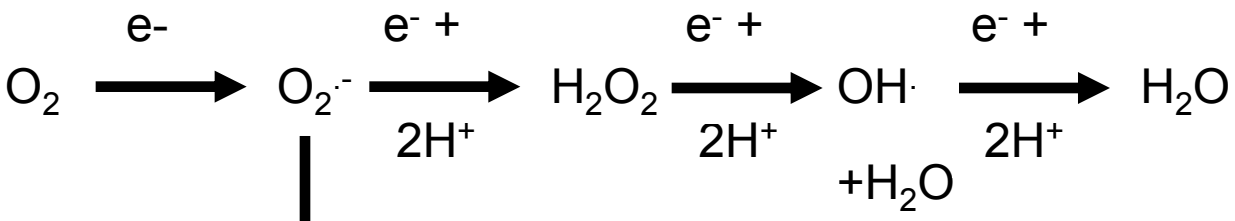
Monitored using HPLC methods

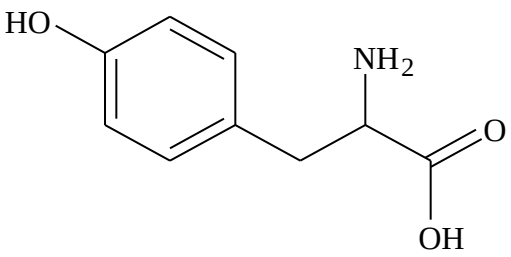
ELISA kits also available

Protein Oxidation – susceptible residues

Cys	many modifications – see slides to follow
Met	Methionine sulfoxide
Tyr	Dityrosine, nitrotyrosine, chlorotyrosines, dopa
Trp	Hydroxy-and nitro-tryptophans, kynurenines
Phe	Hydroxyphenylalanines
Val,Leu	Hydroperoxides
His	2-Oxohistidine, asasparagine, aspartate, HNE-His
Glu	Oxalic acid, pyruvic acid
Pro	Hydroxyproline, pyrrolidone, glutamic semialdehyde
Thr	2-Amino-3-ketobutyric acid
Arg	Glutamic semialdehyde, chloramines
Lys	MDA-Lys, HNE-Lys, acrolein-Lys, CML

Peroxynitrite ($OONO^-$) and nitrotyrosine formation



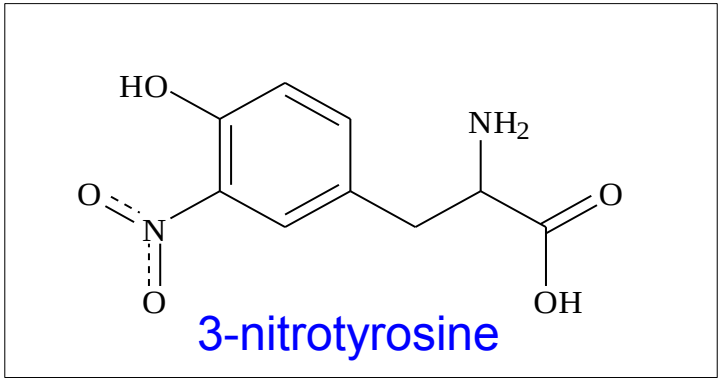


tyrosine

NO·

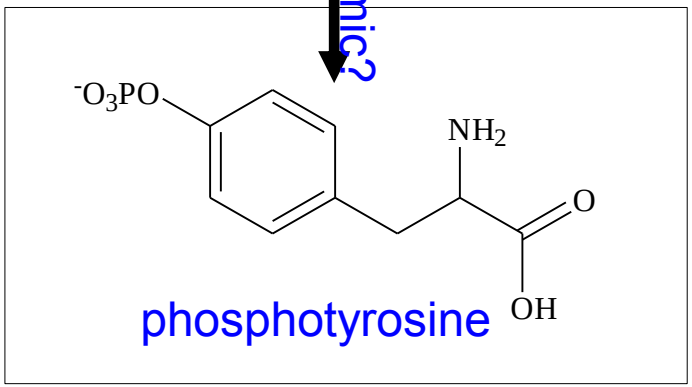
OONO-

denitrase?

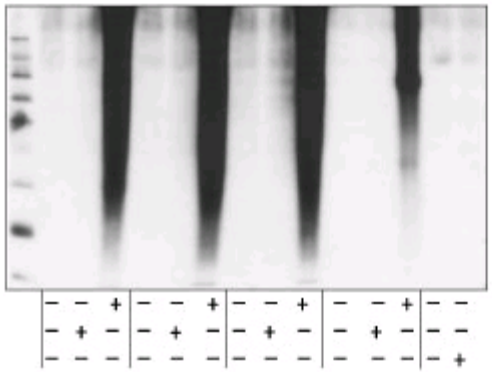


3-nitrotyrosine

mimic?



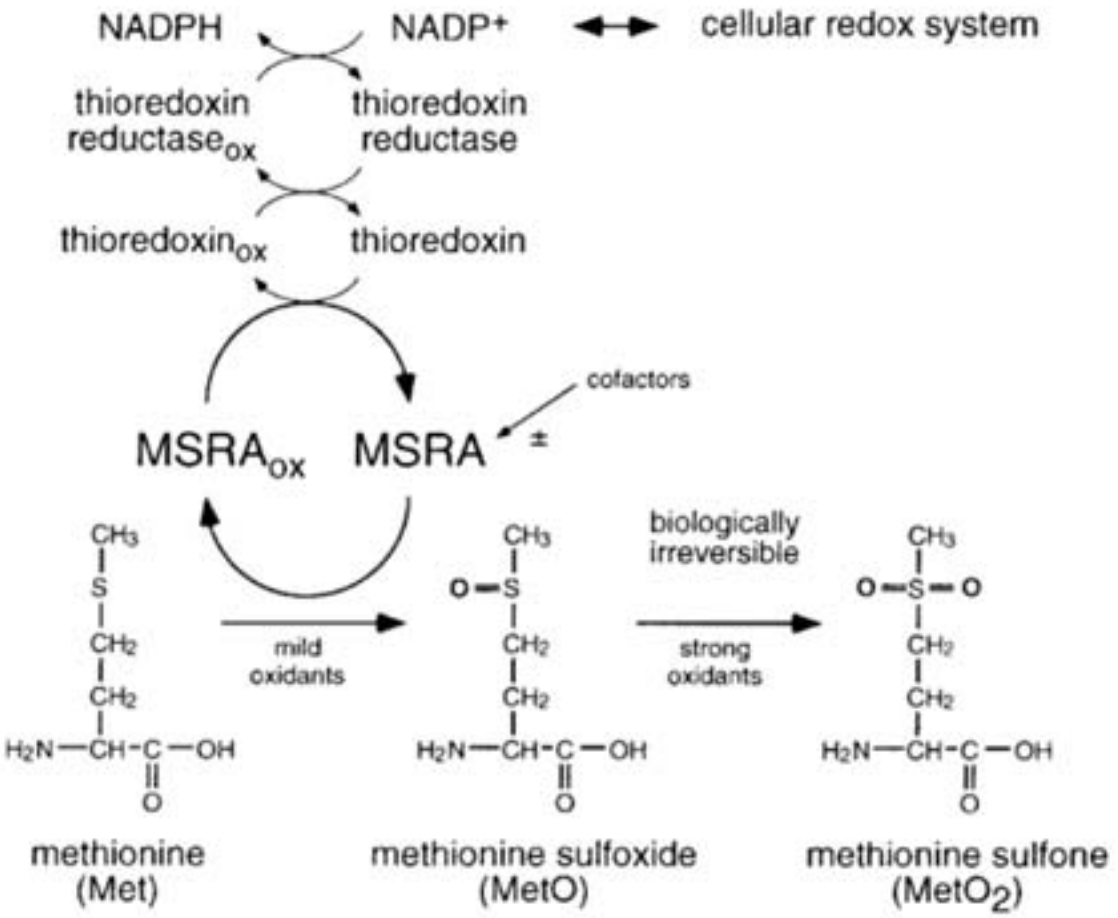
phosphotyrosine



Nitro-Tyrosine
Polyclonal

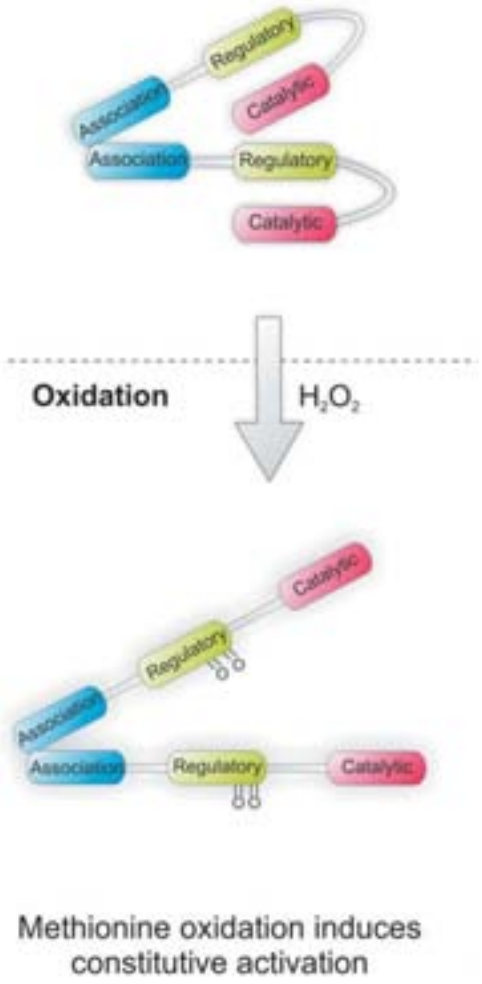
Peroxynitrite
Degraded Peroxynitrite
Pervanadate

Methionine oxidation



Hoshi T , and Heinemann S H J Physiol 2001;531:1-11

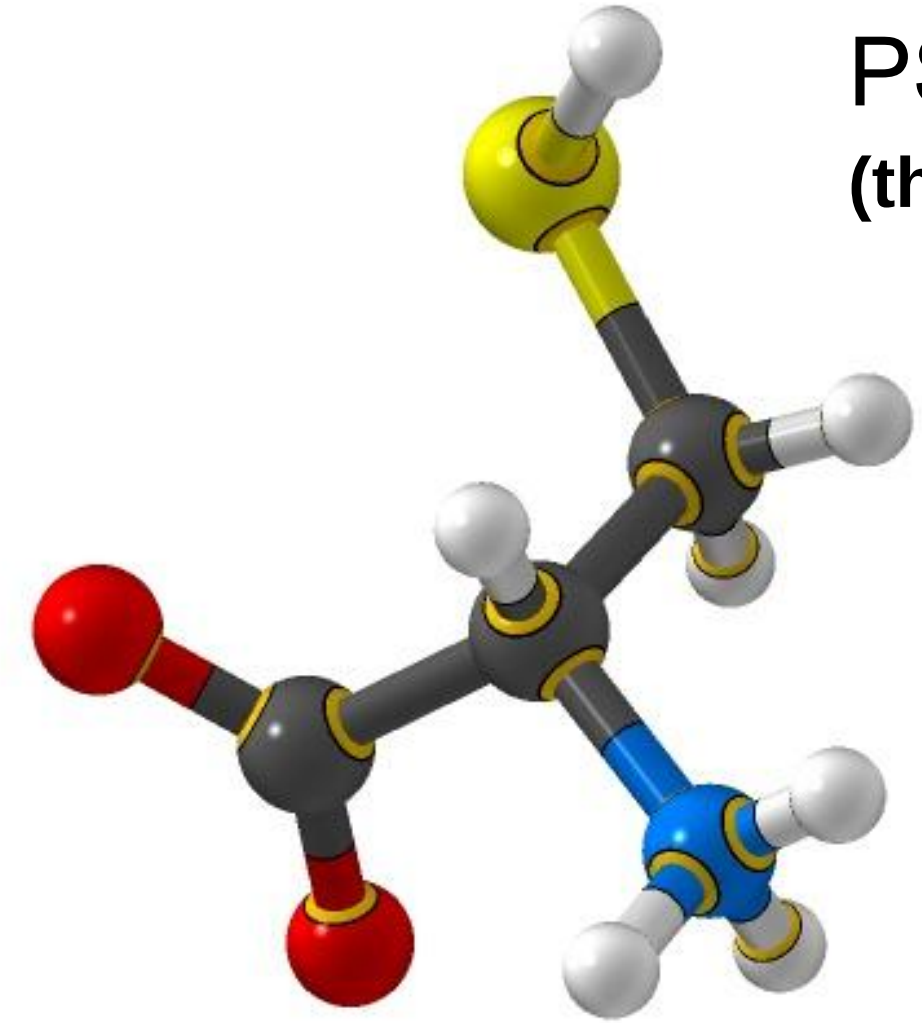
Methionine oxidation in cardiac CaMKII



Methionine oxidation induces constitutive activation

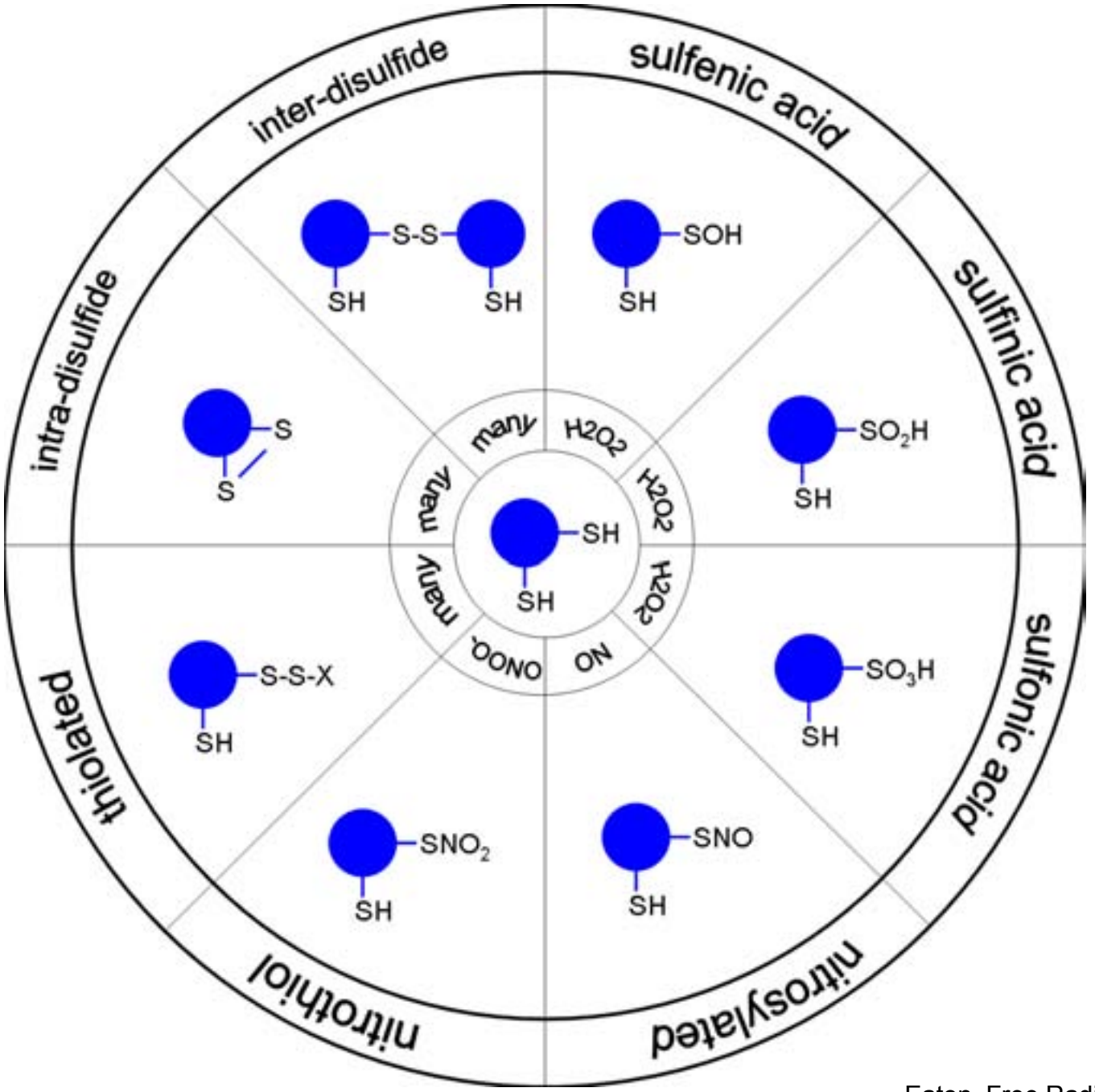
cardiac dysfunction

Cysteine thiol – potential oxidant sensor

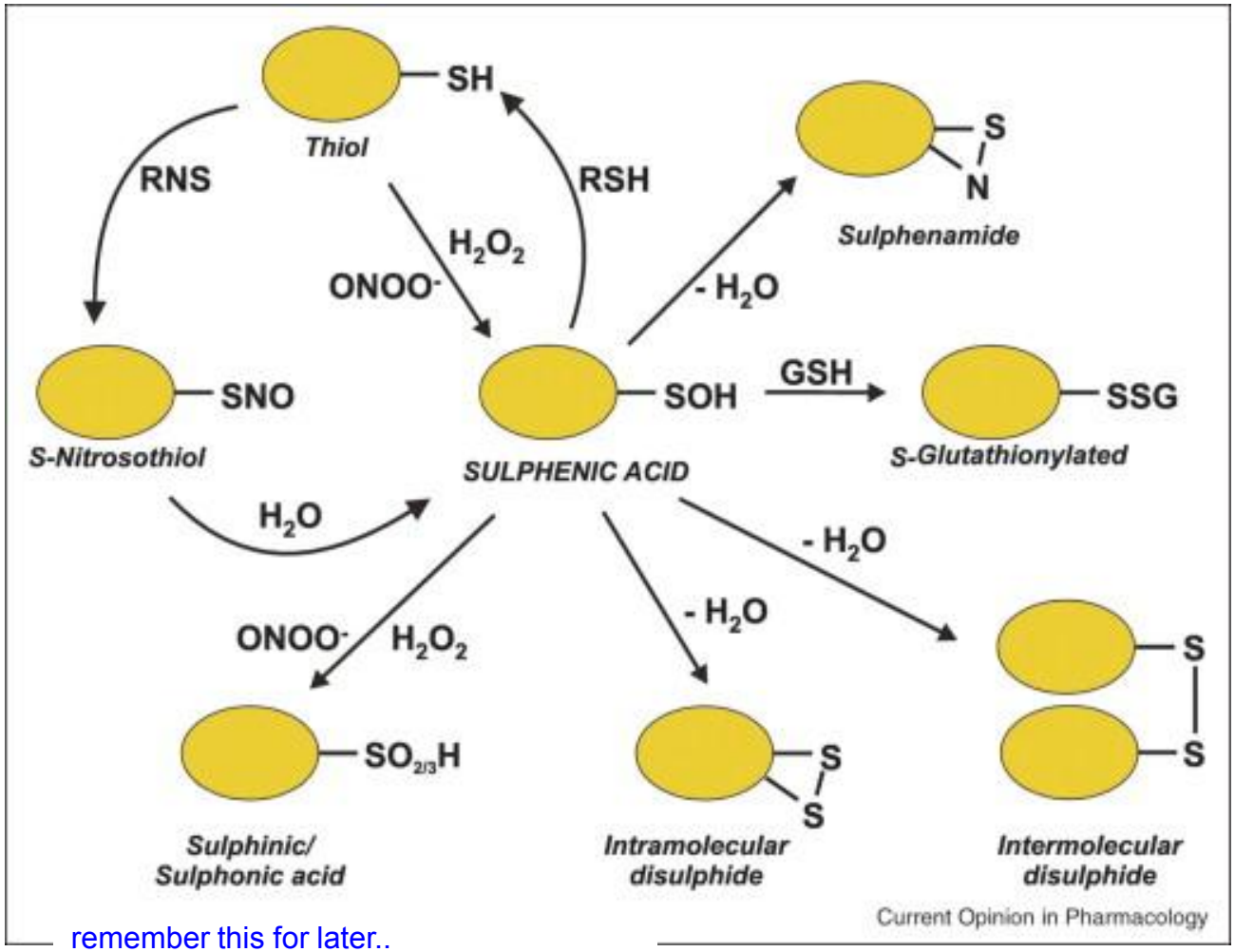


$PSH \longrightarrow PS^-$

(thiol) **(thiolate anion)**



Eaton, Free Radic Biol Med 2006



remember this for later..
... peroxiredoxin proteins and „floodgate"

Schröder and Eaton, 2008

How can selective / specific oxidant-signalling occur when there are so many thiols in the cell?

1-5mM glutathione plus other small thiols intracellular

1000‟s different protein thiols

Doesn‟t the oxidant just react with all these thiol targets?

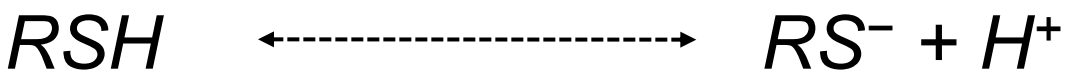
not all thiols are „equal‟

some are more nucleophilic than other

nucleophiles react with electrophiles (oxidants)

this provides a molecular basis for selective oxidant signalling

Thiol pKa determines its reactivity



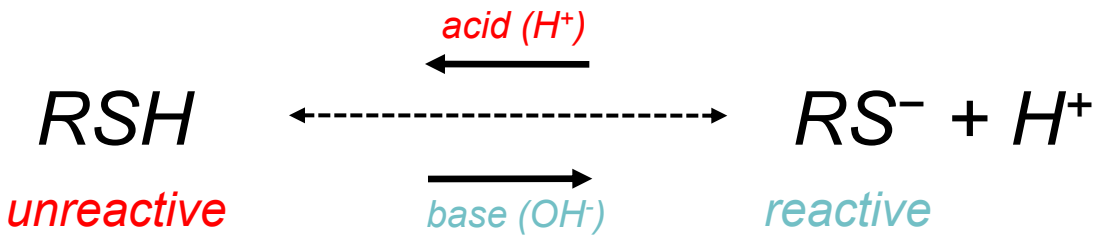
pKa is the pH at which [RSH] = [RS-]

or pH when the thiol is 50% ionised

Oxidants (e.g. H_2O_2) will reacts very, very slowly with RSH..

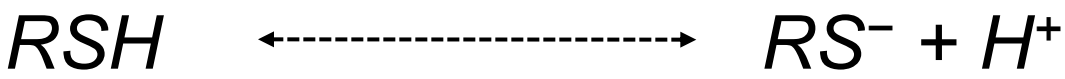
… but very, very fast with RS^-

Thiol pKa determines its reactivity



pKa = acid dissociation constant (pH at which the molecule ionises)

Thiol pKa determines its reactivity

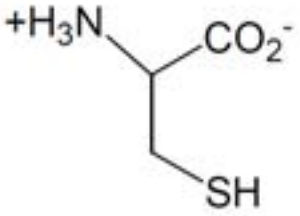


pKa is the pH at which [RSH] = [RS-]

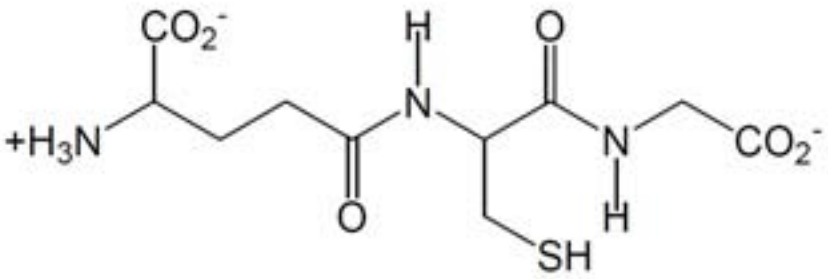
or pH when the thiol is 50% ionised

Thiol molecule	**p*Ka***	***%RS⁻ at pH 7.4***
Cysteine	~8·5	~4.5%
glutathione	~8·9	~2.5%
Peroxiredoxin	~4·5	~100%

Thiol pKa determines its reactivity



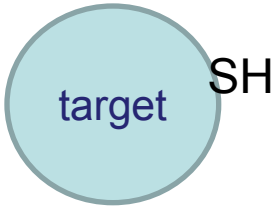
Cysteine

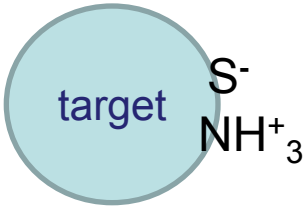


Glutathione

Thiol molecule	**p*Ka***	***%RS⁻ at pH 7.4***
Cysteine	~8·5	~4.5%
glutathione	~8·9	~2.5%

Thiol pKa determines its reactivity





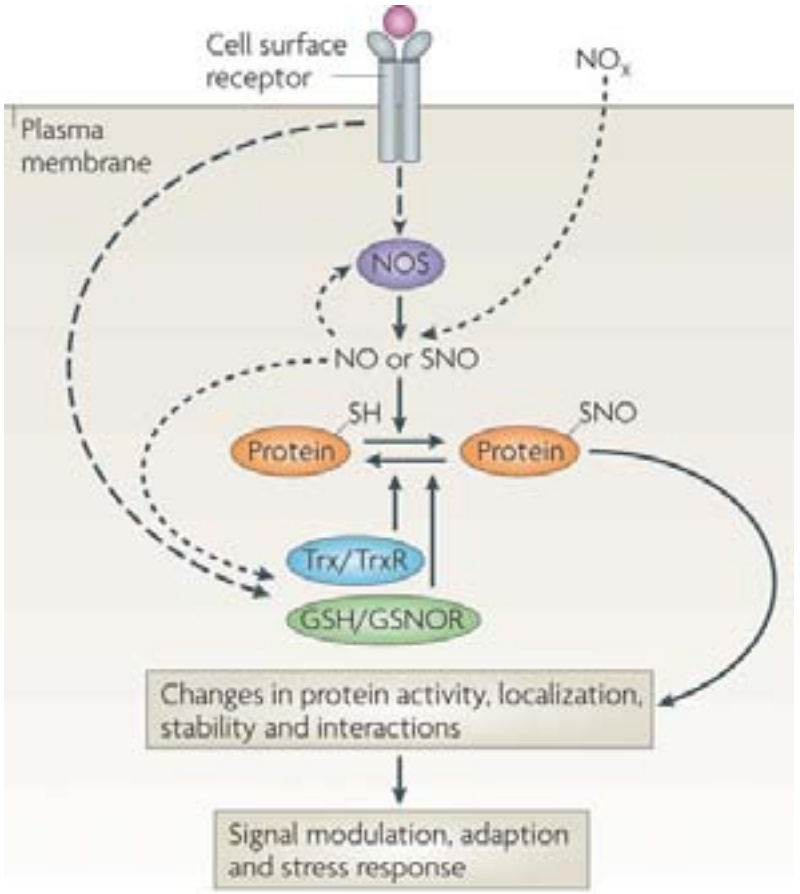
Thiol molecule	**p*Ka***	***%RS⁻ at pH 7.4***
Peroxiredoxin	~4·5	~100%

Protein S-nitrosylation (-SNO formation)

covalent adduction of nitric oxide (NO)

aka S-nitrosation

aka nitrosothiol



Protein S-nitrosylation

Table 1 | **Enzymatic activities that are involved in *S*-nitrosylation/de-nitrosylation**

Enzyme	Substrate(s)	Action/Product	Mechanism
Ceruloplasmin (other multi-Cu^{2+} proteins including laccase)	Glutathione	GSNO	1) CPCu(II) + NO → CPCu(II)–NO + GSH → CPCu(II) + GSNO + $1H^+$ + $1e^-$ 2) CP[Cu cluster]ox + $4e^-$ → $CP[Cu\ cluster]_{red}$ 3) $CP[Cu\ cluster]_{red}$ + $4H^+$ + O_2 → $CP[Cu\ cluster]_{ox}$ + $2H_2O$ Net: $4NO + 4GSH + O_2 \rightarrow 4GSNO + 2H_2O$
	Glycipan-1	SNO-glycipan-1	
Superoxide dismutase	Haemoglobin	Hb[β-Cys93-NO]	
Haemoglobin-FeNO	Self (auto-*S*-nitrosylation)	Hb[β-Cys93-NO]	1) $Hb([Fe(II)NO][Fe(II)_3])Cys + 4O_2 \leftrightarrow (Hb([Fe(II)O_2]_4)CysNO + 1e^-$ 2) $Hb([Fe(III)NO][Fe(II)_3])Cys + 4O_2 \leftrightarrow Hb([Fe(II)O_2]_4)CysNO$
Thioredoxin/ thioredoxin reductase	SNO-protein (NO-synthase; PKC?) GSNO	De-nitrosylation De-nitrosylation	
GSNO-reductase	GSNO	GSNO metabolism (GSNO ↔ SNO-protein equilibrium)	1) Protein-SNO + GS^- ↔ protein-S^- + GSNO 2) GSNO + NADH + H^+ $\xrightarrow{GSNOR}$ GSNHOH + NAD^+
NOS	NOS (auto-*S*-nitrosylation)	Inhibition of NO production	
	S-nitrosylation of multiple substrates	Regulation of substrate function, location and protein–protein interactions	

Hess et al, Nature Reviews Molecular Cell Biology 6, 150-166 (2005)

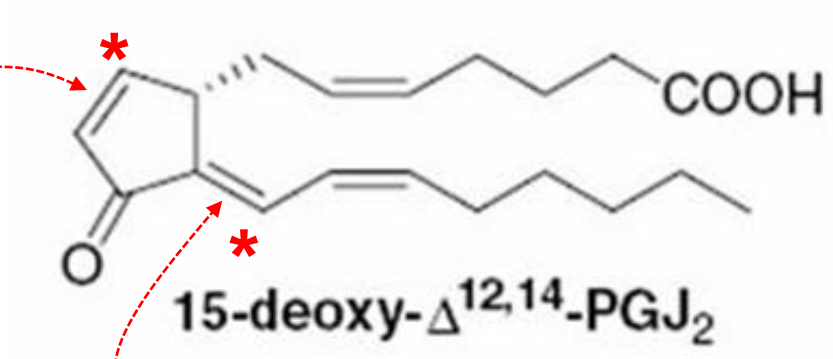
Regulated Protein Denitrosylation by Cytosolic and Mitochondrial Thioredoxins

Moran Benhar,[1] Michael T. Forrester,[2] Douglas T. Hess,[1] Jonathan S. Stamler[1,2]*

SCIENCE VOL 320 23 MAY 2008

S-nitrosylation has the similar enzymatic control mechanisms as phosphorylation

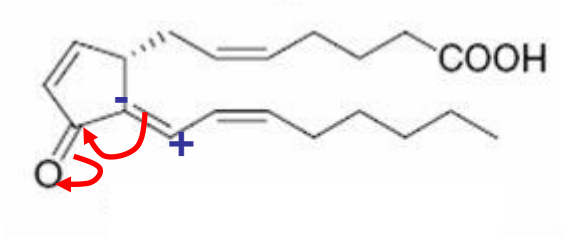
More complex biomolecules can also react with thiolate



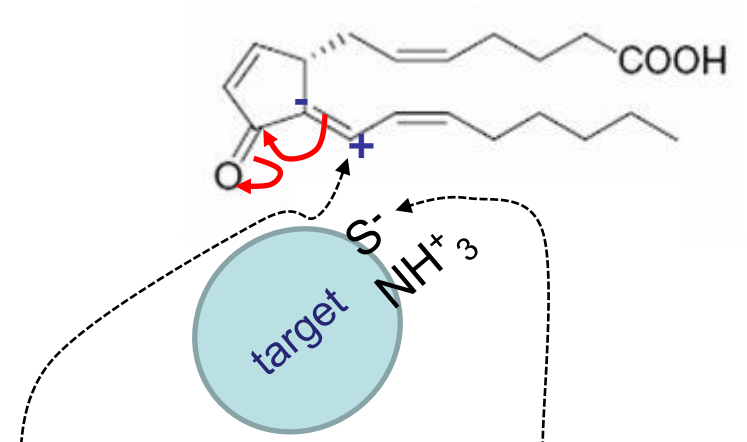
alpha beta unsaturated carbonyls

* = electrophilic carbons

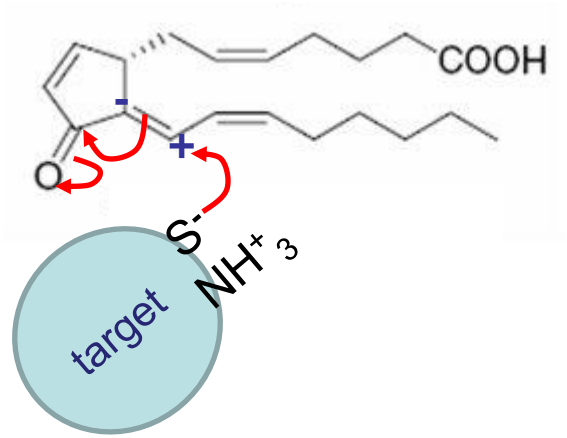
more selective oxidant-signalling Compared to H_2O_2, HNO, HOCl etc?



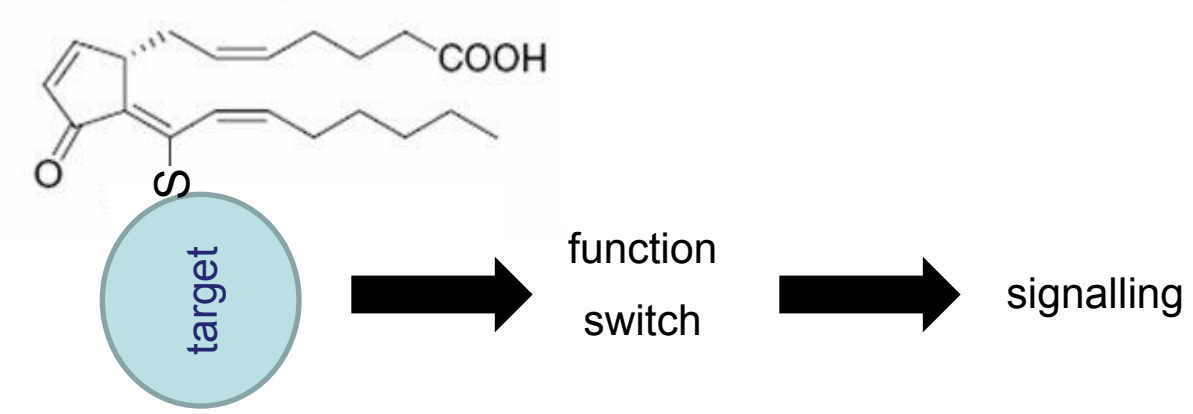
electronegative oxygen atom pulls electrons from the double bond



Resulting „electrophilic carbon" is very reactive with protein thiolate

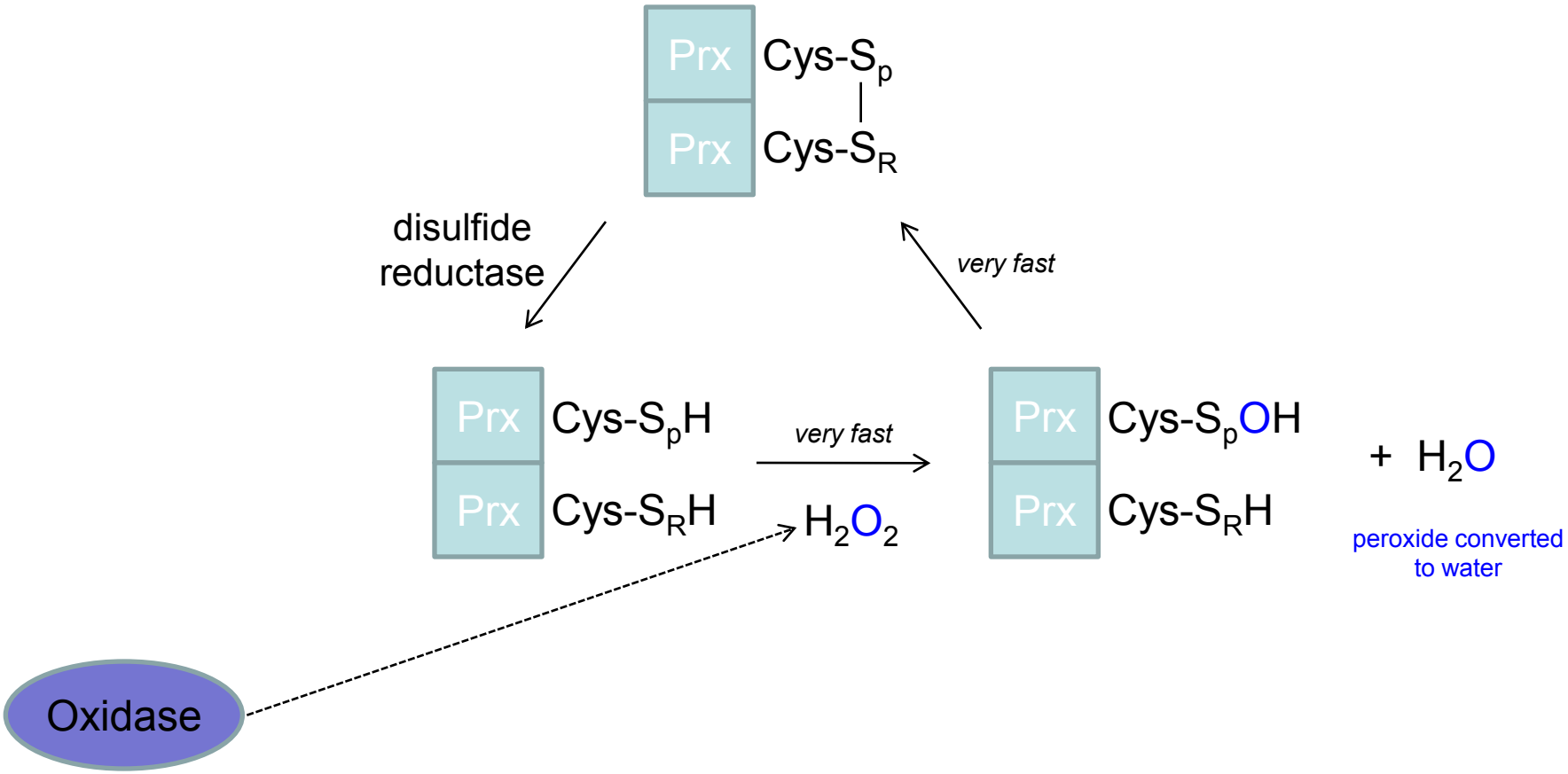

COOH
O
-
+
S-
NH+ 3
target

Michael reaction or Michael addition



Conjugation (adduction of lipid to protein cysteine)

Reaction cycle of Peroxiredoxins (decomposition of H_2O_2)



S_p = peroxidatic thiol (pKa ~4·5 = very reactive = very fast reaction)

S_R = resolving thiol

Examples of proteins that are modified / regulated by H_2O_2

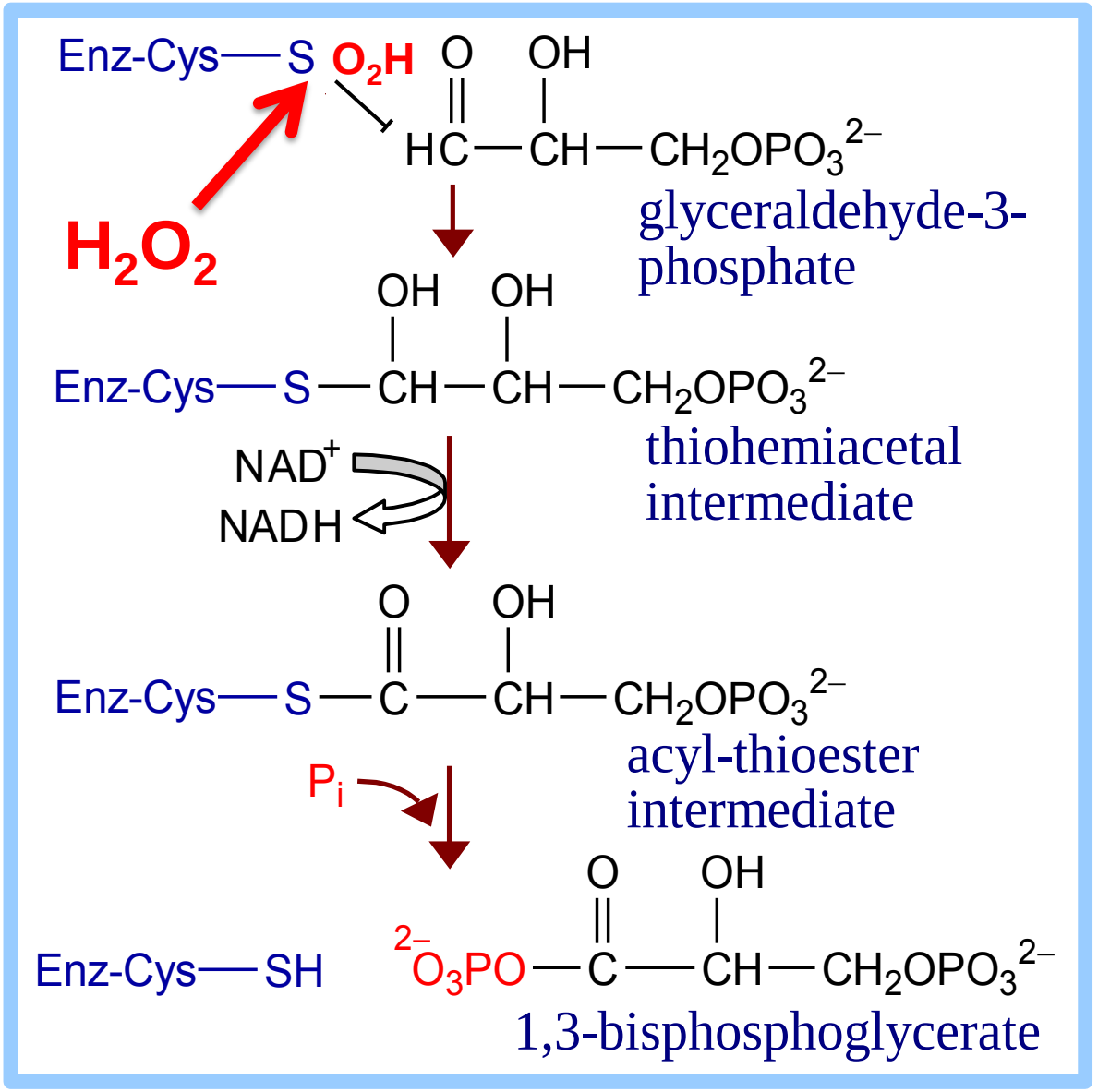
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

The aldehyde of glyceraldehyde-3-phosphate reacts with the cysteine thiol to form a **thiohemiacetal**

Oxidation to a carboxylic acid (in a ~ **thioester**) occurs, as NAD^+ is reduced to **NADH**

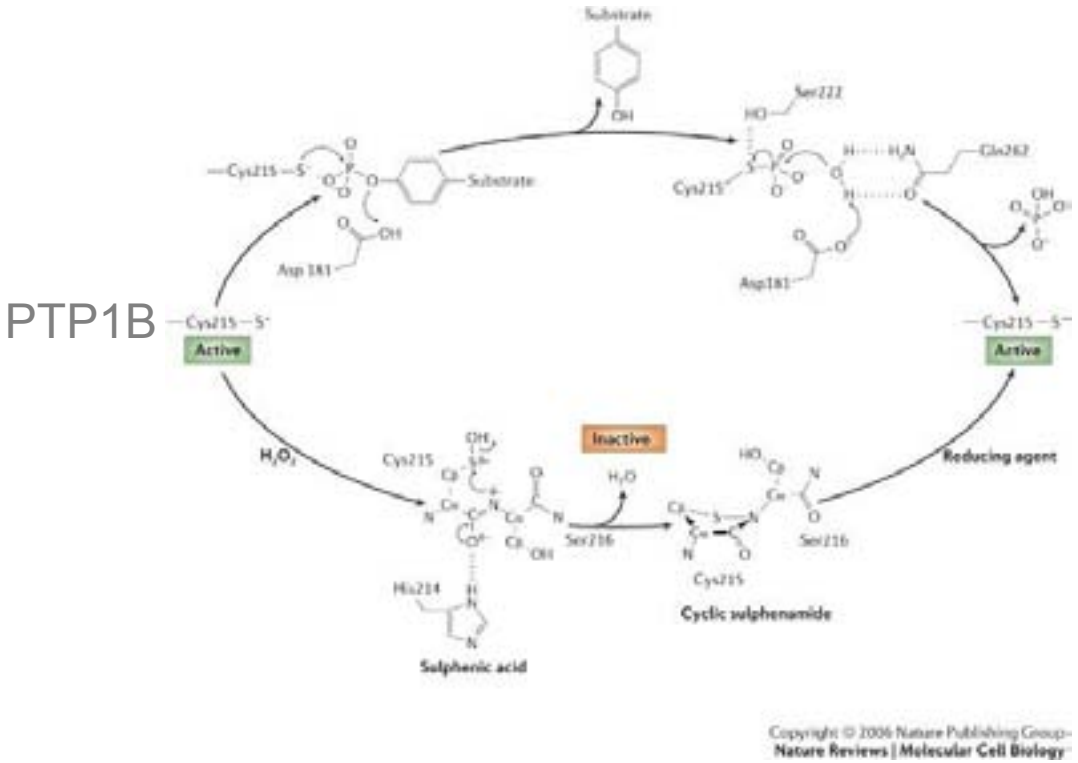
The "high energy" acyl thioester is attacked by P_i to yield the acyl phosphate (**~P**) product

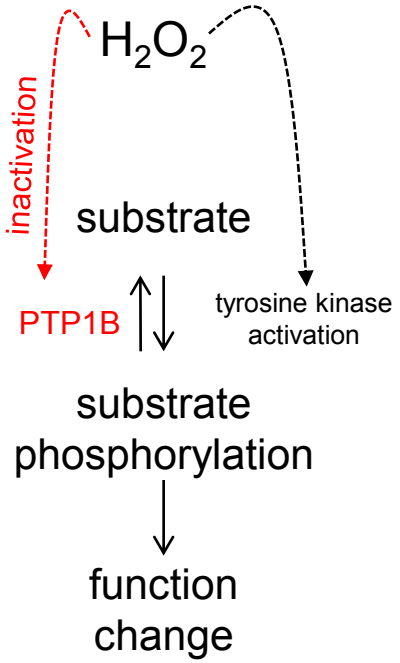
H_2O_2 inactivates GAPDH by thiol oxidation



Examples of proteins that are modified / regulated by H_2O_2

Protein tyrosine phosphatase 1B (PTP1B)





Tonks *Nature Reviews Molecular Cell Biology* **7**, 833–846 (November 2006) | doi:10.1038/nrm2039

nature REVIEWS MOLECULAR CELL BIOLOGY

Comparing thiol targets of H_2O_2

	k_{H2O2} $M^{-1}s^{-1}$	Conc.	% of H_2O_2 reacting with target
Protein tyrosine phosphatases			
PTP1B (pKa ~5·6)	20	1 μM	0.0004
GAPDH (pKa ~5·3)	~500	100 μM	1
Peroxiredoxins (pKa ~4·5)	>100,000	50 μM	99

How can PTP1B or GAPDH ever be stoichiometrically modified by H_2O_2 when peroxiredoxin is so reactive and also abundant ????

adapted from work of Christine Winterbourn

Comparing thiol targets of H_2O_2

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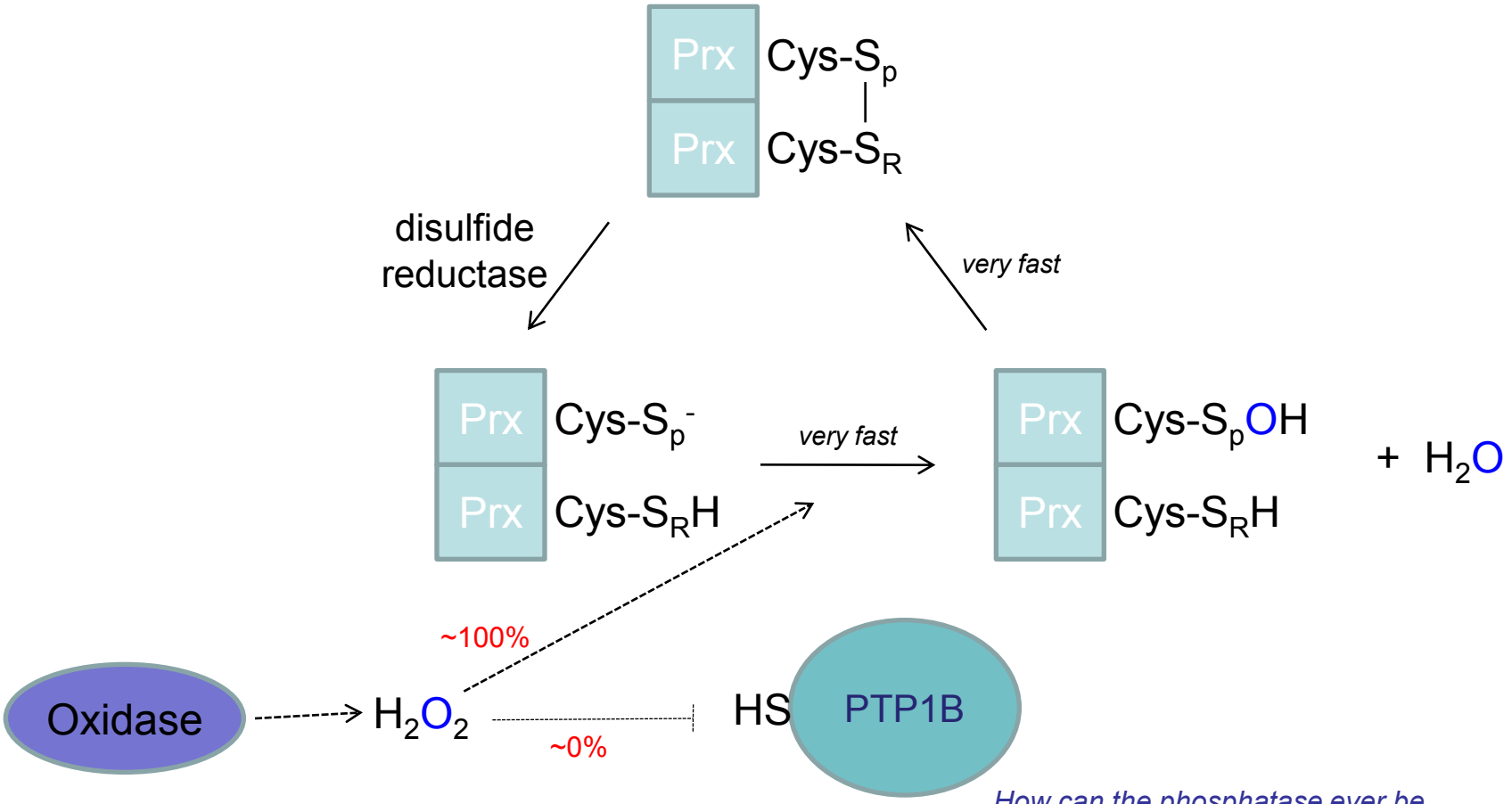
Some proteins that are easily oxidized by H_2O_2 in isolation are unlikely to be directly oxidized in a cell

- until more favourable targets (peroxiredoxins) are oxidized..

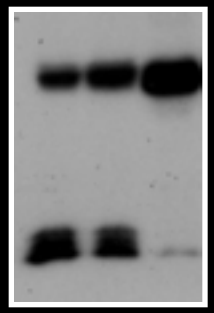
adapted from work of Christine Winterbourn

Peroxiredoxins should prevent oxidation of less abundant / lower pKa thiols



How can the phosphatase ever be stoichiometrically oxidised?

peroxiredoxin peroxidase catalytic cycle



$[H_2O_2]$ 0 10 100 (μM)

Disulfide Reductase

Prx Cys-S_P
Prx Cys-S_R

Prx Cys-S_PH
Prx Cys-S_RH

H_2O_2

Prx Cys-S_POH
Prx Cys-S_RH

H_2O_2

Prx Cys-$S_PO_2^-$
Prx Cys-S_RH

detects only this oxidation state

hyperoxidation and inactivation

anti-Prx SO_2^-

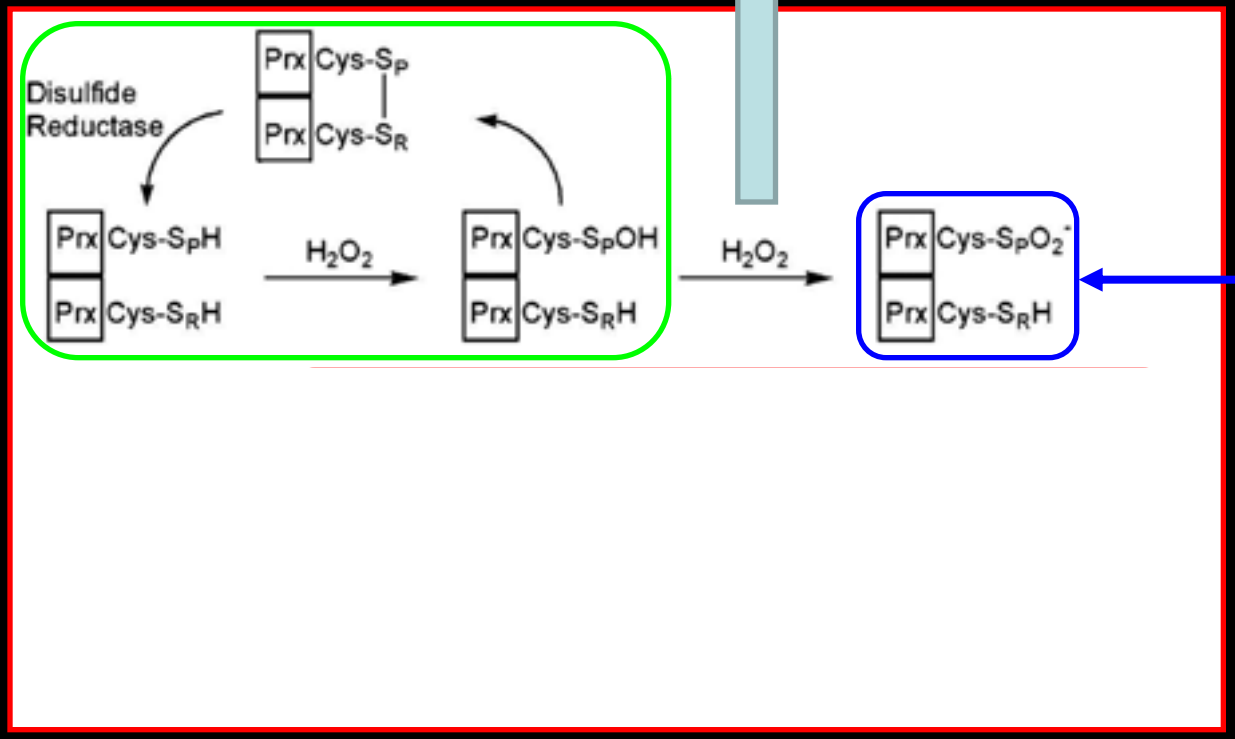
$[H_2O_2]$ / (mM) 0 2 4 6

Jönsson *et al*., 2005, Biochemistry

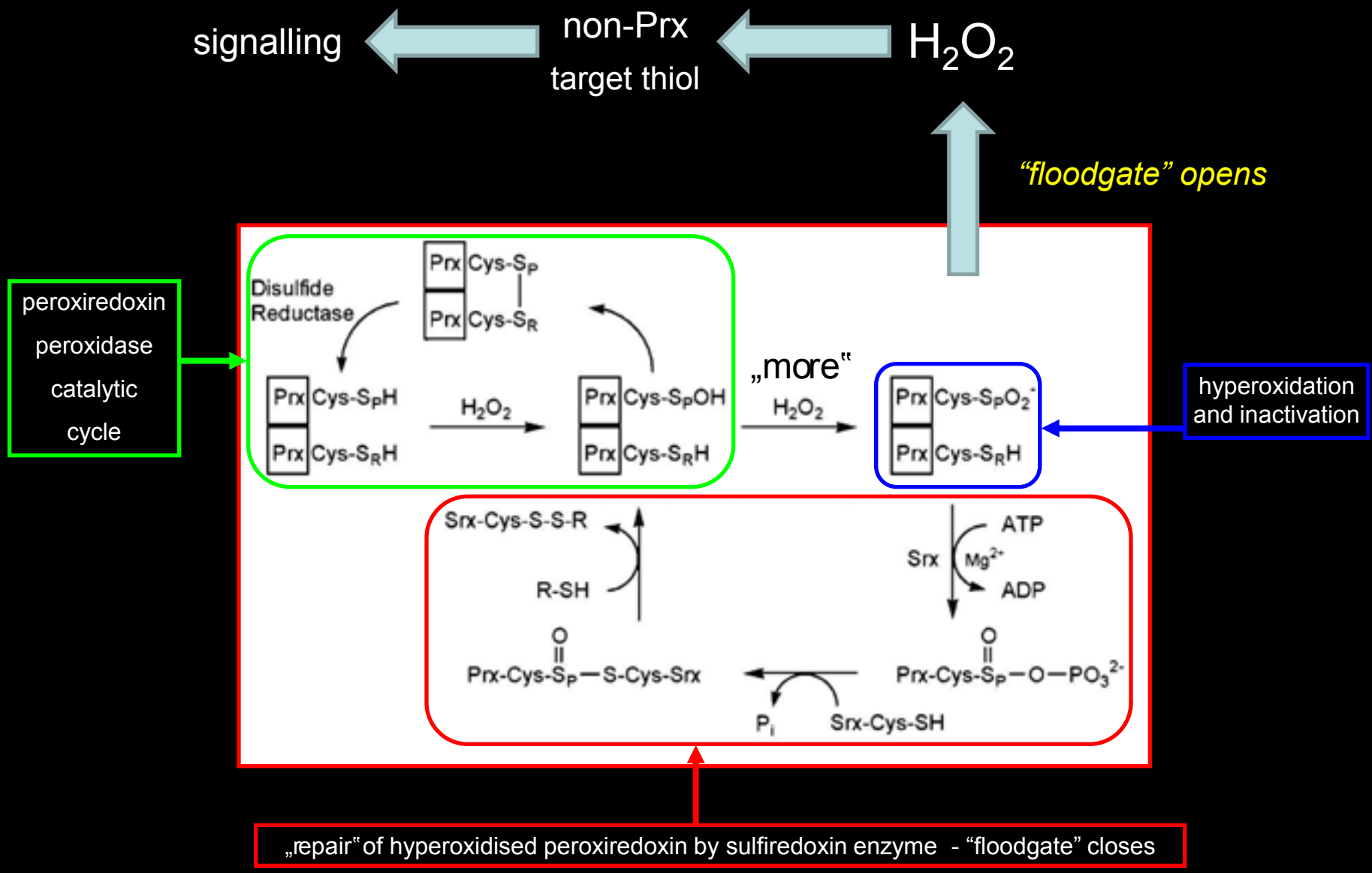
signalling ← non-Prx target thiol ← H_2O_2

Science 2003 (Perspective: *An Overoxidation Journey with a Return Ticket*)
Vol. 300. no. 5619, pp. 592 - 94

"floodgate" opens

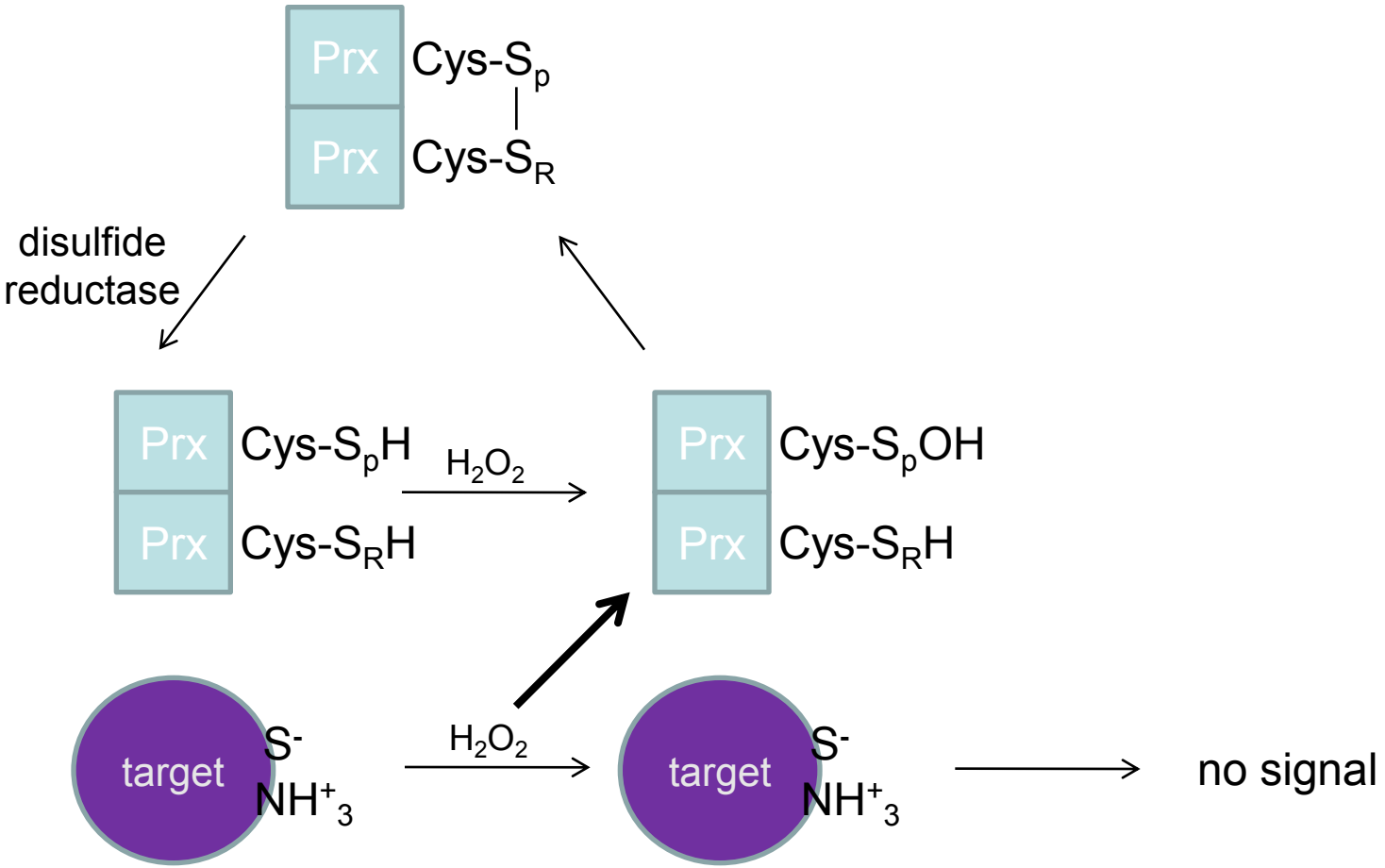


Jönsson *et al*., 2005, Biochemistry

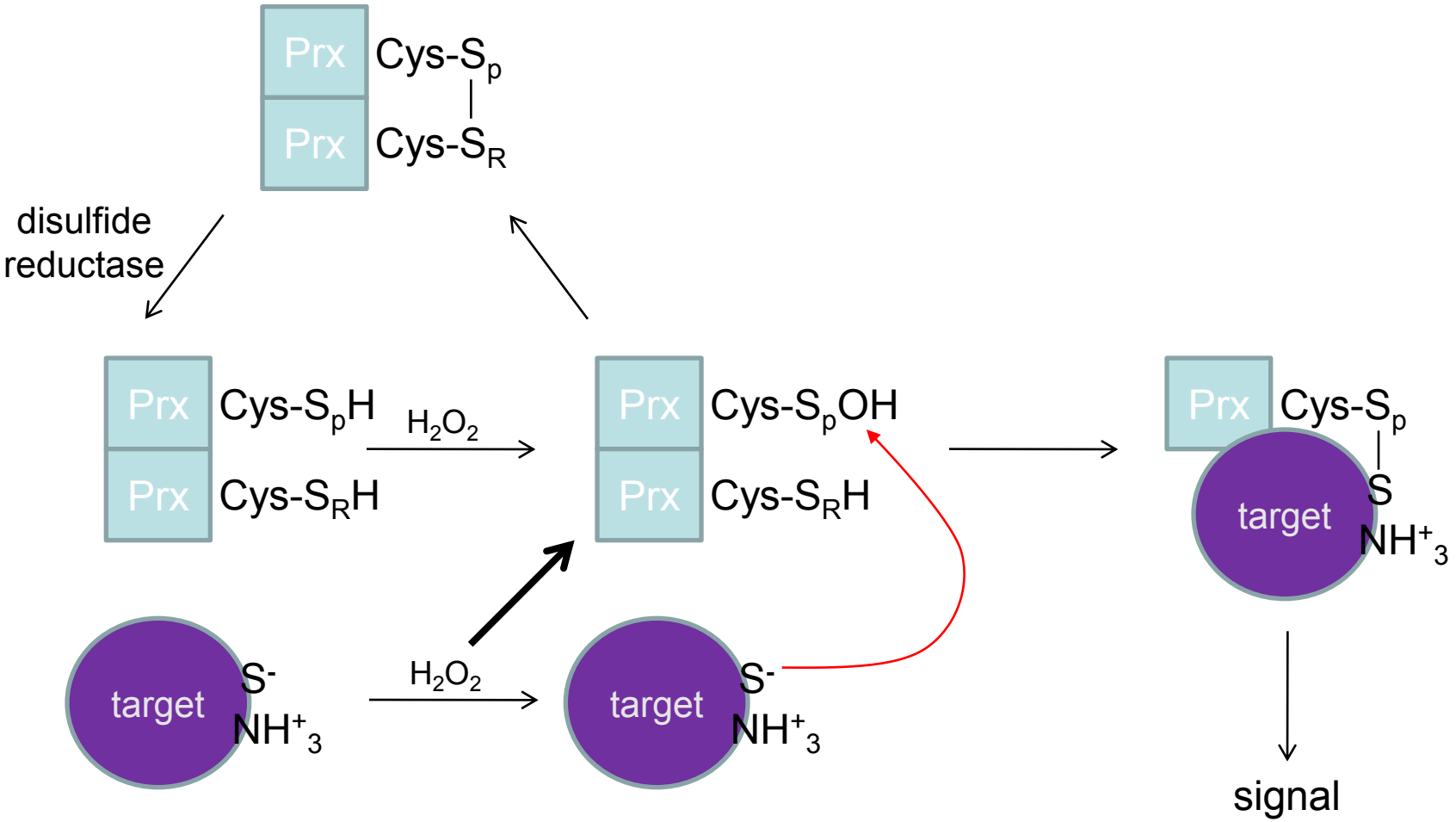


Jönsson *et al.*, 2005, Biochemistry

Alternative to floodgate



Alternative to floodgate



Alternative to floodgate