

Chalcogen ('kal-ka-jen) chemistry and biochemistry: The many faces of O, S, and Se in proteins and enzymes

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What is a Chalcogen ?

Chalcogen

Pronunciation: 'kal-ka-jen; ('kal-kə-jən)

Function: *noun*

Etymology: International Scientific Vocabulary *chalk-bronze, ore* (from Greek *chalkos*) + *-gen*; from the occurrence of oxygen and sulfur in many ores

Date: circa 1961

: any of the elements oxygen, sulfur, selenium, and tellurium

From - *Merriam-Webster's Collegiate® Dictionary*

What is a Chalcogen ?

Group 16

Periodic Table of the Elements

1	IA																0	
1	1											2						
2	3	4	IIA										5	6	7	8	9	10
3	11	12											13	14	15	16	17	18
4	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
5	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
6	55	56	57	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86
7	87	88	89	104	105	106	107	108	109	110								

* Lanthanide Series

+ Actinide Series

58	59	60	61	62	63	64	65	66	67	68	69	70	71
Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
90	91	92	93	94	95	96	97	98	99	100	101	102	103
Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

Chalcogens, essential in biology

Oxygen 42 700 g (in std 70 kg human)

Sulfur 140 g

Selenium 0.0035 g

Tellurium 0 g (toxic)

8 O $1s^2 2s^2 2p^4$

16 S $1s^2 2s^2 2p^6 3s^2 3p^4$

34 Se $1s^2 2s^2 2p^6 3s^2 3p^6 3d^{10} 4s^2 4p^4$

Some Chalcogen Oxidation States in Biology

Oxidation state	Oxygen	Sulfur	Selenium
-2	Oxides H ₂ O	Sulfides H ₂ S Thiols Cys-SH, GSH Thioethers R-S-R Methionine	Selenides H ₂ Se Selenocysteine Cys-SeH
-1	Peroxides H-O-O-H R-O-O-H Oxyl radical RO [•]	Disulfides RSSR Thiyl radical RS [•]	Selenylsulfides Cys-Se-S-Cys Selenyl radical RSe [•]
-0.5	Superoxide O ₂ ^{•-}	Disulfide radical anion RSSR ^{•-}	

Chalcogen Oxidation States,

continued

0	Oxygen O ₂	Sulfur S Sulfenic acid R-SOH, (R-S ⁺) Sulfoxide RS(O)R	Selenium Se Selenocysteine selenol Cys-SeOH,
+2		Sulfinic acid RS(O)OH	Selenocysteine selenic acid Cys-Se(O)OH
+4		Sulfonic acid R-S(O) ₂ OH Sulfone R-S(O) ₂ R	

Reduction of Dioxygen



One electron at a time:



$$E^\circ \zeta = -160 \text{ mV}$$

or (-330 mV)



$$\text{p}K_a = 4.7$$

$\text{O}_2^{\cdot-}$ is, in general, reducing

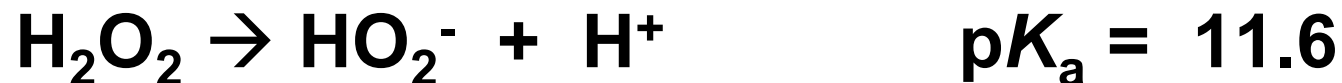
HO_2^{\cdot} is, in general, oxidizing

$$E^\circ \zeta = +1060 \text{ mV}$$

Reduction of Dioxygen, the 2nd electron



The second electron:



H_2O_2 is, in general, oxidizing $E^\circ_{2\text{e}^-} = +1320 \text{ mV}$

Reduction of Dioxygen, the 3rd electron



The 3rd electron:



$\text{HO}\cdot$ is very oxidizing

$$E^\circ\zeta = +2310 \text{ mV}$$



$$\text{p}K_a = 11.9$$

Reduction of Dioxygen, the 4th electron



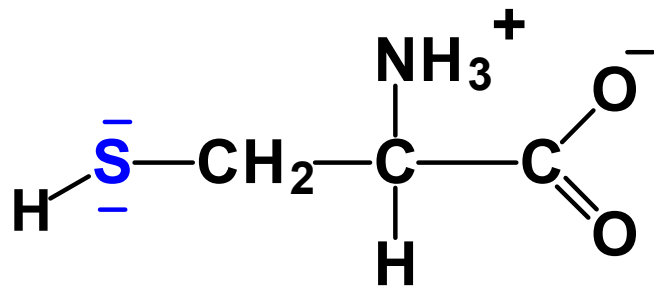
The 4th electron:



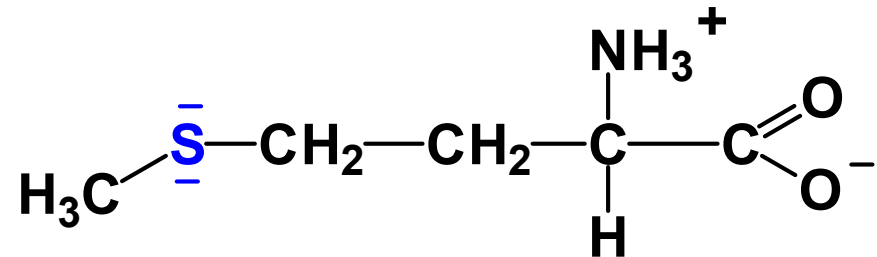
H₂O is quite stable.

Sulfur in Biology

Found in two amino acids: Cysteine and Methionine



cysteine



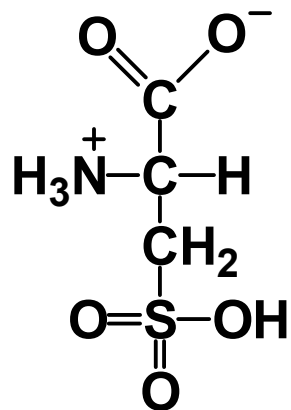
methionine

$$\text{p}K_a (\text{S-H}) = 8.3$$

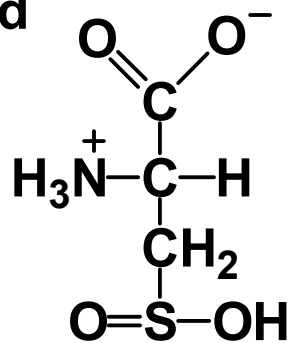
Some oxidation states of sulfur

Formula	Name	Ox state of S
R-SH	thiol	-2
RS [•]	thiyl radical	-1
RSSR	disulfide	-1
RSSR ^{•-}	disulfide radical anion	-0.5
R-SOH (RS ⁺)	sulfenic acid	"0"
R-S(O)OH	sulfinic acid	+2
R-S(O) ₂ OH	sulfonic acid	+4
H ₂ S	hydrogen sulfide	-2
S	sulfur	0
SO	sulfur monoxide	+2
SO ₂	sulfur dioxide	+4
SO ₃ ²⁻ /H ₂ SO ₃	sulfite/sulfurous acid	+4
SO ₃	sulfur trioxide	+6
SO ₄ ²⁻ /H ₂ SO ₄	sulfate/sulfuric acid	+6

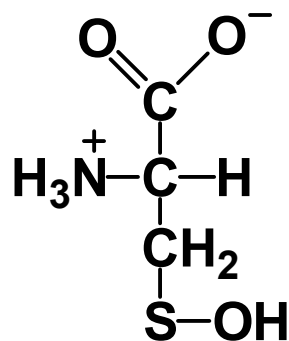
Oxidation of Cysteine



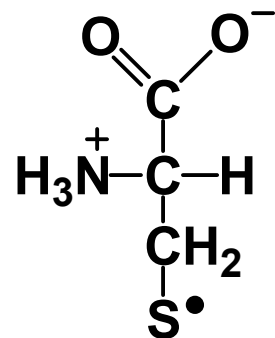
cysteine
sulfonic acid
+4



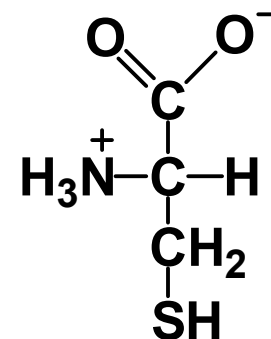
cysteine
sulfinic acid
+2



cysteine
sulfenic acid
0

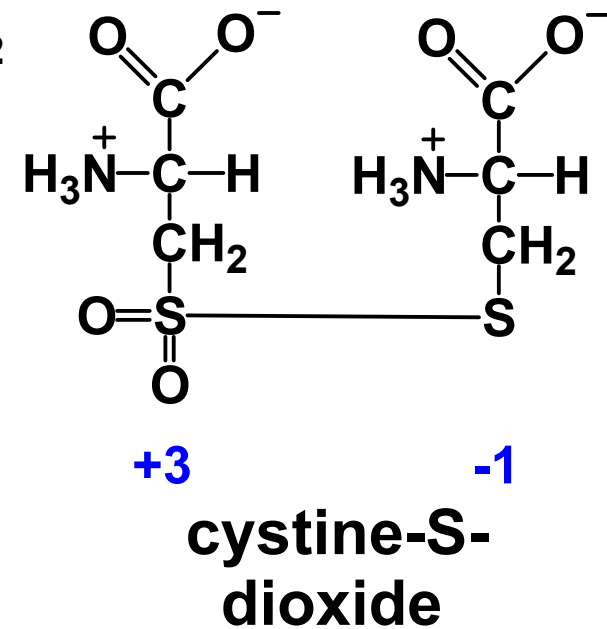
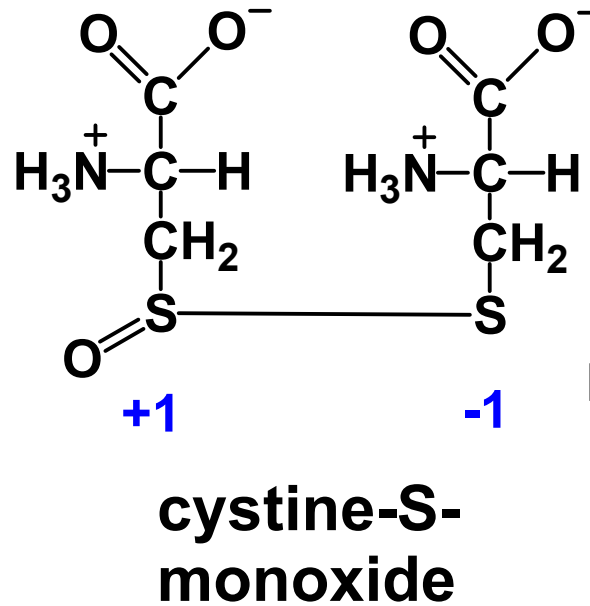
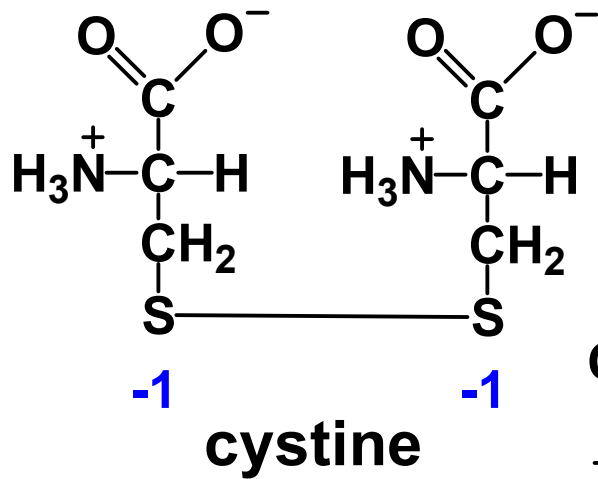


cysteinyl
radical
-1



cysteine
-2

Cystine and its oxidation



Disulfides

Disulfide formation is an important redox reaction of cysteine



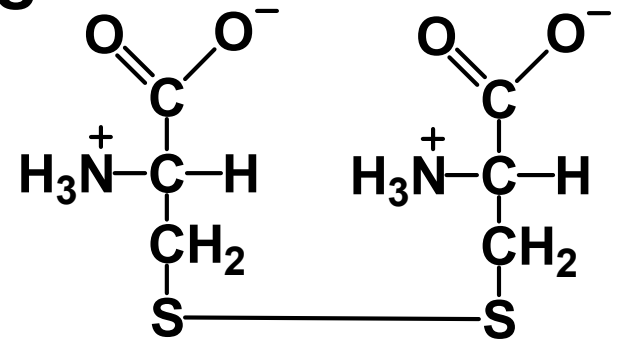
Used:

to form structure of proteins

as redox buffer

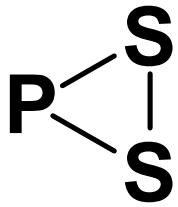
in signaling

to change activity of enzymes



Protein Disulfides

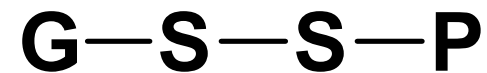
When cysteine residues are oxidized to disulfides, they can form:



**intraprotein
crosslink**



**interprotein
crosslink**

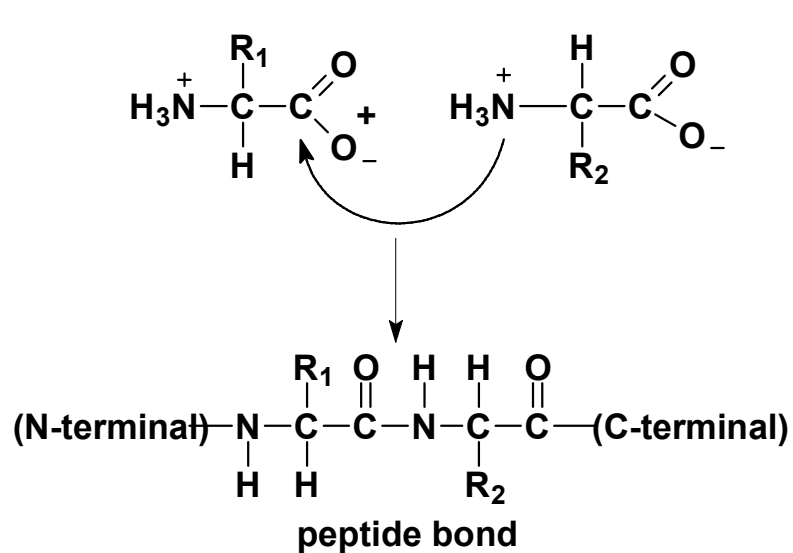


**mixed disulfide
adduct of GSH**

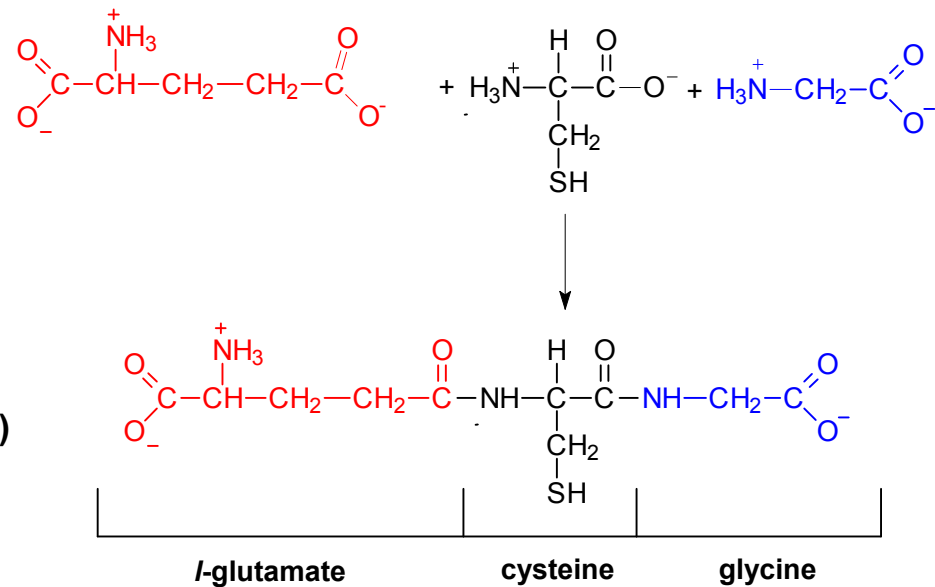
Glutathione, GSH

Note: GSH = *N*-(*N*-*L*- γ -glutamyl-*L*-cysteinyl)glycine
(C.A.S. NUMBER: 70-18-8)

Tripeptide with unusual structure.



Standard Peptide Bond



glutathione
Glutathione (GSH)

Some general reactions of thiols

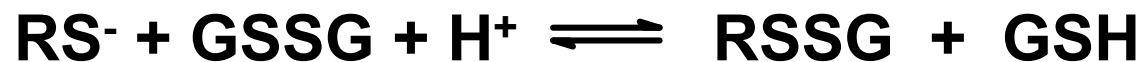


The thiol-disulfide exchange reaction



These reactions are probably the most common reactions of cysteine residues *in vivo*.

However, the actual reaction may be better written as:



It is the ionized form that is often the dominant player in the reaction.

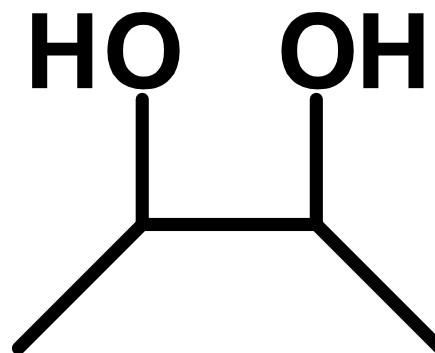
Redox Couple (2-electron reductions)	E° /mV at 25° C
DTTox, 2H ⁺ /DTT	-317 ^a
NADP ⁺ , H ⁺ /NADPH	-315
Lipoic acid, 2H ⁺ /Dihydro-LA acid	-290
Trx(SS), 2H ⁺ /Trx(SH) ₂	-270
GSSG, 2H ⁺ /2GSH	-240
Grx-1(SS), 2H ⁺ /Grx-1(SH) ₂	-233
Cys-S-S-Cys, 2H ⁺ /2Cys-SH	-230
Grx-3(SS), 2H ⁺ /Grx-3(SH) ₂	-198
PDI(SS), 2H ⁺ /PDI(SH) ₂	-125
DHA, H ⁺ /Ascorbate	+54
CoQ, 2H ⁺ /(CoQH ₂)	+84

**Some two-
electron
reduction
potentials**

Vicinal thiols, Vicinal

Vicinal: from Latin *vicinalis*, neighboring.

In chemistry, vicinal means neighboring groups, e.g. vicinal alcohol groups.



Vicinal thiols

In a protein, vicinal has been translated to mean two intervening amino acids, *e.g.*, **-CXXC-** would be two cysteines separated by two intervening amino acids.

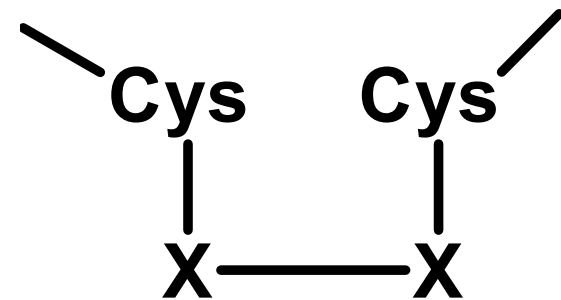
The **-CXXC-** motif of thiol:disulfide oxidoreductases is now widely recognized as being essential for the catalysis of thiol redox reactions:

Thioredoxin -Cys-Gly-Pro-Cys-

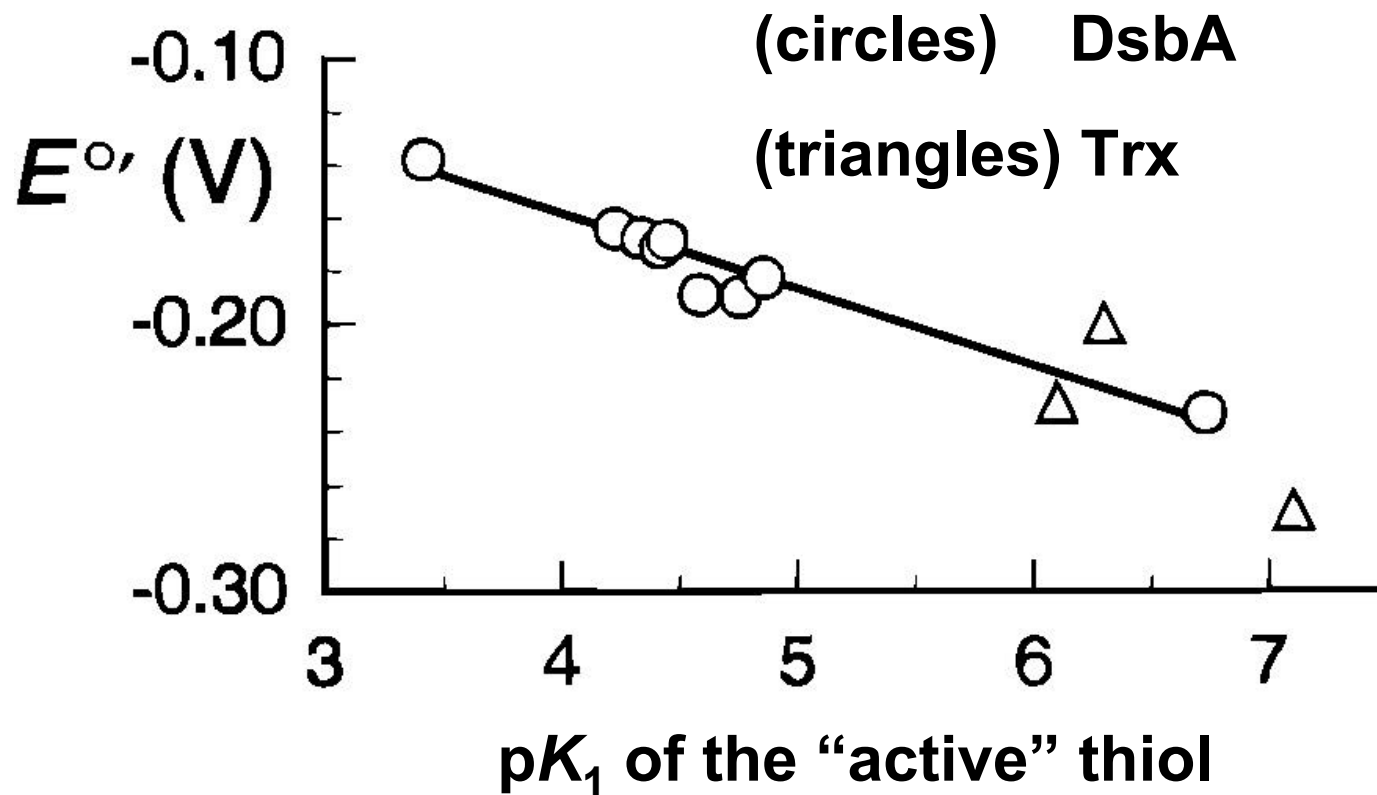
Glutaredoxin -Cys-Pro-Trp-Cys-

PDI -Cys-Gly-His-Cys-

DsbA -Cys- Pro-His-Cys-



The reduction potential and the pK_a of the active thiol is key in the function of di-thiol proteins

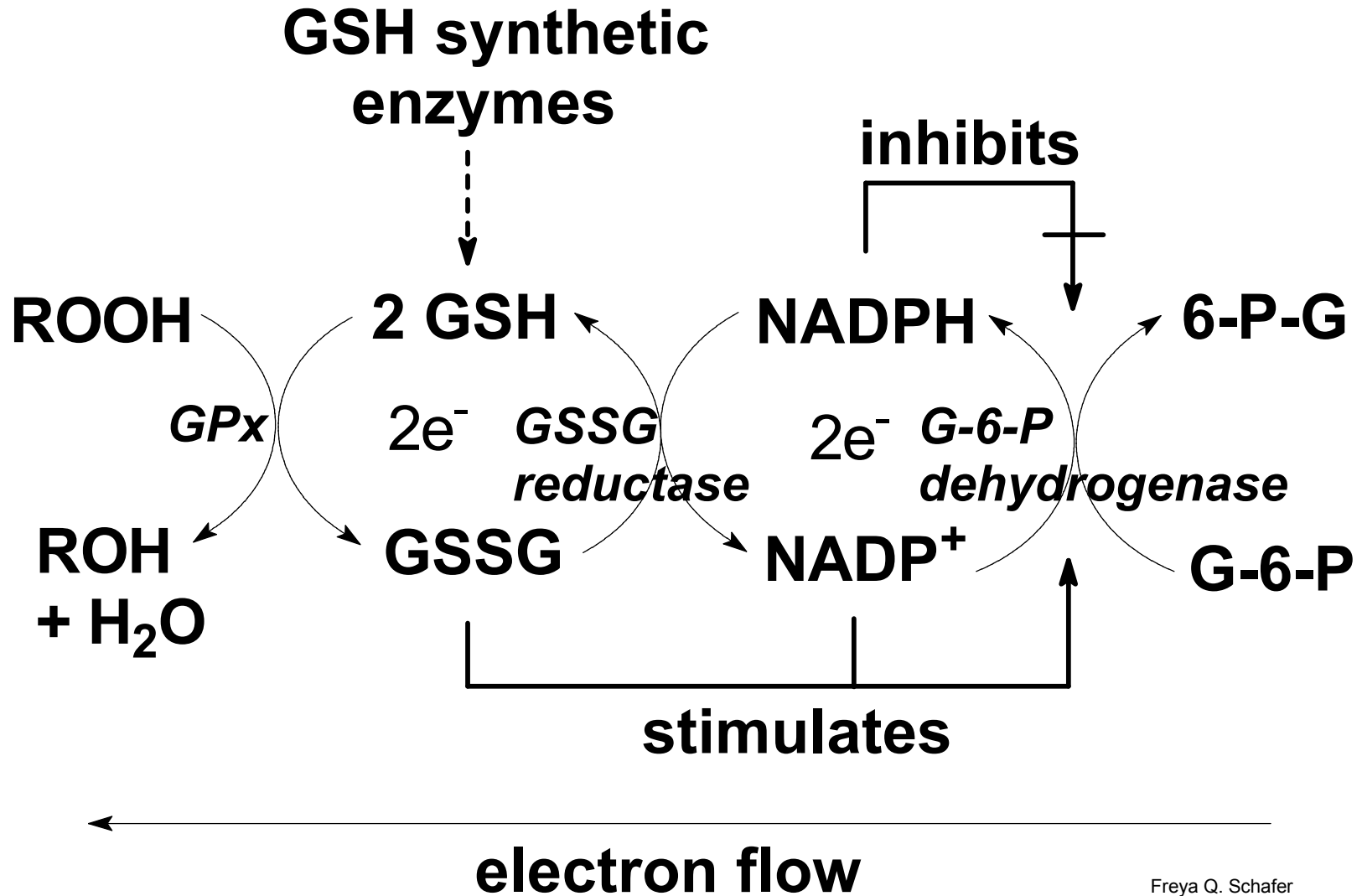


Chivers PT, Prehoda KE, Raines RT. (1997) The CXXC motif: a rheostat in the active site. *Biochemistry*. **36**:4061.

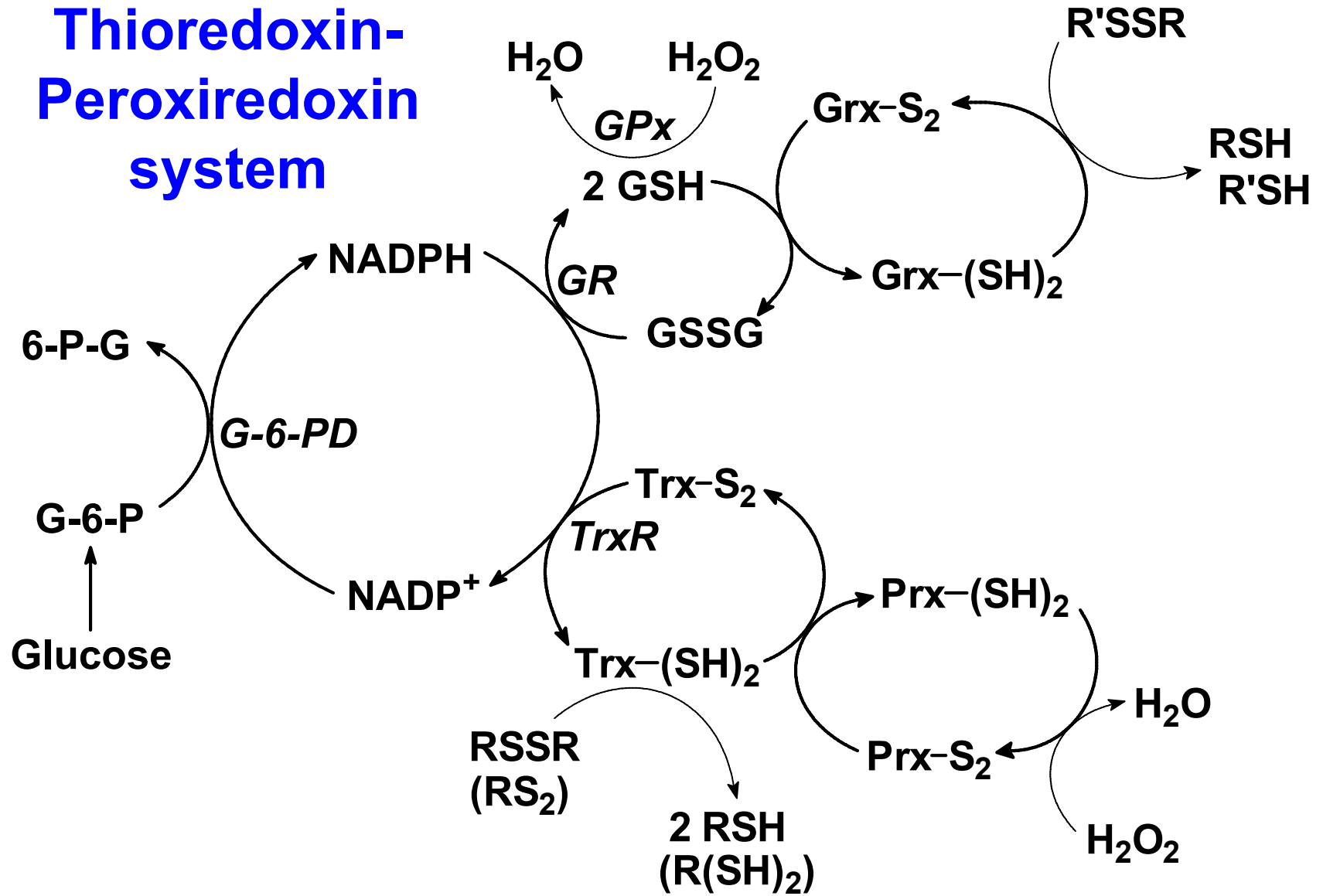
**A primary function of the thiol
system is to remove
hydroperoxides**



GSH system and peroxide removal



Thioredoxin- Peroxioredoxin system



Thiols as switches

Thiols are major contributors to the redox environment of cells.

They are involved in the function and control of many proteins.

Thiols and disulfides as nano-switches

A nano-switch is a very small, operating on a nanometer scale.

- For example, the **distance between two sulfhydryls** in a protein with two intervening amino acids, such as in thioredoxin.
- The redox environment of the cell, or regions within a cell, could be viewed as a means to activate a **cellular switchboard**, thereby changing the message of cellular signals.
- By changing the reduction potential, a series of nano-switches are activated/de-activated that move the cell from proliferation through various stages of proliferation, differentiation or into apoptosis.
- Necrosis is the complete loss of the ability to activate/de-activate and respond to changes in these nano-switches.

Type I switch



$$K = \frac{[\text{GSH}] [\text{PSSG}]}{[\text{GSSG}] [\text{PSH}]}$$

For the GSSG, 2H⁺/2GSH couple

$$E_{\text{hc}} = -240 - (59.1/2) \log ([\text{GSH}]^2/[\text{GSSG}]) \text{ mV} \quad \text{at } 25^\circ\text{C, pH } 7.0$$

If [GSH] = 5 mM and [GSSG] = 25 μM, then $E_{\text{hc}} = -240$ mV.

If [GSH] decreases to 2.5 mM and [GSSG] increases to 100 μM, a +35 mV change will occur resulting in $E_{\text{hc}} = -205$ mV.

The [PSSG]/[PSH] ratio will change by a factor of 8, resulting in an 8-fold change in the status of the switch.

Type II switch



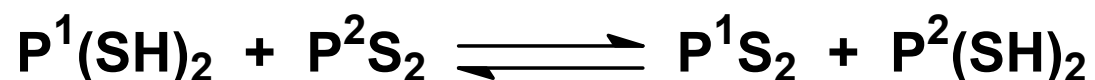
$$K = \frac{[\text{GSH}]^2 [\text{PSS}]}{[\text{GSSG}] [\text{P(SH)}_2]}$$

Using the example for the Type I switch, this same +35 mV change in the GSSG/2GSH couple will result in a change of the [PSS]/[P(SH)₂] ratio by a factor of 16. Thus, a Type II switch translates changes in reduction potential into a 2-fold greater change in the status of the nano-switch, compared to a Type I switch.

Schafer FQ, Buettner GR. (2001) Redox state of the cell as viewed through the glutathione disulfide/glutathione couple. *Free Radic Biol Med.* **30**:1191-1212.

Schafer FQ, Buettner GR. (2003) Redox state and redox environment in Biology. In *Signal Transduction by Reactive Oxygen and Nitrogen Species: Pathways and Chemical Principles*. Eds Forman HJ, Torres M, Fukuto J. Kluwer academic Publishers, Dordrecht, Netherlands, Chapter 1, pp. 1-14.

Summary



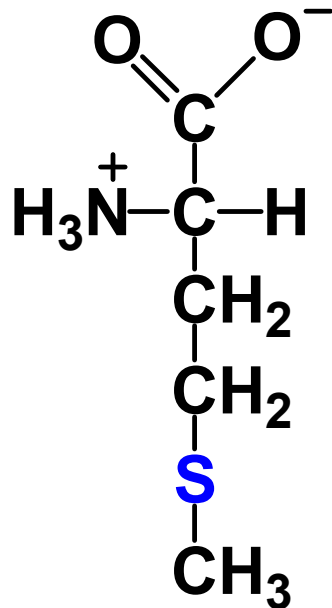
In a cell there is are many copies of proteins, and several components to a signal. Thus, the “state” of a switch is due to the ensemble. It is the ensemble that results in an action like a rheostat.

Overviews:

Nathan, Carl (2003) Specificity of a third kind: reactive oxygen and nitrogen intermediates in cell signaling. *J. Clin. Invest.* **111**:769–778.

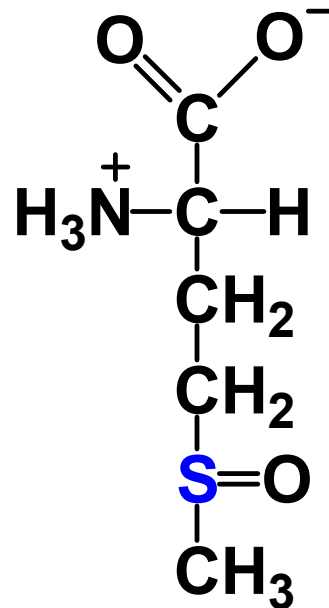
Cooper CE, Patel RP, Brookes PS, Darley-Usmar, VM, (2002) Nanotransducers in cellular redox signaling: modification of thiols by reactive oxygen and nitrogen species, *Trends in Biochemical Sciences.* **27**: 489-492.

Methionine



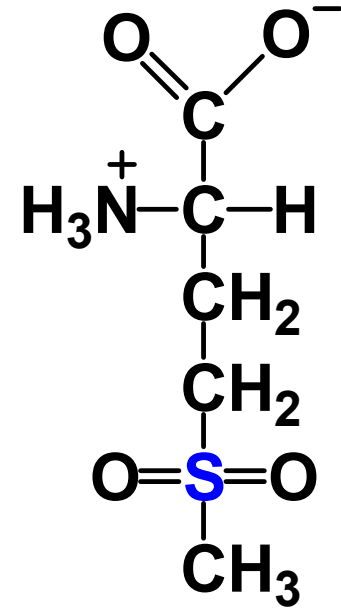
(-2)

methionine



(0)

methionine
sulfoxide



(+2)

methionine
sulfone

Oxidation



Oxidation of methionine

The start codon AUG for protein synthesis encodes methionine, thus this amino acid is found in most proteins.

Methionine is considered to be an antioxidant. Methionine residues on protein surfaces can protect against oxidation of other amino acids that are important for the function of the proteins.

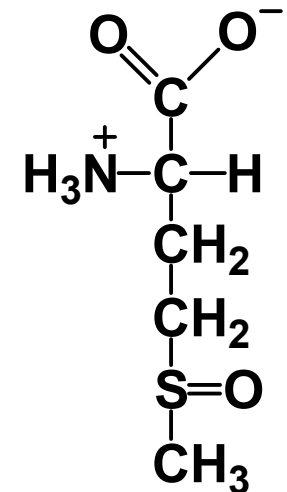
If a protein with a spherical diameter of 60 Å has 8 methionine residues, then the concentration of methionine in that volume is **>100 mM** (cellular [GSH] is 1 – 10 mM).

Jacob C, Giles GI, Giles NM, Sies H. (2003) Sulfur and Selenium: The Role of Oxidation State in Protein Structure and Function. *Angewandte Chemie International Edition*. **42**: 4742-4758.

Oxidation of methionine

In some enzymes methionine can play the role of a **sacrificial lamb**, e.g. glutamine synthetase; oxidation of 8 of the 16 methionine residues to Met(O) did not effect the activity of the enzyme. They were on the surface and appeared to guard the active site of the enzyme.

Methionine residues can also be essential for the activity of proteins.

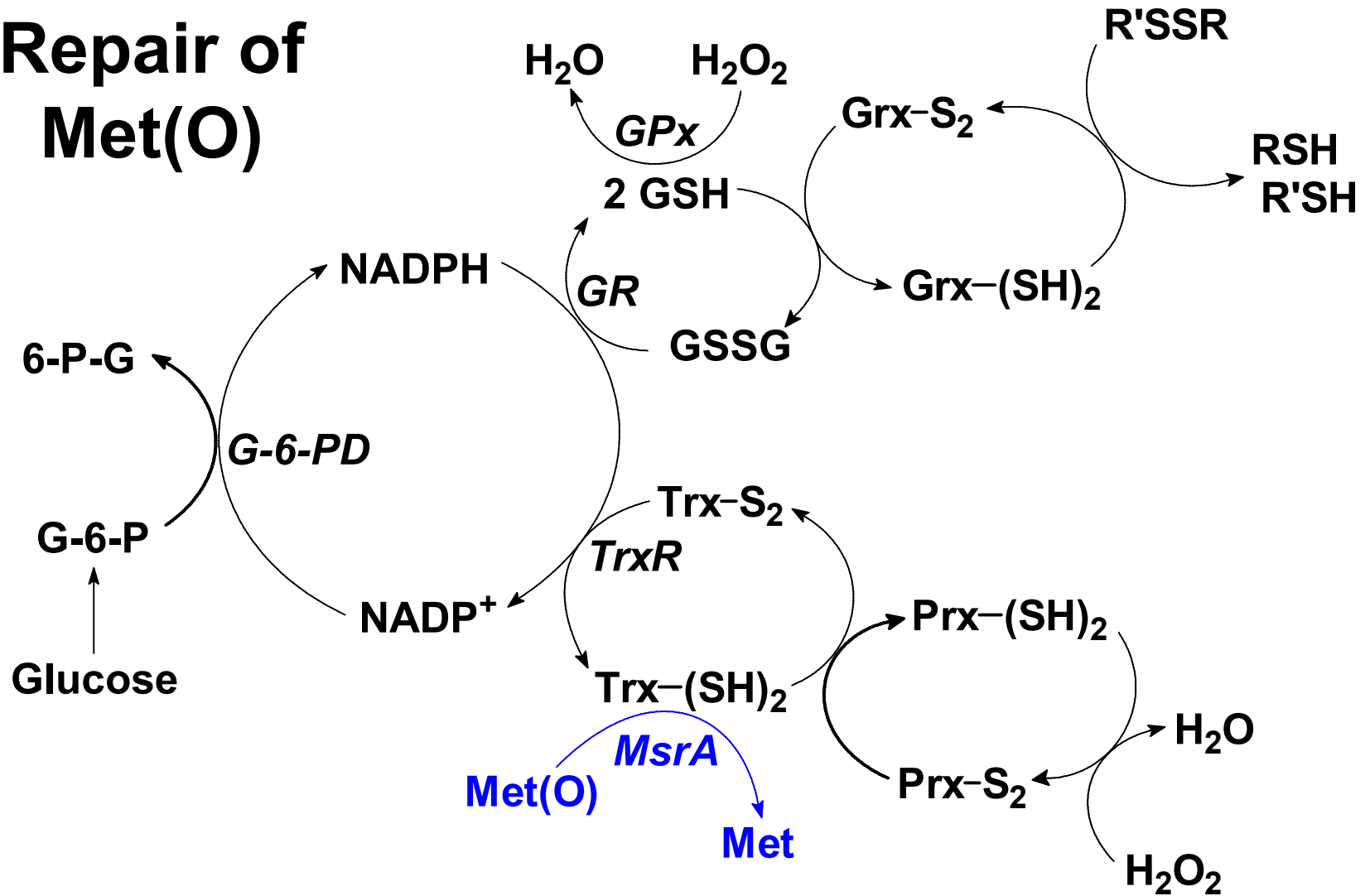


**methionine
sulfoxide**

Met(O)

Levine RL, Mosoni L, Berlett BS, Stadtman ER. (1996) Methionine residues as endogenous antioxidants in proteins. *PNAS*. **93**:15036-40.

Repair of Met(O)

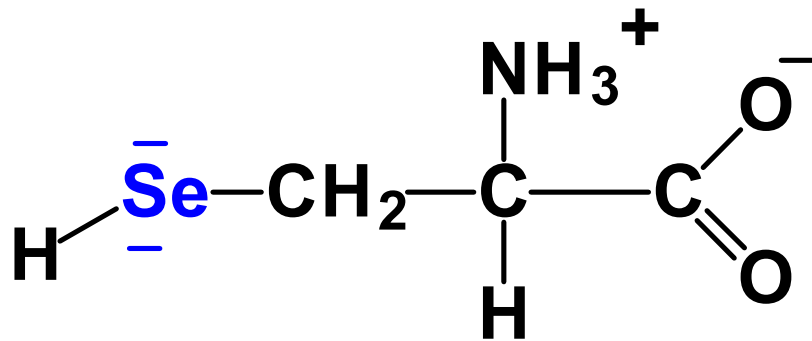


Methionine sulfoxide reductase, MsrA and MsrB

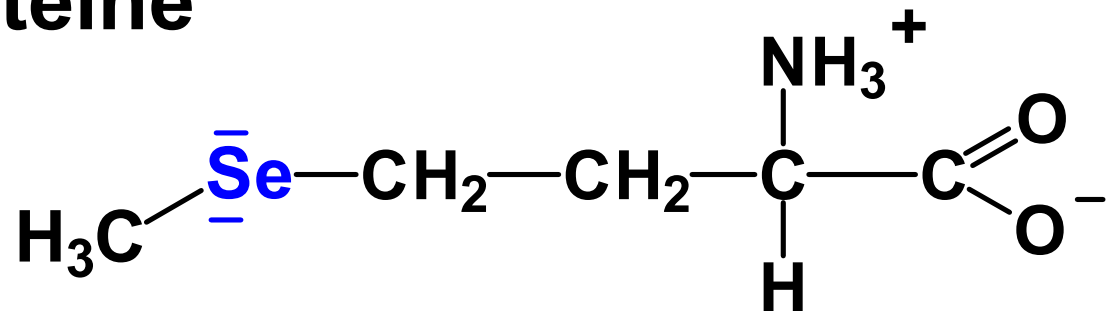
Freya Q. Schafer
2003

Weissbach H, Etienne F, Hoshi T, Heinemann SH, Lowther WT, Matthews B, St John G, Nathan C, Brot N. (2002) Peptide methionine sulfoxide reductase: structure, mechanism of action, and biological function. *Arch Biochem Biophys.* **397**:172-8.

Selenium in proteins



seleno-cysteine



seleno-methionine

Selenocysteine

Selenocysteine: Cys-SeH



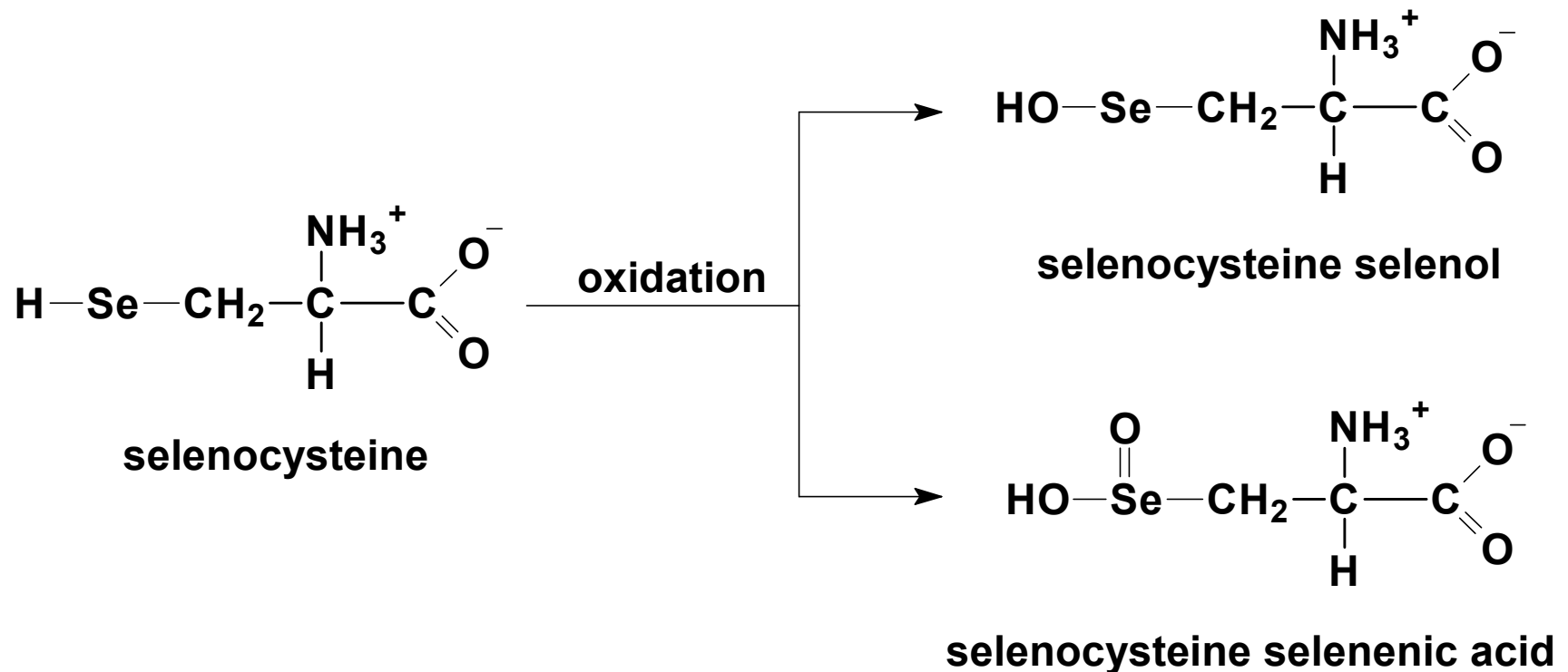
The low $\text{p}K_a$, and the much greater nucleophilicity of Cys-Se^- renders selenocysteine catalytically much more reactive than Cys-SH.

Cys-Se⁻ is at the active site of **GPx, TrxR, ...**

At pH 7.4 - Cys-SH \gg 90%, Cys-Se⁻ \gg 99.9%

Oxidation of selenocysteine

When oxidized, selenocysteine forms a selenol or selenenic acid rather than di-selenides.



Selenylsulfides

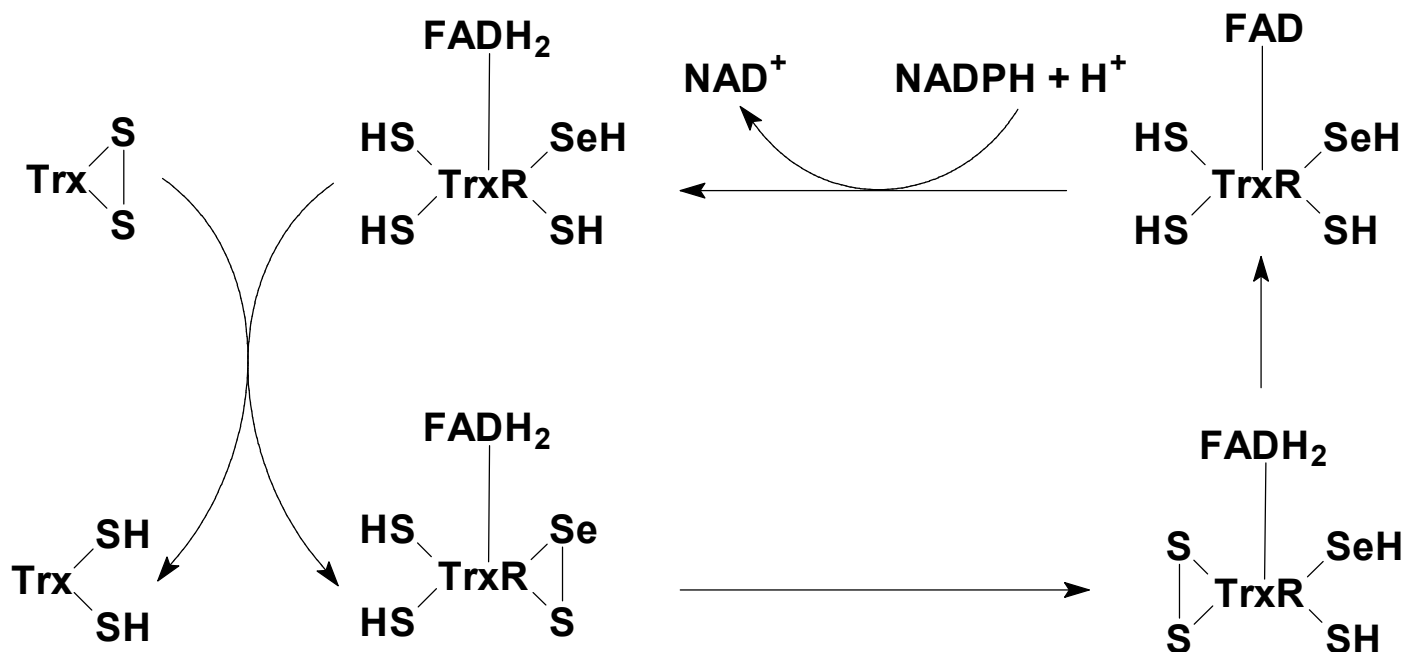


However, selenylsulfides (-Cys-Se-S-Cys-) are found in proteins, e.g. GPx and TrxR.

They are intermediates formed during enzyme function. For example, with GPx the intermediate with GSH is,



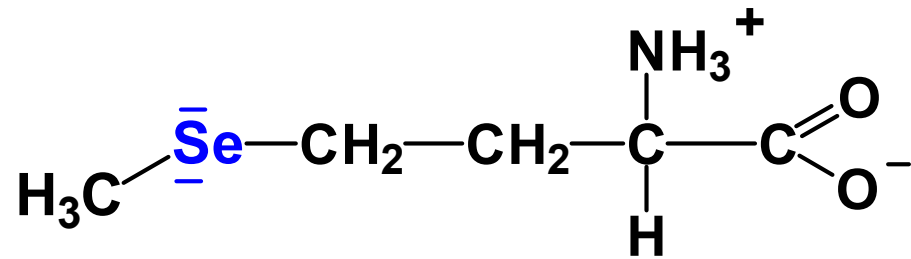
Reduction of a seleno-cysteine



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

Jacob C, Giles GI, Giles NM, Sies H. (2003) Sulfur and Selenium: The Role of Oxidation State in Protein Structure and Function. *Angewandte Chemie International Edition*. **42**: 4742-4758.

Selenometh



seleno-methionine

Selenomethionine is randomly found in proteins and not specifically incorporated as is selenocysteine.

Intake of selenomethionine in animals increases the amount of selenomethionine-containing proteins.

Oxidized to selenosulfoxide or selenosulfones, which can be reduced spontaneously by GSH.

Thus, selenomethionine it might be a defense against permanent protein damage.

Reactive Species

ROS - Reactive **O**xygen **S**pecies

RNS - Reactive **N**itrogen **S**pecies

RSS - Reactive **S**ulfur **S**pecies

The difference is in what element undergoes changes in oxidation state,

O? N? or S?

Reactive Sulfur Species (RSS)

Sulfur can exist in higher oxidation states in biological systems than the -2 of Cys and Met.

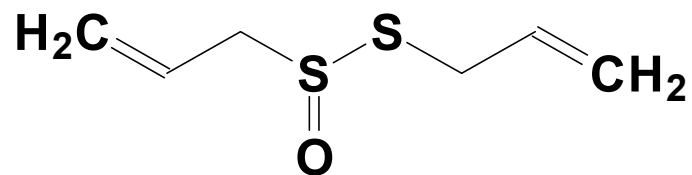
Sulfenic acids

Sulfenic acids (Cys-SOH) found in various proteins, such as GR, FOS and Jun.

Formation of Cys-SOH is reversible (role in signal transduction, oxygen metabolism and transcriptional regulation).

Sulfenic acids seem to be transient intermediates. They react with other thiols to form disulfides.

Reactive Sulfur Species (RSS)



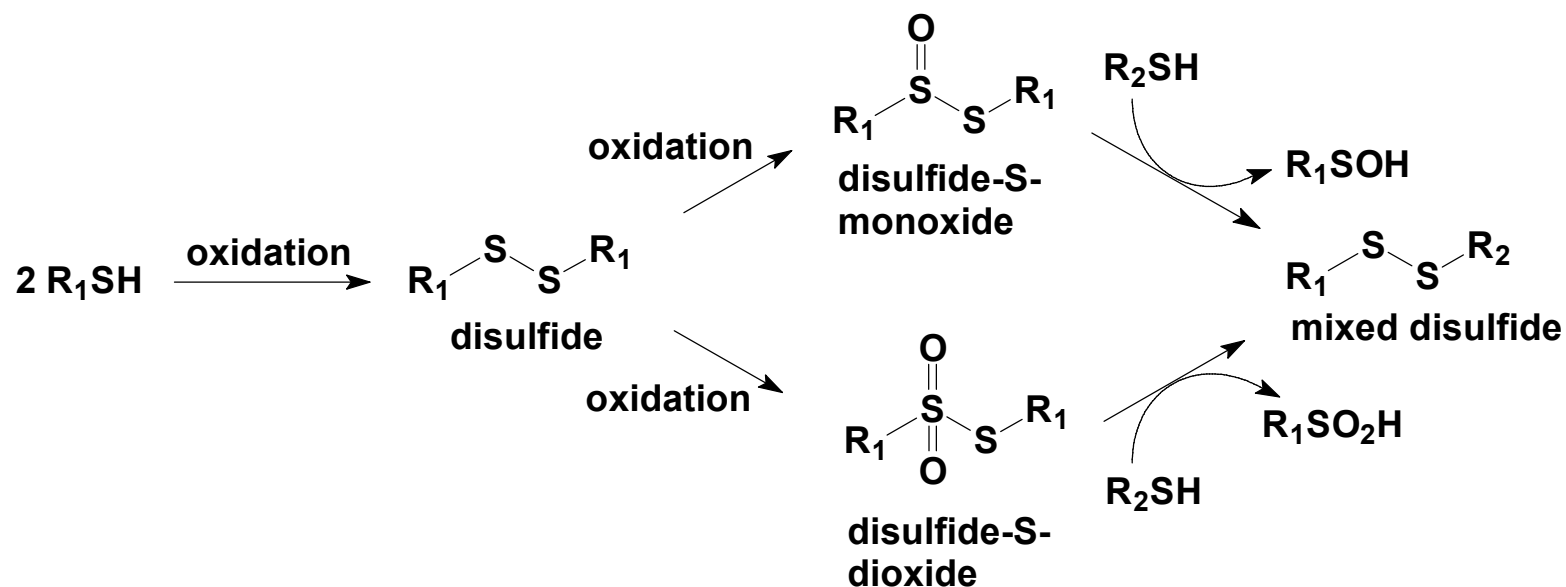
diallyldisulfide-S-monoxide

Disulfide-S-oxides

One sulfur of a disulfide bond can be further oxidized increasing the reactivity of the compound towards sulfur-sulfur exchange reaction.

The garlic component allicin contains a diallyldisulfide-S-monoxide that is thought to be responsible for the antimicrobial properties of garlic.

Reactive Sulfur Species (RSS)



Formation of disulfide-S-oxide and subsequent sulfur-sulfur exchange. These RSS can be formed by reaction with various ROS and RNS (e.g. peroxides, peroxynitrite). The oxidation of one sulfur in the disulfide makes the bond more labile and enhances the reaction with a reduced thiol.

Thiol reagents as research tools

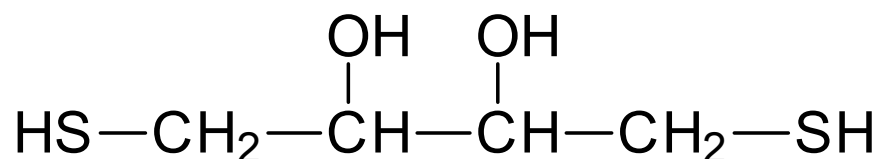
DTT

Diamide

Lipoic acid

Arsenic

Thiol reagents, Dithiothreitol

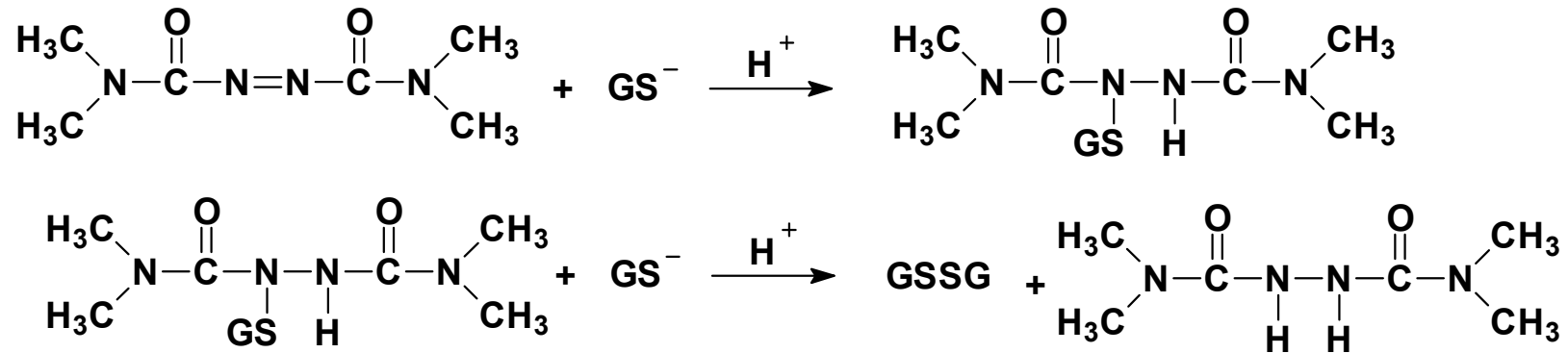


dithiothreitol
DTT

Dithiothreitol, also known as Cleland's Reagent, is one of the most common agents used to reduce disulfide bonds. DTT is used at low concentrations to stabilize enzymes, antibodies *etc.* that have thiol groups.

At high concentrations it is used to cleave disulfide bonds and thereby denature proteins. However, reagents that react faster than DTT are now available.

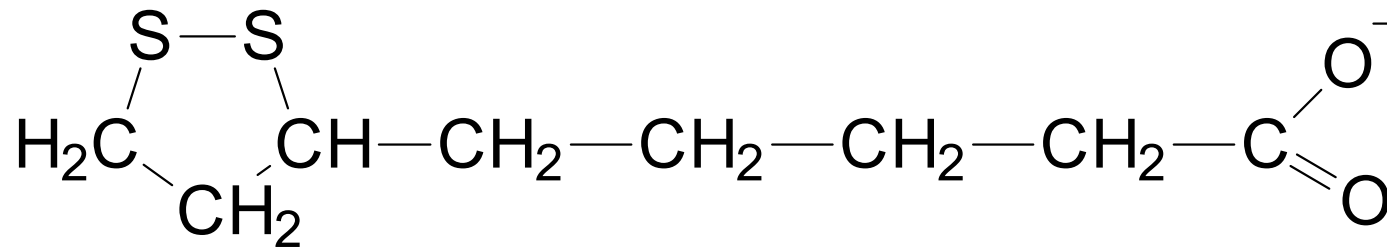
Thiol reagents, diamide



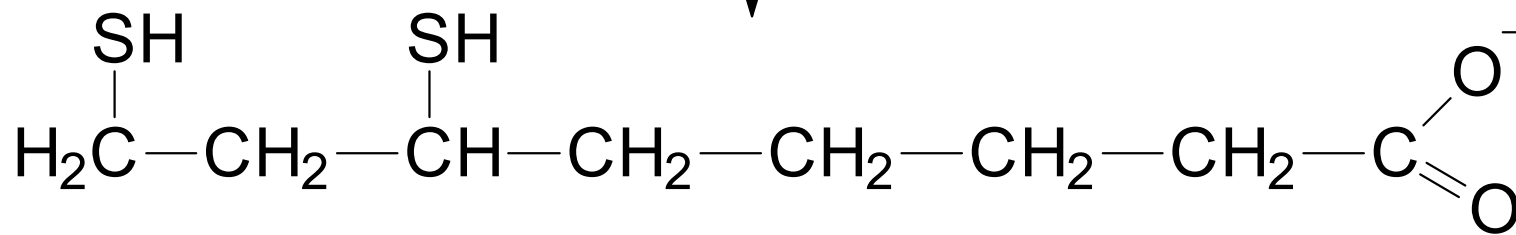
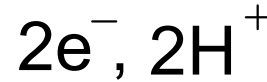
Acidic, low molecular weight thiols are oxidized in preference to protein thiols. Protein thiols are in general less acidic and sterically hindered. Some proteins such as hemoglobin A or albumin, react very slowly with diamide if at all, while other proteins such as thioredoxin or rat hemoglobin react with diamide or the diamide-SG intermediate to form disulfides or mixed disulfides.

Diamide penetrates cell membranes within seconds. The activation energy for the reaction of thiols with diamide is low. Thus, diamide can be used at low temperatures. Glutathione, as the major non-protein thiol in cells, will preferentially react with diamide. At higher diamide concentration protein thiols will also be oxidized.

Lipoic acid is not quite a vicinal thiol



Lipoate



Dihydrolipoic acid

Arsenite, a vicinal thiol reagent

Arsenite (As(III)):

Arsenic has been used as a therapeutic agent and poison for over 2000 years. The solid form As_4O_6 , generally referred to as arsenic trioxide, dissolves in base to yield arsenite ions such as $[\text{AsO}(\text{OH})_2]^-$, $[\text{AsO}_2(\text{OH})]^{2-}$, and $[\text{AsO}_3]^{3-}$.

(Arsenic (group 15 element) is under N and P in the periodic table.)

Arsenite binds to vicinal thiols and this may well be central to its mechanism of action.

The End

H₂O Essential for life; we swim in it.

H₂S **IDLH*** » **100 ppm**

H₂Se **IDLH** » **1 ppm**

What a difference a few electrons make.

*IDLH = Immediately dangerous to life and health