



**2008 Sunrise Free Radical School  
Presentation by:  
Neil Hogg, Ph.D.**

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# The ABCs of Thiol Biological Chemistry

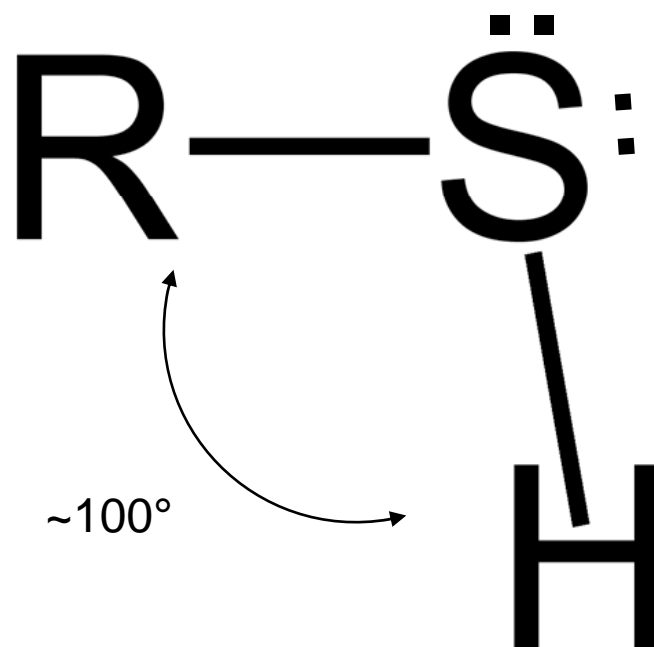
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Neil Hogg

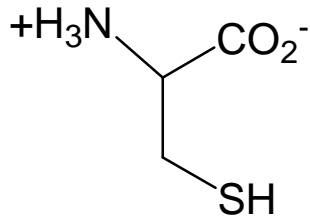
Medical College of Wisconsin

# What is a Thiol:

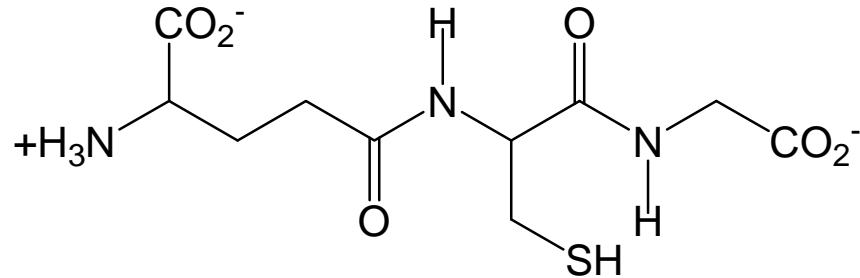
- A **thiol** is a compound that contains the functional group composed of a sulfur atom and a hydrogen atom (-SH). Being the sulfur analogue of an alcohol group (-OH), this functional group is referred to either as a *thiol group* or a *sulfhydryl group*. Old name: **mercaptans** due to affinity for mercury.



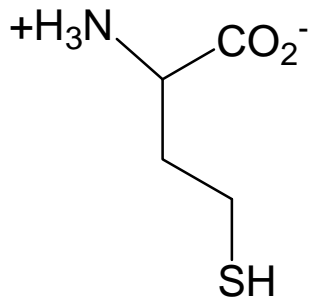
# Thiols of Interest to Biology



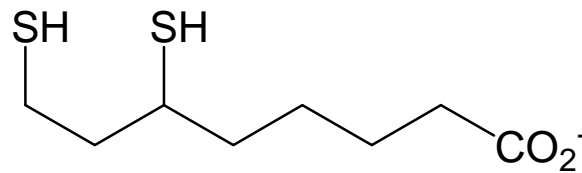
Cysteine



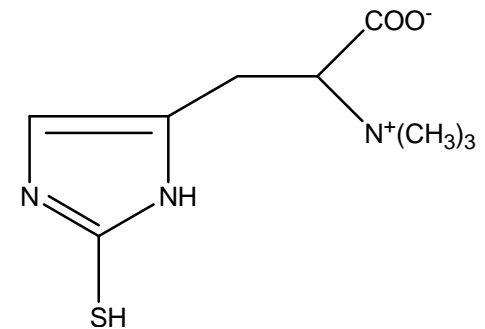
Glutathione



Homocysteine



Lipoic acid (a dithiol)



Ergothionine

# Thiol pKa is a primary determinant of reactivity



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

thiol	pKa	%RS <sup>-</sup> at pH 7.4
H <sub>2</sub> S	~7.1 (to form HS <sup>-</sup> )	~67%
cysteine	~8.5, 8.9 (-NH <sub>3</sub> <sup>+</sup> , -NH <sub>2</sub> )	~4.5%
glutathione	~8.9, 9.1 (-NH <sub>3</sub> <sup>+</sup> , -NH <sub>2</sub> )	~2.5%

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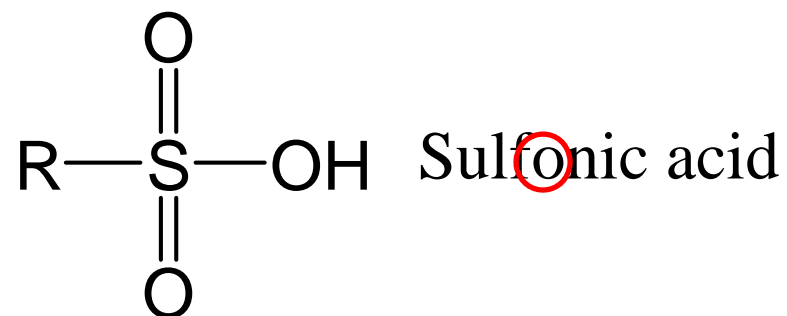
# Why is pKa so important?

- Relative nucleophilicity:



- Thiolates can react many times faster than their corresponding thiols with electrophilic targets.
  - Thiolates are the most nucleophilic functional groups in proteins and are important in catalytic activity, substrate recognition and allosteric redox regulation.
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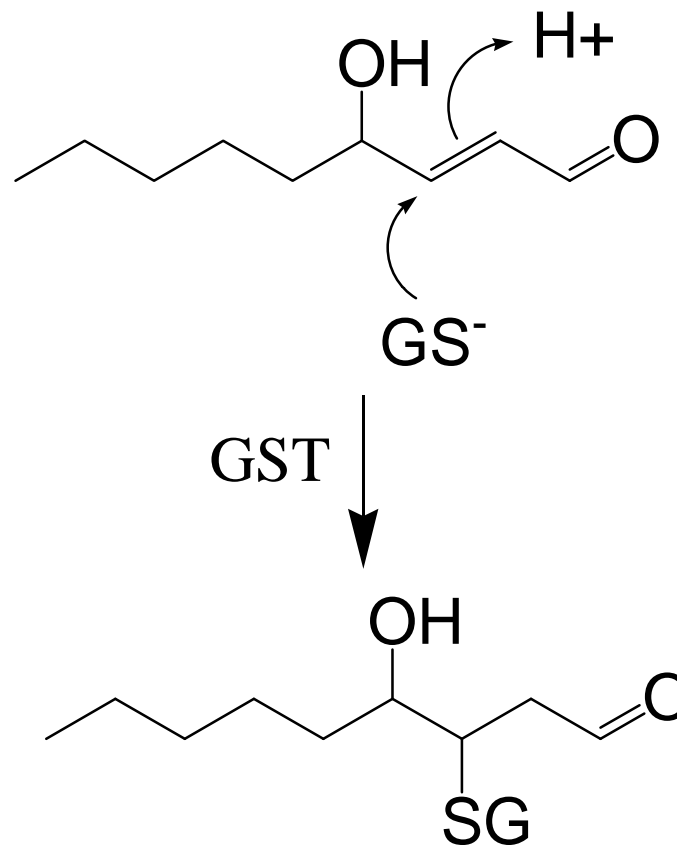
# Thiol oxidation products



Some of the more important reactions of thiols in biology (I):

## Electrophile adduction and detoxification

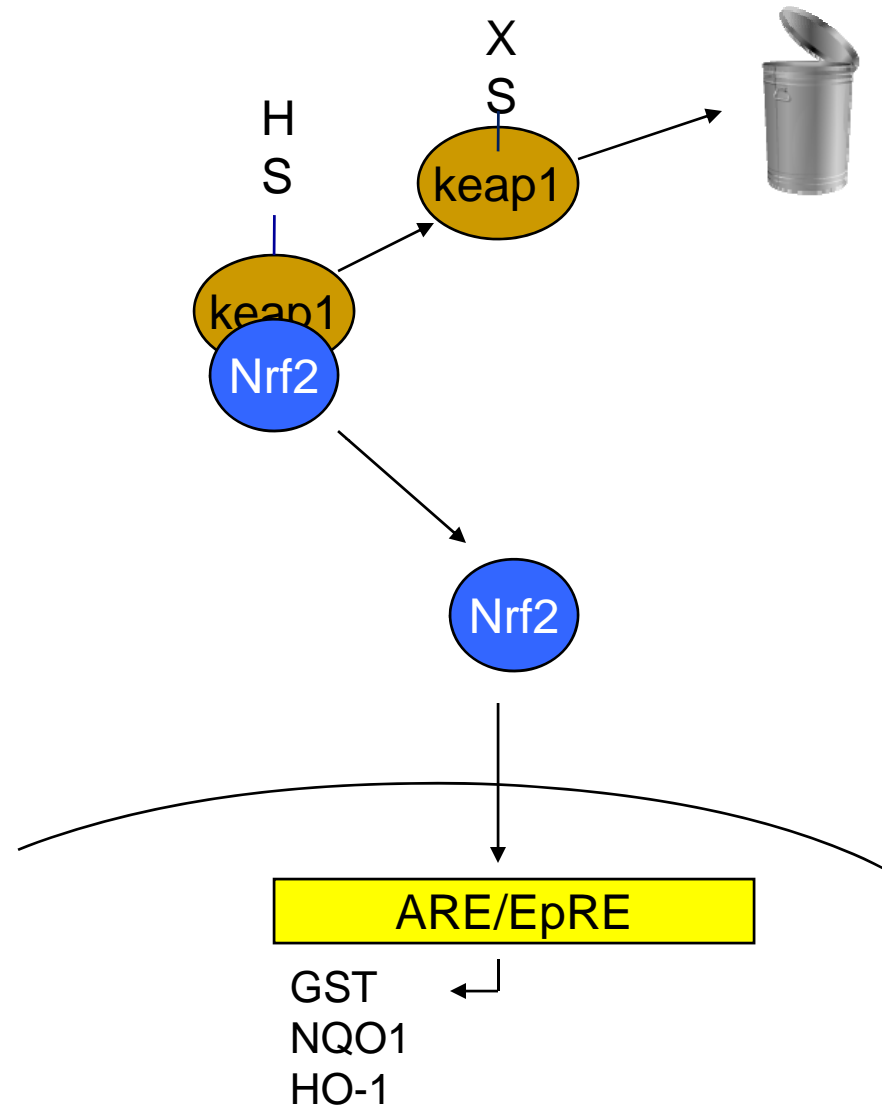
- Glutathione-S-transferase catalyzed xenobiotic/oxidation product removal (e.g 4-hydroxynonenal).
- Protect cells from “electrophilic stress” – addition of electrophiles (xenobiotic metabolites and lipid oxidation products) to nucleophilic centers of proteins and DNA





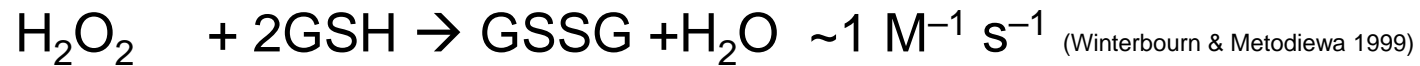
# Electrophile adduction and detoxification

- Keap1 is a 'sentinel' protein for electrophilic stress.

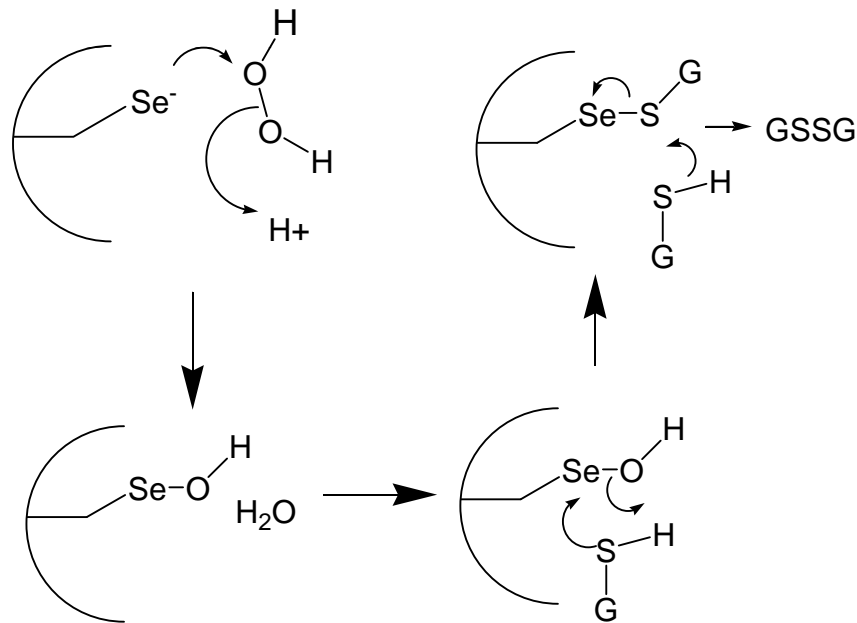


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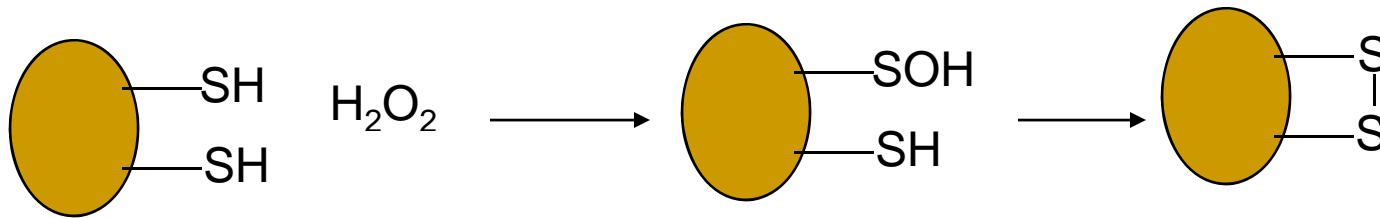
Some of the more important reactions of thiols in biology (II):  
**Hydrogen peroxide removal/detection**



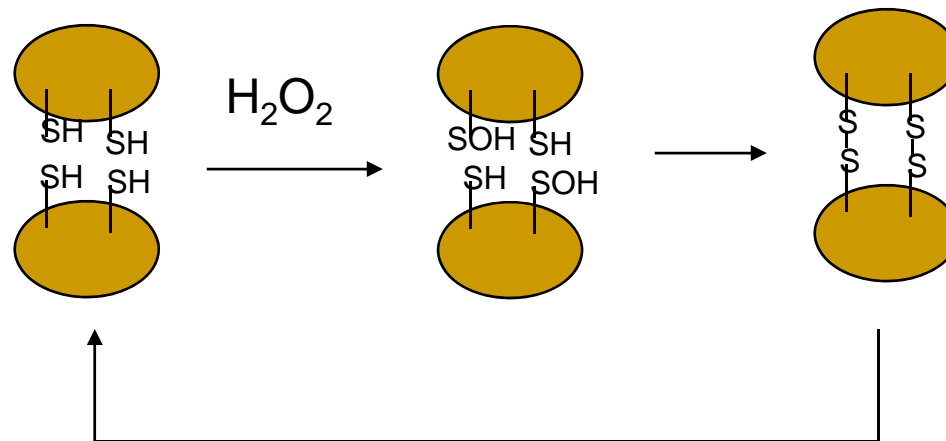
Biologically too slow without catalysis by glutathione peroxidase (a selenate)



# Dithiol oxidation:



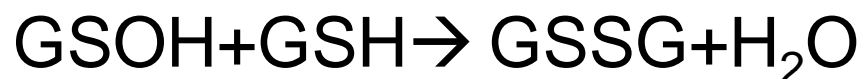
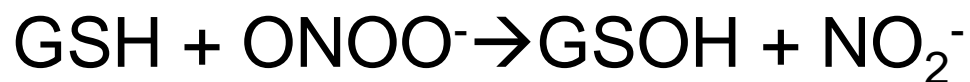
2 Cys Peroxyredoxins



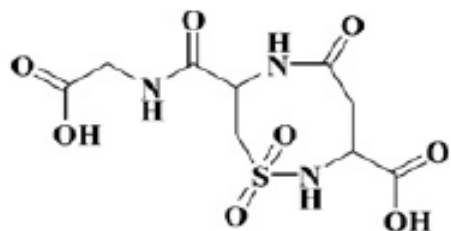
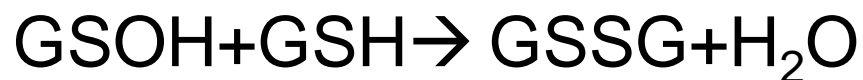
Thioredoxin

## Some of the more important reactions of thiols in biology (III): Other non-radical oxidant scavenging reactions

- Peroxynitrite ( $k \approx 10^3 \text{ M}^{-1}\text{s}^{-1}$ )



- Hypochlorite ( $k \approx 10^7 \text{ M}^{-1}\text{s}^{-1}$ )



Glutathione sulfonamide (GSA)



(Harwood et al, Biochem J, 2006)

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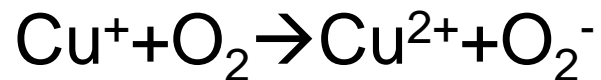
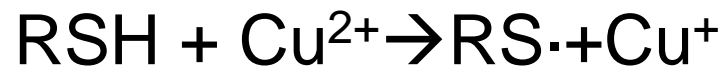
Some of the more important reactions of thiols in biology (III):

## Thiol auto-oxidation

- Thiols as a source of oxidants



- Requires transition metal catalysis



- Relevance to biology/pathology depends availability of redox active metal ions.
  - Probably major culprit in thiol toxicity to cells in culture where metal ions are not well controlled in the cell culture medium (e.g. cellular studies on Homocysteine toxicity)
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# Thiol mixtures auto-oxidize at the speed of the most reactive thiol due to thiol disulfide exchange

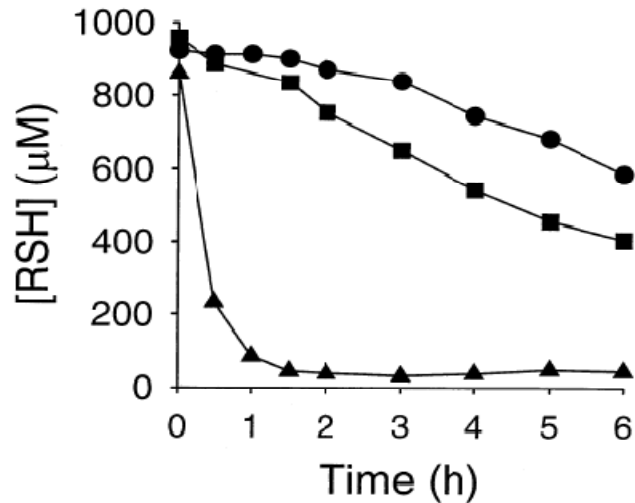


Fig. 1. Autoxidation of thiols. Glutathione (●, 1 mM), homocysteine (■, 1 mM), and cysteine (▲, 1 mM) were each incubated in phosphate buffer (50 mM, pH 7.4) at 37°C. Aliquots were removed at various time intervals and thiol content was determined using the DTNB assay.

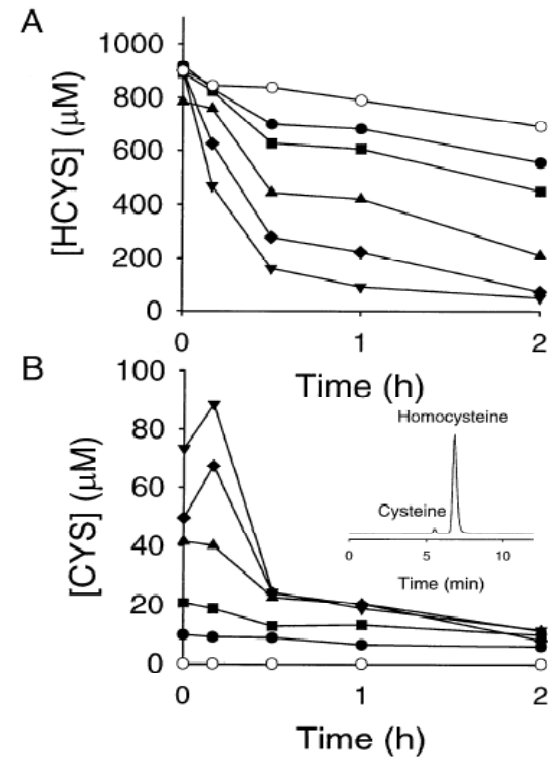
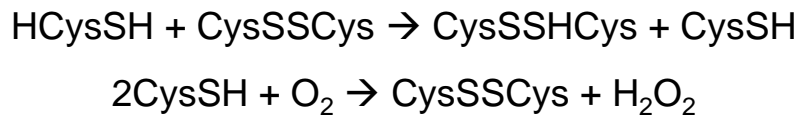


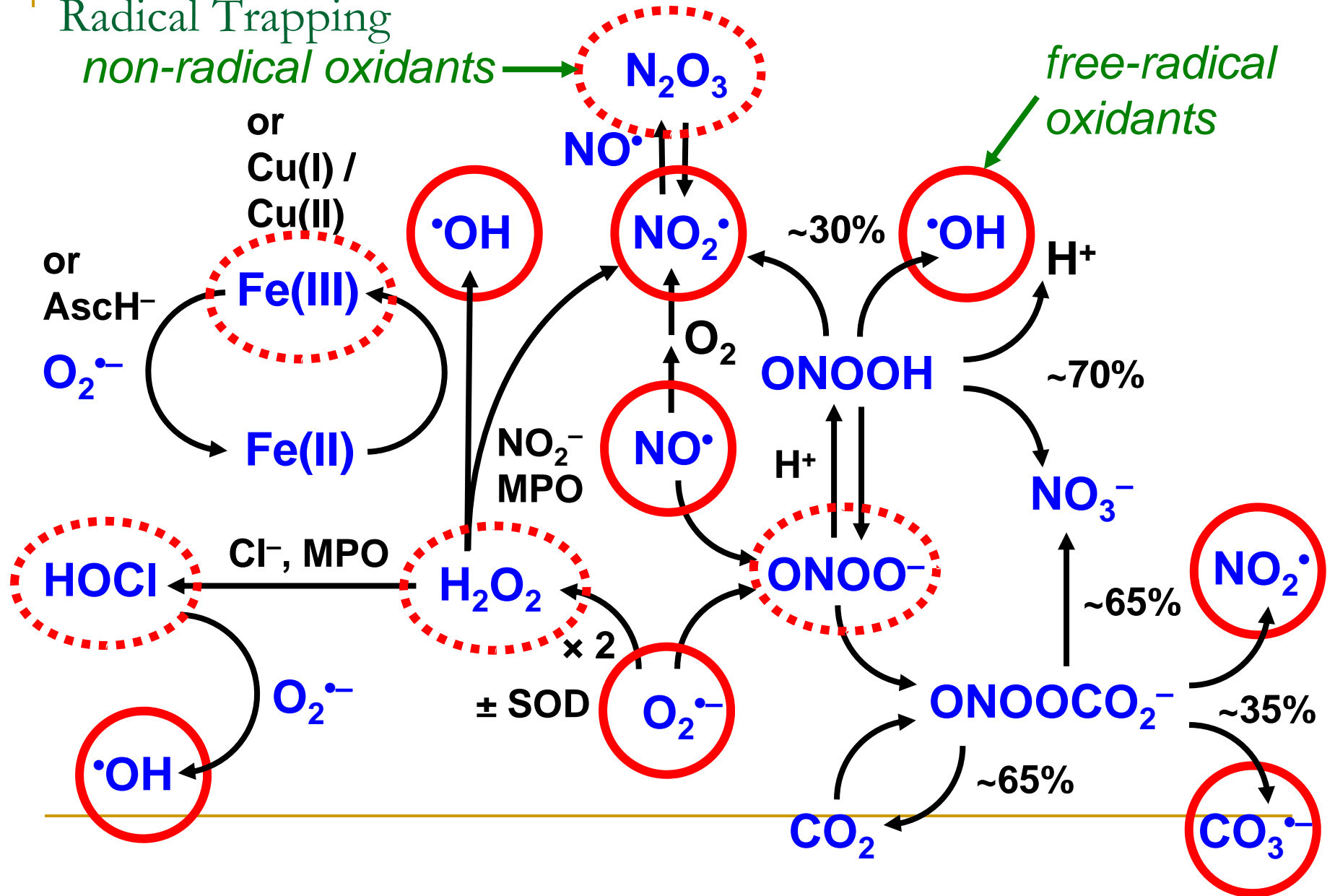
Fig. 4. Autoxidation of homocysteine in the presence of cysteine. Homocysteine (1 mM) was incubated with cysteine (○, 0; ●, 5; ■, 10; ▲, 25; ◆, 50 and ▼, 100 (M) in phosphate buffer (50 mM, pH 7.4) at 37°C. Aliquots were removed at various time intervals and the homocysteine (A) and cysteine (B) content were determined by high-performance liquid chromatography (HPLC) using 7-fluorbenzo-2-oxa-1,3-diazole-4-sulfonic acid (SBDF). Inset: Typical HPLC trace for analysis of cysteine (CYS) and homocysteine (HCYS) using SBDF.

Some of the more important reactions of thiols in biology (IV):

Radical Trapping

*non-radical oxidants*

*free-radical oxidants*



# How fast?



Oxidant	Rate constant / $\text{M}^{-1} \text{s}^{-1}$ (glutathione) at pH 7.4, room temperature	
$\cdot\text{OH}$	$1.3 \times 10^{10}$	(Quinitiliani <i>et al.</i> 1977)
$\text{NO}_2\cdot$	$1.9 \times 10^7$	(Ford <i>et al.</i> 2002)
$\text{CO}_3^{\cdot-}$	$5.3 \times 10^6$	(Chen & Hoffman 1973)
$\text{O}_2^{\cdot-}$	$2.2 \times 10^2$	(Jones <i>et al.</i> 2002)
( $\text{NO}\cdot$	$\sim 1 \times 10^{-1}$ )	(Hogg <i>et al.</i> , 1996)

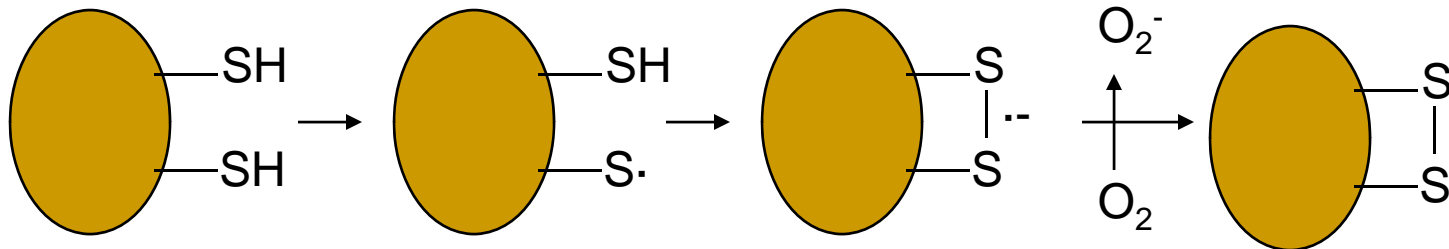
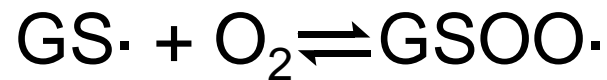


# Reactions of thiyl radicals

“Repair”



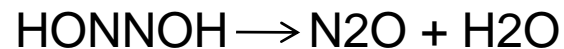
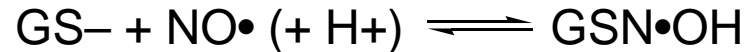
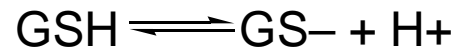
“Pro-oxidant”



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Some of the more important reactions of thiols in biology (V):  
Nitric Oxide

- The direct reaction is very slow



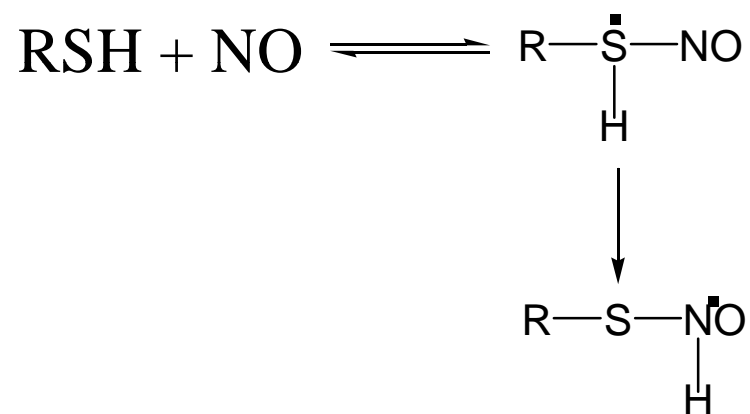
The rate is then proportional to  $[\text{NO} \cdot]^2[\text{GSH}]$  (Folkes & Wardman 2004):

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# Is $\text{RSNOH}\cdot$ an important NO metabolite

- Crystal structure of Hb infused with NO appears to form  $\text{RSNHO}\cdot$  radicals (Yi-Lei Zhao and K. N. Houk, JACS, 2006)

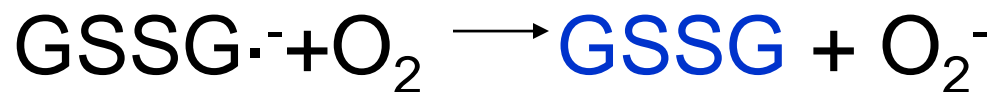
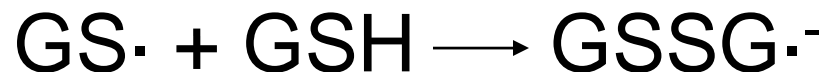
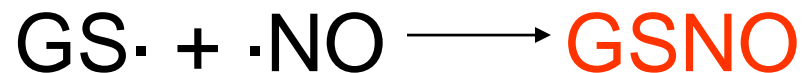
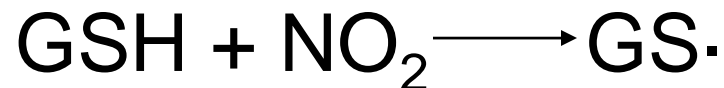
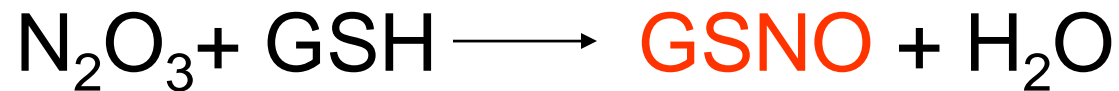
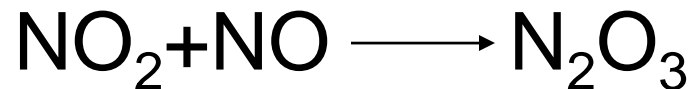
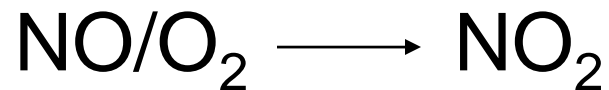


- Evidence needed that this is stable in solution: Should have a very nice and easy to see EPR signal!?
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# How are S-nitrosothiols formed?

NO, Oxygen and Thiols:

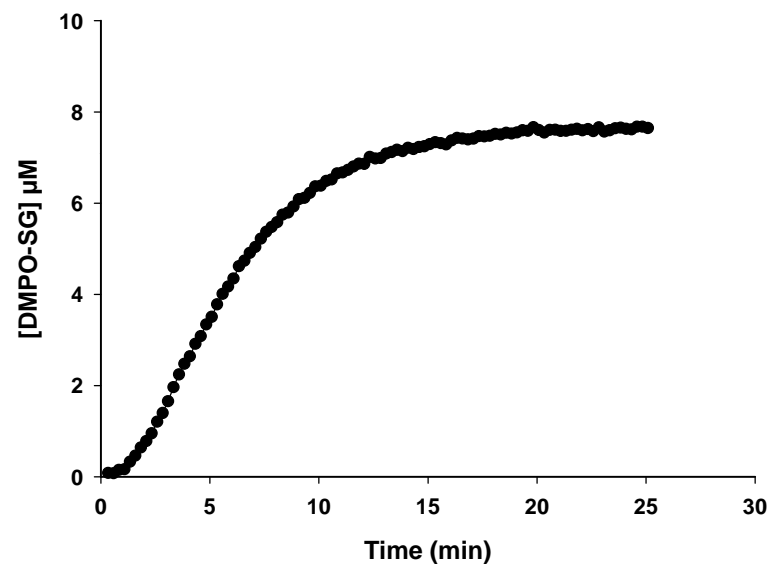
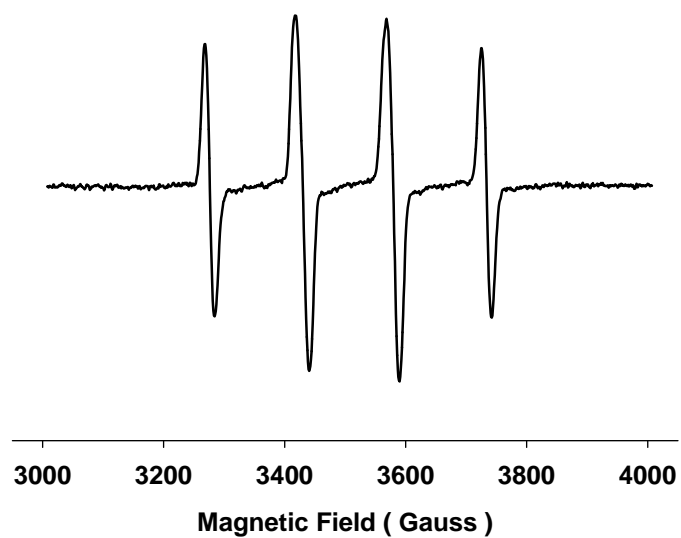


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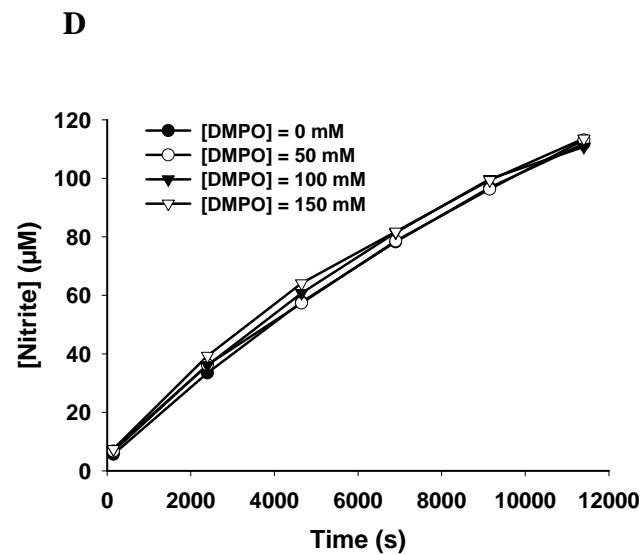
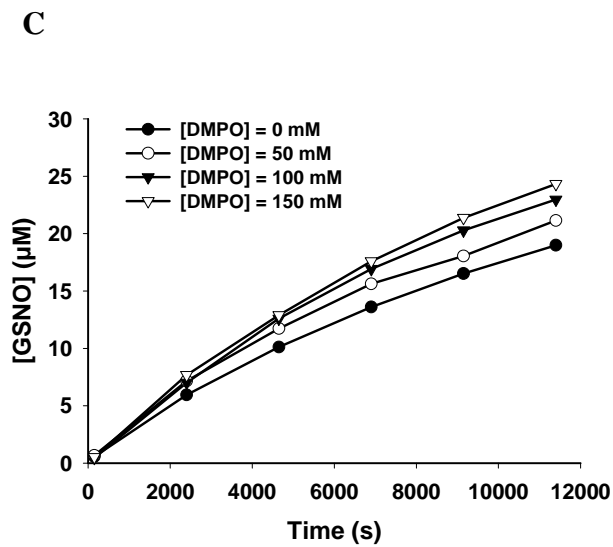
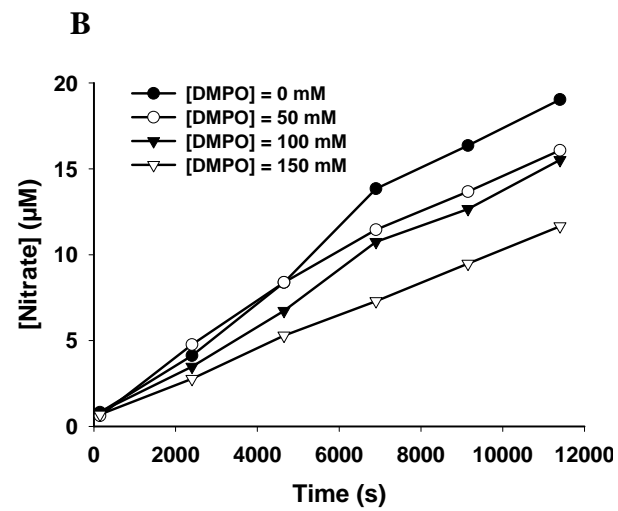
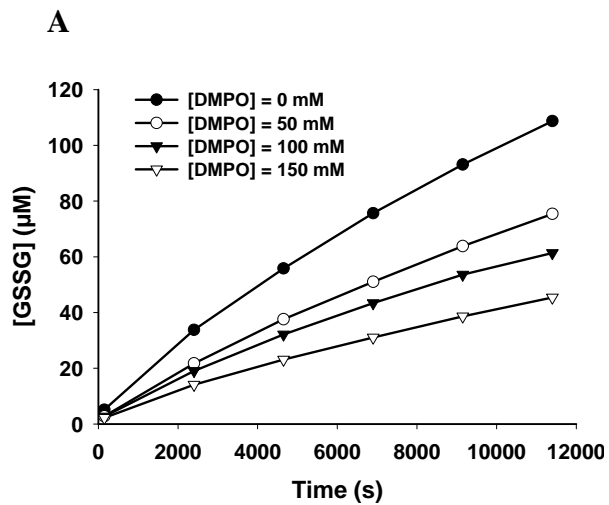
# How do we test which mechanism forms GSNO

- DMPO to trap thiyl radicals
  - Phosphate to catalyze  $\text{N}_2\text{O}_3$  hydrolysis
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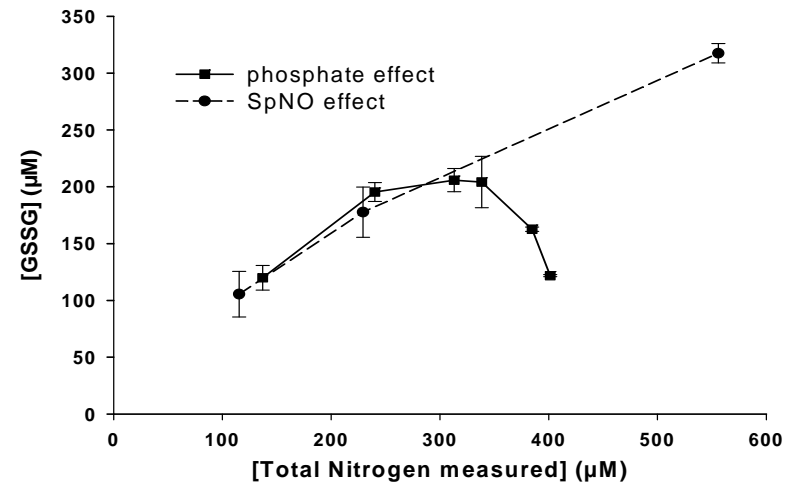
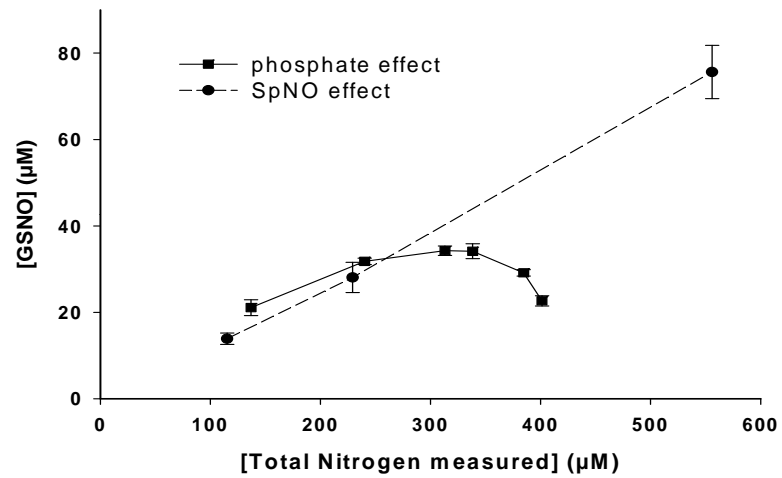
# Spin-trapping thiyl radicals during the NO/O<sub>2</sub> reaction



# However! DMPO enhances GSNO formation

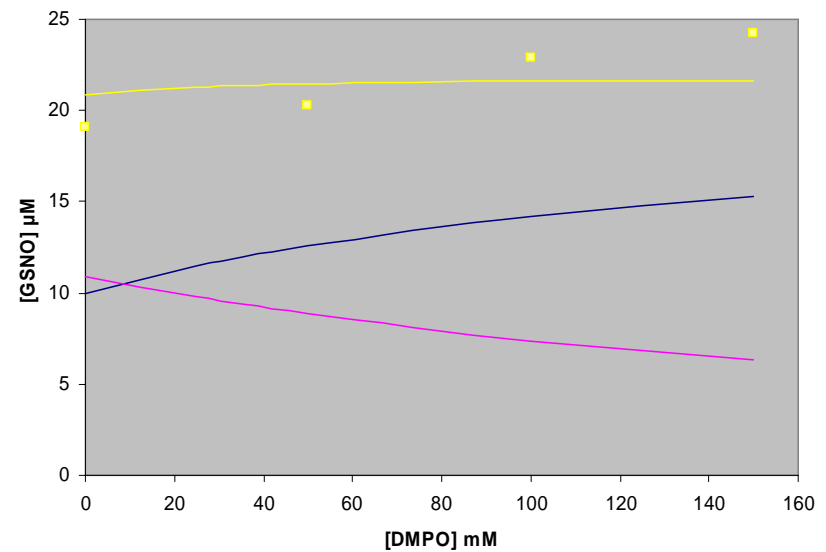
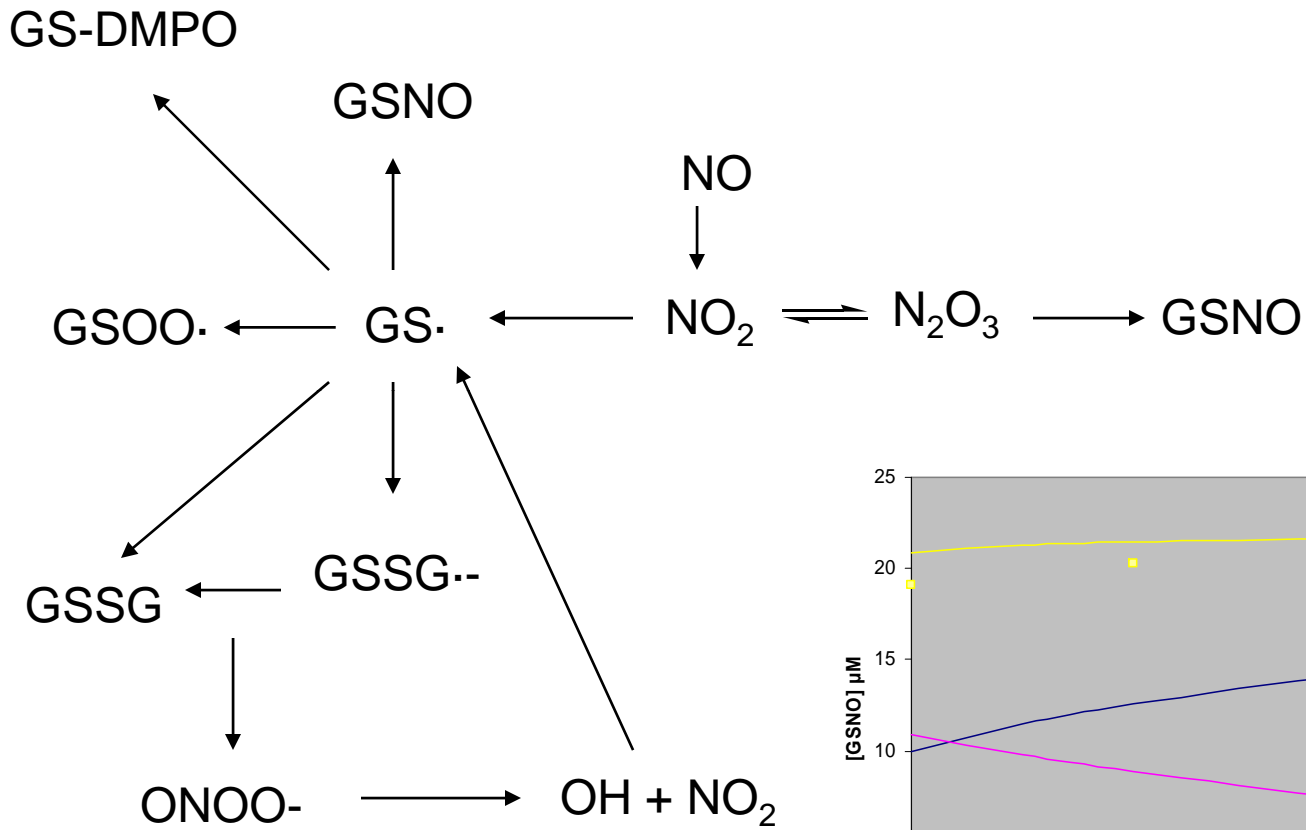


# The effect of phosphate





# Both pathways are operative

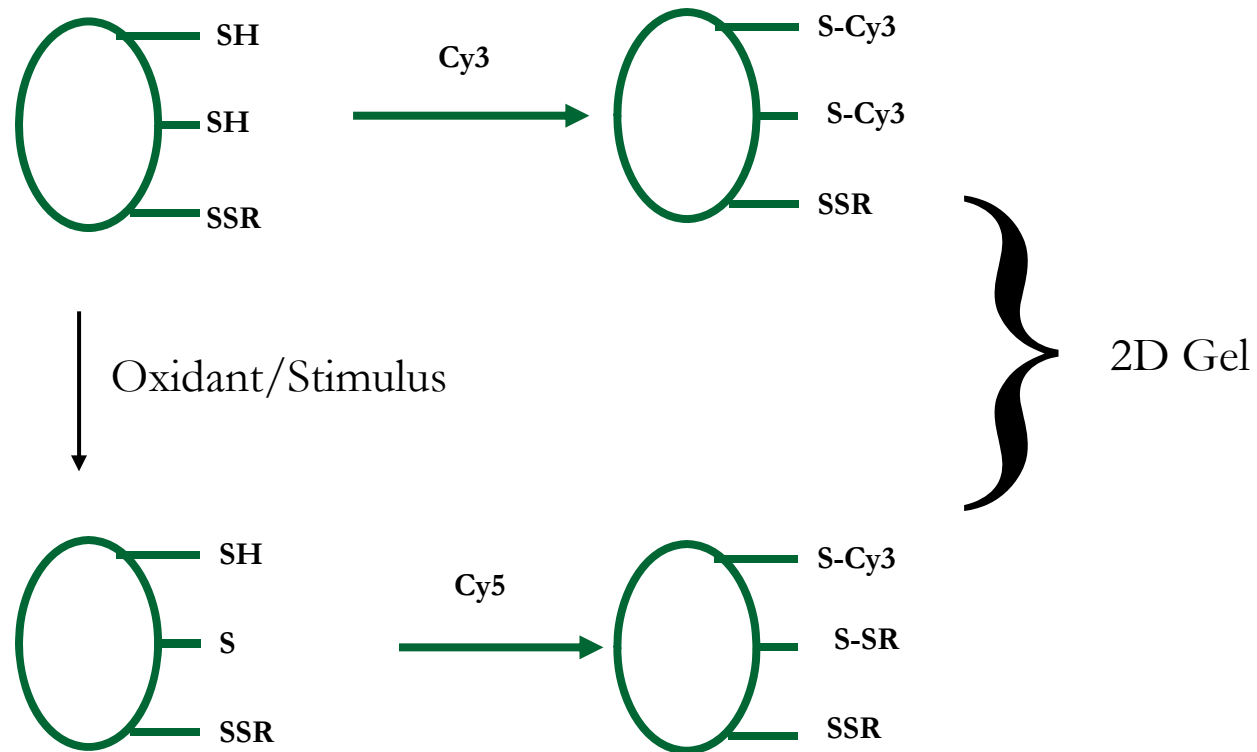


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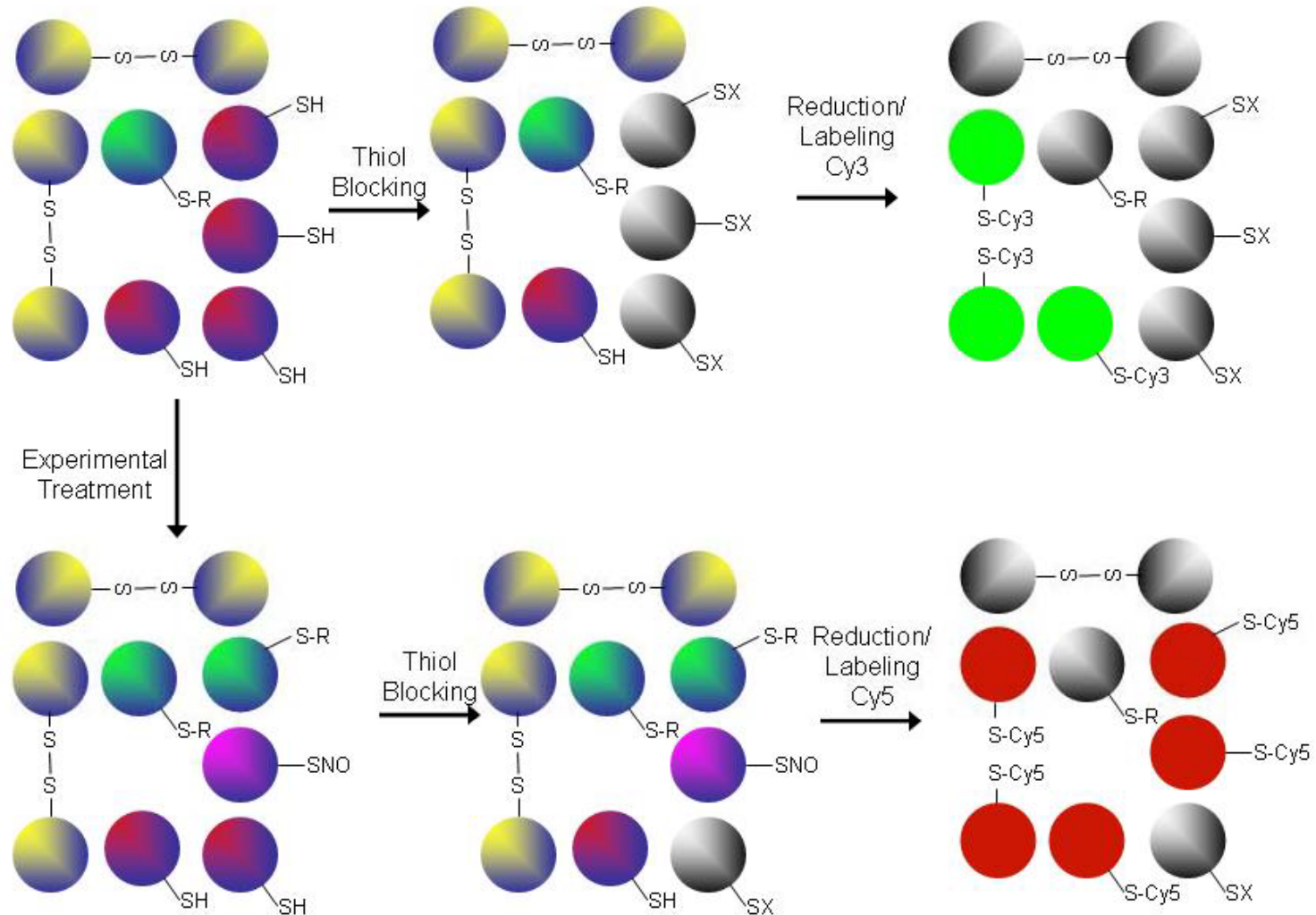
# Which pathway in vivo?

- Kinetically, the GS $\cdot$  pathway is more attractive – there are potentially many ways to get to GS $\cdot$ .
  - Testing this is tricky as interference with one pathway will also interfere with the other pathway.
  - If RSNO are formed via a RS $\cdot$  radical mechanism then RSNO formation is likely rate limited by the rate of RS $\cdot$  formation and not by the rate of NO formation (unless the rate of RS $\cdot$  formation also depends on NO). Is RSNO formation really NO signaling?
  - S-Nitrosothiol formation from NO in cells is a very low efficiency pathway.
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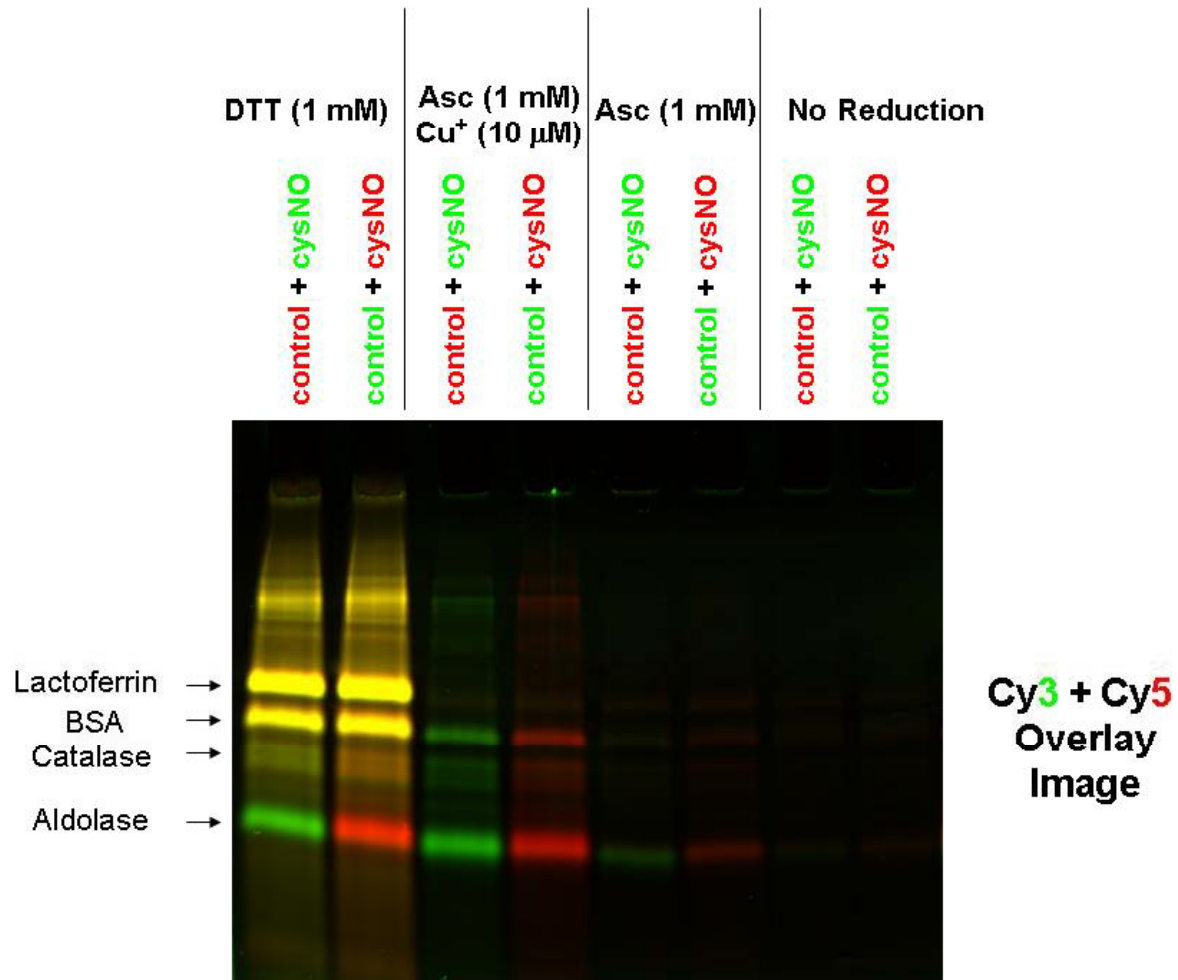
# Proteomic methods for the detection of thiol redox state: Redox DIGE



# Cye-Dye Switch for S-Nitrosation



# Protein S-nitrosothiols



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# Acknowledgements

- Kasia Broniowska
  - Nick Kettenhofen
  - Agnes Keszler
  - Yanhong Zhang
  
  - Peter Wardman
  - Mark Gladwin
  - Xunde Wang
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