

A misty lake scene with trees and a small boat. The background is a soft, hazy landscape with a body of water in the foreground. The text is overlaid on this scene.

# Hydrogen Sulfide: A Mediator and Modulator of Conditioning

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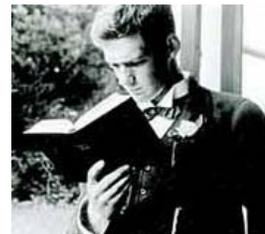
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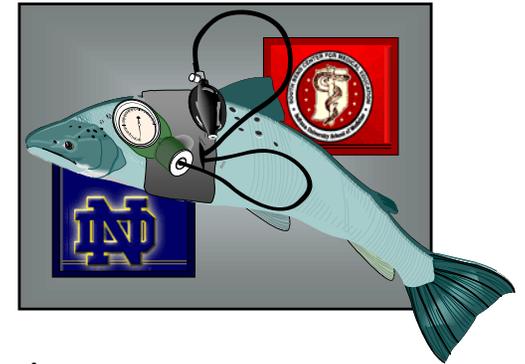


*Am. Heart Association*

**APS Fellow: Barb Behnke**



*John Angus Erskine Fund (NZ)*



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**Disclaimer**

**No Financial Interest**

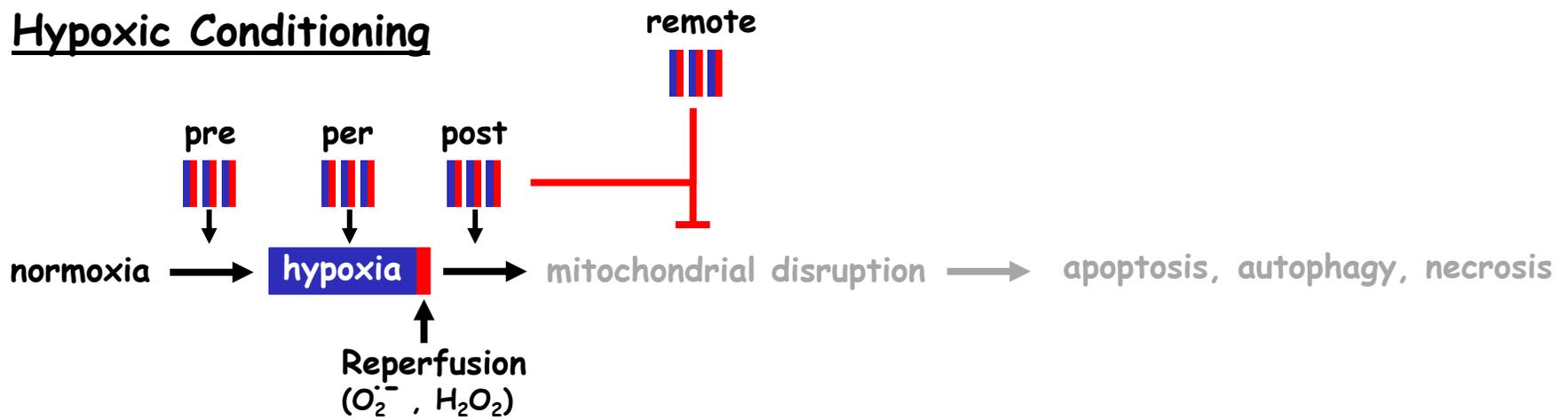
# Ischemia Reperfusion Injury (I/R) and Hypoxic Conditioning

Stimulus → Transduction → Activation → Effector Response

## Ischemia Reperfusion Injury (I/R)



## Hypoxic Conditioning



# H<sub>2</sub>S protection in ischemia reperfusion injury (I/R)

## Preconditioning

Heart: mouse, rat

Kidney: mouse

Brain: rat

Liver: mouse, rat

Retina: rat

Heart and kidney: human N-acetylcysteine prior to angioplasty

## I/R (Preconditioning?)

Heart: mouse, rat, pig

Kidney: mouse, rat

Brain: rat

Liver: mouse

## Postconditioning

Heart: rat, pig

Skeletal muscle: mouse

Intestine: rat

## Remote conditioning?

Liver ischemia, heart and kidney protected: rat

## Nutrient conditioning?

Liver ischemia, heart and kidney protected: mouse, C.elegans, others



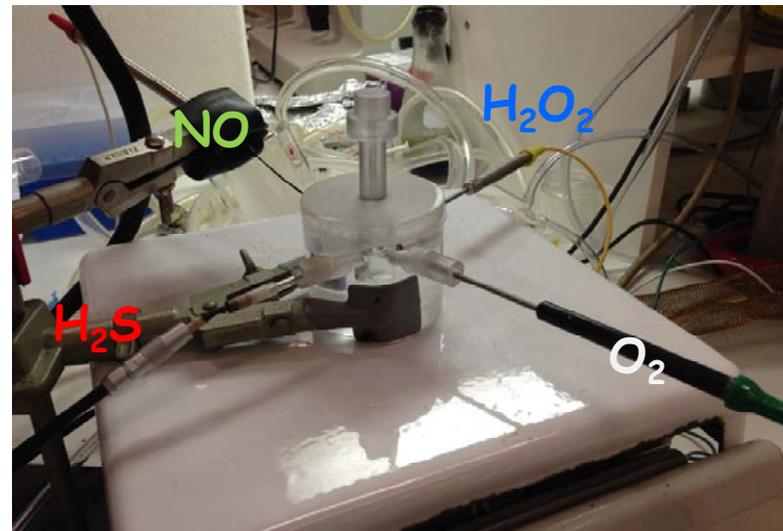
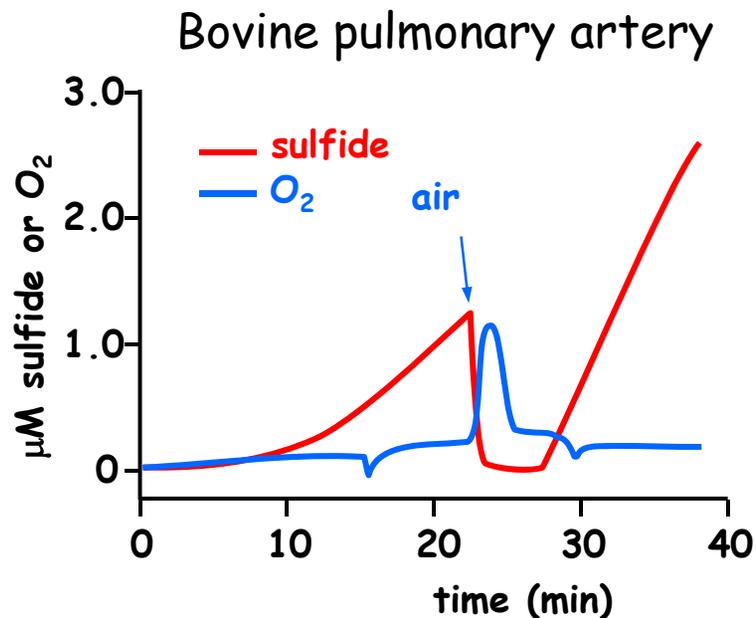
# Ischemia Reperfusion Injury (I/R) and H<sub>2</sub>S Conditioning

Stimulus → **Transduction** → **Activation** → **Effector Response**

**H<sub>2</sub>S** "hormetic" couple between O<sub>2</sub>  
and **activation/effector response**

**In both I/R and Conditioning**

H<sub>2</sub>S is constitutively produced by all tissues but consumed by O<sub>2</sub>

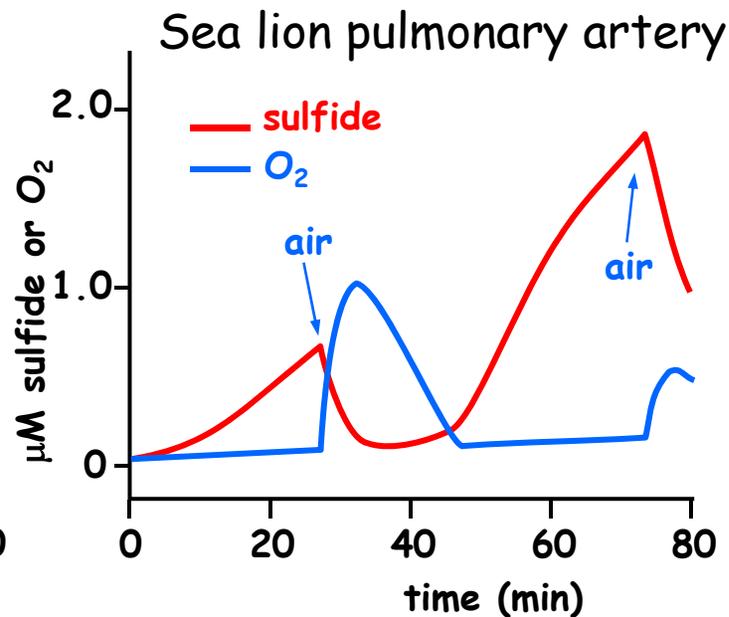
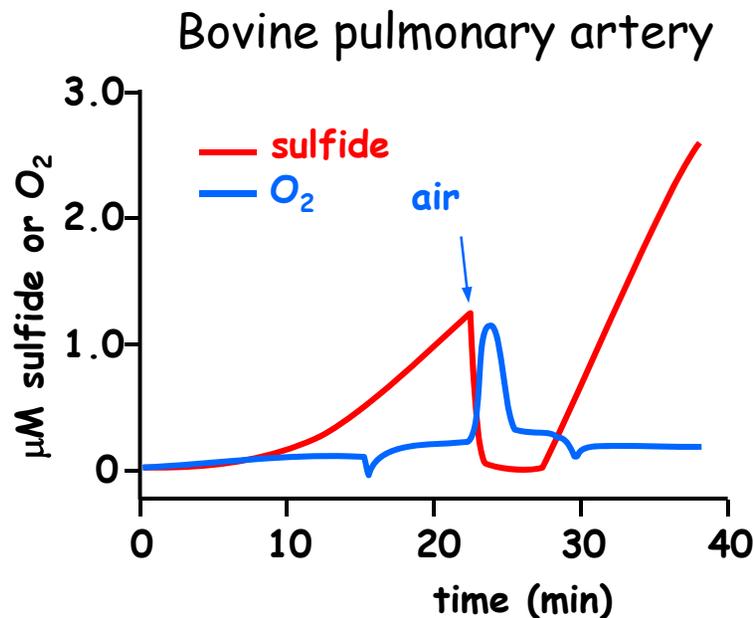


# Ischemia Reperfusion Injury (I/R) and H<sub>2</sub>S Conditioning

Stimulus → **Transduction** → **Activation** → **Effector Response**

**H<sub>2</sub>S** "hormetic" couple between O<sub>2</sub>  
and **activation/effector response**  
**In both I/R and Conditioning**

H<sub>2</sub>S is constitutively produced by all tissues but consumed by O<sub>2</sub>



How does sulfide ( $\text{H}_2\text{S}$ ,  $\text{HS}^-$ ,  $\text{S}^{2-}$ ) transduce the hypoxic signal?

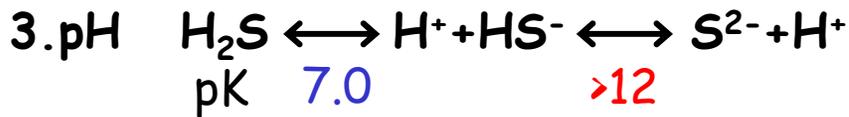
1. Chemistry/Biochemistry
2. Signaling
3. Evolution
4. ROS or RSS?
5. Sulfide Donating Compounds



# Chemistry

1. Volatile:  $t_{1/2}$  5 min tissue culture, 3 min myograph,  
Langendorff ~1 circulation

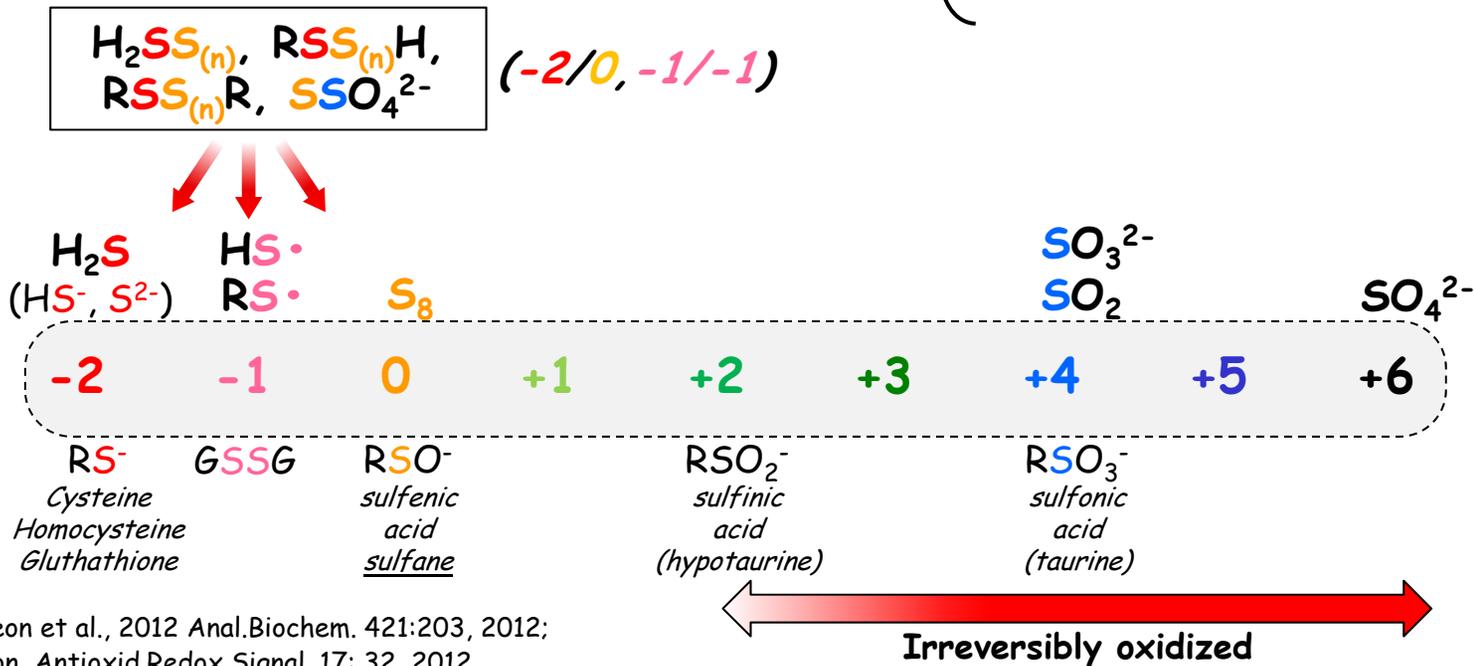
2. Water & Lipid soluble



**HS<sup>-</sup>/H<sub>2</sub>S**

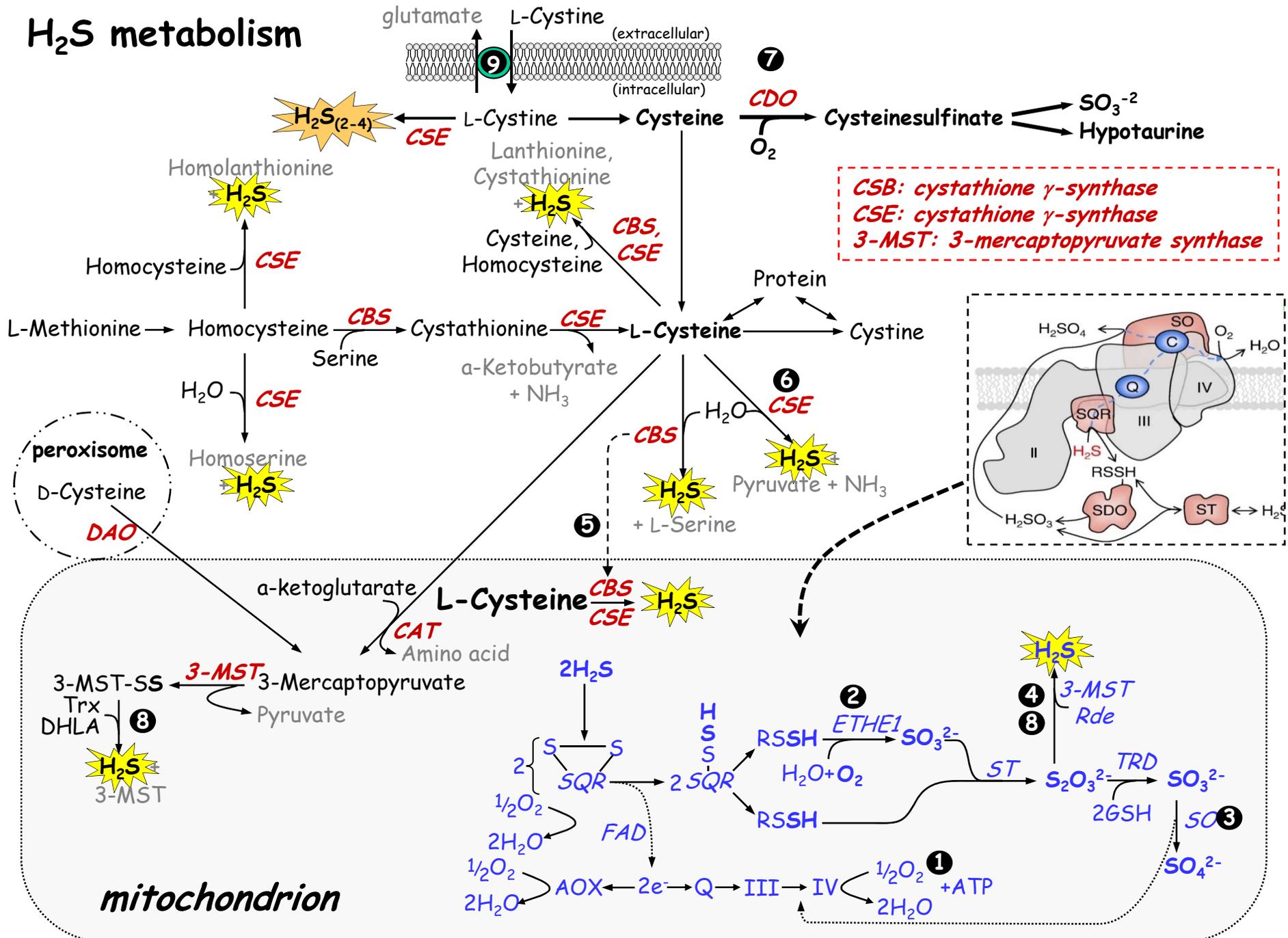
Plasma (7.4):	<b>3.2</b>
Cytosol (6.9):	1
Mitochondrion (8.0):	<b>12.6</b>
Golgi (6.5):	0.3
Lysosome (4.7):	0.006

4. Reactivity (redox)



DeLeon et al., 2012 Anal.Biochem. 421:203, 2012;  
Olson, Antioxid.Redox Signal. 17: 32, 2012

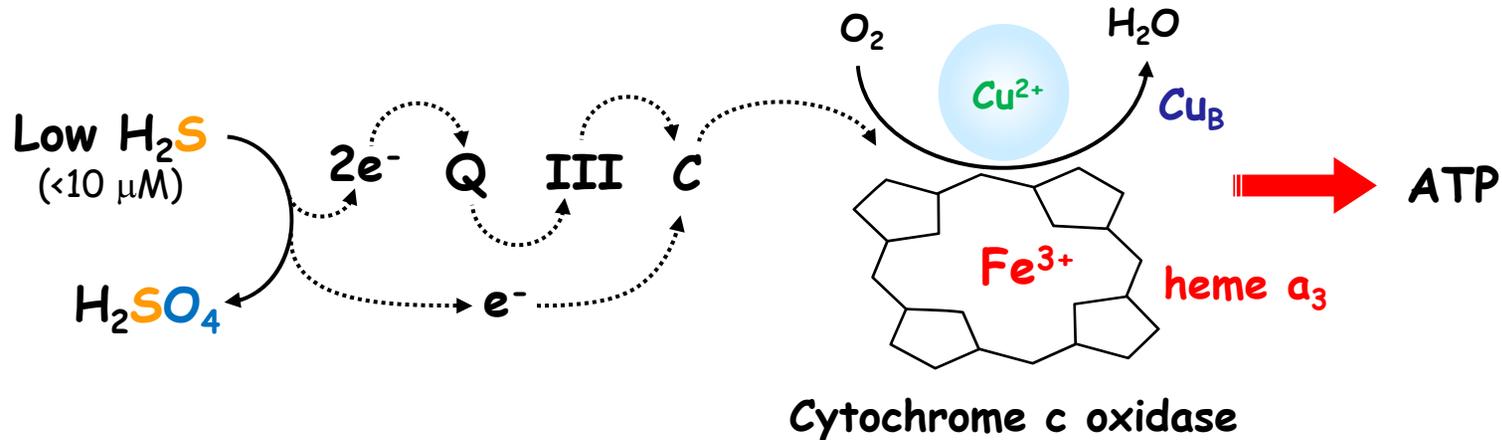
# H<sub>2</sub>S metabolism



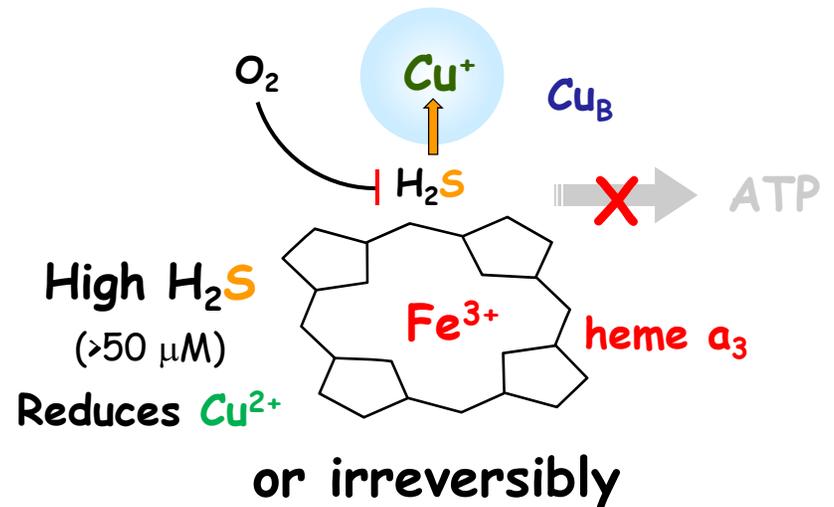
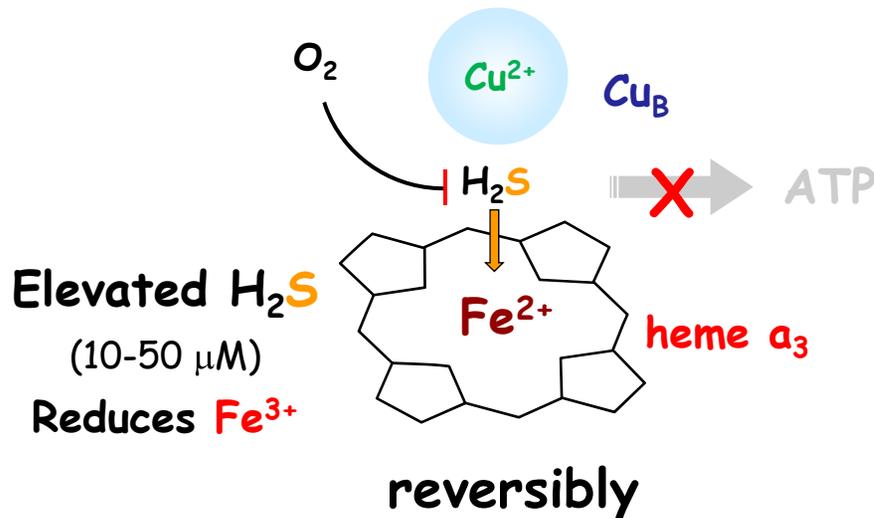
# H<sub>2</sub>S signaling

## 1. As a Metabolic Regulator

H<sub>2</sub>S stimulates ATP production...



...or inhibits it



## 2. As an anion ( $\text{HS}^-$ )

A. antiporters ( $\text{Na-HCO}_3\text{-Cl}$ ,  $\text{HCO}_3\text{-Cl}$ )

B. symporters: ( $\text{Na-K-2Cl}$ )

C. channels ( $\text{Cl}^-$ )

## 3. $\text{H}_2\text{S}$ interactions with $\text{N}$

A. Novel S-nitrosothiol



B. Reaction with  $\text{ONOO}^-$



(Filipovic et al Biochem J 441:609, 2012)

C. Reaction with  $\text{NO}_2^-$



(Miljkovic et al. Angew Chem Int Ed 52:12061, 2013)

## 4. $\text{H}_2\text{S}$ interactions with $\text{H}_2\text{O}_2$

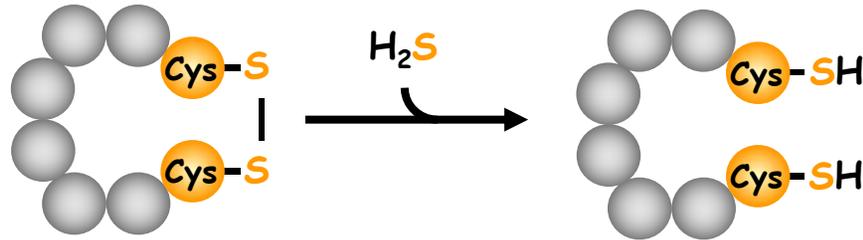


## 5. $\text{H}_2\text{S}$ interactions with $\text{Zn}^{2+}$ (angiotensin converting enz)

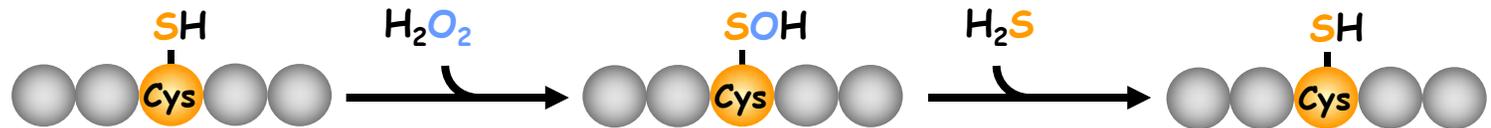
(Laggner et al. J Hypertens 25:2100, 2007)

# Potential mechanisms of H<sub>2</sub>S signaling via protein cysteine

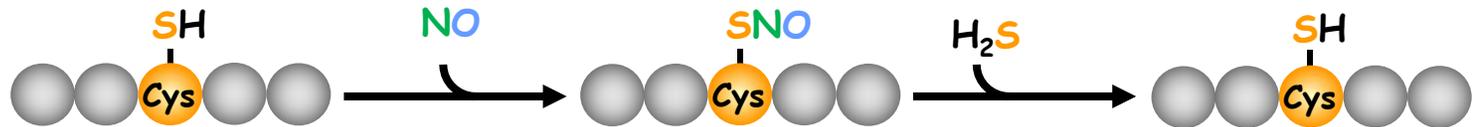
## 1. Disulfide reduction



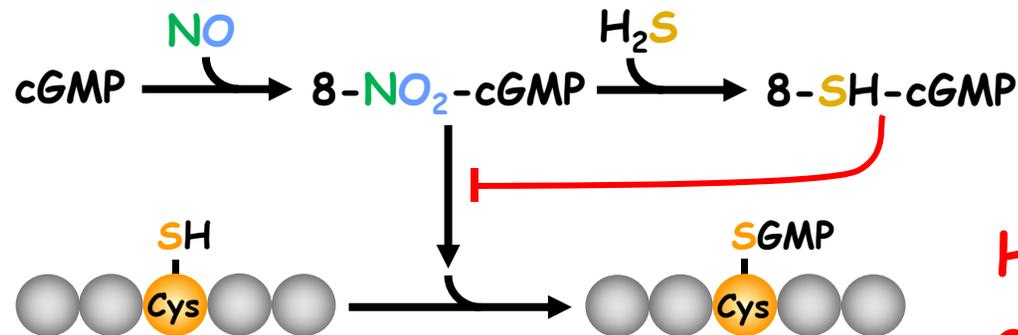
## 2. Sulfenyl reversal



## 3. S-nitrosothiol reversal



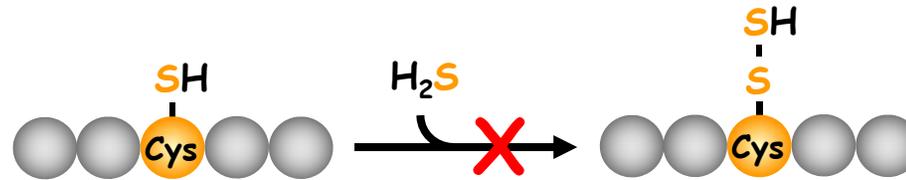
## 4. Prevention of S-guanylation



However, H<sub>2</sub>S is a weak reductant

# H<sub>2</sub>S signaling via protein cysteine (cont)

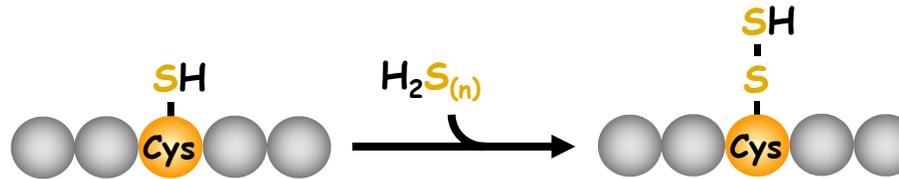
## 5. Sulphydration



Mustafa et al. Sci.Signal 2:ra72, 2009

Need 2e<sup>-</sup>  
oxidation  
of H<sub>2</sub>S

## 5. Sulphydration



Persulfide, H<sub>2</sub>S<sub>2</sub> (n=2)



Polysulfide, H<sub>2</sub>S<sub>n</sub> (n=3-8)



ANTIOXIDANTS & REDOX SIGNALING  
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DOI: 10.1089/ars.2012.5041

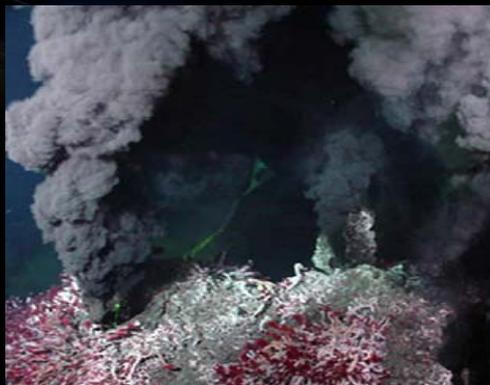


ORIGINAL RESEARCH COMMUNICATION

### Polysulfides Link H<sub>2</sub>S to Protein Thiol Oxidation

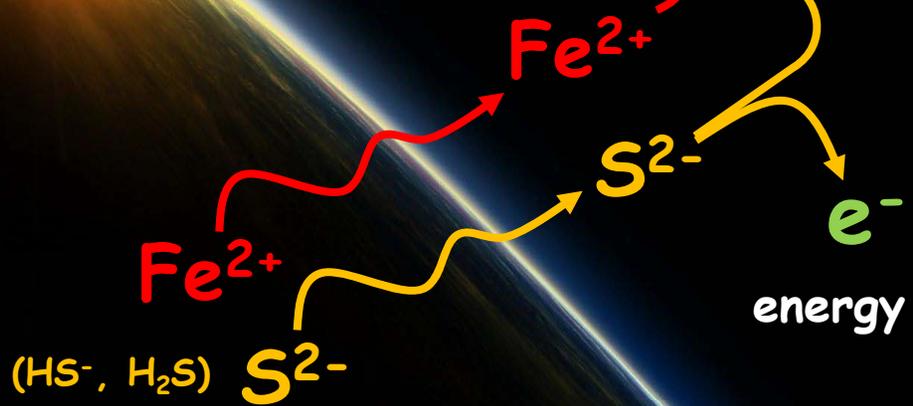
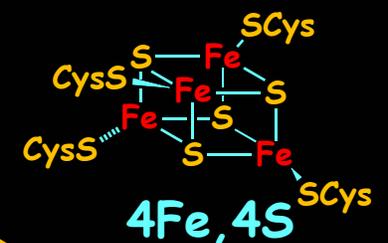
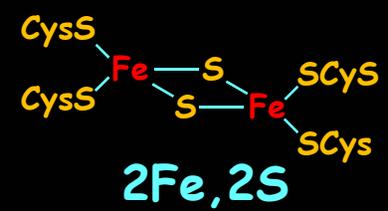
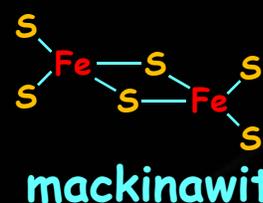
Lipid phosphatase (PTEN)

Romy Greiner,<sup>1</sup> Zoltán Pálkás,<sup>2</sup> Katrin Bäsell,<sup>3</sup> Dörte Becher,<sup>3</sup>  
Haïke Antelmann,<sup>3</sup> Péter Nagy,<sup>2</sup> and Tobias P. Dick<sup>1</sup>



catalysts & membranes

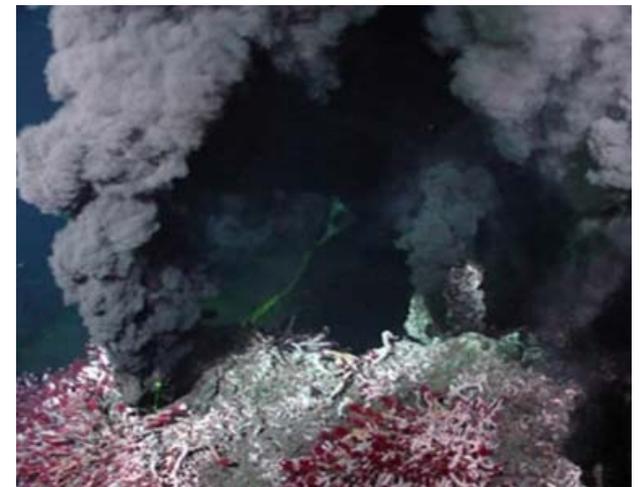
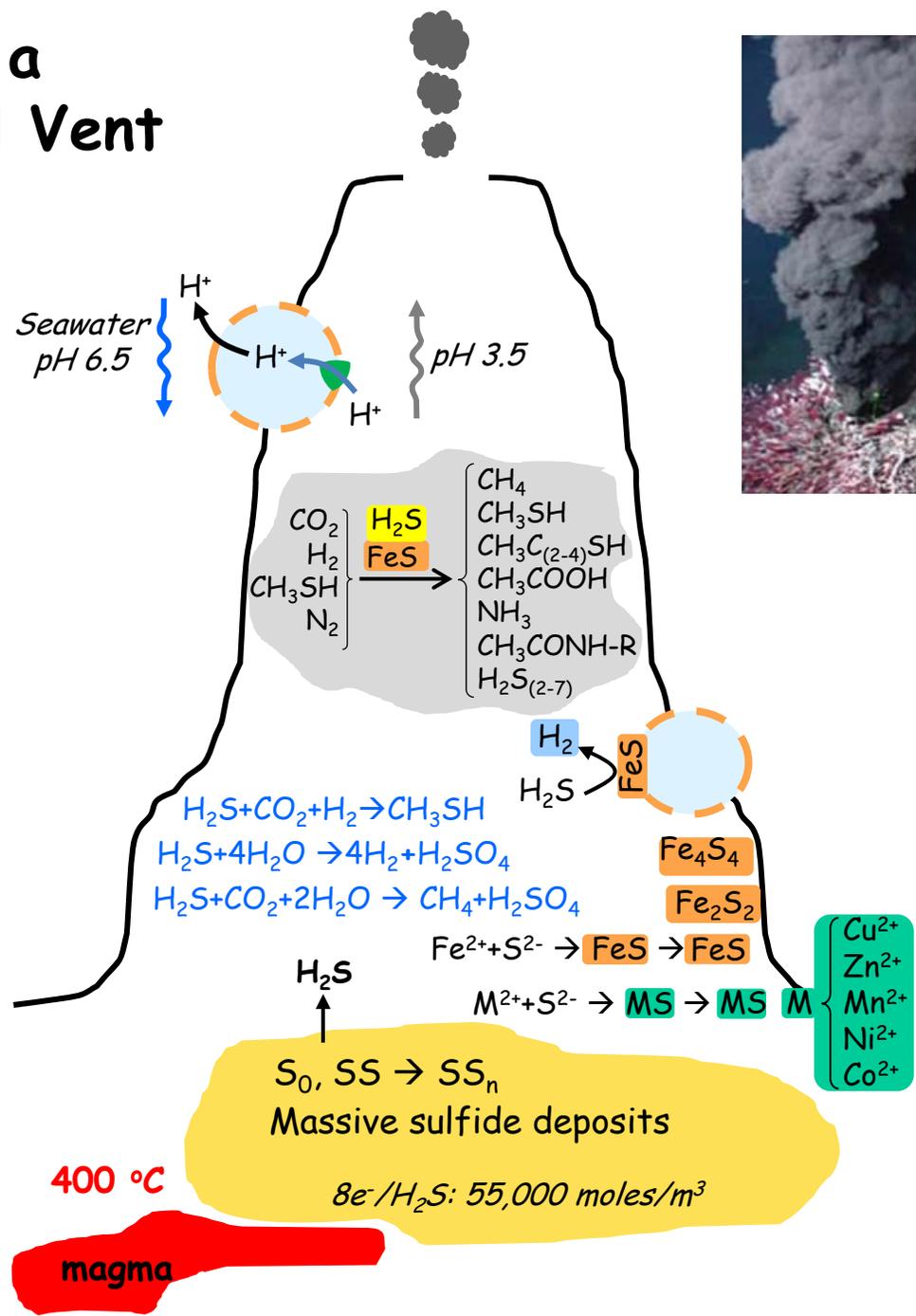
**FeS**



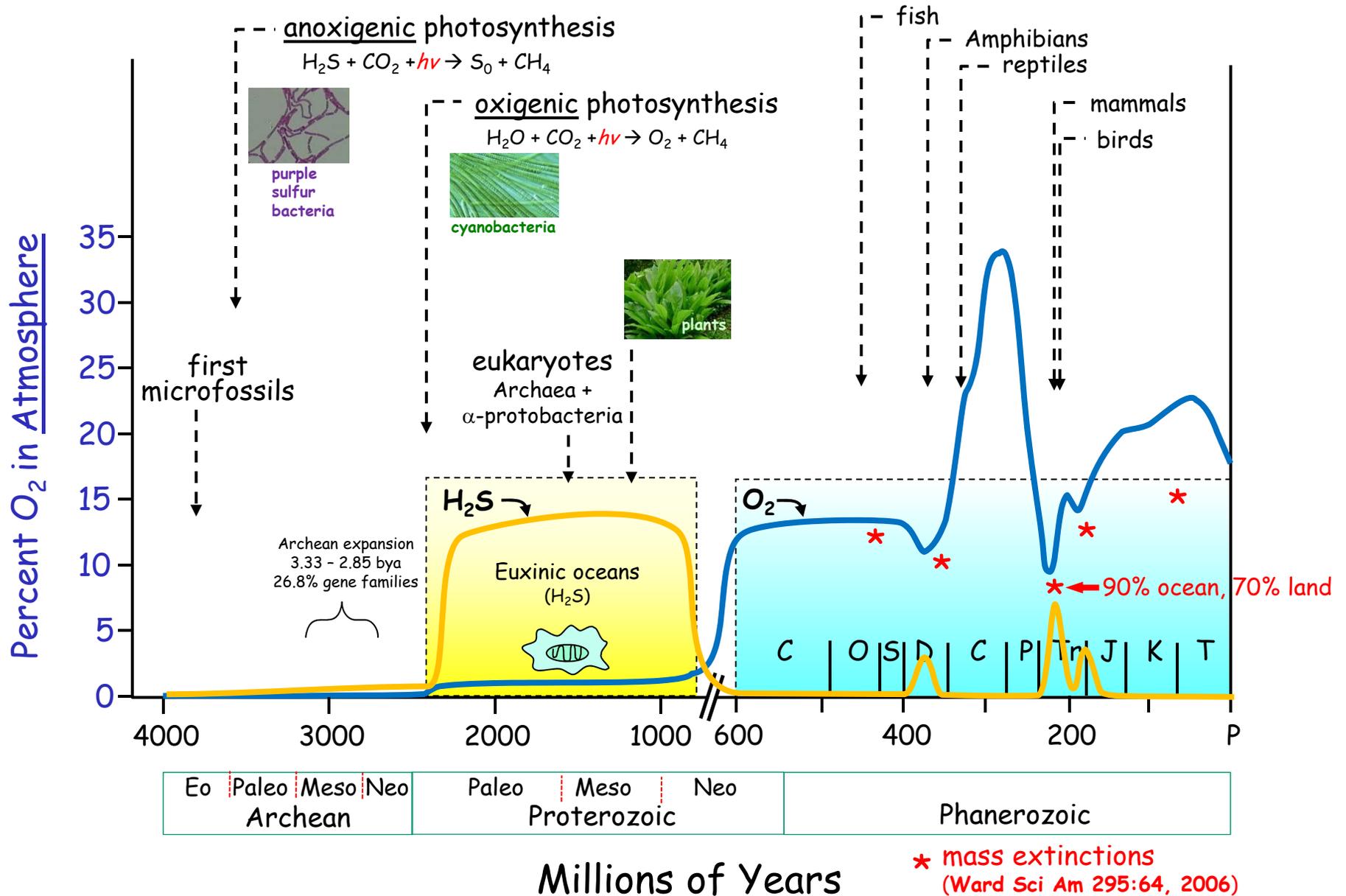
In the beginning there was no oxygen

Pratt, Artif. Life 17:203, 2011  
 Martin & Russell, Phil Trans R Soc Lond 358:59, 2003  
 The "iron-sulfur world" Wächtershäuser, Microbiol.Rev.52:452, 1988

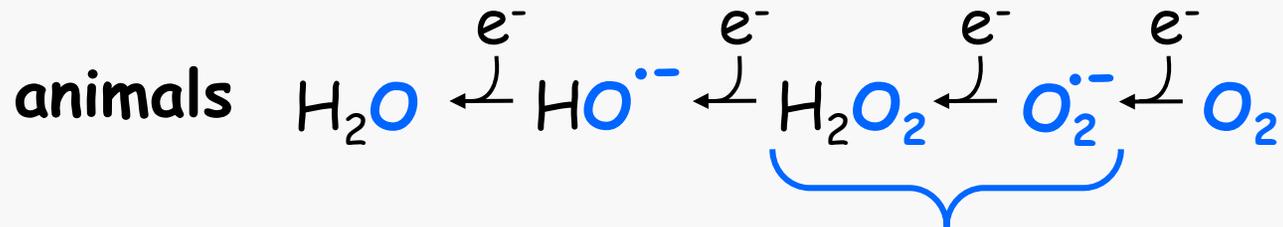
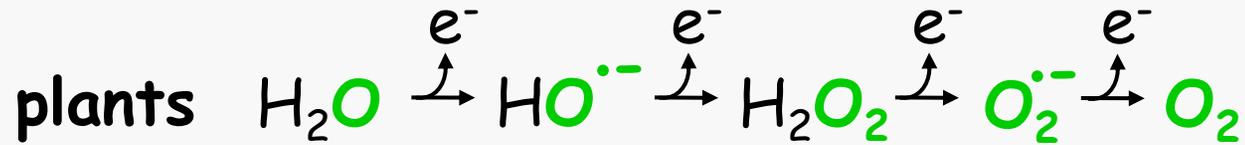
# Chemistry at a Hydrothermal Vent



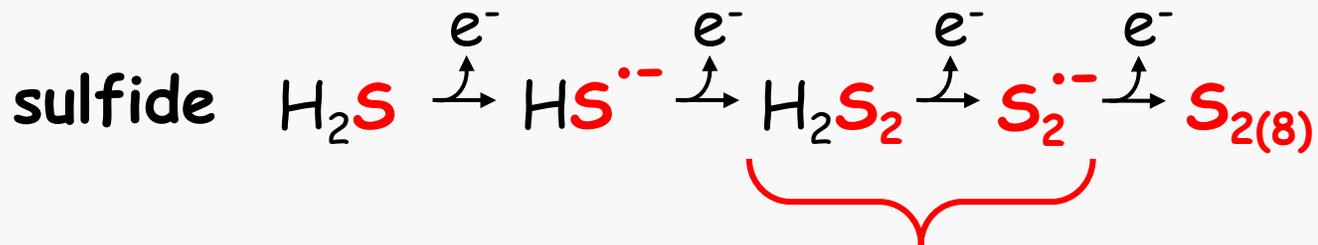
# H<sub>2</sub>S - Oxygen and Evolution



# Evolution, Reactive Oxygen Species (ROS) and I/R injury



Reactive Oxygen Species (ROS)  $H_2O_2 + O_2^{\bullet-}$



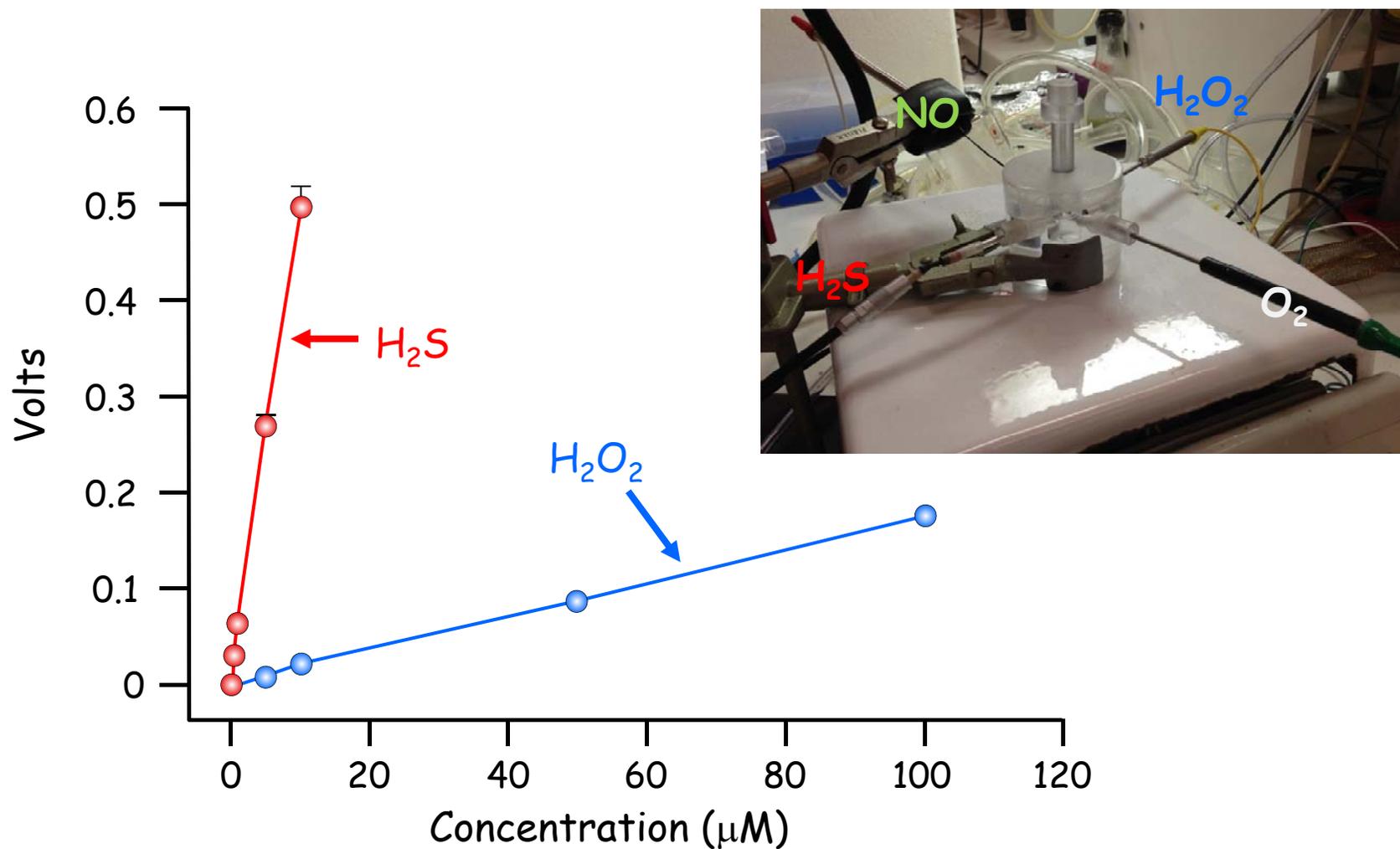
Reactive Sulfide Species: (RSS)  $H_2S_2 + S_2^{\bullet-}$



**RSS:** ancient, long evolutionary history, promiscuous electrons.

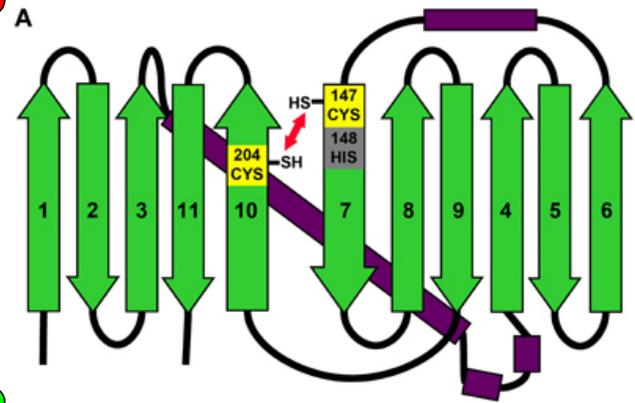
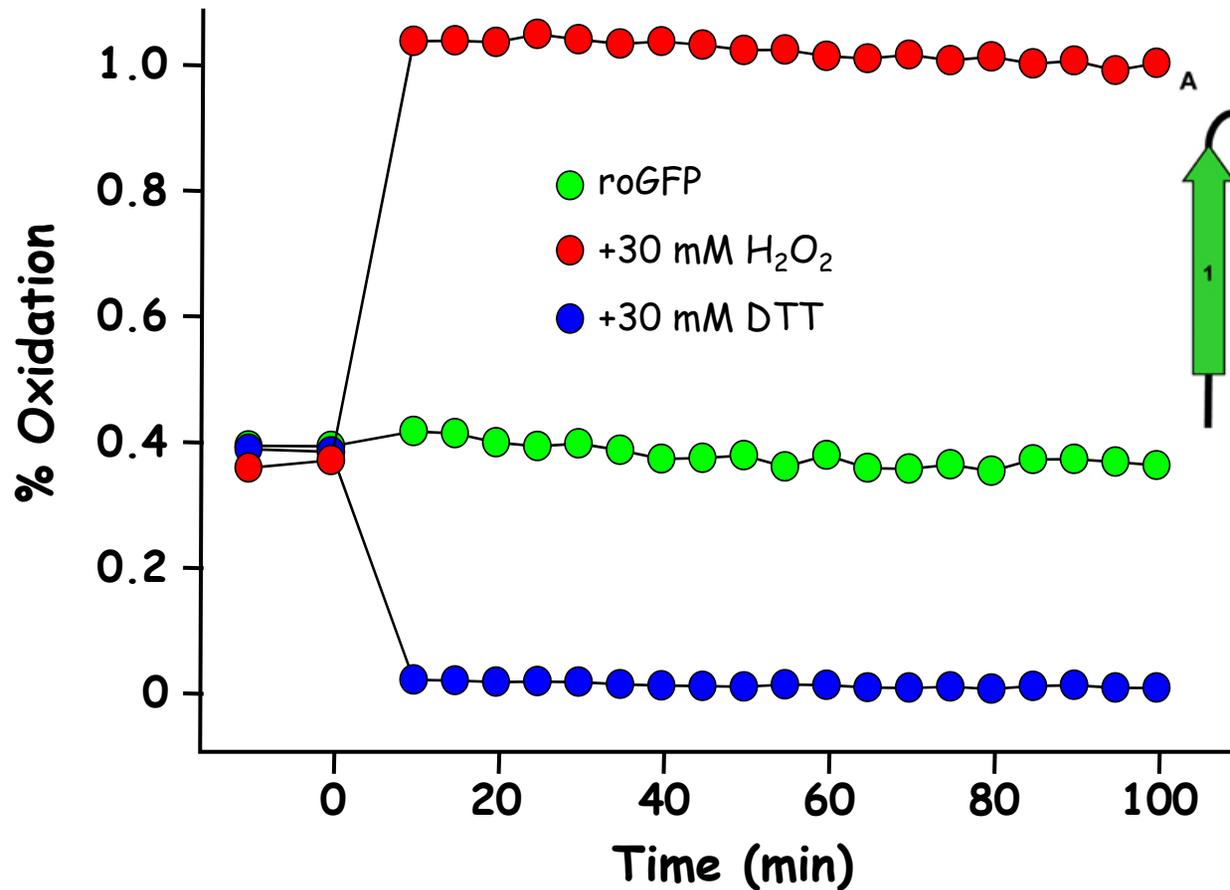
1. Are **RSS** more reactive/physiological than **ROS**?
2. Are we sure we are measuring **ROS** not **RSS**?

Commercial amperometric "H<sub>2</sub>O<sub>2</sub>" electrodes are 25x more sensitive to H<sub>2</sub>S than H<sub>2</sub>O<sub>2</sub>

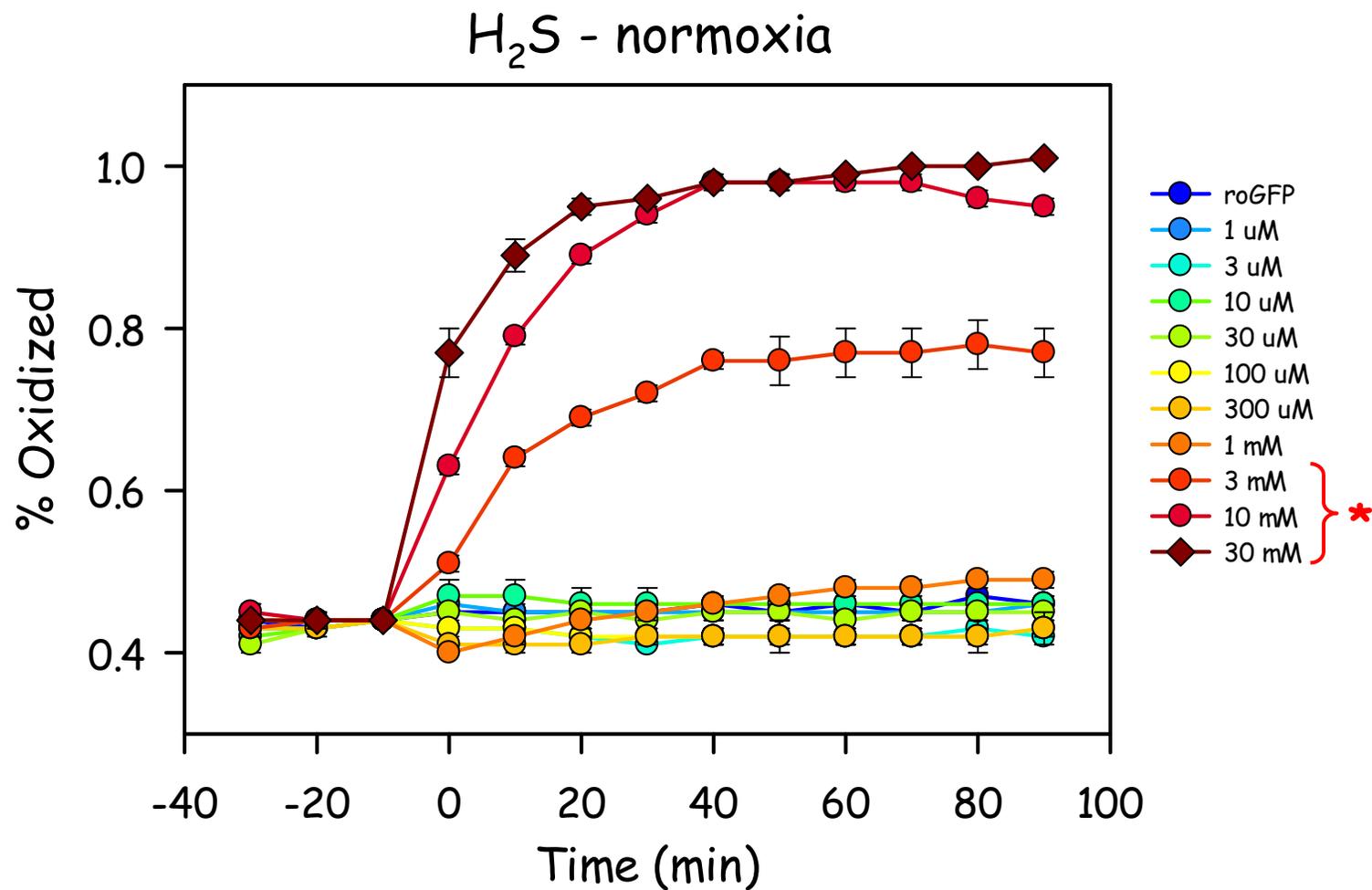


Calibration curves for H<sub>2</sub>O<sub>2</sub> and H<sub>2</sub>S with H<sub>2</sub>O<sub>2</sub> electrode

# Redox-sensitive Green Fluorescent Protein (roGFP)

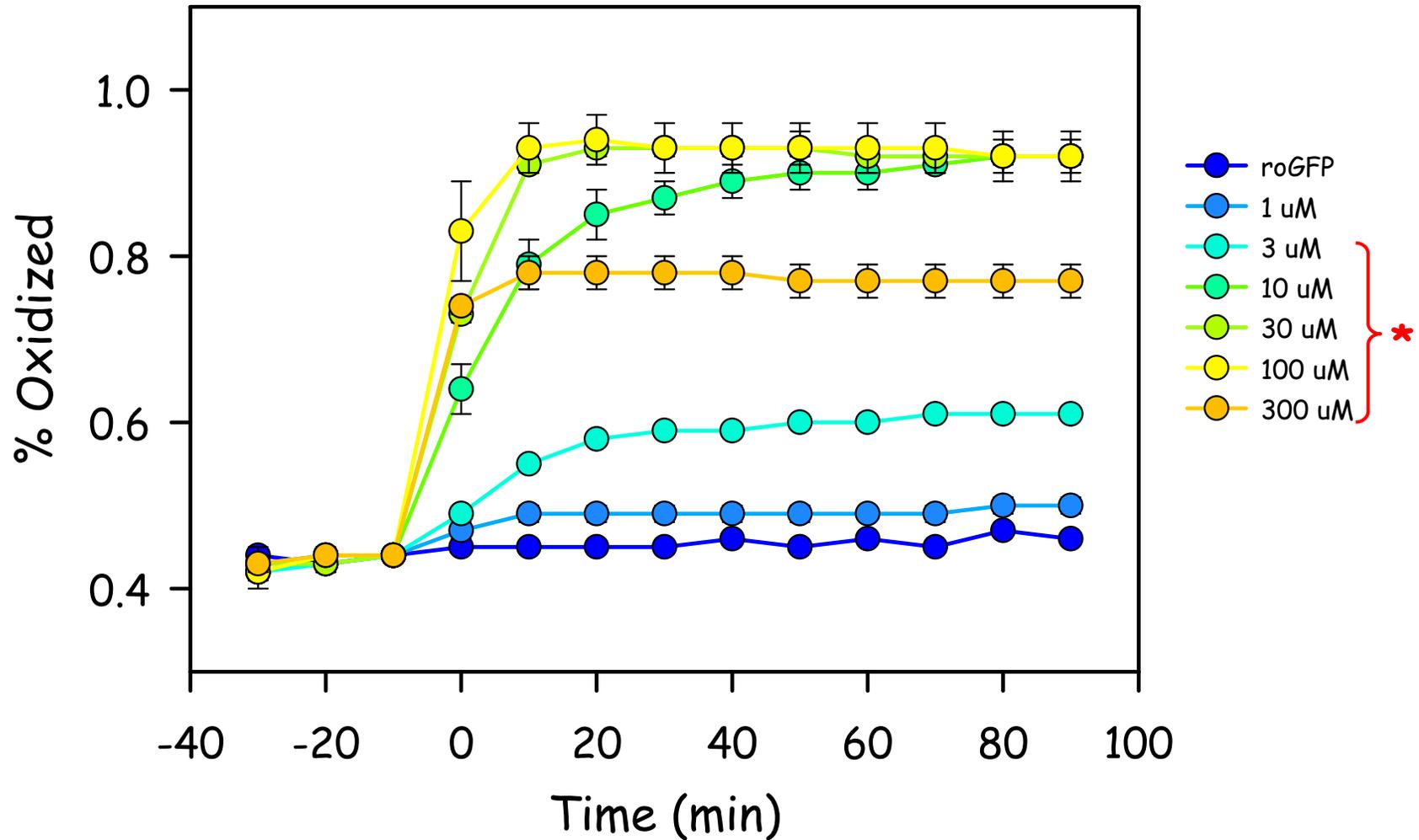


# H<sub>2</sub>S > 1 mM oxidizes roGFP



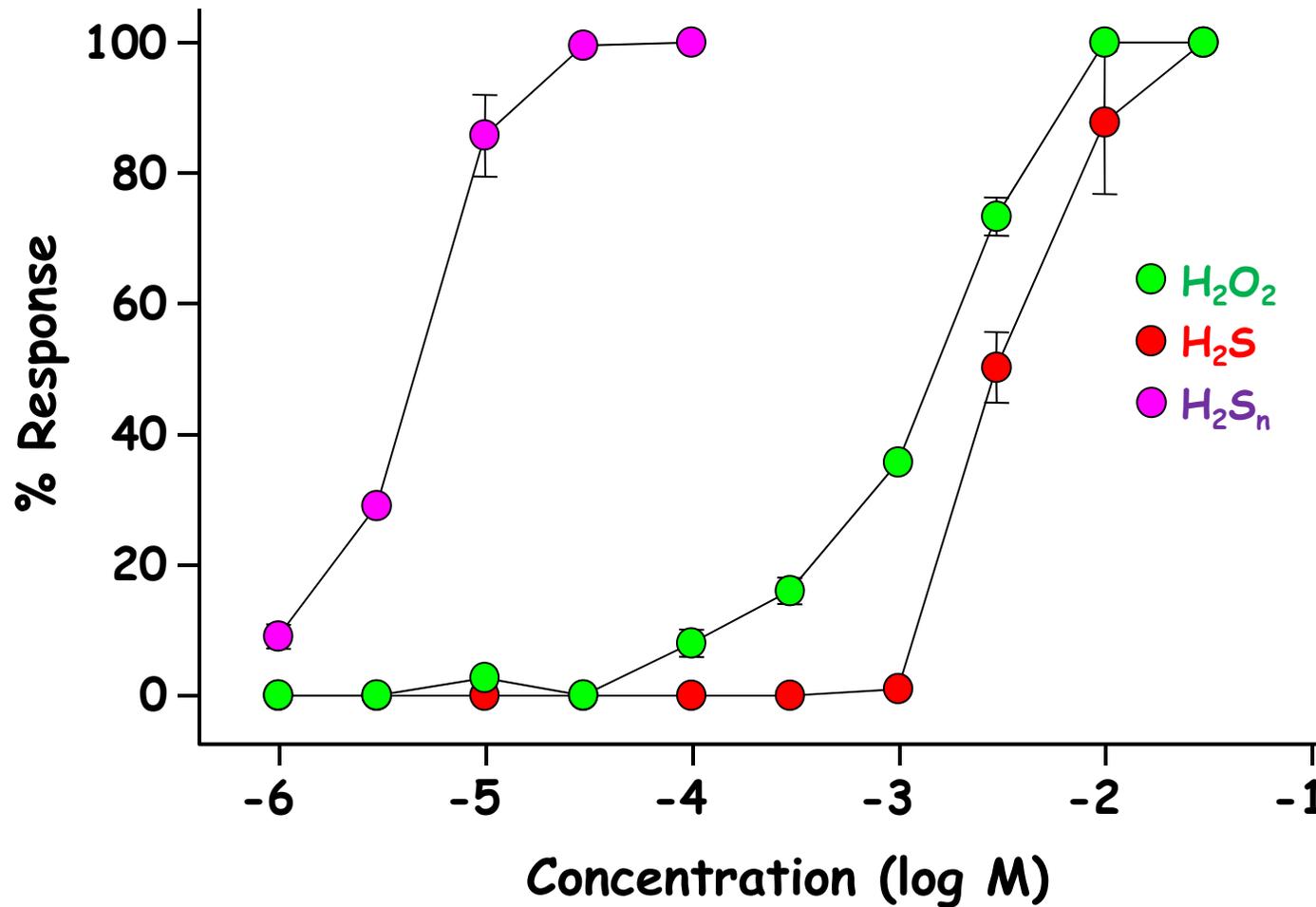
# Polysulfides $\geq 3 \mu\text{M}$ oxidize roGFP

Polysulfides ( $\text{K}_2\text{S}_n$ ) normoxia



$\text{K}_2\text{S}_n$  mixed polysulfides  $\text{S}_1, \text{S}_2, \text{S}_3 \dots \text{S}_7$  (~40%  $\text{K}_2\text{S}$ )

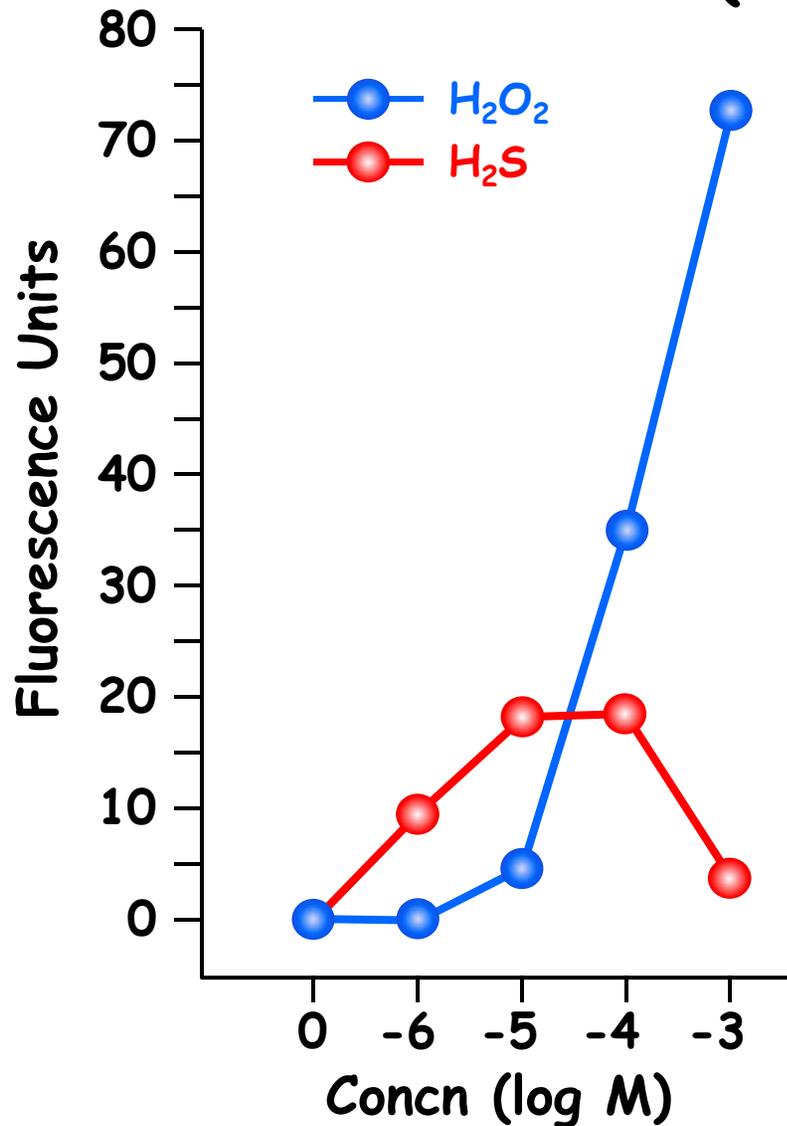
# Sensitivity of roGFP oxidation



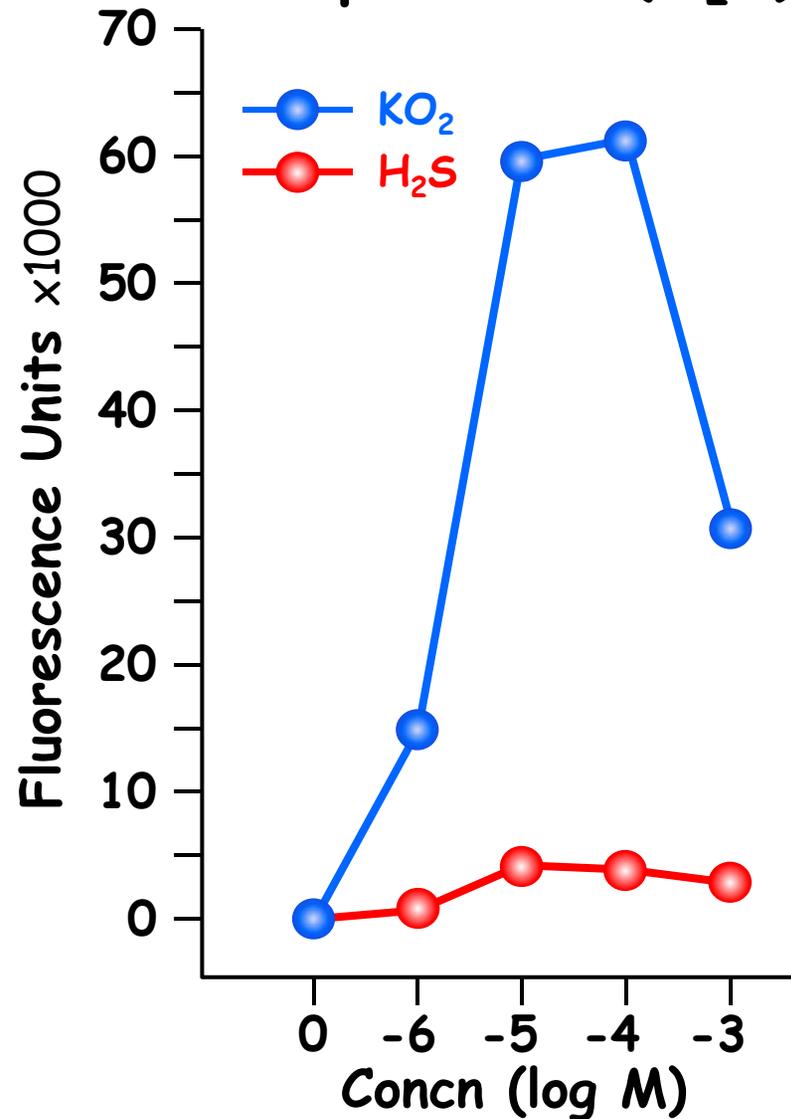
$H_2S_n > 600X H_2O_2$  or  $H_2S$

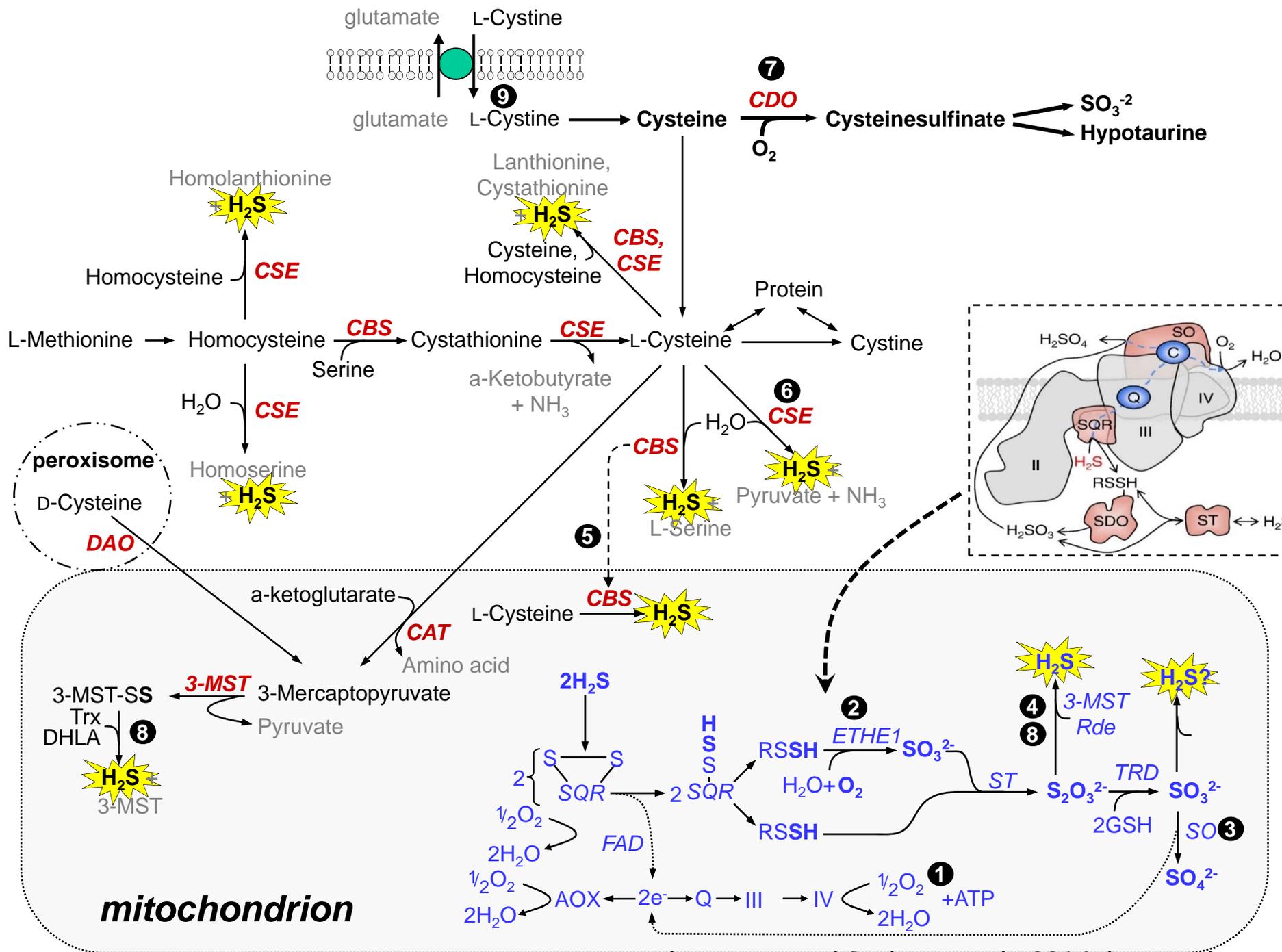
# H<sub>2</sub>S is detected by "Redox-Specific" Fluorescence dyes

## MitoSOX Red (H<sub>2</sub>O<sub>2</sub>)



## Amplex Red (O<sub>2</sub><sup>•-</sup>)

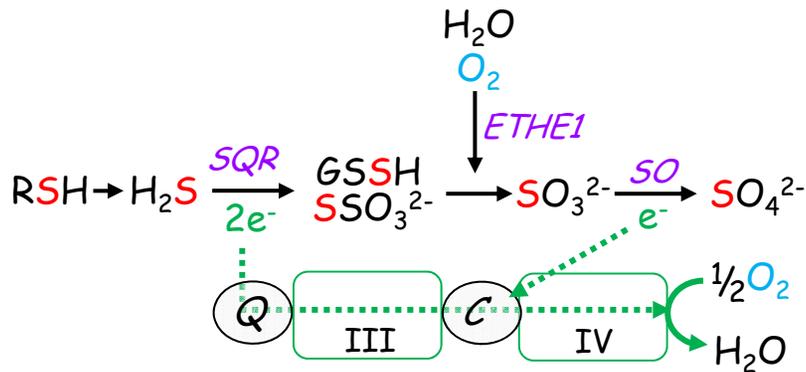




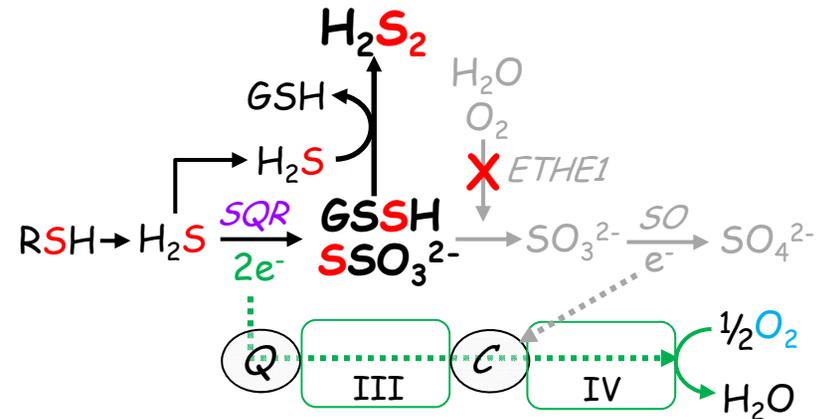
Olson, Antioxid.Redox Signal. 2014 (in press)

# H<sub>2</sub>S, I/R and conditioning - a hypothesis

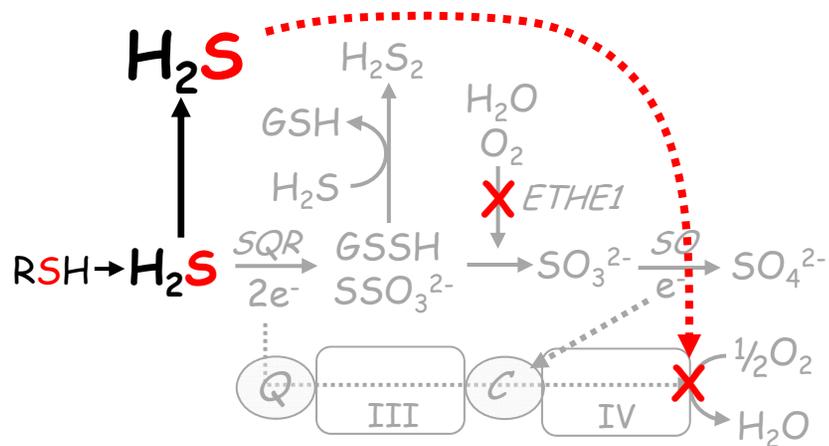
## NORMOXIA



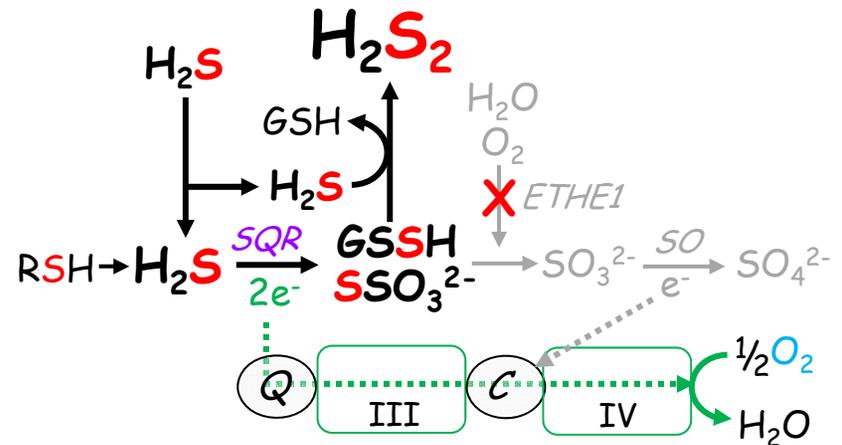
## TRANSIENT HYPOXIA (Conditioning)



## PROLONGED HYPOXIA (I/R)



## REOXYGENATION (sulfide paradox?)

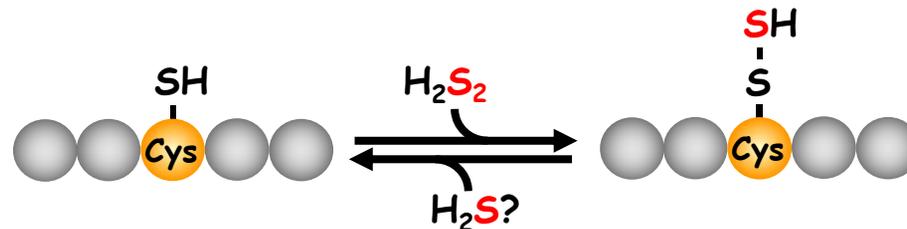


SQR: sulfur:quinone oxidoreductase

ETHE1: mitochondrial sulfur dioxygenase

SO: sulfite oxidase

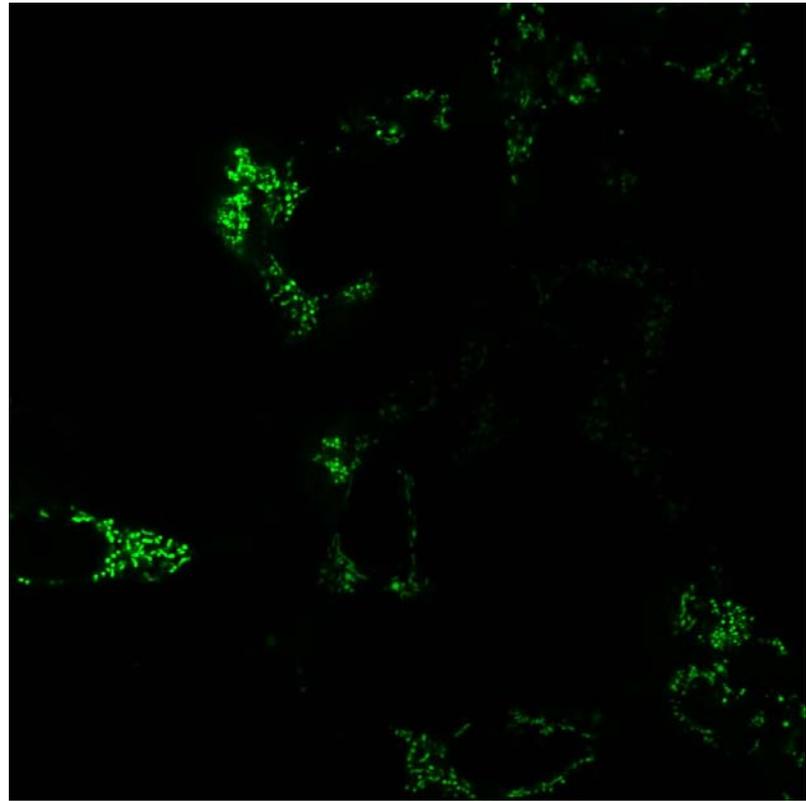
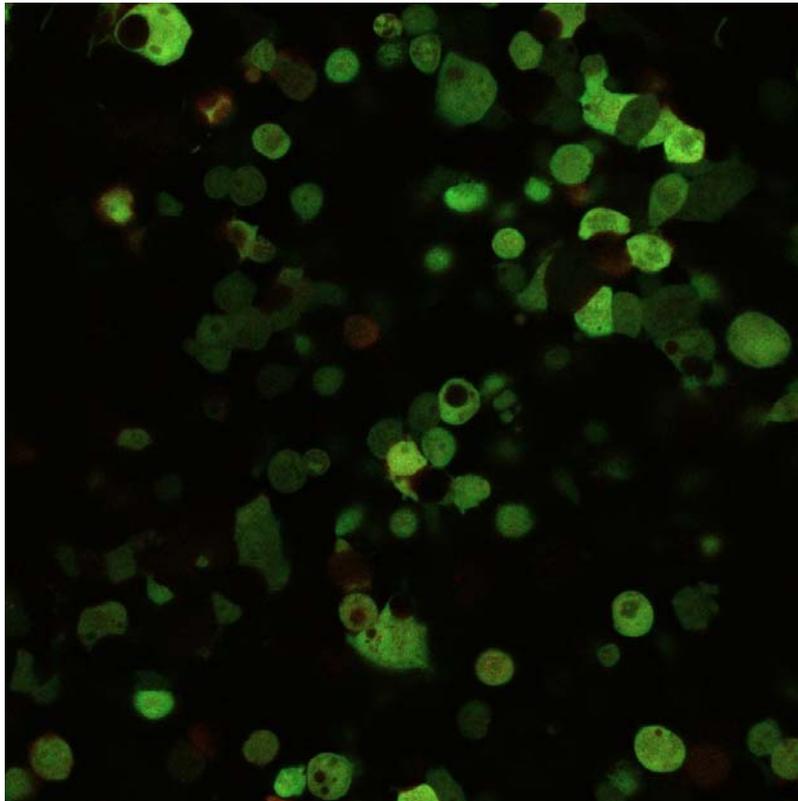
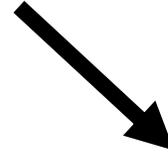
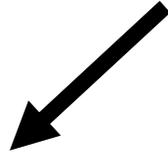
H<sub>2</sub>S vs H<sub>2</sub>S<sub>2</sub> signaling?



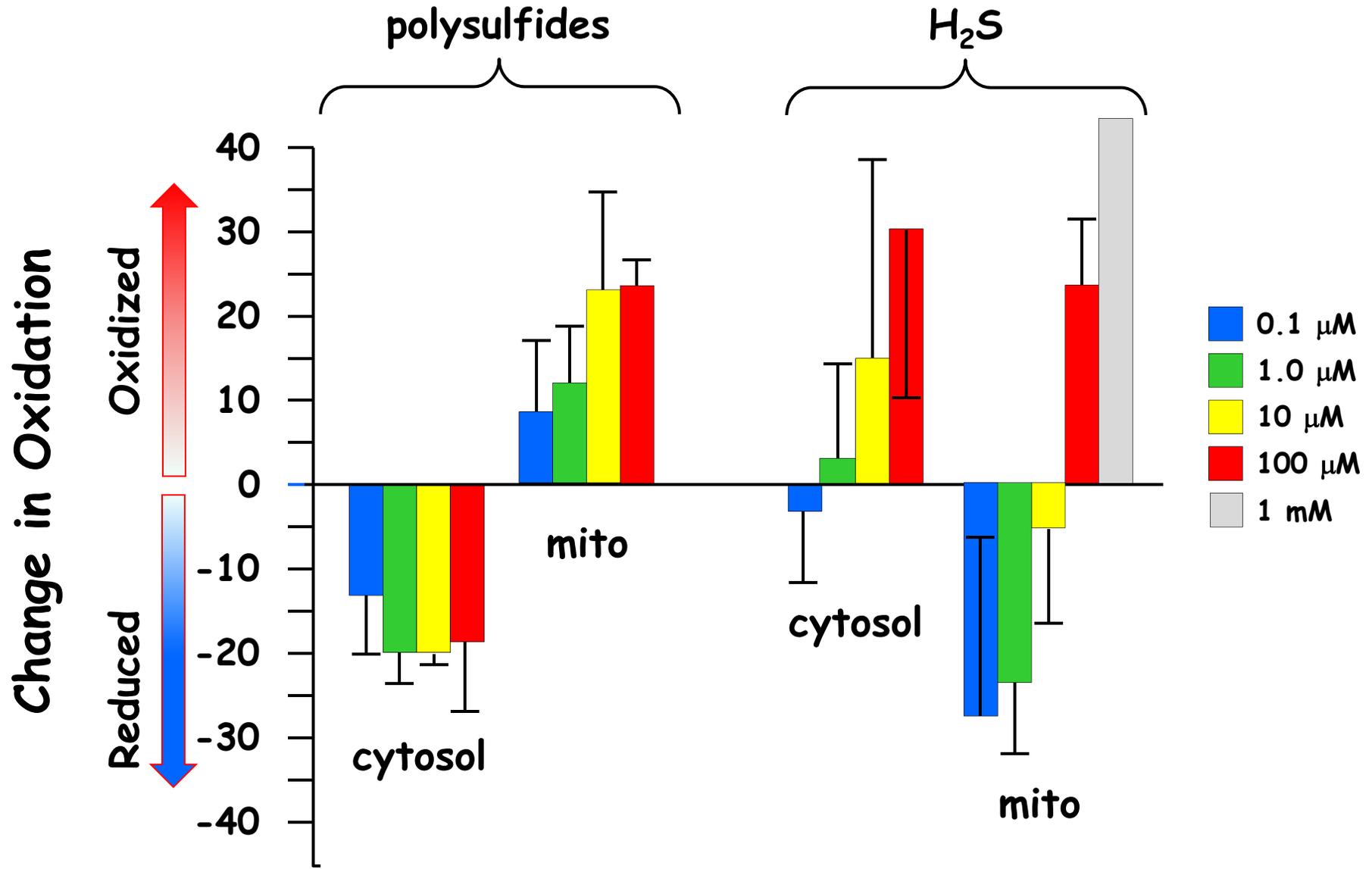




roGFP localized to cytosol or mitochondria in HEK293 cells



# Exogenous polysulfides and H<sub>2</sub>S differentially affect cytosolic and mitochondrial REDOX in HEK 293 cells

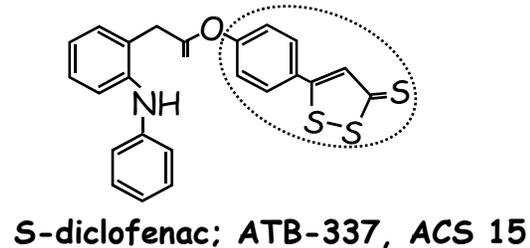
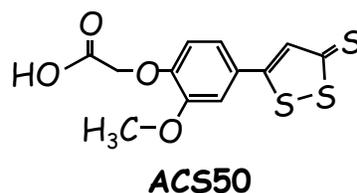
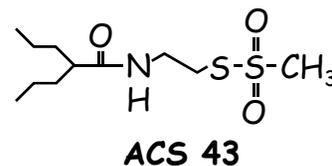
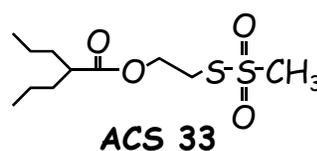
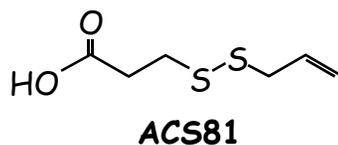
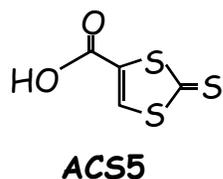
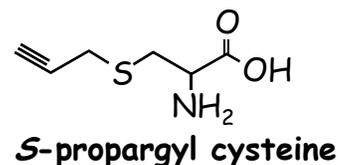
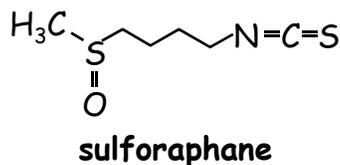
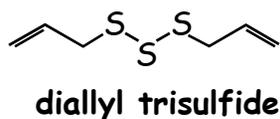
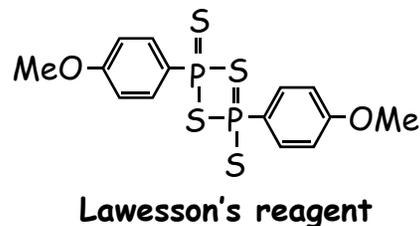
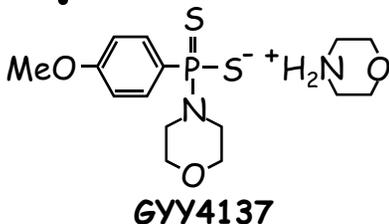


roGFP localized to cytosol or mitochondria in HEK293 cells

(DeLeon unpub.)

# H<sub>2</sub>S “donating” compounds

NaHS Na<sub>2</sub>S



ATB-429 (mescalamine) Inflammatory Bowel Disease (IBD): first stage clinical trials

ATB-284 (?) Irritable Bowel Syndrome (IBS): pre-clinical

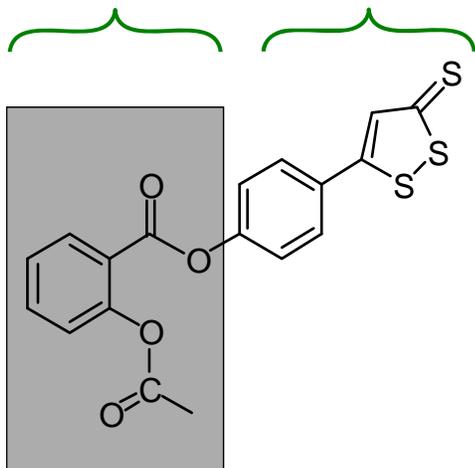
ATB-346 (?) joint pain pre-clinical

ATB: Antibe Therapeutics

ACS: CTG Pharma

Olson, Am.J.Physiol. 301:R297,2010

Traditional NSAID      H<sub>2</sub>S releasing moiety

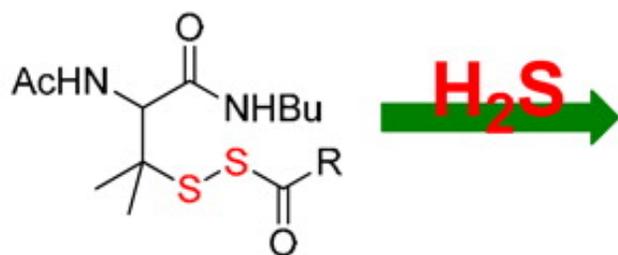
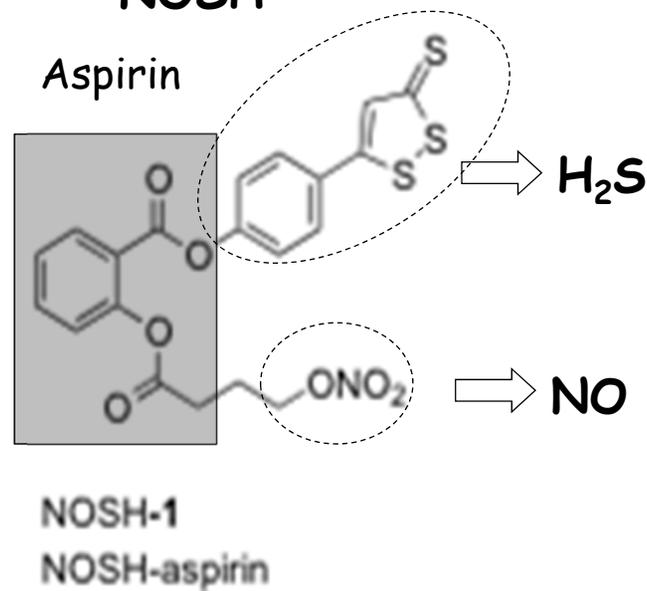


HS-Aspirin

**H<sub>2</sub>S+NSAIDs**  
 ADT-OH + ...  
 aspirin  
 sulindac  
 Naproxin  
 indomethacin  
 ibuprofen  
 mescalamine

**H<sub>2</sub>S+other**  
 ADT-OH + ...  
 latanoprost  
 valproate  
 sildenafil  
 losartan  
 L-DOPA  
 leonurine

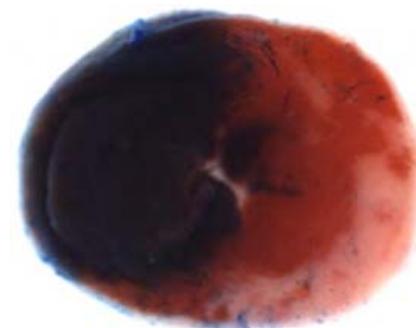
**"NOSH"**



Perthiol-based H<sub>2</sub>S donor



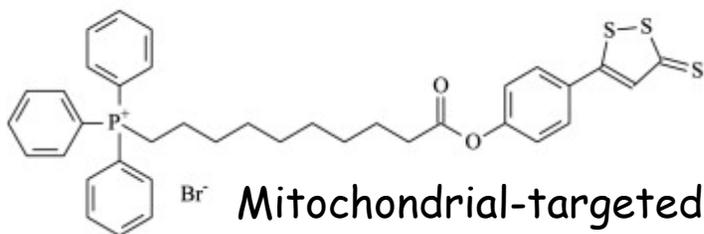
Vehicle



H<sub>2</sub>S Donor

Controllable hydrogen sulfide donors and the activity against myocardial ischemia-reperfusion injury.  
 Zhao et al., ACS Chem.Biol. 8:1283. 2013

**AP39**



Mitochondrial-targeted H<sub>2</sub>S donor

# CONCLUSIONS:

## $H_2S$ and polysulfides

1. Physiologically versatile signaling molecules
2. Evolutionarily ancient
3. Key in  $O_2$  sensing
4. Are RSS the endogenous ROS?
5.  $H_2S$  “donating” drugs may allow greater control over  $H_2S$  delivery, turnover and site of action.