H_2S and systemic inflammation

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National and Kapodistrian University of Athens, Greece

Outline

1. Introduction
2. Production and degradation
3. Signaling, target and physiological functions
4. Agents regulating H_2S levels
5. H_2S, bacteria and antibiotics
6. H_2S and infection

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Gasotransmitter Definition

Gasotransmitter is a gaseous messanger molecule involved in signaling processes

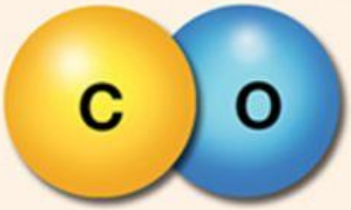
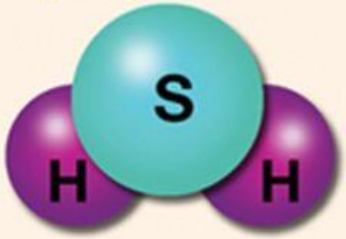
To be a gasotransmmiter, a molecule should:

- Be a small molecules of gas
- Be freely permeable to membranes. Its effects do not rely on cognate membrane receptors
- Be endogenously and enzymatically generated; the generation is regulated
- Have well-defined specific functions at physiologically relevant concentrations; functions of this endogenous gas can be mimicked by its exogenously applied counterpart
- Its cellular effects may or may not be mediated by second messengers, but the molecule should have specific cellular and molecular targets

Wang R, FASEB J, 16:1792-1798, 2002

Gasotransmitters

ENDOGENOUS GASOTRANSMITTERS

	Nitric Oxide	Carbon Monoxide	Hydrogen Sulfide
			
Enzymatic Production	nNOS iNOS eNOS	HO-1	CBS CSE (CGL) 3MST
Blood Concentration	low nM	nM-μM	high nM – low μM
Half-life (*in vivo*)	seconds	minutes	seconds – minutes
Year of Discovery as a Physiological Modulator	1987	1991	1996

Polhemus & Lefer, Circ Res 114:730–737, 2014

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Comparison of gasotransmitter properties

	NO	CO	H_2S
Biological sources	• NO synthases • Non-enzymatic processes (for example, via conversion from nitrite) • Conversion from nitrite by several bacteria (for instance, in the oral cavity)	• Haem oxygenases	• Produced in mammalian cells from L-cysteine by at least three distinct enzymes • Produced from D-cysteine in certain tissues (for example, the kidneys) • Non-enzymatic processes • Produced by enteral bacterial flora (for example, in the oral cavity and intestines)
Chemical properties	• A diffusible and labile free-radical gas	• A diffusible and labile gas	• A diffusible and labile gas
Biological half-life	• Short (a few seconds)	• Long (minutes)	• Medium (seconds to minutes)
Elimination	• Mainly via the urine as nitrite and nitrate • A small amount is exhaled	• Mainly unaltered, in the exhaled air	• Via the urine as sulfite, sulfate and thiosulfate • A small amount is exhaled
Key biological reactions	• Reacts with haem iron centres in various proteins • Reacts with protein cysteines to initiate S-nitrosylation. • Has multiple reactions with oxygen free radicals (for example, with superoxide, to yield peroxynitrite) • Reacts with haemoglobin to yield nitrosyl-haemoglobin and met-haemoglobin	• Binds to haem iron centres • Reacts with haemoglobin to yield carboxyhaemoglobin	• Binds to protein cysteines to initiate sulfhydration • Reacts with oxygen free radicals • Can form persulfides and polysulfides • Reacts with haemoglobin to yield sulfhaemoglobin
Selected signalling pathways	• Activates guanylyl cyclase to increase cGMP levels • Post-transcriptional protein modification via nitrosylation and reactions with haem groups • Activates (opens) K_{ATP} channels	• Reactions with haem groups • Activates guanylyl cyclase (less potently than NO), which then forms cGMP • Activates (opens) K_{Ca} channels	• Post-transcriptional protein modification via sulfhydration • Activates (opens) K_{ATP} channels • Inhibits cGMP and cAMP phosphodiesterases

Szabo, Nat Rev Drug Discov. 15:185-203, 2016

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H_2S toxicity

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H_2S toxicity

0.00047 ppm recognition threshold

< 10 ppm, exposure limit 8hr/d

50–100 ppm eye damage

100–150 ppm olfactory nerve paralysis

320–530 ppm leads to pulmonary edema

530–1000 ppm CNS stimulation, loss of breathing

800 ppm LC_{50} for 5min exposure

> 1000 ppm immediate collapse with loss of breathing

Dangerous Japanese 'Detergent Suicide' Technique Creeps Into U.S.

By Kevin Poulsen ✉ March 13, 2009 | 1:55 pm | Categories: Threats

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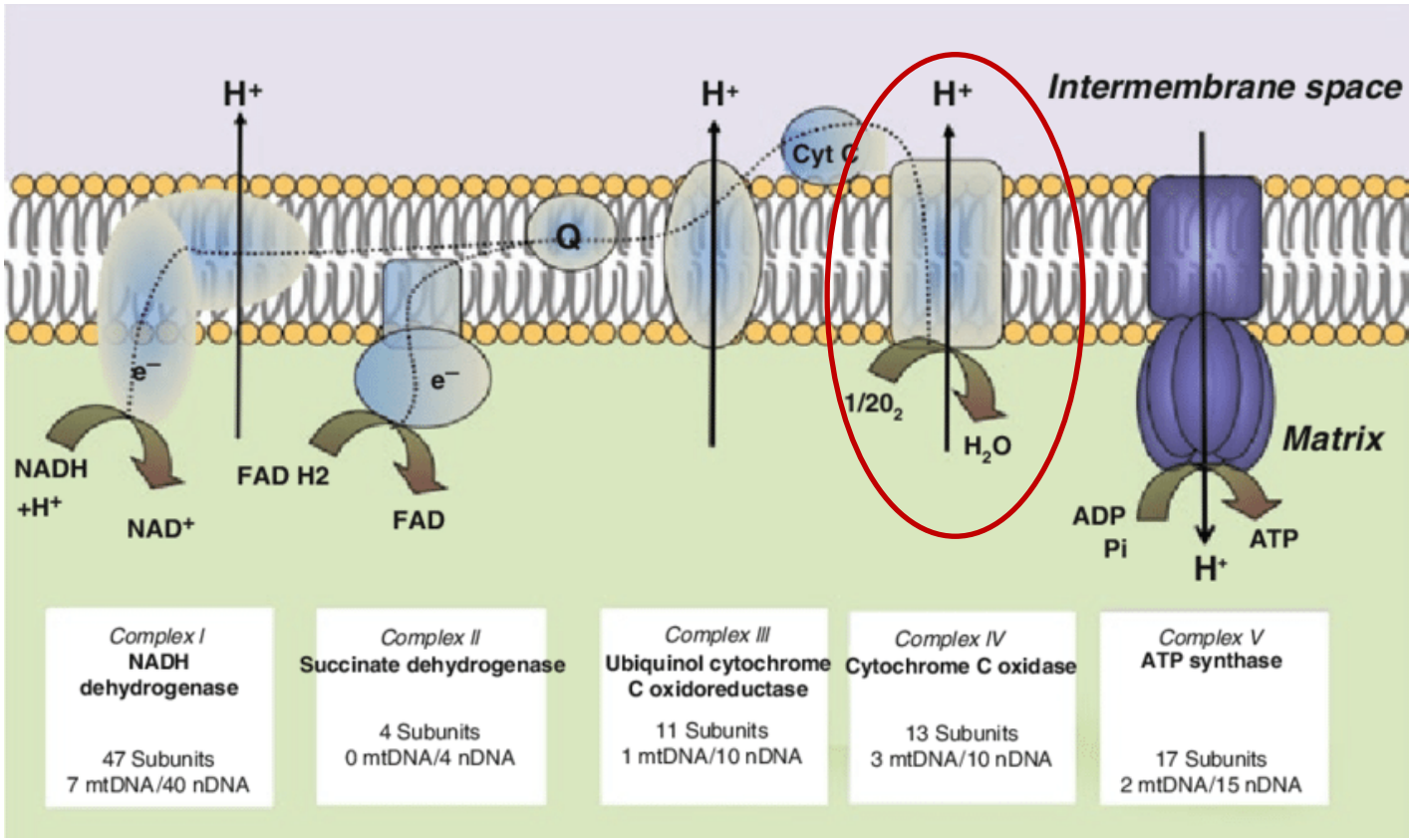
Gasotransmitters target cytochrome C oxidase



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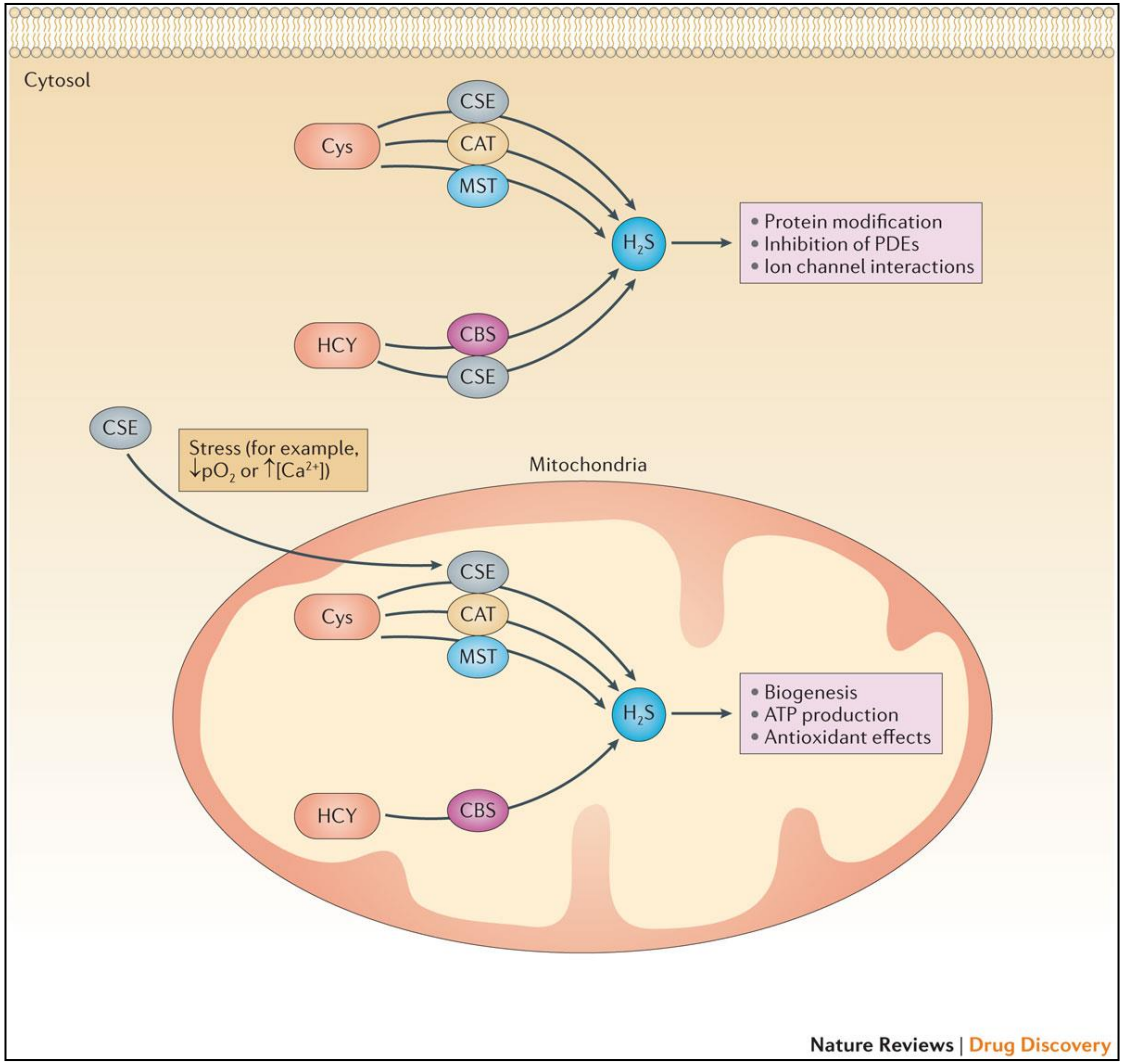
H_2S biosynthesis



Wang & Wallace, Nat. Rev. Drug Discov, 14:329-45, 2015

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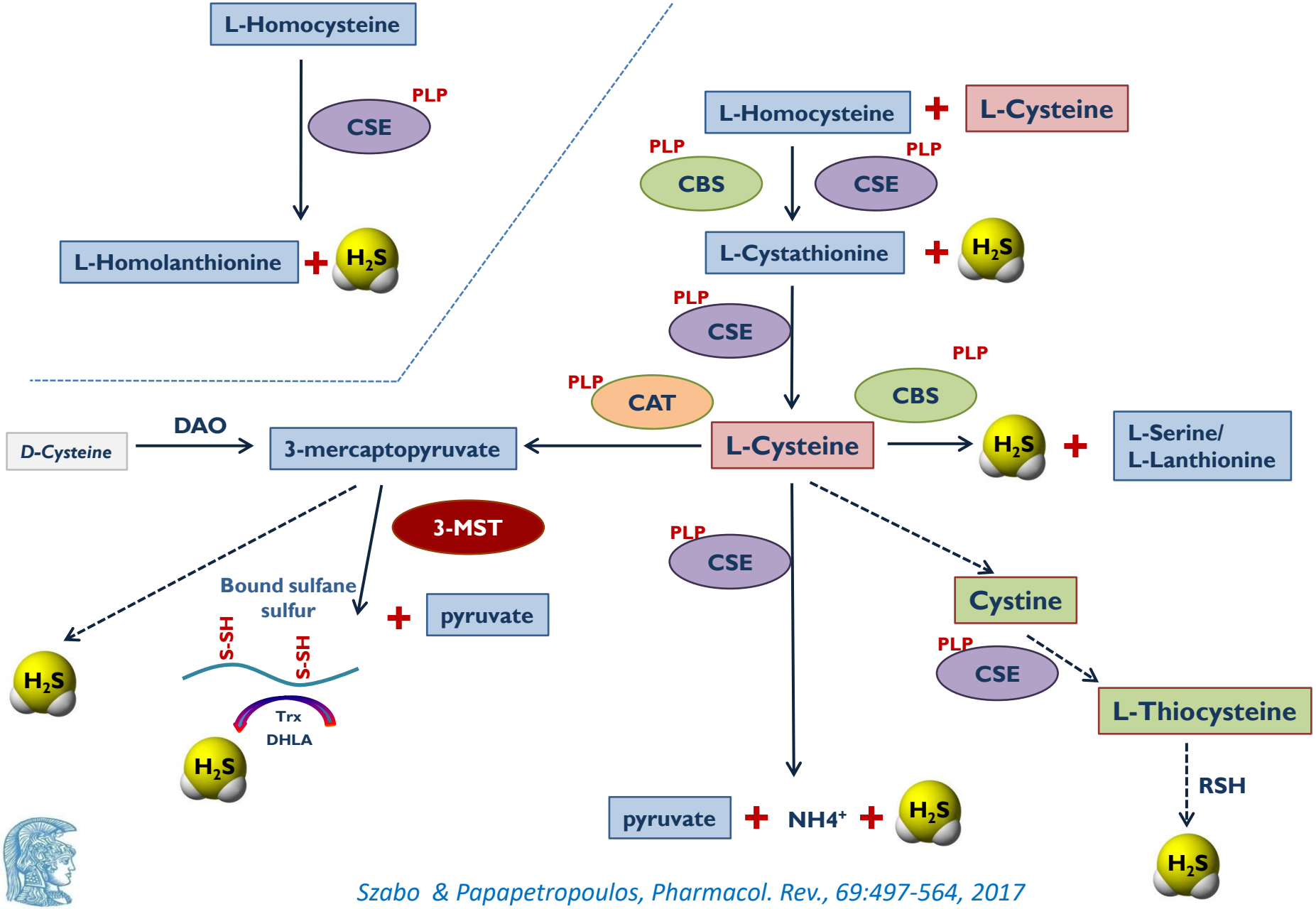
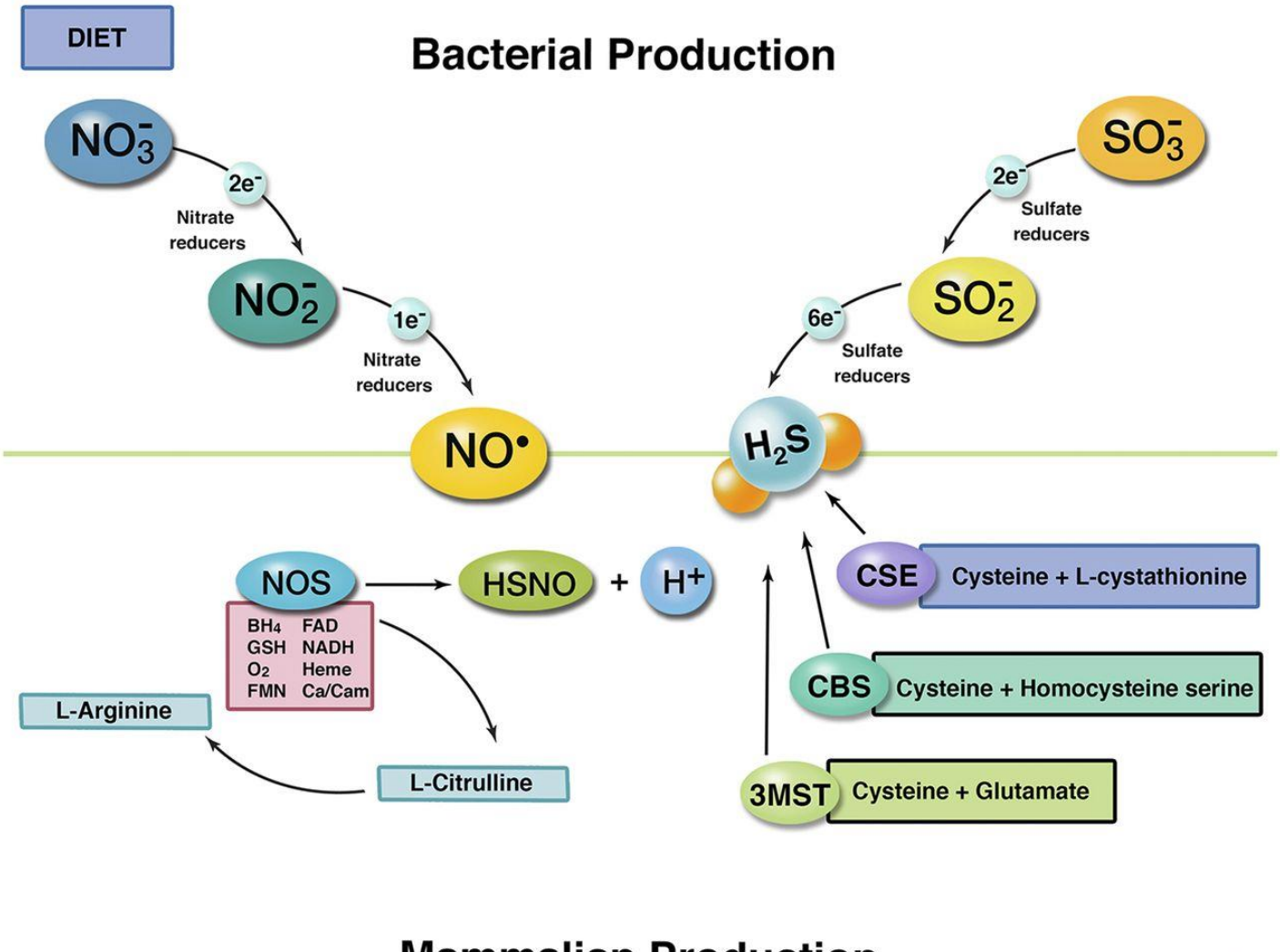
H_2S synthesis



Szabo & Papapetropoulos, Pharmacol. Rev., 69:497-564, 2017

H_2S synthesis in bacteria



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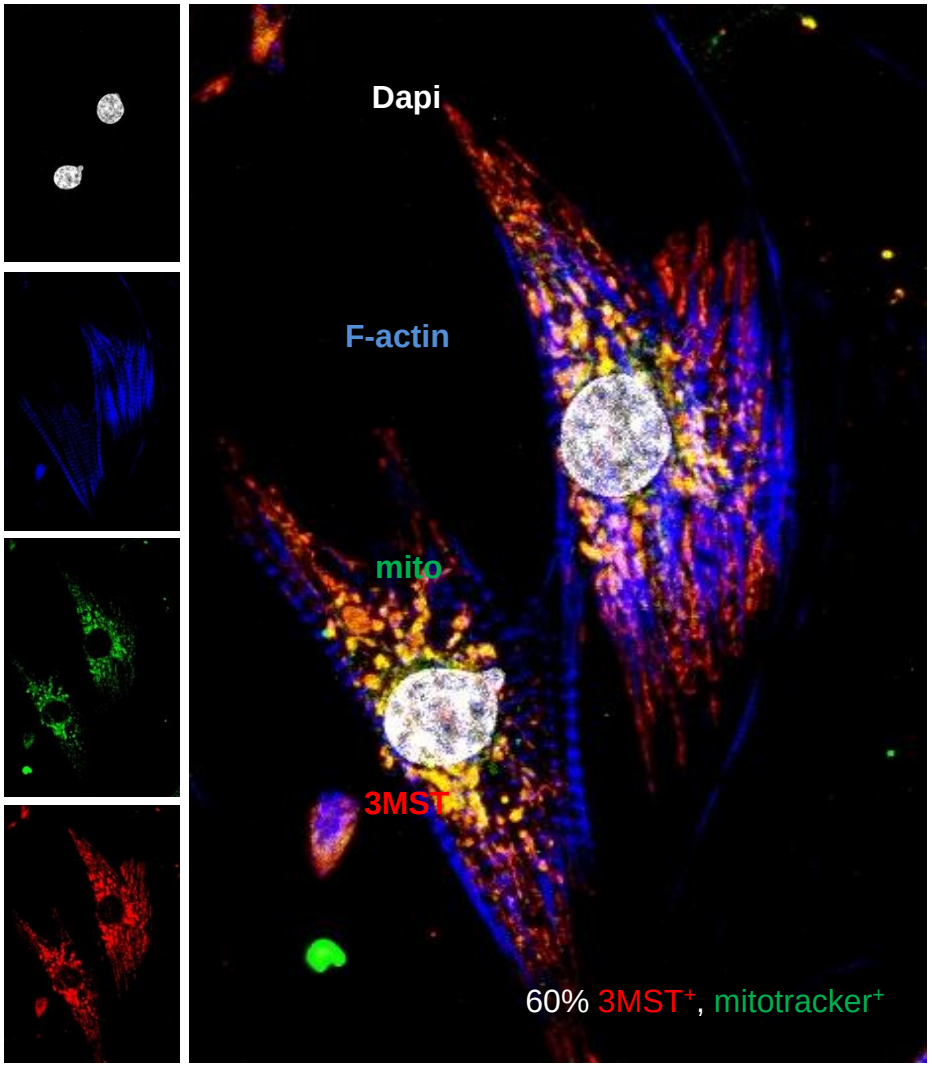
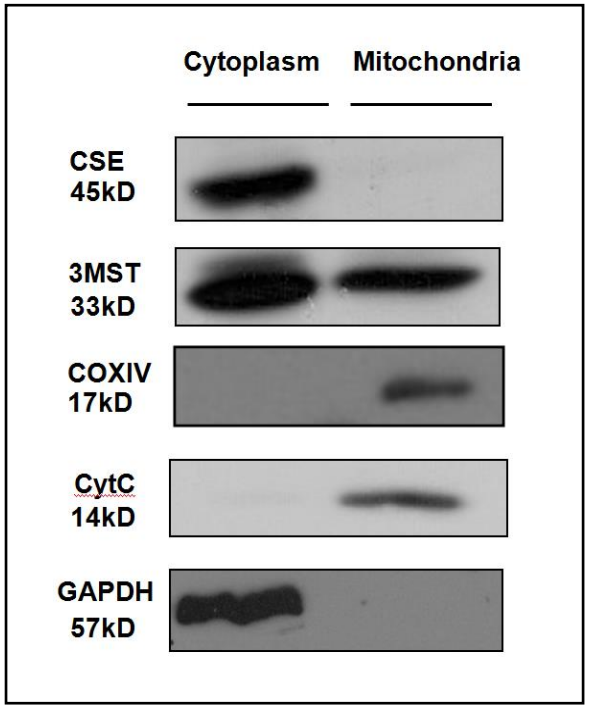
H_2S-synthesizing enzyme localization





Peleli et al, Biochem Pharm, 176:113833, 2020

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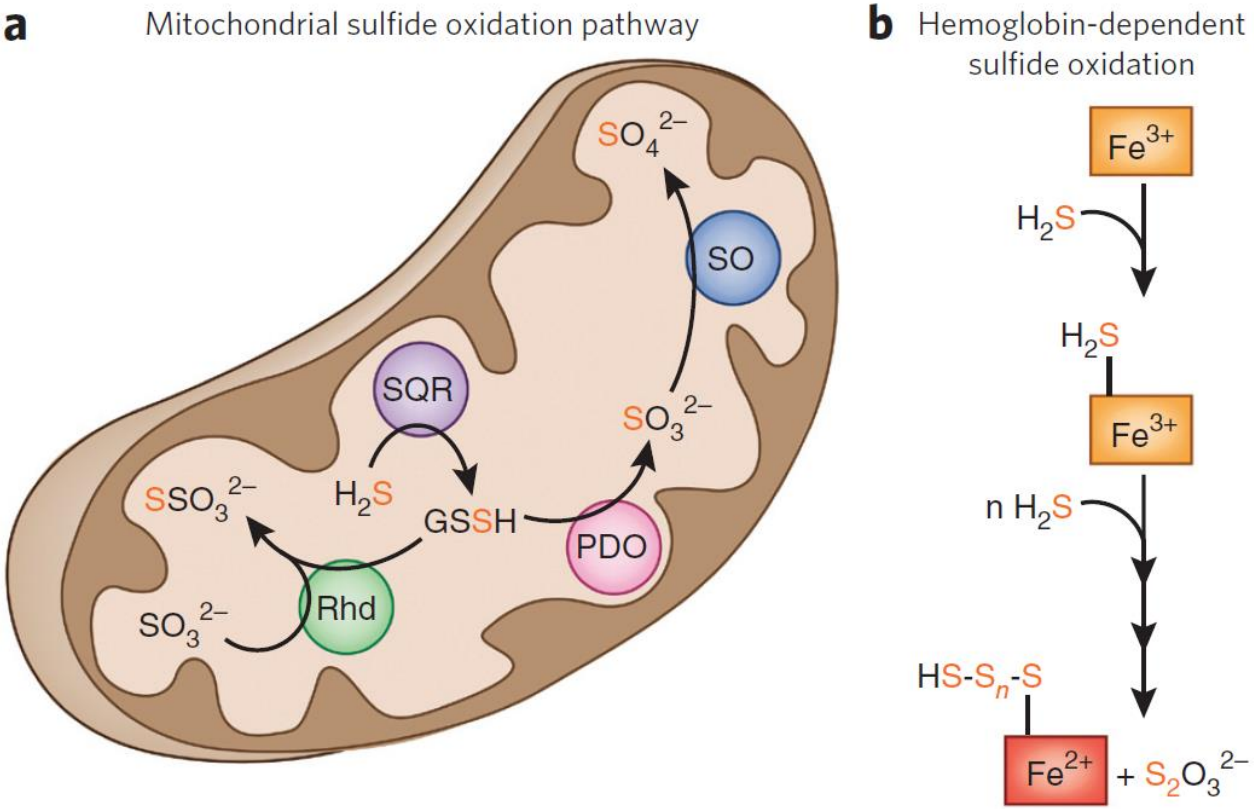
H_2S degradation



Mishanina et al., Nature Chem Biol, 11:457-464, 2015

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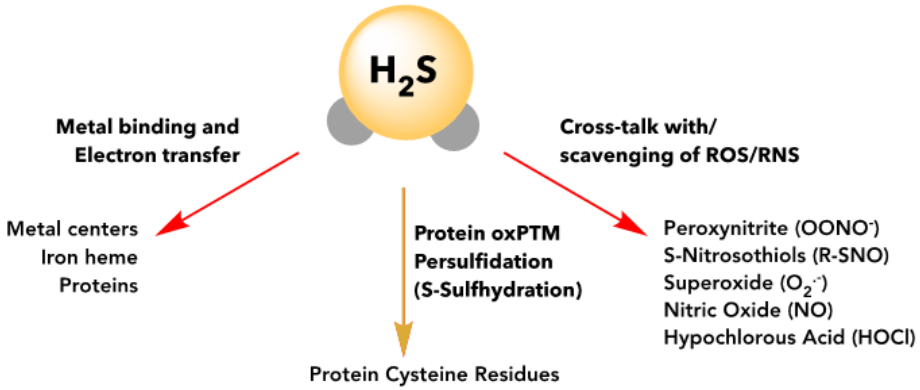
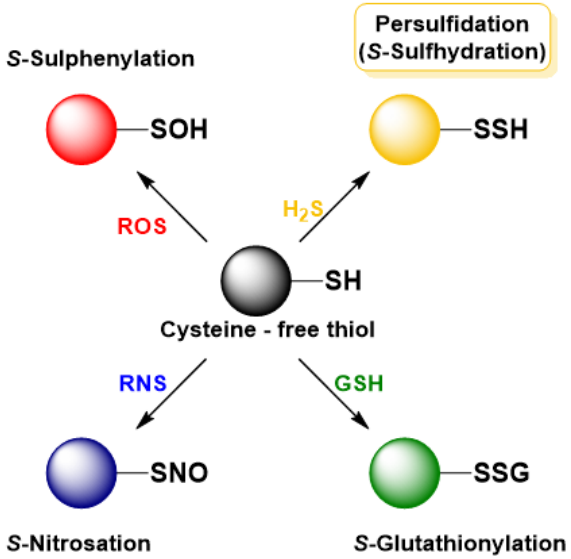
H_2S signaling



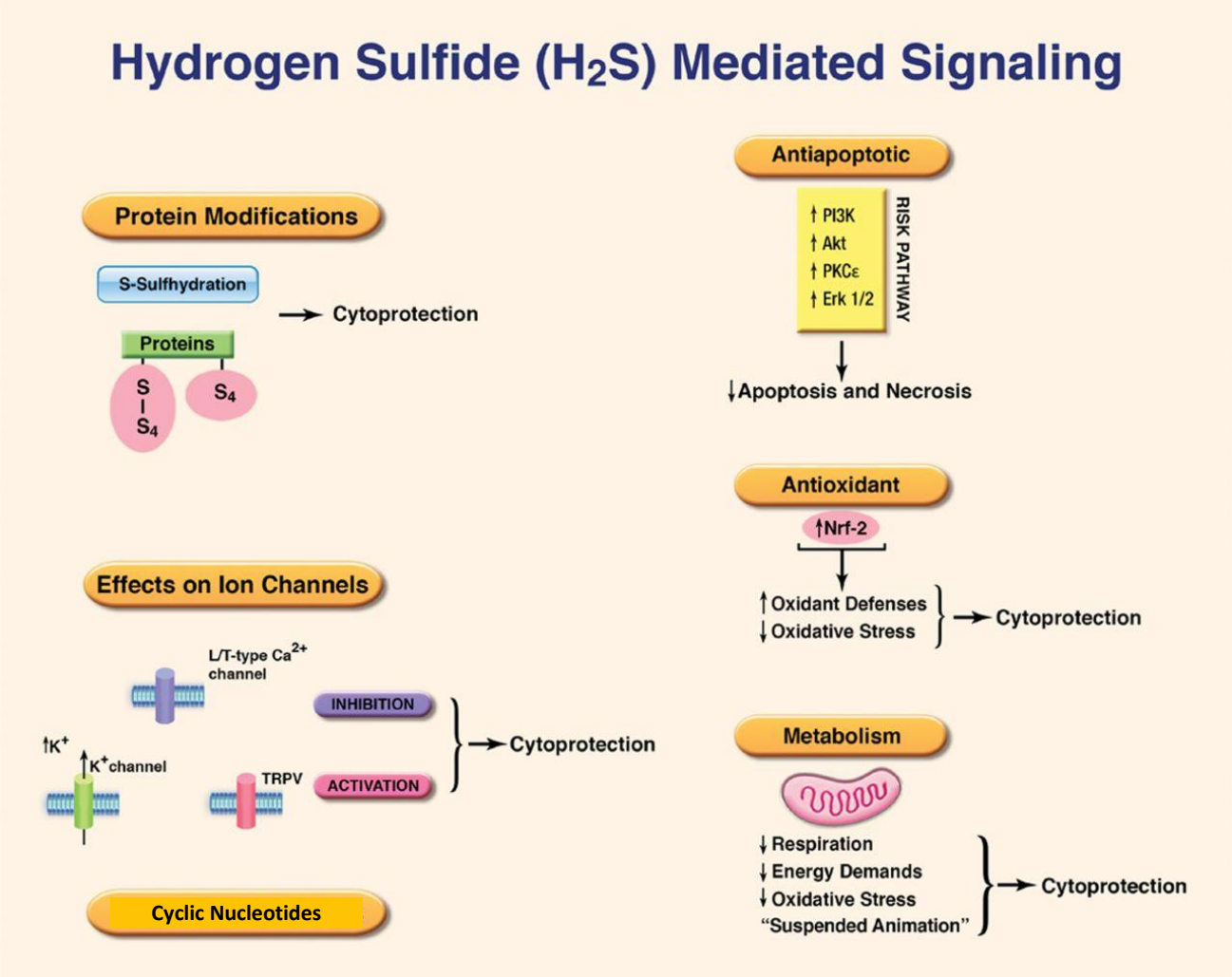
Persulfidation

A means of explaining biological effects of H_2S through the modulation of cysteine residues?



Oxidative Posttranslational modifications (oxPTM) of Cysteines

Examples of biological targets of H_2S



Adapted from Polhemus D J , and Lefer D J Circ Res 114:730-737, 2014

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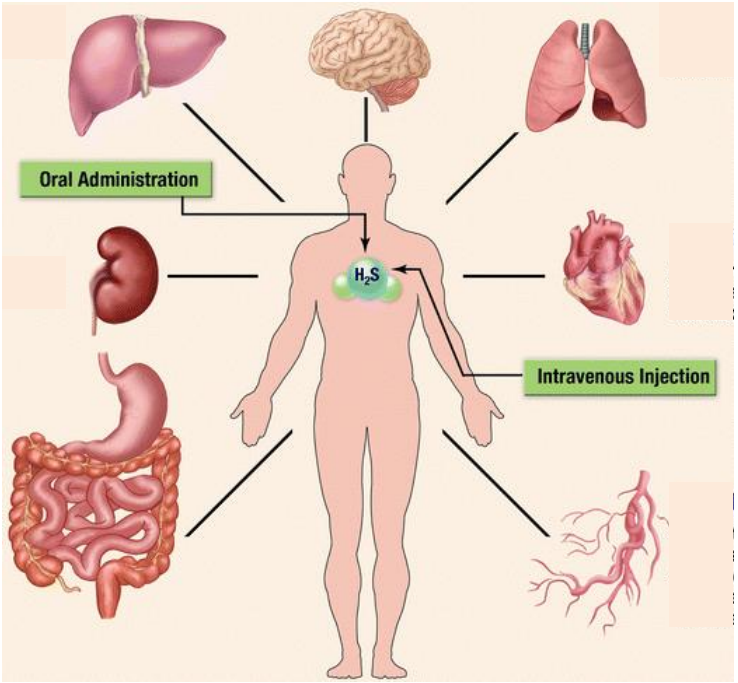
Physiological effects of H_2S

- Antioxidant
- Anti-apoptotic
- Angiogenesis stimulator
- Bronchodilation
- Cardioprotective
- Glucose and lipid homeostasis
- Inhibits atherosclerosis
- Inhibits fibrosis
- Inhibits inflammation
- Promotes physiological calcification (bone)
- Smooth muscle relaxation
- Vasorelaxation



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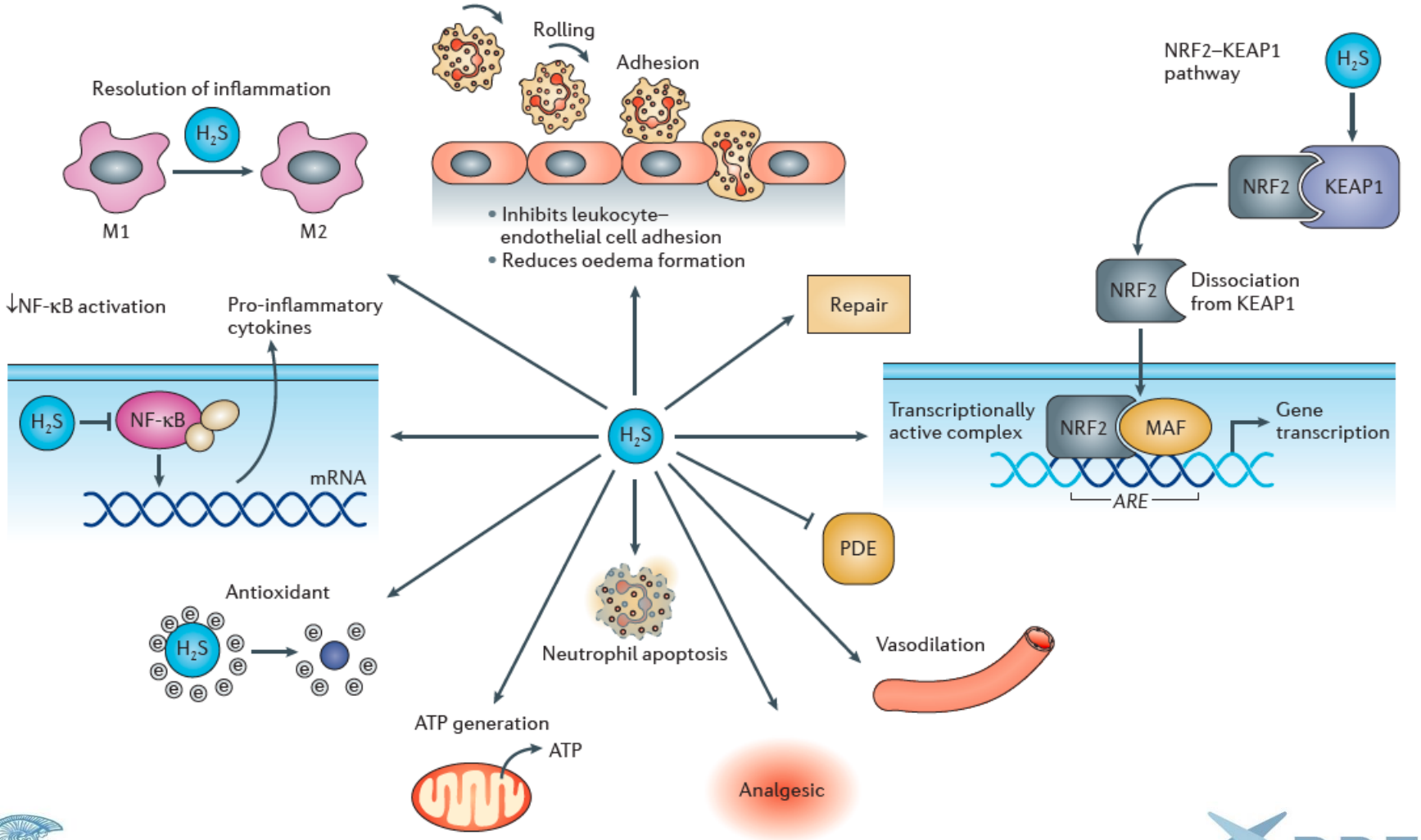
Hydrogen Sulfide is an Anti-Inflammatory Molecule



Wang & Wallace, Nat Rev Drug Discov, 14:329-45, 2105

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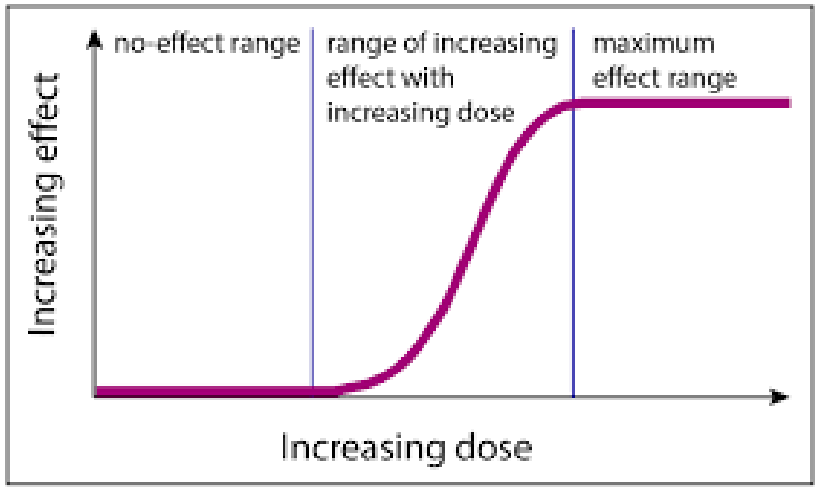
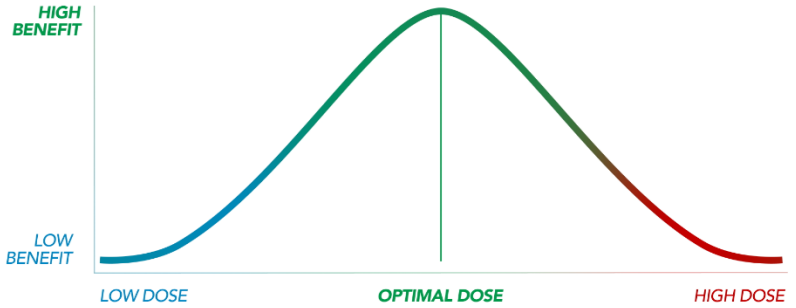
Concentration-response curves of gasotransmitters





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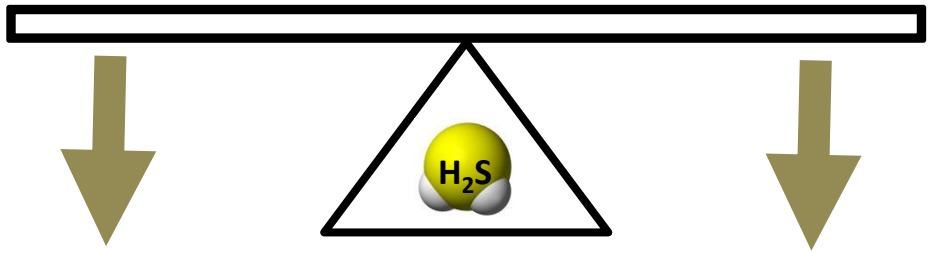
"H_2S-poor" state

Aging
Ischemia
Heart failure
Liver disease
Hypertension
Atherosclerosis
Obesity
Endothelial dysfunction
Diabetic complications
(cardiovascular system)
Preeclampsia
Alzheimer's disease
Huntington's disease

Circulatory shock
Burns
Cancer
Sleep apnea
Down Syndrome
Diabetes onset
(beta cells)
Stroke
Schizophrenia

"H_2S-rich" state



Increased H_2S consumption and/or Downregulation of H_2S producing enzymes

H_2S donors

Upregulation of H_2S producing enzymes

CBS/CSE inhibitors

Szabo & Papapetropoulos, Pharmacol Rev. 69:497-564, 2017

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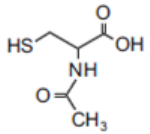
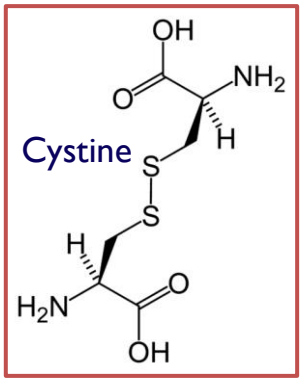
Properties & Differences of H_2S donors

- Source (naturally occurring, synthetic)
- Sulfide salts (Na_2S, NaHS)-not really donors
- Mode of H_2S release: spontaneous vs controlled (Cys-activated, ROS-activated, pH-activated, esterase-activated)
- Rate of release
- Targeted delivery (AP39)
- Hybrid or bi-functional donors (ATB-346, adenine-H_2S, many others)
- Clinically used (NAC, cystine, zofenopril)

H_2S donors/precursors approved for human use

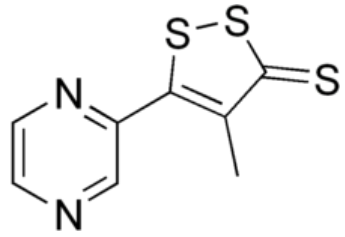


N-acetylcysteine



thiosulfate
$S_2O_3^{2-}$

Food Supplement



oltipraz

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Beneficial effects of H_2S in traditional medicine

PNAS

Hydrogen sulfide mediates the vasoactivity of garlic

Gloria A. Benavides*†, Giuseppe L. Squadrito*†, Robert W. Mills*, Hetal D. Patel*, T. Scott Isbell*§, Rakesh P. Patel*§, Victor M. Darley-Usmar*§, Jeannette E. Doeller*†, and David W. Kraus*†¶

Departments of *Environmental Health Sciences, †Biology, and §Pathology and ¶Center for Free Radical Biology, University of Alabama at Birmingham, Birmingham, AL 35294

Edited by Solomon H. Snyder, Johns Hopkins University School of Medicine, Baltimore, MD, and approved September 12, 2007 (received for review June 18, 2007)

The consumption of garlic is inversely correlated with the progression of cardiovascular disease, although the responsible mechanisms remain unclear. Here we show that human RBCs convert garlic-derived organic polysulfides into hydrogen sulfide. Searcy and Lee (11), corroborated by ourselves (data not shown), have demonstrated that human RBCs produce H_2S when provided with elemental sulfur (S_8) or inorganic polysulfides (S_2^{2-} and S_2^{2-}). However, because inorganic polysulfides

SEE COMMENTARY

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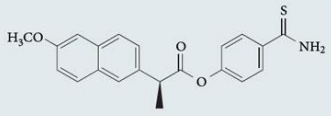
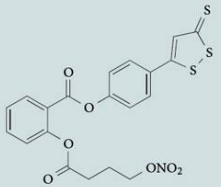
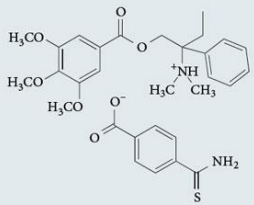
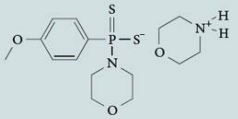
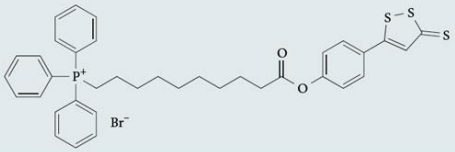
Translation of H_2S donors

Institution (location)	Structure	Clinical indications	Lead drug	Comment	Stage of development
Antibe Therapeutics (Toronto, Ontario, Canada)	 ATB-346	Osteoarthritis	ATB-346	Naproxen derivative	Phase I
		Acute pain	ATB-352	Ketoprofen derivative	Preclinical
		Veterinary (pain)	ATB-338	Diclofenac derivative	Preclinical
		Thrombosis	ATB-350	Aspirin derivative	Preclinical
City University of New York (New York, USA)		Cancer	NBS-1120	Aspirin derivative	Preclinical
Glcare Pharma (Montreal, Quebec, Canada)		Colonic pain	GIC-1001	Trimebutine salt; licensed from Antibe Therapeutics	Phase II for analgesia during colonoscopy*
National University of Singapore (Singapore)		Hypertension, inflammation, cancer	GYY4137	Slow-releasing H_2S donor	Unknown
Sova Pharmaceuticals (La Jolla, California, USA	No structure available	Pain, metabolic disorders	Unknown	Inhibitor of CSE activity	Unknown
SulfaGENIX (New Orleans, Louisiana, USA)		Oxidative stress	SG-1002	Polyvalent sulfur	Phase II for heart failure‡
University of Exeter (Exeter, UK)		Inflammation, oxidative stress	AP39	Mitochondrion-targeted H_2S release	Preclinical

CSE, cystathionine γ-lyase; H_2S, hydrogen sulfide. *ClinicalTrials.gov identifiers: NCT01926444 and NCT02276768. ‡ClinicalTrials.gov identifier: NCT01989208.

Wang & Wallace, Nat Rev Drug Discov, 14:329-45, 2105

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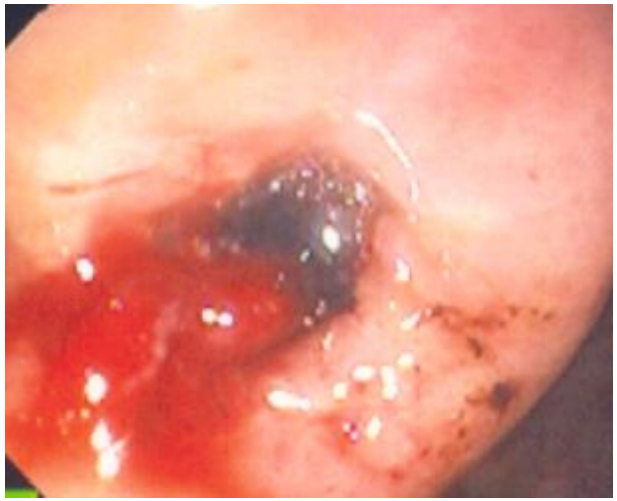
H_2S-Releasing Anti-Inflammatory Drugs



- **30 million people take NSAIDs on a daily basis**
- **15.3 deaths per 100,000 users**

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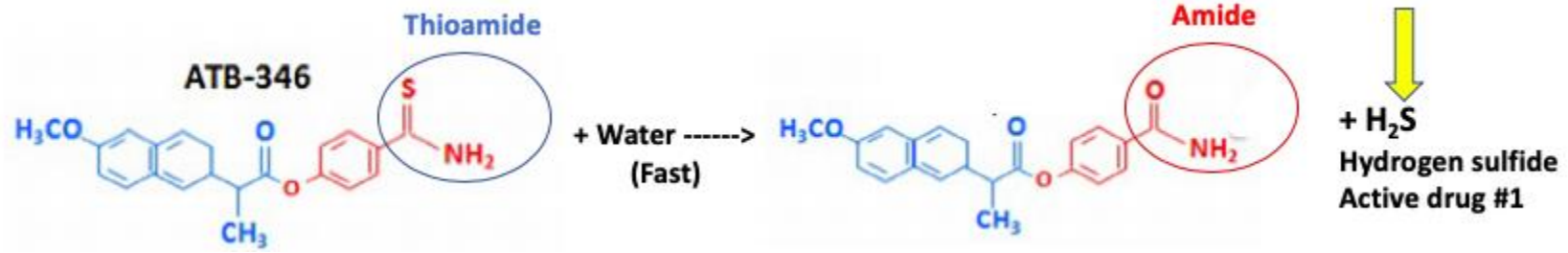
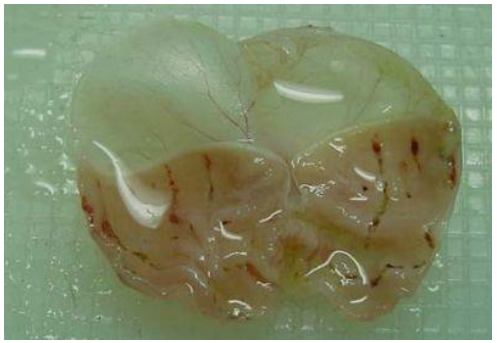
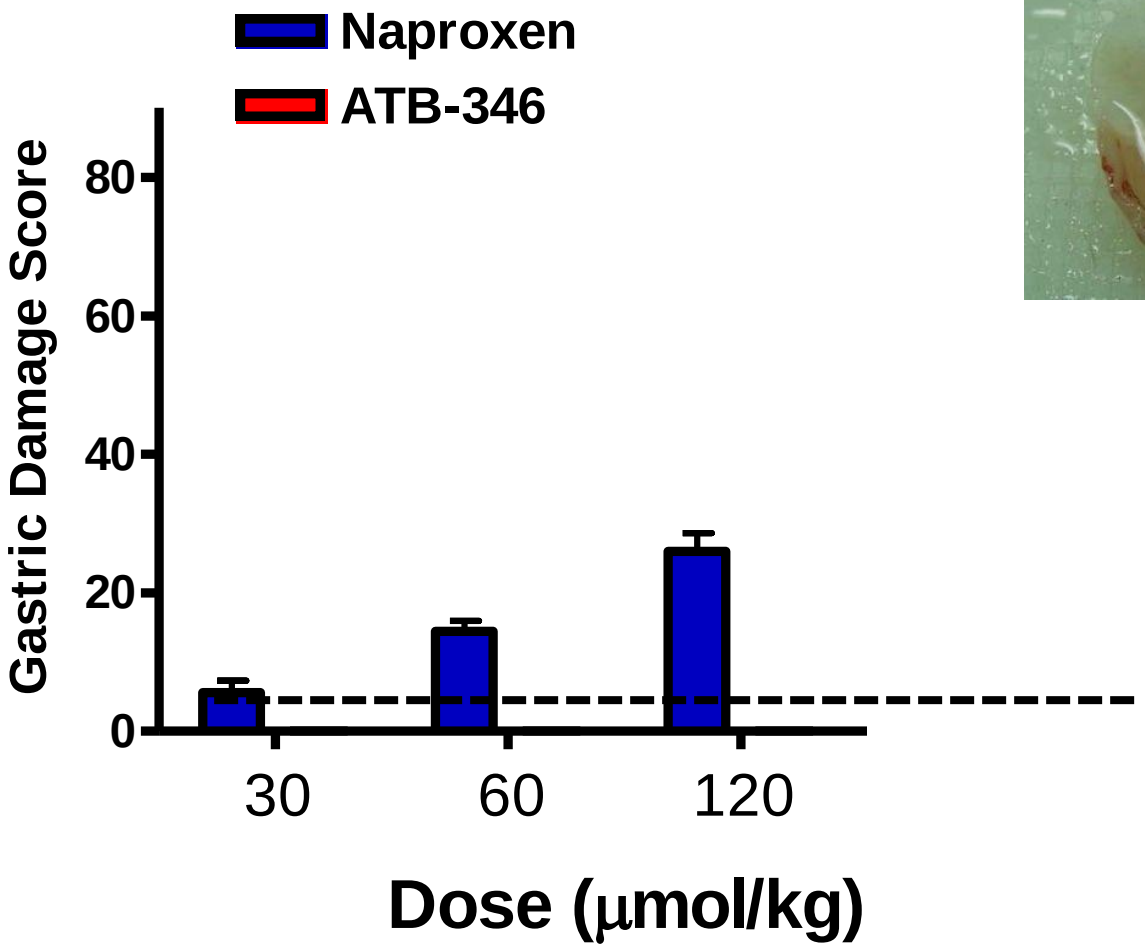
Otenaproxesul (ATB-346)



ATB-346 Causes Negligible Gastric Damage





Wallace et al., Br J Pharmacol. 159: 1236–1246, 2010

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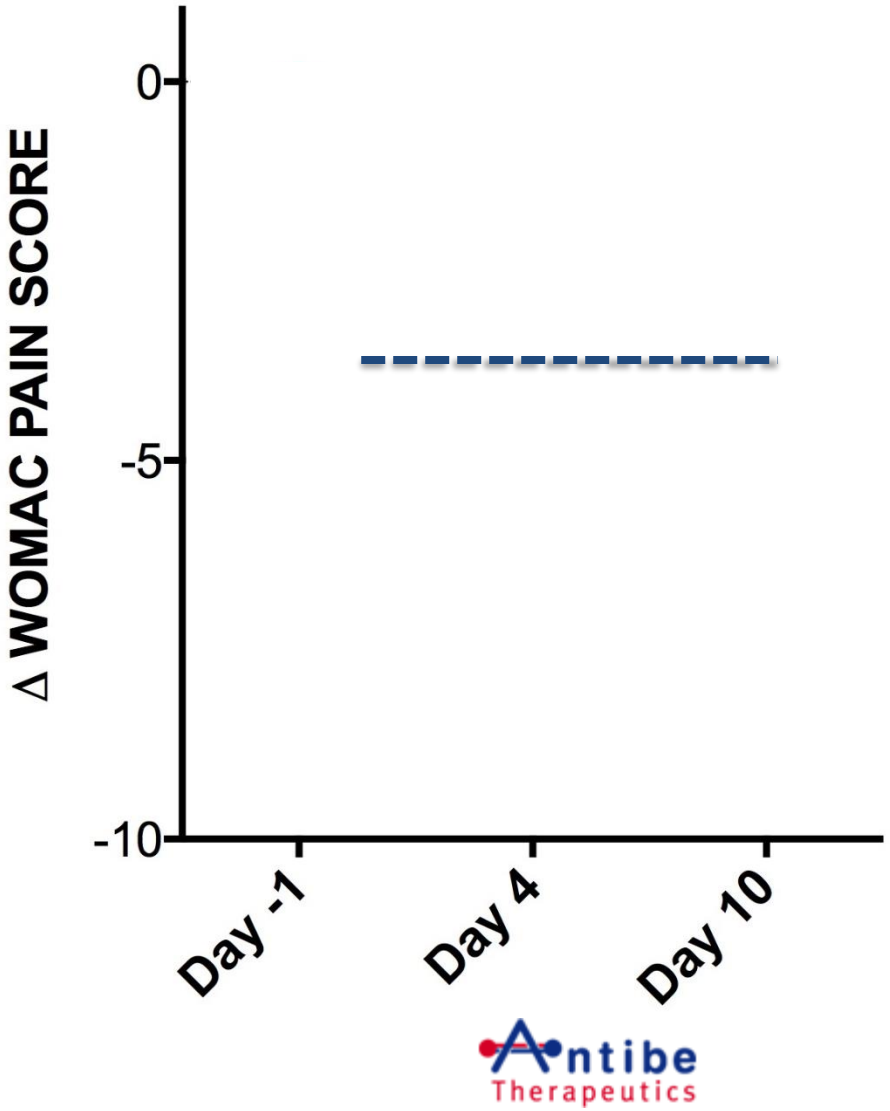
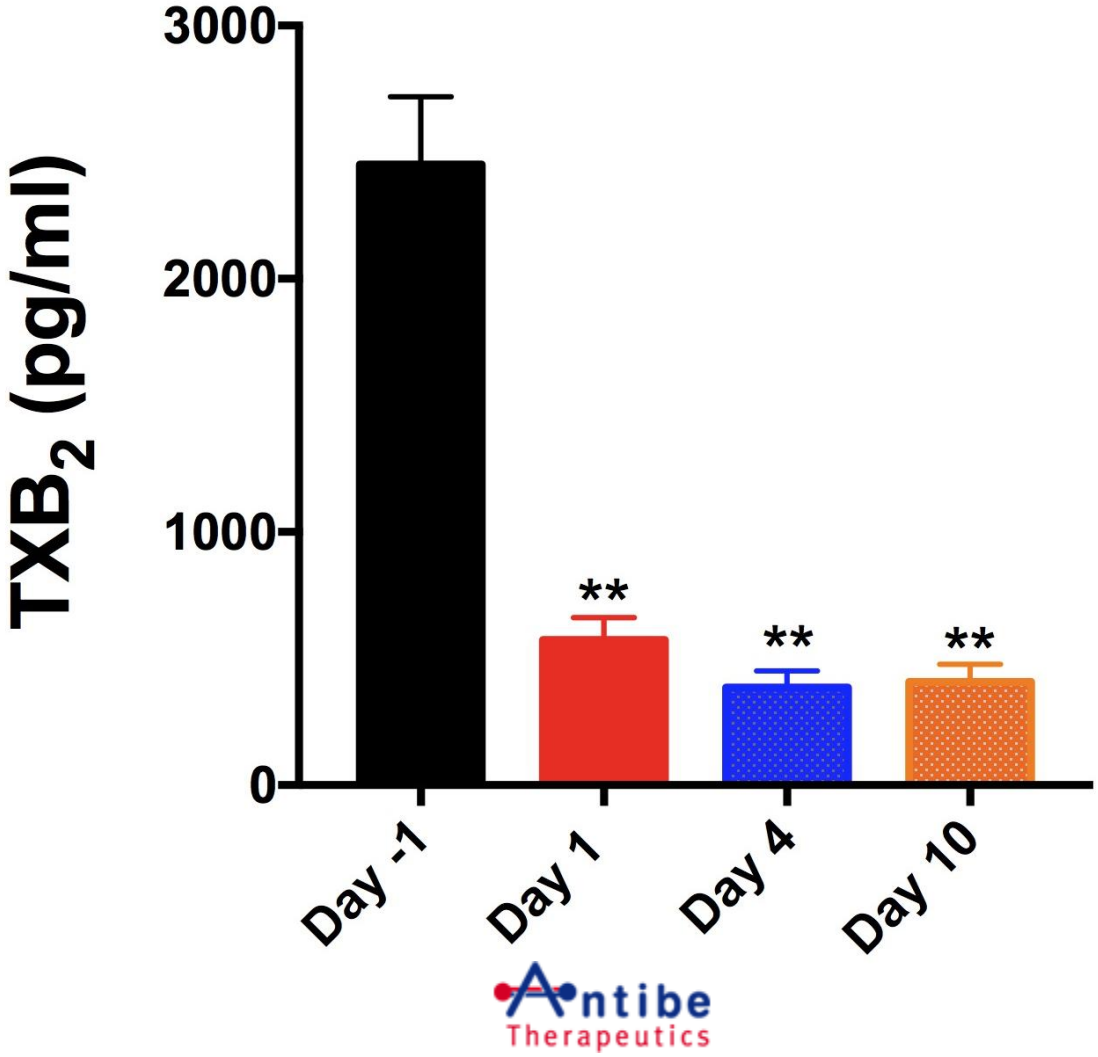
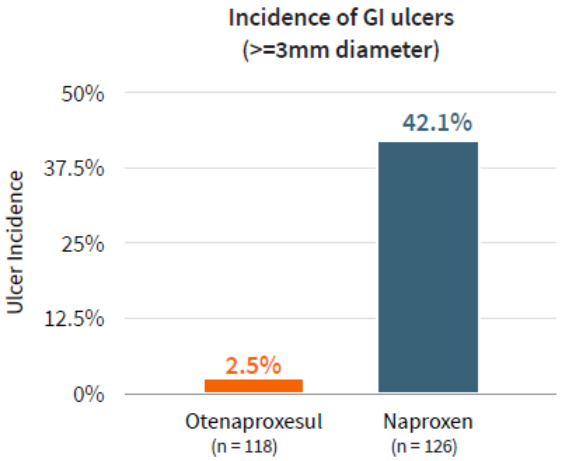
Otenaproxesul reduces pain in patients with OA

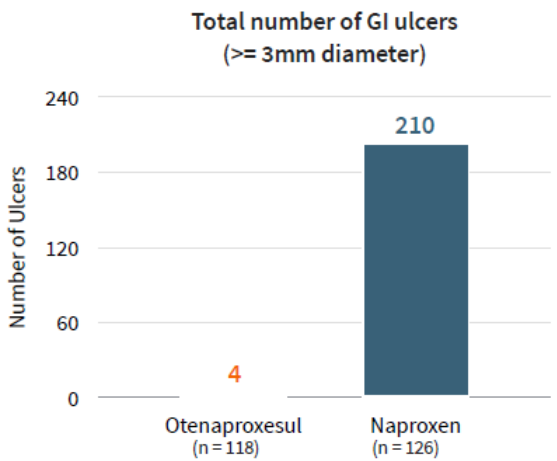


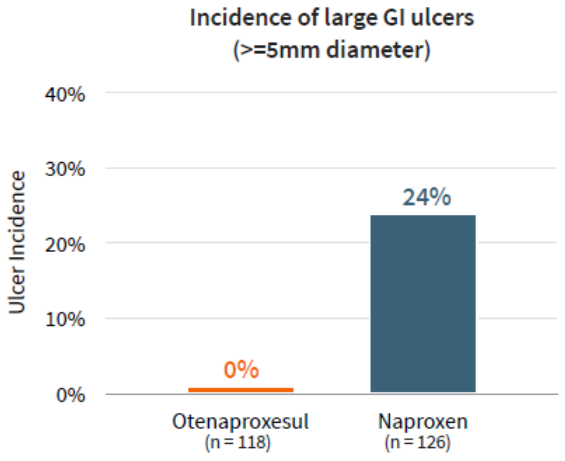
Otenaproxesul reduces prostaglandin synthesis in patients with OA



GI safety of otenaproxesul







Antibe Therapeutics

H_2S synthesis inhibitors

Compounds	CSE, IC_{50} (μM)	CBS, IC_{50} (μM)
PAG	40.0 ± 8.0	–
BCA	14.0 ± 0.16	–
HA	4.83 ± 0.31	278.0 ± 22.0
AOAA	1.09 ± 0.12	8.52 ± 0.71
Trifluoroalanine	289.0 ± 7.0	66.0 ± 9.0
AVG	1.0 ± 0.1	–

Asimakopoulou et al, Br. J. Pharmacol, 169: 922–932, 2013

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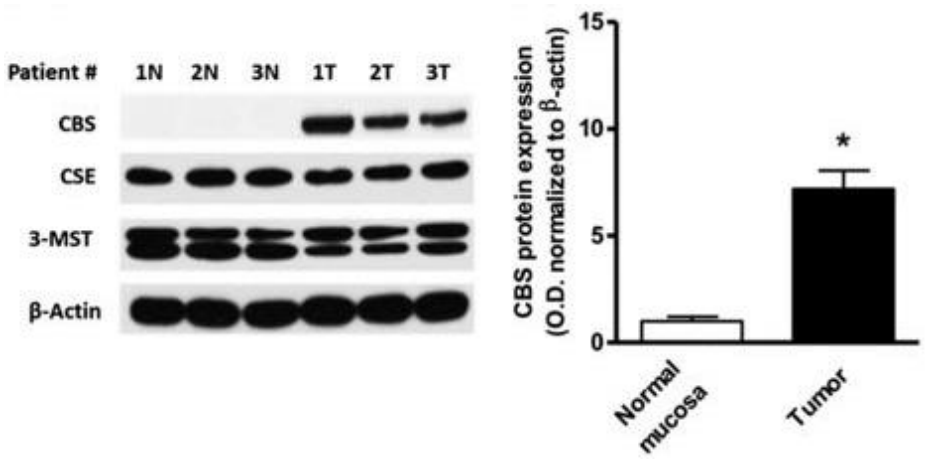
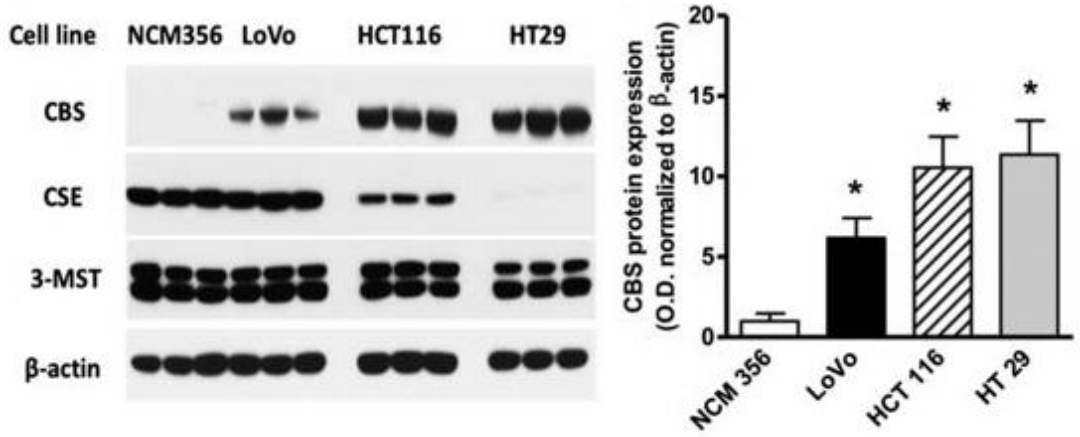
CBS is over expressed in human colorectal cancer





Szabo C et al. PNAS, 110:12474-12479, 2013

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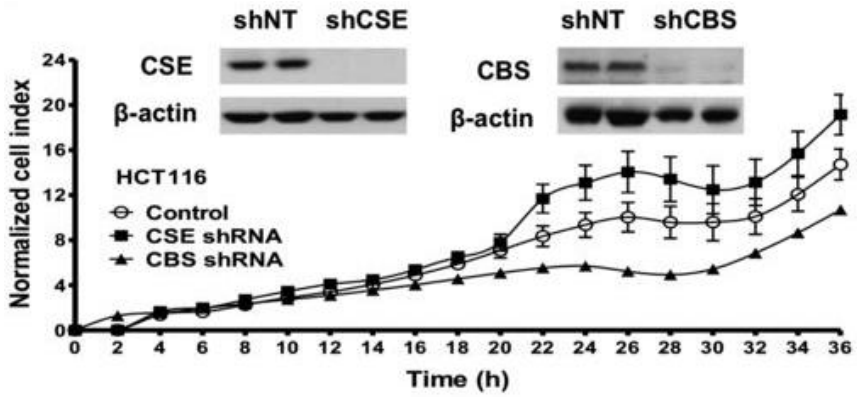
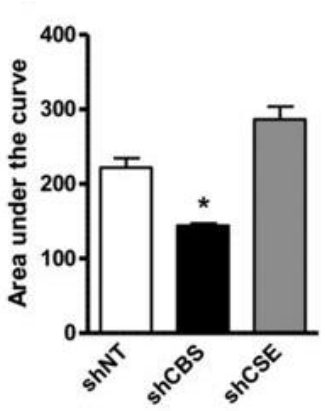
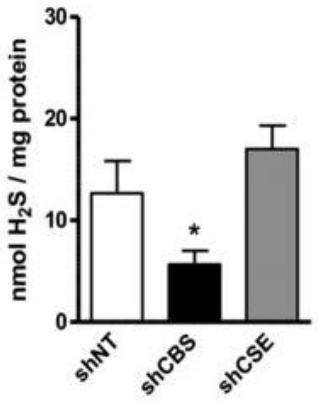
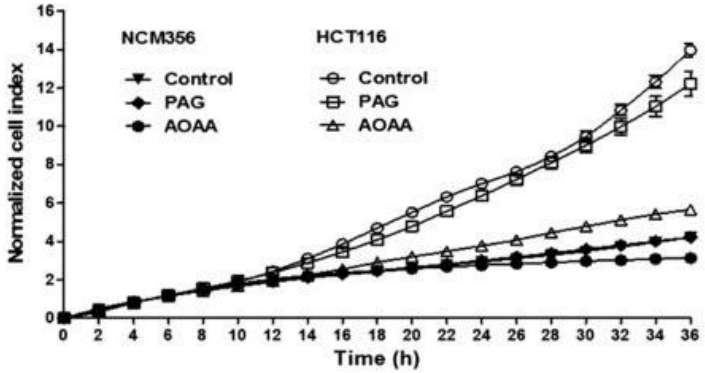
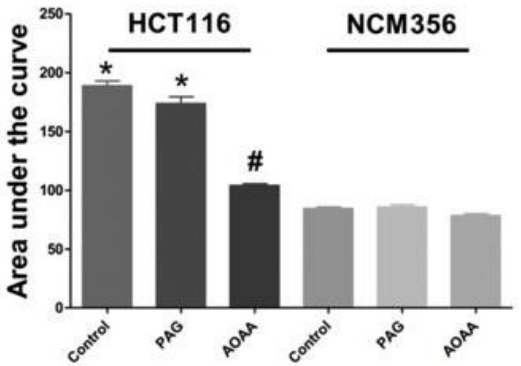
Down-regulation or pharmacological inhibition of CBS inhibits proliferation of HCT116 cells











Szabo C et al. PNAS, 110:12474-12479, 2013

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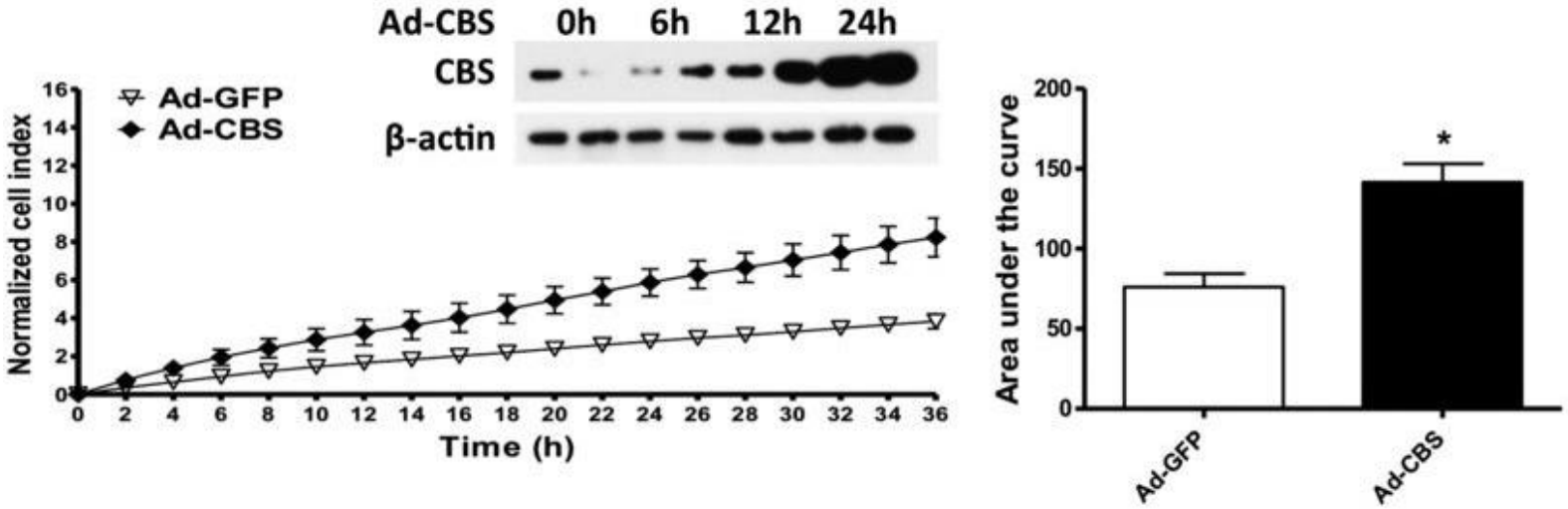
Adenoviral-mediated CBS overexpression enhances the proliferation rate of NCM356 cells



Szabo C et al. PNAS, 110:12474-12479, 2013

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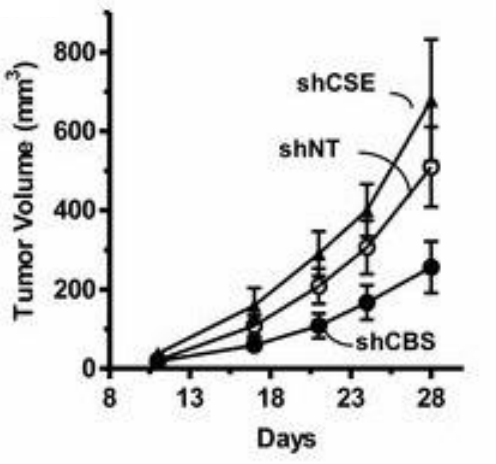
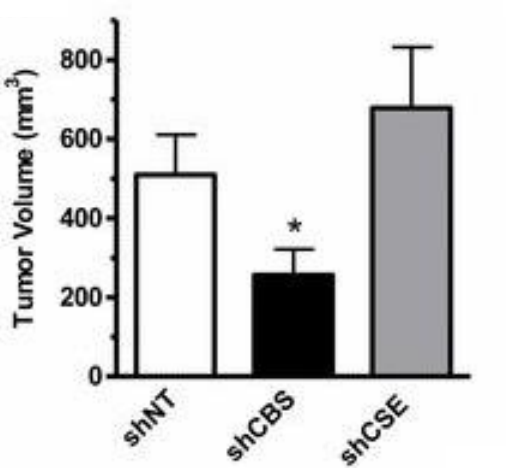
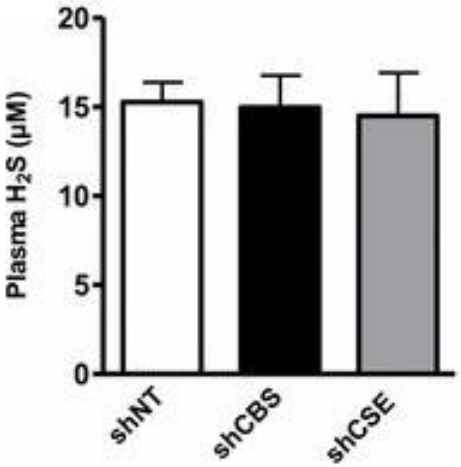
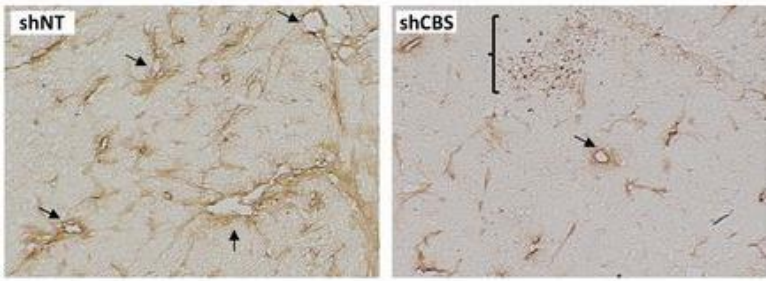
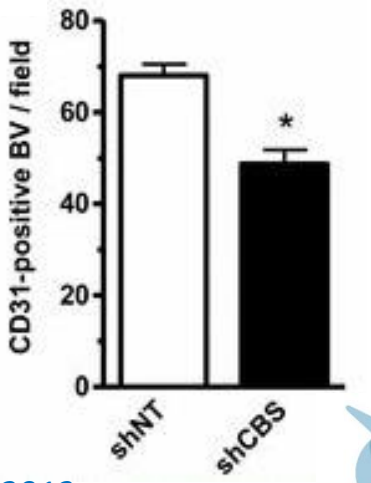
ShRNA-mediated CBS down-regulation inhibits colon cancer growth in vivo











Szabo C et al. PNAS, 110:12474-12479, 2013

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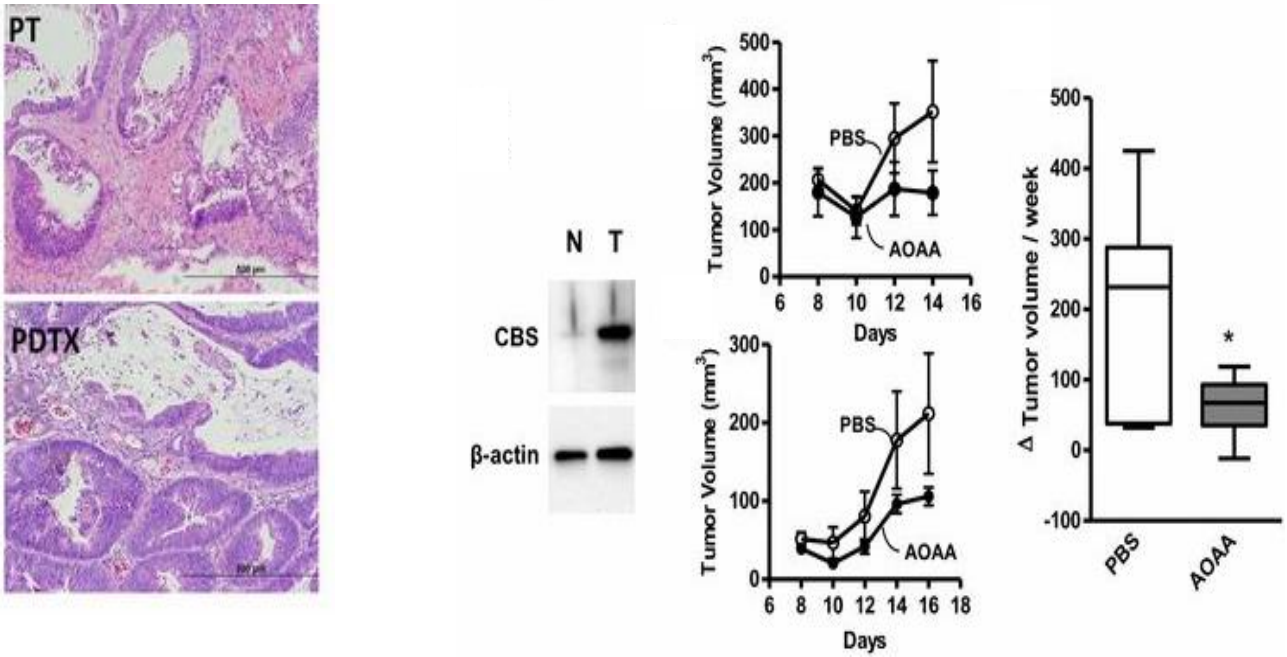
AOAA inhibits colon cancer growth and tumor angiogenesis in vivo



Szabo C et al. PNAS, 110:12474-12479, 2013

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Outline

1. Introduction
2. Production and degradation
3. Signaling, target and physiological functions
4. Agents regulating H_2S levels
5. **H_2S, bacteria and antibiotics**
6. H_2S and infection

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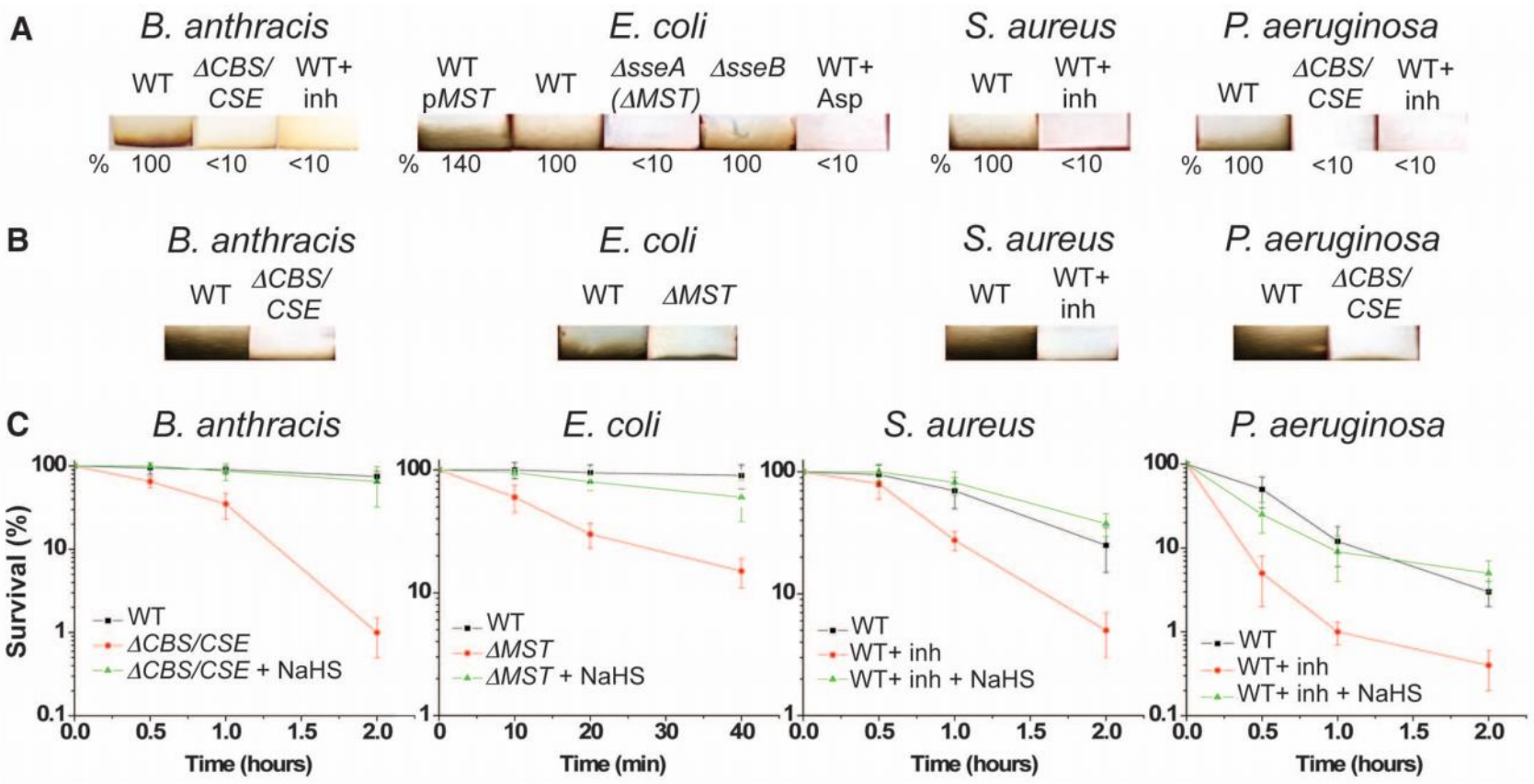
Inhibition of H_2S production renders bacteria sensitive to antibiotics



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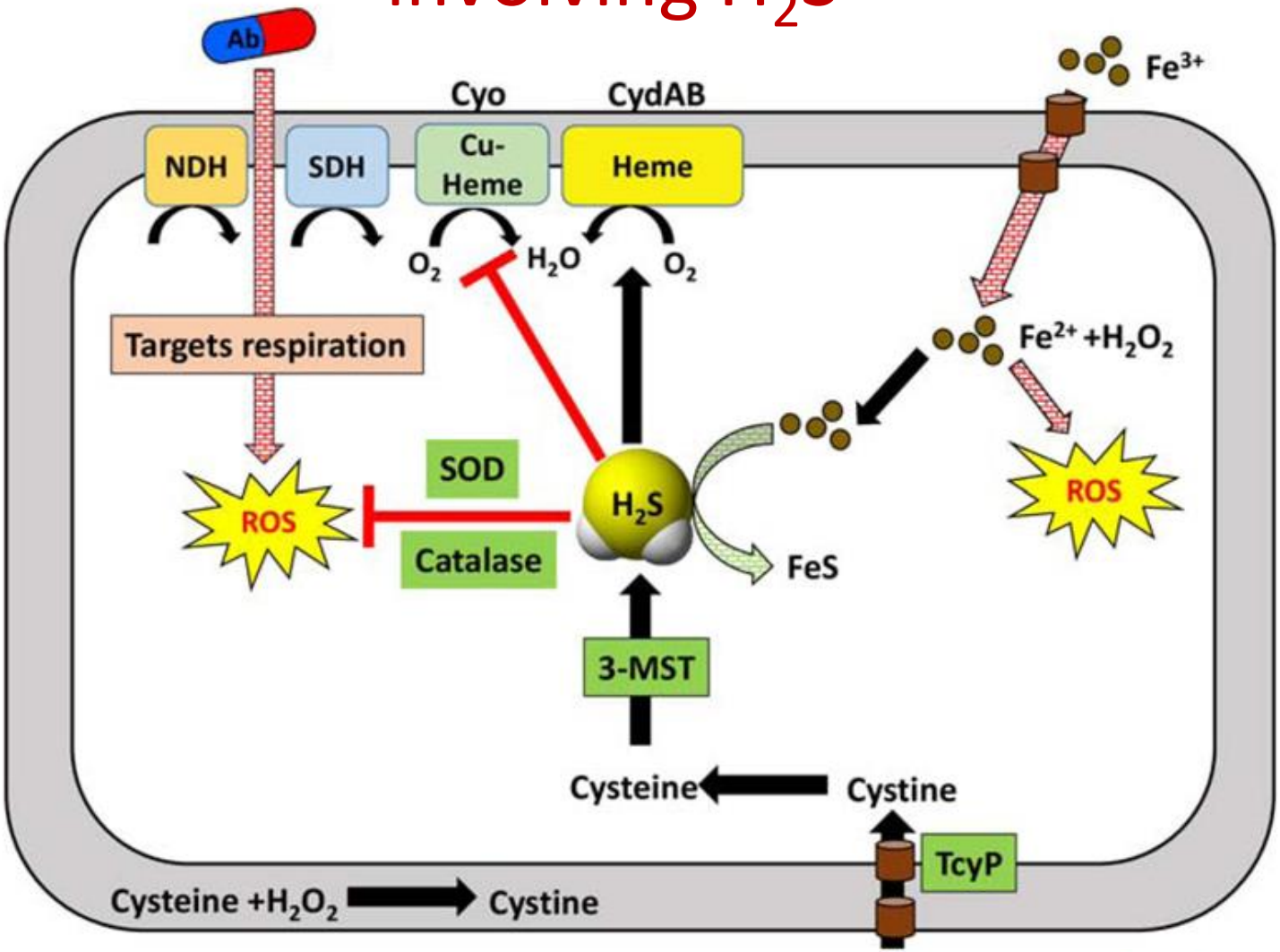
Mechanisms of antibiotics resistance involving H_2S



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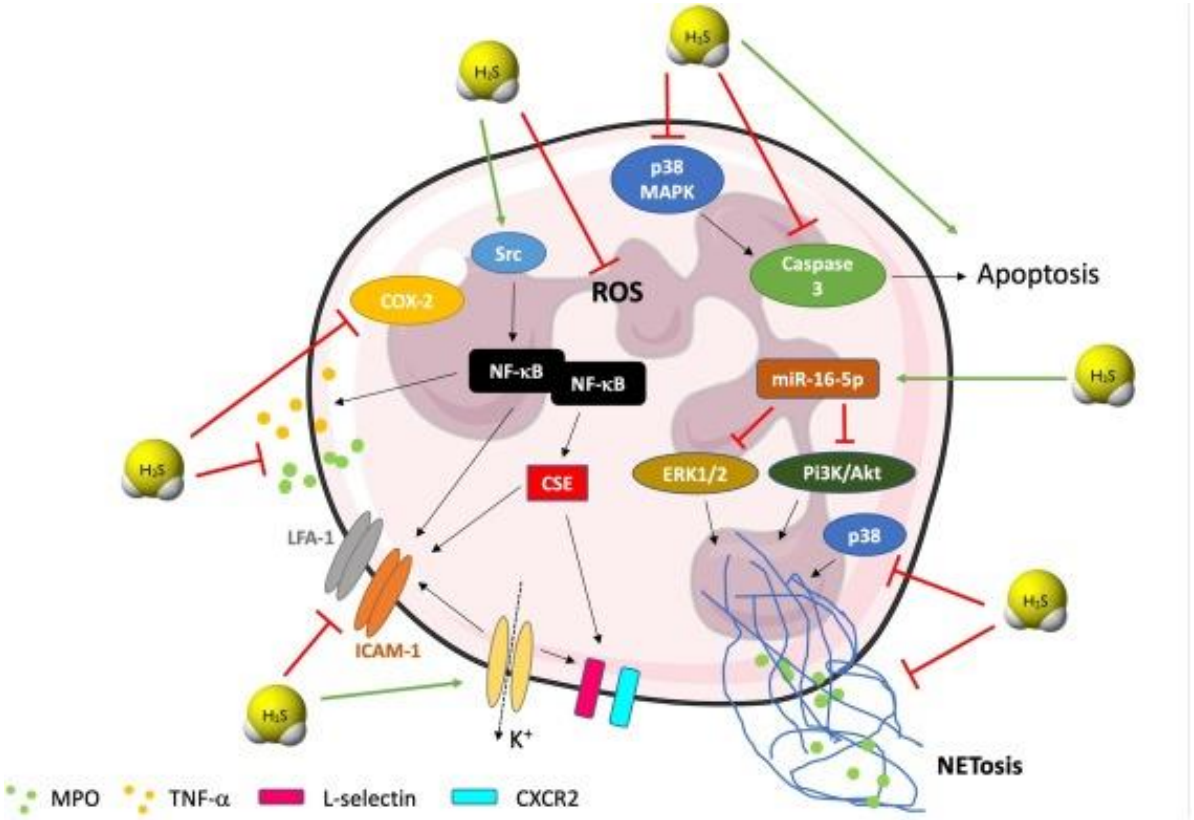
H_2S and neutrophils



Dilek et al., Pharmacol Res, 161:105119, 2020

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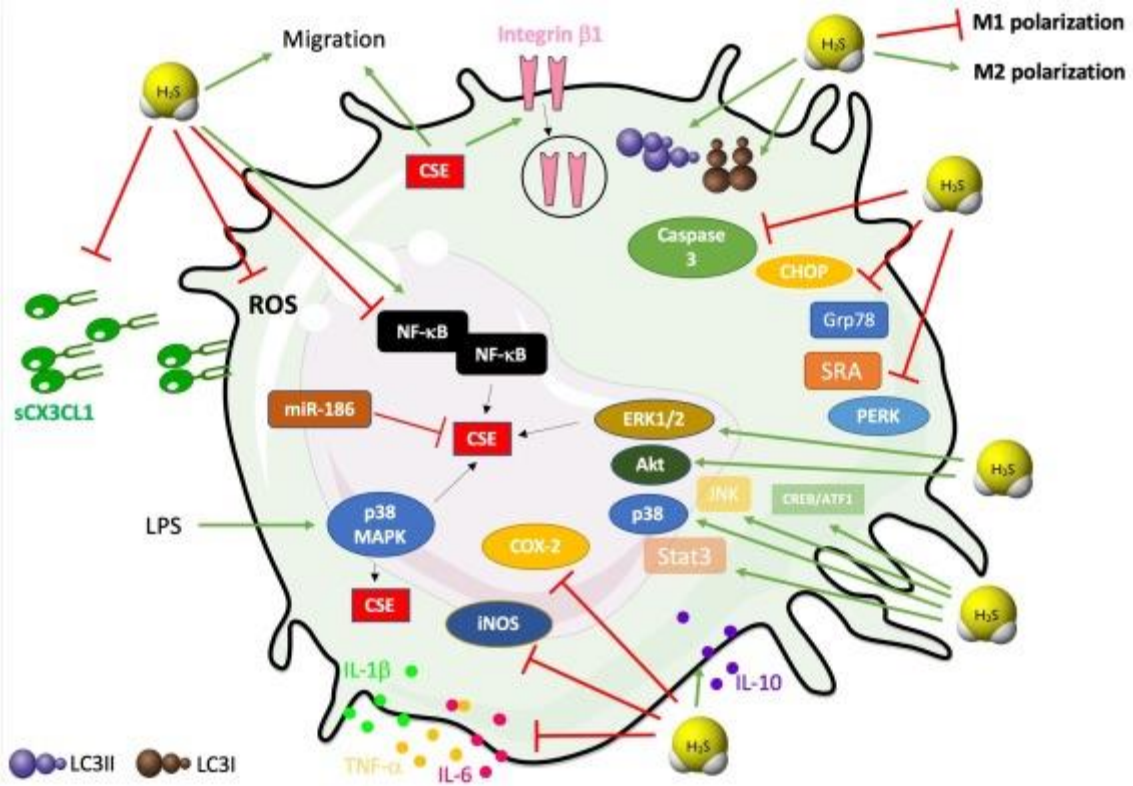
H_2S and macrophages



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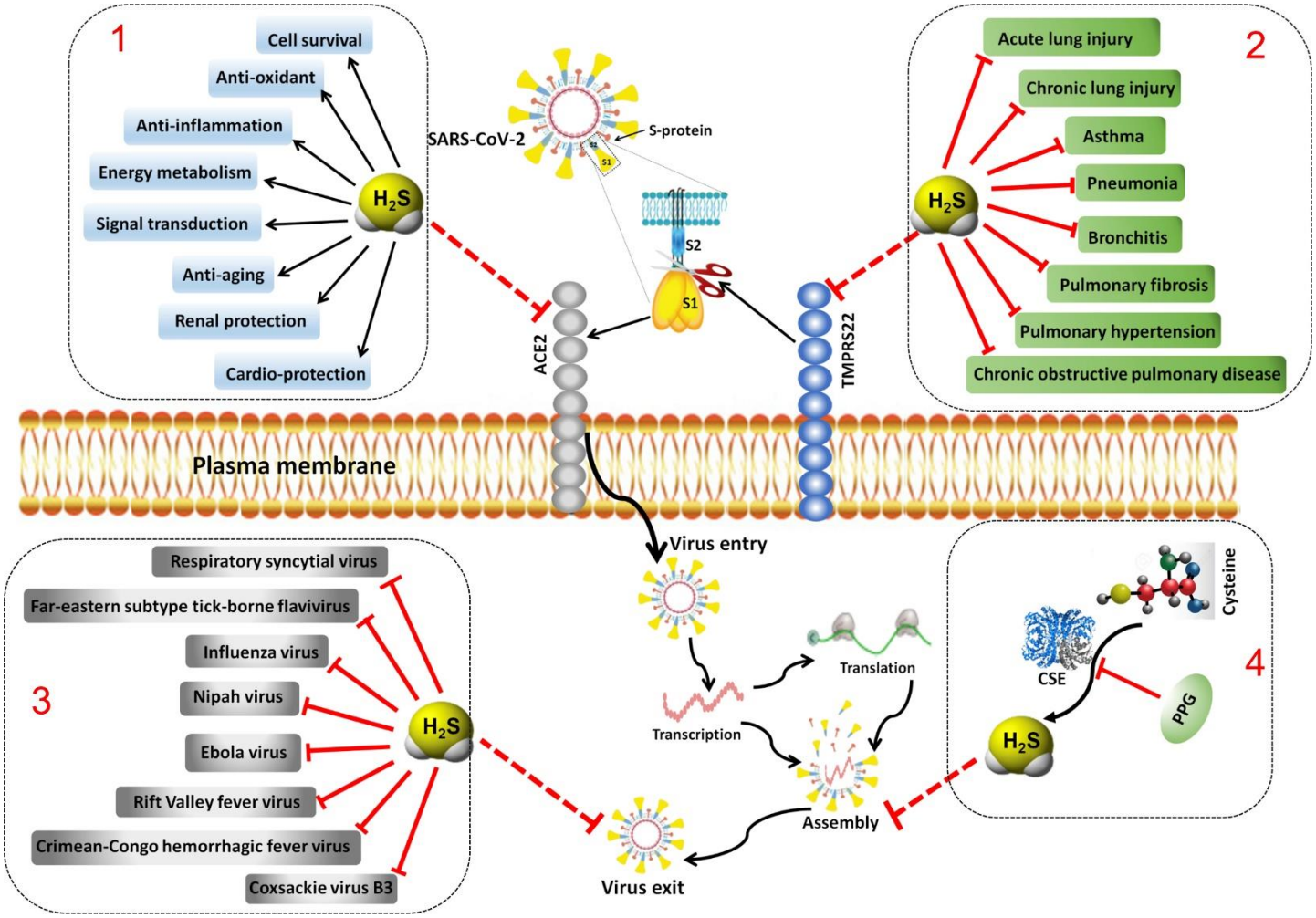
H_2S and Covid-19



Yang, Am J Physiol Heart Cic Physiol, 319: C244-C249, 2020

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