Nitric Oxide Dioxygenase (NOD): A •NO Detoxification Enzyme

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•NO is ubiquitous.
It can be a lethal poison.

Various life forms have evolved strategies for •NO detoxification

•NO is ubiquitous

Common Biological Sources:

1. Oxidation-oxygenation of amines
   •NO synthases (L-arginine)
   / immune defense (animals + plants)
   organic combustions/ cigarette smoke (lung)

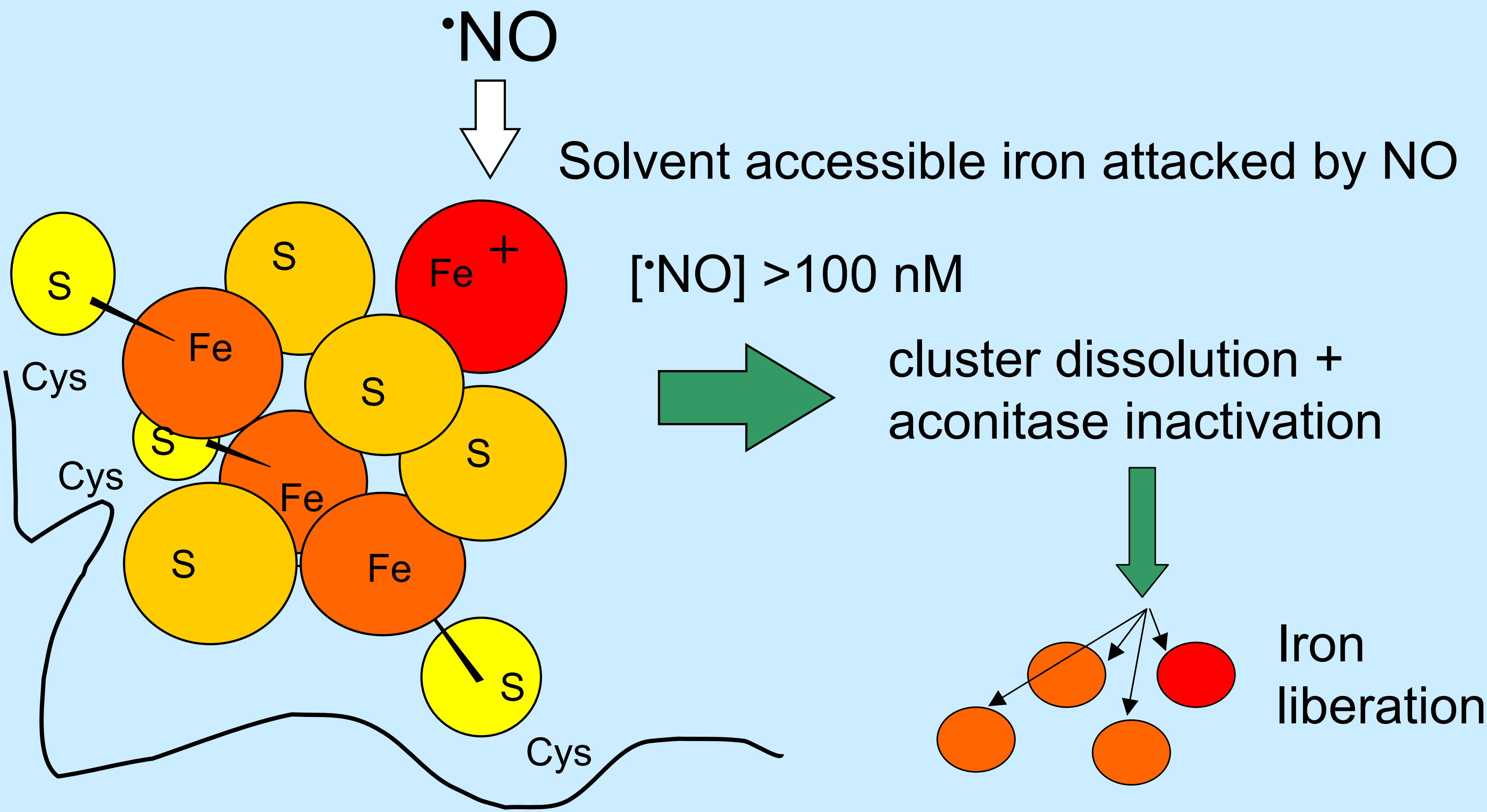
2. Reduction of nitrogen oxides (nitrate and nitrite)
   microbial denitrification pathways (soil)
   nitrite reduction by oxidoreductases (gut)

•NO Can Be a Lethal Poison

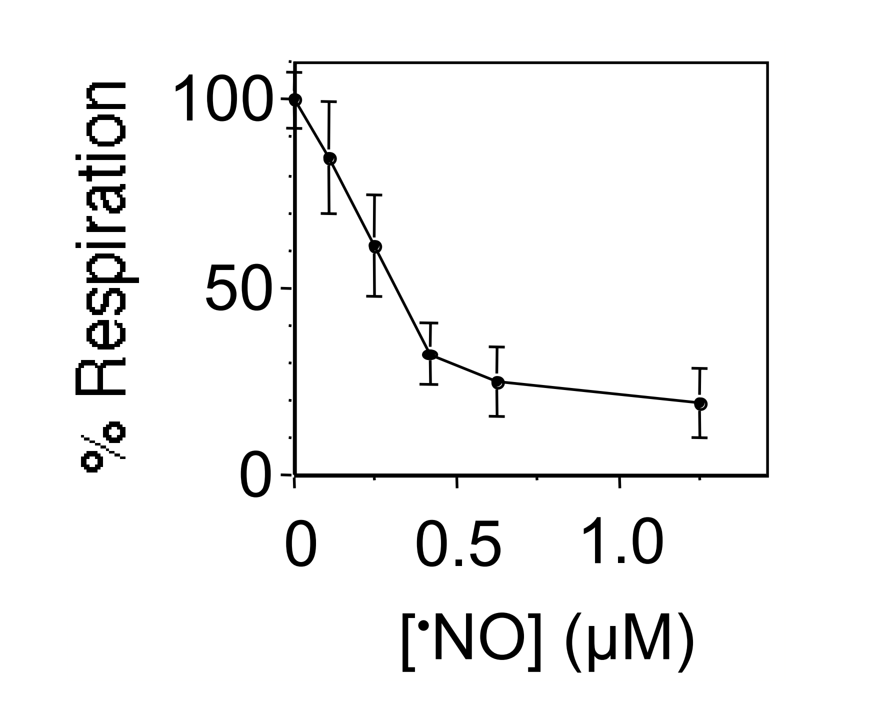
•NO Can Poison Cell Energy Production

Sensitive Targets are:
Aconitase and
Cytochrome Oxidase that affect Respiration.

Aconitase, a citric acid cycle enzyme, is a sensitive and critical target of •NO

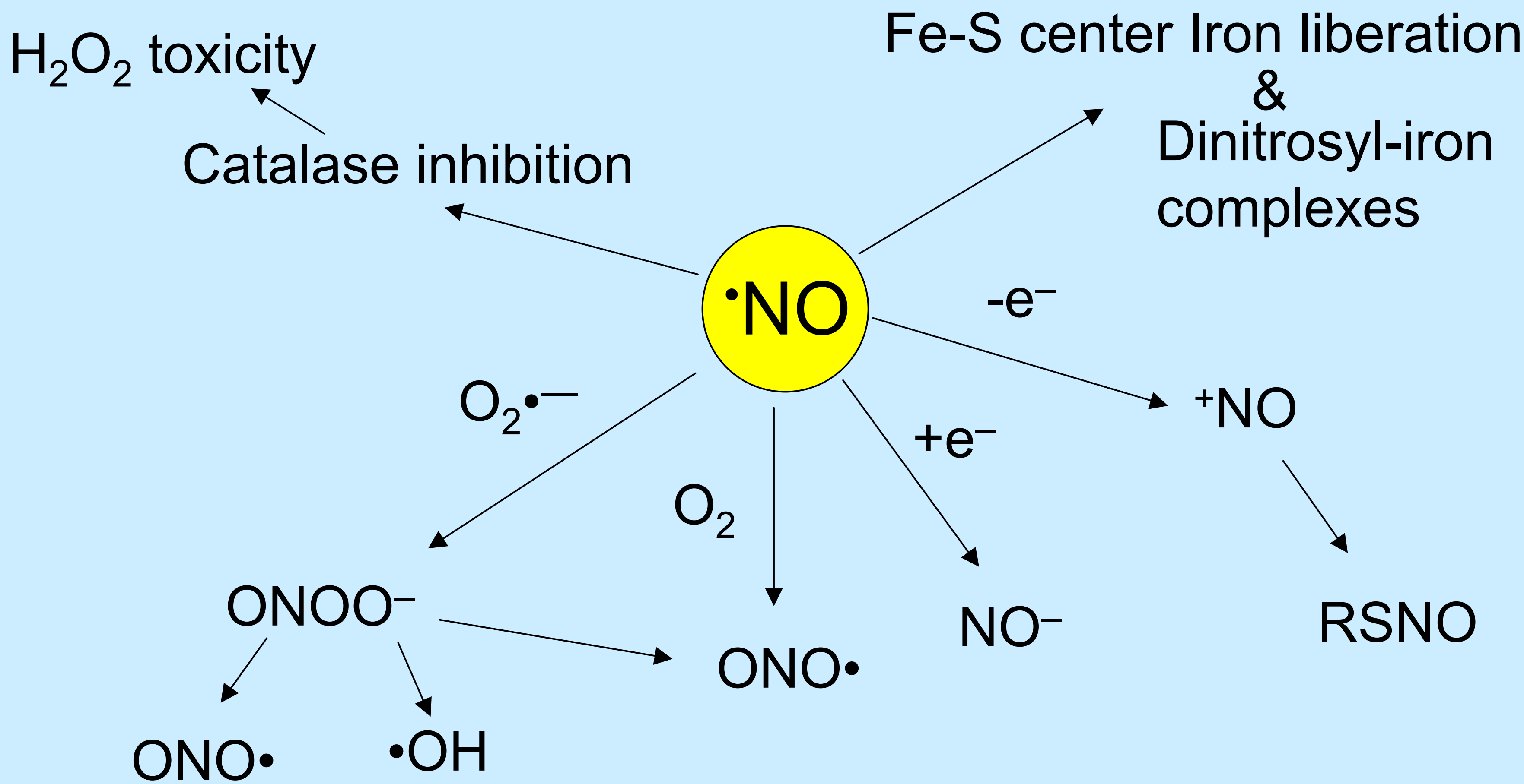


${}^{\bullet}NO$ rapidly inhibits cytochrome oxidase and thereby inhibits mitochondrial respiration



Human A549 lung cells respiring with a physiological level of O_2 (5 µM) are poisoned by submicromolar ${}^{\bullet}NO$ levels. Gardner *et al.* 2001 FRBM 31, 191.

Multiple Secondary Mechanisms for •NO Toxicity



Cellular Strategies for $^{\bullet}$NO Detoxification-Metabolism

1. Non-enzymatic and enzymatic ‘Oxidations’ ($^{\bullet}$NO oxidases)

$$^{\bullet}NO + O_2^{\bullet -}/O_2/H_2O_2 \longrightarrow \{ONOO^-, ONO^{\bullet}, \text{ or } ONO^-\}$$

Toxic products?

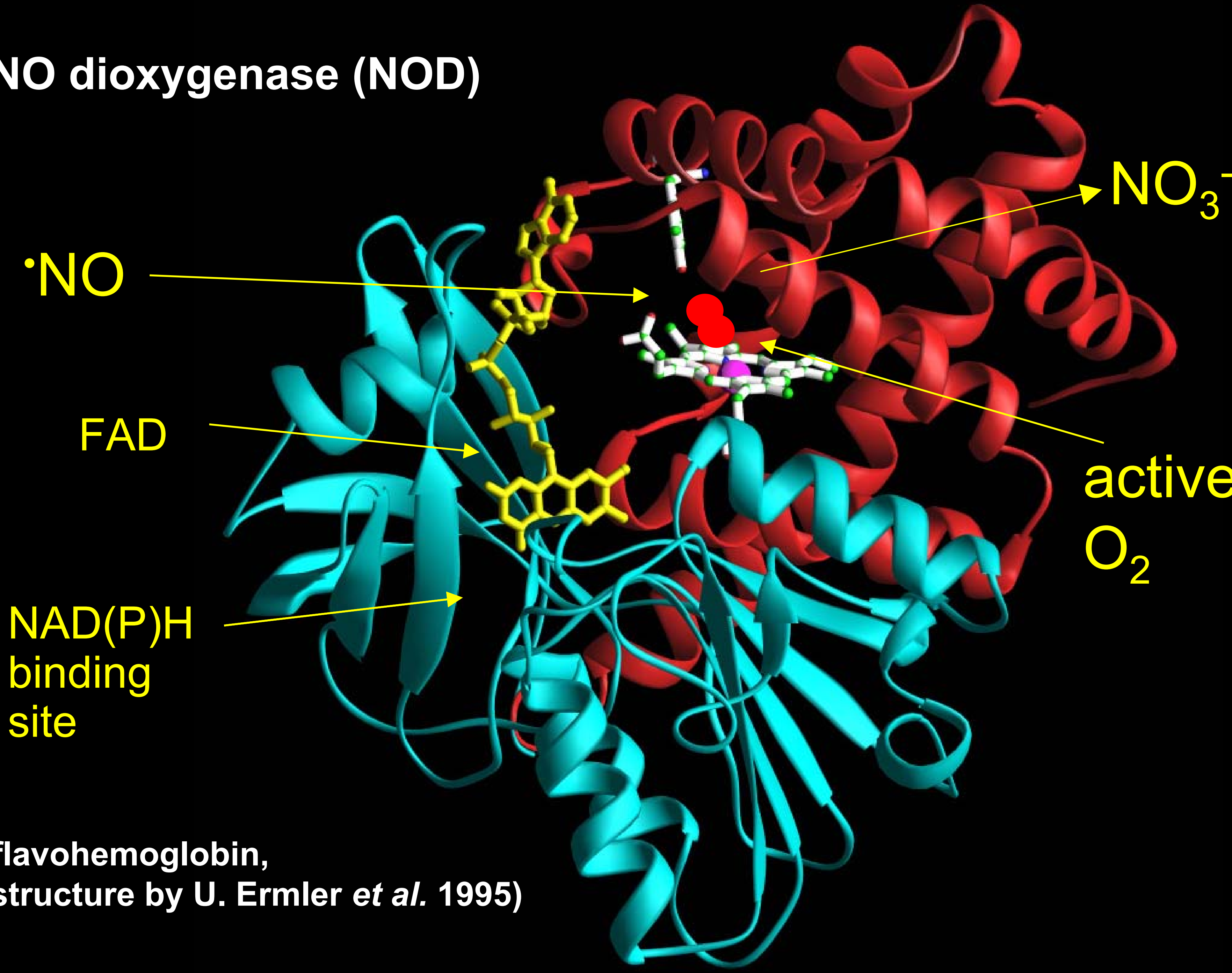
2. Reduction ($^{\bullet}$NO reductases)

$$2\ ^{\bullet}NO + 2\ e^- \xrightarrow{NOR} N_2O \text{ (nitrous oxide)}$$

3. Dioxygenation ($^{\bullet}$NO dioxygenases)

$$^{\bullet}NO + O_2 + e^- \xrightarrow{NOD} \underset{O}{ONO^-} \text{ (nitrate)}$$

•NO dioxygenase (NOD)



(flavohemoglobin,
structure by U. Ermler *et al.* 1995)

Proposed $^{\bullet}$NO Dioxygenase Rxn Mechanism

$$2\ {}^{\bullet}NO + 2\ O_2 + NAD(P)H \longrightarrow 2\ NO_3^{-} + NAD(P)^{+} + H^{+}$$

FAD reduction $NAD(P)H + FAD + H^{+} \longrightarrow NAD(P)^{+} + FADH_2$

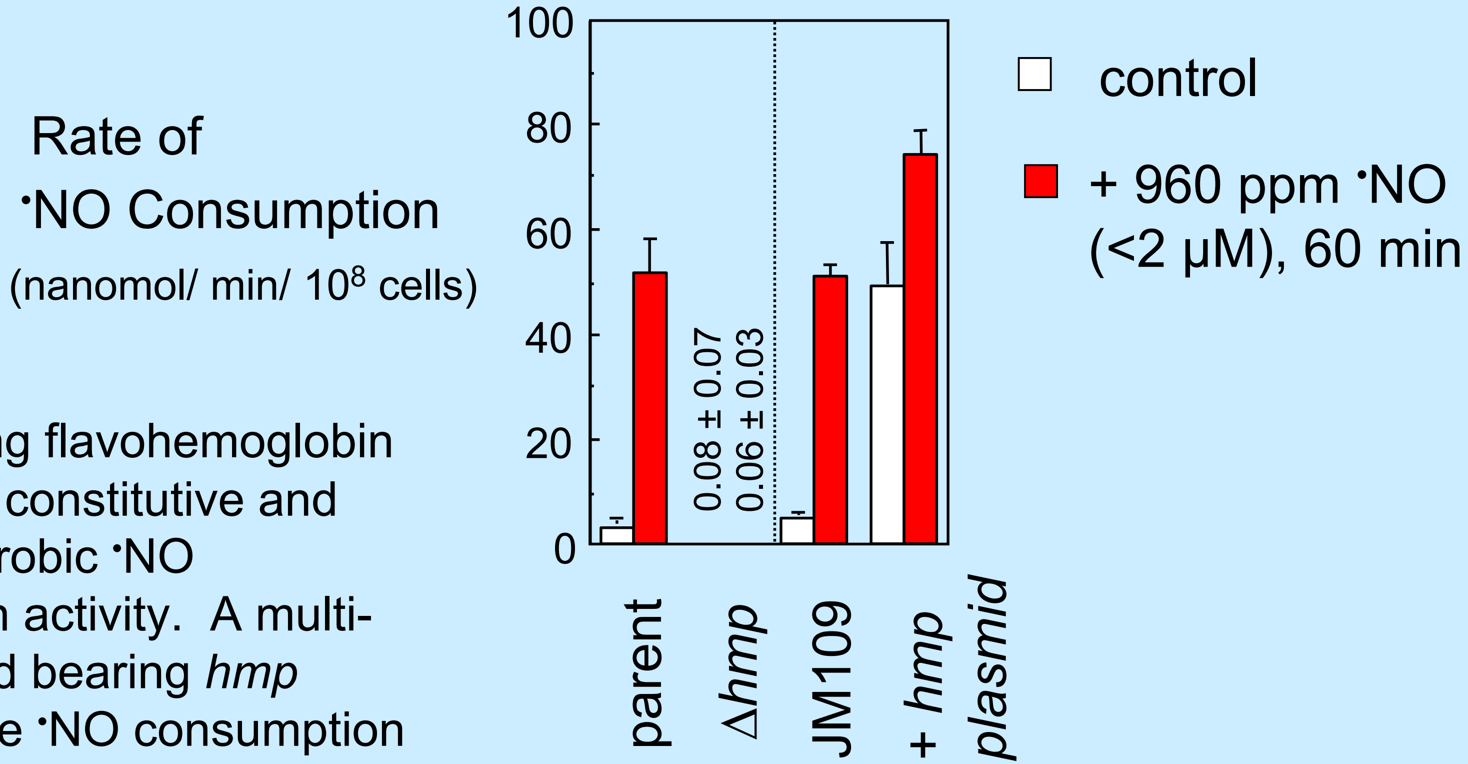
iron reduction $FADH_2 + Fe^{3+} \xrightarrow{(-)\ CN^{-}} Fe^{2+} + FADH^{\bullet} + H^{+}$

O_2 binding $Fe^{2+} + O_2 \xrightarrow{(-)\ {}^{\bullet}NO,\ CO} Fe^{3+}(O_2^{\bullet -})$

•NO dioxygenation $Fe^{3+}(O_2^{\bullet -}) + {}^{\bullet}NO \longrightarrow Fe^{3+} + NO_3^{-}$

iron reduction $FADH^{\bullet} + Fe^{3+} \xrightarrow{(-)\ CN^{-}} FAD + Fe^{2+} + H^{+}$

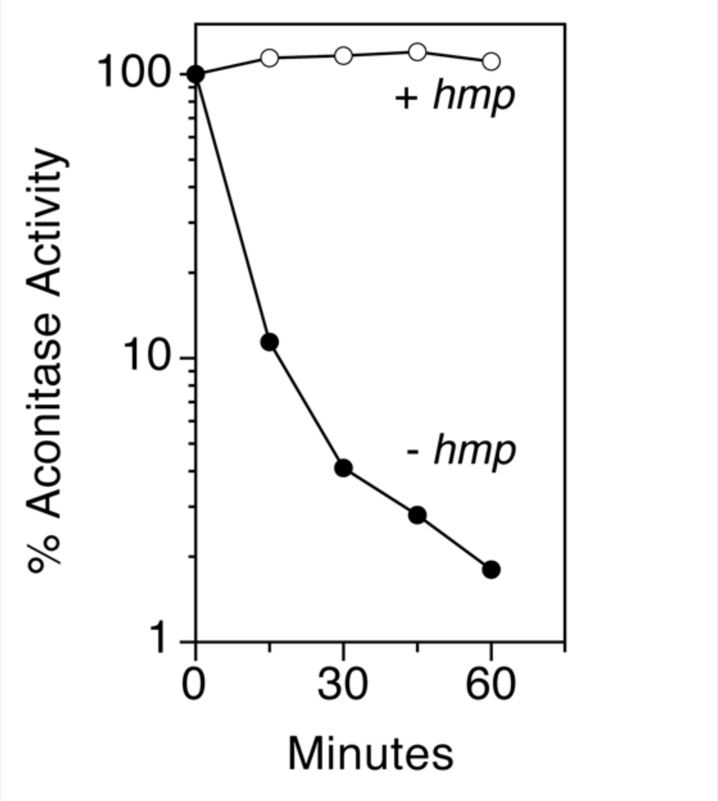
Flavohemoglobin (*hmp*) catalyzes constitutive and <u>inducible</u> aerobic •NO consumption in *Escherichia coli*



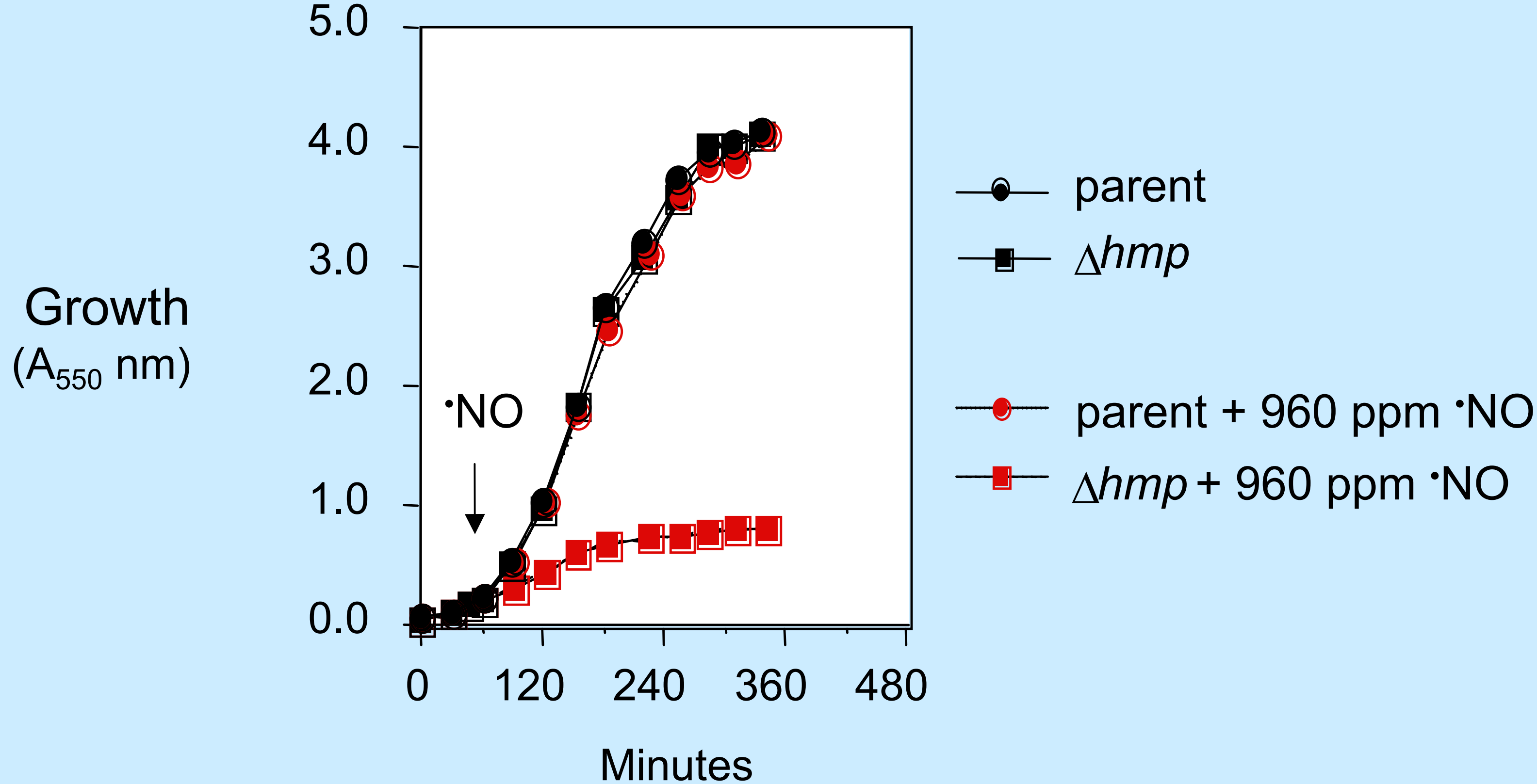
E. coli lacking flavohemoglobin (*Δhmp*) lack constitutive and inducible aerobic •NO consumption activity. A multi-copy plasmid bearing *hmp* increases the •NO consumption activity in host JM109. Gardner *et al.* 1998 PNAS <u>95</u>, 10378.

FlavoHb (*hmp*) protects aconitase in aerobic *Escherichia coli*

Aconitase is rapidly inactivated in *E. coli* lacking NOD (*hmp*) when exposed to an aerobic atmosphere containing 960 ppm $^{\bullet}NO$ ($\leq$ 2 μM in solution). NOD (*hmp*) protects aconitase. Gardner *et al.* 2002 JBC 277, 8166.



FlavoHb protects aerobic *E. coli* against •NO-mediated growth inhibition



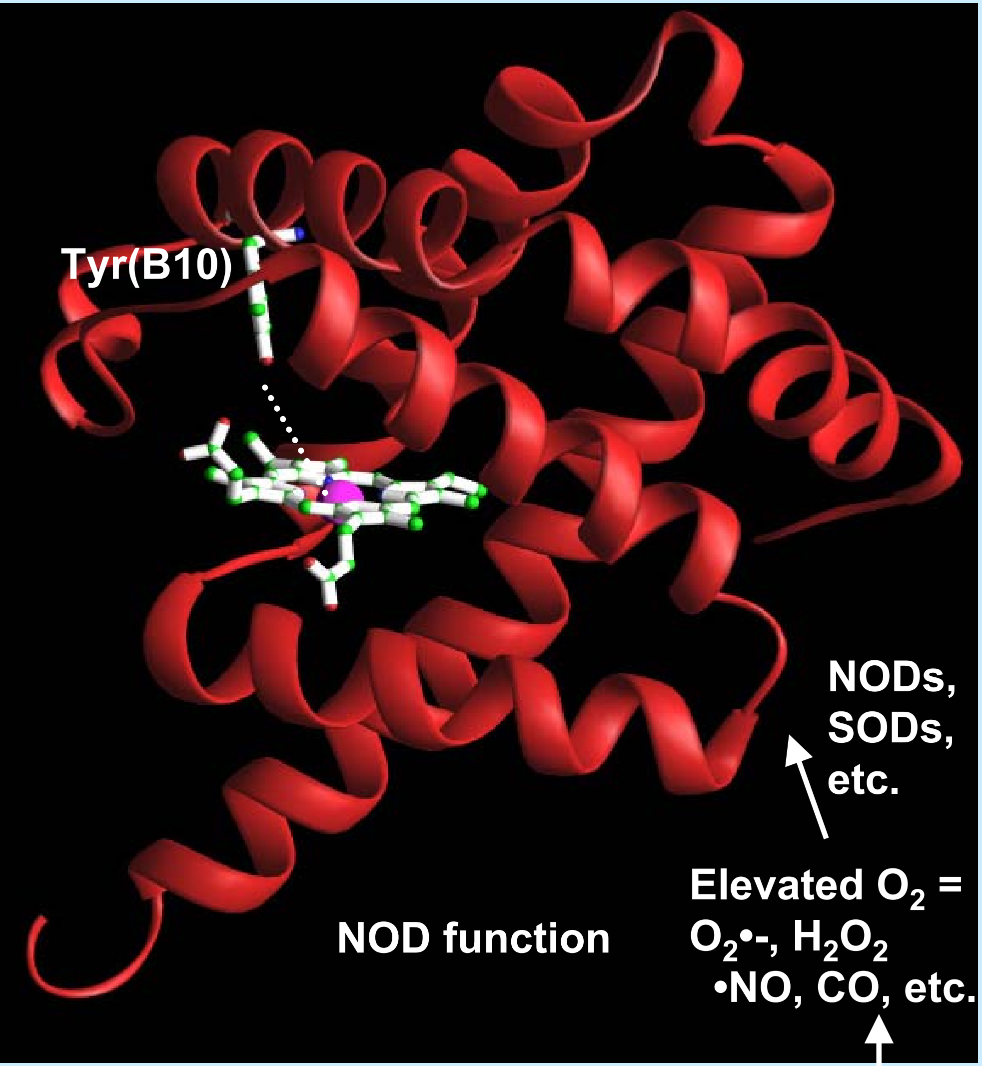
E. coli lacking NOD (Δ*hmp*) that are exposed to an aerobic atmosphere containing •NO do not grow well. Gardner *et al.* 1998 PNAS 95, 10378; 2002 JBC 277, 8166, 8172.

$^{\bullet}$NO dioxygenation is a primal (1.8 billion year old) function of hemoglobin/myoglobin

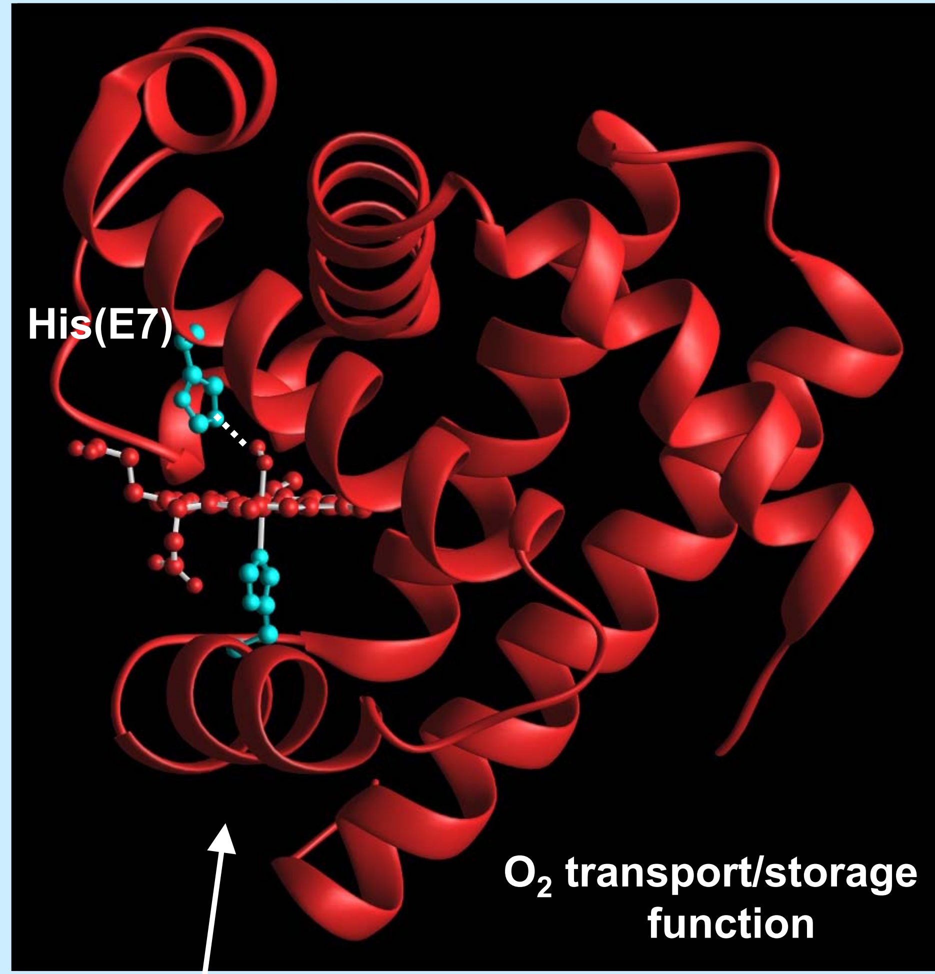
NOD $\rightarrow$ O_2 storage/transport

The first hemoglobin/myoglobin most likely functioned as an enzyme utilizing bound ‘activated’ O_2 to dioxygenate NO, or other substrates in microbes. Multicellular organisms that benefit from the O_2 storage-transport functions of hemoglobin/myoglobin appeared much later. Gardner *et al.* 1998 PNAS 95, 10378.

Microbial flavohemoglobin (Hb domain)



Muscle Myoglobins & RBC Hemoglobins



~ 3.6 Billion years of life on earth

beginning | 1.8 By | 3.0 By | today

Structure and Kinetics Control Diverse Hemoglobin and Myoglobin Functions

	<u>flavoHbs*</u>	<u>Sperm Whale Mb</u>
V_{max} NOD	$\boxed{112\text{-}670\ s^{-1}}$	--
k_{on} O_2	$1.7\text{-}5.0 \times 10^7\ M^{-1}\ s^{-1}$	$1.7 \times 10^7\ M^{-1}\ s^{-1}$
k_{off} O_2	$0.2\text{-}0.6\ s^{-1}$	$15\ s^{-1}$
K_d O_2	4-36 nM	800 nM
k_{ox} $^{\bullet}NO$	$\boxed{0.9\text{-}2.9 \times 10^9\ M^{-1}\ s^{-1}}$	$3.4 \times 10^7\ M^{-1}\ s^{-1}$
k_{on} $^{\bullet}NO$	$1.0\text{-}2.6 \times 10^7\ M^{-1}\ s^{-1}$	$2.2 \times 10^7\ M^{-1}\ s^{-1}$
k_{off} $^{\bullet}NO$	$0.0002\ s^{-1}$	$0.0001\ s^{-1}$
K_M (O_2)	60-90 μM	--
K_M $(^{\bullet}NO)$	100-250 nM	--

**E. coli*, *S.cerevisiae* and *A. eutrophus;*
Gardner *et al.* 2000 JBC <u>275</u>, 12581, 31581

Mammalian Cells Produce a flavoHb-like NOD Activity for $^{\bullet}NO$ Metabolism-Detoxification

$$2\,^{\bullet}NO + 2\,O_2 + NADPH \longrightarrow 2\,NO_3^{-} + NADP^{+} + H^{+}$$

Human Intestinal Epithelial Cells (CaCo-2)

20-30 nmol $^{\bullet}NO/min/10^7$ cells

Apparent K_m (O_2) = 17 μM

Apparent K_m ($^{\bullet}NO$) = 0.2 μM

CO sensitive K_i (CO) = 3 μM (heme-dependent)

Cyanide sensitive K_i (CN^-) ≈ 20 μM (heme-dependent)

Diphenylene iodonium sensitive (flavin-dependent)

Gardner *et al.* 2001 FRBM 31, 191

Key Points:

1) $^{\bullet}$NO can be a potent toxin;

2) $^{\bullet}$NO dioxygenase (NOD) is one enzyme that efficiently detoxifies $^{\bullet}$NO in bacteria, fungi, and mammals; and

3) $^{\bullet}$NO dioxygenation is an ancient function for the hemoglobin/myoglobin family.

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Acknowledgements

Drs. John Olson and Yi Dou are gratefully acknowledged for their contribution of hemoglobin structure graphics. This work was supported by grants from the Children's Hospital Research Foundation Trustees, the American Heart Association (9730193N), and the National Institutes of Health (R01 GM65090).