ABCs OF REACTIVE NITROGEN SPECIES AND THEIR SCAVENGERS

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Sunrise Free Radical School Free Radical Biology: Back to the Basics
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SUNRISE FREE RADICAL SCHOOL

ABCs of Reactive Nitrogen Species and their Scavengers

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INCT de Processos Redox em Biomedicina

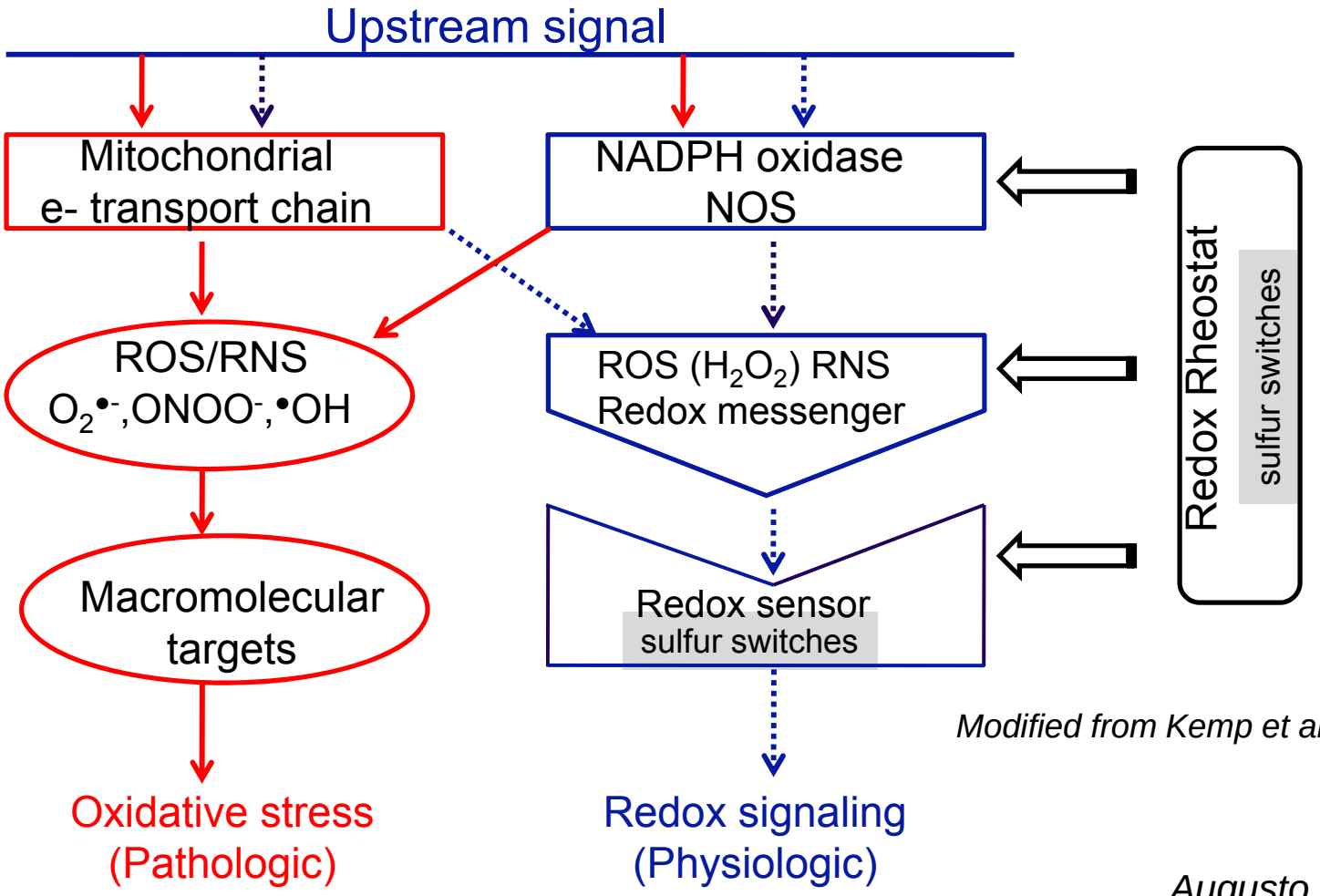
Redoxoma

FAPESP

CNPq
Conselho Nacional de Desenvolvimento Científico e Tecnológico

PRESENT GOAL OF FREE RADICAL RESEARCH

-enhance our understanding of the mechanisms by which oxidants and radicals can act as mediators of physiological and pathophysiological networks.



Modified from Kemp et al FRBM 2008

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TIME TO BE MORE SPECIFIC

The terms *ROS (reactive oxygen species)* and *antioxidants* are appropriate for describing general classes of compounds but are counterproductive for understanding mechanisms.

-*ROS* encompasses the range of oxidants [including *RNS (reactive nitrogen species)*] encountered by cells/organisms with little attention to the fact that these species varies widely in reactivity (strong & weak oxidants, one-electron (radicals) & two-electron oxidants non radical species), etc).

-Compounds classified as *antioxidants* do not all have the same generic action.

modified from Winterbourn Nature Chem Biol 2008
Winterbourn& Hampton FRBM 2008

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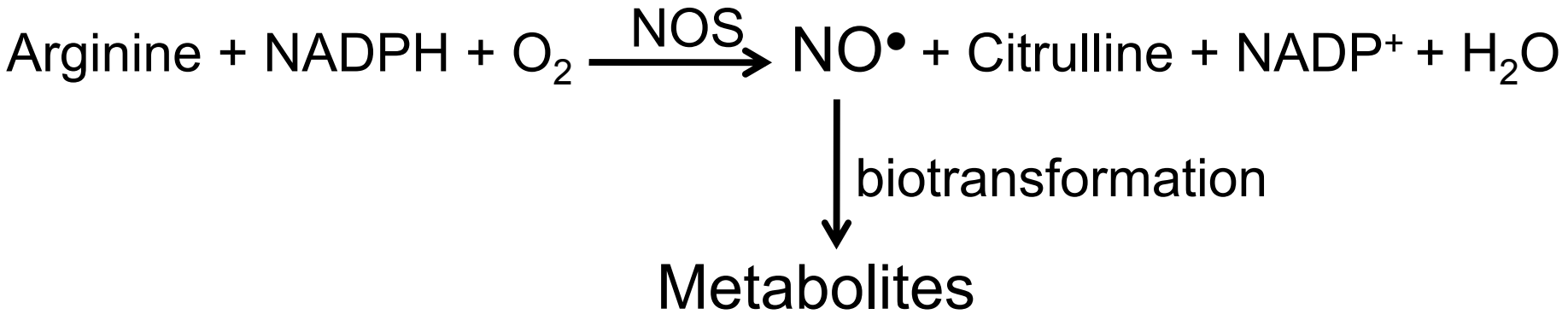
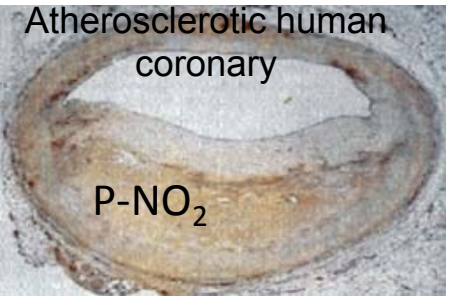
RNS & NO• METABOLITES

Nitric oxide is a signal transducing radical



Nitric oxide synthase (NOS): constitutive eNOS (endothelial) and nNOS (neuronal) and inducible (iNOS)



Beckman & co-workers BC Hoppe-Seyler 1994

NO• metabolites more reactive towards biomolecules than NO• could participate in many diseases and became known as: *RNS* (reactive nitrogen species), *nitric oxide-derived oxidants*, *nitric oxide-derived species.*

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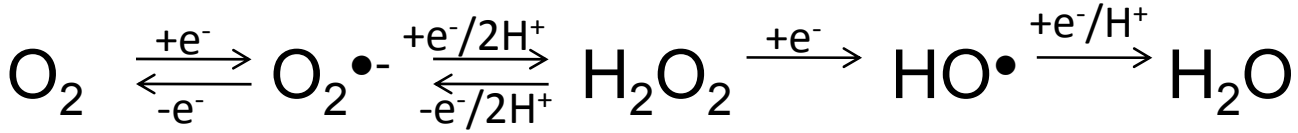
REDOX SPECIES

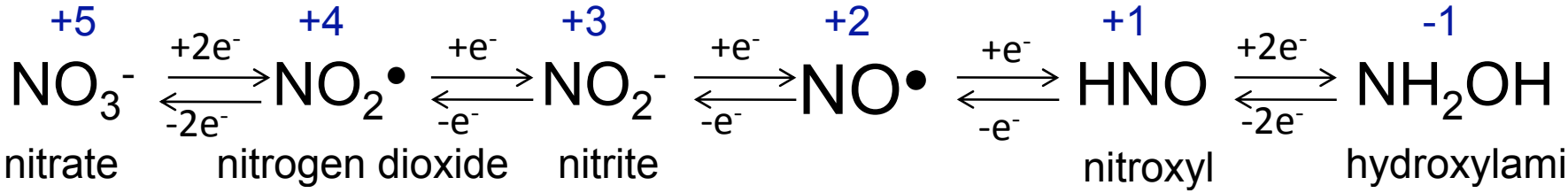
-Molecular oxygen (O_2)

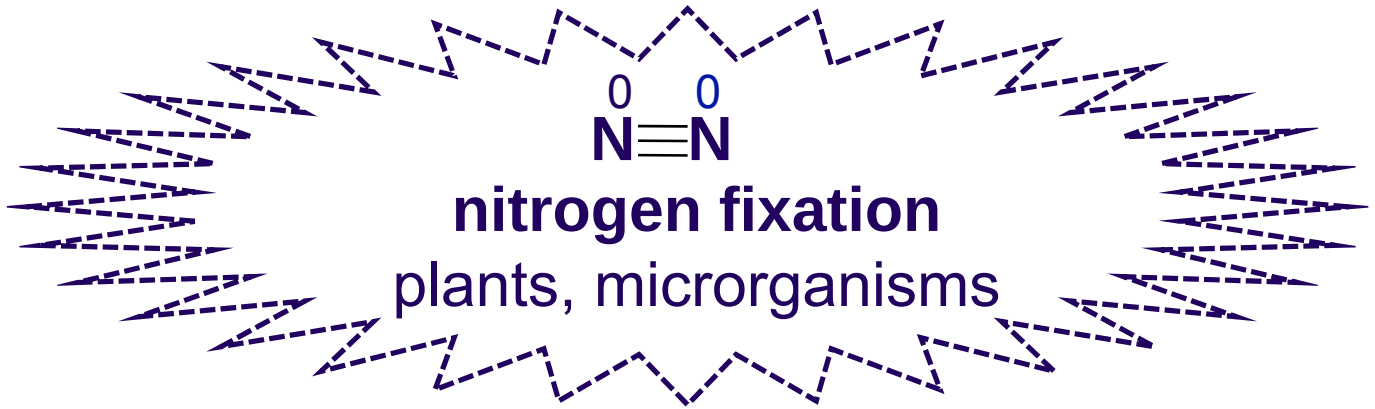


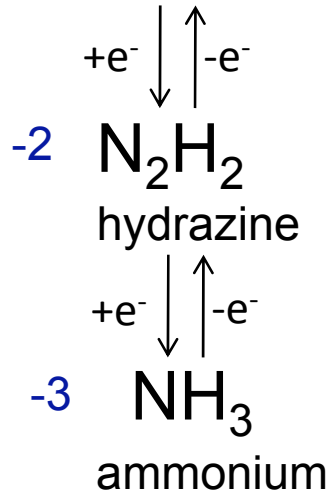
-Nitric oxide ($NO^{\bullet}$)

protons ommited

oxidation states







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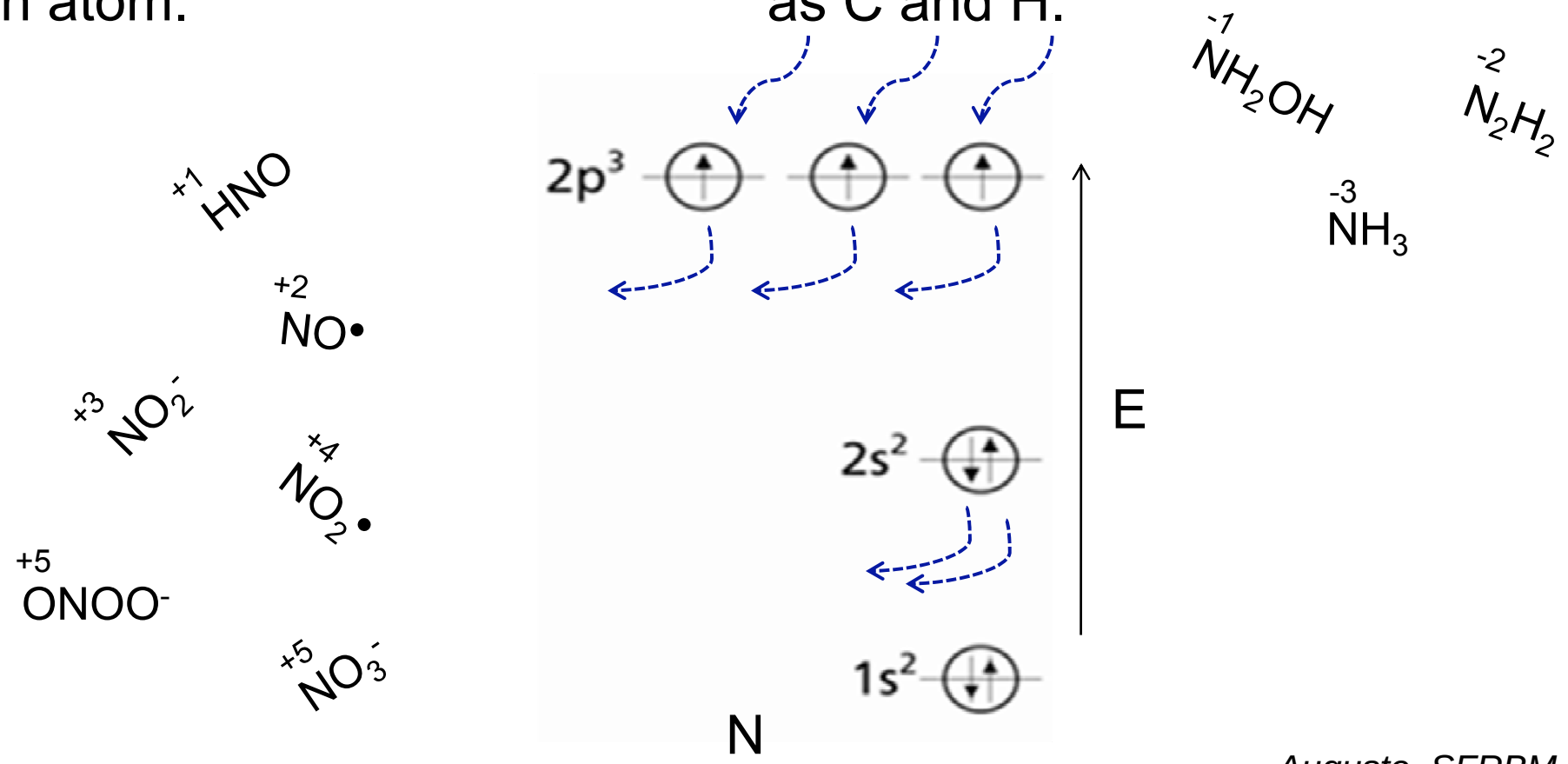
VERSALITY OF NITROGEN COMPOUNDS

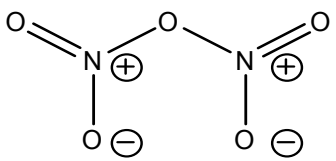
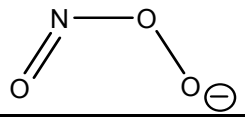
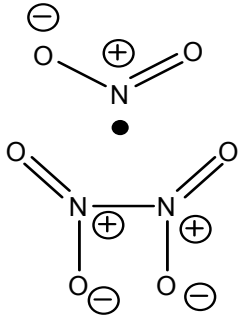
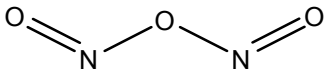
Oxidation states (+5 to -3)

-Nitrogen atom can donate up to 5 e^- to the electronegative oxygen atom.

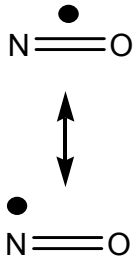
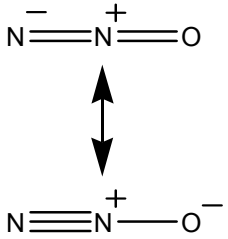
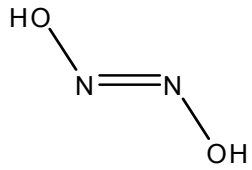
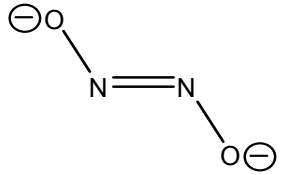
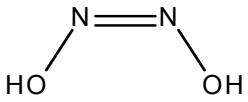
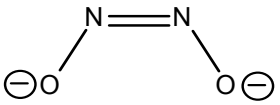
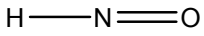
or gain up to 3 e^- from more electropositive atoms such as C and H.



Summary of the redox properties of nitrogen oxides, acids and ions.

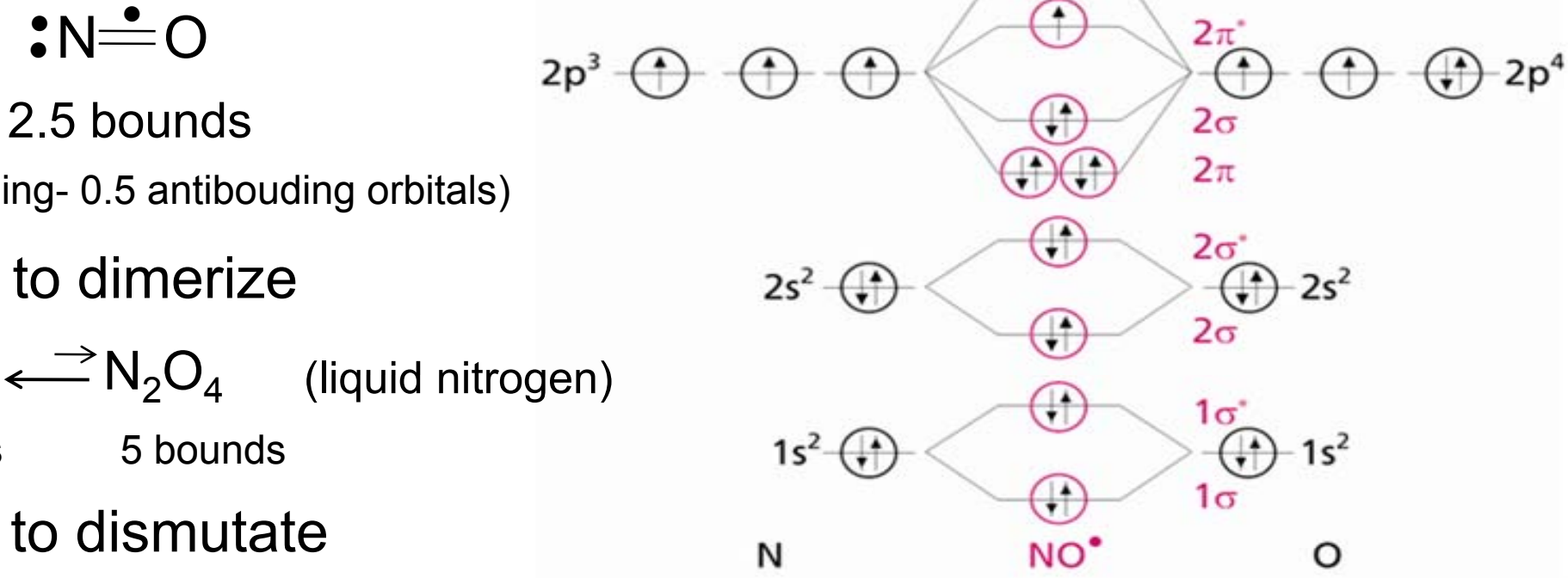
Oxidation Number	Oxide	Acid	Ions	Names	Redox Properties
+5		HNO_3	NO_3^-	nitric anhydride, nitrate	oxidant
+5		ONOOH	$ONOO^-$	peroxinitrite	powerful oxidant
+4				nitrogen dioxide	
				nitrogen tetroxide	dismutates in solution
+3		HO–N=O	NO_2^-	nitrous anhydride, nitrite	oxidant and reducer, cannot dismutate except in acidic media
			NO^+	Nitrosonium	

Summary of the redox properties of nitrogen oxides, acids and ions (continued).

Oxidation Number	Oxide	Acid	Ions	Names	Redox Properties
+2				**nitric oxide or nitrogen monoxide**	**oxidant and reducer, reacts rapidly with O_2**
+1				nitrous oxide	neither oxidant nor reducer
				cis and trans hyponitrite	does not dismutate
			NO^-	nitroxyl	

Henry, Guissani, Ducastel "Nitric oxide research from chemistry to biology: EPR spectroscopy of nitrosylated compounds"

NITROGEN MONOXIDE (NITRIC OXIDE)



2.5 bounds

(3 bounding- 0.5 antibouding orbitals)

-unlikely to dimerize

$2\ NO^{\bullet} \rightleftharpoons N_2O_4$ (liquid nitrogen)

5 bounds 5 bounds

-unlikely to dismutate

$4\ NO^{\bullet} + H_2O \longrightarrow 2\ HNO_2 + N_2O$ (Me^{n+} catalyzed)

(in contrast with most radicals, such as $O_2^{\bullet -}$, Ascorbyl•, SQ•)

-high reactivity towards species with unpaired e^-(O_2, radicals, Me^{+n})

$[P—Me^{(n+1)+}—NO^-] \longleftrightarrow [P—Me^{n+}—NO^{\bullet}] \longleftrightarrow [P—Me^{(n-1)+}—NO^+]$

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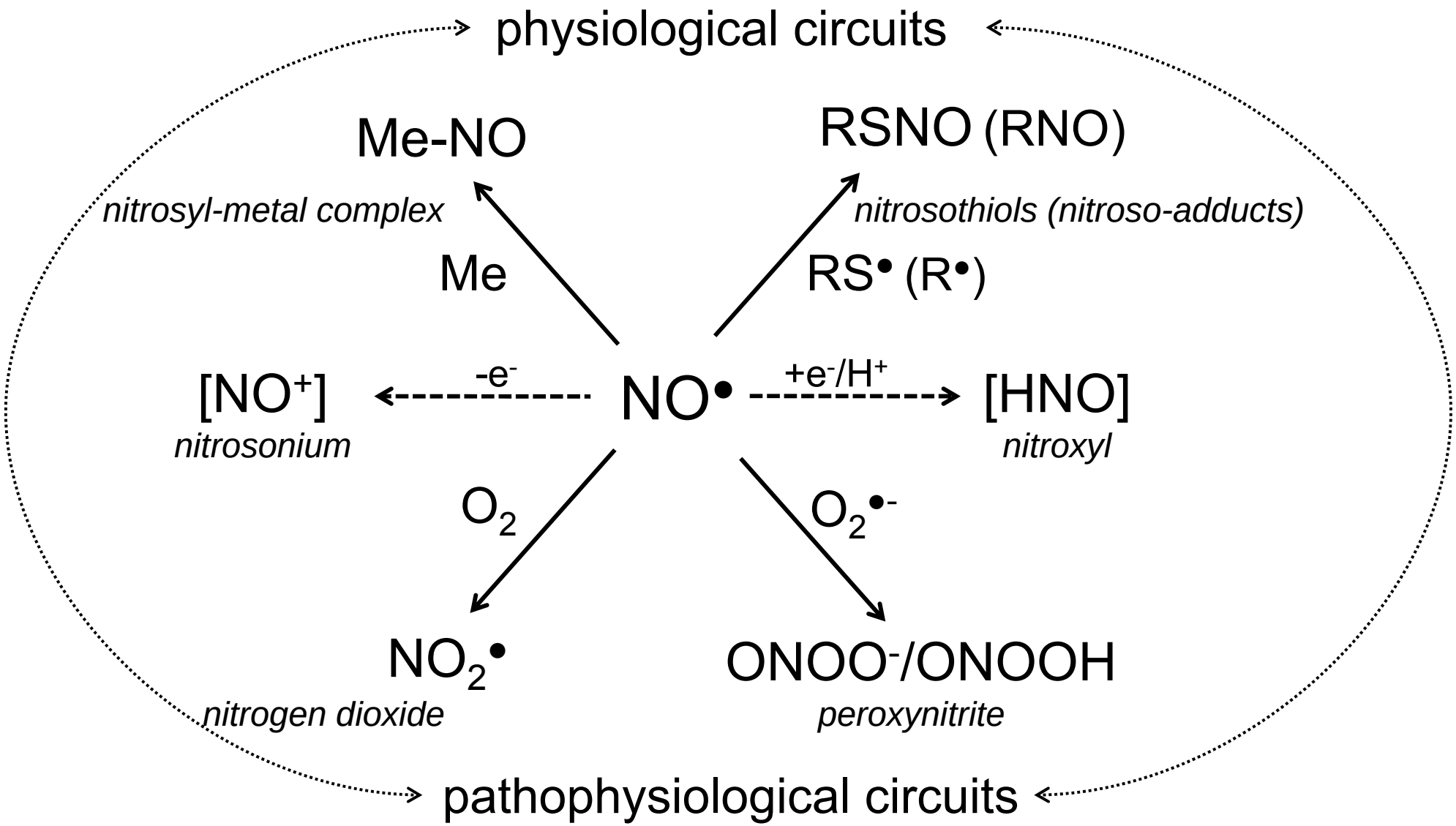
MOST STUDIED NO• REACTIONS AND SPECIES



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NITROXYL

HNO- nitroxyl (nitrosyl hydride or hydrogen oxonitrite)

-pharmacological actions: anti-alcoholic drug cyanamide

cardiovascular actions diverse from $NO^{\bullet}$ donors

-pKa~ 11.4

$$^1HNO \rightleftharpoons {}^3NO^- + H^+$$

Shafirovich & Lymar PNAS 2002

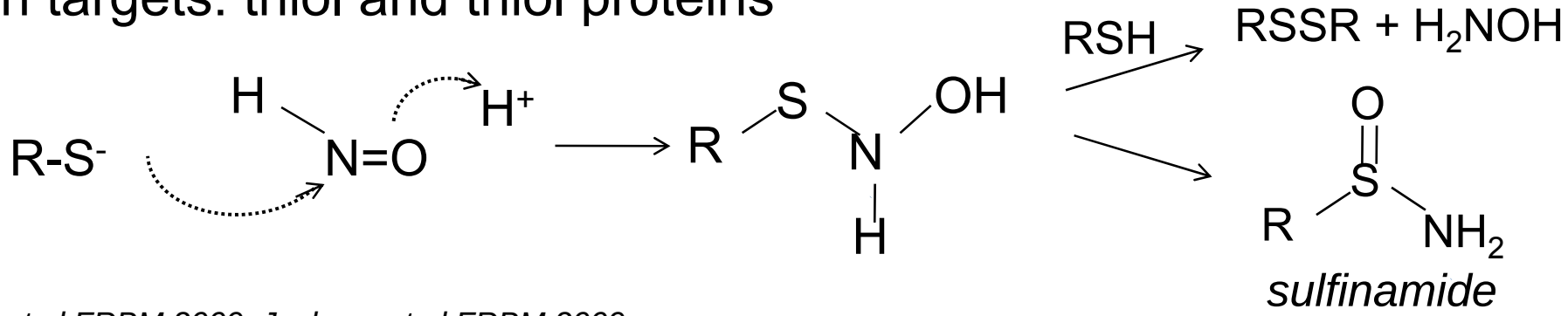
-unstable

$$HNO + HNO \longrightarrow HON{=}NOH \longrightarrow N_2O + H_2O$$

-donor (Angeli´s anion)

$$HN_2O_3^- \longrightarrow HNO + NO_2^-$$

-main targets: thiol and thiol proteins



Fukuto et al FRBM 2009; Jackson et al FRBM 2009; Donzelli et al FRBM 2008; Fukuto et al 2005

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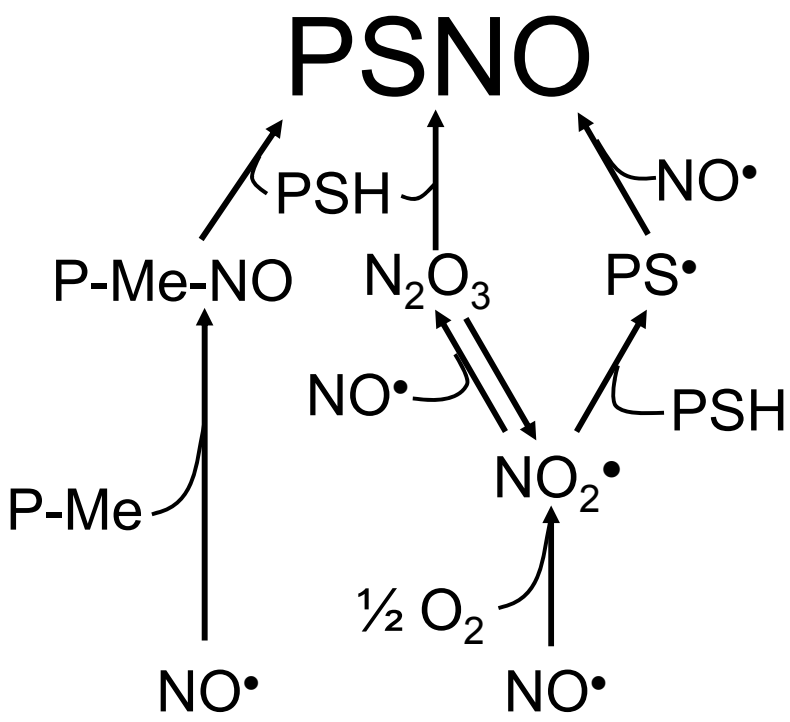
PROTEIN-CYSNO FORMATION FROM NO•

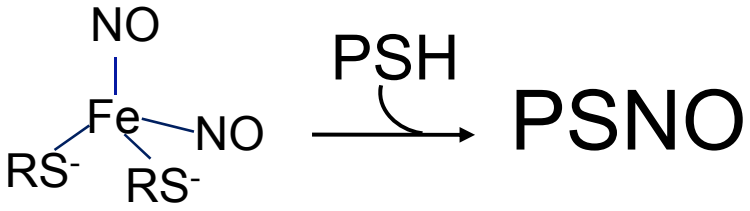
Protein S-nitrosation (P-Cys-SNO) -ubiquitous posttranslational modification
(Stamler & co-workers, others)
-signaling & cytotoxicity
-*in vivo* formation mechanism debatable

Vanin et al, PNAS 2005
Weischsei et al PNAS 2005



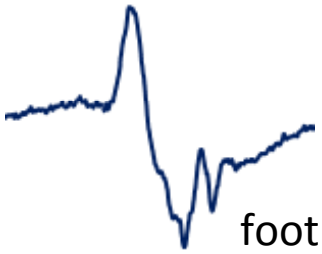
Jourd'heuil et al JBC 2003
Schrammel et al FRBM 2003
Fernandes FRBM 2005

Lancaster & coworkers
PNAS 2009; JBC 2008



Dinitrosyl iron complexes of chelatable cellular iron

Linares et al, FRBM 2001



footpad EPR spectra of
mice infected with *Leishmania*

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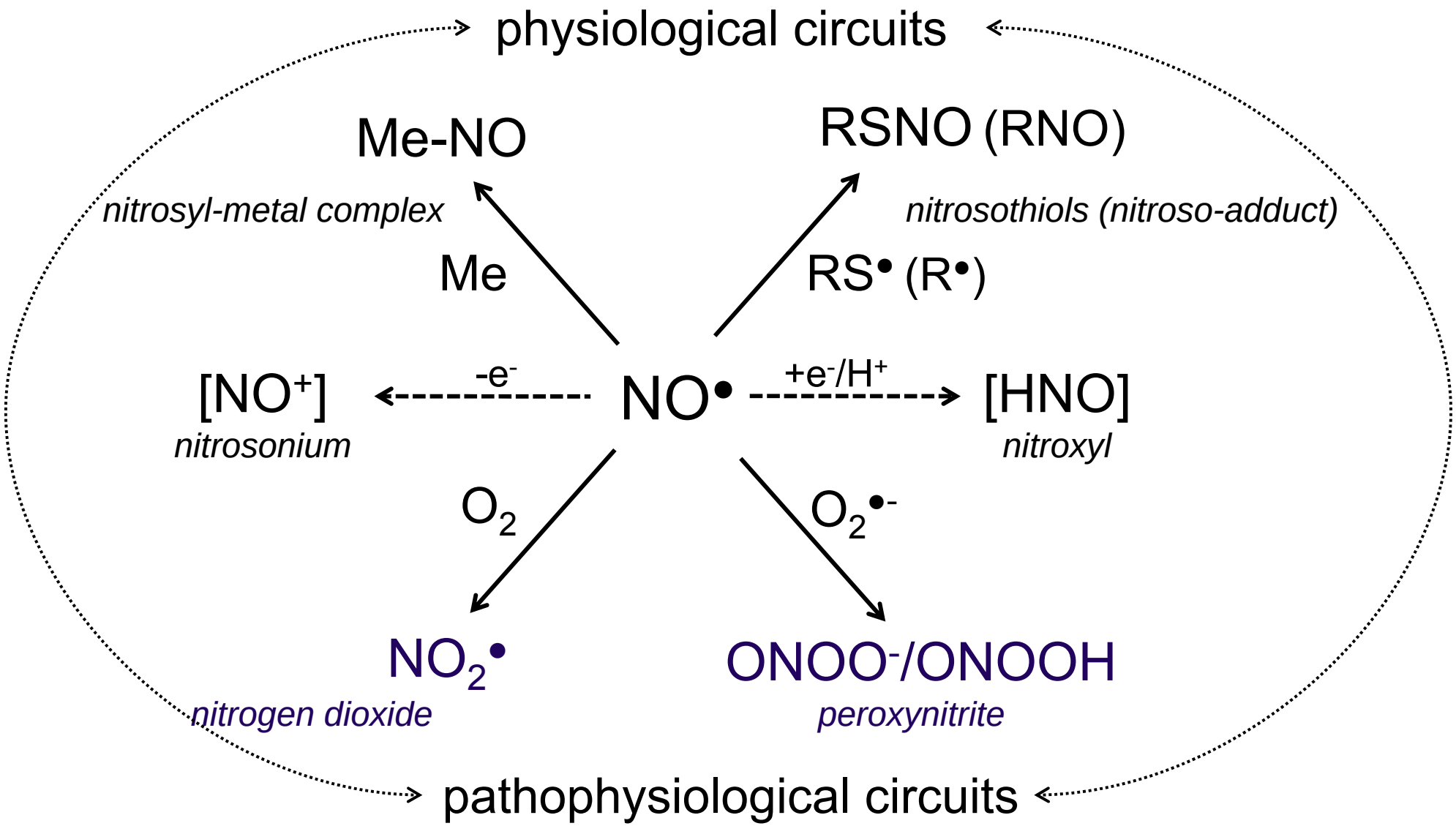
MOST STUDIED NO• REACTIONS AND SPECIES



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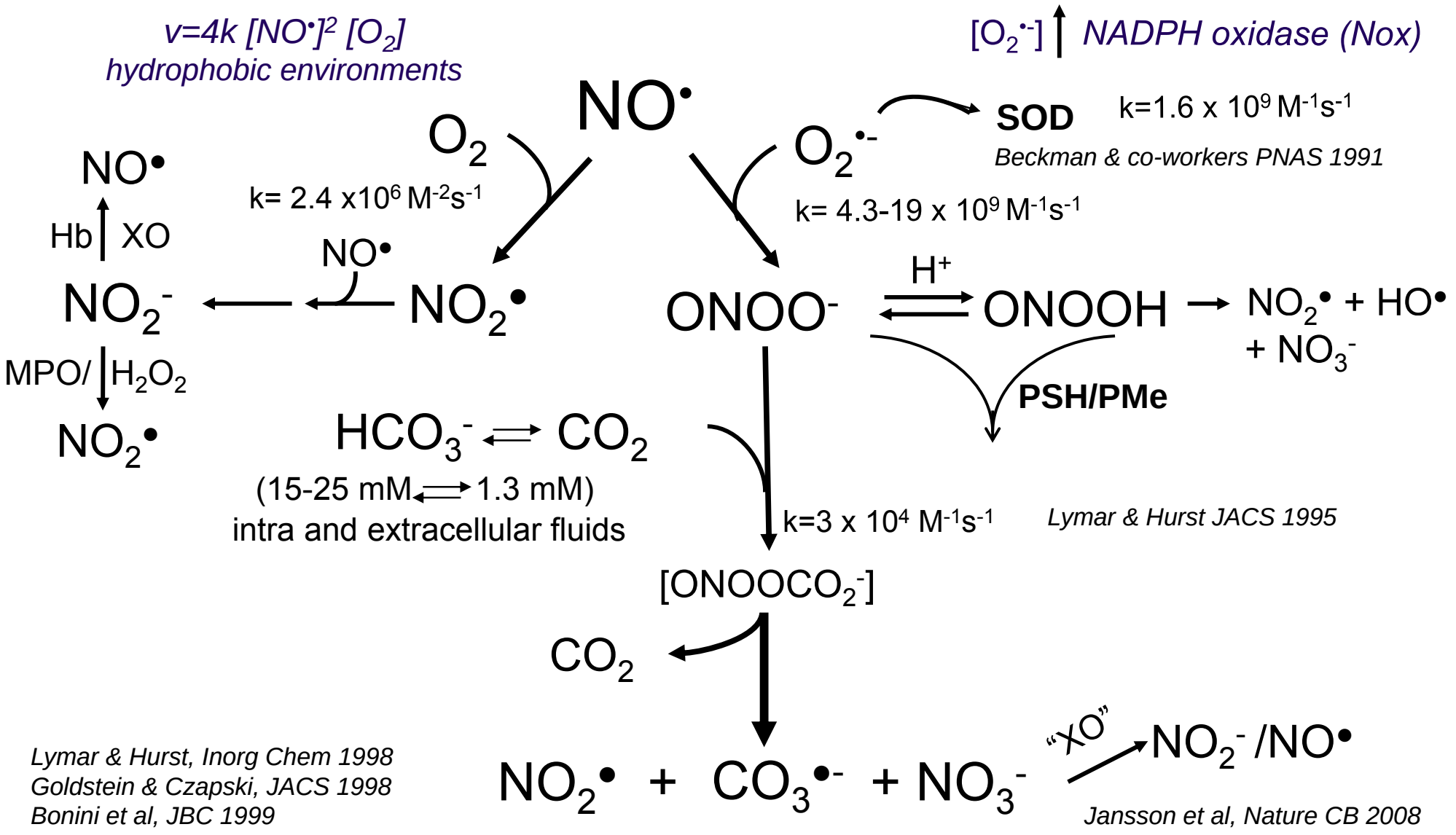
$NO_2^{\bullet}$ & PEROXYNITRITE PRODUCTION FROM $NO^{\bullet}$



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REDUCTION POTENTIAL & REACTION RATES

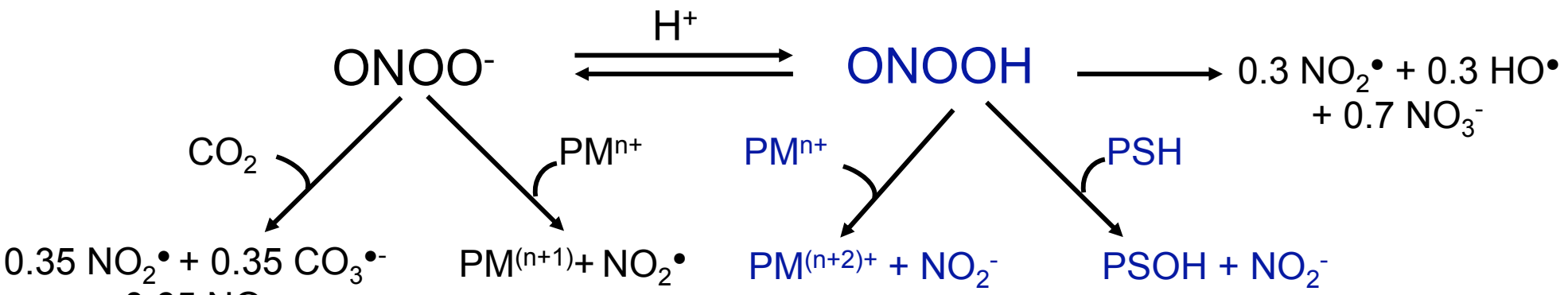
Species	Reduction potential (Eo´) Volts, pH 7.0		Reaction types
$NO^{\bullet}$	+ 0,39	oxidant power ↓	Me nitrosylation/Me oxidation/ Radical recombination
$ONOO^{-}$/ONOOH	+ 0,80		oxidation/nitration (via derived radicals)
$NO_2^{\bullet}$	+ 0,99		oxidation/double bond addition (nitration)
$CO_3^{\bullet -}$	+ 1,80		oxidation
$HO^{\bullet}$	+ 2,30		oxidation/double bound addition (hydroxylation)

Second order rate constant of $NO_2^{\bullet}$,$CO_3^{\bullet -}$ and $HO^{\bullet}$ reactions

Collected in Augusto et al, FRBM 2002

Target	Species	k (M^{-1} s^{-1})
Tyr	$NO_2^{\bullet}$	3.2×10^5
	$CO_3^{\bullet -}$	4.5×10^7
	$HO^{\bullet}$	1.3×10^{10}
Trp	$NO_2^{\bullet}$	3.2×10^6
	$CO_3^{\bullet -}$	7.0×10^8
	$HO^{\bullet}$	1.3×10^{10}
Cys	$NO_2^{\bullet}$	5.0×10^7
	$CO_3^{\bullet -}$	4.6×10^7
	$HO^{\bullet}$	1.9×10^{10}
Urate	$NO_2^{\bullet}$	1.8×10^7
	$CO_3^{\bullet -}$	~ 8.0×10^8
	$HO^{\bullet}$	7.2×10^9

PEROXYNITRITE MEDIATES 1 & 2e$^-$ OXIDATIONS



PM^{n+}= hemeproteins

RSH= RCysSH= thiols & thiol proteins

(likely role in signaling)

Radi et al FRBM 2001
Augusto et al FRBM 2002
Radi & Alvarez AA 2003

Second order rate constant of peroxynitrite reaction with selected proteins

Target	k (M^{-1} s^{-1})	Reference
Myeloperoxidase	6.2 x 10^6	*Floris et al BJ 1993*
Lactoperoxidase	3.3 x10^5	*Floris et al BJ 1993*
HbO_2(monomer)	1.0 x10^4	*Denicola et al PNAS1998*
c-TPx1 *(S. cerevisiae)*	1.0 x10^6	*Ogususcu et al FRBM 2007*
Human Prx5	7.0 x10^{7*}	*Dubuisson et al FEBS 2004*
	1.2 x 10^{8**}	*Trujillo et al ABB 2007*

* pH 7.8 ; ** pH 7.4

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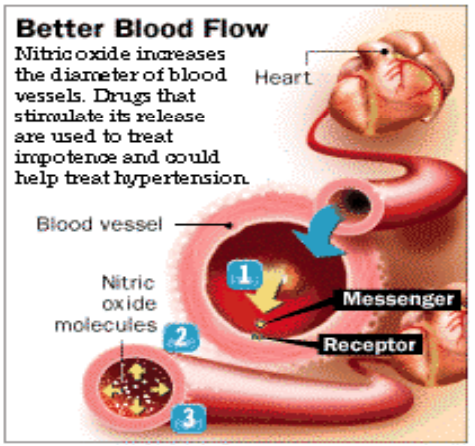
SIMPLIFIED VIEW OF NO• PHYSIOLOGY & PATHOPHYSIOLOGY



[NO•]~ nM

(NOs I pathway)

(Ca^{+2}-calmodulina)

eNOs
nNOs

Arg → NO•

P-Heme → HbO2 → NO_3^-

P-Heme → GC → signaling

iNOs (NOs II pathway)

(inflammatory/
infectious conditions)

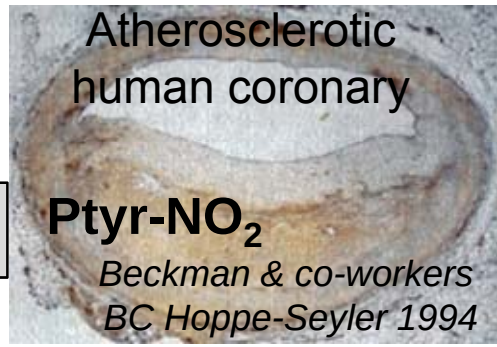
derived oxidants → *scavengers*

BM_{ox}
$BM\text{-}NO_2$
BM-SNO

→ cell
tissue damage

biomarkers

[NO•]~μM



SCAVENGERS OF "INFLAMMATORY" OXIDANTS

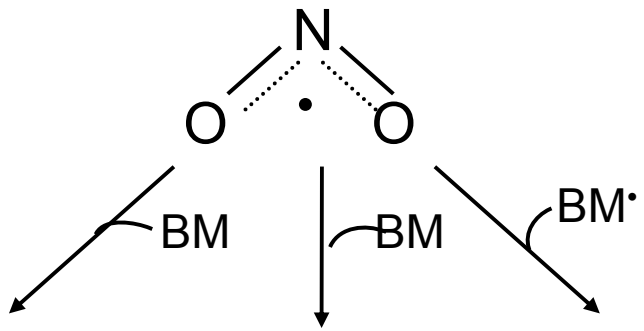
-Protein-TyrNO_2 levels inversely correlate with inflammatory injury

-Nitrated proteins and nitrated lipids consistently detected in animal models and human patients reveals $NO_2^{\bullet}$ production

Typical reactions:

$NO_2^{\bullet}$

E^o = 0.99 V



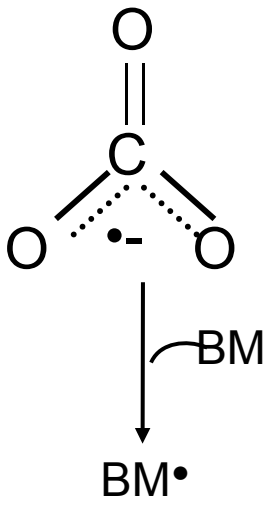
BM• oxidation

[BM-NO_2]• addition

BM-NO_2/BM-ONO recombination

$CO_3^{\bullet-}$

E^o = 1.78 V



Myeloperoxidase (MPO)

E^o = 1.35 V MPO-I

0.97 V MPO-II

peroxidase cycle

$MPO + H_2O_2 \longrightarrow$ MPO-I

MPO-I + BM $\longrightarrow$ MPO-II + BM•

MPO-II + BM $\longrightarrow$ MPO + BM•

chlorinating cycle

$MPO + H_2O_2 \longrightarrow$ MPO-I

MPO-I + Cl^- + $H^+ \longrightarrow$ MPO + HClO

Augusto et al FRBM 2002
Radi PNAS 2004
Davies et al ARS 2008

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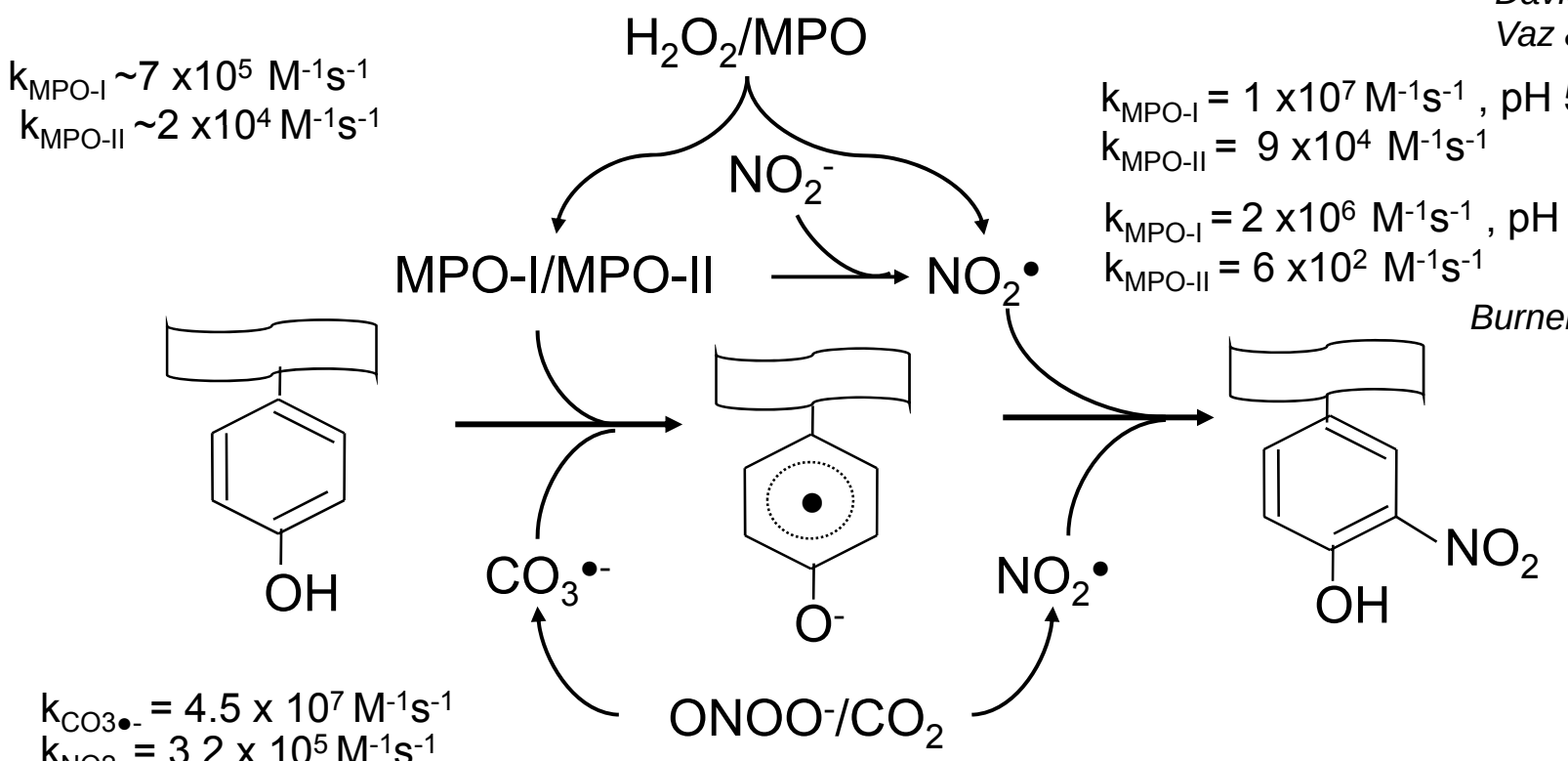
PROTEIN-TYRNO_2 PRODUCERS

$PTyr^{\bullet} + NO_2^{\bullet} \longrightarrow PTyrNO_2$ $k \sim 3 \times 10^9\ M^{-1}s^{-1}$

$PTyr + NO_2^{\bullet} \longrightarrow PTyr^{\bullet}$ $k \sim 3.2 \times 10^5\ M^{-1}s^{-1}$

Augusto et al FRBM 2002
Radi PNAS 2004
Davies et al ARS 2008
Vaz & Augusto ABB 2009



$k_{MPO\text{-}I} \sim 7 \times 10^5\ M^{-1}s^{-1}$
$k_{MPO\text{-}II} \sim 2 \times 10^4\ M^{-1}s^{-1}$

$k_{MPO\text{-}I} = 1 \times 10^7\ M^{-1}s^{-1}$, pH 5.0, 15°C
$k_{MPO\text{-}II} = 9 \times 10^4\ M^{-1}s^{-1}$

$k_{MPO\text{-}I} = 2 \times 10^6\ M^{-1}s^{-1}$, pH 7.4, 15°C
$k_{MPO\text{-}II} = 6 \times 10^2\ M^{-1}s^{-1}$

Burner et al JBC 2000

$k_{CO3\bullet-} = 4.5 \times 10^7\ M^{-1}s^{-1}$
$k_{NO2\bullet} = 3.2 \times 10^5\ M^{-1}s^{-1}$

$k = 2.6 \times 10^4\ M^{-1}s^{-1}$, pH 7.4

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PROTECTION AGAINST INFLAMMATORY TISSUE INJURY

-The protective effects of tempol and urate in animal models of inflammation can be partially due to their reactions with $NO_2^{\bullet}$ and $CO_3^{\bullet -}$

Augusto et al FRBM 2002

-Urate was considered a potent peroxynitrite scavenger but their reaction rate was too low

		k (M^{-1} s^{-1})
Urate +	peroxynitrite	5.1×10^2
+	$NO_2^{\bullet}$	1.8×10^7
+	$CO_3^{\bullet -}$	~ 8.0×10^8
+	MPO I	3.7×10^5

(Kettle & co-workers 2009)

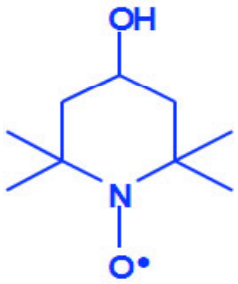
-Clinical trails with inosine (precursor of uric acid) in multiple sclerosis are in progress

Gonsette JNS 2008

-The stable free radical tempol (TPNO$^{\bullet}$) had been extensively studied but its reactions with most oxidants present in inflammatory settings were unknown

Mitchell, Samuni, Krishna & co-workers several studies
Cuzzocrea, Thiemmermann & co-workers several studies

TPNO$^{\bullet}$



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-Selected rate constants:

Goldstein & co-workers
JACS 2003a,b; CRT 200; JPC 2006

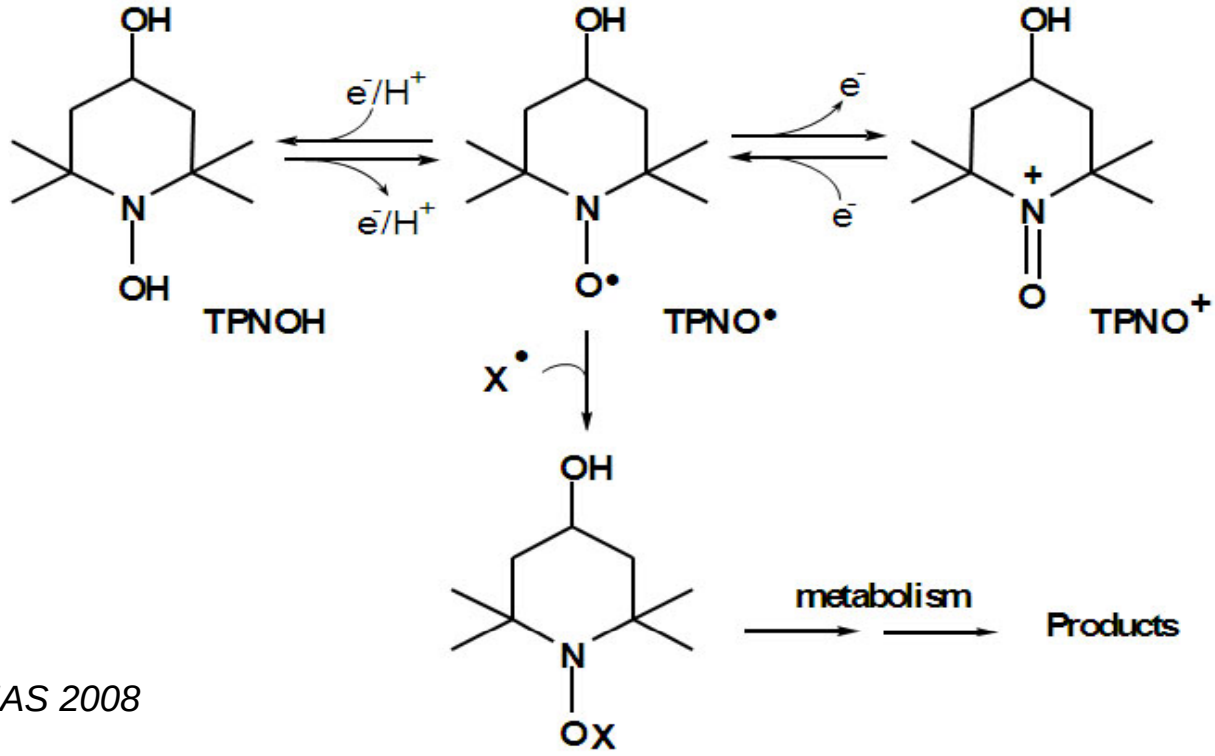
		k ($M^{-1}s^{-1}$)
TPNO• +	NO_2•	8.7×10^8
+	CO_3•-	4.0×10^8
+	MPO I	$\sim 1.0 \times 10^6$

(Vaz & Augusto PNAS 2008)

-Mechanistic studies in test tubes, cells, animals

-multifunctional antioxidant:

after several oxidation/reduction cycles is consumed by recombination reactions with thiyl (RS•) and tyrosyl (Tyr •) radicals (X•) among others.



Recent review Augusto & co-workers AABC 2008
Borisenko et al JACS 2004; JBC 2004
Augusto & co-workers FRBM 2005; FRBM 2008; PNAS 2008
Goldstein et al JPC 2008
Lam et al CRT 2008

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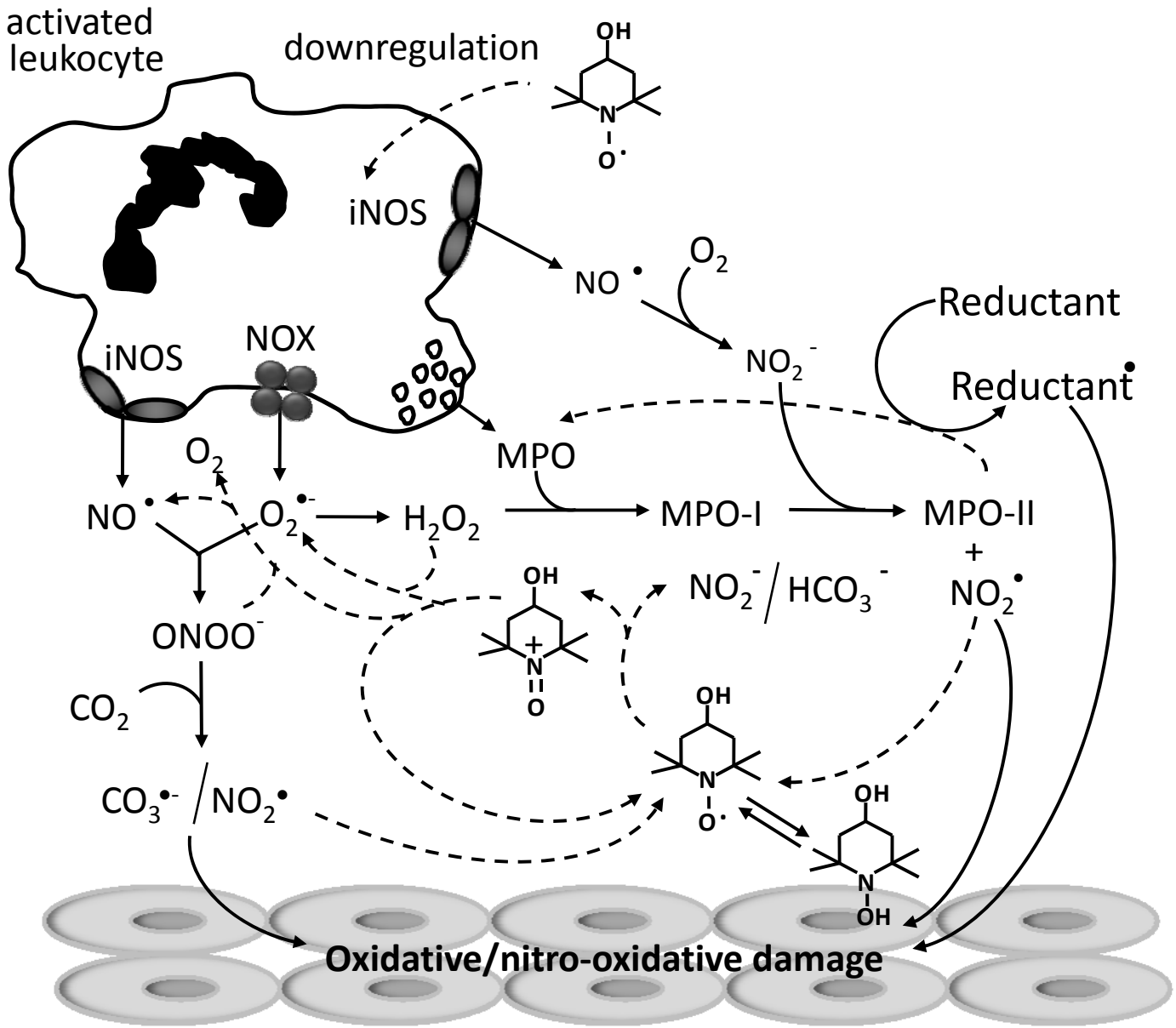

Augusto & co-workers AABC 2008

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