ABCs OF REACTIVE NITROGEN SPECIES AND THEIR SCAVENGERS

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SUNRISE FREE RADICAL SCHOOL

ABCs of Reactive Nitrogen Species and their Scavengers

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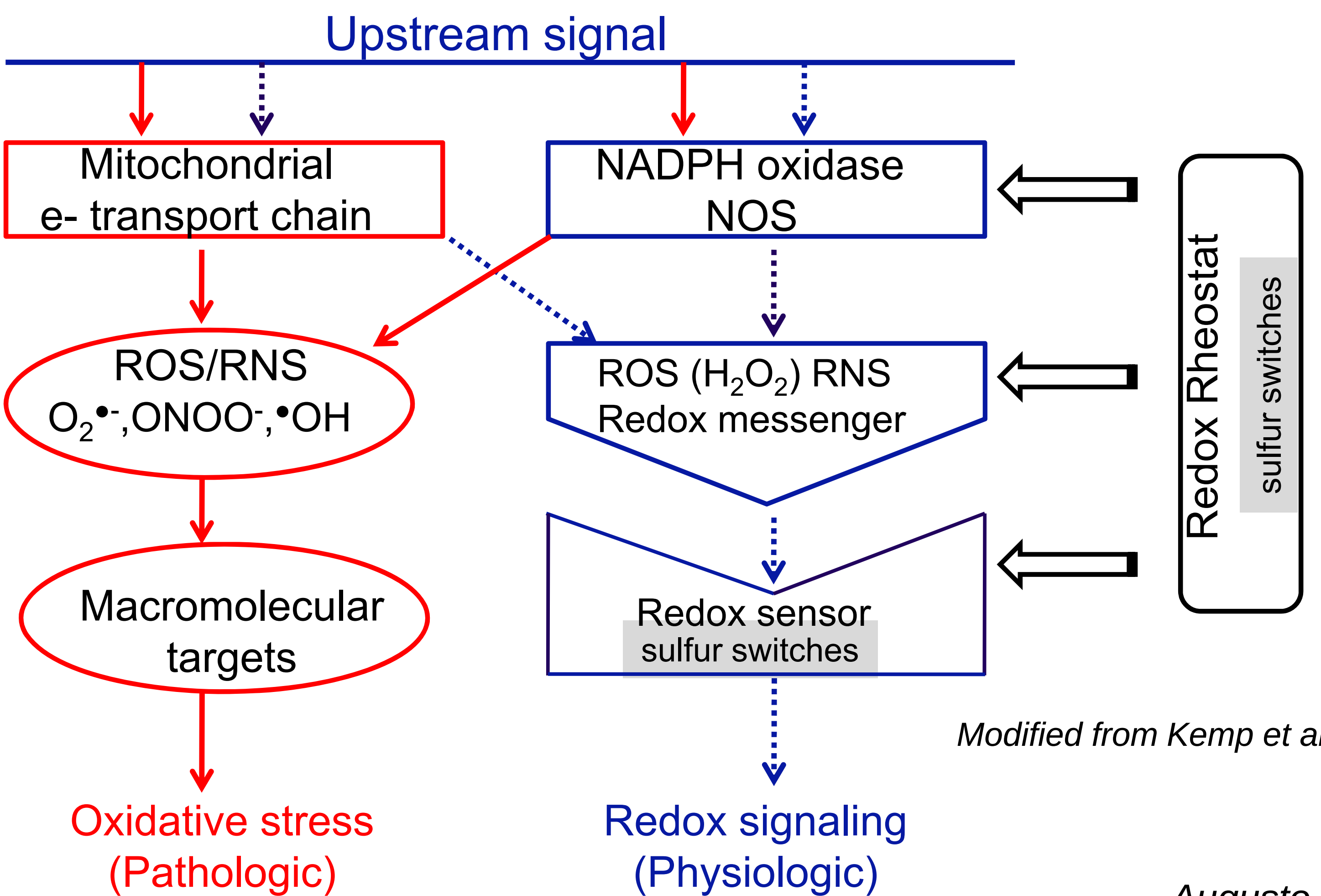
Redoxoma
FAPESP
CNPq

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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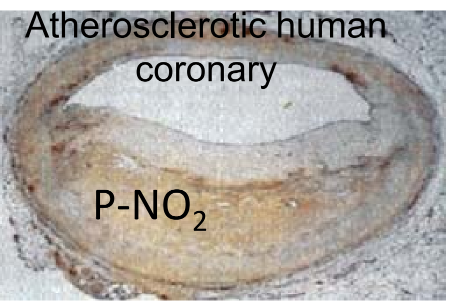
RNS & NO• METABOLITES

Nitric oxide is a signal transducing radical

$$\text{Arginine} + \text{NADPH} + O_2 \xrightarrow{\text{NOS}} \text{NO}^\bullet + \text{Citrulline} + \text{NADP}^+ + H_2O$$

$$\text{NO}^\bullet \xrightarrow{\text{biotransformation}} \text{Metabolites}$$

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

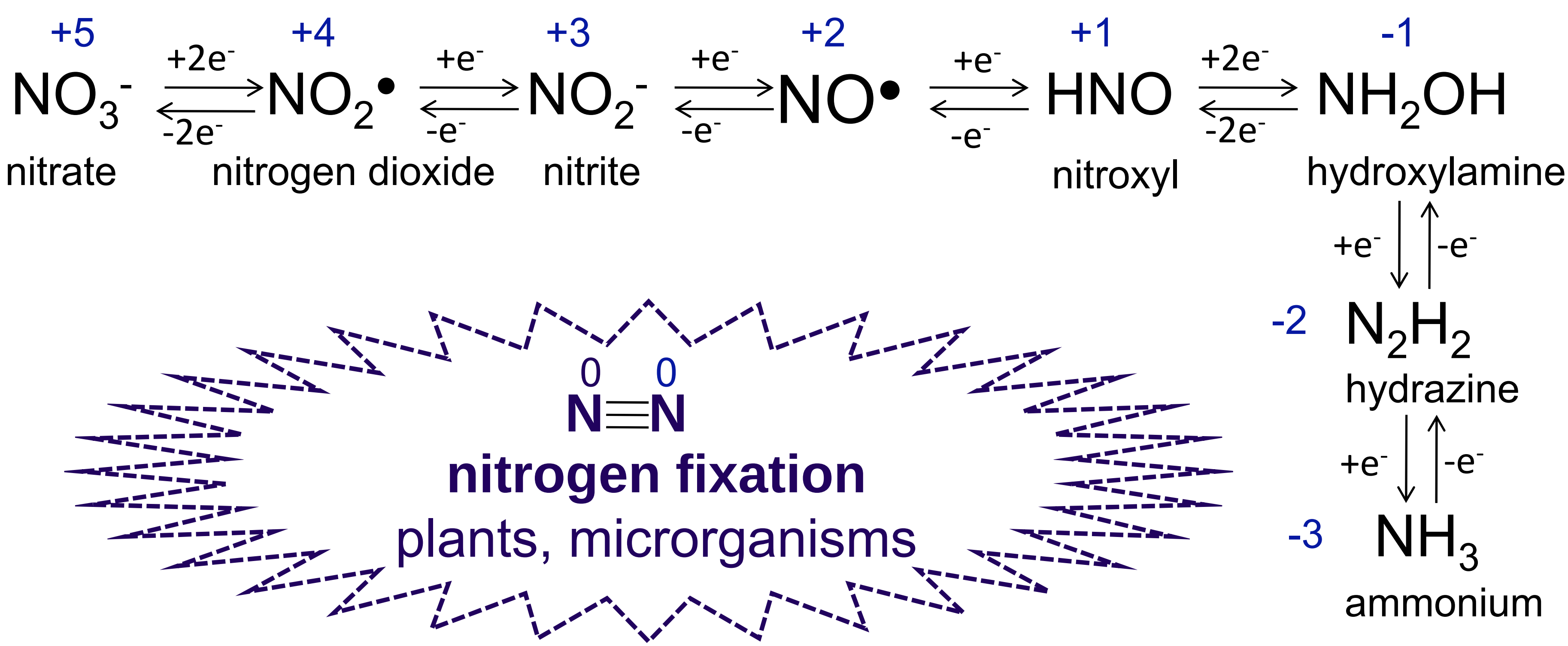
-Molecular oxygen (O_2)

$$O_2 \underset{-e^-}{\overset{+e^-}{\rightleftharpoons}} O_2^{\bullet -} \underset{-e^-/2H^+}{\overset{+e^-/2H^+}{\rightleftharpoons}} H_2O_2 \xrightarrow{+e^-} HO^\bullet \xrightarrow{+e^-/H^+} H_2O$$

-Nitric oxide ($NO^\bullet$)

protons ommited

oxidation states



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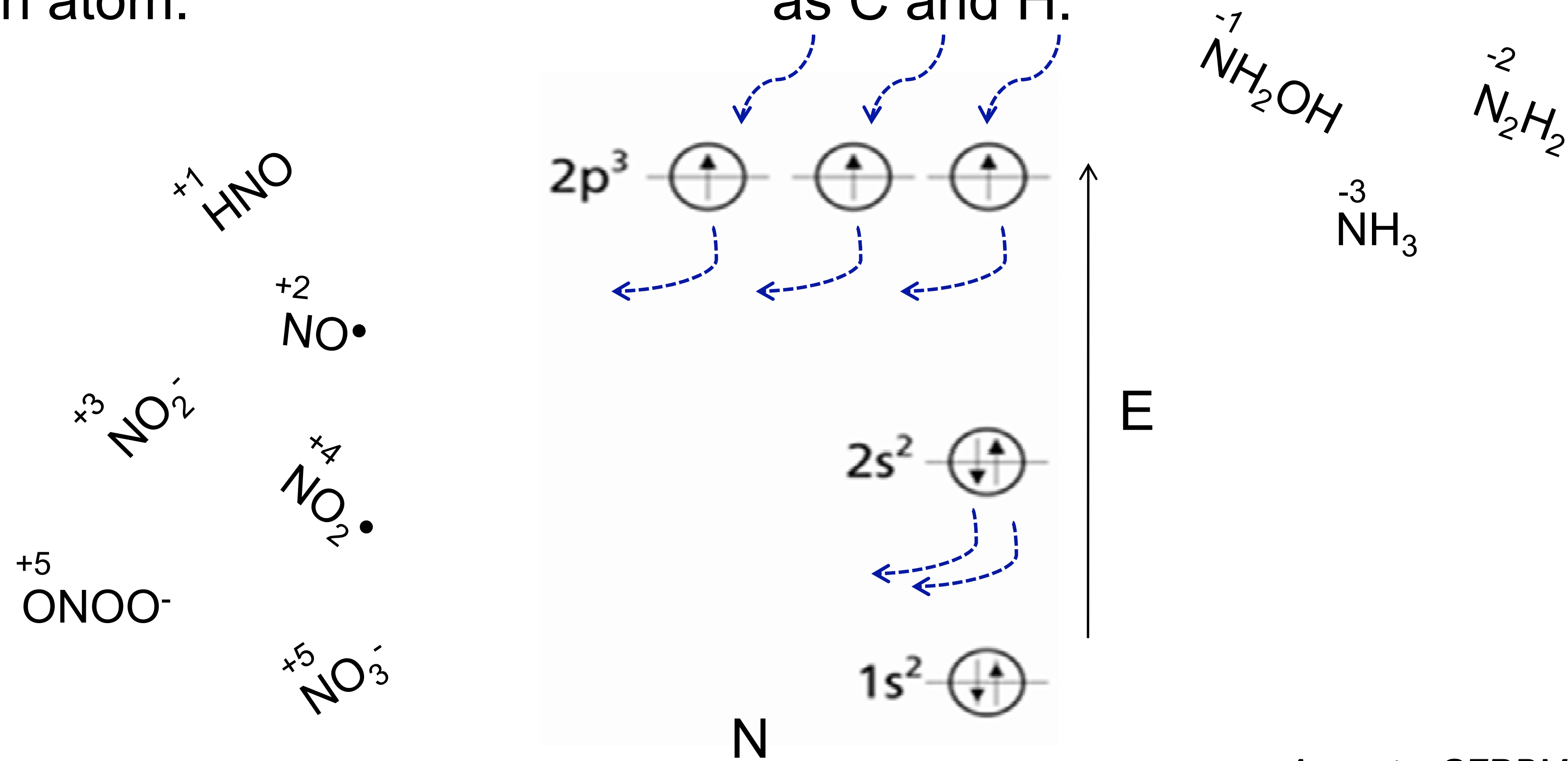
VERSALITY OF NITROGEN COMPOUNDS

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.



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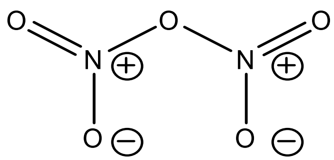
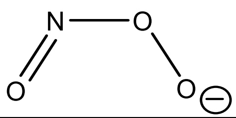
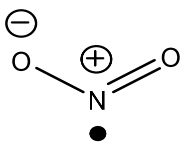
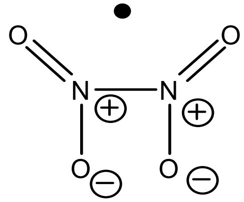
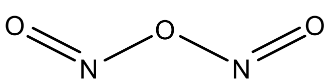
Summary of the redox properties of nitrogen oxides, acids and ions.

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	

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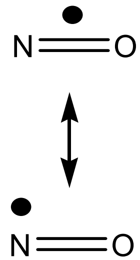
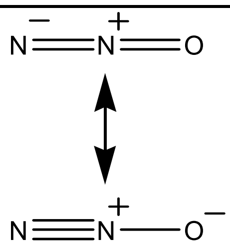
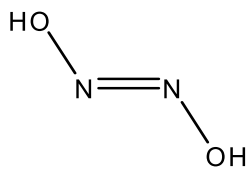
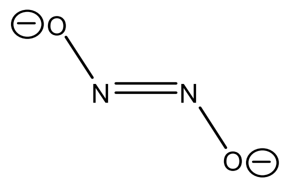
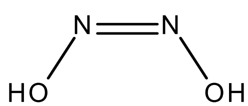
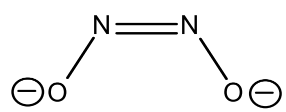
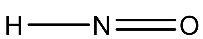
Summary of the redox properties of nitrogen oxides, acids and ions (continued).					
Oxidation Number	**Oxide**	**Acid**	**Ions**	**Names**	**Redox Properties**
+2				**nitric oxide or nitrogen monoxide**	**oxidant and reducer, reacts rapidly with O_2**
+1				nitrous oxide	neither oxidant nor reducer
				cis and trans hyponitrite	does not dismutate
			NO^-	nitroxyl	

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

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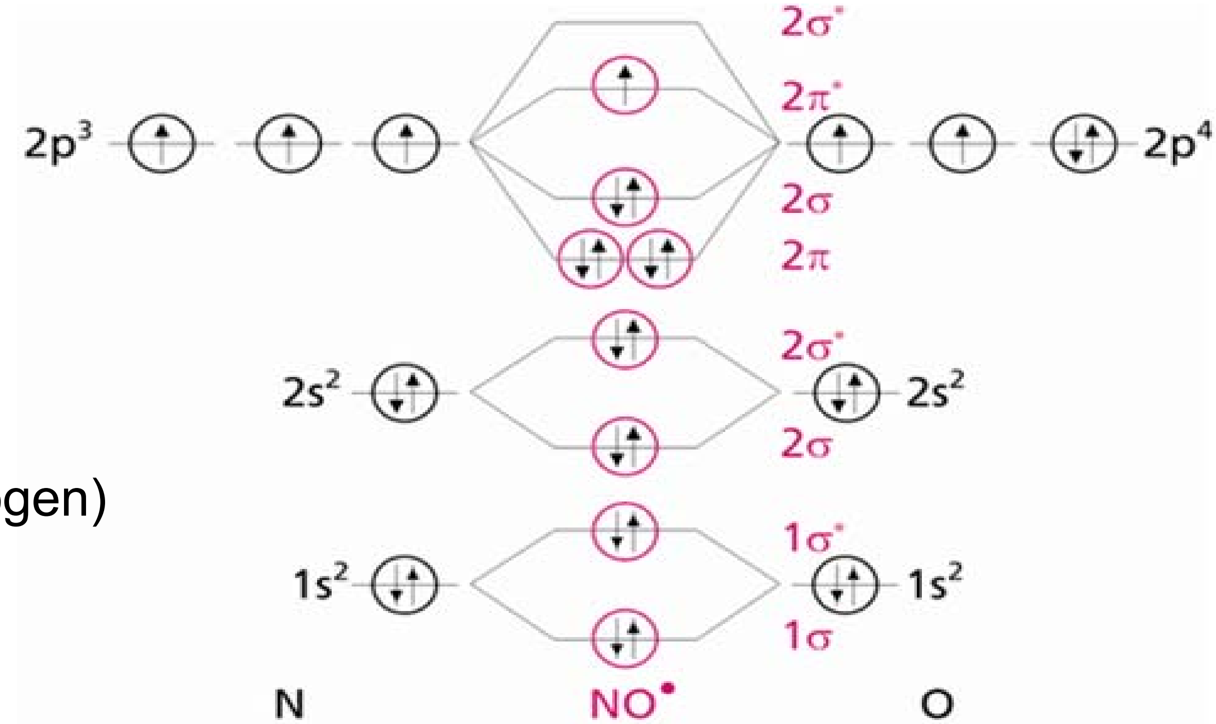
NITROGEN MONOXIDE (NITRIC OXIDE)

$:N\overset{\bullet}{=}O$

2.5 bounds

(3 bounding- 0.5 antibouding orbitals)



-unlikely to dimerize

$2\ NO^{\bullet} \rightleftarrows N_2O_4$ (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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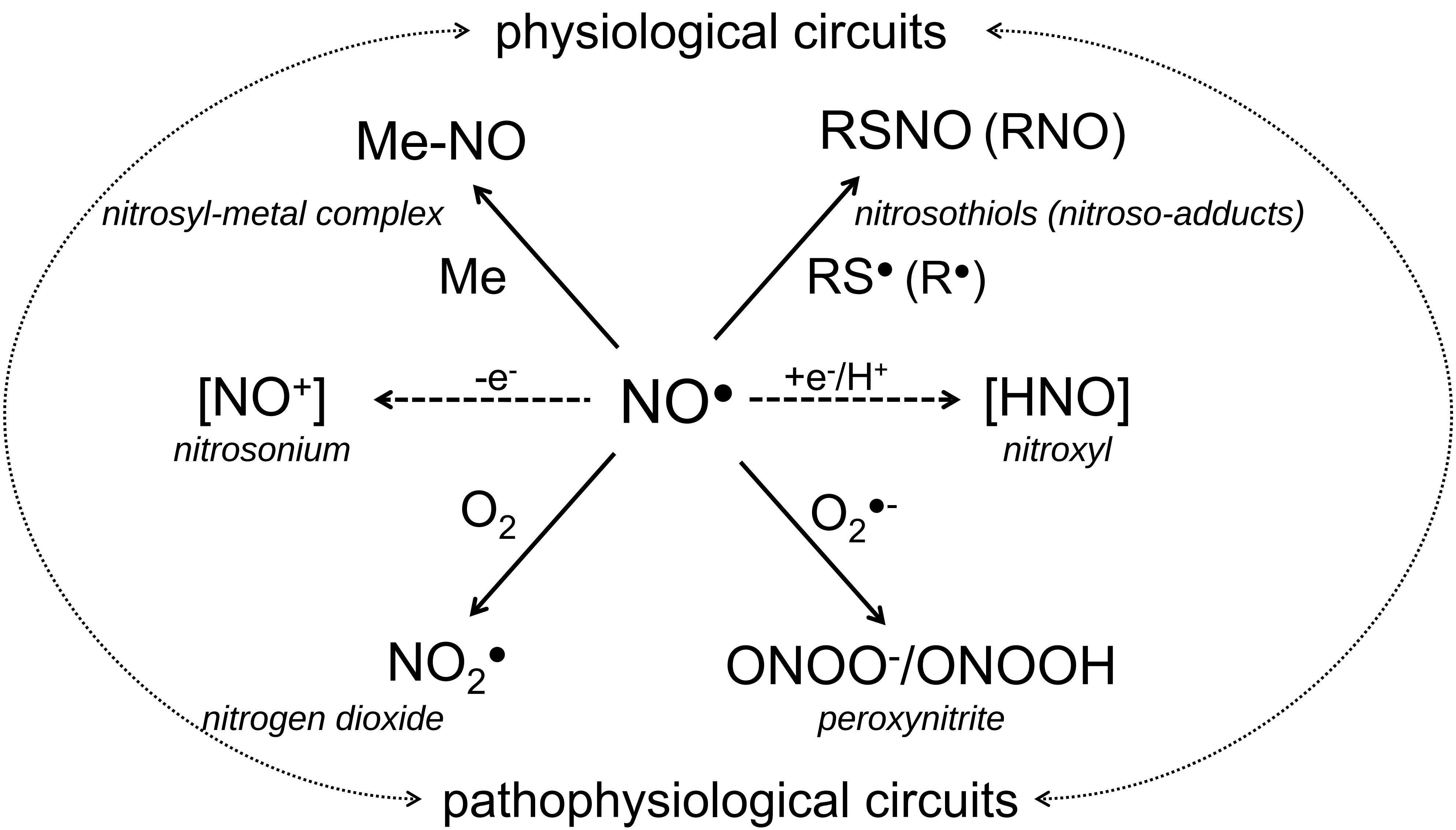
MOST STUDIED NO• REACTIONS AND SPECIES



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NITROXYL

HNO- nitroxyl (nitrosyl hydride or hydrogen oxonitrite)

-pharmacological actions: anti-alcoholic drug cyanamide
cardiovascular actions diverse from NO• donors

-pKa~ 11.4

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

Shafirovich & Lymar PNAS 2002

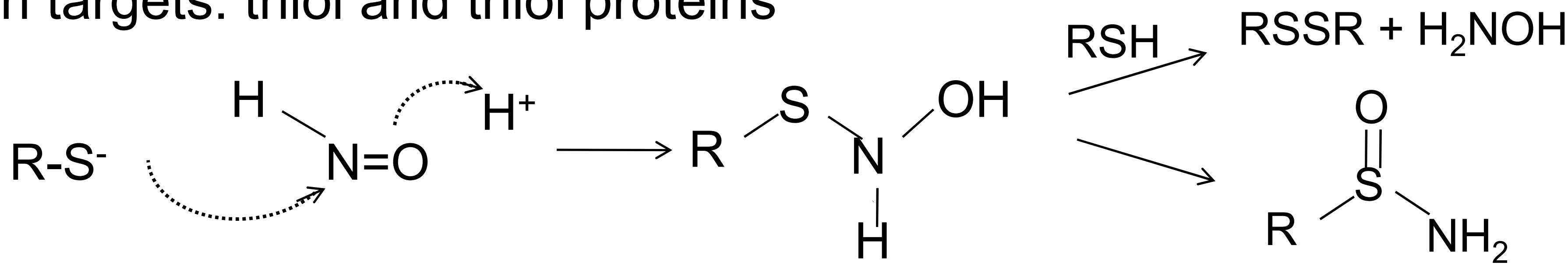
-unstable

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

-donor (Angeli´s anion)

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

-main targets: thiol and thiol proteins



sulfinamide

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

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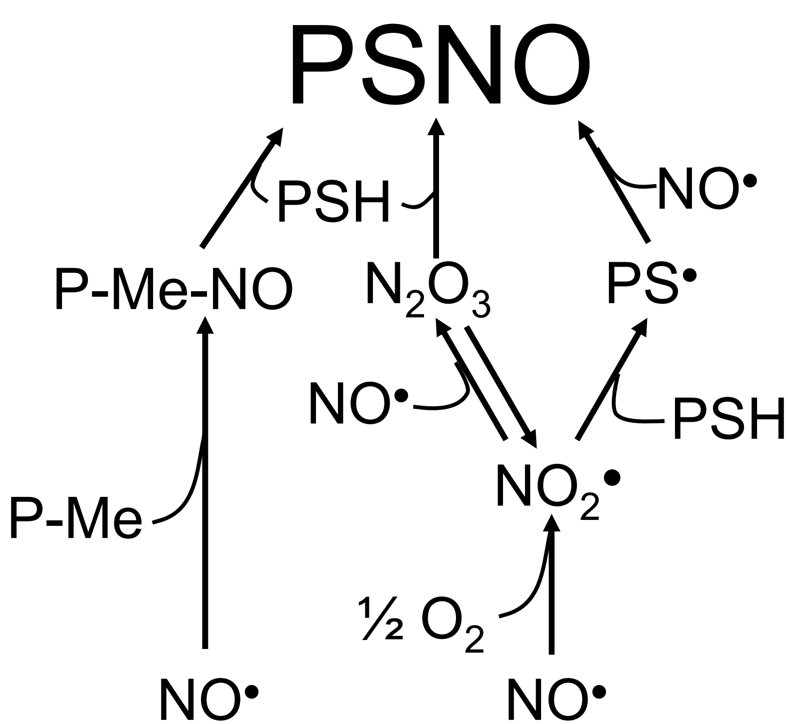
PROTEIN-CYSNO FORMATION FROM NO•

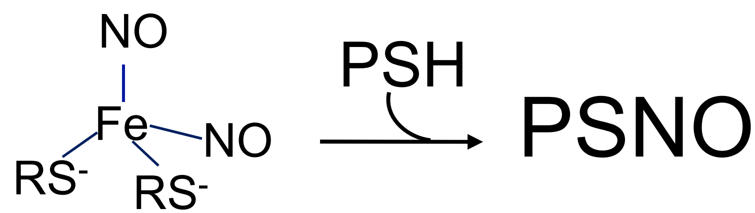
Protein S-nitrosation (P-Cys-SNO) -ubiquitous posttranslational modification
(Stamler & co-workers, others)
-signaling & cytotoxicity
-*in vivo* formation mechanism debatable

Vanin et al, PNAS 2005
Weischsei et al PNAS 2005



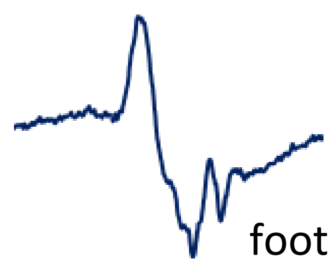
Jourd'heuil et al JBC 2003
Schrammel et al FRBM 2003
Fernandes FRBM 2005

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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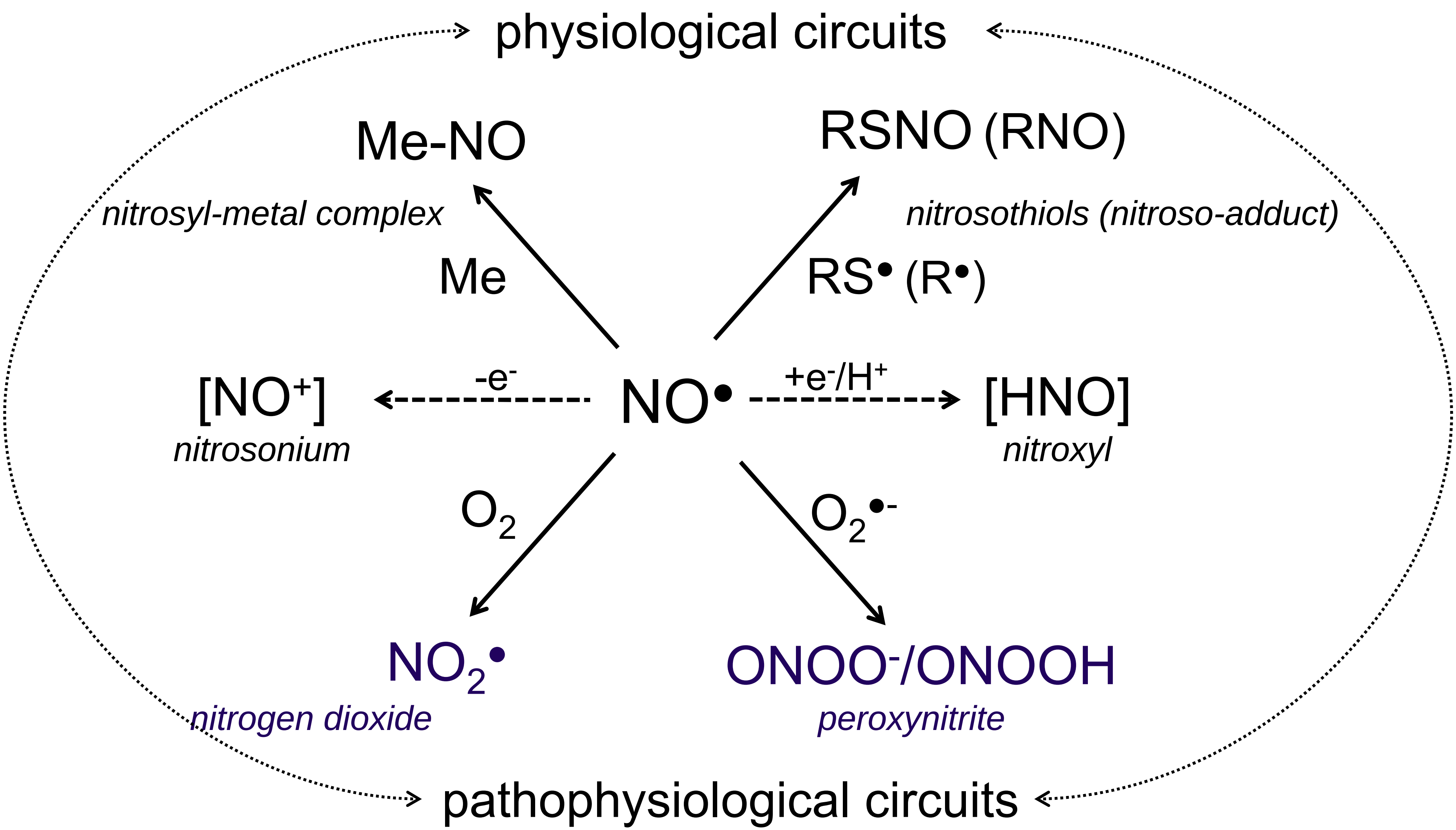
MOST STUDIED NO• REACTIONS AND SPECIES



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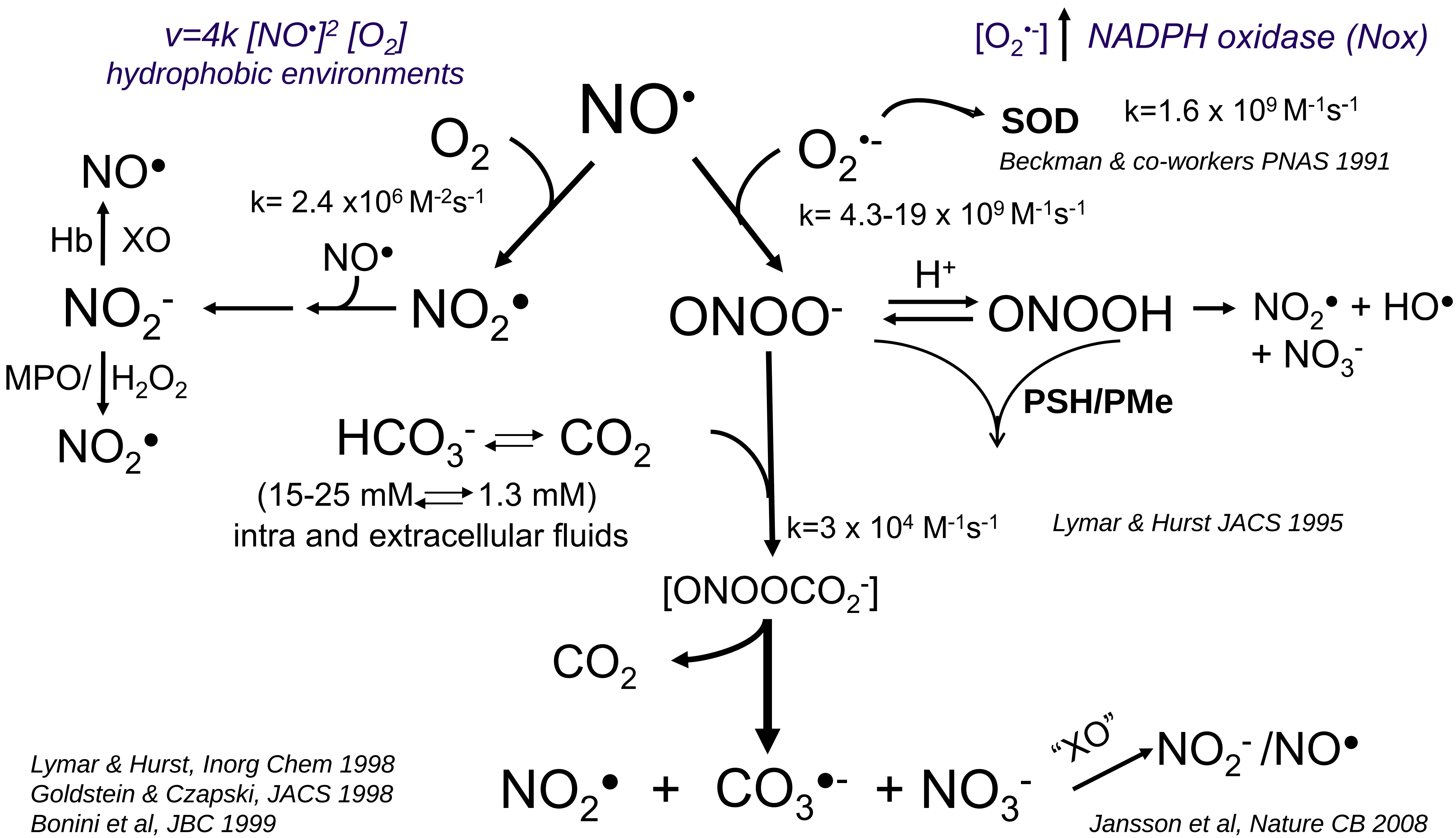
NO_2• & PEROXYNITRITE PRODUCTION FROM NO•



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REDUCTION POTENTIAL & REACTION RATES

Species	Reduction potential (Eo´) Volts, pH 7.0	Reaction types
$NO^{\bullet}$	+ 0,39	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)

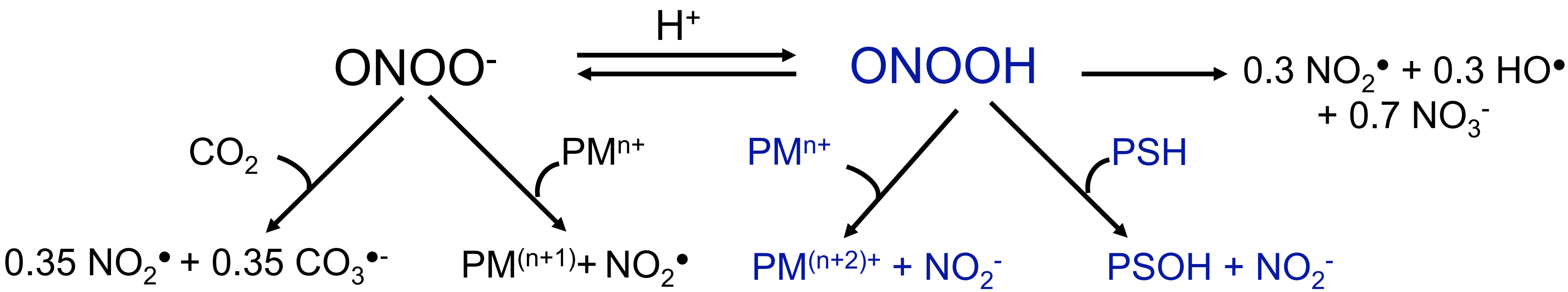
oxidant power

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

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

(likely role in signaling)

Second order rate constant of peroxynitrite reaction with selected proteins

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

* pH 7.8 ; ** pH 7.4

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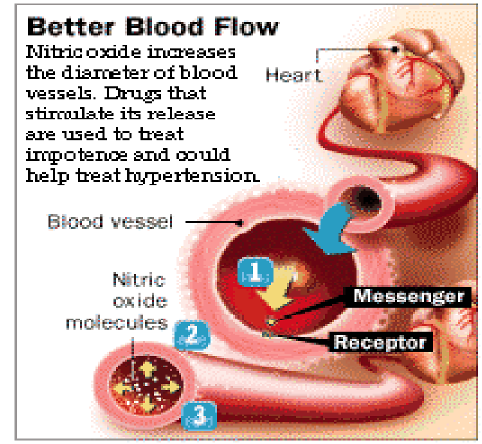
SIMPLIFIED VIEW OF NO• PHYSIOLOGY & PATHOPHYSIOLOGY



[NO•]~ nM

(NOs I pathway)
(Ca^{+2}-calmodulina)

eNOs
nNOs

Arg → NO•

P-Heme →

HbO2 → NO_3^-

GC → signaling

derived oxidants →

iNOs (NOs II pathway)

(inflammatory/
infectious conditions)

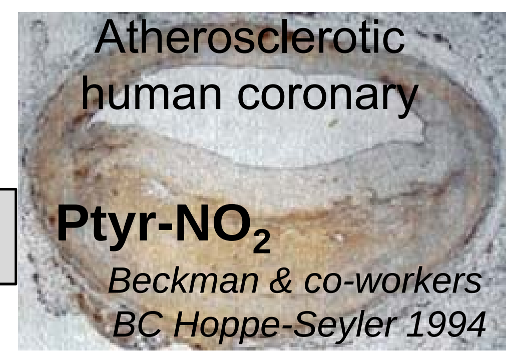
[NO•]~µM

scavengers

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

→ cell
tissue damage

biomarkers



SCAVENGERS OF "INFLAMMATORY" OXIDANTS

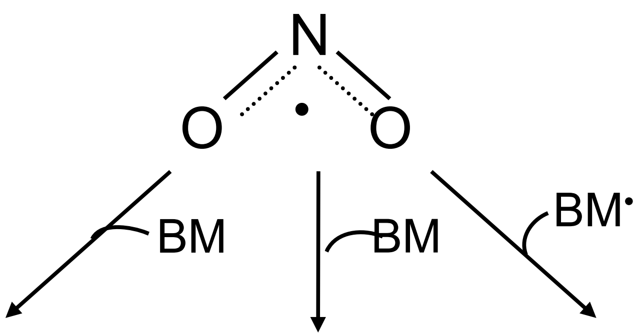
-Protein-TyrNO_2 levels inversely correlate with inflammatory injury

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

Typical reactions:

$NO_2^{\bullet}$

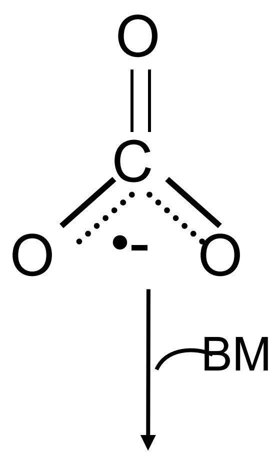
$E^o = 0.99$ V



BM• oxidation | [BM-NO_2]• addition | BM-NO_2/BM-ONO recombination

$CO_3^{\bullet -}$

$E^o = 1.78$ V



BM•

Myeloperoxidase (MPO)

$E^o = 1.35$ V MPO-I
0.97 V MPO-II

peroxidase cycle

MPO + H_2O_2 → MPO-I

MPO-I + BM → MPO-II + BM•

MPO-II + BM → MPO + BM•

chlorinating cycle

MPO + H_2O_2 → MPO-I

MPO-I + Cl^- + H^+ → MPO + HClO

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

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PROTEIN-TYRNO$_2$ PRODUCERS

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

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

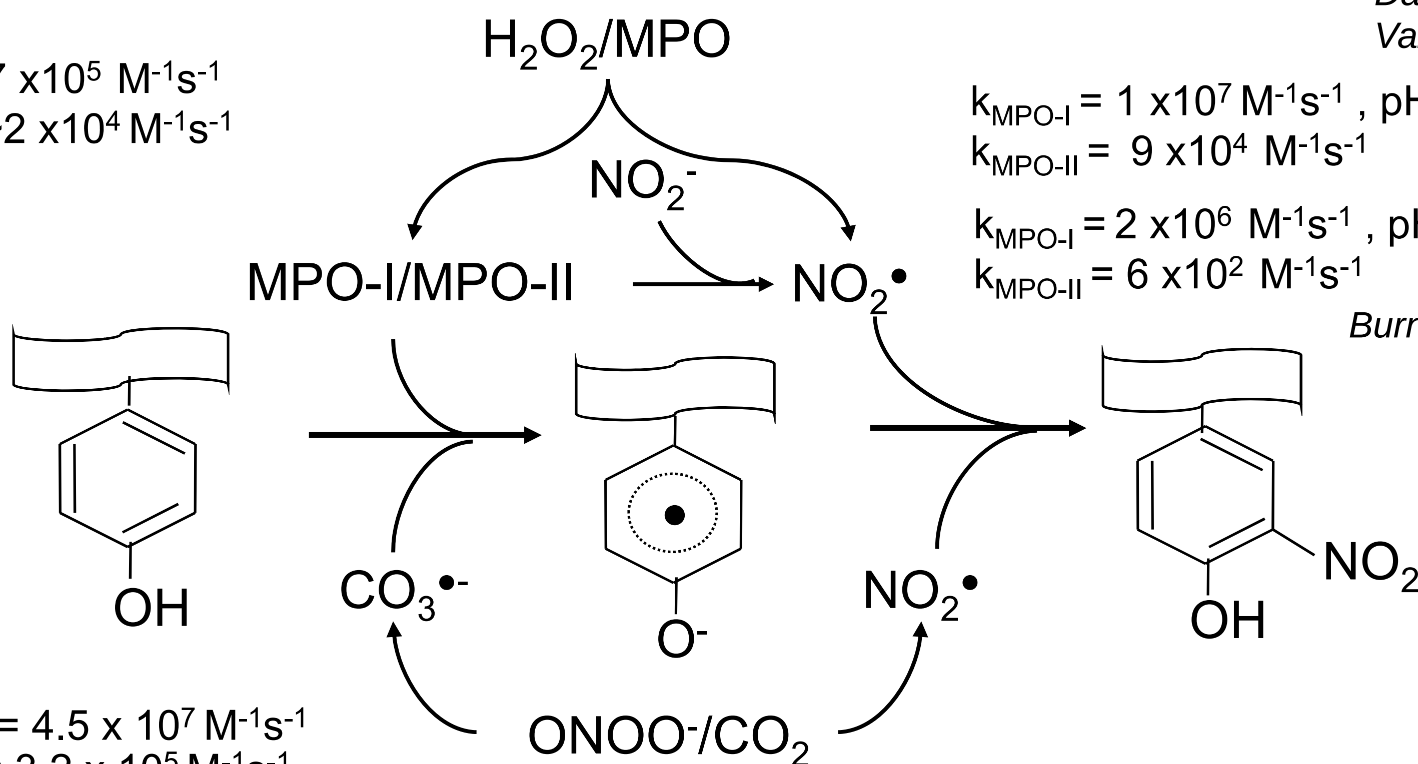
Augusto et al FRBM 2002
Radi PNAS 2004
Davies et al ARS 2008
Vaz & Augusto ABB 2009

$k_{MPO\text{-}I}$ ~7 x10^5 $M^{-1}s^{-1}$
$k_{MPO\text{-}II}$ ~2 x10^4 $M^{-1}s^{-1}$

$k_{MPO\text{-}I}$ = 1 x10^7 $M^{-1}s^{-1}$, pH 5.0, 15^{o}C
$k_{MPO\text{-}II}$ = 9 x10^4 $M^{-1}s^{-1}$

$k_{MPO\text{-}I}$ = 2 x10^6 $M^{-1}s^{-1}$, pH 7.4, 15^{o}C
$k_{MPO\text{-}II}$ = 6 x10^2 $M^{-1}s^{-1}$

Burner et al JBC 2000



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

k = 2.6 x10^4 $M^{-1}s^{-1}$, 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}
+	$CO_3^{\bullet -}$	$\sim 8.0 \times 10^{8}$
+	MPO I	3.7×10^{5}

(Kettle & co-workers 2009)

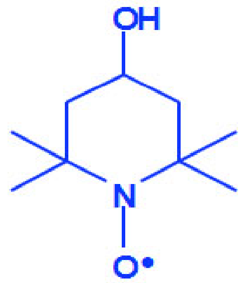
-Clinical trails with inosine (precursor of uric acid) in multiple sclerosis are in progress

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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TEMPOL & PROTECTION AGAINST INFLAMMATORY INJURY

-Selected rate constants:

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

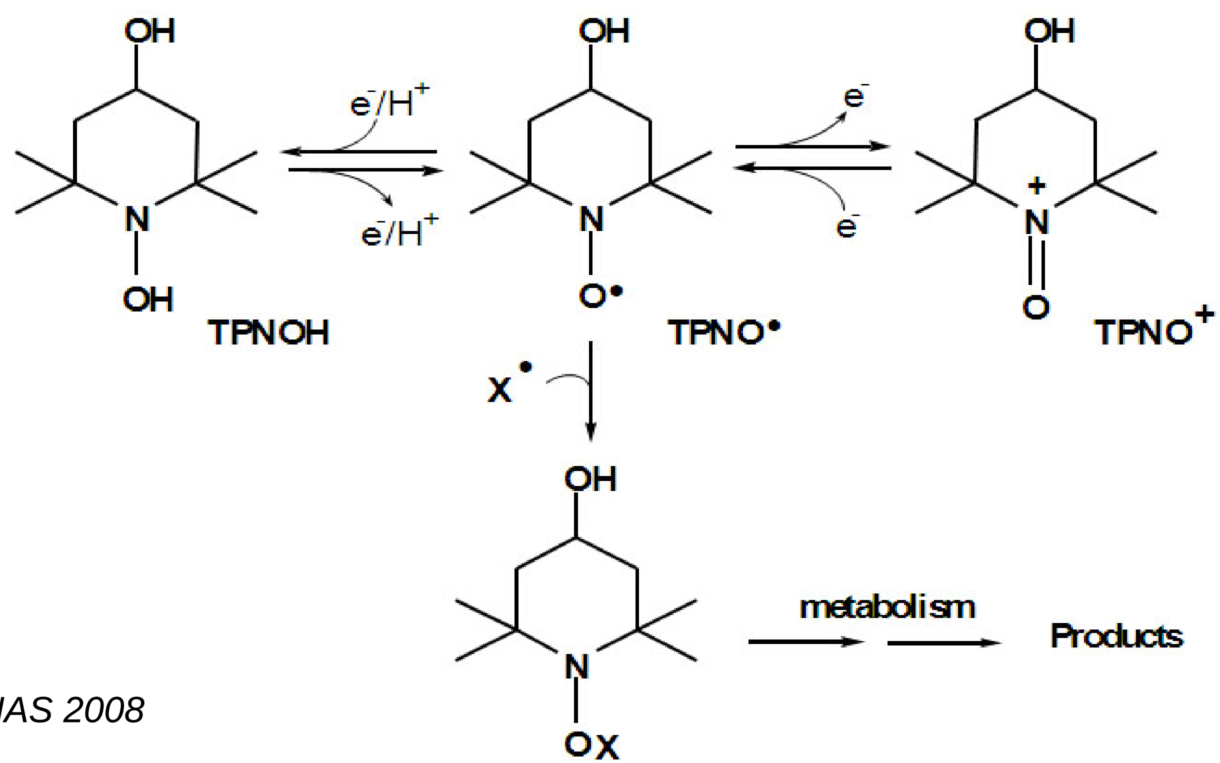
		k ($M^{-1}s^{-1}$)
TPNO•	+ NO_2•	8.7 x 10^8
	+ CO_3•-	4.0 x 10^8
	+ MPO I	~1.0 x 10^6

(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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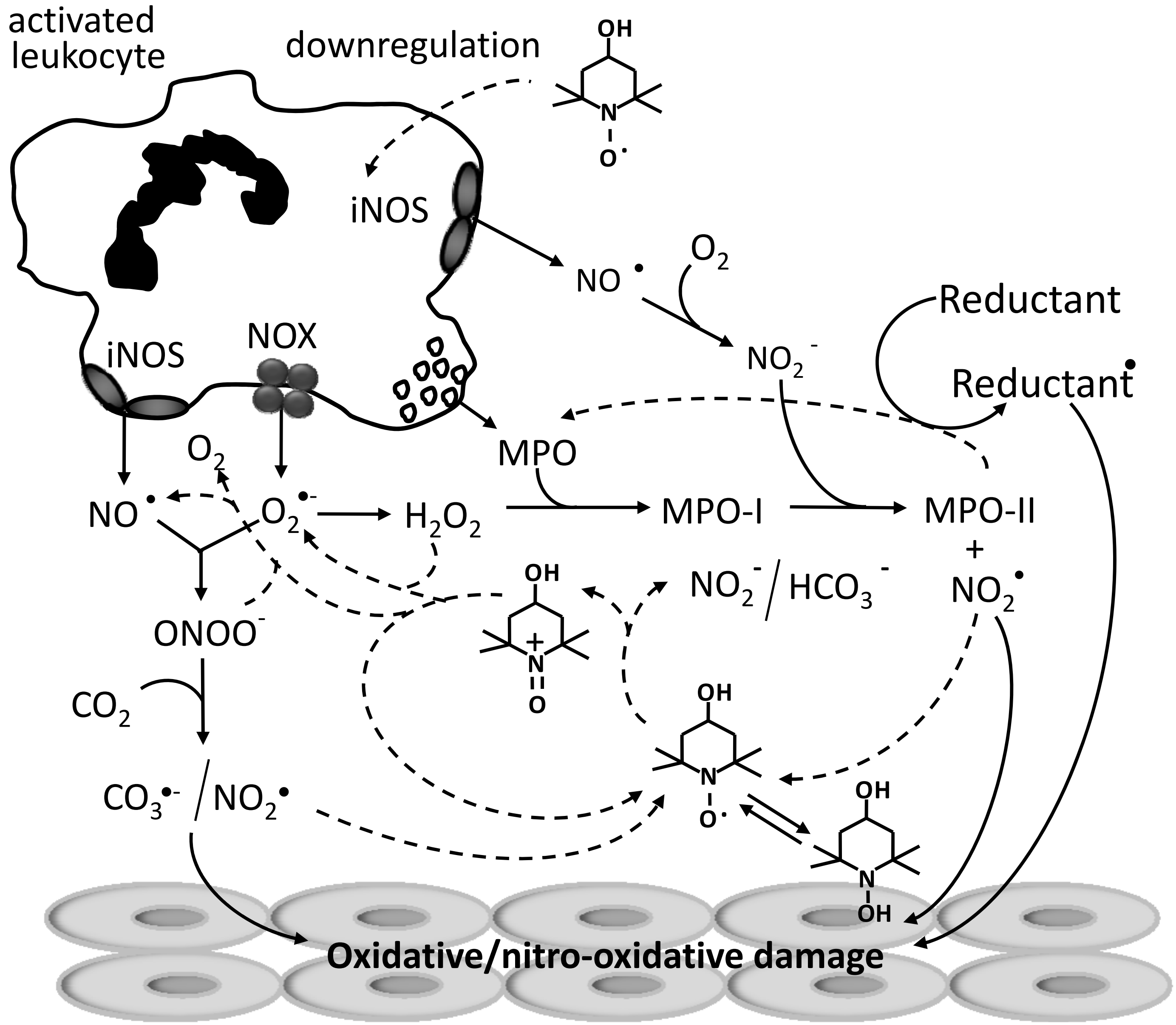
TEMPOL & PROTECTION AGAINST INFLAMMATORY INJURY



Augusto & co-workers AABC 2008

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