Antioxidantes Enzimáticos e Não Enzimáticos

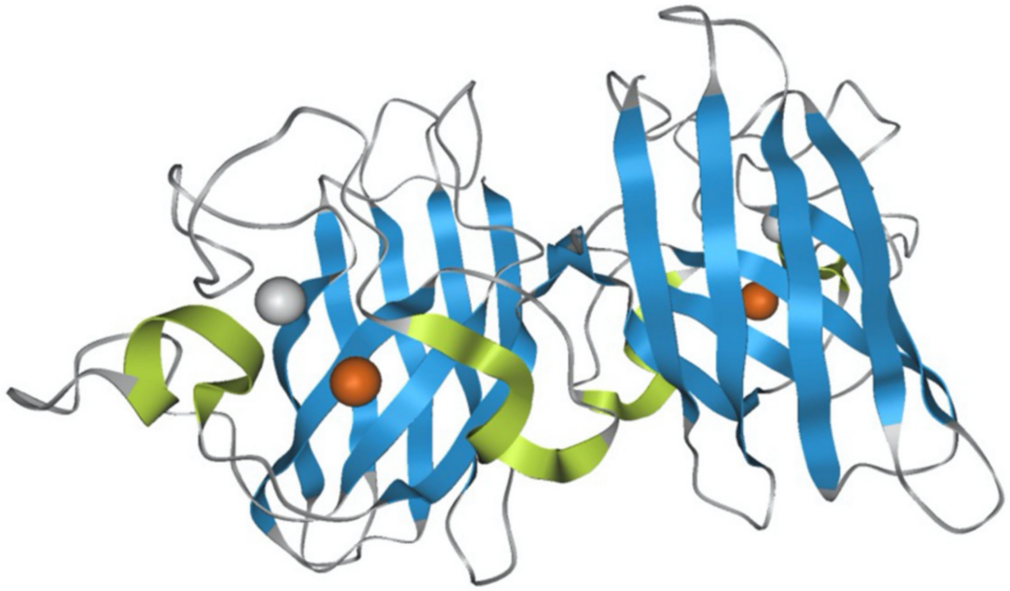
QBQ2509: Bioquímica Redox
QBQ5893: Processos Redox em Bioquímica

Dr. Danilo B. Medinas

Material de estudo para prova

Halliwell: Capítulos 3, 4 e 11

Manuscritos citados



Superoxide Dismutase 1

Tópicos e metas da aula

- **Definição de antioxidantes e mecanismos de ação.**
  - Entender o propósito biológico da existência de antioxidantes e sua diversidade molecular.
- **Descoberta da Superóxido Dismutase.**
  - Reconhecer a importância da descoberta da defesa antioxidante para o entendimento do papel de oxidantes e radicais livres em biologia.
- **Substratos e tipos de enzimas antioxidantes.**
  - Conhecer as diferentes classes de enzimas antioxidantes e sua eficiência catalítica.
- **Acoplamento entre a geração e a eliminação de oxidantes.**
  - Compreender como o sistema de antioxidantes enzimáticos evoluiu.
- **Antioxidantes não enzimáticos.**
  - Identificar moléculas com ação antioxidante.
- **Terapias antioxidantes.**
  - Ser capaz de racionalizar estratégias para o emprego de antioxidantes em doenças.

Antioxidantes

Bom (Ex. Vitaminas)

Ruim ($Ex. No_x$)

antioxidantes — oxidantes

Balança Redox

Antioxidantes

> Living organisms are exposed to much more severe oxidation than is food in a refrigerator. Nevertheless, they do not become rancid until they, in their turn, become food.
> **Graham Burton and Keith Ingold**

No contexto bioquímico,

Qualquer substancia que previne, retarda ou remove modificações oxidativas em moléculas biológicas.

- ✓ **Enzimas e miméticos** (efeito catalítico)
- ✓ **Moléculas pequenas** (estados de oxidação reversíveis)

Estratégias antioxidantes

- ✓ **Agentes catalíticos**
- ✓ **Agentes (estratégias) que limitam (ou impedem) a produção de oxidantes** (complexo IV da cadeia de transporte de elétrons da mitocôndria; proteínas quelantes de metal como transferrina, etc)
- ✓ **Agentes que impedem a oxidação de biomoléculas por diversos mecanismos** (proteínas que revestem o ADN; proteínas que determinam o nível de ácidos graxos poliinsaturados nas membranas da mitocôndria)
- ✓ **Extintores físicos de oxidantes** (decaimento do O_2 singlete por carotenoides)
- ✓ **Evolução de moléculas resistentes a oxidação** (fumarase de *E. coli*)
- ✓ **Agentes de sacrifício** (ascorbato, glutationa, etc)
- ✓ **Reação de oxidantes para formação de produtos citoprotetores** (terminação de peroxidação lipídica)
- ✓ **Compartimentalização de processos bioquímicos**
- ✓ **Sistemas de reparo e degradação**
- ✓ **pO_2**

Halliwell and Gutteridge, Free Radicals in Biology and Medicine, 5th Edition, 2015.

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 244, No. 22, Issue of November 25, pp. 6049-6055, 1969
Printed in U.S.A.

Superoxide Dismutase

AN ENZYMIC FUNCTION FOR ERYTHROCUPREIN (HEMOCUPREIN)*

(Received for publication, June 23, 1969)

JOE M. MCCORD‡ AND IRWIN FRIDOVICH
From the Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27706

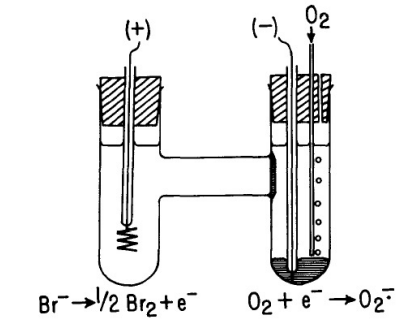


FIG. 1. Cell for the electrolytic reduction of O_2 to $O_2\cdot^-$ in *N,N*-dimethylformamide. Details described under "Experimental Procedure".

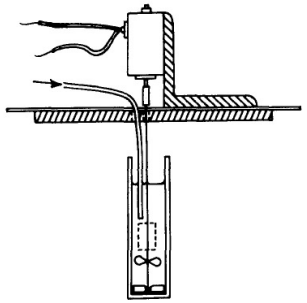


FIG. 2. Cuvette stirrer for infusion experiments. The *dashed line* approximates the light path through the 1-cm cuvette. A lucite bearing in the bottom of the cuvette prevents shaft wobble. See description under "Experimental Procedure".

Science

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ARTICLE

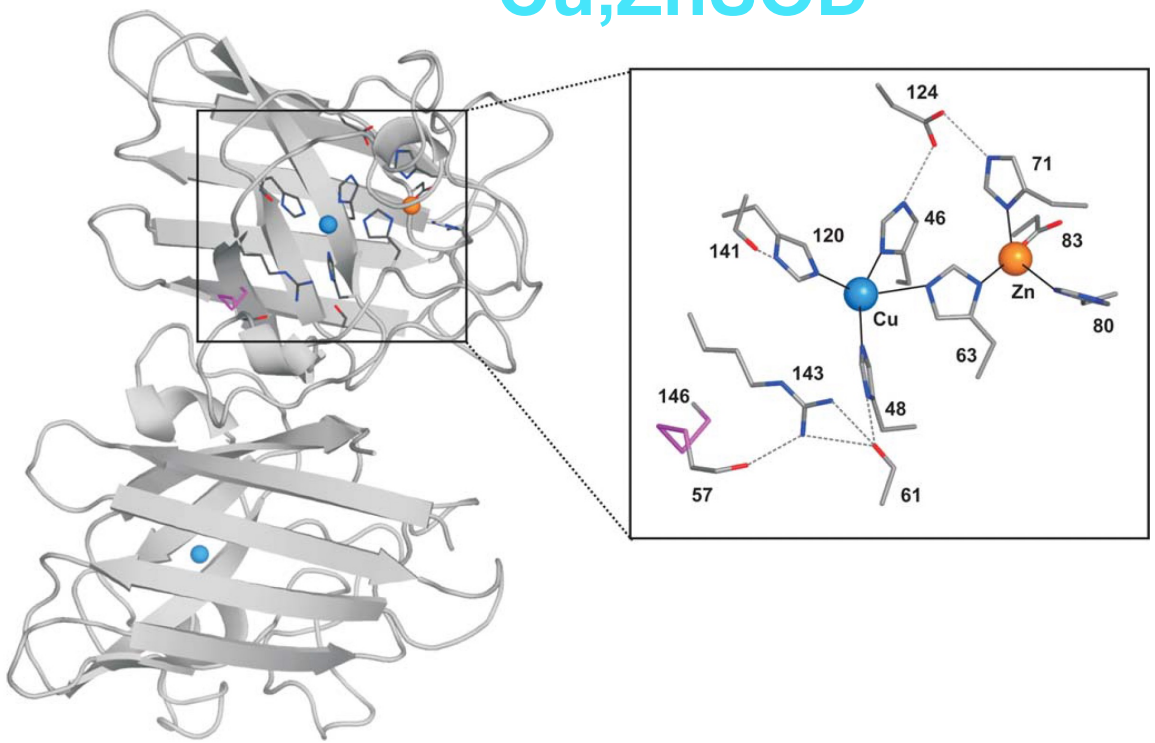
The Biology of Oxygen Radicals: The superoxide radical is an agent of oxygen toxicity; superoxide dismutases provide an important defense.

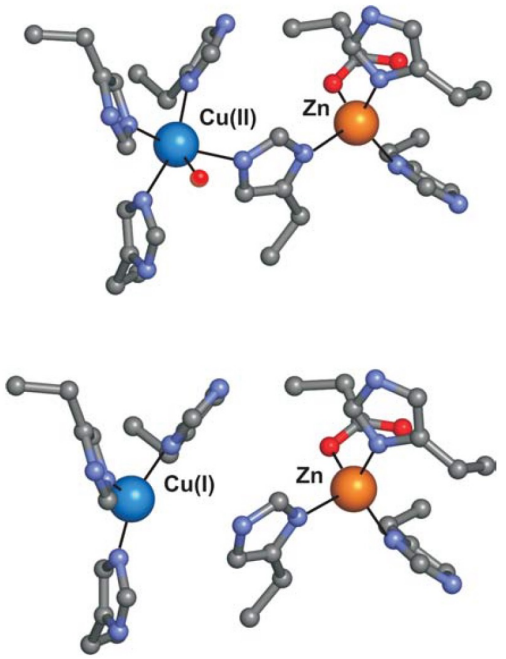
IRWIN FRIDOVICH

Teoria da toxicidade do oxigênio mediada por superóxido.

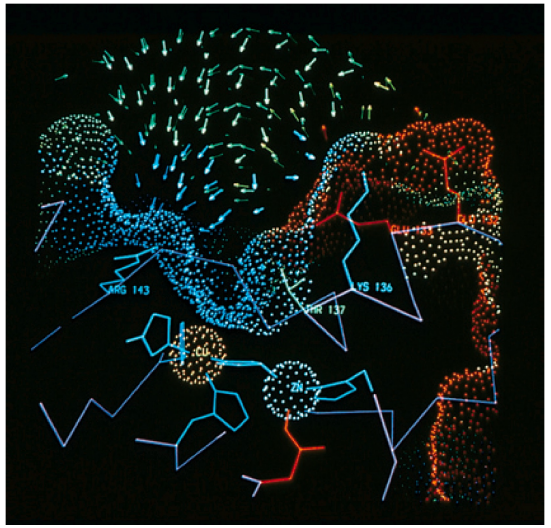
Eliminação do superóxido

Cu,ZnSOD





Limitado por difusão
Caminho eletrostático



$$\text{enzyme-Cu}^{2+} + O_2^{\bullet-} \rightarrow \text{enzyme-Cu}^{+} + O_2$$

$$\text{enzyme-Cu}^{+} + O_2^{\bullet-} + 2H^{+} \rightarrow \text{enzyme-Cu}^{2+} + H_2O_2$$

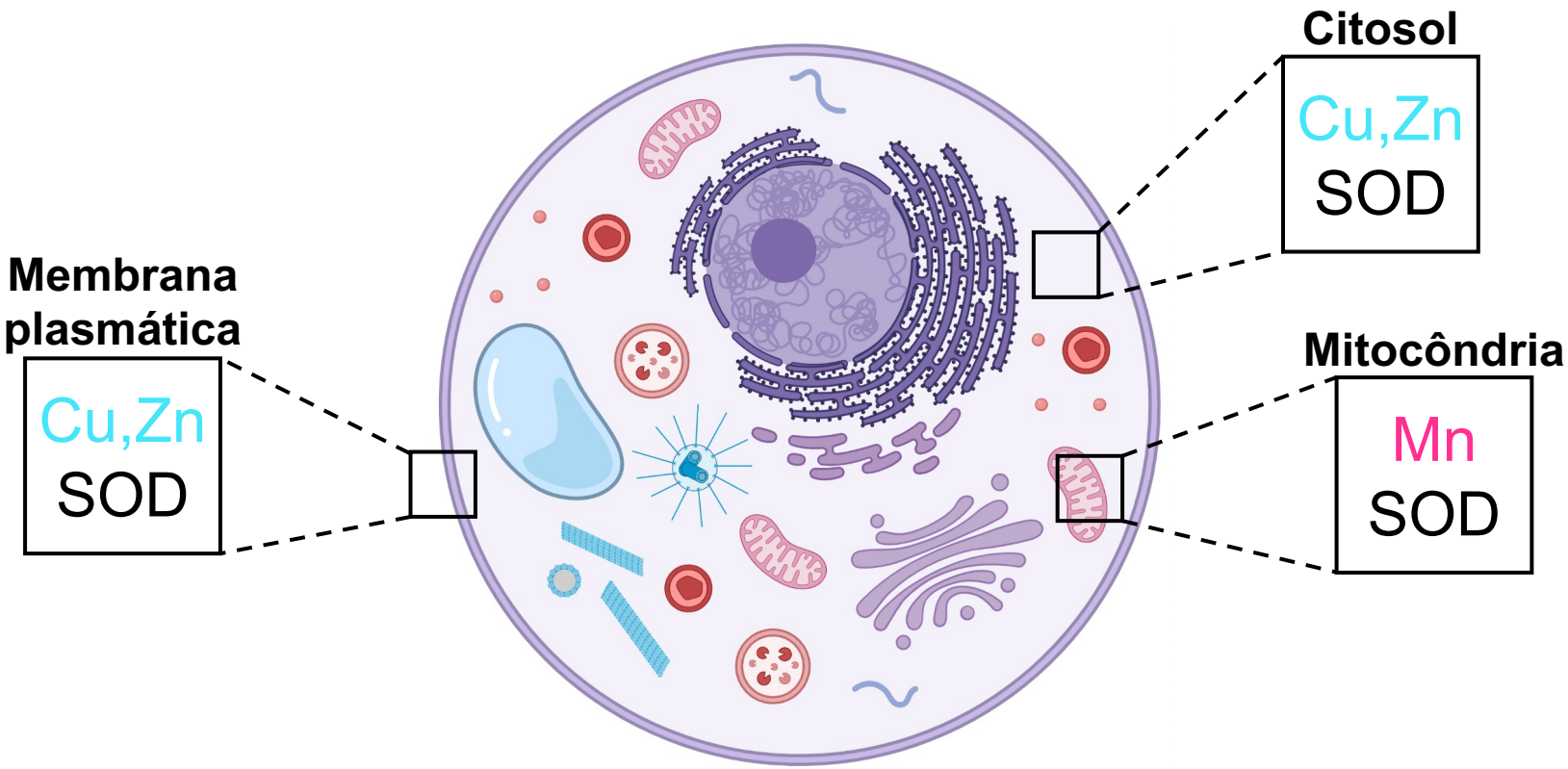
$$\text{Net reaction: } O_2^{\bullet-} + O_2^{\bullet-} + 2H^{+} \rightarrow H_2O_2 + O_2$$

$k1 \sim 1 \times 10^9\ M^{-1}s^{-1}$

$k2 \sim 1 \times 10^9\ M^{-1}s^{-1}$

Halliwell and Gutteridge, Free Radicals in Biology and Medicine, 5th Edition, 2015.
Voet & Voet, Bioquímica, 4ª edição.
Valentine JS, Ann Ver Biochem, 2005.

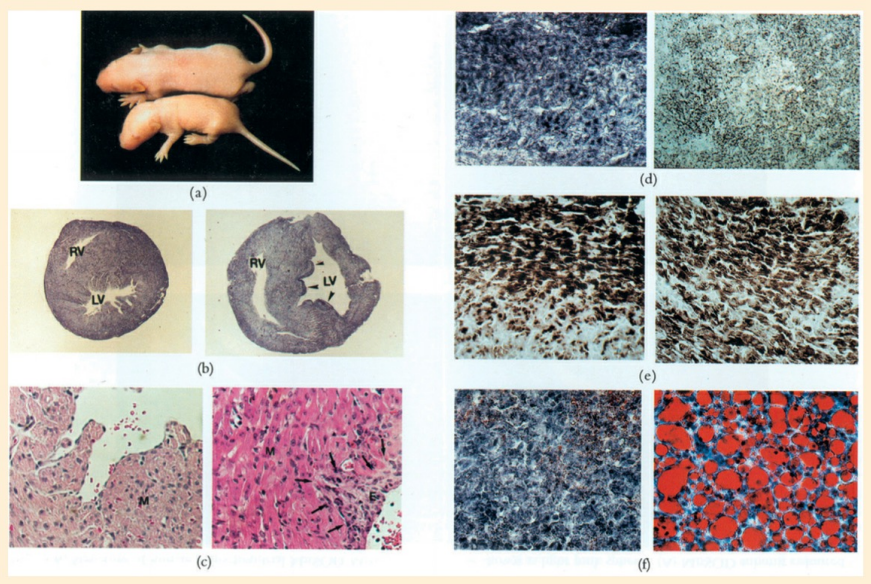
Tipos de Superóxido Dismutase (em eucariotos)



Toxicidade do oxigênio mediada por superóxido?

- ✓ **Sensibilidade de bactérias e leveduras mutantes e nocautes de genes codificando SOD em meio aeróbio.**
- ✓ **Efeitos protetores em camundongos transgênicos que sobreexpressam SOD e deletérios naqueles que são nocaute**

Nocaute de MnSOD



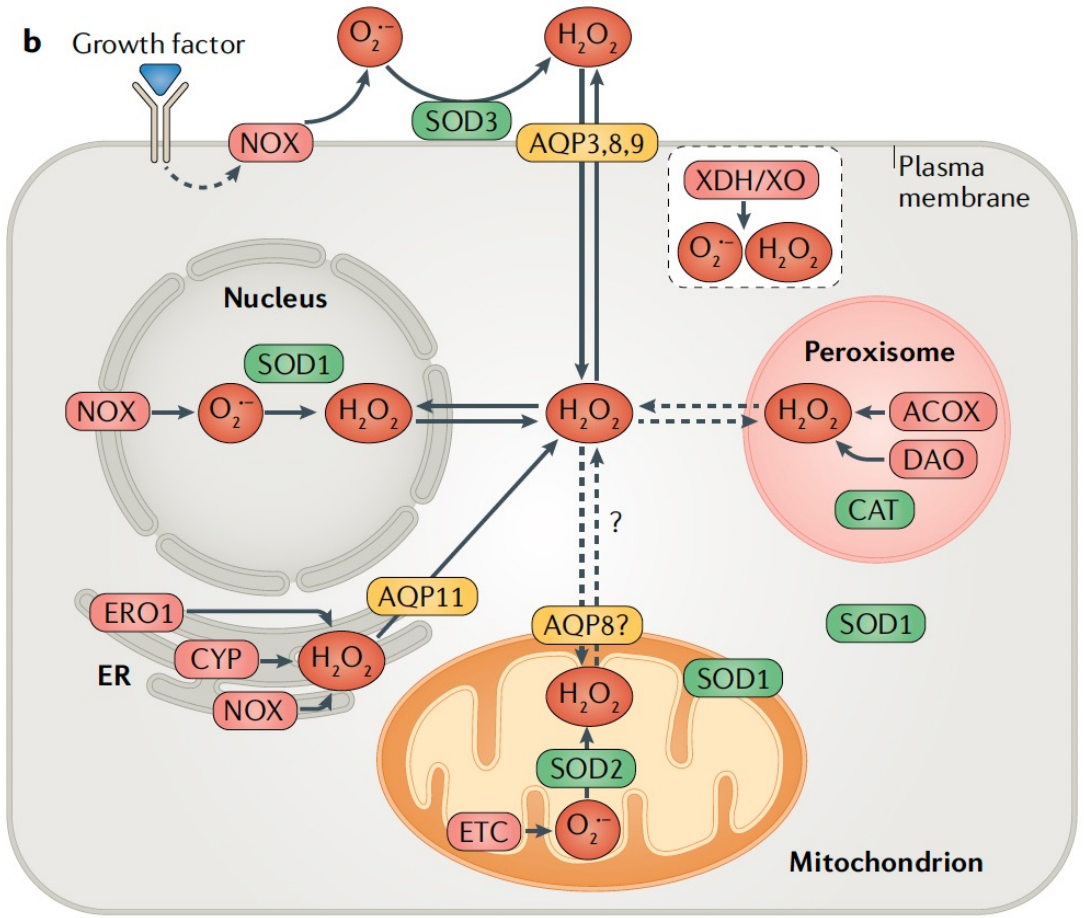
Mecanismos da toxicidade?

$$[4Fe-4S]^{2+} + O_2^{\bullet-} + 2H^+ \rightarrow [4Fe-4S]^{3+} + H_2O_2$$

$$[4Fe-4S]^{3+} \rightarrow [3Fe-4S]^{+} + Fe^{2+}$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe(III) + OH^{\bullet} + OH^{-}$$

Onde são produzidos o $O_2^{\bullet-}$ e o H_2O_2



Sies e Jones, Nat Rev Mol Cel Biol, 2020

Atividade SOD produz H_2O_2? Por quê?

Por que não a evolução de uma superóxido oxidase que devolveria o poder redutor ao metabolismo energético?

A SOD1 possui atividade peroxidásica

SOD1 na esclerose lateral amiotrófica

nature

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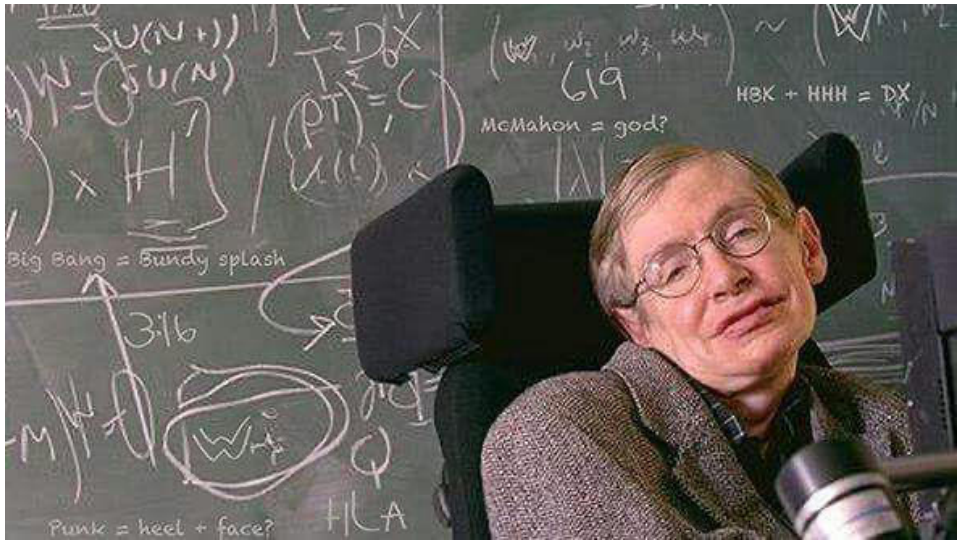
nature › letters › article

Published: 01 March 1993

Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis

Daniel R. Rosen, Teepu Siddique, David Patterson, Denise A. Figlewicz, Peter Sapp, Afif Hentati, Deirdre Donaldson, Jun Goto, Jeremiah P. O'Regan, Han-Xiang Deng, Zohra Rahmani, Aldis Krizus, Diane McKenna-Yasek, Annarueber Cayabyab, Sandra M. Gaston, Ralph Berger, Rudolph E. Tanzi, John J. Halperin, Brian Herzfeldt, Raymond Van den Bergh, Wu-Yen Hung, Thomas Bird, Gang Deng, Donald W. Mulder, Celestine Smyth, Nigel G. Laing, Edwin Soriano, Margaret A. Pericak–Vance, Jonathan Haines, Guy A. Rouleau, James S. Gusella, H. Robert Horvitz & Robert H. Brown Jr — Show fewer authors

Nature **362**, 59–62 (1993) | Cite this article



Articles

Peroxymonocarbonate and Carbonate Radical Displace the Hydroxyl-like Oxidant in the Sod1 Peroxidase Activity under Physiological Conditions

Danilo B. Medinas,[†] José C. Toledo, Jr.,[†,‡] Giselle Cerchiaro,[†,‡] Antonia T. do-Amaral,[§] Leandro de-Rezende,[§] Alberto Malvezzi,[§] and Ohara Augusto*[,†]

Departamento de Bioquímica and Departamento de Química Fundamental do Instituto de Química, Universidade de São Paulo, Caixa Postal 26077, CEP 05513-970, São Paulo, Brazil

Received August 1, 2008

$$\mathrm{SodCu(II) + H_2O_2 \rightleftharpoons SodCu(I) + O_2^{\bullet -} + 2H^+} \quad (1)$$

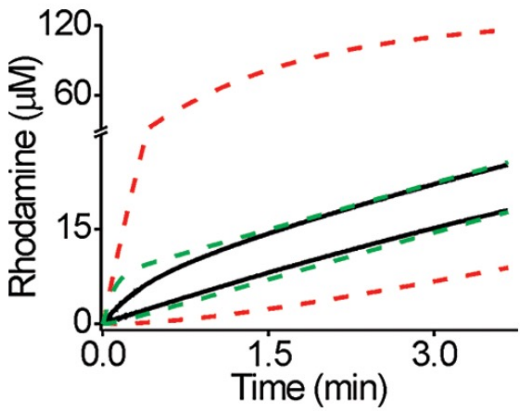
$$\mathrm{SodCu(I) + H_2O_2 \rightarrow SodCu(II)(OH^{\bullet}) + OH^-} \quad (2)$$

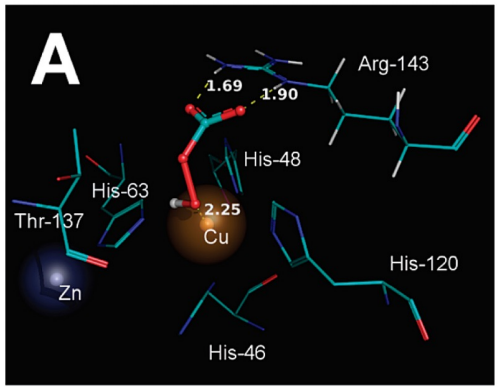
$$\mathrm{SodCu(II)(OH^{\bullet}) + SodHis \rightarrow SodCu(II) + SodHis^{\bullet +} + OH^-} \quad (3)$$

$$\mathrm{SodCu(II)(OH^{\bullet}) + CO_2 \rightarrow SodCu(II) + CO_2^{+} + OH^-} \quad (4)$$

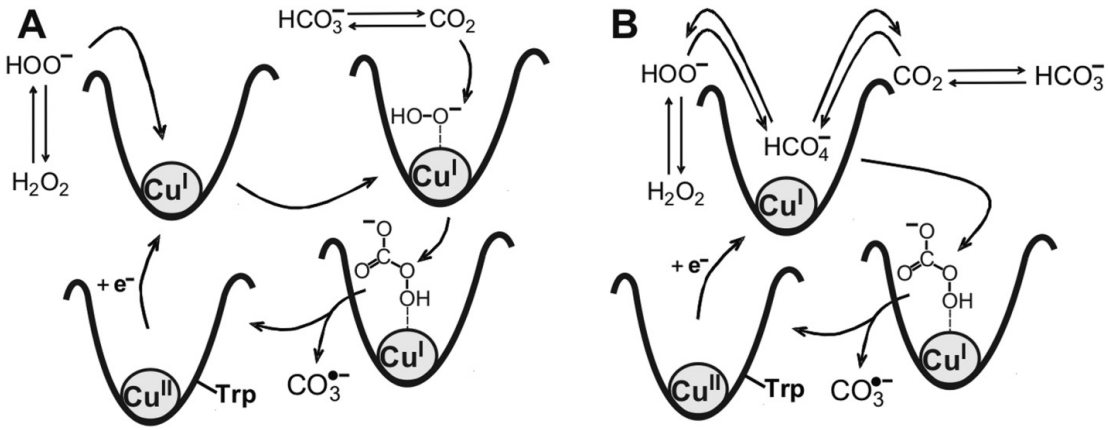
$$\mathrm{CO_2^{+} + OH^- \rightarrow CO_3^{\bullet -} + H^+} \quad (5)$$

$$\mathrm{SodCu(I)(HCO_3^-) + H_2O_2 \rightarrow SodCu(I)(HCO_4^-) + H_2O} \quad (6)$$



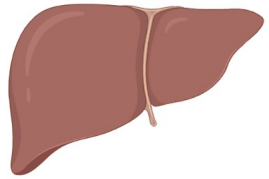


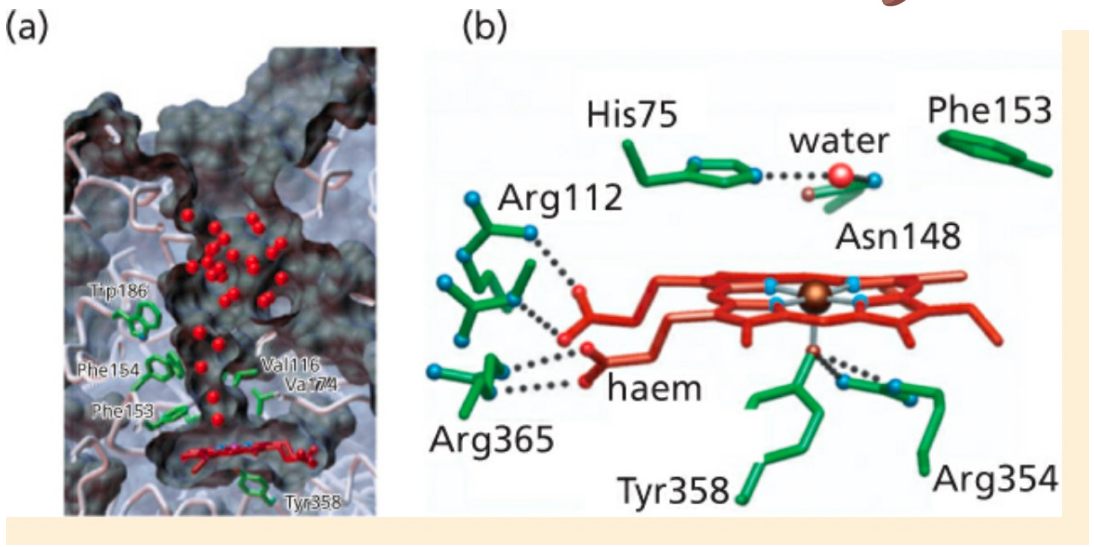
$$\mathrm{HCO_3^- + H_2O_2 \rightleftharpoons HCO_4^-}$$



Eliminação de H_2O_2

Catalase





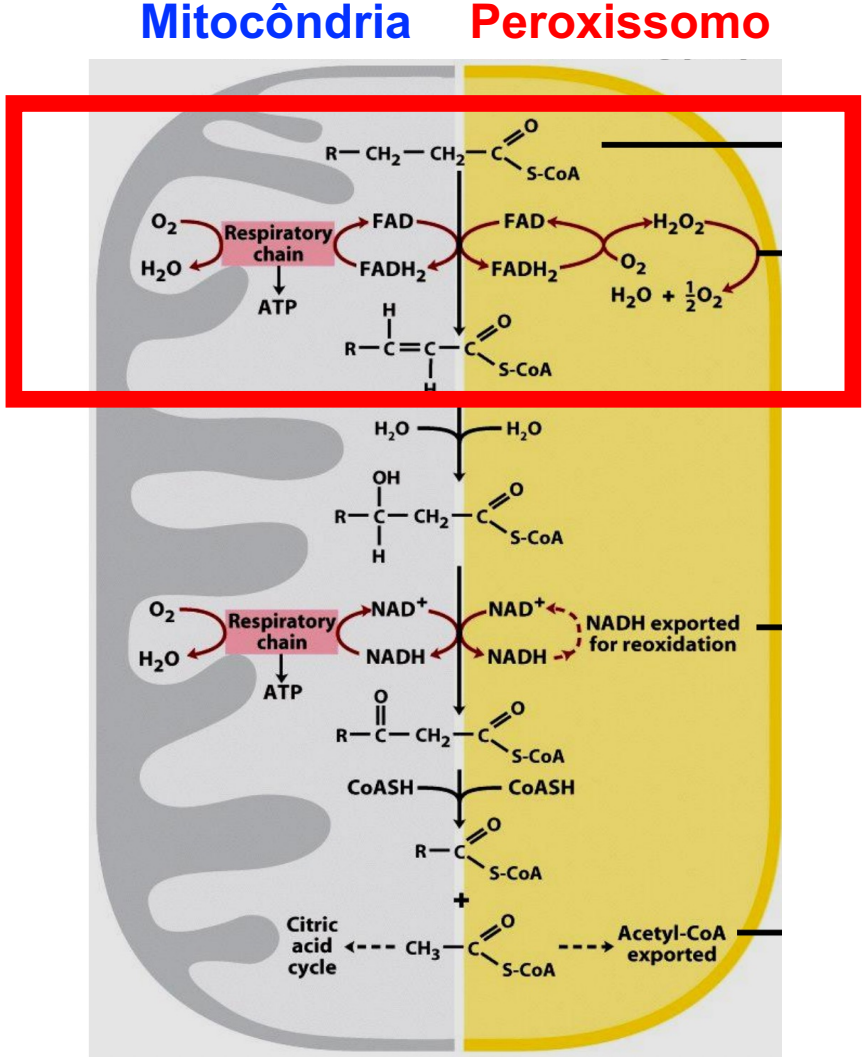
k1 = 1,7 x 10^7 $M^{-1}s^{-1}$

k2 = 2,6 x 10^7 $M^{-1}s^{-1}$

$$\text{catalase-Fe(III)} + H_2O_2 \xrightarrow{k_1} \text{compound I} + H_2O$$

$$\text{compound I} + H_2O_2 \xrightarrow{k_2} \text{catalase-Fe(III)} + H_2O + O_2$$

$$\text{Net } 2H_2O_2 \rightarrow 2H_2O + O_2$$



American Philological Associations, of the list reported by the American Committee in 1886, as starters for more ambitious movements of reform.

The list is as follows:

1. Drop the so-called *subjunctive mood* altogether. It is moribund in much of our best prose, and can be allowed to die out of our

SHORTER ARTICLES.

CATALASE, A NEW ENZYM OF GENERAL OCCURRENCE.

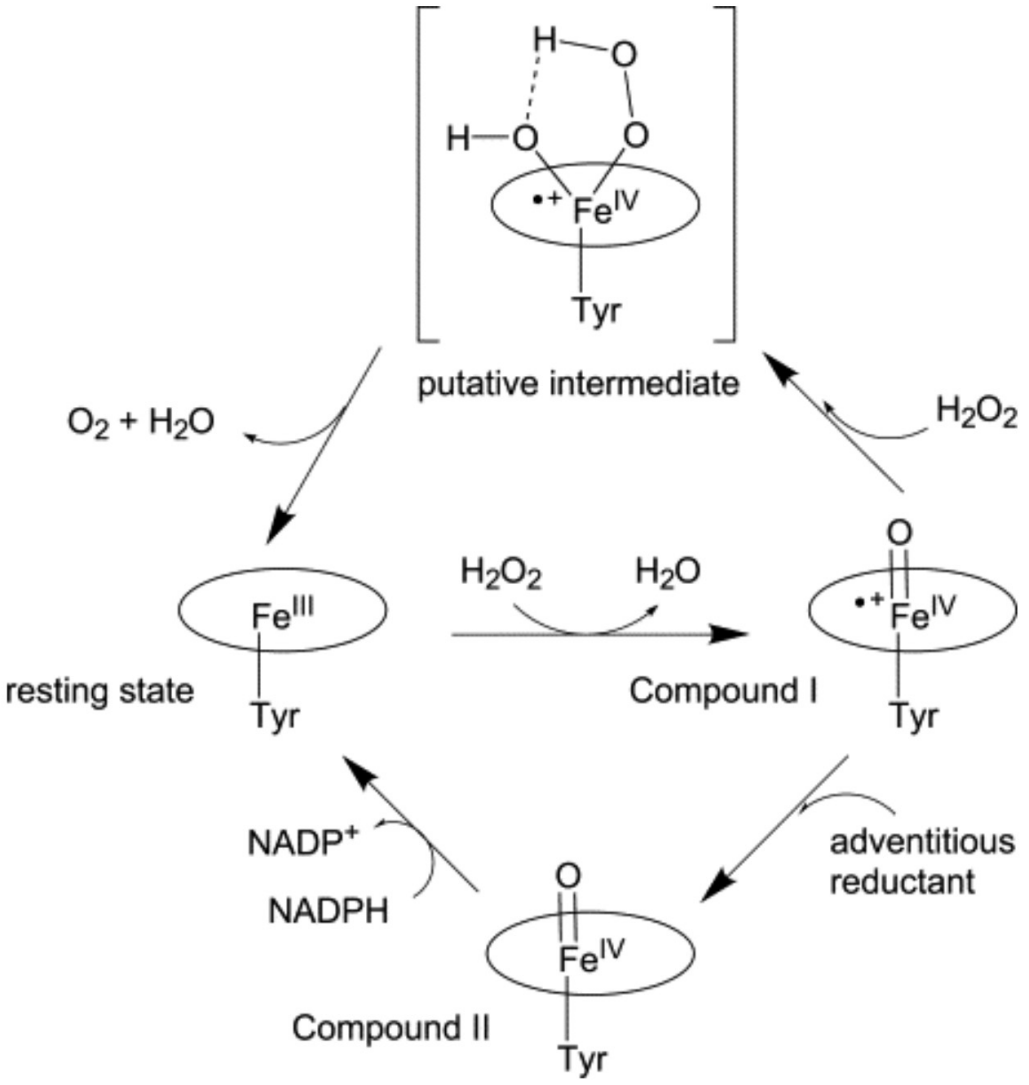
THE study of the enzyms has been pursued with growing interest by a number of scientists during later years. These unorganized ferments being substances of a highly ephemeral

nature, the method of investigating them has departed somewhat from the paths usually followed in determining the composition, effects and rôle, of organic combinations in vegetable and animal organisms. The nature of the enzym is still a matter of much doubt. Of their action we are more sure, and it is along this line that we have become familiar with the nature of some members of this very interesting group of compounds. The rôle of the enzym in the life processes may also in some cases be defined with certitude.

From his studies the author gives the following as the most plausible explanations of the action of catalase in vegetable organism: (1) It destroys instantly the hydrogen peroxid, probably formed in cells during the oxidation caused by the respiration process; (2) it loosens chemical affinities in certain compounds so that the protoplasm can more easily split or oxidize them. "In other words, catalase might represent an aid for fermentative as well as for respirative phenonomena." D. W. MAY.

U. S. DEPARTMENT OF AGRICULTURE.

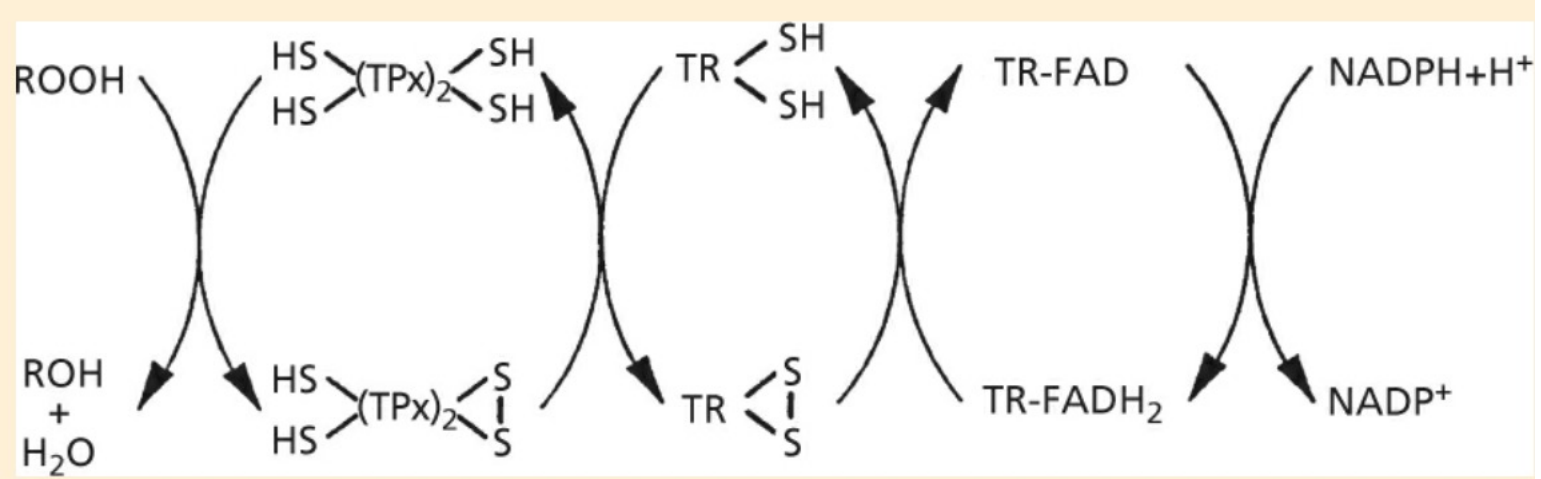
Mecanismo da catalase



Outras rotas para eliminação de H_2O_2 e outros: Bioquímica de Tióis

Peroxiredoxinas

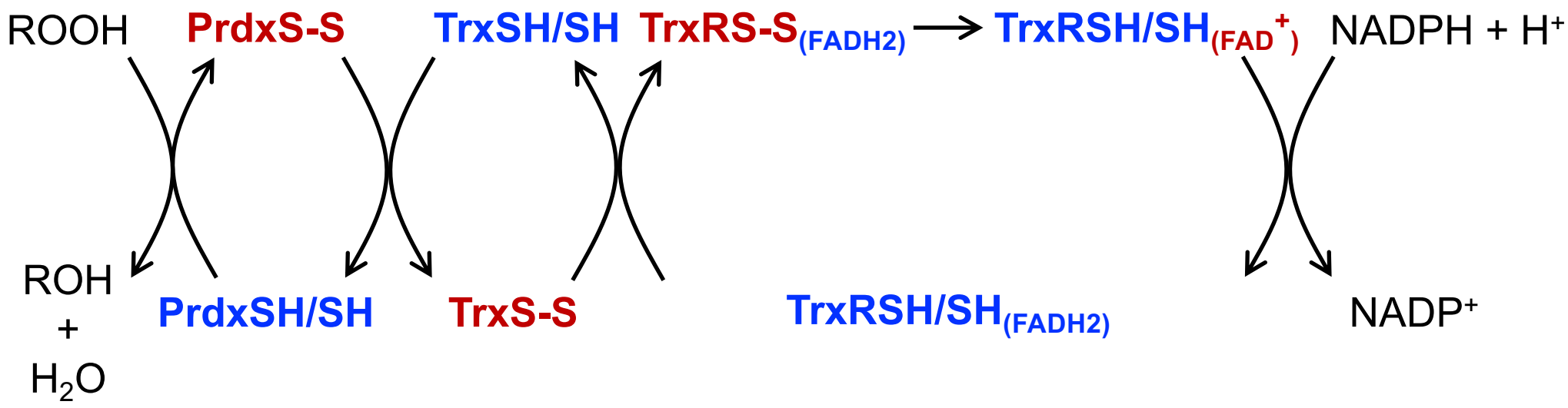
Eliminação de peróxidos às custas do metabolismo energético (via tioredoxinas)



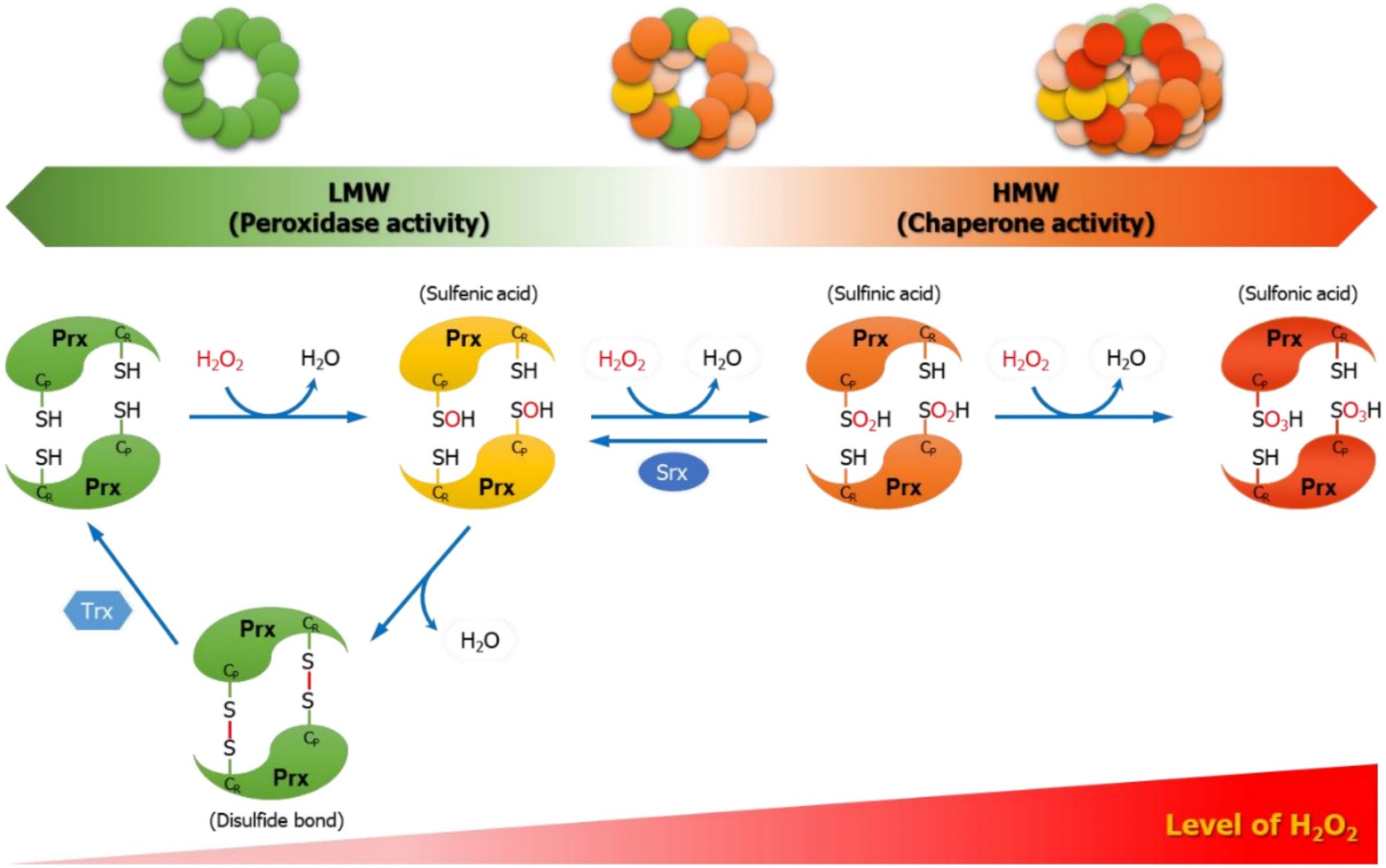
Nicolussi *et al*, Mol and Clin Oncol, 2017

Outras rotas para eliminação de H_2O_2 e outros: Bioquímica de Tióis

Ciclo Peroxirredoxinas (Prdx)/
Tiorredoxinas (Trx)/Tiorredoxinas Redutases (TrxR)

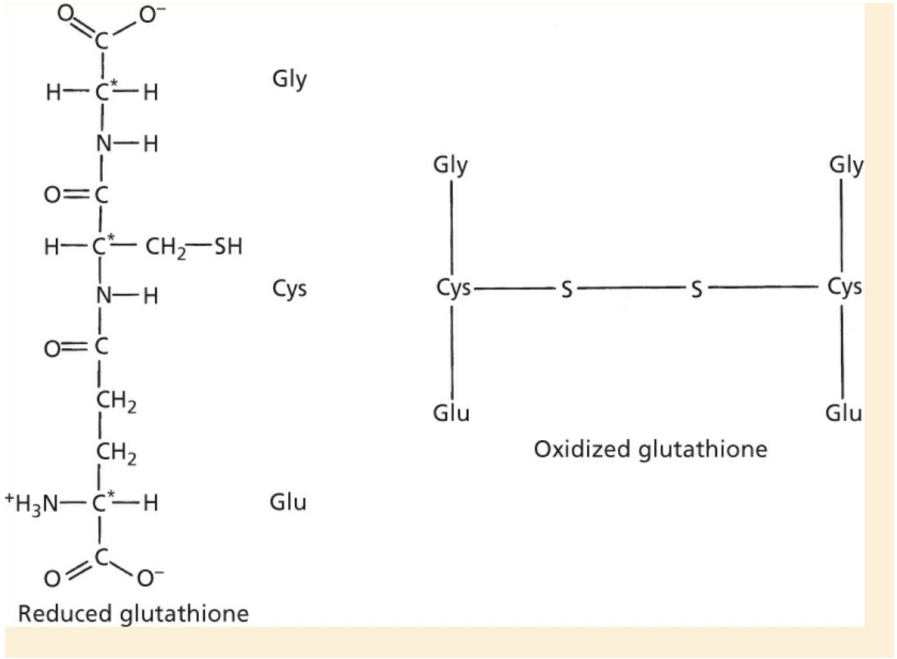


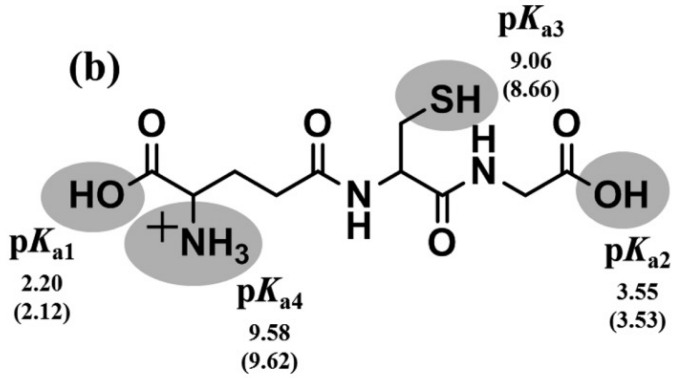
Peroxiredoxinas como sensores de estresse celular?



Kim e Jang, Antioxidants, 2019

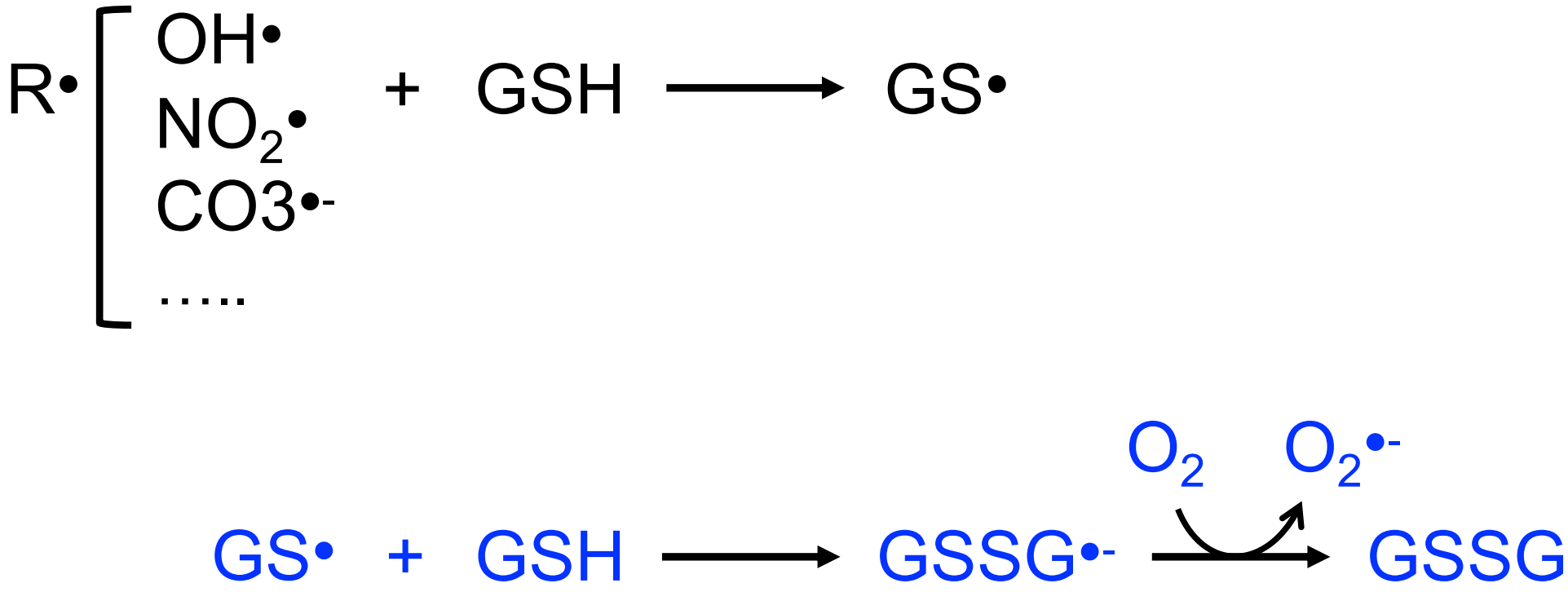
Glutationa: um tiol de baixo peso molecular essencial para as defesas antioxidantes





Tummanapelli e Vasudevan, J Phys Chem B, 2015

Reações entre oxidantes e glutationa



Glutationa Peroxidases: Glutationa na linha de defesa contra oxidantes de 2 elétrons

Fontes de selênio na dieta

$$GPx\text{-}Se^- + ROOH + H^+ \rightarrow ROH + GPx\text{-}SeOH$$

$$GPx\text{-}SeOH\text{-}GSH \rightarrow H_2O + GPx\text{-}Se\text{-}SG$$

$$GPx\text{-}Se\text{-}SG\text{-}GSH \rightarrow GPx\text{-}SeH\text{-}GSSG \rightarrow$$

$$GPx\text{-}Se^- + H^+ + GSSG$$

https://www.hsph.harvard.edu/nutritionsource/selenium/

Article

Cell

Selenium Utilization by GPX4 Is Required to Prevent Hydroperoxide-Induced Ferroptosis

Irina Ingold,[1] Carsten Berndt,[2] Sabine Schmitt,[3] Sebastian Doll,[1] Gereon Poschmann,[4] Katalin Buday,[1] Antonella Roveri,[5] Xiaoxiao Peng,[6] Florencio Porto Freitas,[1] Tobias Seibt,[7] Lisa Mehr,[1] Michaela Aichler,[8] Axel Walch,[8] Daniel Lamp,[9,10] Martin Jastroch,[9,10] Sayuri Miyamoto,[11] Wolfgang Wurst,[1,12,13] Fulvio Ursini,[5] Elias S.J. Arnér,[14] Noelia Fradejas-Villar,[15] Ulrich Schweizer,[15] Hans Zischka,[3,16] José Pedro Friedmann Angeli,[1,17] and Marcus Conrad[1,18,*]

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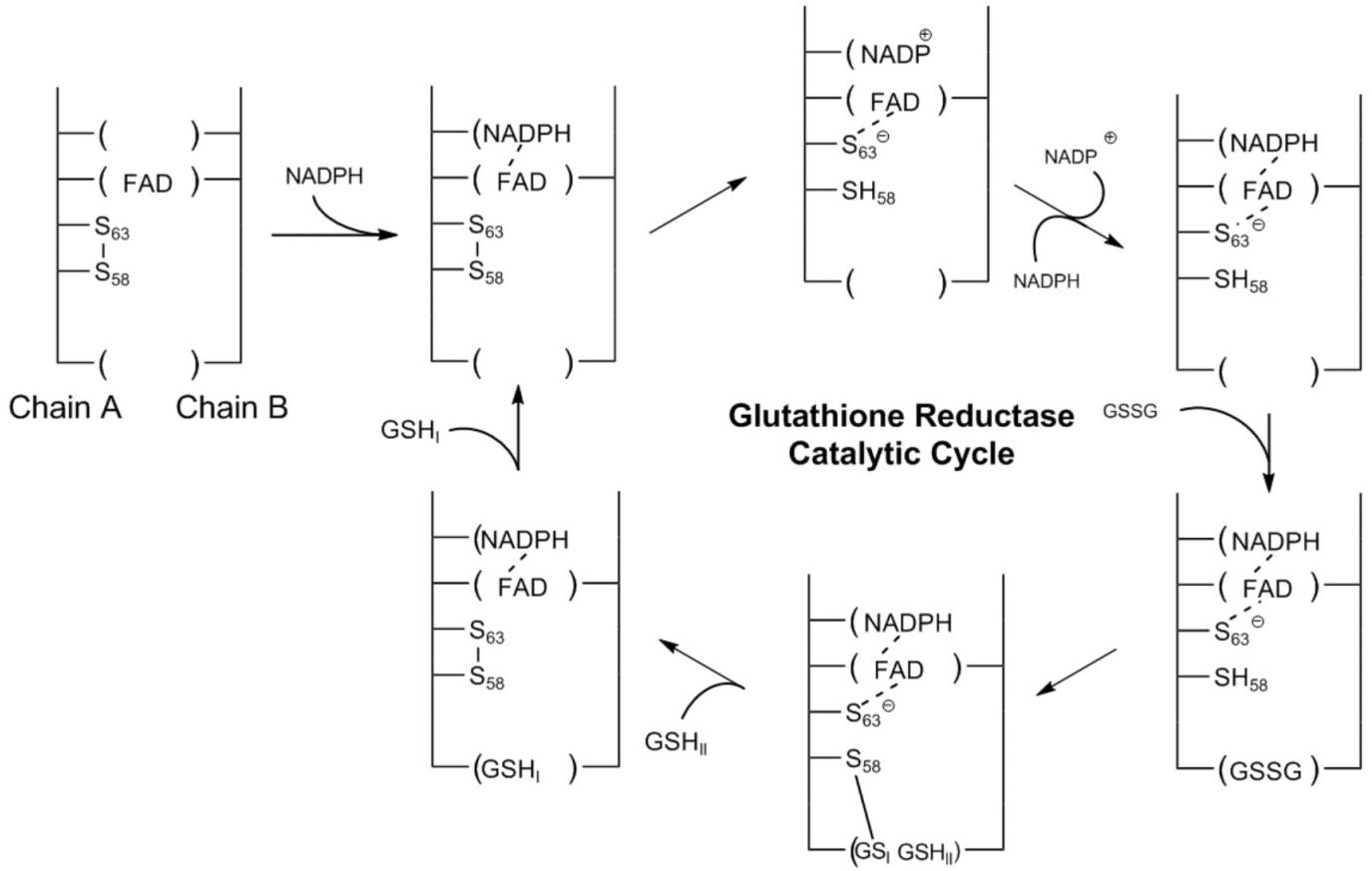
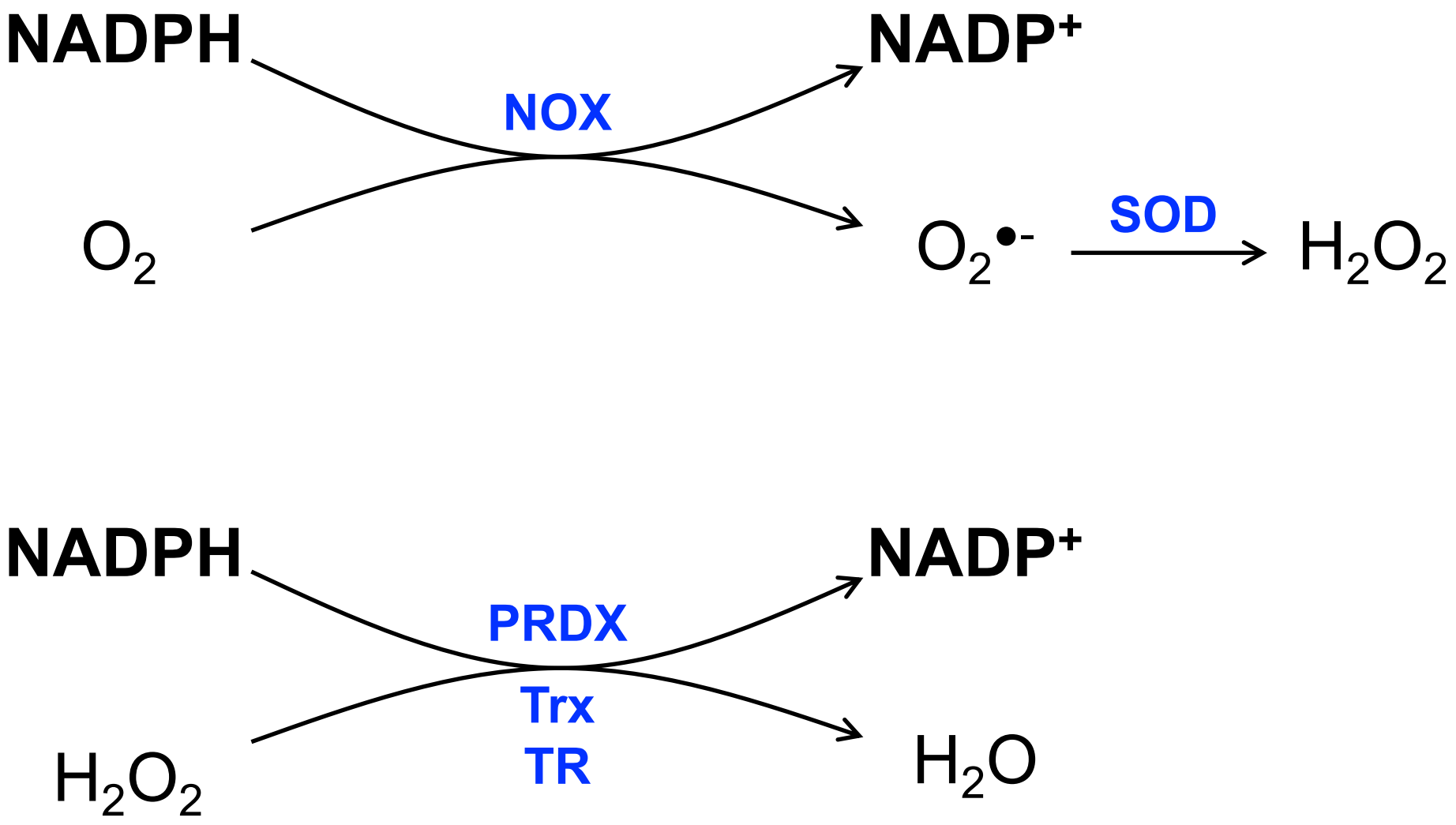
[17]Present address: Rudolf Virchow Center for Experimental Biomedicine, University of Würzburg, 97080 Würzburg, Germany
[18]Lead Contact
*Correspondence: marcus.conrad@helmholtz-muenchen.de
https://doi.org/10.1016/j.cell.2017.11.048

Glutationa Redutase

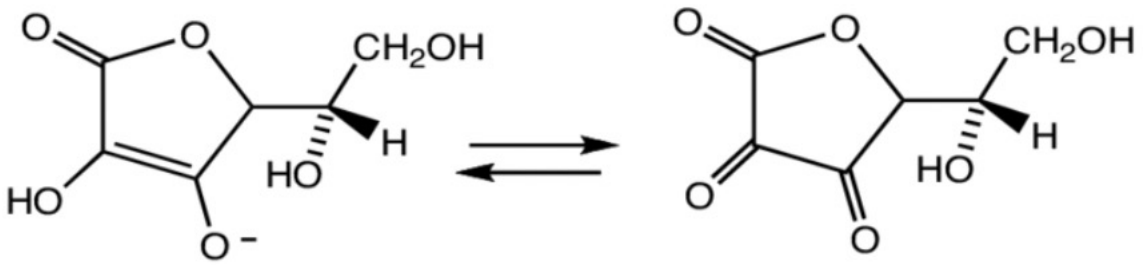
$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$$



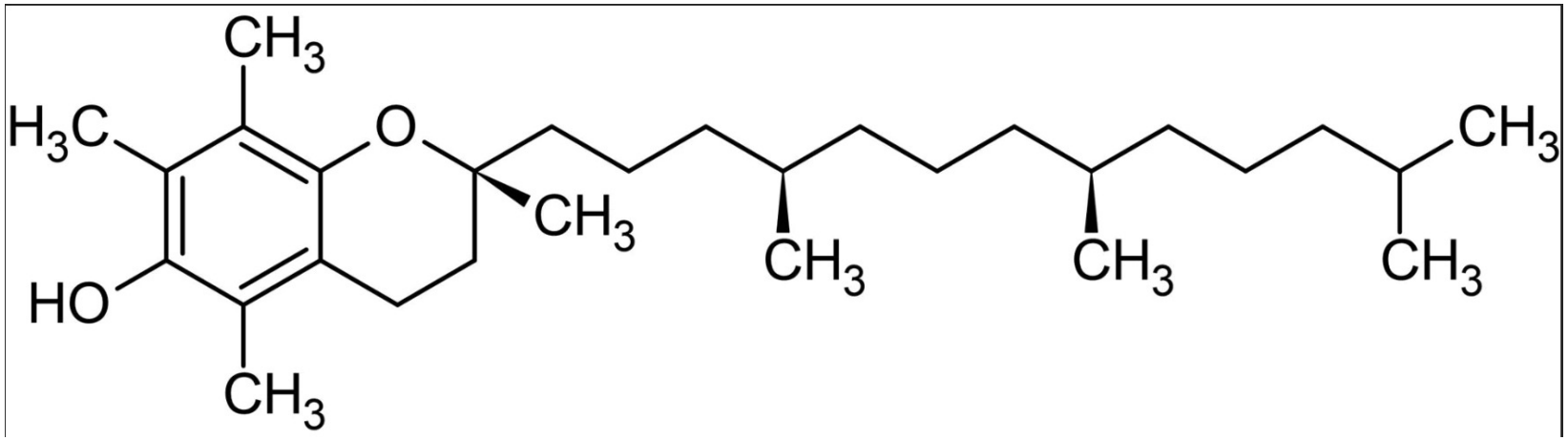
NADPH: via de acoplamento entre a geração e a eliminação de oxidantes?



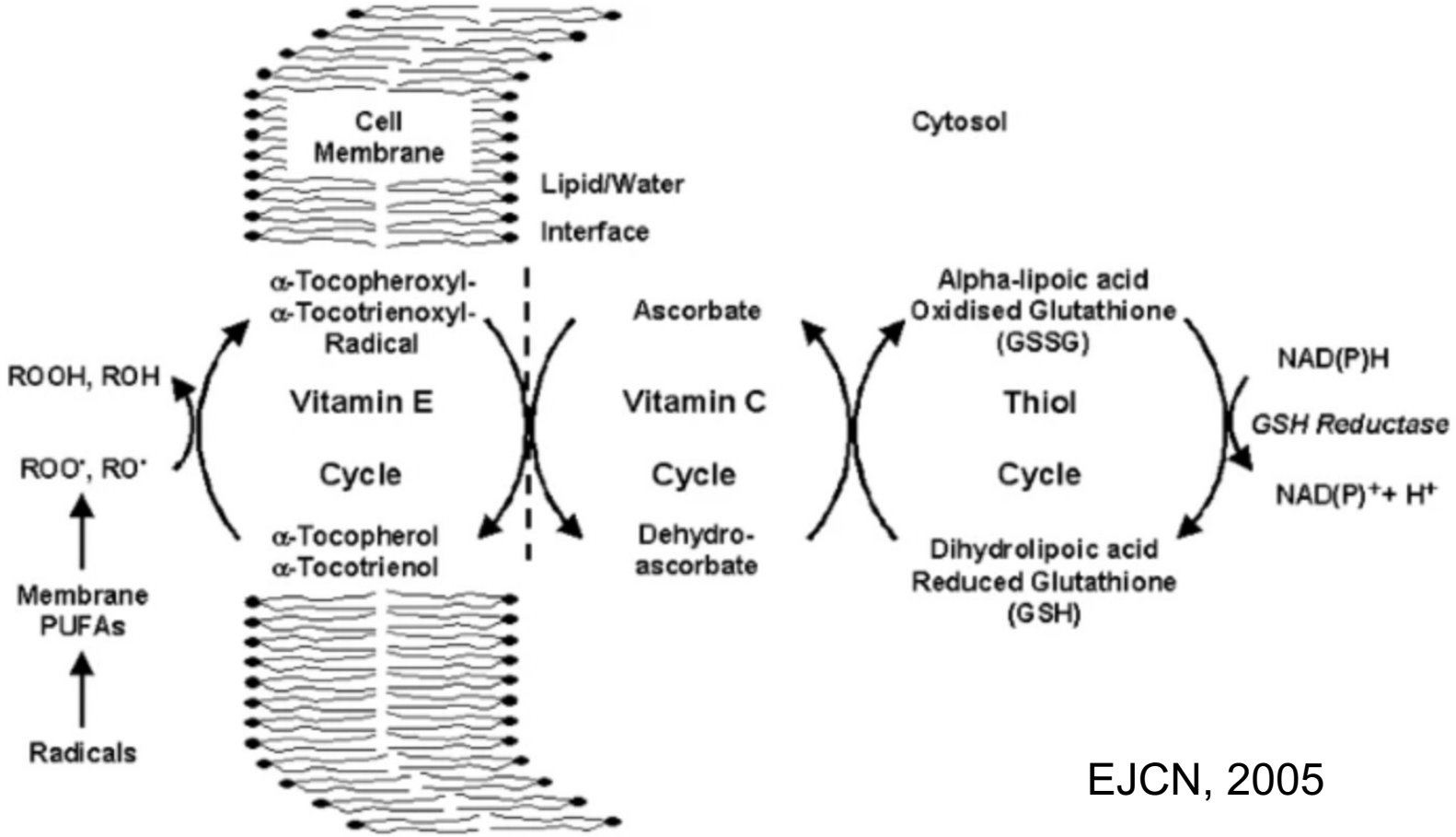
Antioxidantes de baixo peso molecular: ação das vitaminas C e E



L-ascorbate L-dehydroascorbate



Antioxidantes de baixo peso molecular: ação das vitaminas C e E

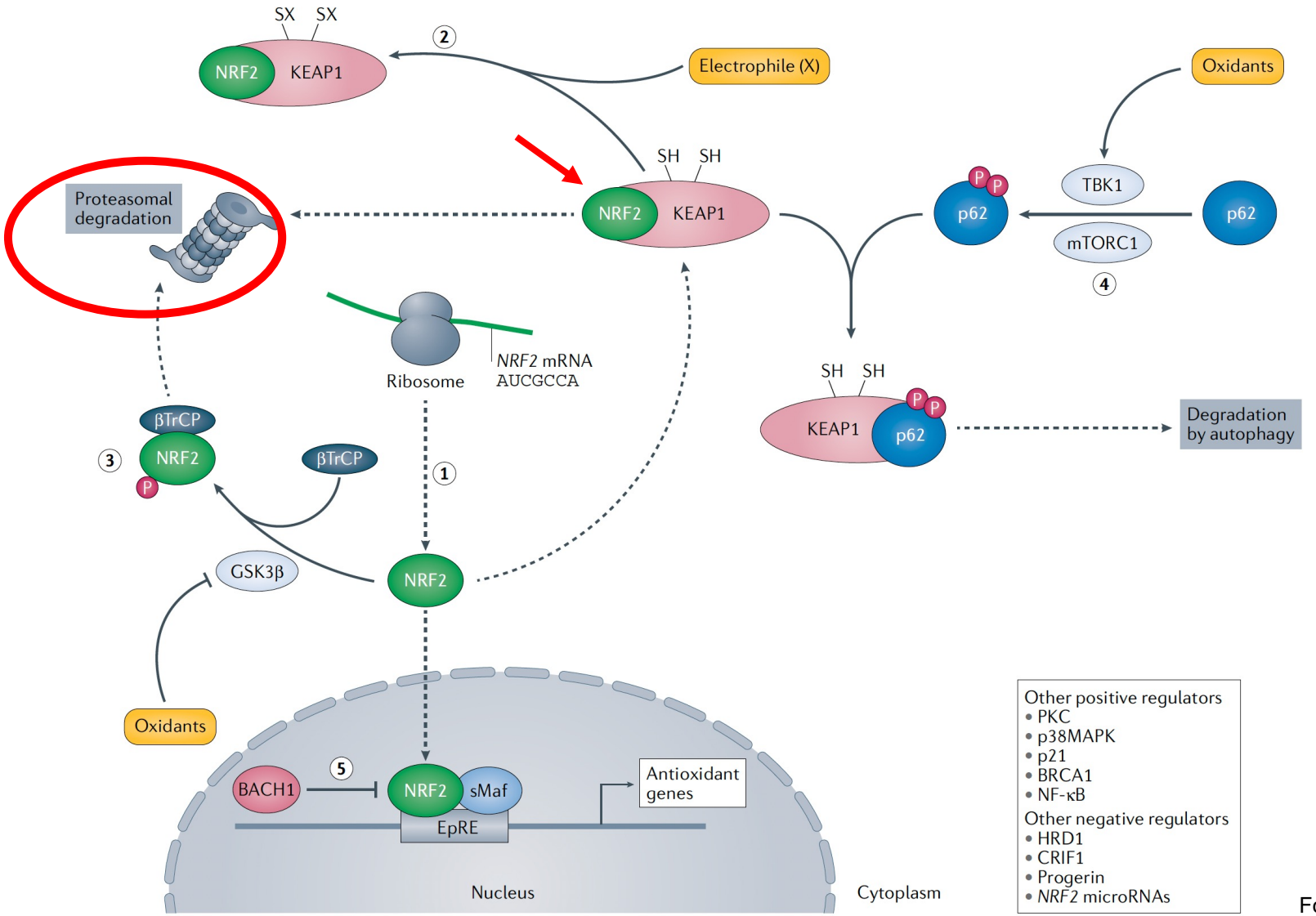


EJCN, 2005

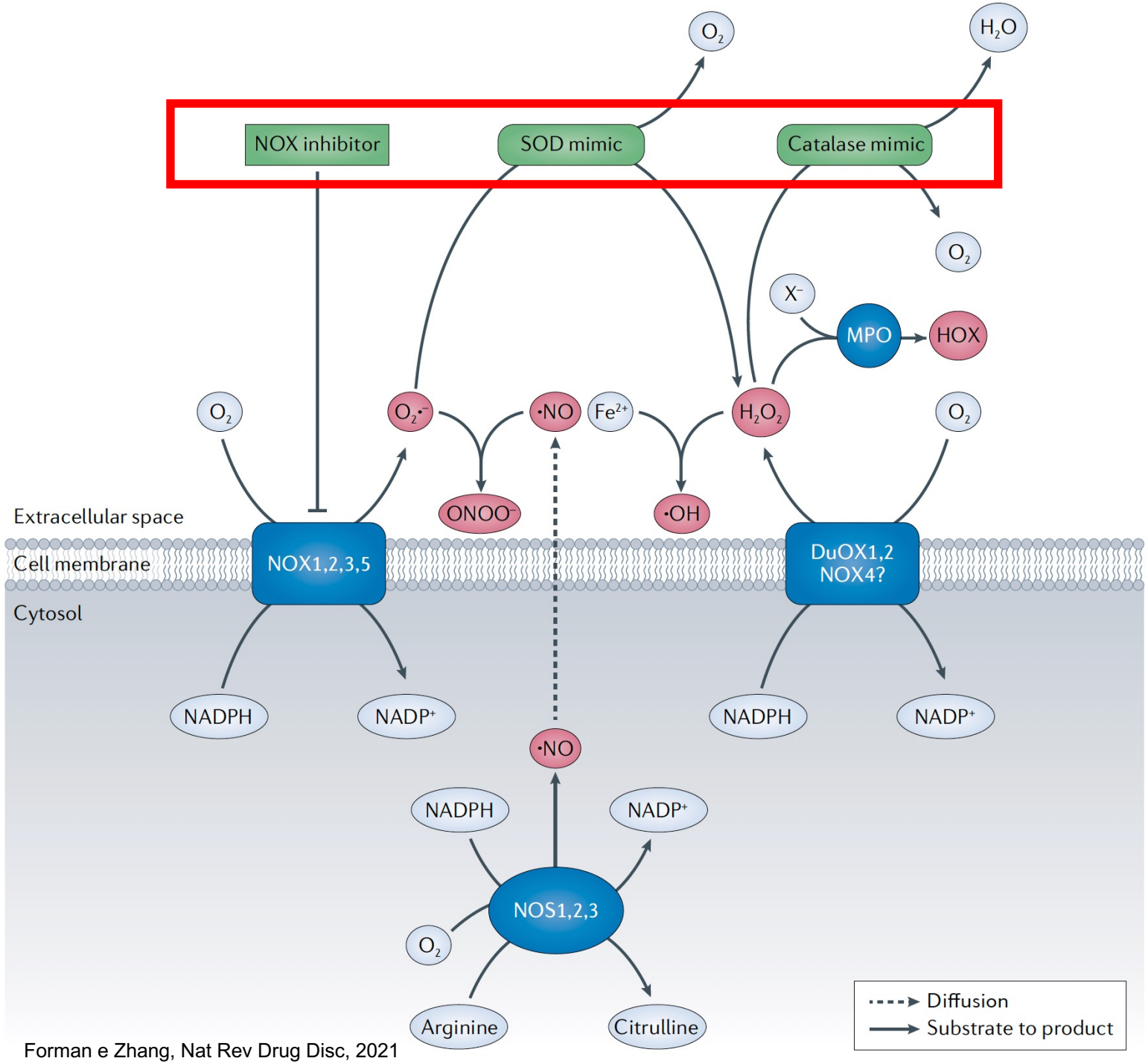
Terapias antioxidantes?

- ✓ **Oxidantes participam de inúmeras doenças, mas são agentes causais?**
- ✓ **Antioxidantes funcionam em modelos pré-clínicos, mas falharam em estudos clínicos.**
- ✓ **Quando, onde e em que extensão oxidantes participam de mecanismos patogênicos?**
- ✓ **Classificar doenças quanto a participação do estresse oxidativo: Primária vs secundária.**
- ✓ **Moléculas terapêuticas: substancias que induzem as defesas antioxidantes enzimáticas (fatores de transcrição como NRF2);**
  - ✓ **Três linhas de defesa antioxidante (linha 1: enzimas que atuam nas espécies oxidante; linha 2: enzimas que reciclam as enzimas da linha 1; linha 3: enzimas de reparo e sistemas de degradação).**
- ✓ **Miméticos e ativadores da resposta antioxidante.**

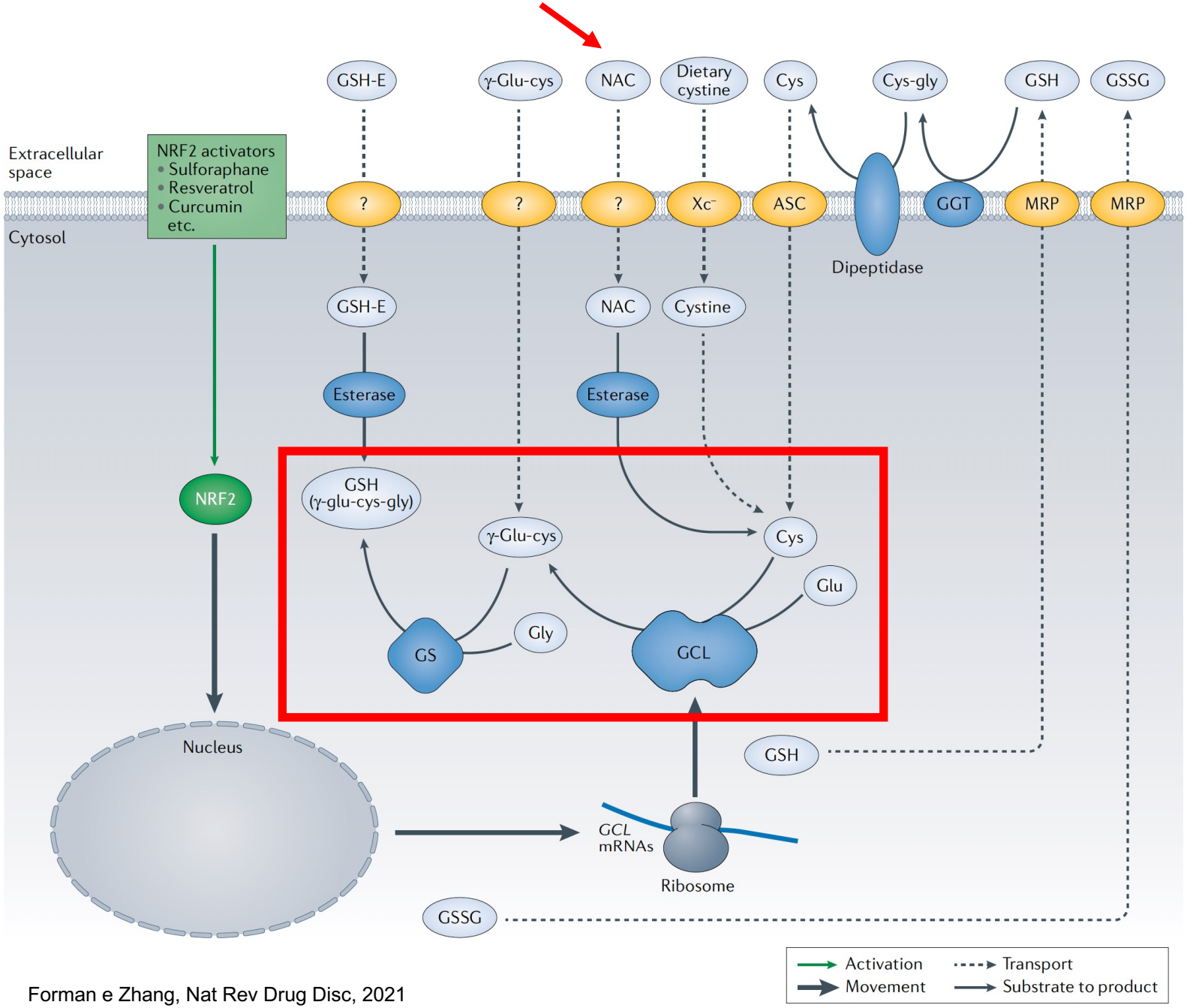
Ativação de NRF2: efeitos bons, mas com limitações (câncer, envelhecimento)



Forman e Zhang, Nat Rev Drug Disc, 2021



Forman e Zhang, Nat Rev Drug Disc, 2021



Forman e Zhang, Nat Rev Drug Disc, 2021

Table 1 | **Clinical status of antioxidant enzyme mimics**

Mimic	Antioxidant	Indications	Clinical trial status and refs
NAC	GSH	Paracetamol toxicity, cystic fibrosis, nephropathy and so on	Phase IV (highest; 529 trials in total)[163]
ALT-2074	GPX	Diabetes, coronary artery disease	NCT00491543, phase II[159]
Ebselen	GPX	Meniere disease, bipolar disorder	NCT02603081, phase II[151,152] NCT03013400, phase II[153,245,246]
GC4419	SOD	Squamous cell cancers of the head and neck	NCT01921426, phase I
AEOL-10150	SOD	Non-human animal models of radiation-induced lung injury and inflammation in stroke	Preclinical[247,248]
EUK-8	SOD and catalase	Non-human animal models of sepsis, heart ischaemia–reperfusion, cardiomyopathy, haemorrhage and ALS	Preclinical[137–141]
EUK-134	SOD and catalase	Non-human animal models of ischaemia–reperfusion injury, sepsis and stroke	Preclinical[142,249,143]
EUK-189	SOD and catalase	Non-human animal models of radiation lung fibrosis, cognitive impairment and hyperthermia	Preclinical[144–147]

ALS, amyotrophic lateral sclerosis; GPX, glutathione peroxidase; GSH, glutathione; SOD, superoxide dismutase.

Forman e Zhang, Nat Rev Drug Disc, 2021

Recapitulando as metas da aula

- Considerações sobre a mecanismos de ação antioxidante.
- Enzimas antioxidantes: eliminação de superóxido.
- Enzimas antioxidantes: eliminação de peroxido de hidrogênio.
- Enzimas antioxidantes em base a tióis e glutationa
- NADPH e bioquímica de tióis: prelúdio para sinalização redox.
- Ação de antioxidantes de baixo peso molecular: vitamina C e E.
- Terapias antioxidantes.

Questões

- ✓ **Por que a sensibilidade de microorganismos deficientes de SOD ao oxigênio pode ser revertida com a suplementação de alguns aminoácidos?**
- ✓ **Discuta a razão biológica pela qual peroxirredoxinas, enzimas antioxidantes, assumam papel de chaperonas.**
- ✓ **Numa doença onde existe evidência para participação de peroxinitrito, que estratégia antioxidante você empregaria para tratamento? Justifique.**
- ✓ **Descreva uma intervenção antioxidante para neutralizar a toxicidade do radical $OH^{\bullet}$.**
- ✓ **Faça uma análise crítica do uso de ativadores de NRF2 como terapia antioxidante.**

Bibliografia

- Halliwell and Gutteridge, Free Radicals in Biology and Medicine, 5th Edition, 2015.
- Manuscritos citados.