Oxidação de Proteínas

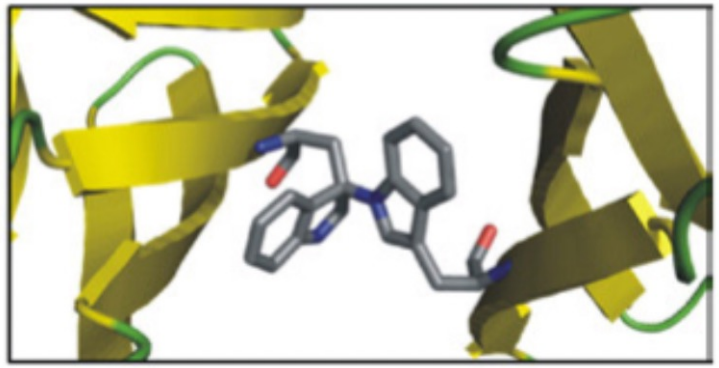
QBQ2509: Bioquímica Redox
QBQ5893: Processos Redox em Bioquímica

Dr. Danilo B. Medinas

Material de estudo para prova

Halliwell: Capítulos 5

Manuscritos citados

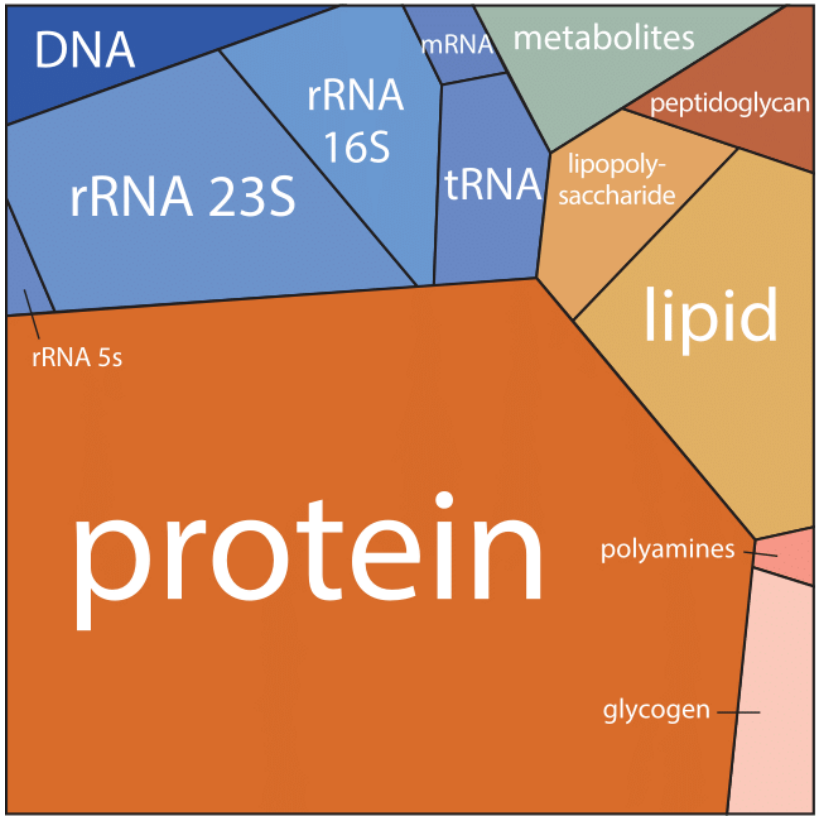


Di-triptofano da SOD1 humana

Tópicos e metas da aula

- **Tipos de modificações oxidativas.**
  - Entender a diversidade química da oxidação de proteínas.
- **Impacto biológico da oxidação de proteínas.**
  - Reconhecer os papéis das modificações oxidativas em condições fisiológicas e patológicas.
- **Oxidação de proteínas como biomarcadores de doenças.**
  - Racionalizar a utilidade de produtos de oxidação de proteínas como biomarcadores.
- **Respostas celulares para manutenção da proteostase (homeostase de proteínas).**
  - Conhecer mecanismos de (re)enovelamento e controle de qualidade de proteínas sob estresse oxidativo.

As proteínas são um importante alvo biológico para oxidação

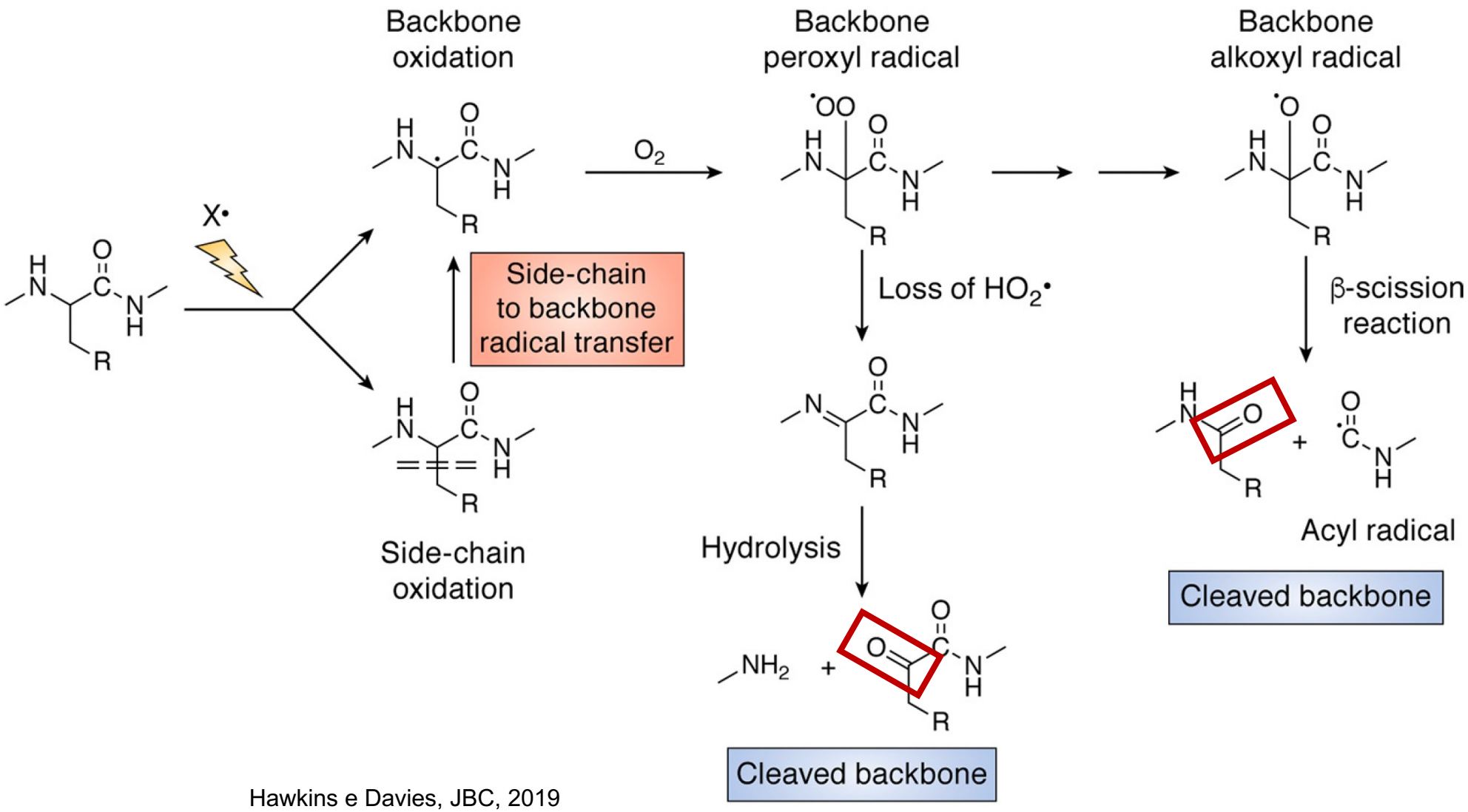


Composição bioquímica da célula

Modificações oxidativas

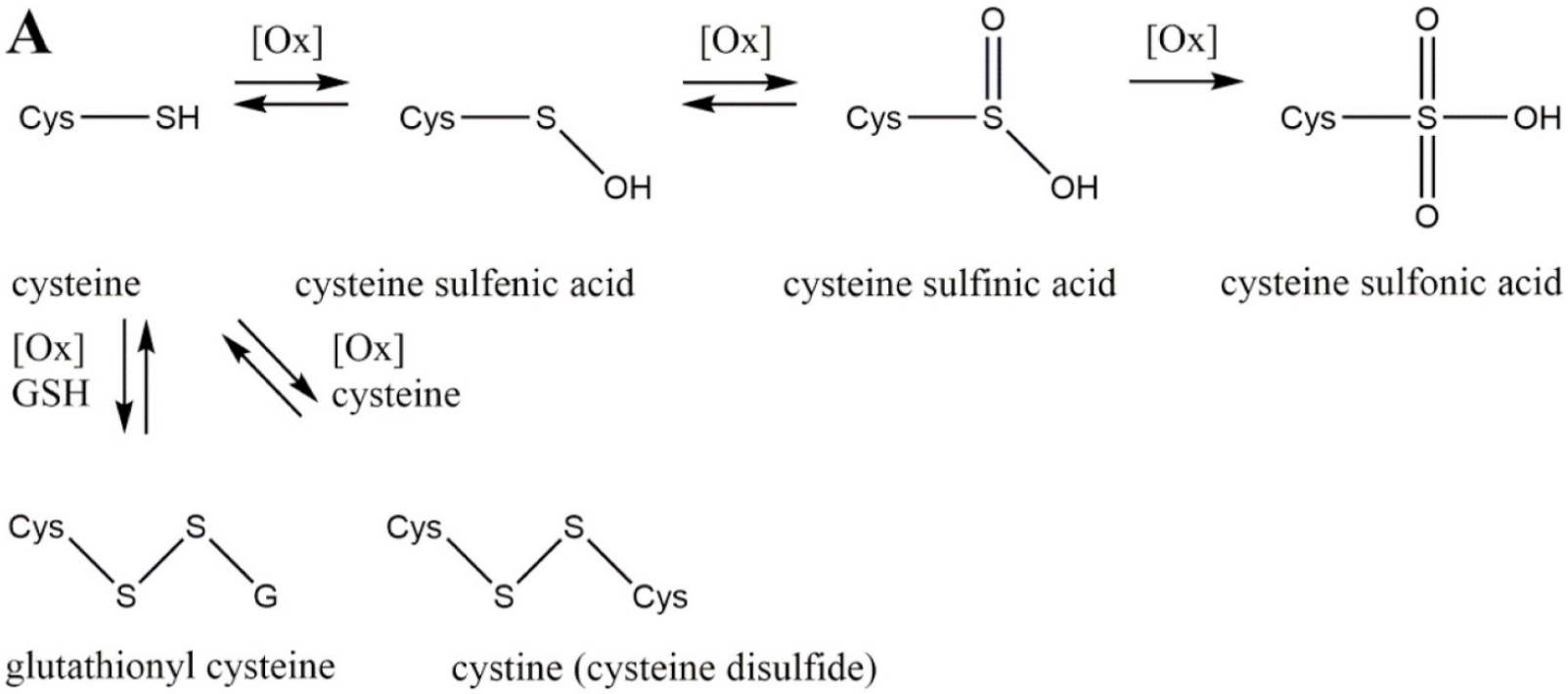
- Esqueleto polipeptídico
- Cadeias laterais
- Reação direta com oxidantes
- Reação com derivados de oxidação de outras biomoléculas

Ataque de radicais no esqueleto polipeptídico

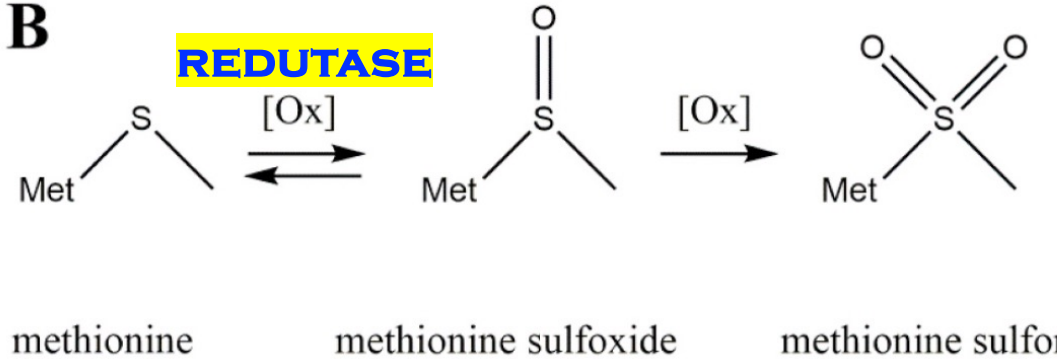


Hawkins e Davies, JBC, 2019

Oxidação de tióis

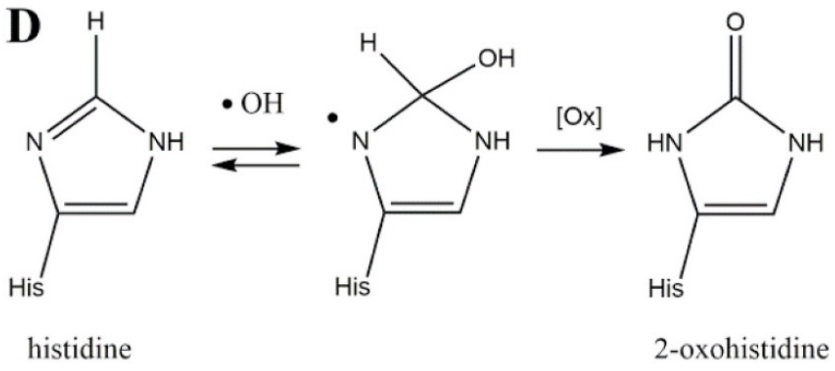


METIONINA SULFÓXIDO REDUTASE



Kehm et al, Redox Biol, 2021

Oxidação de resíduos aromáticos - histidina



Kehm et al, Redox Biol, 2021

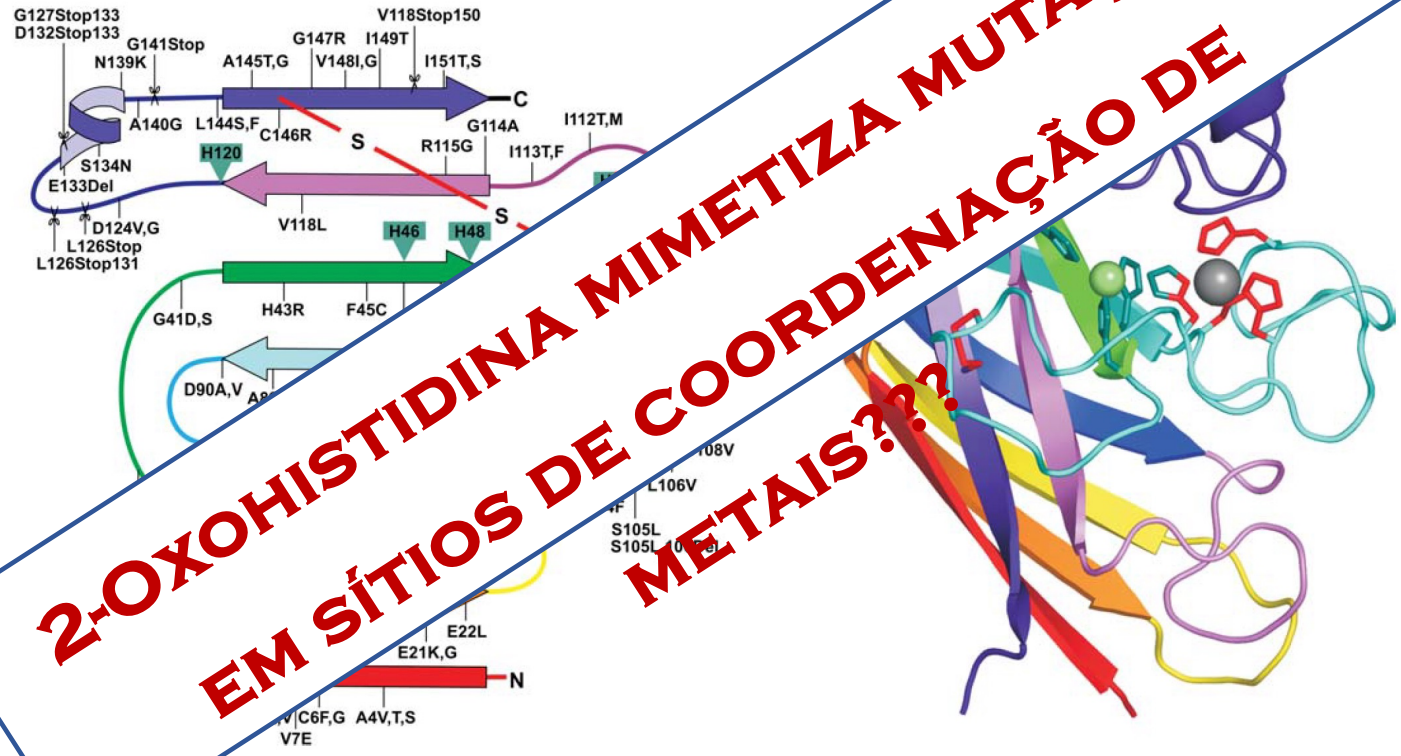
Atividade Peroxidásica da SOD1

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

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

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

Mutações da SOD1 em Esclerose Lateral Amiotr



2-Oxohistidina mimetiza mutações em sítios de coordenação de metais???

Valentine, Ann Rev Biochem, 2005

Oxidação de resíduos aromáticos - triptofaano

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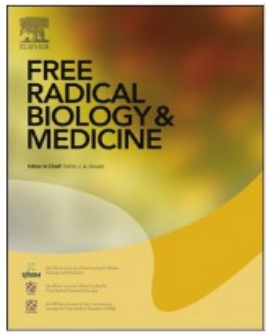

Original article

Human cataractous lenses contain cross-links produced by crystallin-derived tryptophanyl and tyrosyl radicals

Verônica Paviani[a], Paulo Junqueira de Melo[b], Amaryllis Avakin[b], Paolo Di Mascio[a], Graziella Eliza Ronsein[a,**], Ohara Augusto[a,*]

[a] *Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Av. Lineu Prestes 748, 05508-000, São Paulo, Brazil*
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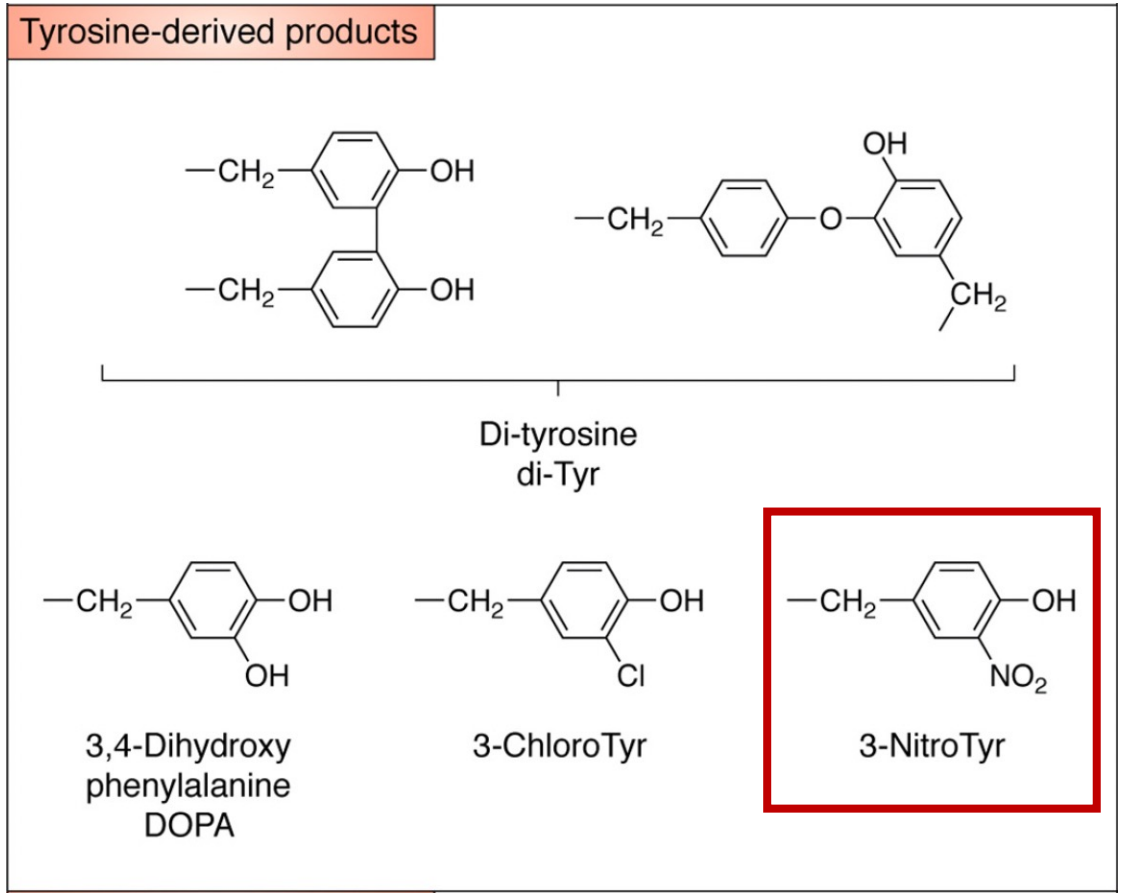
Oxidação de resíduos aromáticos - tirosina



Hawkins e Davies, JBC, 2019

???

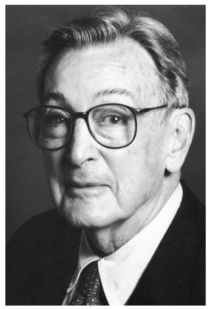
Histone H1.2 is a substrate for denitrase, an activity that reduces nitrotyrosine immunoreactivity in proteins

Yasuyuki Irie*†, Makio Saeki‡, Yoshinori Kamisaki‡, Emil Martin*, and Ferid Murad*§

*Department of Integrative Biology and Pharmacology and the Institute of Molecular Medicine, University of Texas Medical School, 6431 Fannin, MSB 4.100, Houston, TX 77030; †Department of Pharmacology A6, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan; and ‡Department of Pharmacology, Graduate School of Dentistry, Osaka University, 1-8 Yamadaoka, Suita, Osaka 565-0871, Japan

Contributed by Ferid Murad, March 26, 2003

The Nobel Prize in Physiology or Medicine 1998



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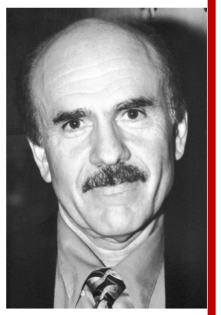
Robert F. Furchgott
Prize share: 1/3



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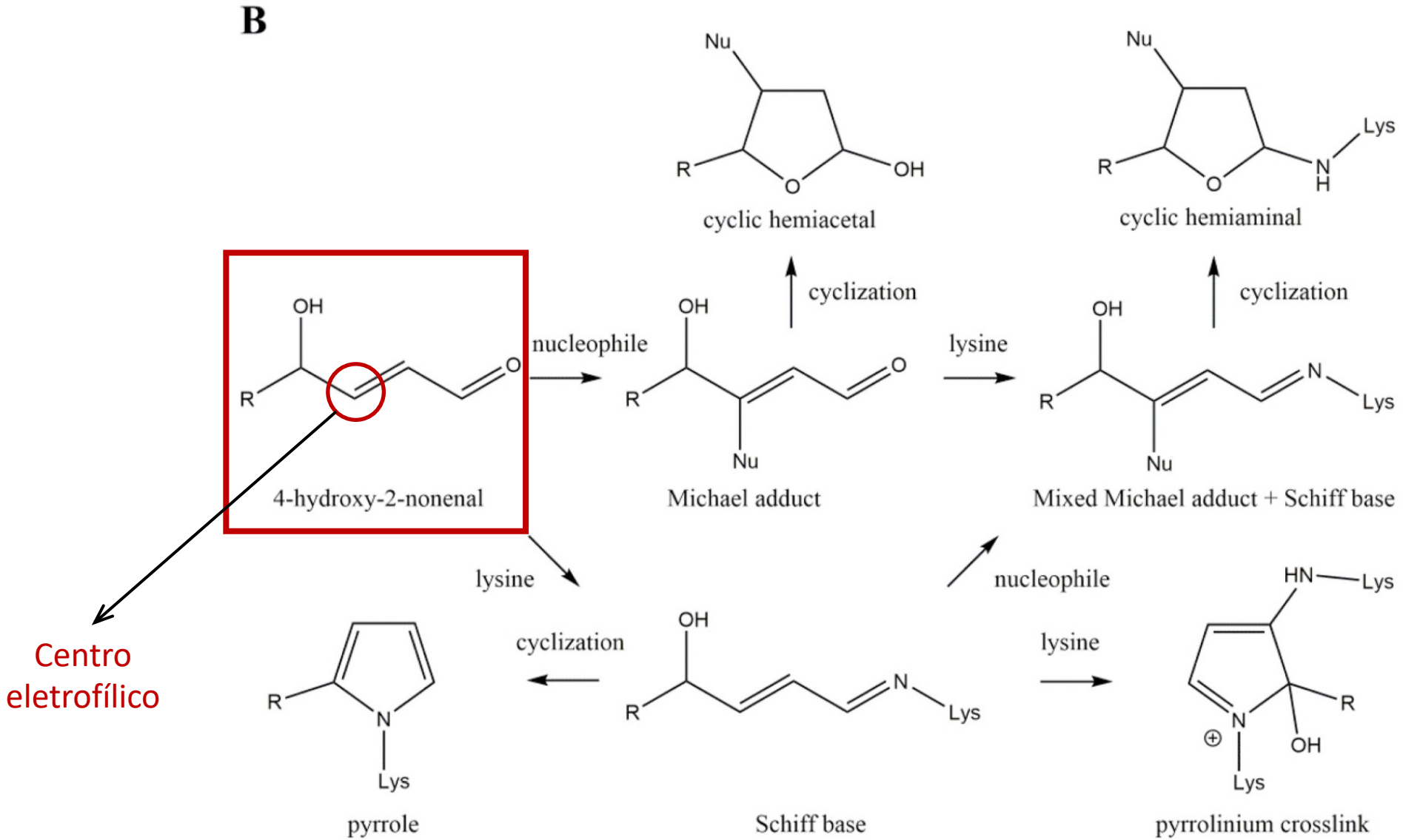
Modificação por derivados da peroxidação lipídica

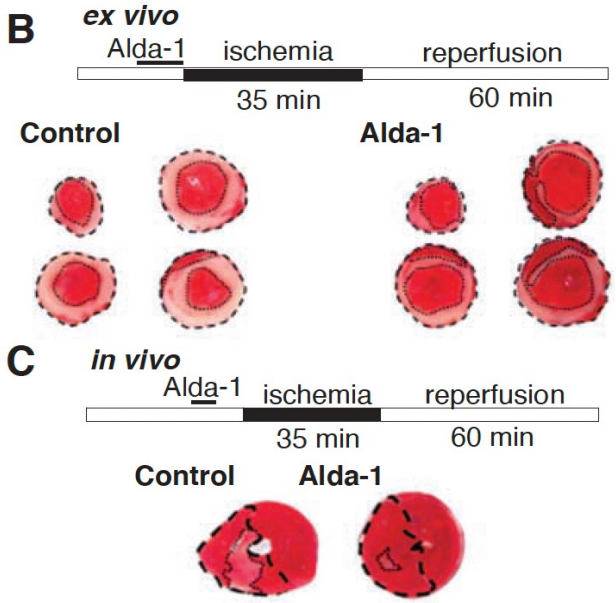


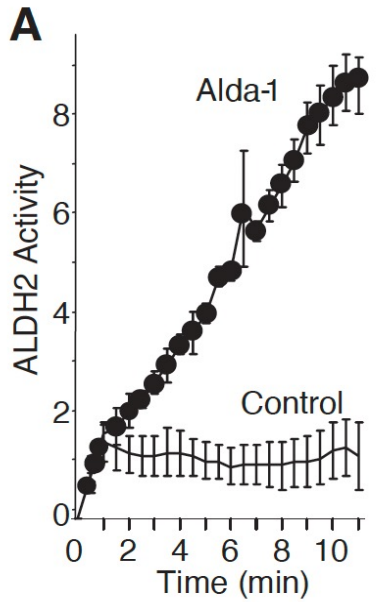
Kehm et al, Redox Biol, 2021

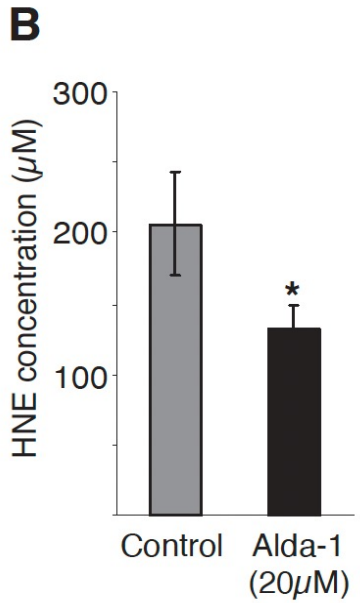
Modificação por derivados da peroxidação lipídica – moléculas protetoras

Activation of Aldehyde Dehydrogenase-2 Reduces Ischemic Damage to the Heart
Che-Hong Chen *et al.*
Science **321**, 1493 (2008);
DOI: 10.1126/science.1158554

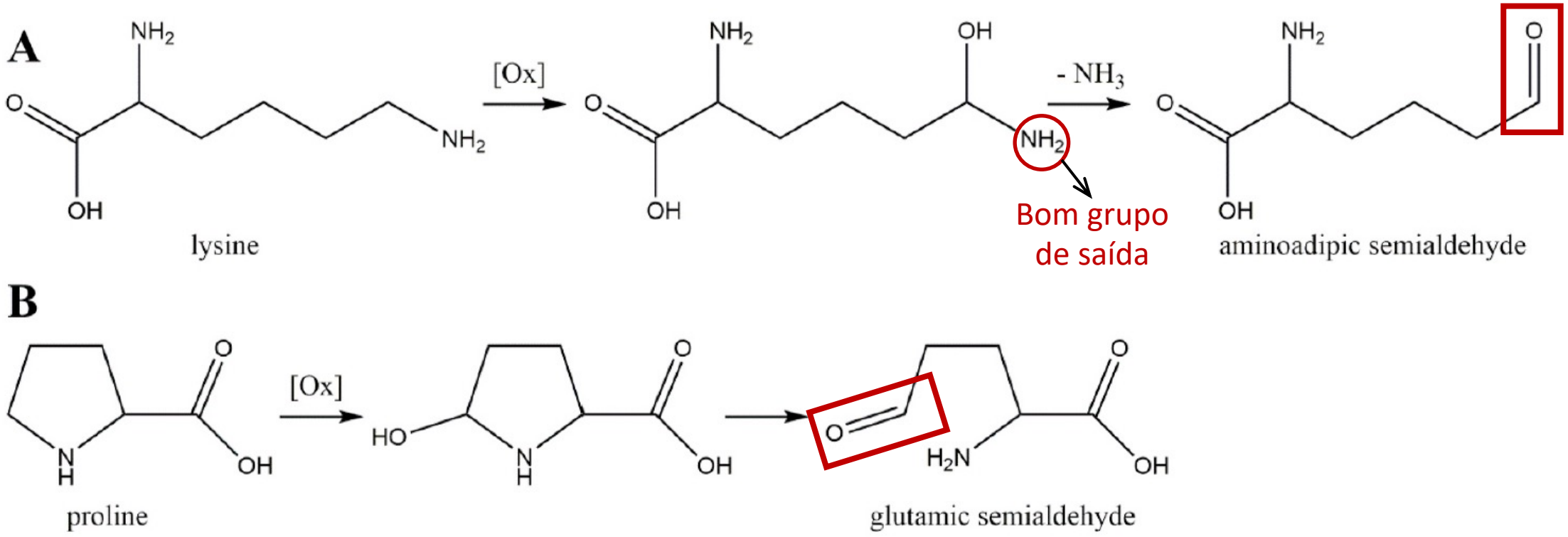
ALDA-1







Formação de carbonilas por oxidação de cadeias laterais



Kehm et al, Redox Biol, 2021

Produtos de Oxidação em Proteínas como Biomarcadores

Produtos de oxidação em tióis

Table 2
Selected studies using modifications of sulfur-containing amino acids as biomarkers of protein oxidation in clinical settings.

Disease/aging	Specimen	Study population	Method	Main outcome	Reference
Cystine and cysteine sulfonic acid					
Coronary artery disease (CAD)	Plasma	N = 247 patients (of n = 1411) experienced primary outcome of death	HPLC	Higher cystine levels associated with increased risk for all-cause mortality in CAD patients	[208]
Lethal prostate cancer	Serum	Control subjects vs. lethal prostate cancer patients (n = 523 per group)	LC-MS/MS	Higher serum cystine levels are associated with reduced risk for lethal prostate cancer	[209]
PD	Serum	Control subjects (n = 30) vs. PD patients (n = 20)	UPLC-MS	Higher cystine levels in PD patients	[210]
HIV	Serum	HIV-negative (n = 21) vs. HIV-positive (n = 113) patients	GC-MS	Increased levels of cystine in HIV-positive patients	[211]
Sickle-cell disease and sepsis	Plasma	Healthy donors (n = 18) vs. sickle-cell disease (n = 9) and sepsis patients (n = 6)	UPLC-MS/MS	Elevated levels of cysteine disulfides (protein-bound cysteine) in disease patients	[124]
T2D	Plasma	Healthy subjects vs. T2D patients (n = 8 per group)	LC-MS/MS	Higher cysteine sulfonic acid levels in T2D patients	[125]
Methionine sulfoxide					
T2D and diabetic complications	Serum (serum albumin)	Healthy subjects (n = 18) vs. T2D patients with (n = 23) and w/o (n = 12) renal failure	LC-MS	Higher levels of methionine sulfoxide in T2D patients with and w/o renal failure compared to controls	[212]
AD	Plasma	Healthy subjects (n = 23) vs. patients with mild-cognitive impairment (MCI) (n = 13) or AD (n = 25)	WB	Elevated levels of methionine sulfoxide in plasma of AD patients compared to other groups	[130]

Kehm et al, Redox Biol, 2021

Carbonilas protéicas

Table 4
Selected studies using protein carbonyls as a biomarker of protein oxidation in clinical settings.

Disease/aging	Specimen	Study population	Method	Main outcome	Reference
T2D with vascular complications	Serum	Healthy individuals (n = 94) vs. T2D patients with vascular complications (n = 94)	ELISA	Higher protein carbonyl levels in T2D patients with vascular complications	[149]
T2D with NASH	Serum	Control subjects (n = 50) vs. T2D patients with NASH (n = 60) or T2D w/o NAFLD (n = 50)	ELISA	Elevated protein carbonyl levels in T2D patients with NASH compared to other groups	[218]
T2D with and w/o CKD	Serum	Healthy subjects vs. T2D patients with and w/o CKD (n = 50 per group)	Spectrophotometry/ HPLC	Higher protein carbonyl levels in T2D patients with and w/ o CKD compared to control subjects	[219]
Diabetic nephropathy	Serum	Control subjects (n = 142) vs. T2D patients with diabetic nephropathy (n = 153)	ELISA	Elevated levels of protein carbonylation in patients with diabetic nephropathy	[150]
CAD	Plasma	Control subjects vs. premature CAD or normal CAD (n = 30 per group)	ELISA	Higher plasma protein carbonyl levels in premature and normal CAD patients compared to control groups	[220]
Acute coronary syndrome or chronic periodontitis	Saliva	Control subjects vs. patients with acute coronary syndrome, chronic periodontitis or both (n = 24 per group)	Spectrophotometry	Higher protein carbonyl levels in all disease groups compared to controls	[221]
Alcoholic liver cirrhosis (ALC)	Serum	Healthy control subjects (n = 130) vs. ALC patients (n = 57)	Spectrophotometry	No difference between control and ALC groups	[222]
Normal and end stage ALD	Hepatic tissue	Normal ALD (n = 8) vs. end stage ALD (n = 9)	IHC, WB	Increased protein carbonylation in liver sample of end stage ALD patients	[223]
PD and AD	Plasma	Control subjects (n = 34) vs. AD (n = 40) and PD patients (n = 70)	ELISA	Higher protein carbonyl levels in male AD patients compared to PD patients and controls	[151]

Kehm et al, Redox Biol, 2021

Modificações derivadas da peroxidação lipídica

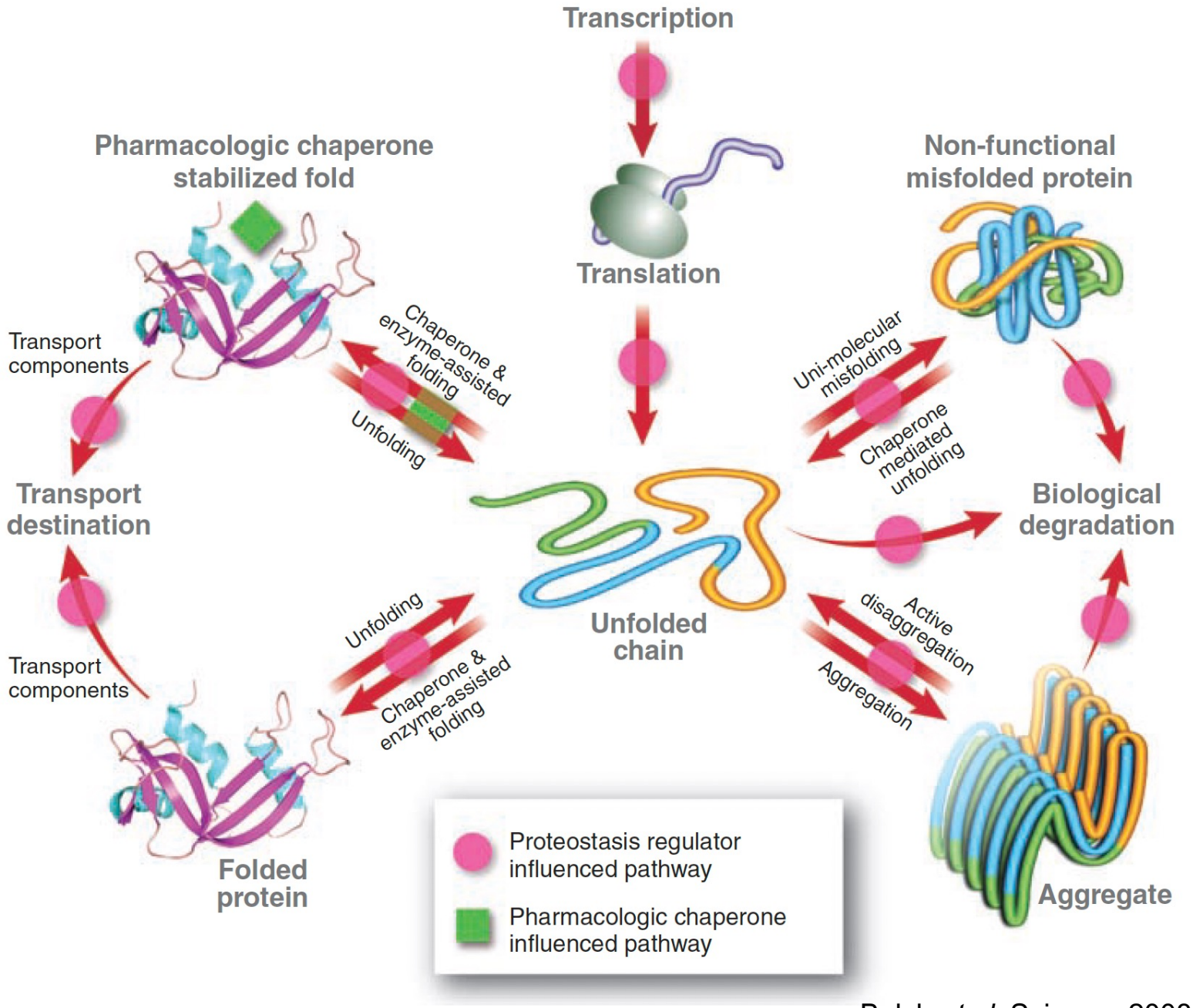
Table 6
Selected clinical studies using 4-HNE and MDA as biomarkers in different diseases.

Disease	Specimen	Study population	Method	Main outcome	Reference
4-HNE					
T2D	Serum	Control subjects (n = 9) vs. T2D patients (n = 11)	ELISA	Higher levels of 4-HNE in patients with T2D	[240]
Alcoholic liver disease (ALD)	Human hepatic tissue	Normal ALD (n = 8) vs. end stage ALD (n = 9)	IHC	Elevated 4-HNE in liver sample of ALD patients	[223]
Lung cancer	Serum	Control subjects (n = 40) vs. lung cancer patients (n = 92)	ELISA	Higher levels of 4-HNE in male and female lung cancer patients	[183]
RA	Plasma, urine	Healthy subjects vs. RA patients (n = 73 per group)	GC-MS, ELISA	Elevated levels of free and protein-bound 4-HNE in RA patients	[177]
Thick-born encephalitis	CSF, plasma, urine	Healthy subjects (n = 56) vs. patients with thick-born encephalitis (n = 60)	GC-MS, ELISA	Elevated free and protein-bound 4-HNE levels in plasma of thick-born encephalitis patients	[178]

Kehm et al, Redox Biol, 2021

Respostas celulares ao estresse proteotoxico

A rede da proteostase

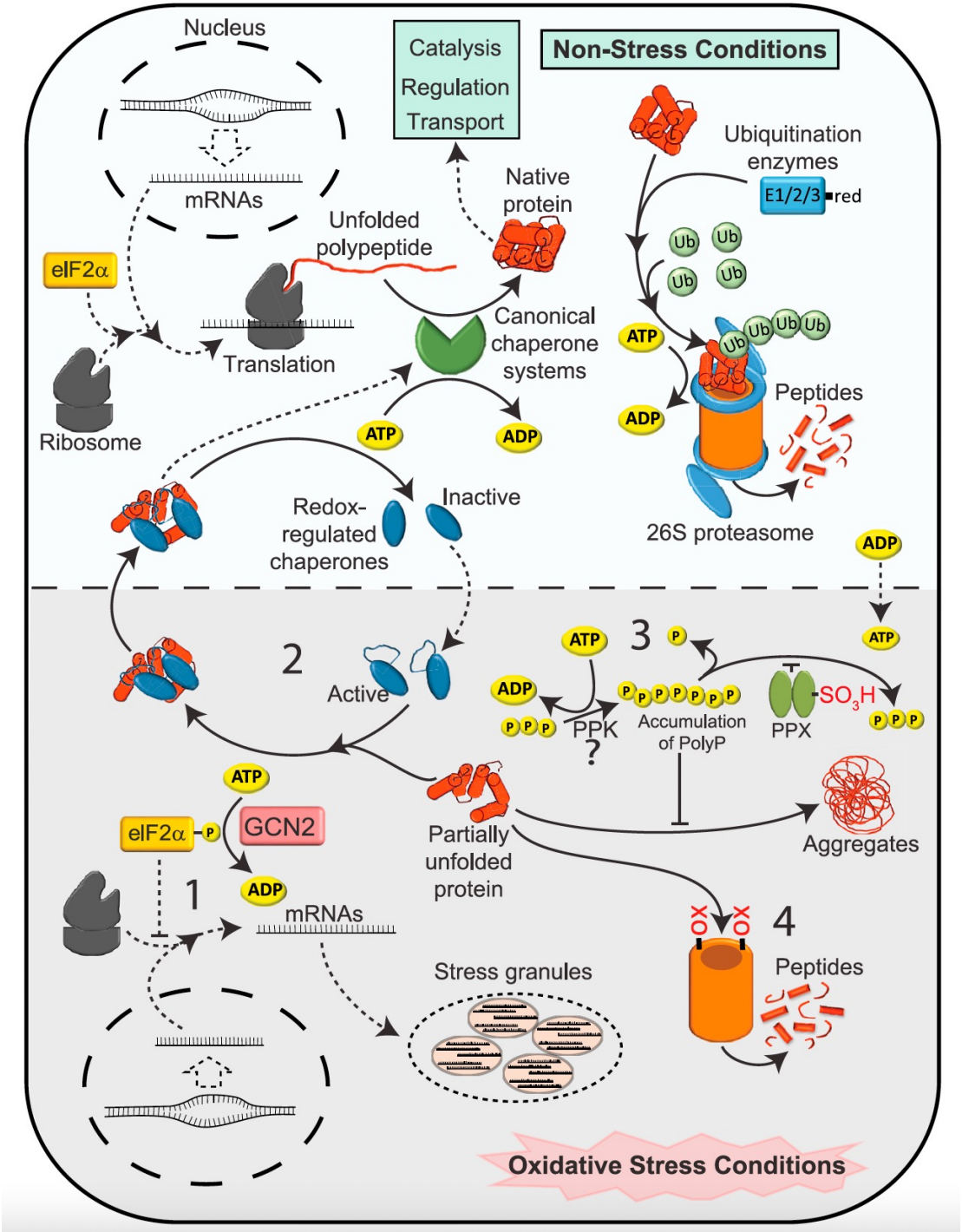


Balch *et al*, Science, 2008

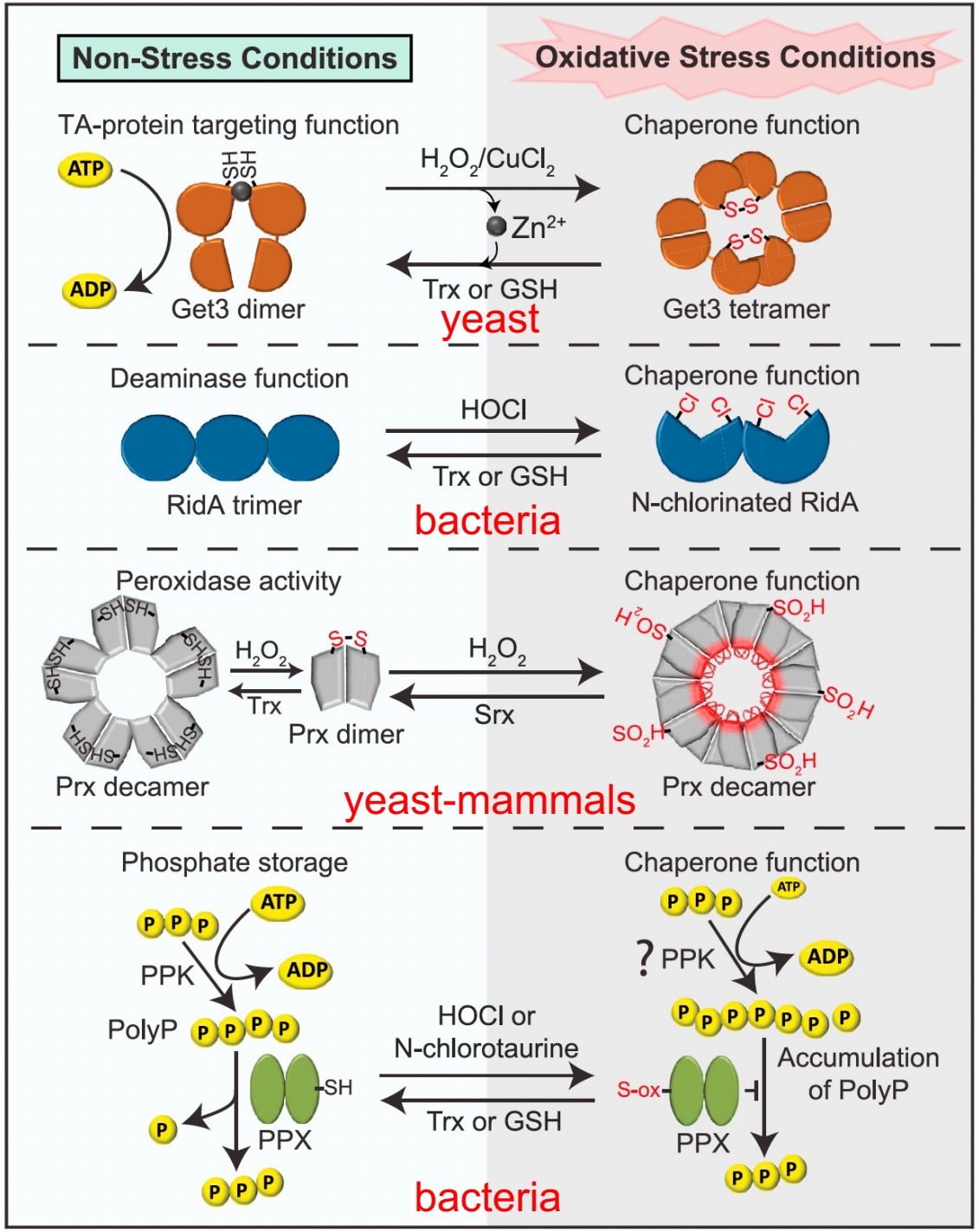
A rede da proteostase reage ao estresse oxidativo

Reichmann *et al*, Mol Cell, 2018

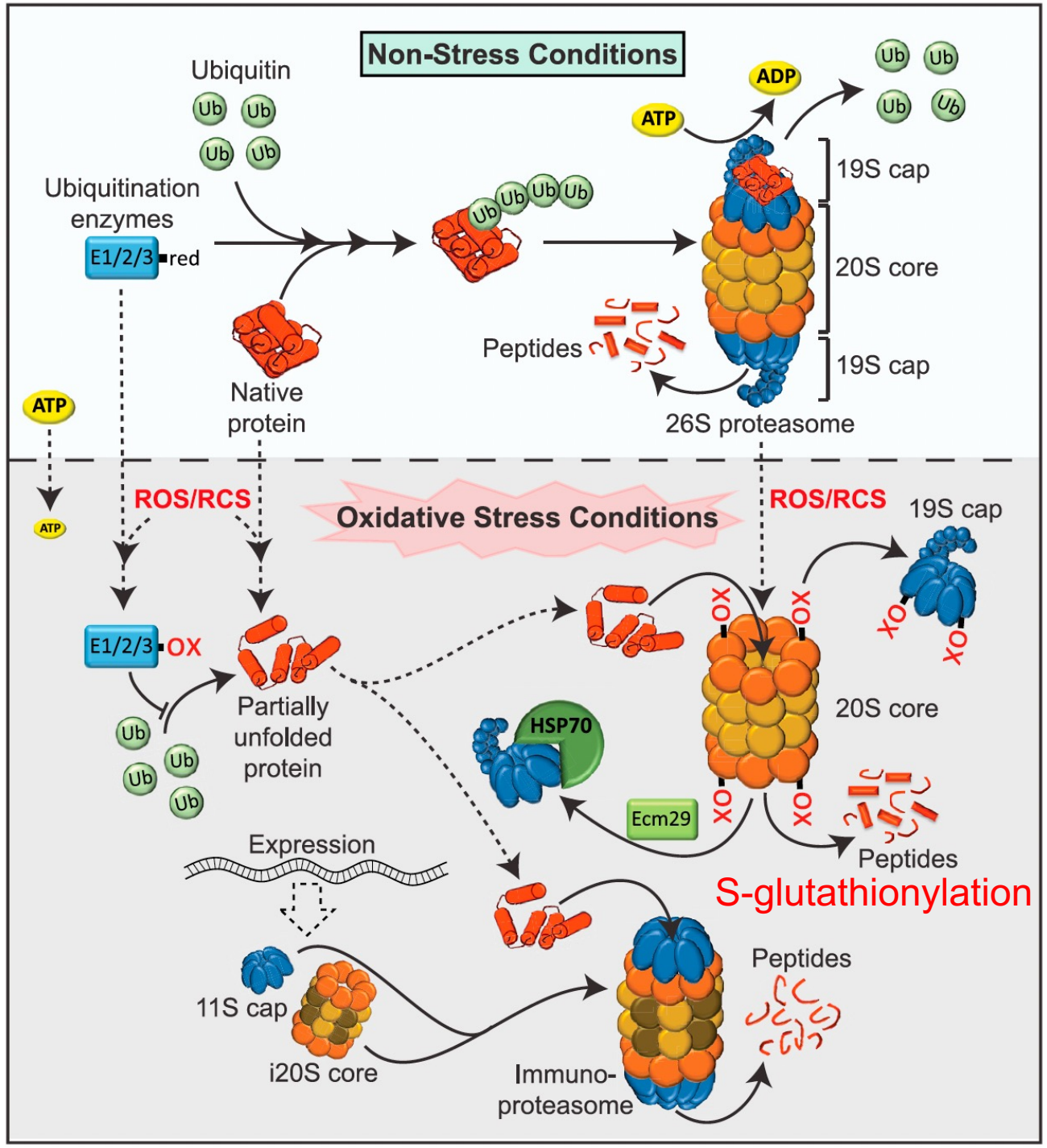
Visão geral


Nucleus
mRNAs
Catalysis
Regulation
Transport
Non-Stress Conditions
Native protein
Unfolded polypeptide
eIF2α
Translation
Ribosome
Canonical chaperone systems
ATP
ADP
Ubiquitination enzymes
E1/2/3
red
Ub
Peptides
26S proteasome
Redox-regulated chaperones
Inactive
Active
2
3
PPK
?
Accumulation of PolyP
PPX
SO_3H
GCN2
Partially unfolded protein
Aggregates
1
4
Stress granules
Oxidative Stress Conditions

Ativação de chaperonas



Remodelamento do Proteassomo



Recapitulando as metas da aula

- Oxidação da cadeia polipeptídica e cadeias laterais.
- Diferentes oxidantes podem oxidar proteínas, mas existe importante convergência de produtos finais.
- Biomarcadores correlacionam estresse oxidativo a varias doenças, por exemplo diabetes tipo 2, mas a aplicação clínica ainda é limitada.
- As respostas celulares a oxidantes ocorrem em vários níveis, de ativação de chaperonas não convencionais ao remodelamento de sistemas proteolíticos.

Bibliografia

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

Questões de Acompanhamento

1. Mencione ao menos três possibilidades para a produção de carbonilas em proteínas.

2. Por que não é possível definir biomarcadores definitivos para a produção de determinado oxidante? Exemplifique.

3. Explique os mecanismos e propósito biológico para a ativação de chaperonas em condições de estresse oxidativo.

4. Cite algumas razões pelas quais o proteassomo muda seu comportamento regulatório em condições de estresse oxidativo.