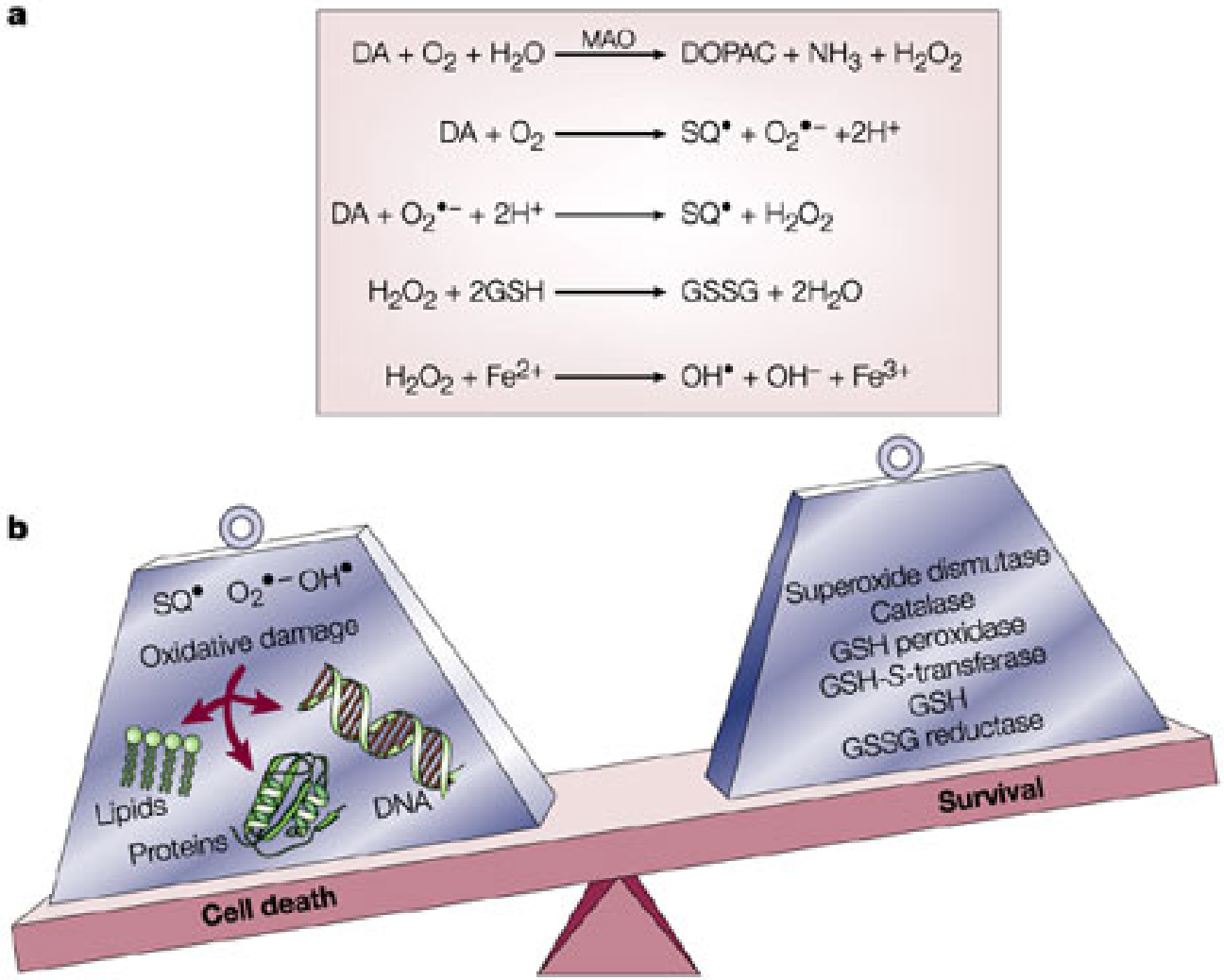
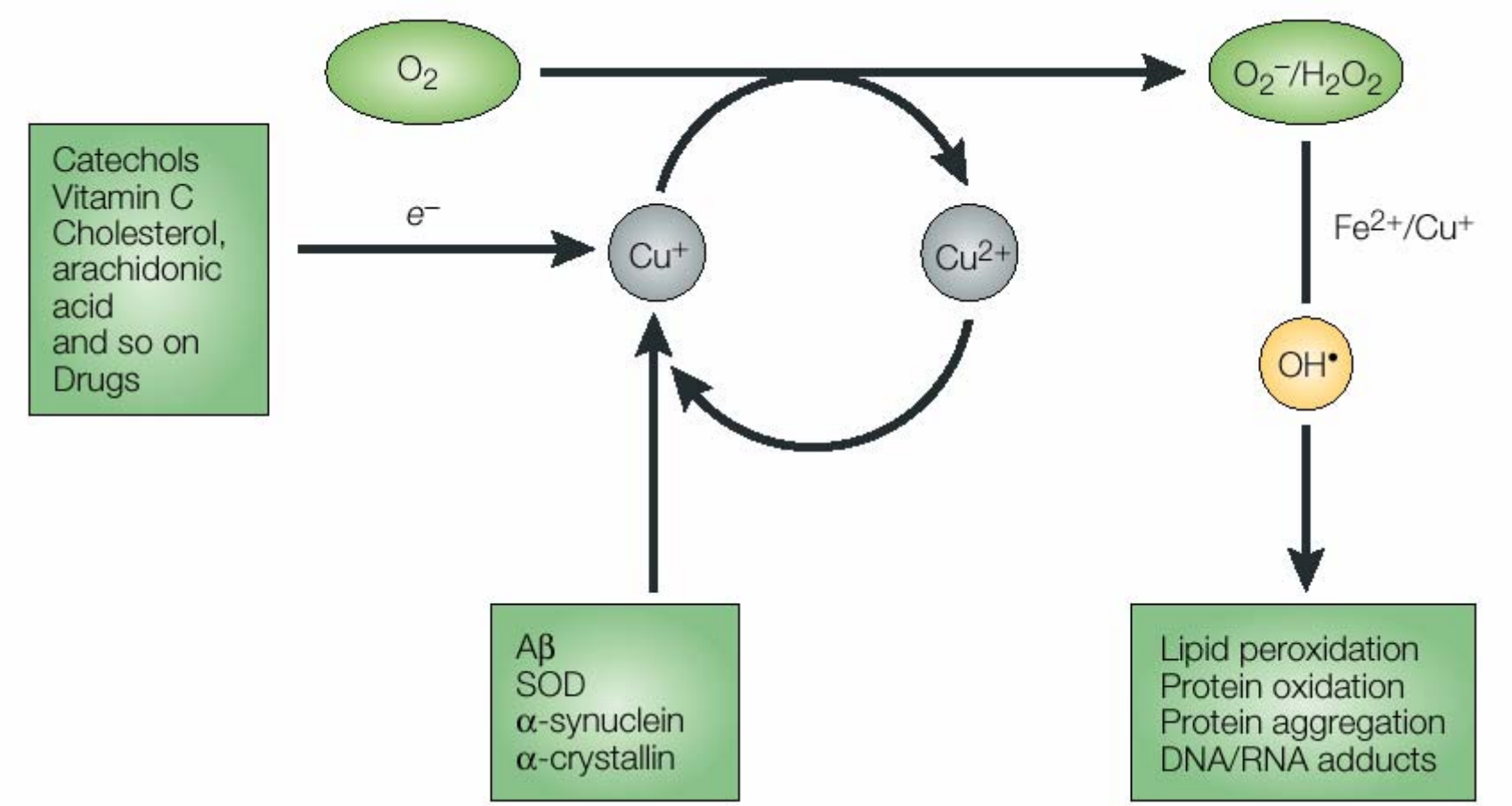
Stress Ossidativo



La produzione di ROS è catalizzata da metalli legati a proteine



Reazioni di Fenton e di Haber-Weiss

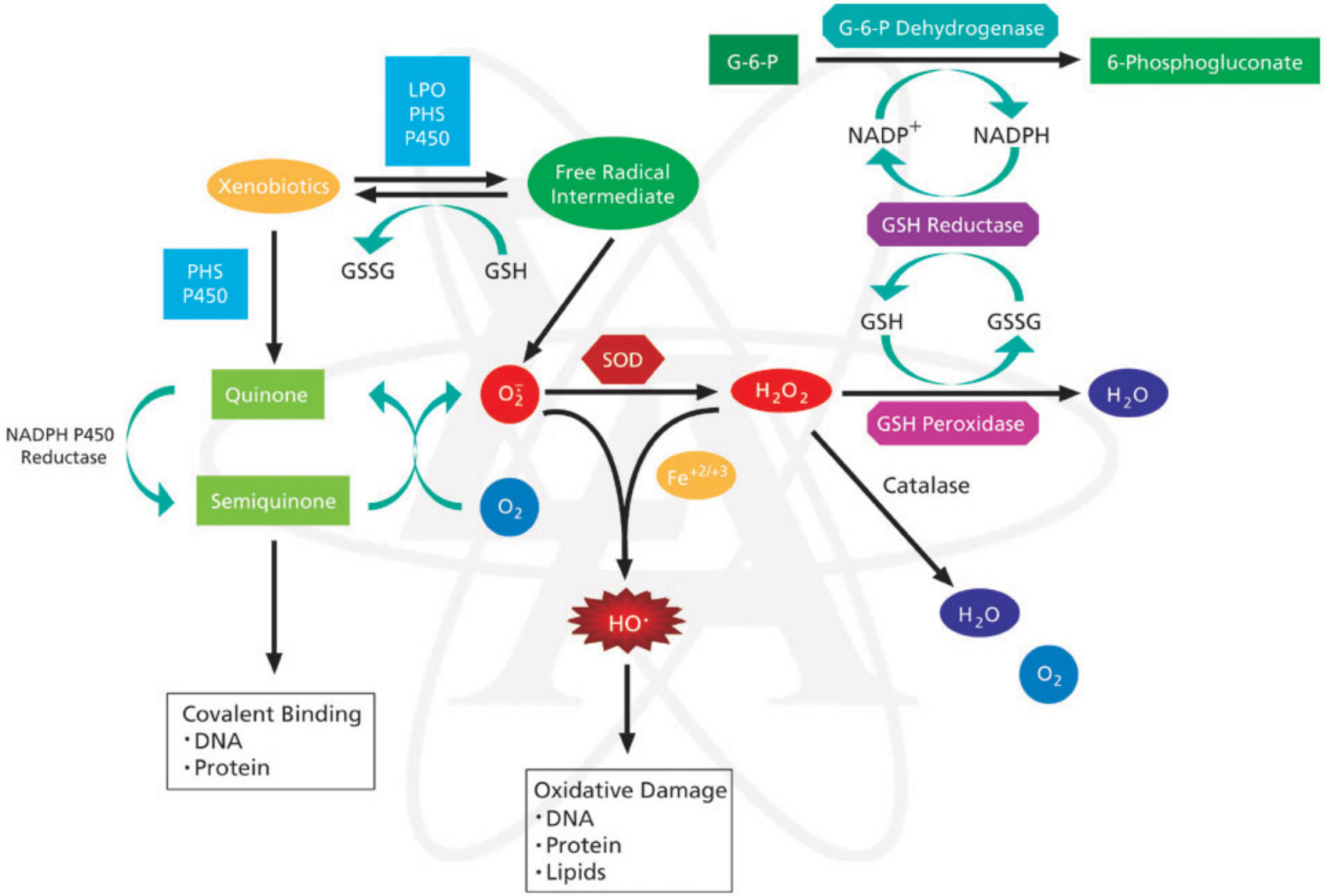
$$O_2^{-\bullet} + Fe^{3+} \rightarrow Fe^{2+} + O_2$$

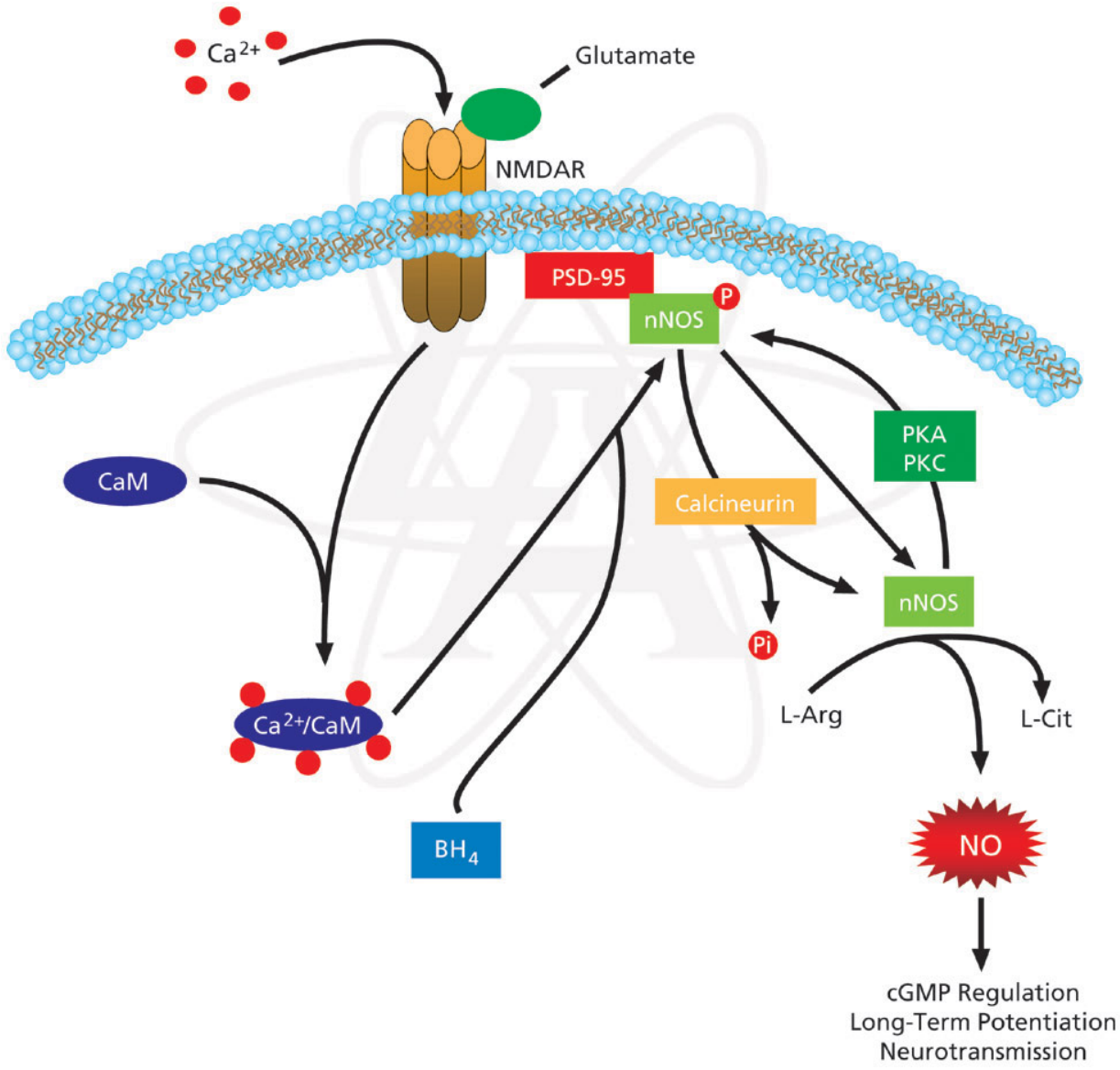
$$Fe^{2+} + H_2O_2 \rightarrow OH^- + Fe^{3+} + OH^\bullet$$

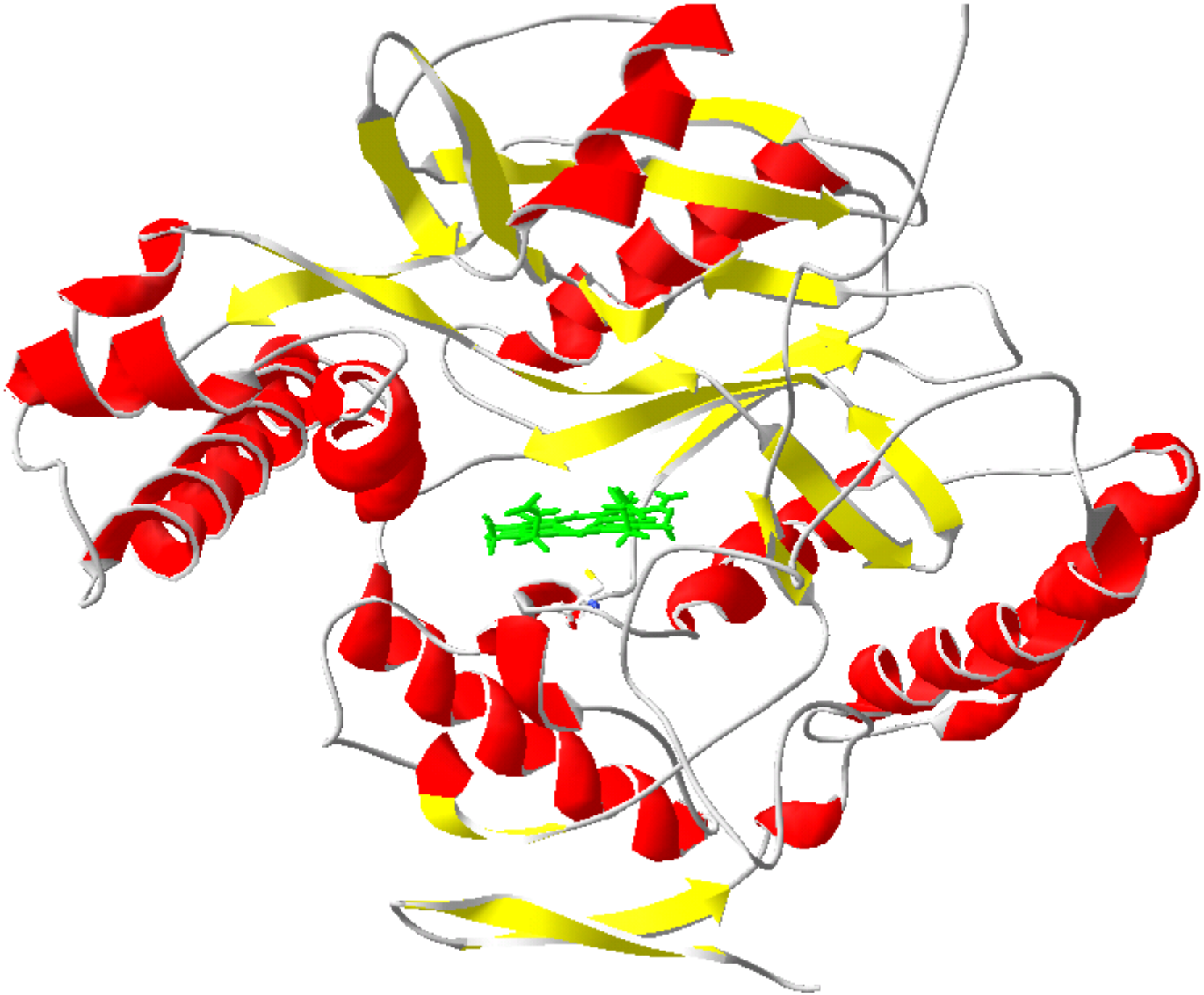
$$O_2^{-\bullet} + H_2O_2 \rightarrow OH^- + O_2 + OH^\bullet$$

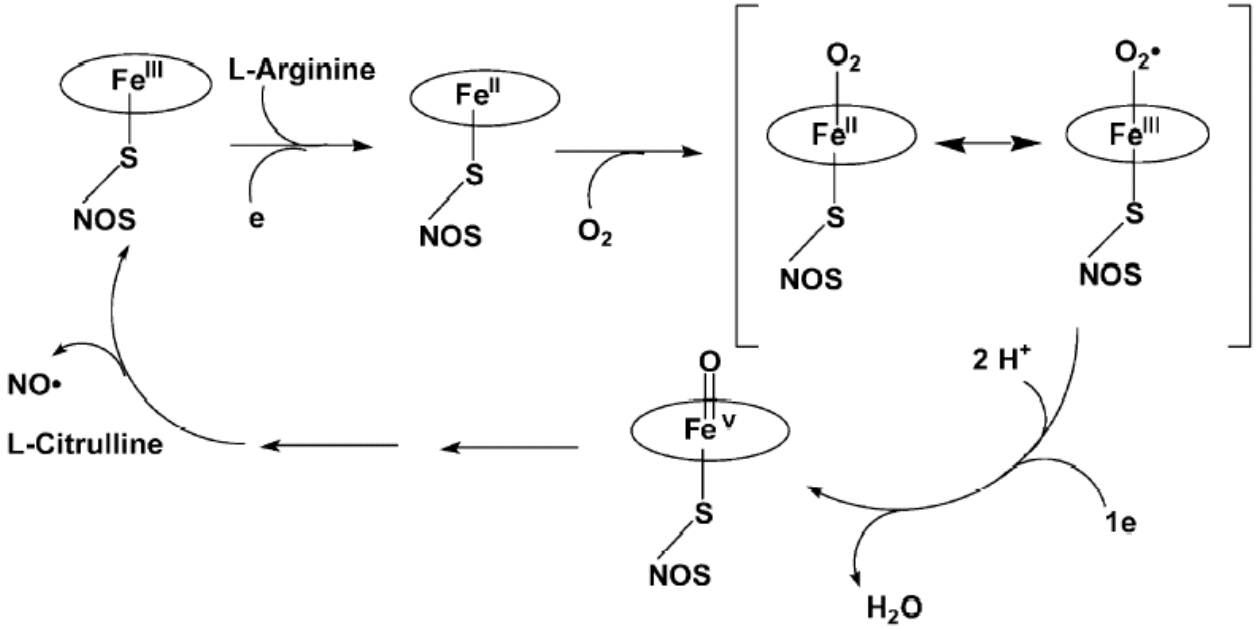
Enzimi coinvolti nell'omeostasi dei ROS

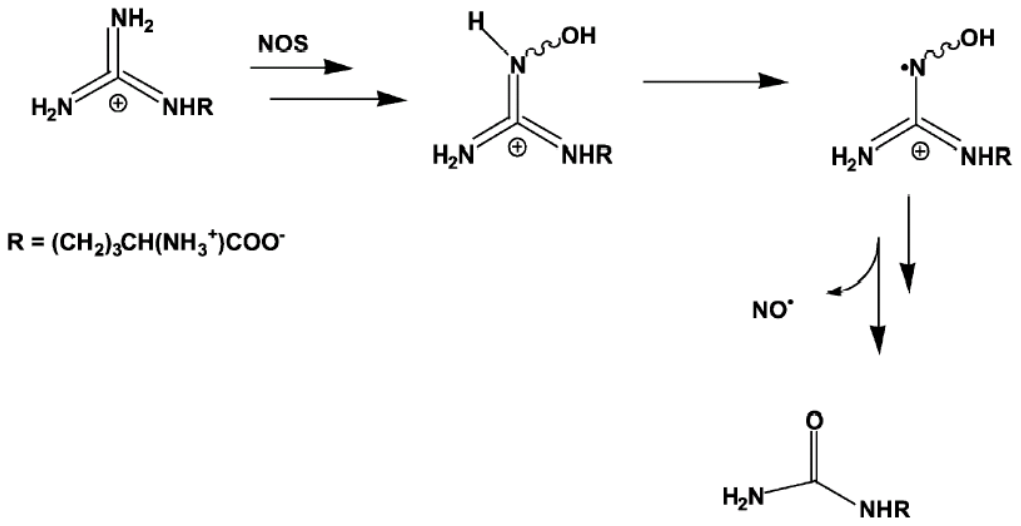
- Superossido dismutasi (Mn e Cu/Zn)
- Catalasi (Fe)
- Glutatione perossidasi (Se)
- Glutatione S-transferasi
- GSSG reduttasi (NADPH)
- NO sintasi (Fe)

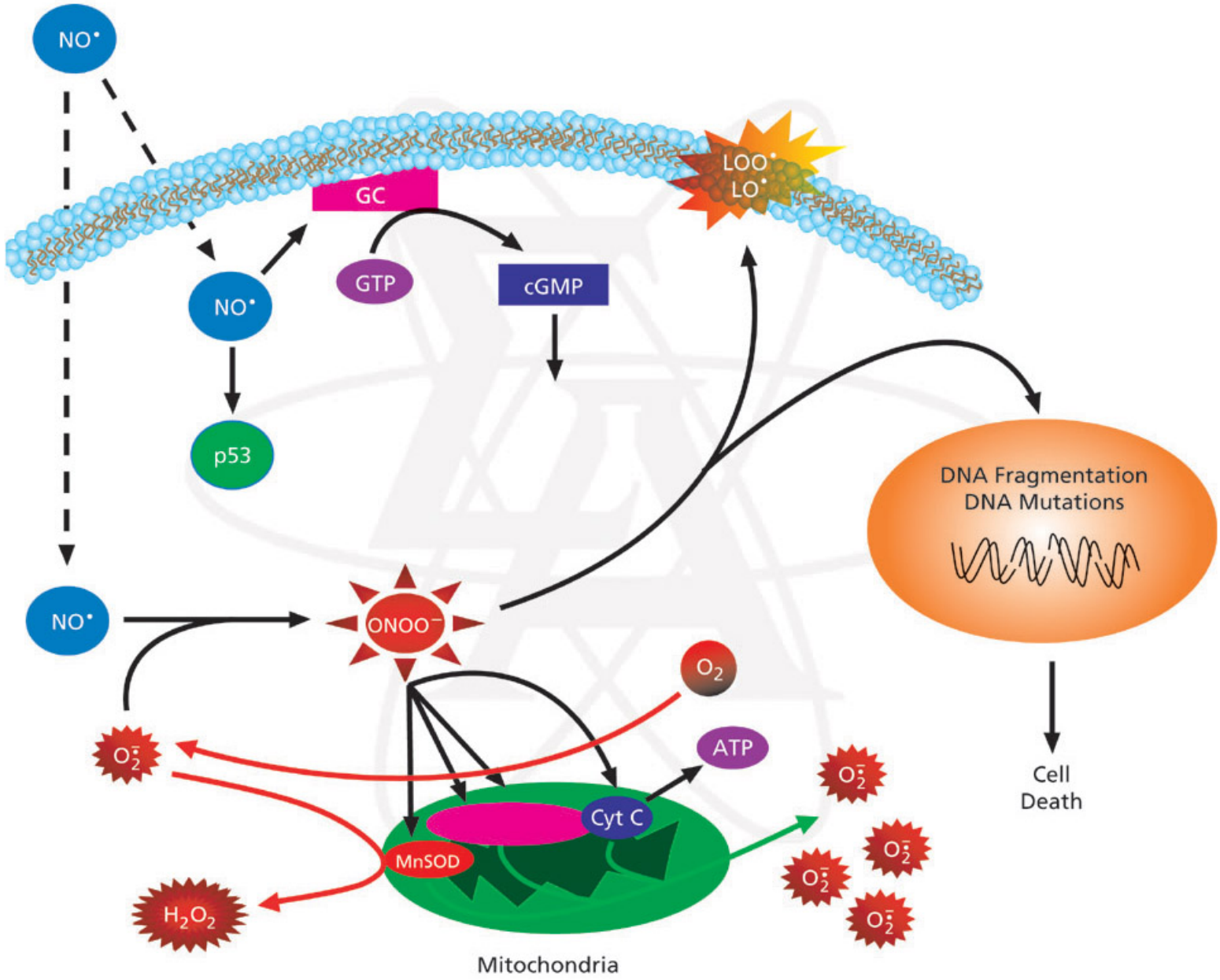

G-6-P
G-6-P Dehydrogenase
6-Phosphogluconate
NADP+
NADPH
LPO
PHS
P450
Xenobiotics
Free Radical
Intermediate
GSH Reductase
GSSG
GSH
PHS
P450
GSH
GSSG
SOD
Quinone
O_2^-
H_2O_2
H_2O
NADPH P450
Reductase
GSH Peroxidase
$Fe^{+2/+3}$
Catalase
Semiquinone
O_2
HO·
H_2O
O_2
Covalent Binding
• DNA
• Protein
Oxidative Damage
• DNA
• Protein
• Lipids

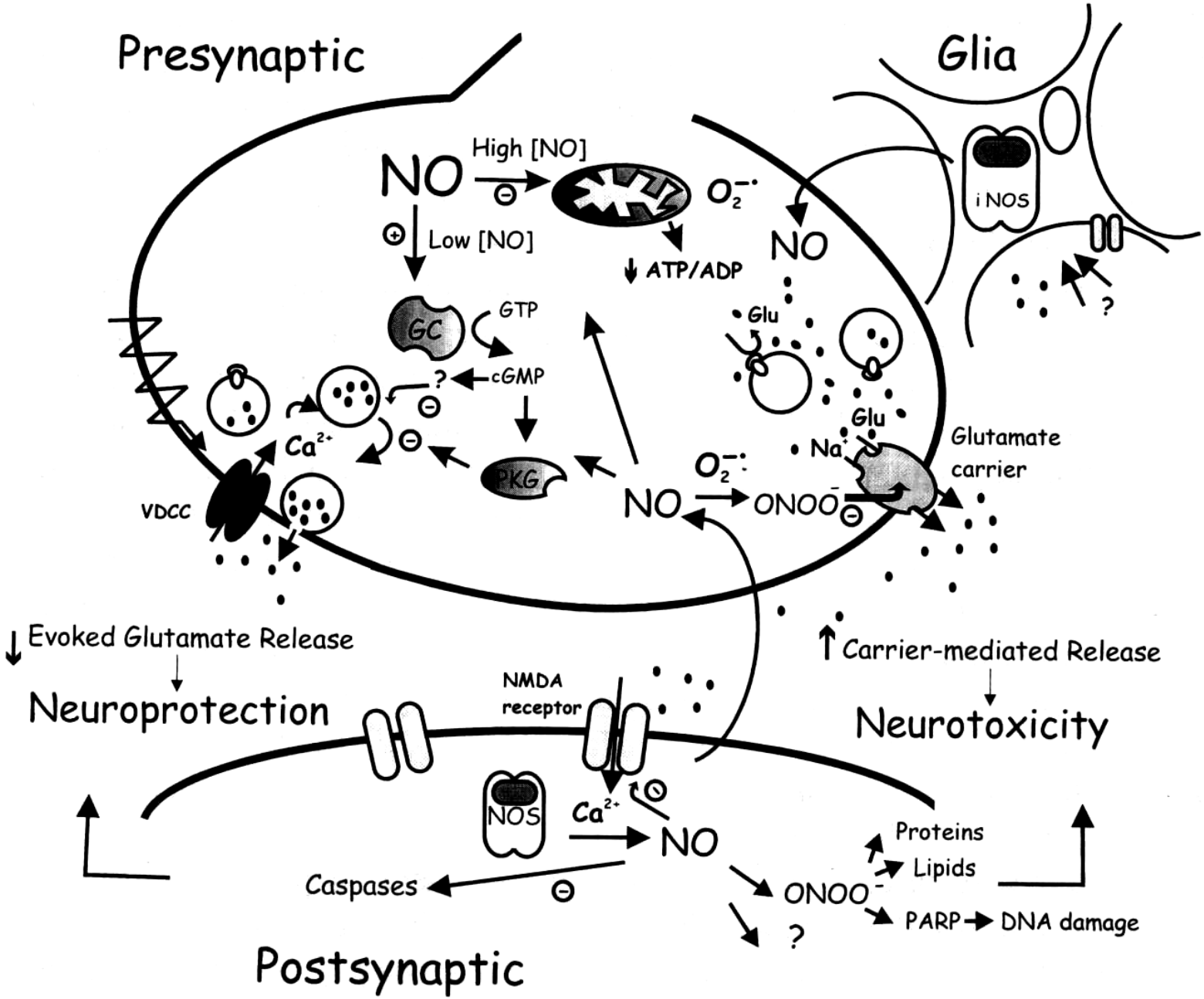

Ca2+
Glutamate
NMDAR
PSD-95
nNOS
P
CaM
PKA
PKC
Calcineurin
nNOS
Pi
Ca2+/CaM
L-Arg
L-Cit
BH4
NO
cGMP Regulation
Long-Term Potentiation
Neurotransmission

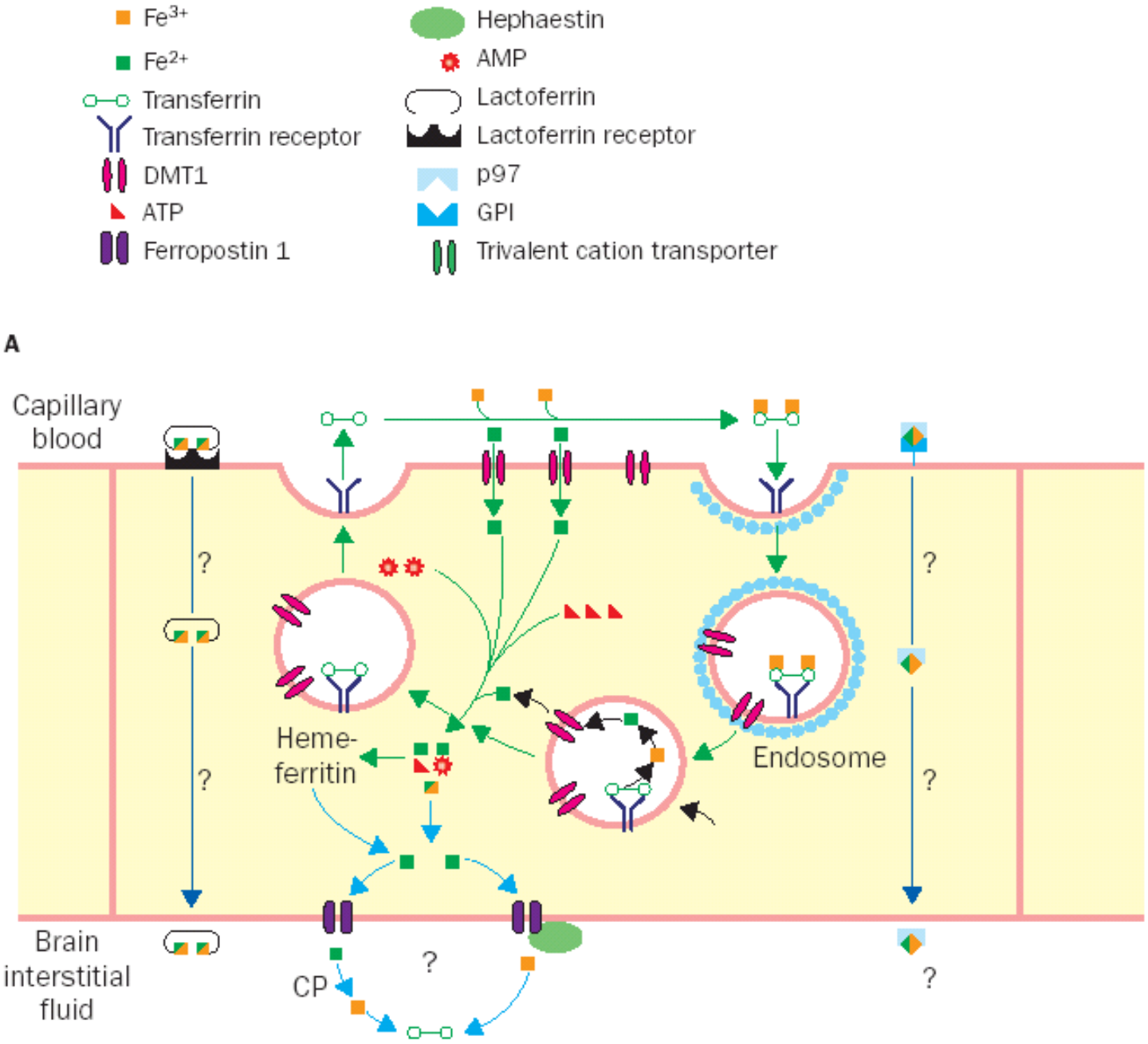


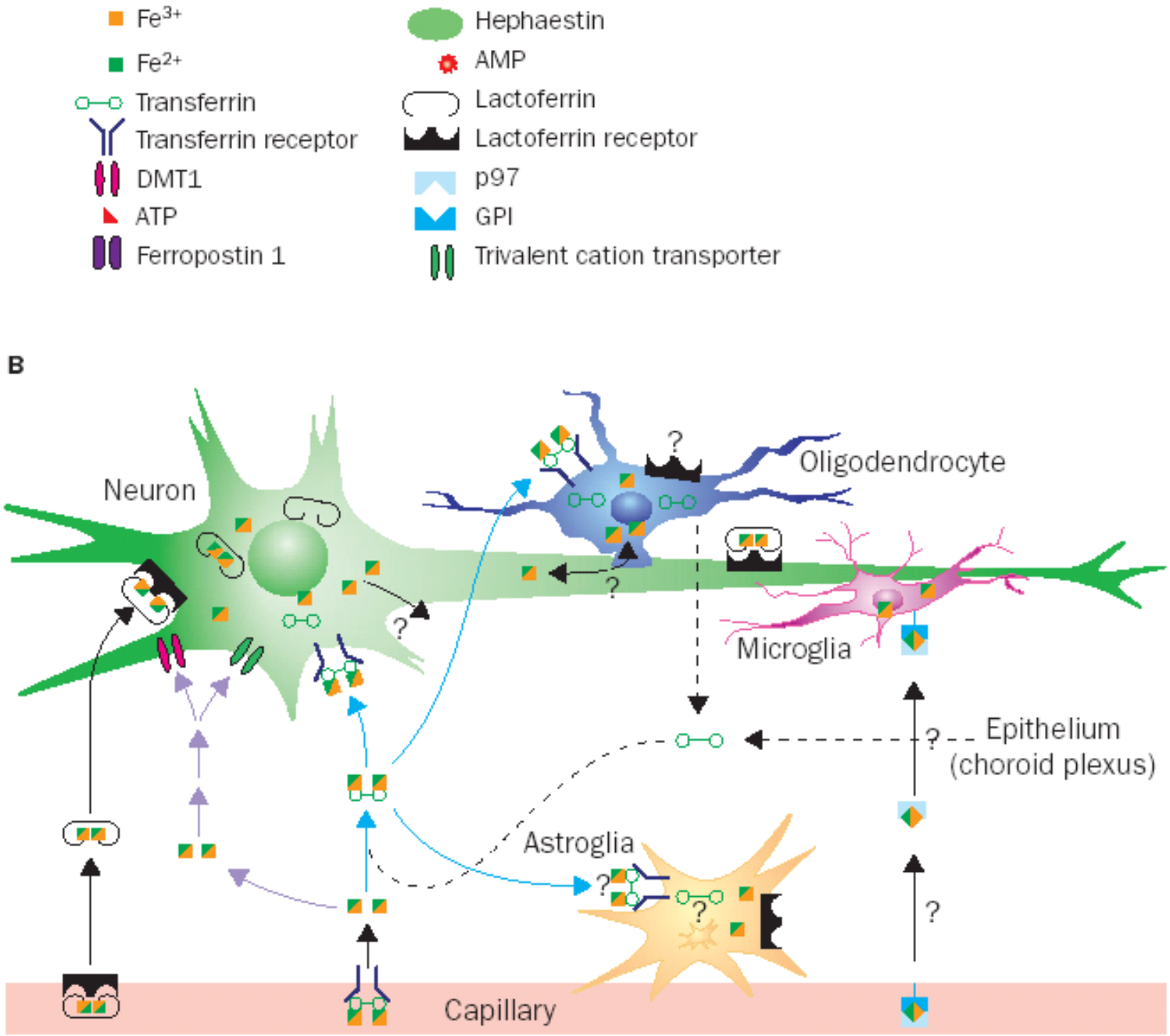

Fe^III
S
NOS
L-Arginine
e
Fe^II
S
NOS
O2
O2
Fe^II
S
NOS
O2•
Fe^III
S
NOS
2 H+
1e
H2O
O
Fe^V
S
NOS
NO•
L-Citrulline


NH2
H2N
NHR
NOS
H
N
OH
H2N
NHR
•N
OH
H2N
NHR
R = (CH2)3CH(NH3+)COO-
NO•
O
H2N
NHR

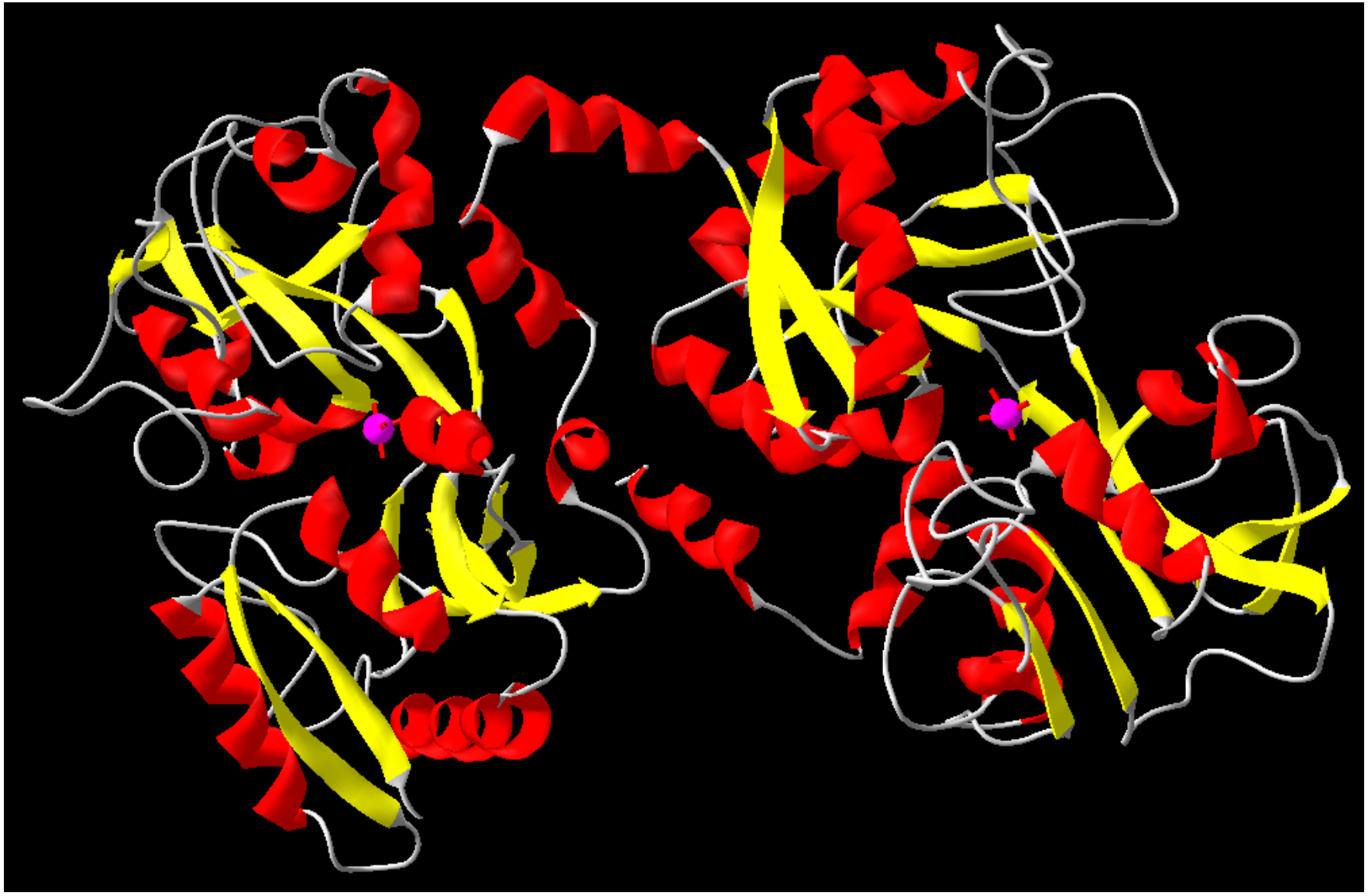

NO•
GC
LOO•
LO•
GTP
cGMP
NO•
p53
DNA Fragmentation
DNA Mutations
NO•
ONOO⁻
O2
ATP
O2•⁻
Cyt C
MnSOD
H2O2
Cell
Death
Mitochondria


Presynaptic
Glia
NO
High [NO]
Low [NO]
$O_2^{-\cdot}$
ATP/ADP
iNOS
NO
?
Glu
GTP
GC
cGMP
?
Ca^{2+}
PKG
VDCC
Glu
Na^+
Glutamate carrier
$O_2^{-\cdot}$
NO
$ONOO^-$
↓ Evoked Glutamate Release
Neuroprotection
↑ Carrier-mediated Release
Neurotoxicity
NMDA receptor
NOS
Ca^{2+}
NO
Caspases
Proteins
Lipids
$ONOO^-$
PARP → DNA damage
?
Postsynaptic

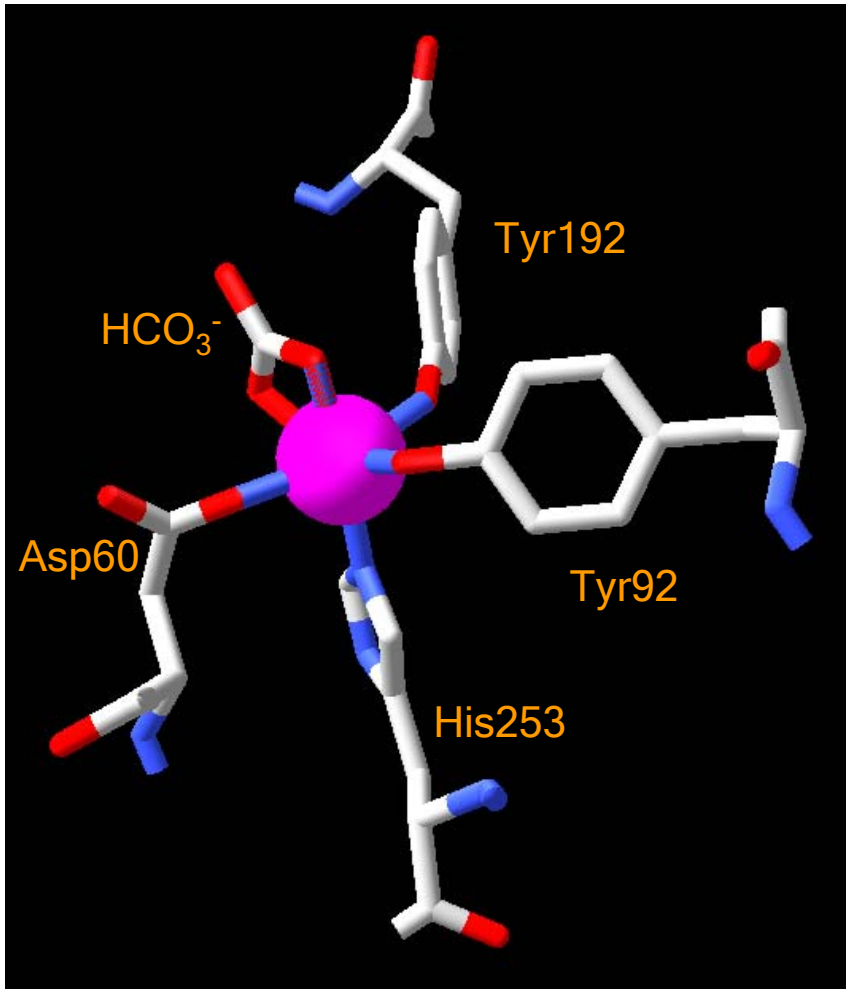

Fe3+
Fe2+
Transferrin
Transferrin receptor
DMT1
ATP
Ferropostin 1
Hephaestin
AMP
Lactoferrin
Lactoferrin receptor
p97
GPI
Trivalent cation transporter
A
Capillary blood
Brain interstitial fluid
Heme-ferritin
Endosome
CP
?


Fe3+
Fe2+
Transferrin
Transferrin receptor
DMT1
ATP
Ferropostin 1
Hephaestin
AMP
Lactoferrin
Lactoferrin receptor
p97
GPI
Trivalent cation transporter
B
Neuron
Oligodendrocyte
Microglia
Epithelium
(choroid plexus)
Astroglia
Capillary
?

Transferrina



Transferrina



Stress ossidativo nel SNC

Pro-ossidanti

Ossigeno e glucosio

PUFA

Metalli di transizione

Anti-ossidanti

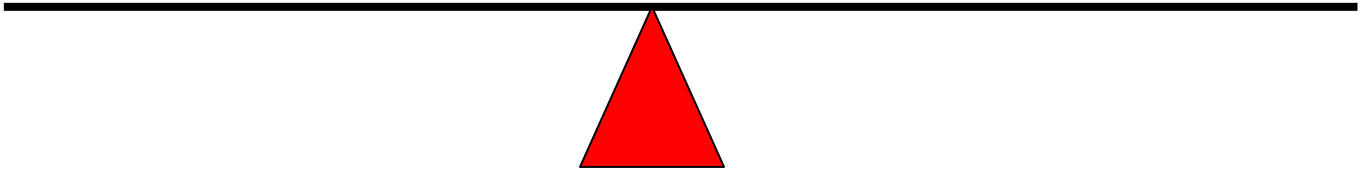
Catalasi

SOD

GSH Px; GSH

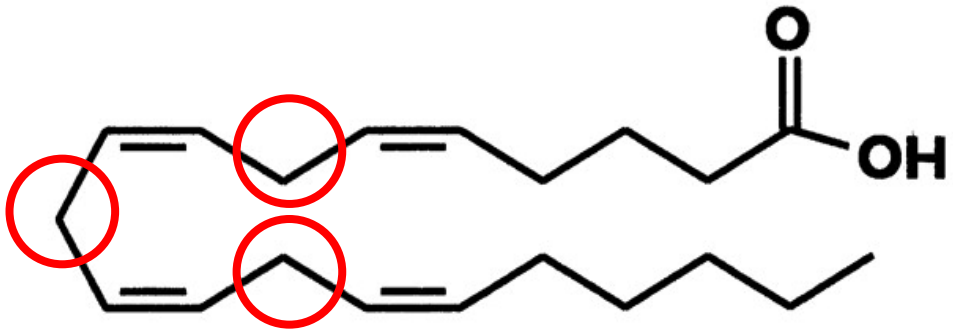
Vitamine C / E

Acido urico

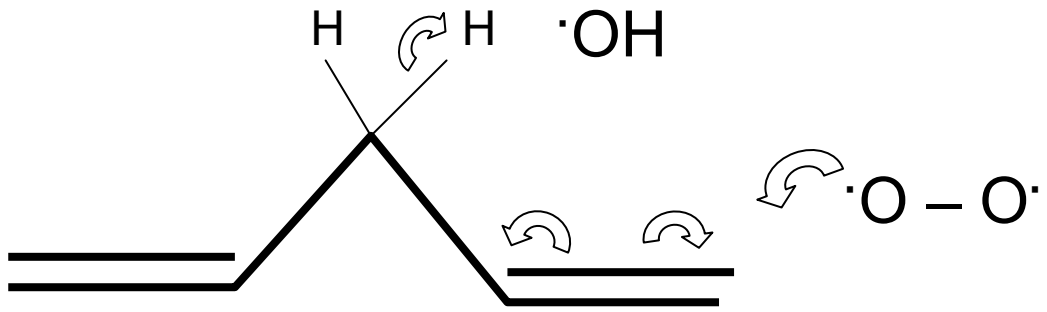


Perossidazione lipidica

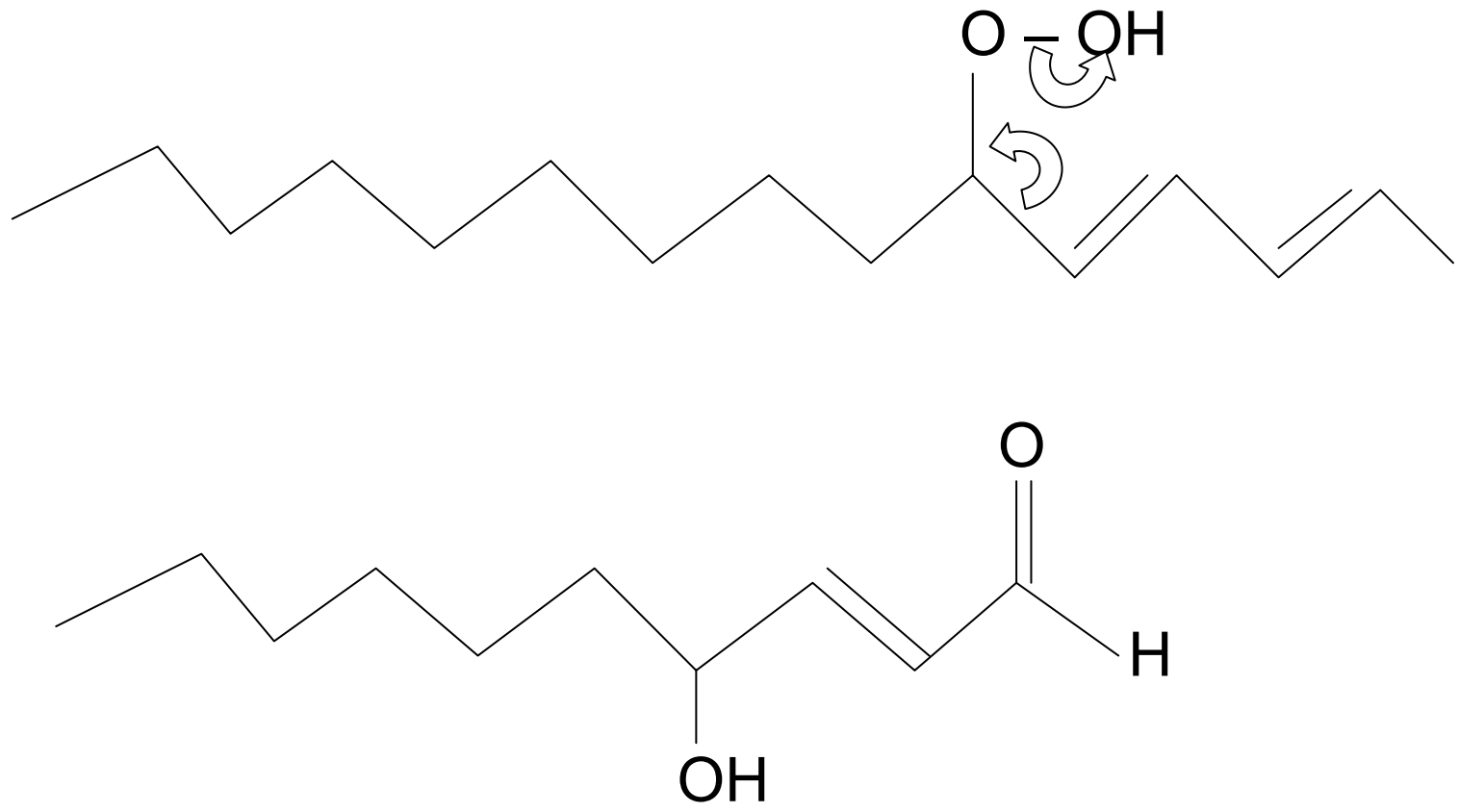
- PUFA (Es. ac. arachidonico)



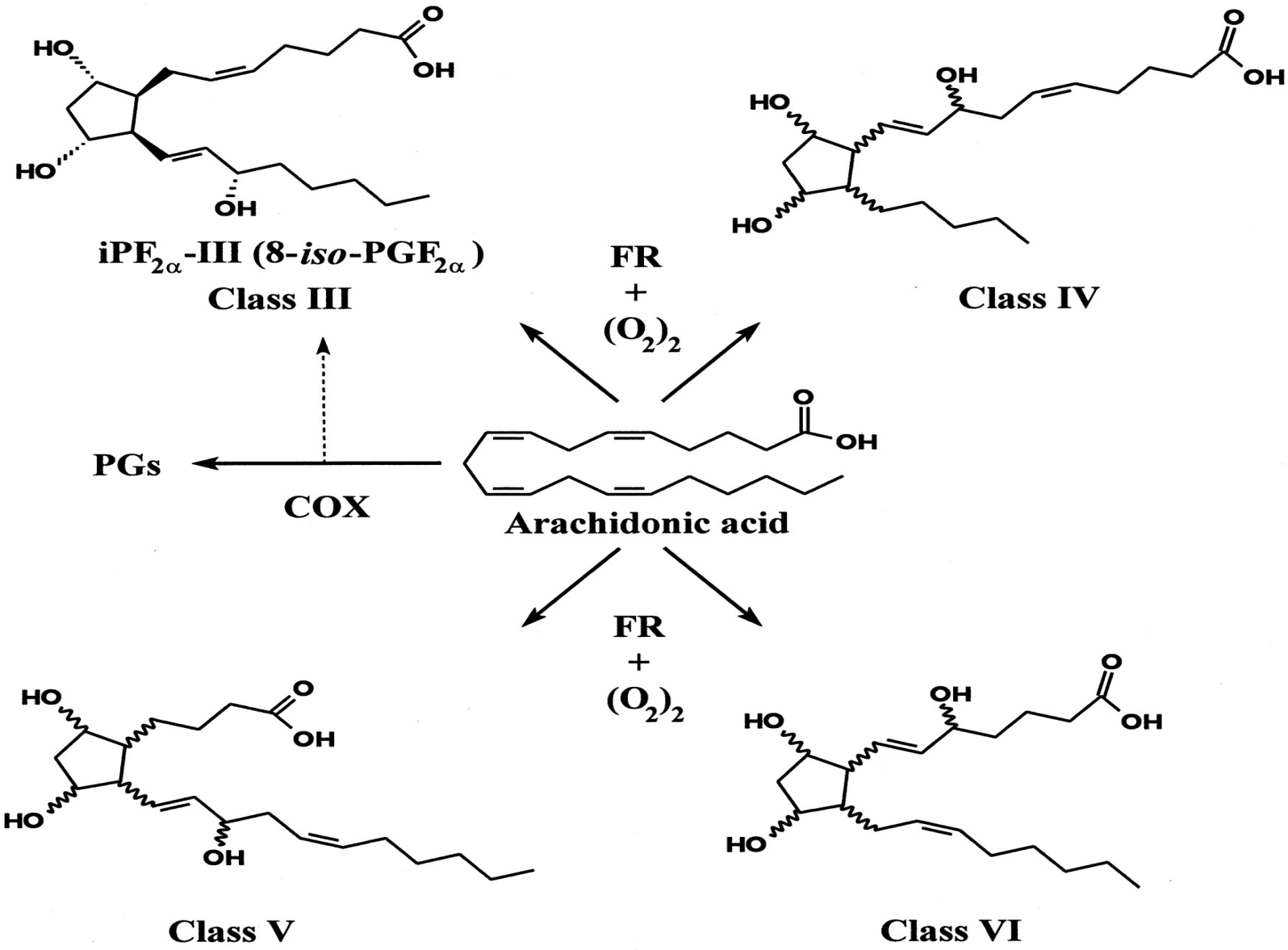
Posizione
bis-allilica



da lipidi-idroperossido a HNE



Isoprostani



Ossidazione proteica

- Modificazione covalente di una proteina indotta da ROS o intermedi dello stress ossidativo

Ossidazione proteica

- Causata da:
  - agenti chimici
    (H_2O_2, Fe^{2+}, Cu^{+}, glutatione, HOCl, HOBr, $^1O_2$, $ONOO^-$)
  - Fagociti attivati (oxidative burst activity)
  - luce UV, ozono
  - Lipidi idroperossidi (HNE, MDA, acroleina)
  - Mitocondri (intermedi della catena respiratoria)
  - Ossidoreduttasi
  - Farmaci e loro metaboliti

Ossidazione proteica

- Cys e Met sono gli aminoacidi più suscettibili di ossidazione
- Possono essere reversibili se le cellule possiedono l'opportuno sistema enzimatico
  - metionina solfossido reduttasi
  - *tioredossine, ecc.*
- possibile funzione regolatoria
- "sacrificial scavengers"

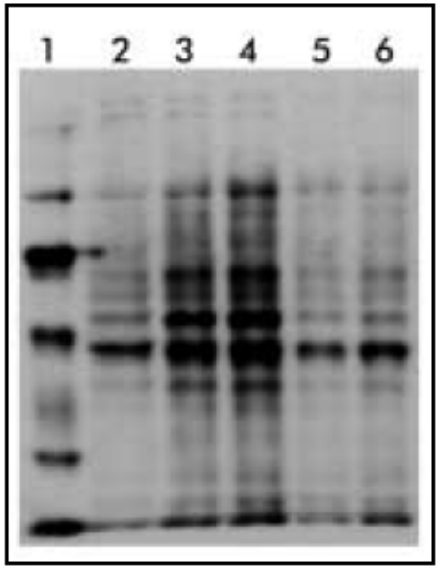
Ossidazione proteica

- Aminoacidi ossidati da ioni metallici:
  - Pro (γ-glutammilsemialdeide)
  - Arg (γ-glutammilsemialdeide)
  - Lys (semialdeide amminoadipica)
  - Thr (amminochetobutirrato)

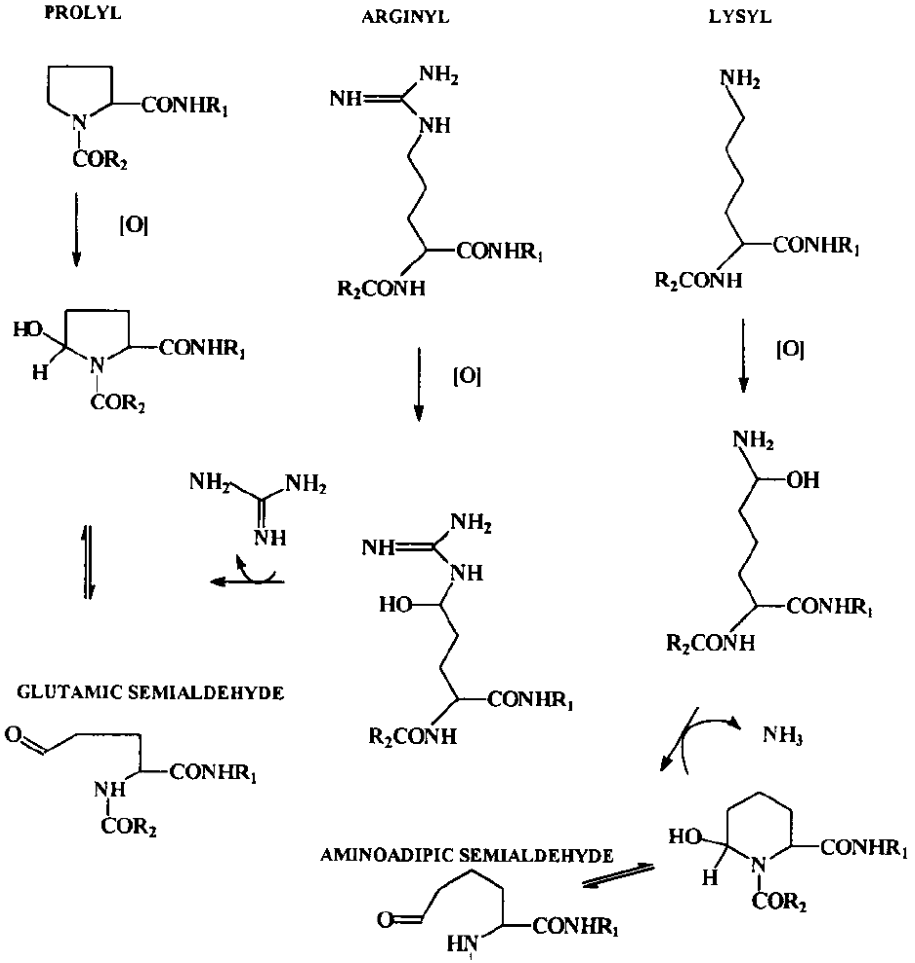
Glutamic and aminoadipic semialdehydes are the main carbonyl products of metal-catalyzed oxidation of proteins

Jesús R. Requena, Chien-Chung Chao, Rodney L. Levine, and Earl R. Stadtman*

Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Building 3, Room 222, 3 Center Drive, Bethesda, MD 20892



Lane 1: Molecular Weight Standard
Lane 2: HeLa Control Cells – DNPH Derivatized
Lane 3: HeLa H_2O_2 (150 μM) 30 min. – DNPH Derivatized
Lane 4: HeLa H_2O_2 (150 μM) 30 min. + 3 hr. incubation – DNPH Derivatized
Lane 5: HeLa Control – No DNPH, Derivatization Control
Lane 6: HeLa H_2O_2 (150 μM) 30 min. – No DNPH, Derivatization Control



Ossidazione proteica indotta da HOCl

- Formazione di clorotirosine e ditirosine
- Dipende solo dalla mieloperossidasi, riflettendo l'attività di neutrofili e monociti
- E' un marker dello stress ossidativo di tipo infiammatorio

Ossidazione proteica e perossidazione lipidica

- Addizione covalente di HNE, MDA, acroleina a residui Lys, Cys e His
- Origine aspecifica, prevalentemente ioni metallici e radiazioni ionizzanti
- Importante nelle malattie neurodegenerative

ELSEVIER

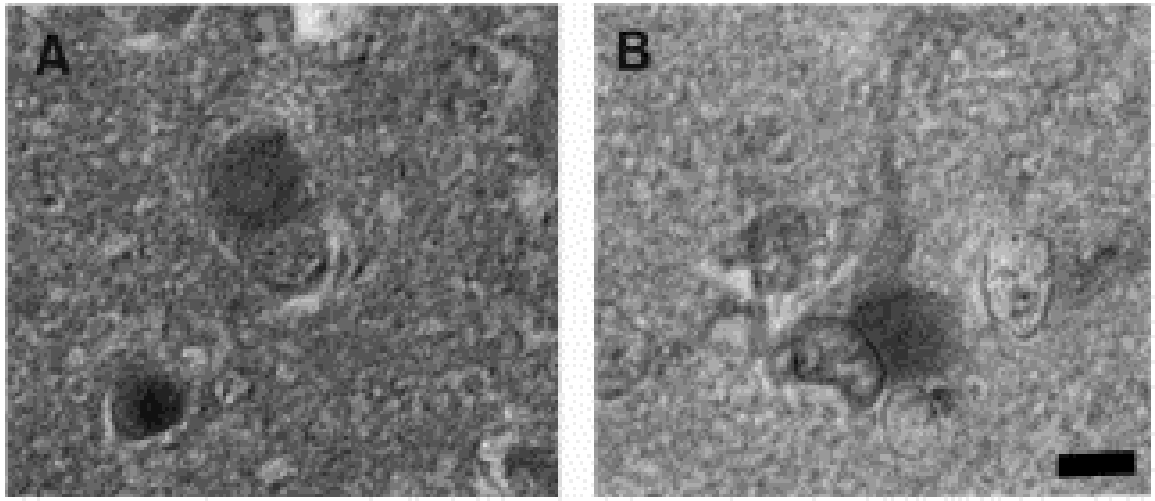
Neuroscience Letters 319 (2002) 25–28

Neuroscience Letters

www.elsevier.com/locate/neulet

Hydroxynonenal adducts indicate a role for lipid peroxidation in neocortical and brainstem Lewy bodies in humans

Rudy J. Castellani[a], George Perry[a,*], Sandra L. Siedlak[a], Akihiko Nunomura[b], Shun Shimohama[c], Jing Zhang[d], Thomas Montine[d], Lawrence M. Sayre[e], Mark A. Smith[a]



Lewy bodies in cortical neurons of DLBD demonstrate hydroxynonenal (A) and carboxymethyllysine (B) immunoreactivity. Scale bar, 20 μm.

Fattori genetici che causano stress ossidativo in malattie neurodegenerative

• Catena leggera della ferritina	➢ *Neuroferritinopatia*
• loss-of-function pantotenato-chinasi	➢ *Sindrome di Hallervorden-Spatz*
• Ceruloplasmina	➢ *Aceruloplasminemia*
• Fratassina	➢ *Atassia di Friedreich*
• BACE, preseniline	➢ *Malattia di Alzheimer*
• ATP7B	➢ *Malattia di Wilson*
• α-Sinucleina	➢ *Malattia di Parkinson*
• gain-of-function Cu,Zn-SOD	➢ *Sclerosi laterale amiotrofica familiare*

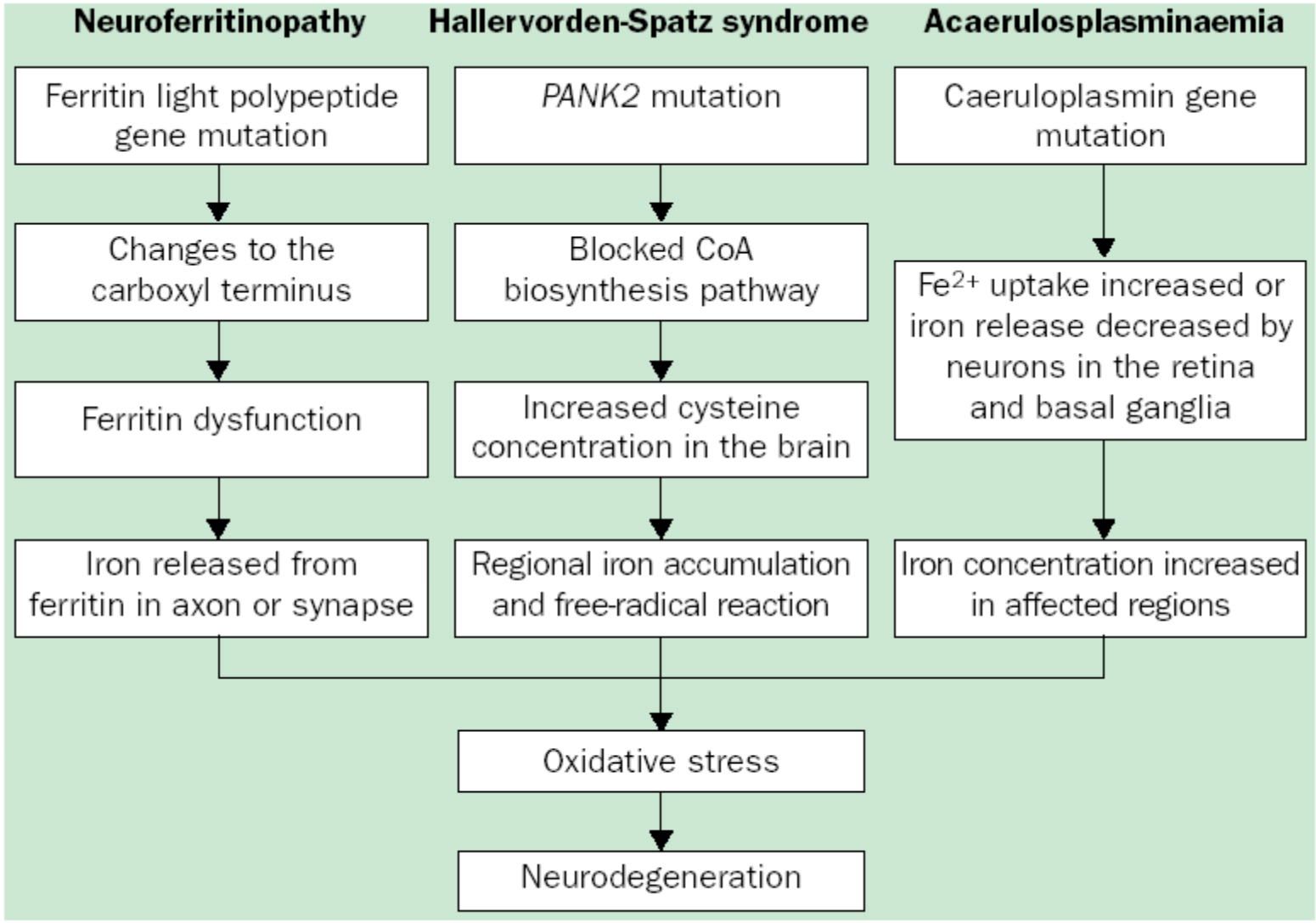
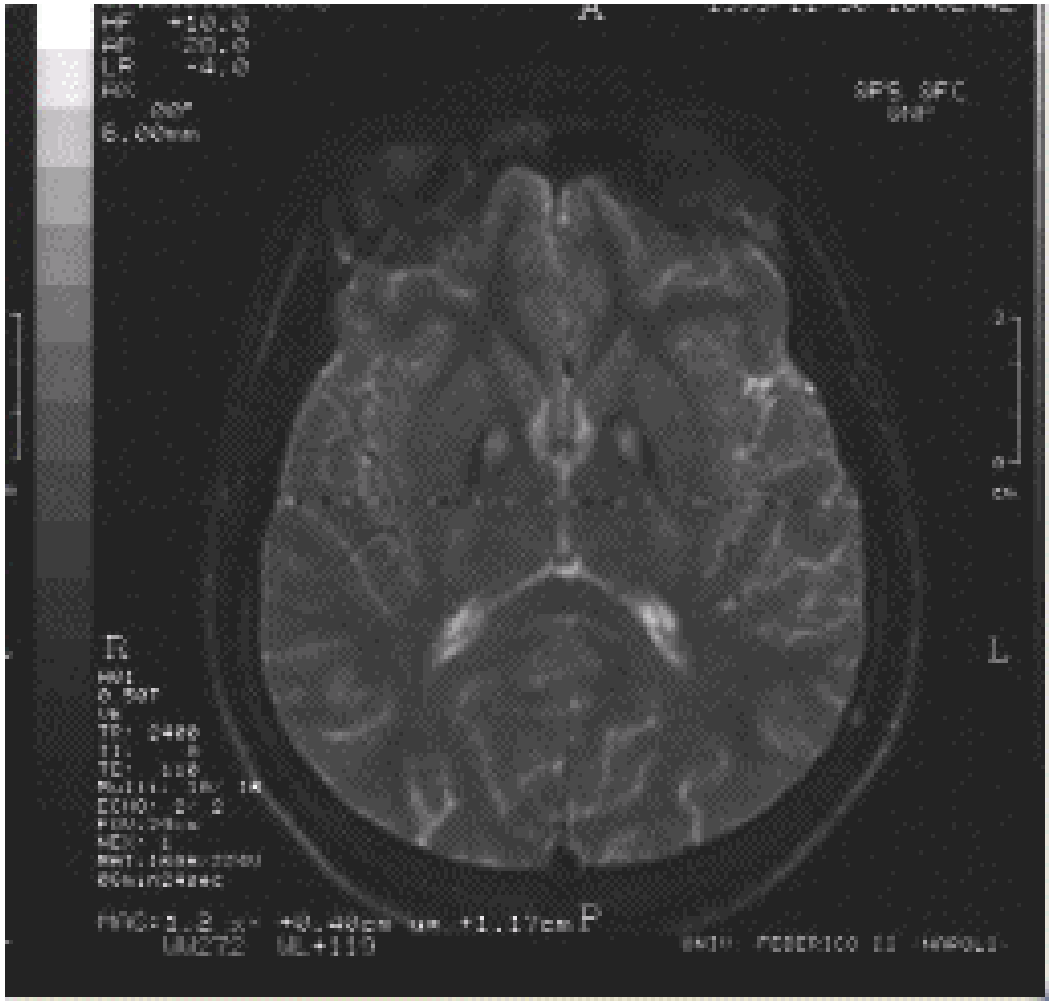


Figure 1. The role of genetic factors in iron misregulation in the development of neurodegenerative disorders.

Sindrome di Hallervorden-Spatz (NBIA-1)

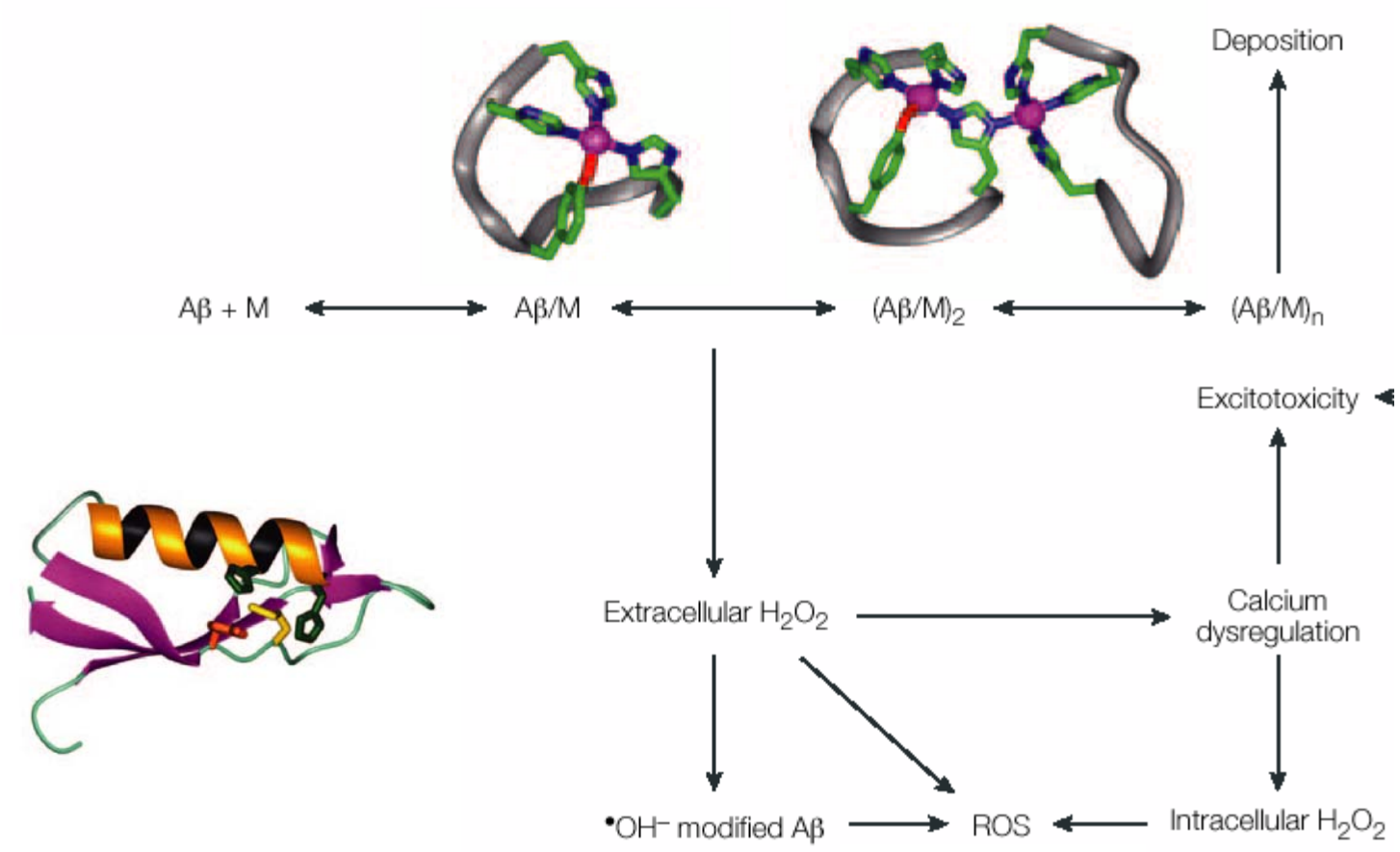


Fratassina
(Atassia di Friedreich)

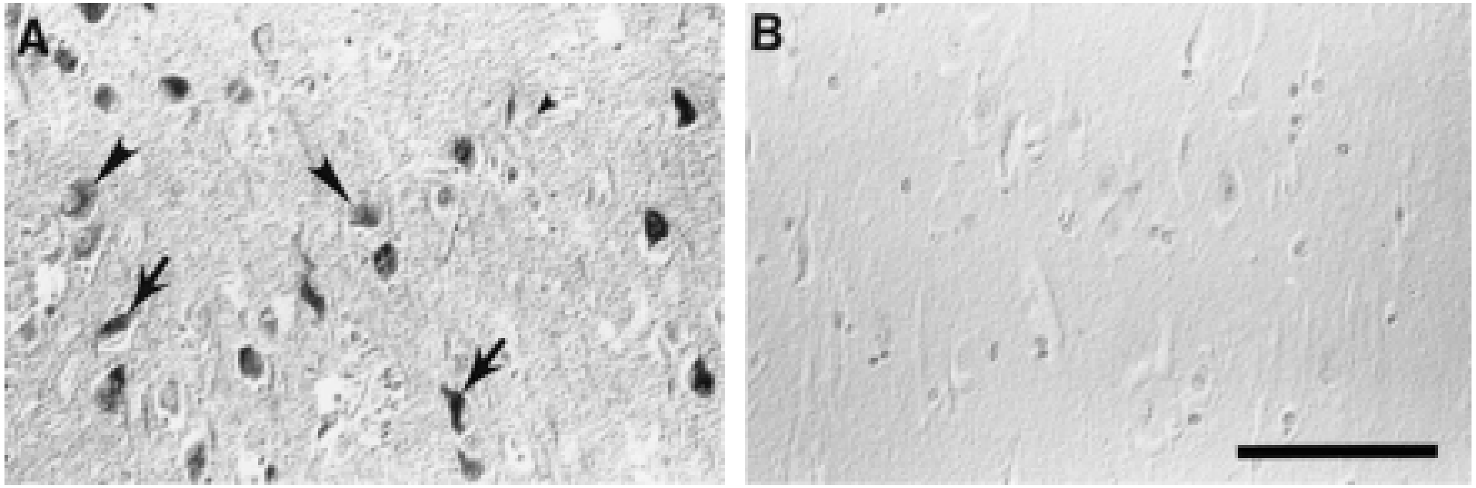
- Espansione GAA nel primo introne
- Accumulo di Fe mitocondriale
- Ridotta funzionalità proteine Fe/S

  - Neurodegenerazione
  - Cardiomiopatia

Aβ lega Cu^{2+}

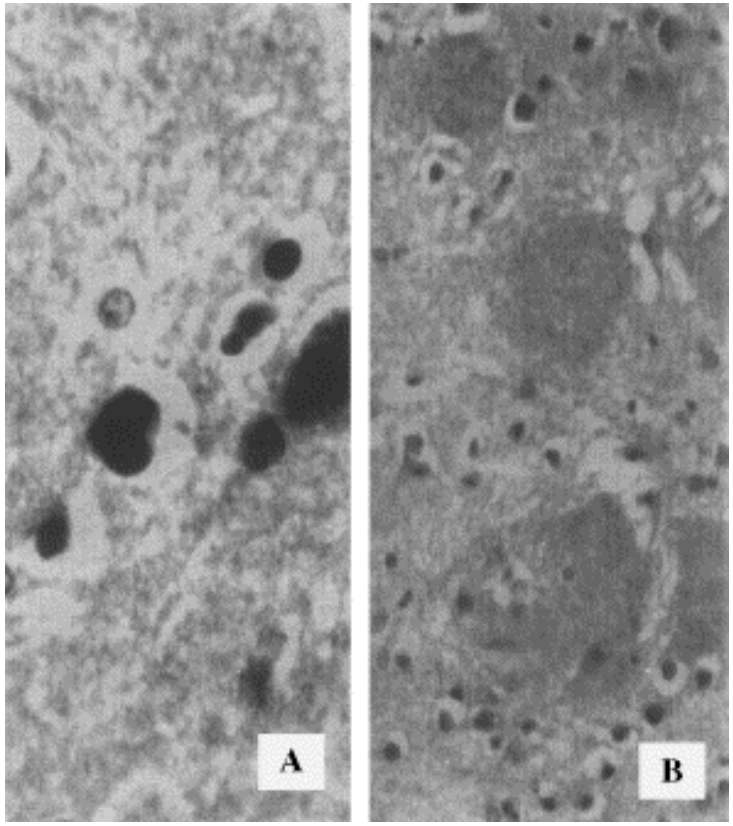


Gruppi carbonilici (ossidazione proteica) nella malattia di Alzheimer

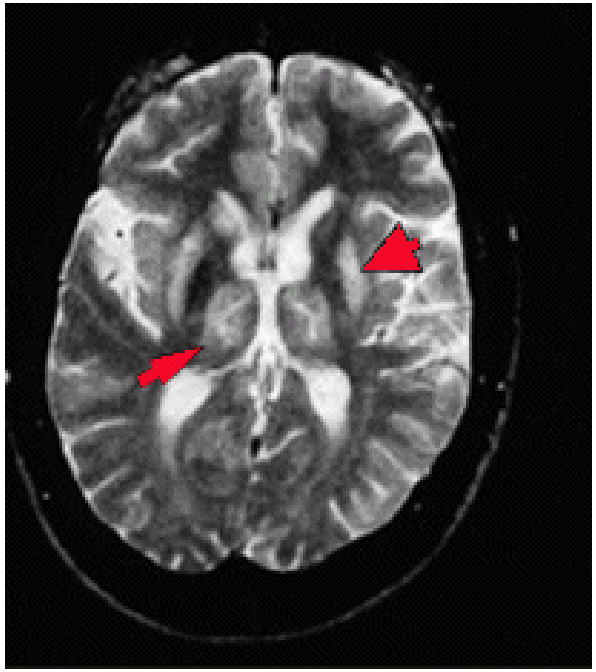


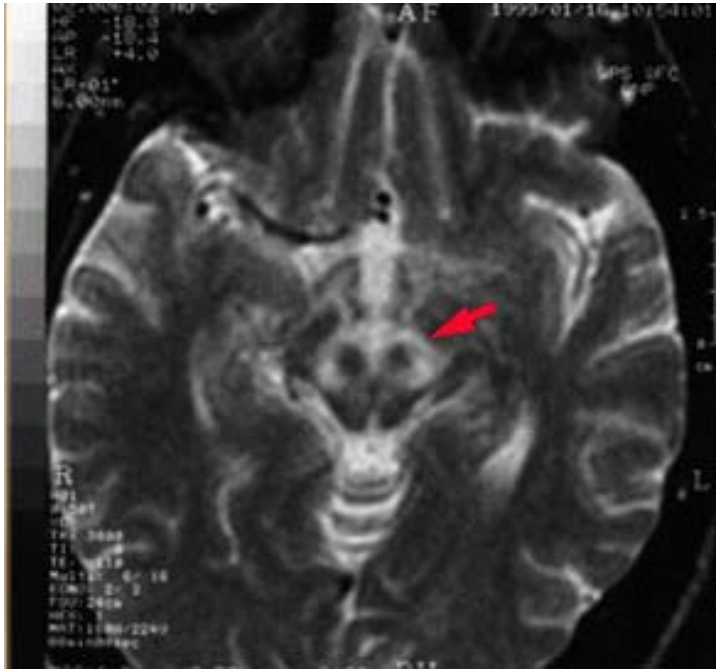
26, 1998

Addizione di HNE (perossidazione lipidica) nella malattia di Alzheimer

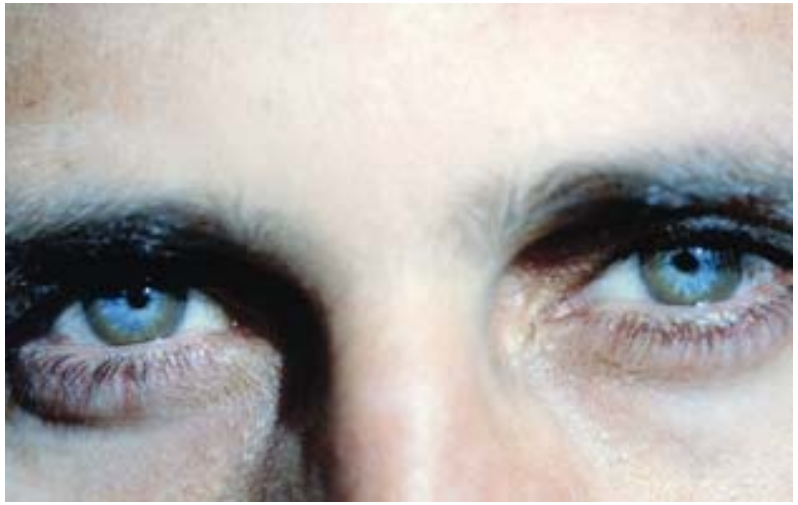


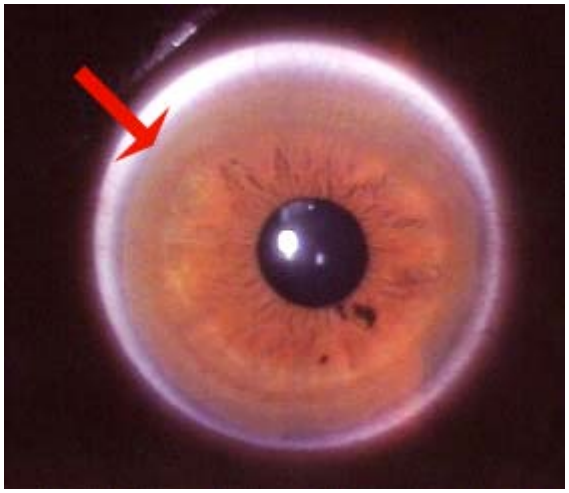
Malattia di Wilson





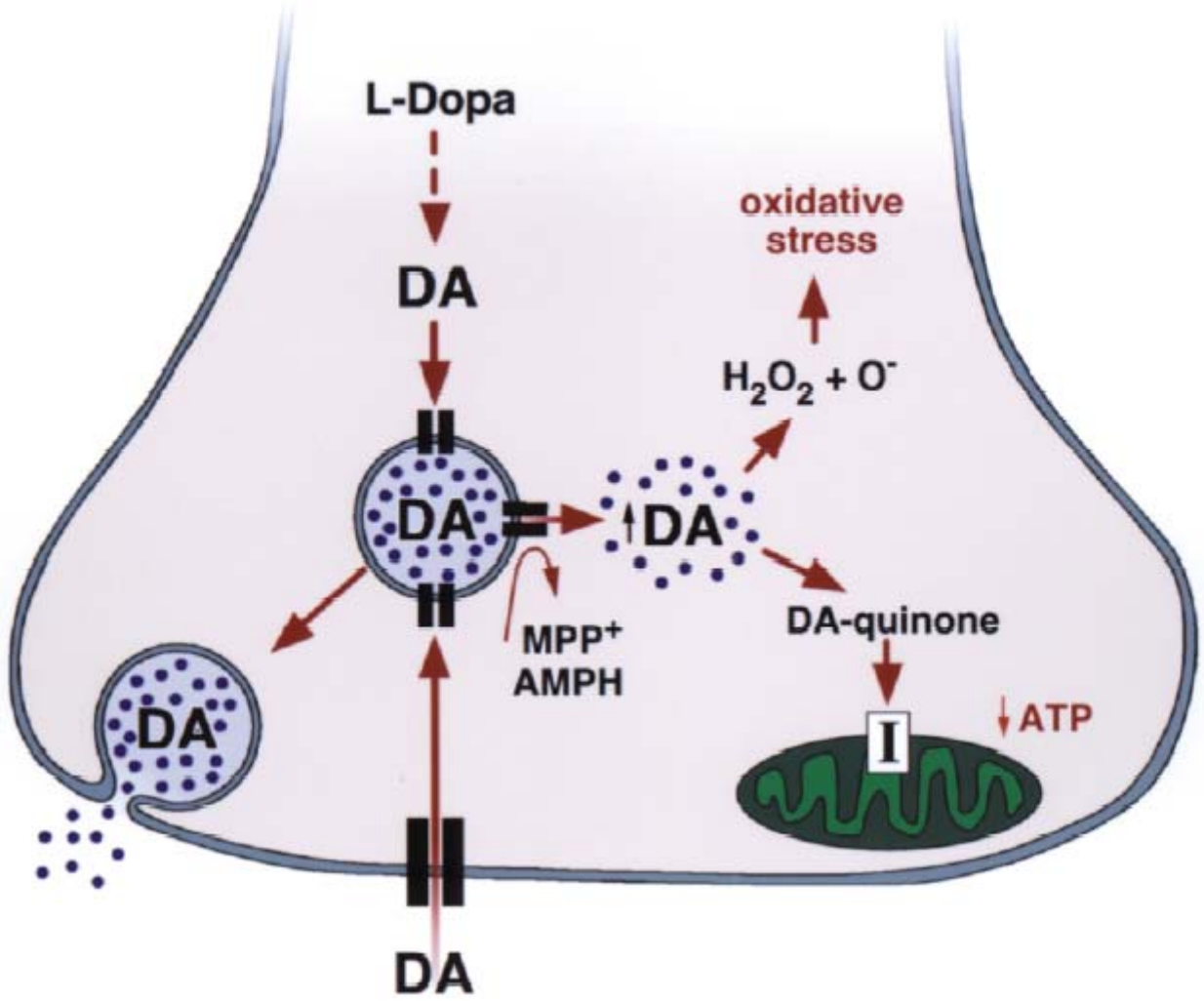
Malattia di Wilson



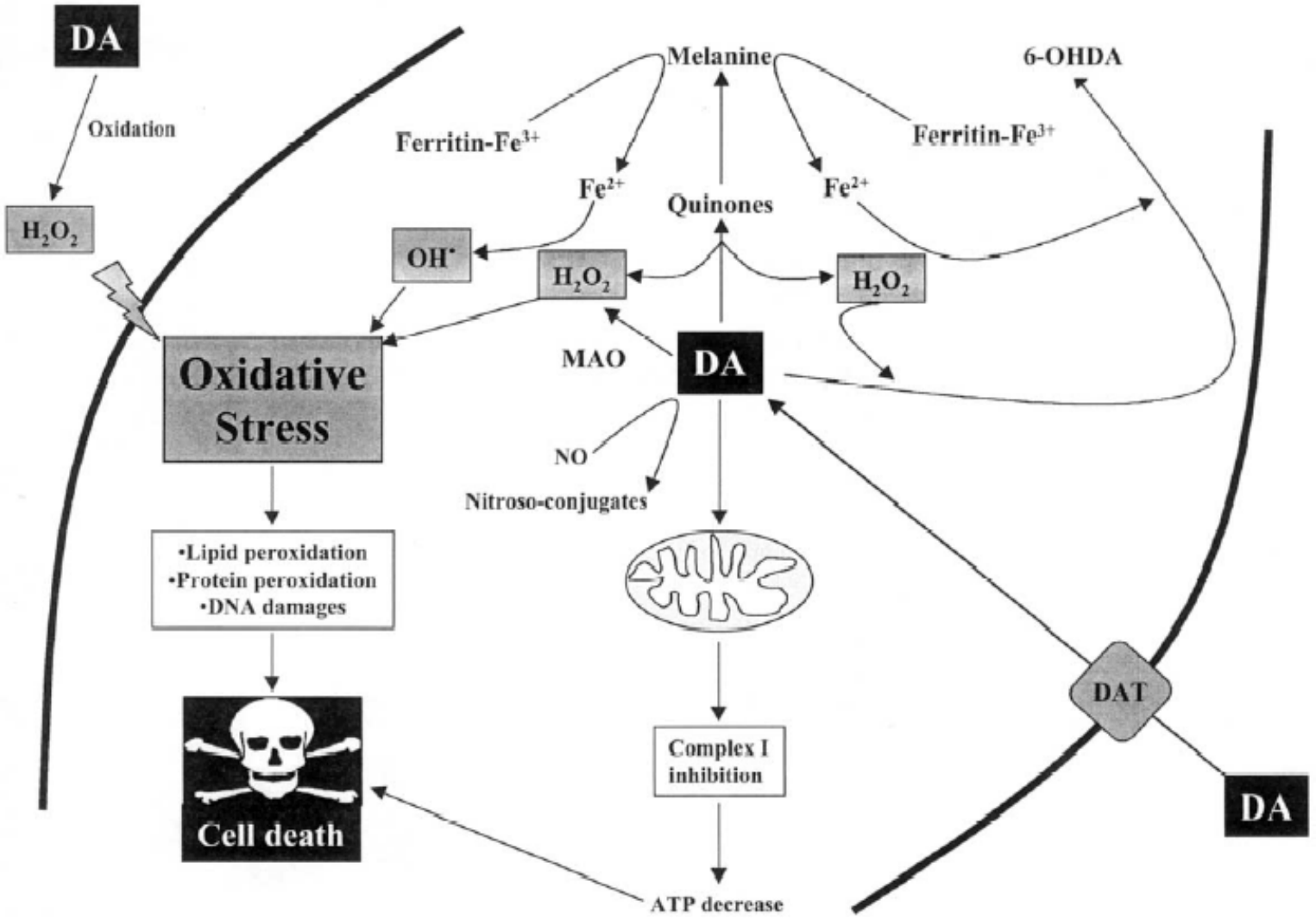


Anello di Kayser-Fleischer

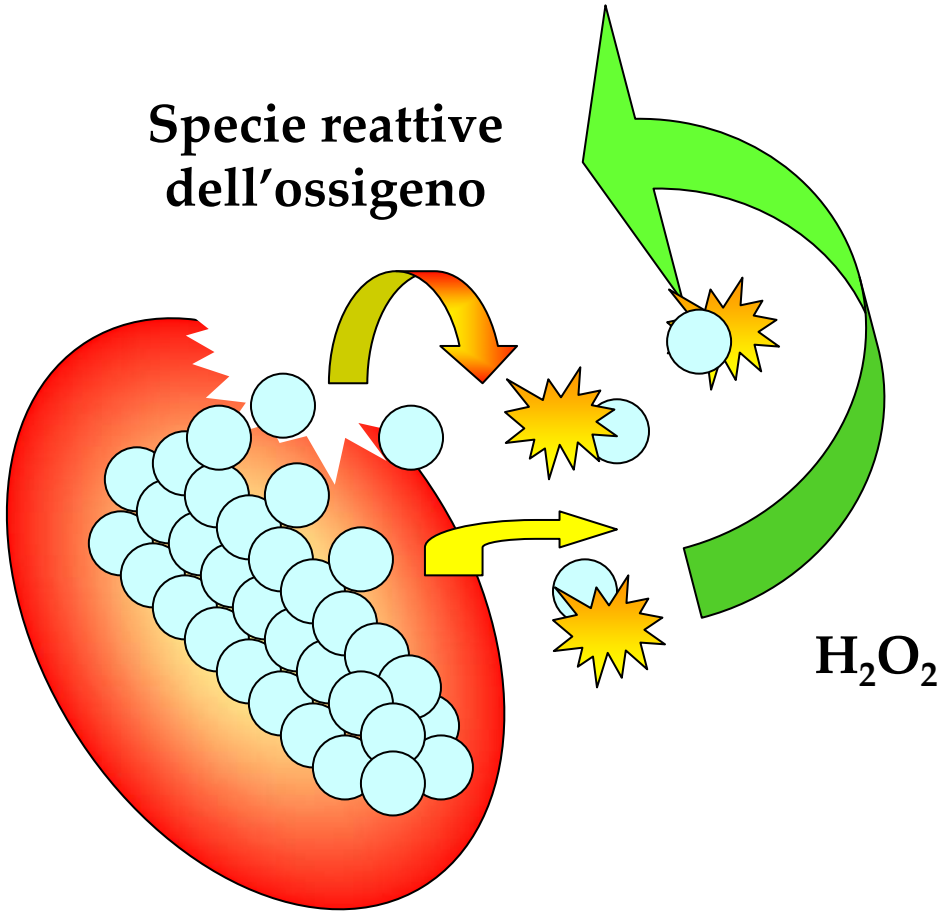
Stress ossidativo nel terminale nigrostriatale



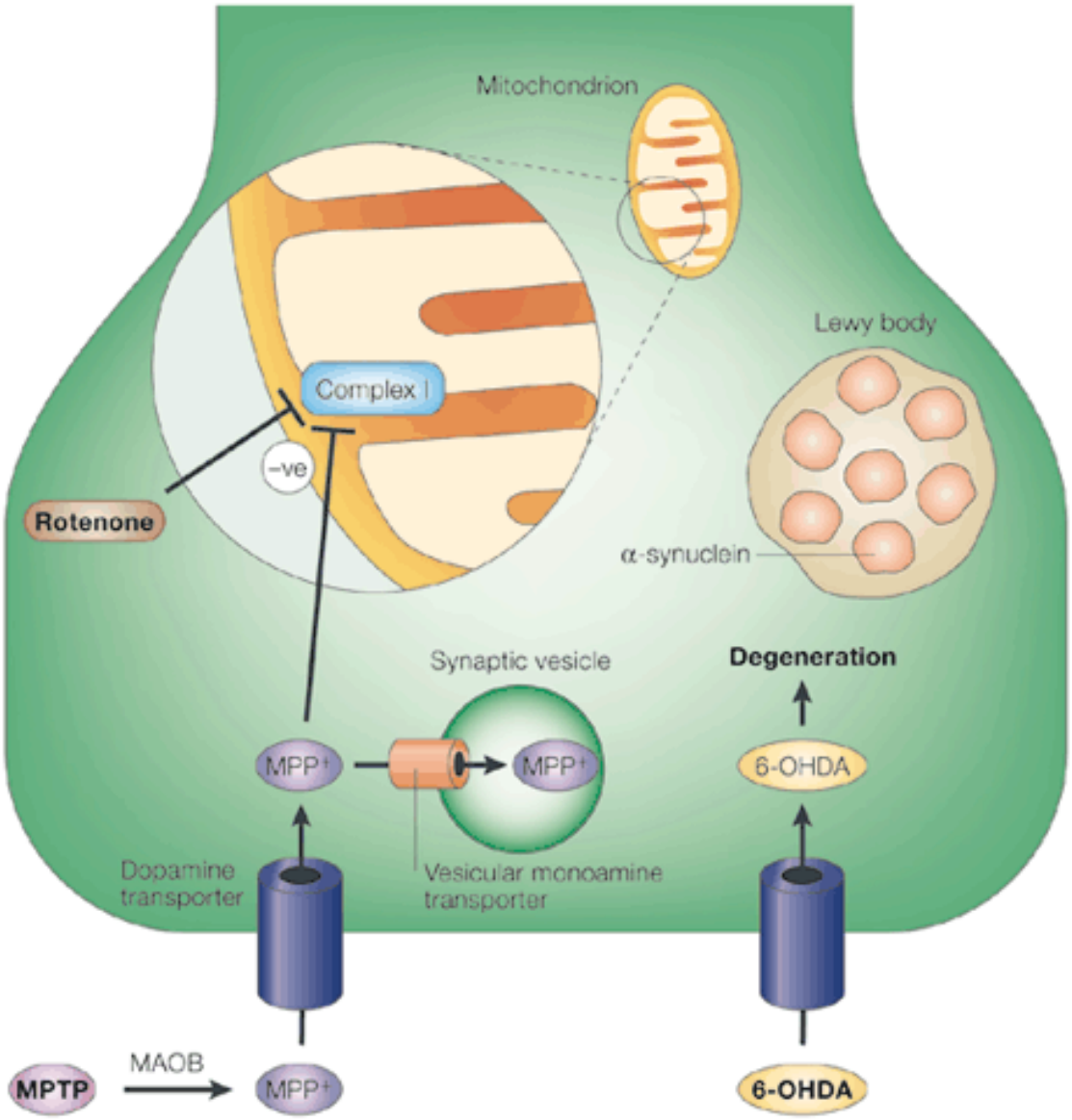
Lotharius & Brundin, Hum. Mol. Genet. 2002


DA
Oxidation
H_2O_2
Melanine
6-OHDA
Ferritin-Fe^{3+}
Ferritin-Fe^{3+}
Fe^{2+}
Fe^{2+}
Quinones
$OH^{\cdot}$
H_2O_2
H_2O_2
Oxidative Stress
MAO
DA
NO
Nitroso-conjugates
•Lipid peroxidation
•Protein peroxidation
•DNA damages
DAT
Complex I inhibition
Cell death
ATP decrease
DA

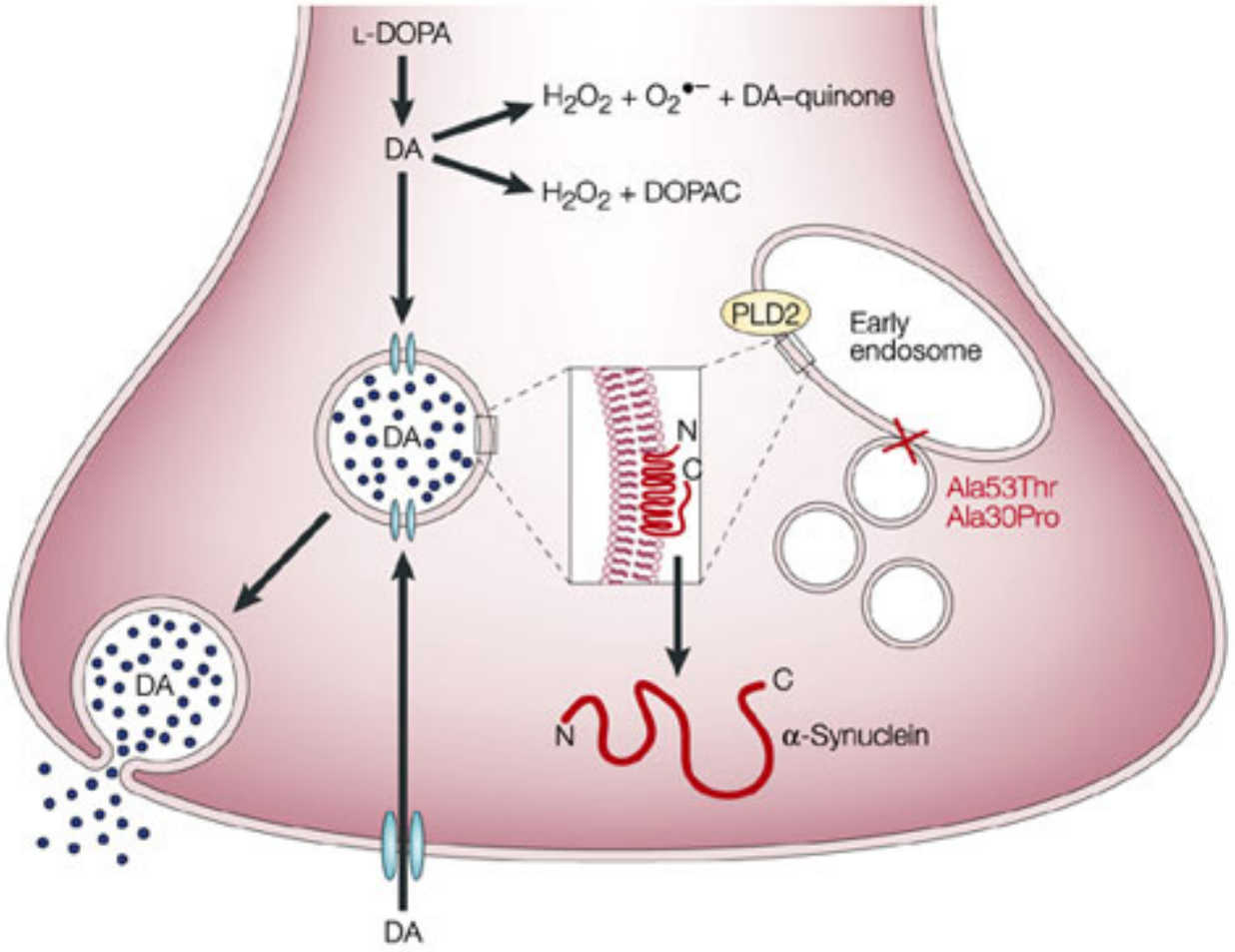
Il sistema ferro-neuromelanina amplifica lo stress ossidativo



Aime, Bergamasco, Casu, Digilio, Fasano, Giraudo, Lopiano, *Movement Disorders*, 2000.

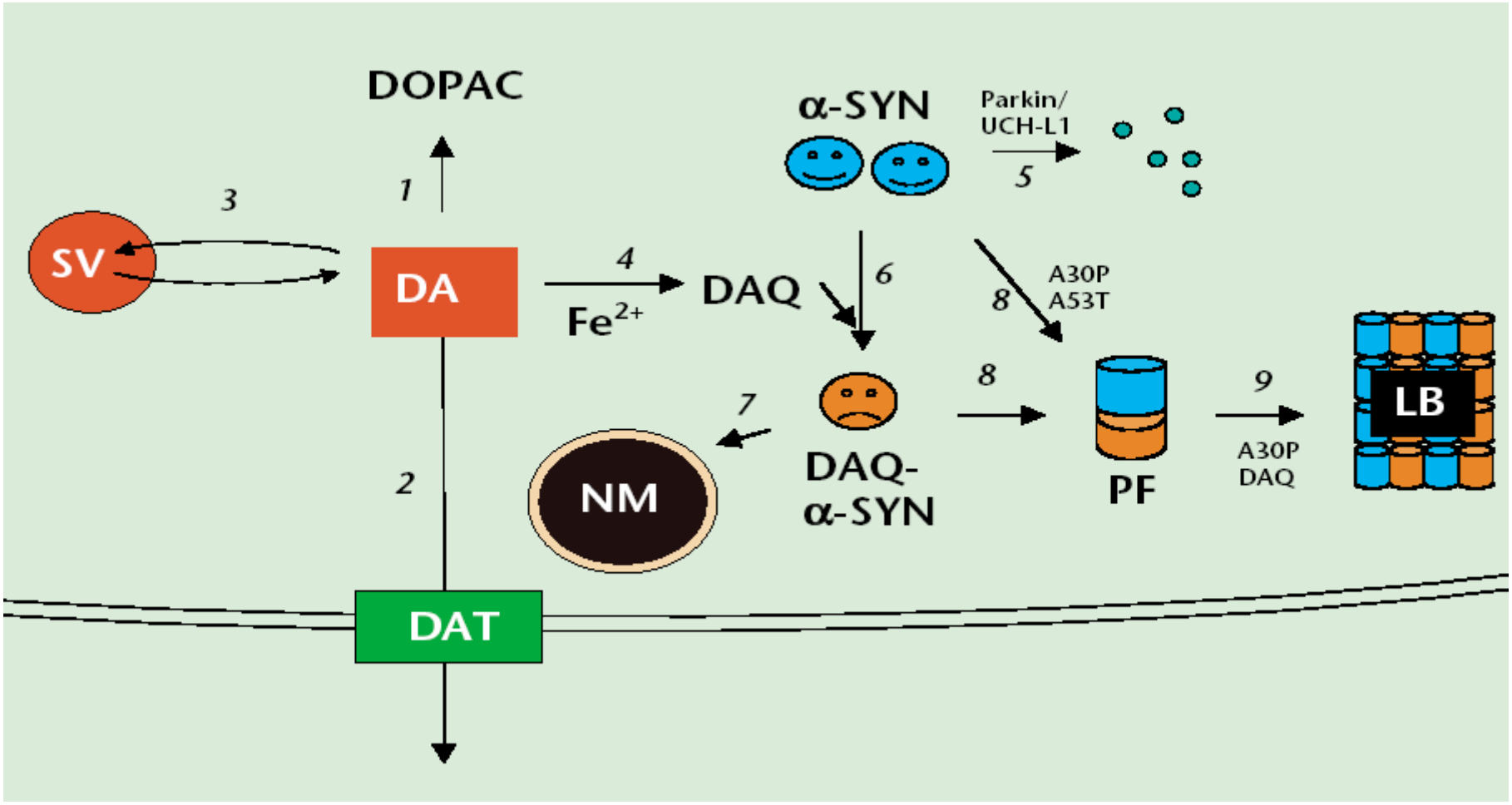


Nature Reviews | Neuroscience



Nature Reviews | Neuroscience

Interazione tra dopamina ossidata (DAQ) e α-sinucleina



Sulzer, Nature Med. 2001

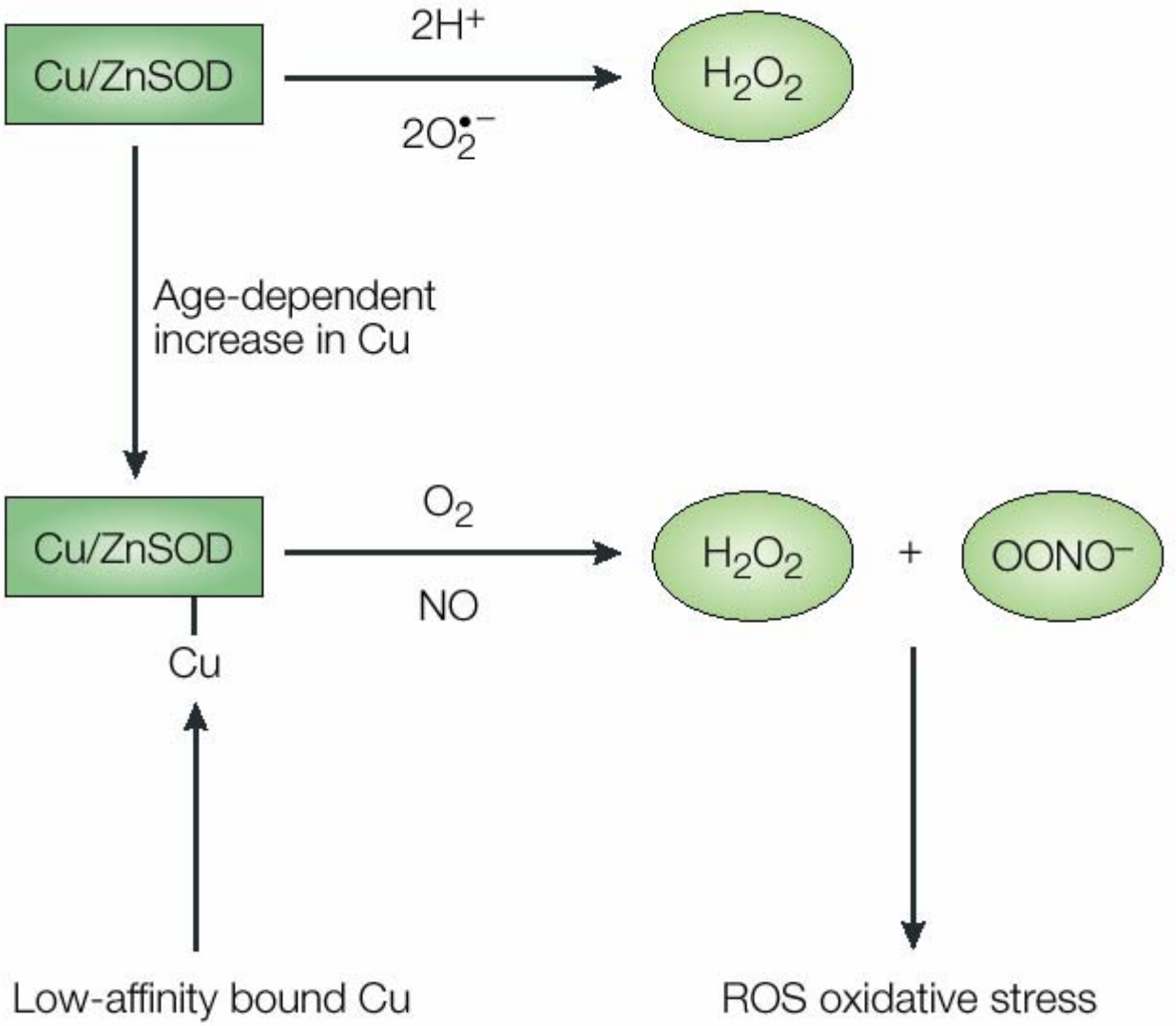


Figure 4 | **Oxidative stress in amyotrophic lateral sclerosis.**

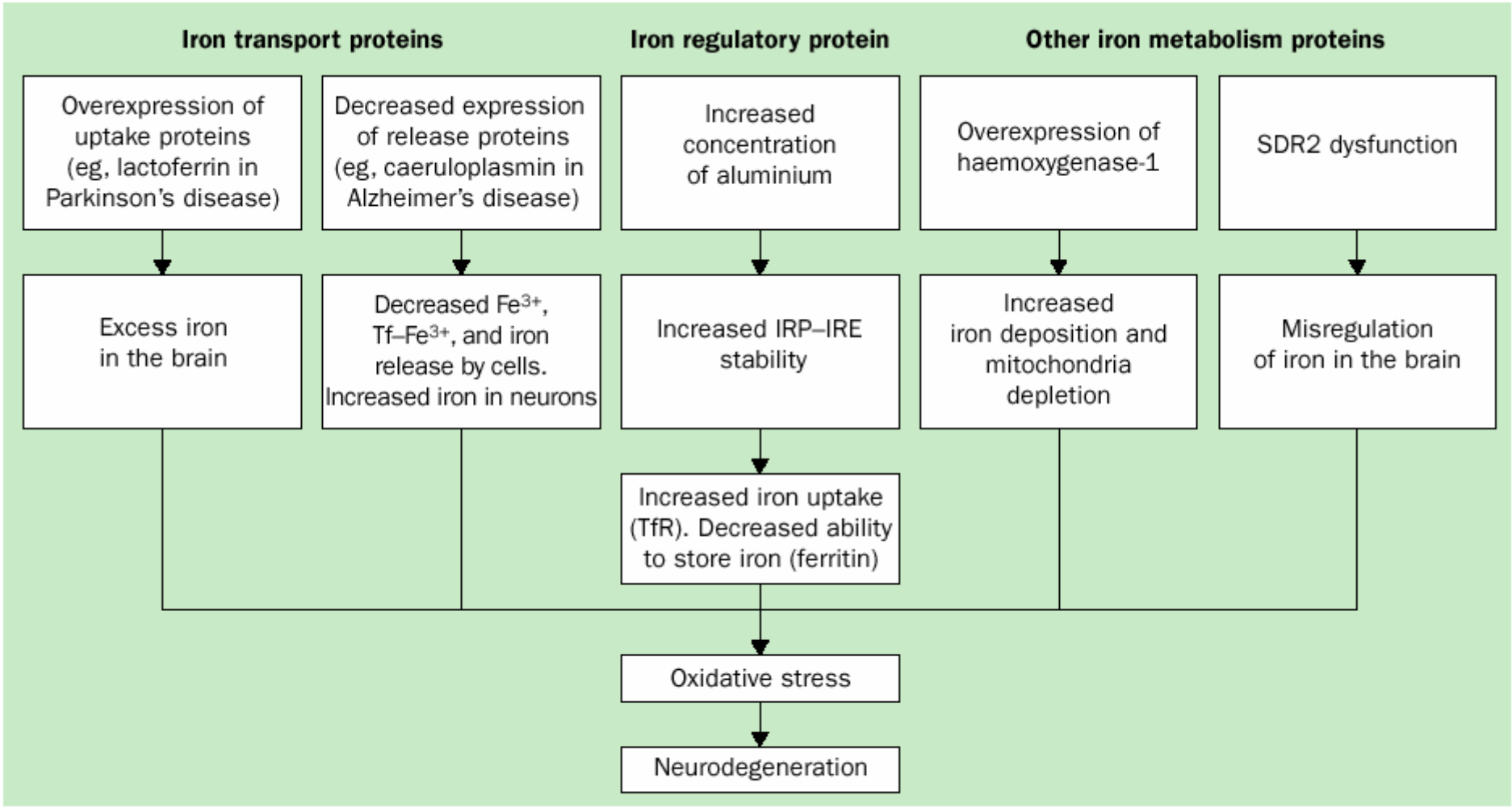
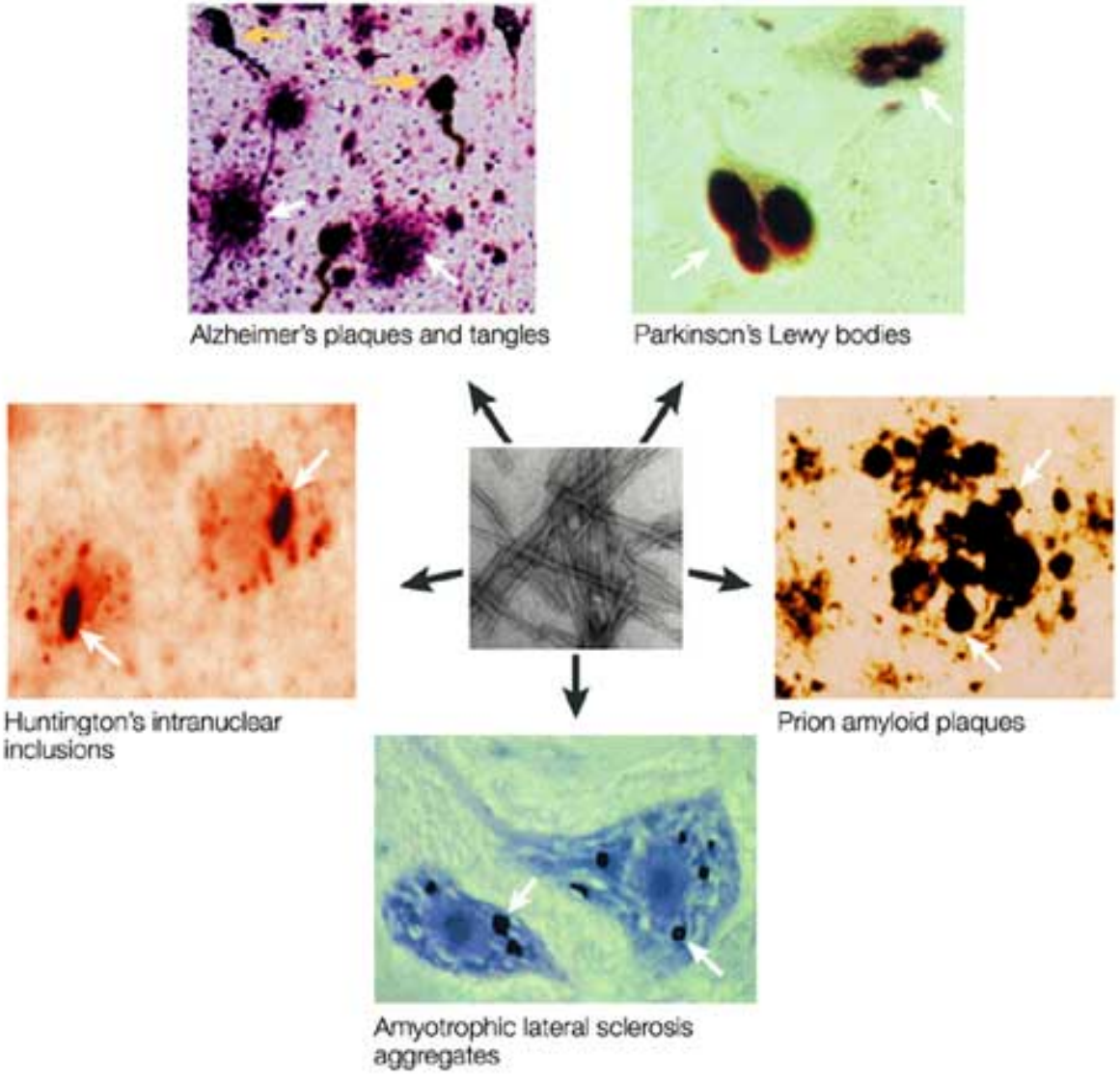


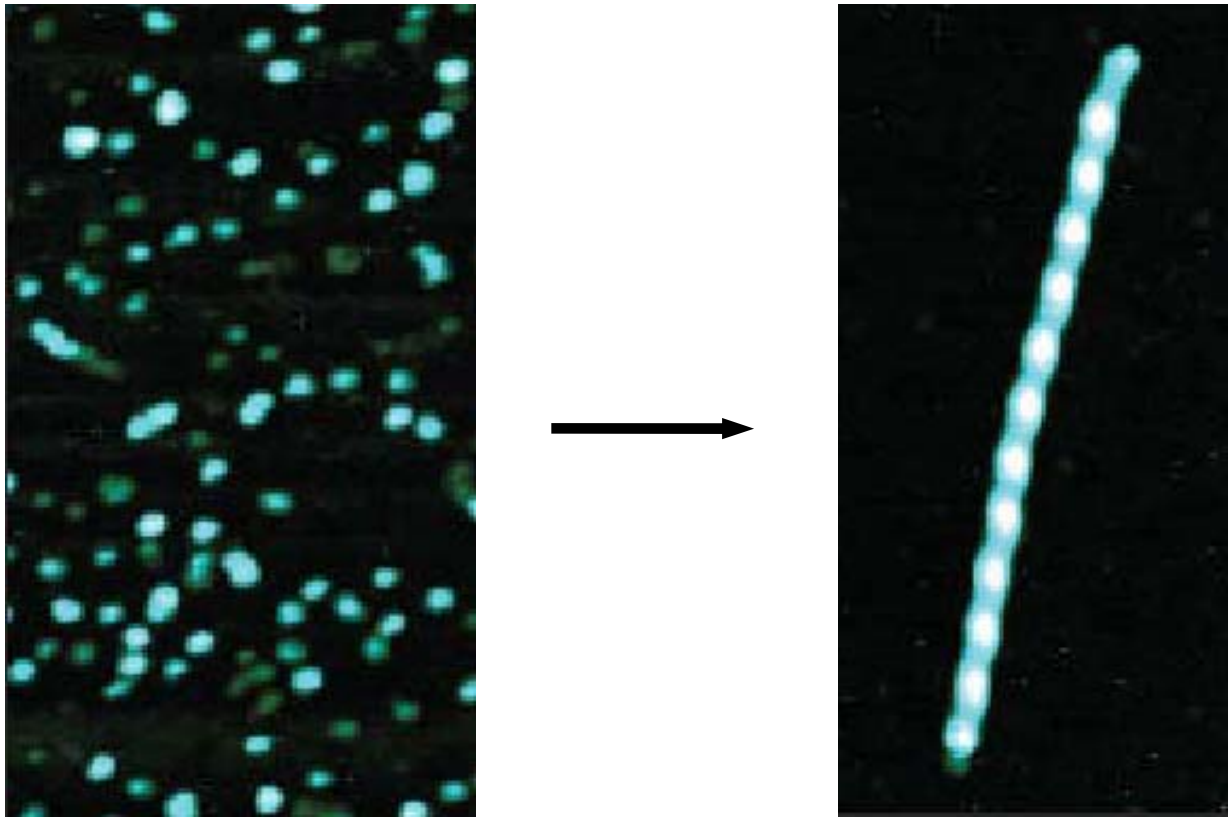
Figure 4. The possible role of non-genetic factors-induced misexpression of iron metabolism proteins in the development of some neurodegenerative disorders.

Patologie da aggregazione proteica

• Aβ	➢ *Malattia di Alzheimer*
• α-Sinucleina	➢ *Malattia di Parkinson*
• Huntingtina	➢ *Corea di Huntington*
• Proteina Prionica	➢ *Malattia di Creutzfeld-Jacob*

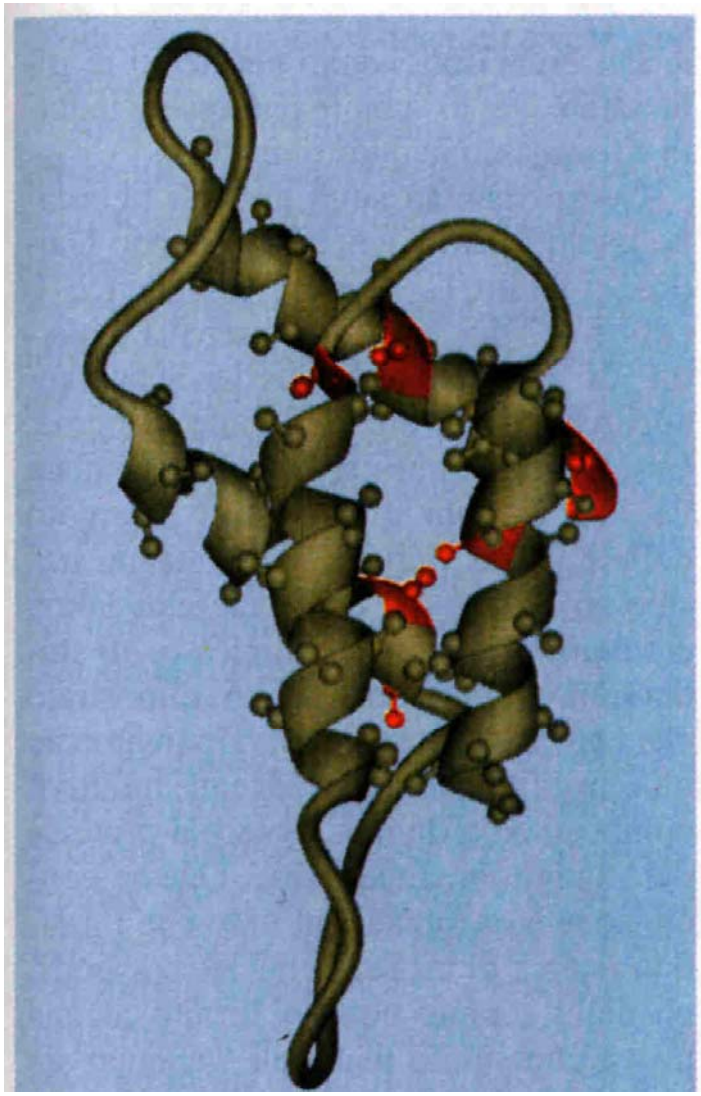

Alzheimer's plaques and tangles
Parkinson's Lewy bodies
Huntington's intranuclear inclusions
Prion amyloid plaques
Amyotrophic lateral sclerosis aggregates

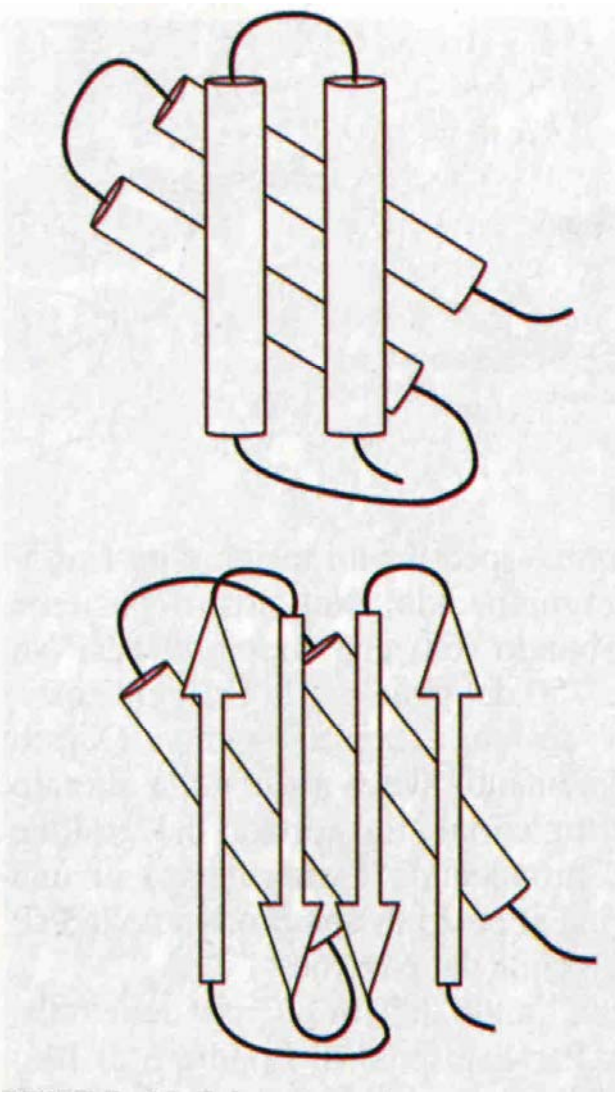
Fibrille di α-sinucleina (M. di Parkinson)



Conway et al., Science 2001

Proteina Prionica





Designed protein tetramer zipped together with a hydrophobic Alzheimer homology: A structural clue to amyloid assembly

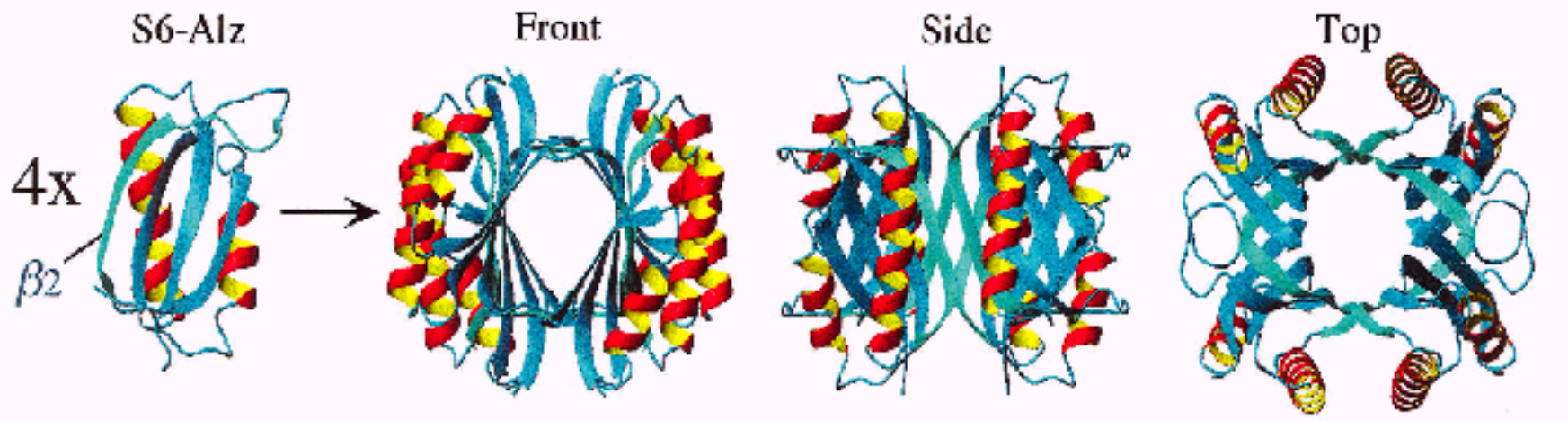
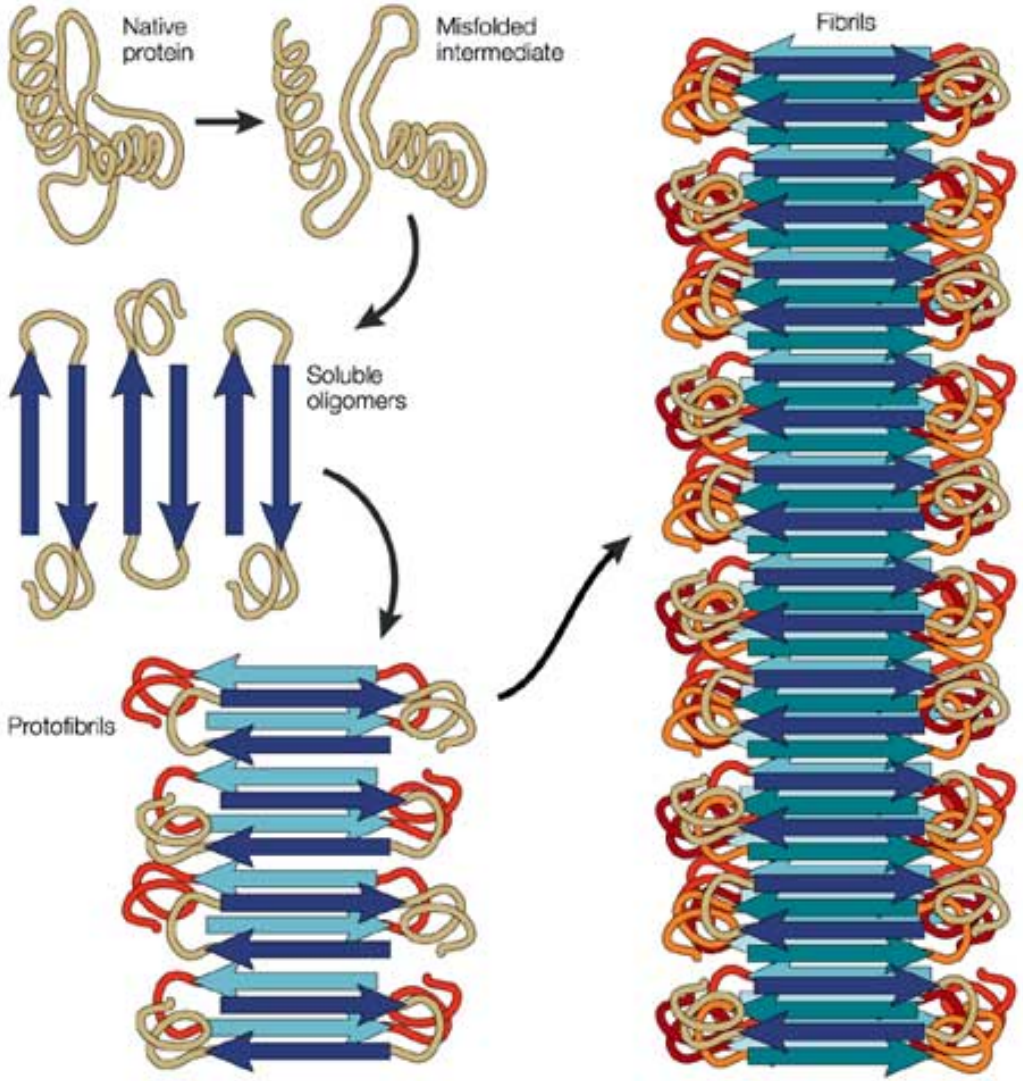


Fig. 3. The build-up of the S6-Alz tetramer. The monomers are joined by the β-AP homology in strand 2 (β2), forming intermolecular β-sheets.



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