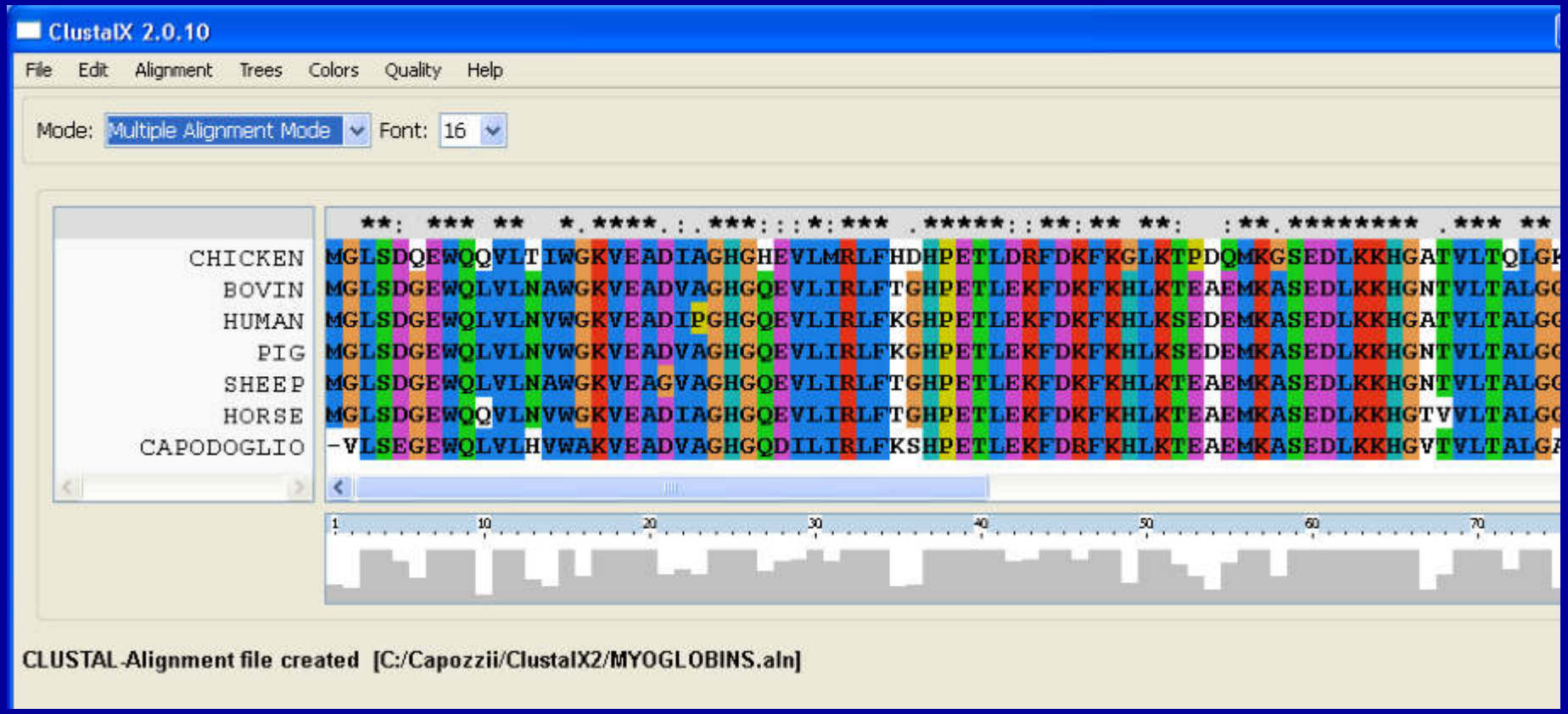
Chimica strutturale delle proteine

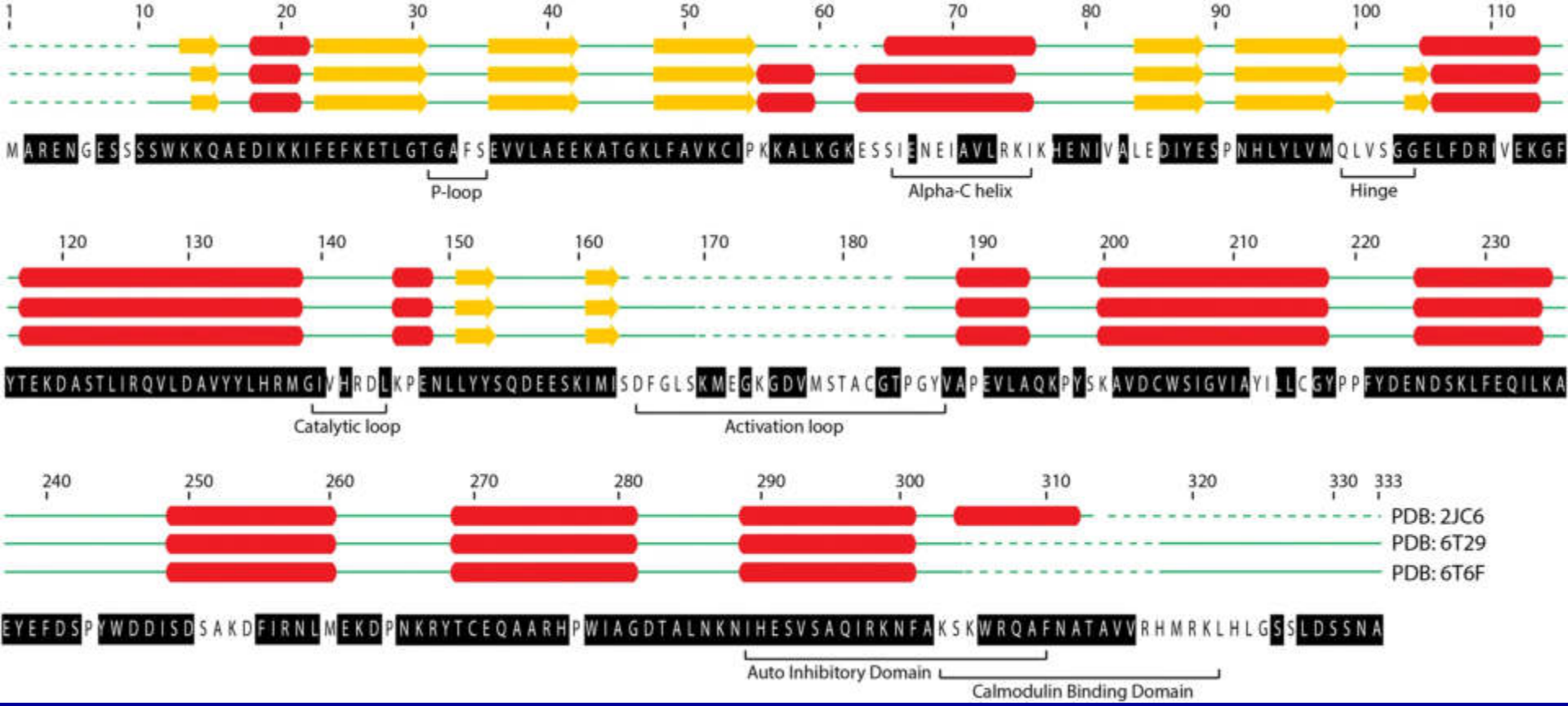
"Nel nostro corpo esistono almeno duecentomila tipi diversi di proteine e, secondo alcuni, abbiamo capito la funzione solo del due per cento di esse."

(da "Breve storia di (quasi) tutto" di "Bill Bryson").

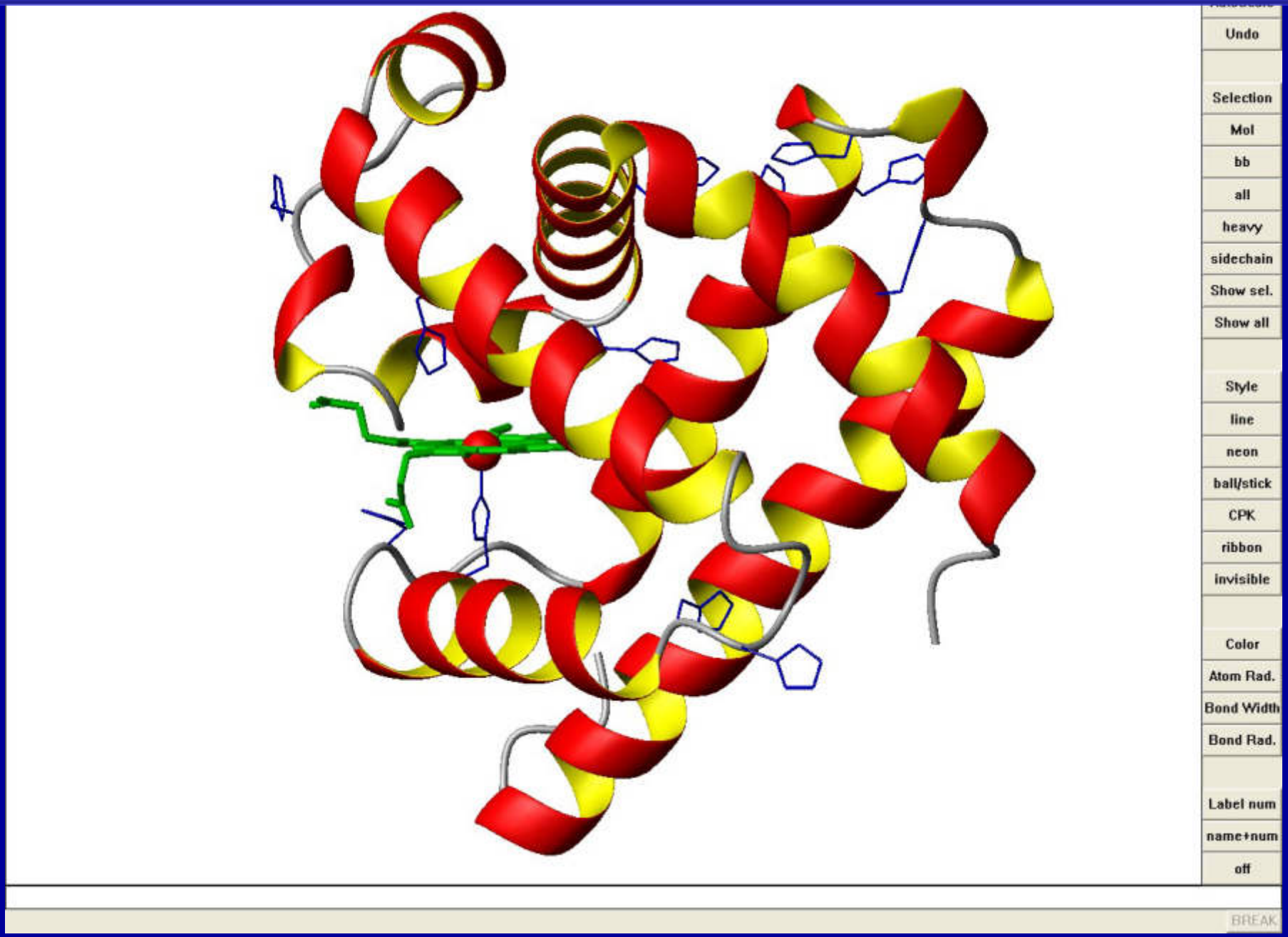
Struttura primaria delle proteine



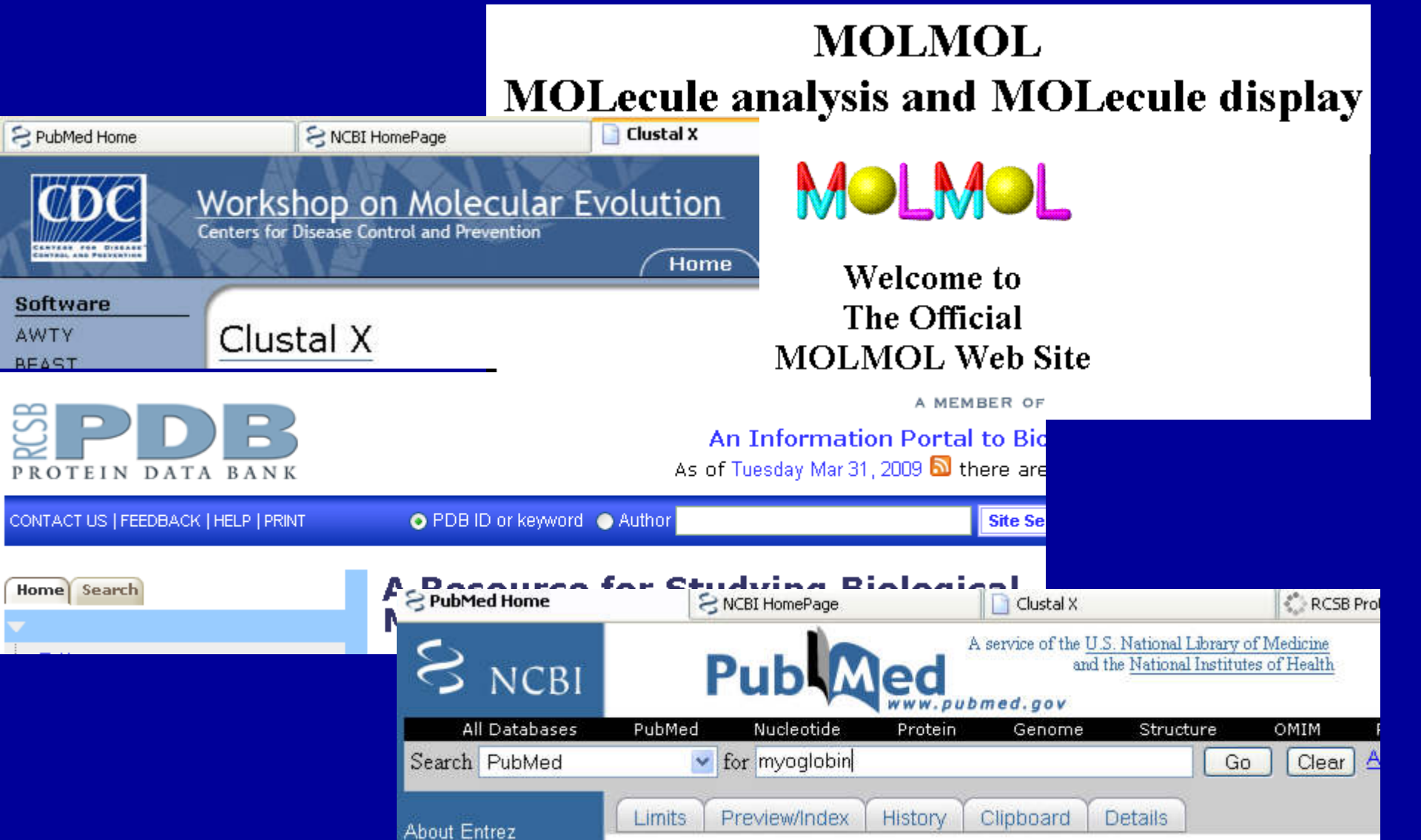
Struttura secondaria delle proteine

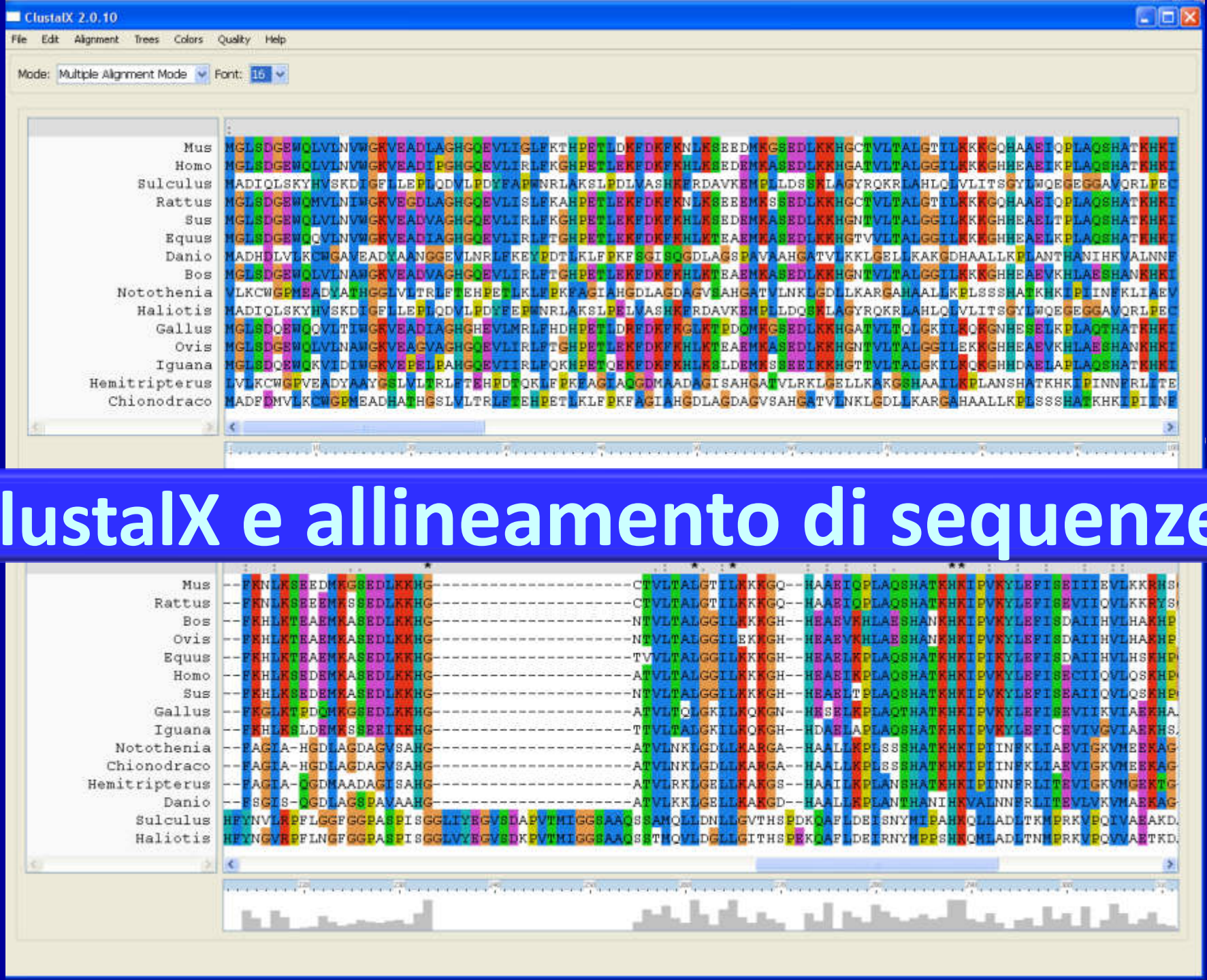


Struttura terziaria delle proteine



Bioinformatica




ClustalX 2.0.10
File Edit Alignment Trees Colors Quality Help
Mode: Multiple Alignment Mode
Font: 16
Mus
Homo
Sulculus
Rattus
Sus
Equus
Danio
Bos
Notothenia
Haliotis
Gallus
Ovis
Iguana
Hemitripterus
Chionodraco
ClustalX e allineamento di sequenze
Mus
Rattus
Bos
Ovis
Equus
Homo
Sus
Gallus
Iguana
Notothenia
Chionodraco
Hemitripterus
Danio
Sulculus
Haliotis

Protein data bank @ rcsb.org

1pit.pdb - WordPad

File Modifica Visualizza Inserisci Formato ?

```
REMARK   1  AUTH   G.WAGNER,K.WUTHRICH
REMARK   1  TITL   SEQUENTIAL RESONANCE ASSIGNMENTS IN PROTEIN 1H
REMARK   1  TITL 2 NUCLEAR MAGNETIC RESONANCE SPECTRA.  BASIC
REMARK   1  TITL 3 PANCREATIC TRYPSIN INHIBITOR
REMARK   1  REF    J.MOL.BIOL.                   V. 155   347 1982
REMARK   1  REFN   ASTM JMOBAK  UK ISSN 0022-2836                 070
REMARK   2
REMARK   2 RESOLUTION. NOT APPLICABLE.  SEE REMARK 4.
REMARK   3
REMARK   3 REFINEMENT.  NONE.
REMARK   3
REMARK   3 THREE-DIMENSIONAL STRUCTURE IN AQUEOUS SOLUTION AS
REMARK   3 DETERMINED BY NUCLEAR MAGNETIC RESONANCE AND DISTANCE
REMARK   3 GEOMETRY. DATA WERE COLLECTED AT PH 4.6, AND A TEMPERATURE
REMARK   3 OF 36 DEGREES CELSIUS. INPUT DATA CONSISTS OF 642 UPPER
REMARK   3 DISTANCE LIMIT CONSTRAINTS FROM NOE DATA; 41 PHI, 41 PSI,
REMARK   3 AND 33 CHI1 DIHEDRAL ANGLE CONSTRAINTS; 9 UPPER AND
REMARK   3 9 LOWER DISTANCE LIMIT CONSTRAINTS TO ENFORCE THE THREE
REMARK   3 DISULFIDE BONDS. THESE INPUT DATA ARE ALSO AVAILABLE
REMARK   3 FROM THE PROTEIN DATA BANK. A TOTAL OF 36 STEREOSPECIFIC
REMARK   3 PROTON RESONANCE ASSIGNMENTS WERE MADE.
REMARK   4
```

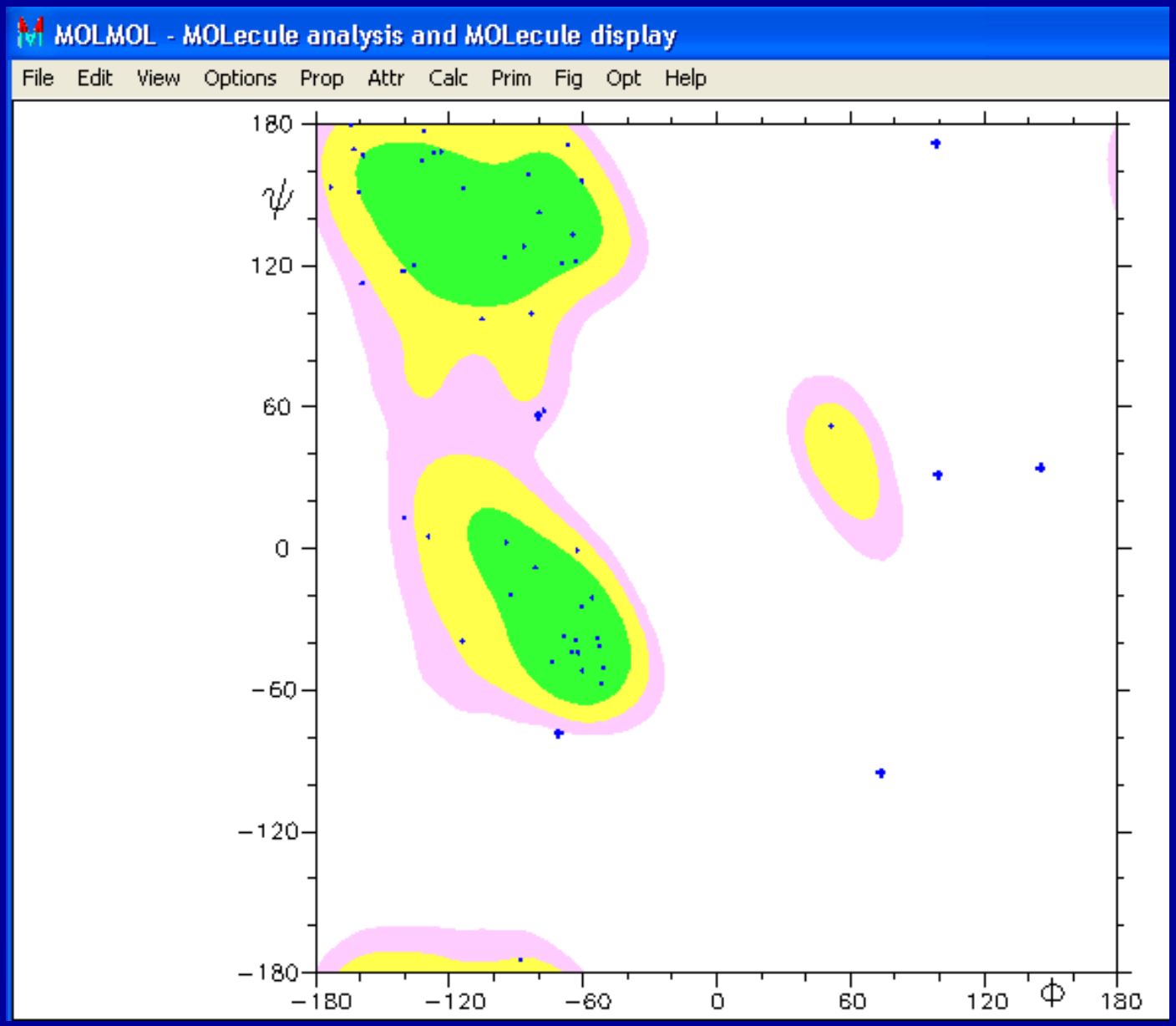
Coordinate di Proteine in PDB

1pit.pdb - WordPad

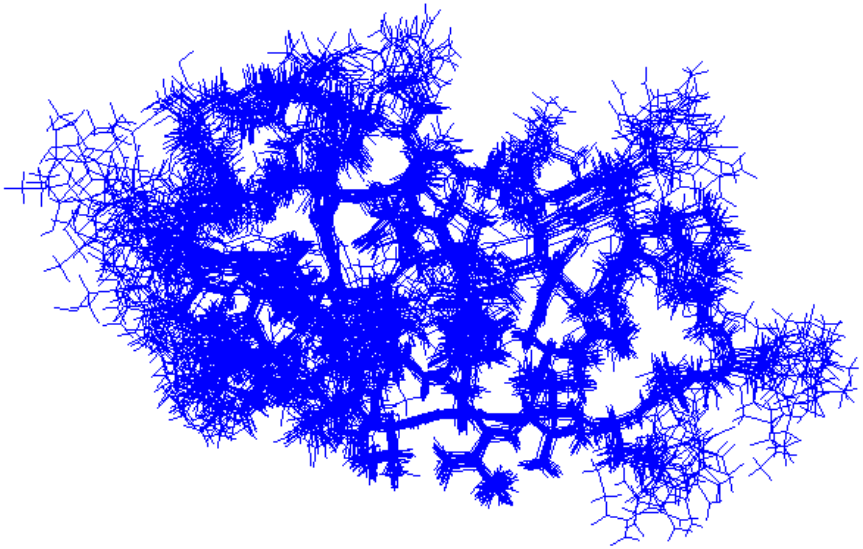
File Modifica Visualizza Inserisci Formato ?

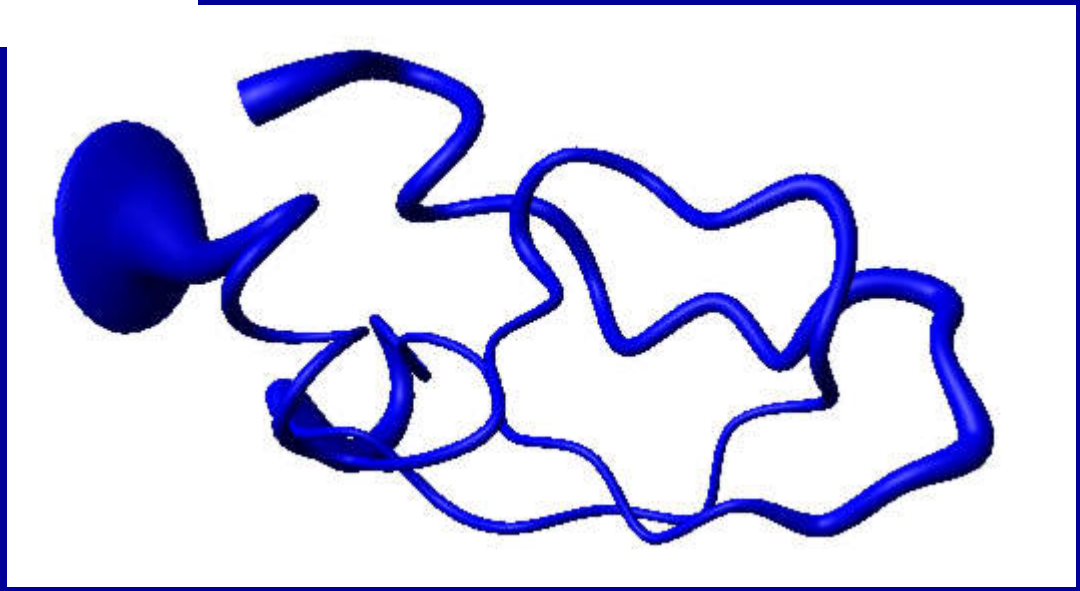
```
MODEL        1
ATOM      1  N   ARG     1      -8.544   3.578  14.046
ATOM      2  CA  ARG     1      -7.776   3.484  12.790
ATOM      3  C   ARG     1      -8.492   4.333  11.742
ATOM      4  O   ARG     1      -9.713   4.432  11.844
ATOM      5  CB  ARG     1      -7.640   2.025  12.309
ATOM      6  CG  ARG     1      -8.996   1.382  11.955
ATOM      7  CD  ARG     1      -8.875  -0.093  11.554
ATOM      8  NE  ARG     1      -8.120  -0.280  10.303
ATOM      9  CZ  ARG     1      -7.792  -1.484   9.802
ATOM     10  NH1 ARG     1      -8.140  -2.588  10.477
ATOM     11  NH2 ARG     1      -7.139  -1.609   8.640
ATOM     12  H   ARG     1      -8.193   3.022  14.799
ATOM     13  HA  ARG     1      -6.780   3.877  12.993
ATOM     14 1HB  ARG     1      -6.995   2.010  11.428
ATOM     15 2HB  ARG     1      -7.148   1.441  13.089
ATOM     16 1HG  ARG     1      -9.660   1.426  12.819
ATOM     17 2HG  ARG     1      -9.473   1.918  11.131
ATOM     18 1HD  ARG     1      -8.402  -0.641  12.369
ATOM     19 2HD  ARG     1      -9.887  -0.479  11.411
ATOM     20  HE  ARG     1      -7.881   0.558   9.793
ATOM     21 1HH1 ARG     1      -8.653  -2.502  11.341
ATOM     22 2HH1 ARG     1      -7.954  -3.514  10.126
ATOM     23 1HH2 ARG     1      -6.921  -0.841   8.004
ATOM     24 2HH2 ARG     1      -6.870  -2.518   8.306
ATOM     25  QB  ARG     1      -7.072   1.726  12.259
```

Ramachandran Plot

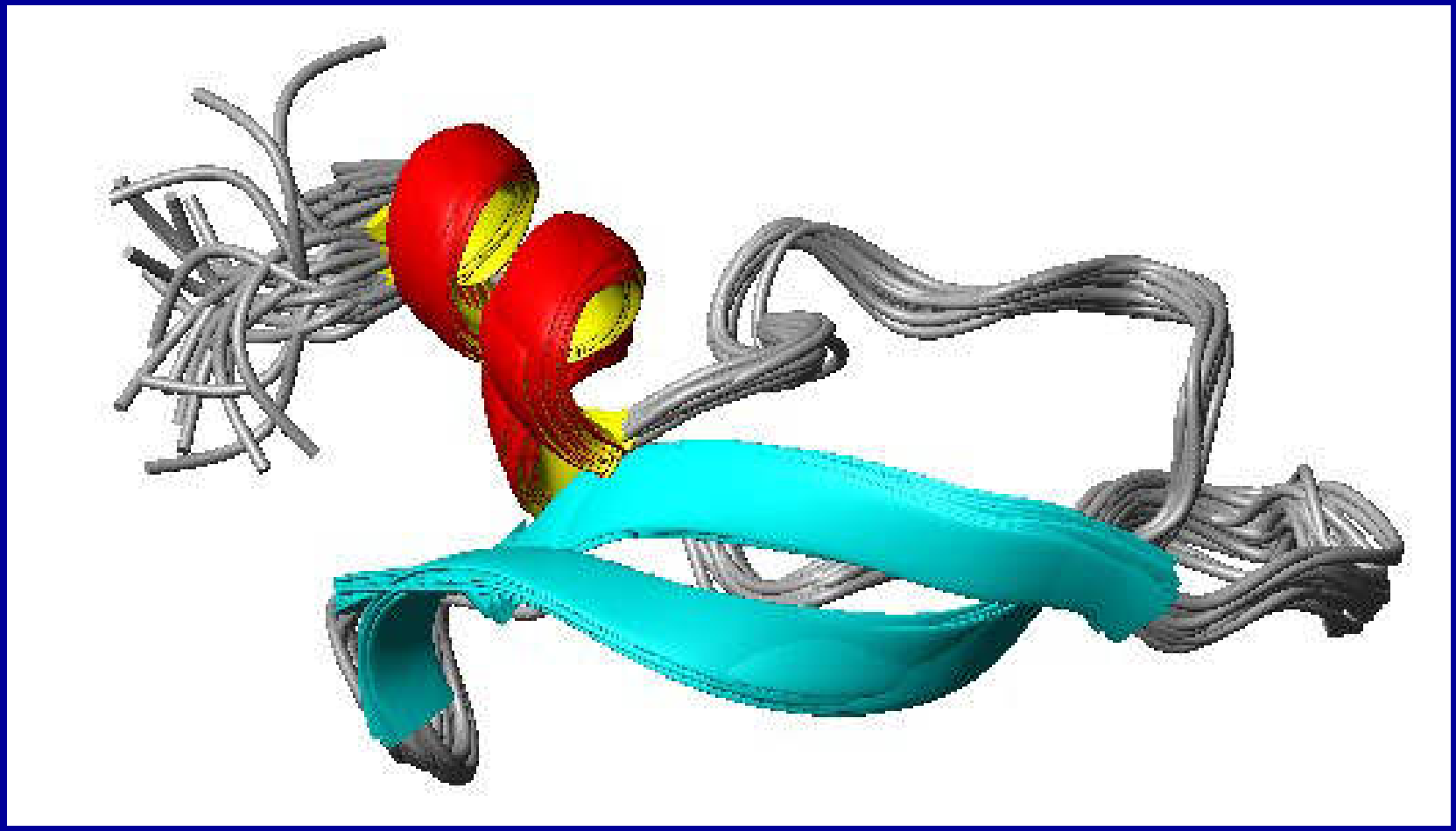


Famiglie di strutture NMR e rappresentazione grafica





Struttura secondaria e terziaria: flessibilità delle proteine



Classificazione strutturale e funzionale: attività e meccanismo di azione

Calpain

From Wikipedia, the free encyclopedia

A **calpain** (/ˈkælpeɪn/;[1] EC 3.4.22.52, EC 3.4.22.53) is a protein belonging to the family of calcium-dependent, non-lysosomal cysteine proteases (proteolytic enzymes) expressed ubiquitously in mammals and many other organisms. Calpains constitute the C2 family of protease clan CA in the MEROPS database. The calpain proteolytic system includes the calpain proteases, the small regulatory subunit CAPNS1, also known as CAPN4, and the endogenous calpain-specific inhibitor, calpastatin.

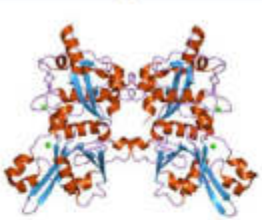
Discovery [edit]

The history of calpain's discovery originates in 1964, when calcium-dependent proteolytic activities caused by a "calcium-activated neutral protease" (CANP) were detected in brain, lens of the eye and other tissues. In the late 1960s the enzymes were isolated and characterised independently in both rat brain and skeletal muscle. These activities were caused by an intracellular cysteine protease not associated with the lysosome and having an optimum activity at neutral pH, which clearly distinguished it from the cathepsin family of proteases. The calcium-dependent activity, intracellular localization, and the limited, specific proteolysis on its substrates, highlighted calpain's role as a regulatory, rather than a digestive, protease.

When the sequence of this enzyme became known,[2] it was given the name "calpain", to recognize its common properties with two well-known proteins at the time, the calcium-regulated signalling protein, calmodulin, and the cysteine protease of papaya, papain. The activity was found to be attributable to two main isoforms, dubbed μ ("mu")-calpain and m-calpain (or calpain I and II), that differed primarily in their calcium requirements *in vitro*. Their names reflect the fact that they are activated by micro- and nearly millimolar concentrations of Ca^{2+} within the cell, respectively.[3]

To date, these two isoforms remain the best characterised members of the calpain family. Structurally, these two heterodimeric isoforms share an identical small (28 kDa) subunit (CAPNS1 (formerly CAPN4)), but have distinct large (80 kDa) subunits, known as calpain 1 and calpain 2 (each encoded by the *CAPN1* and *CAPN2* genes, respectively).

Calpain



Crystal structure of the peptidase core of Calpain II.

Identifiers	
Symbol	Calpain
Pfam	PF00648
Pfam clan	CL0125
InterPro	IPR001300
SMART	CysPc
PROSITE	PDOC50203
MEROPS	C2
SCOP2	1mdw / SCOPe / SUPFAM
Available protein structures:	[show]

calpain-1

Identifiers	
EC no.	3.4.22.52
CAS no.	689772-75-6
Databases	
IntEnz	IntEnz view
BRENDA	BRENDA entry
ExPASy	NiceZyme view
KEGG	KEGG entry
MetaCyc	metabolic pathway
PRIAM	profile
PDB structures	RCSB PDB PDBe PDBsum

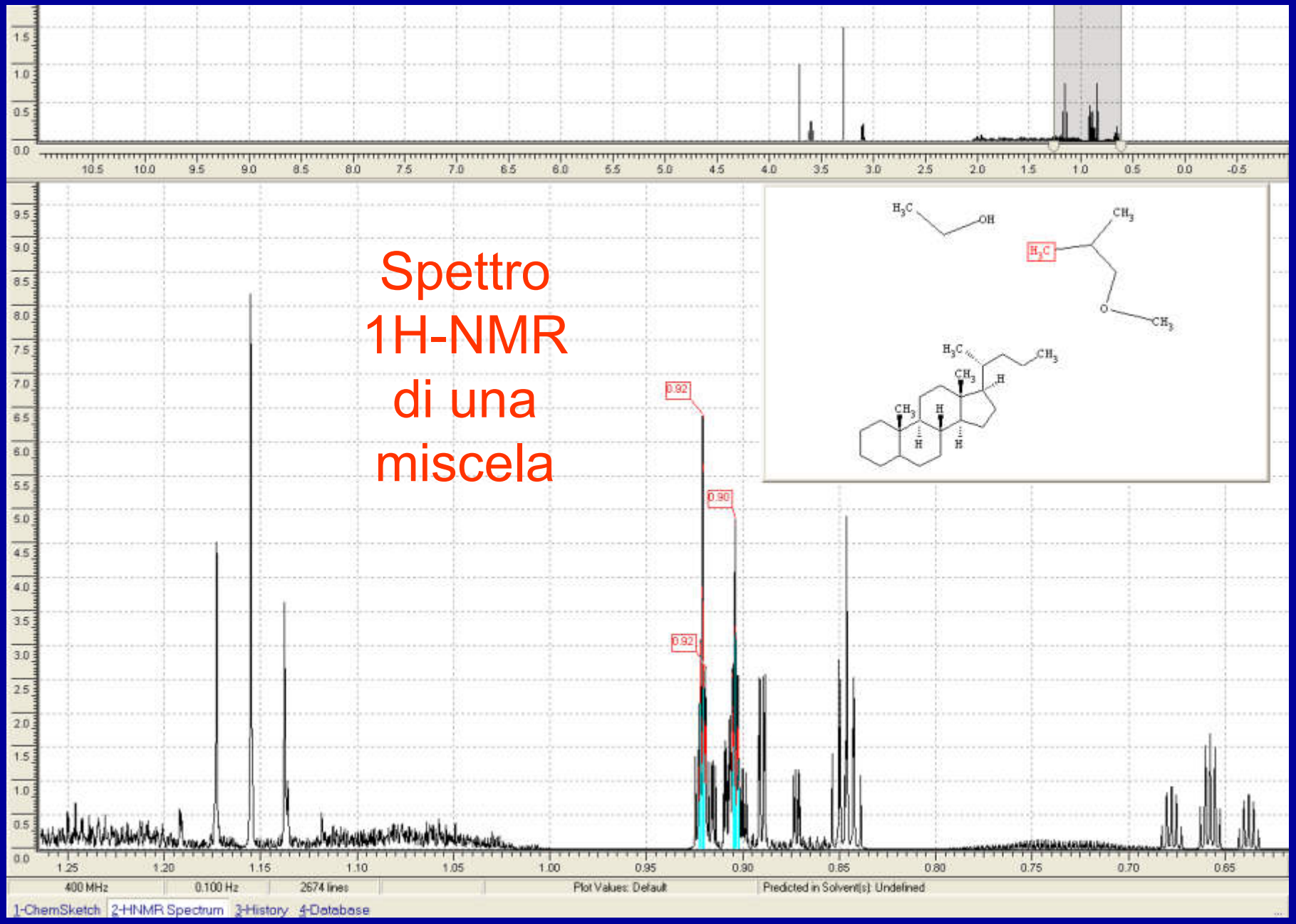
Informazioni da Spettroscopia NMR

- Chemical Shift (δ, ppm)

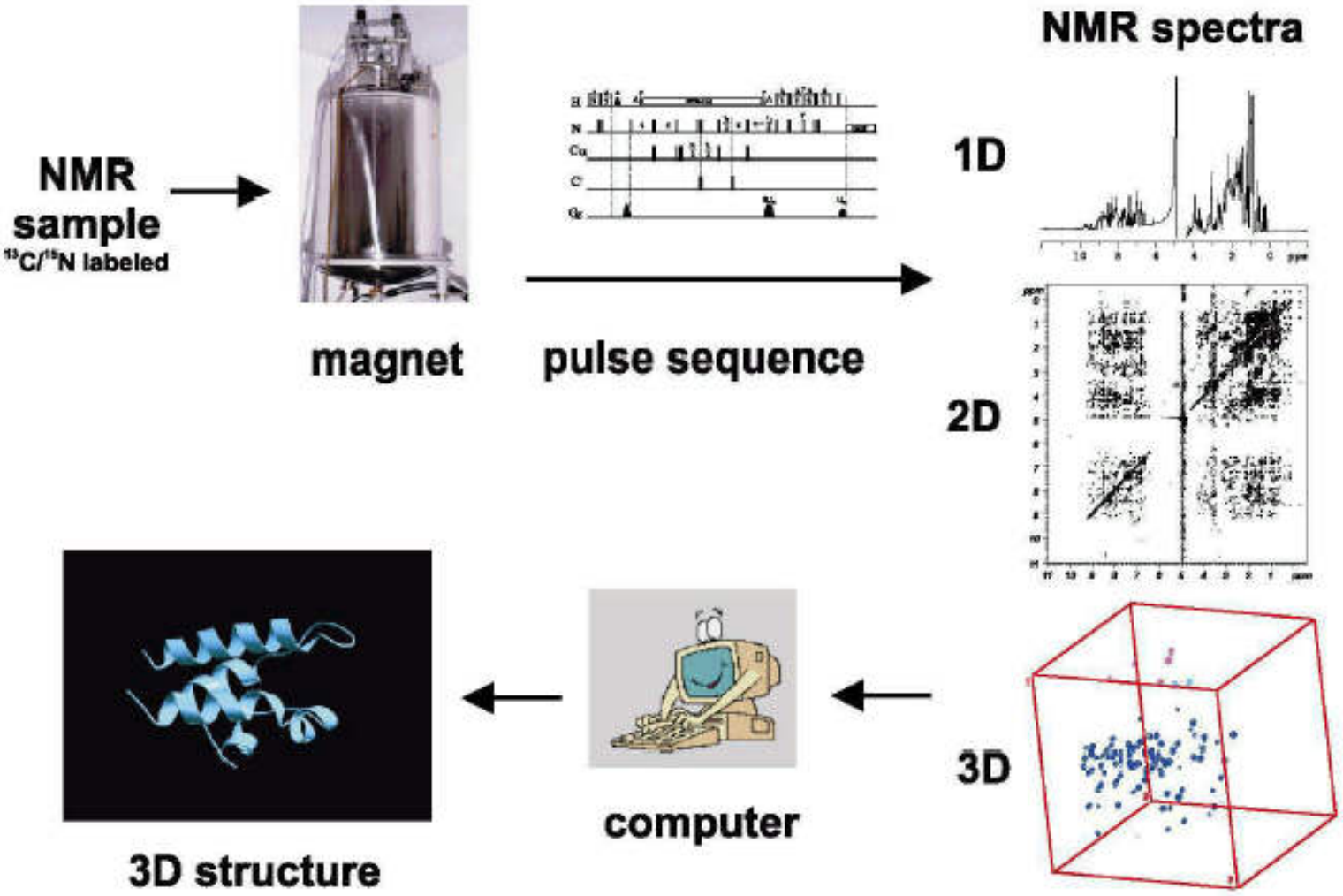
  tipico di ogni nucleo in un particolare intorno chimico
- Molteplicita del segnale

  dipende dal numero dei nuclei magneticamente attivi adiacenti nella stessa molecola
- Relaxation Rates (s^{-1})

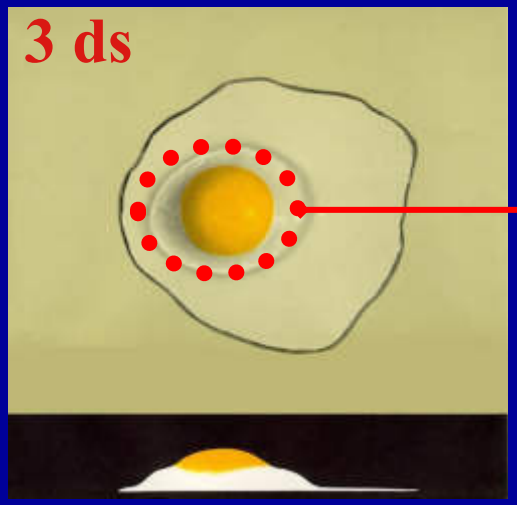
  dipende dall'efficienza di scambio energetico con l'ambiente: maggiore nel solido che allo stato liquido

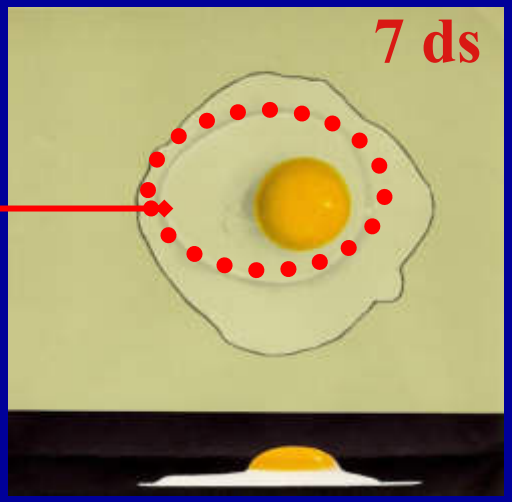

Spettro
1H-NMR
di una
miscela
0.92
0.90
0.92
400 MHz
0.100 Hz
2674 lines
Plot Values: Default
Predicted in Solvent(s): Undefined
1-ChemSketch
2-HNMR Spectrum
3-History
4-Database

Delucidazione di strutture 3D mediante spettroscopia NMR

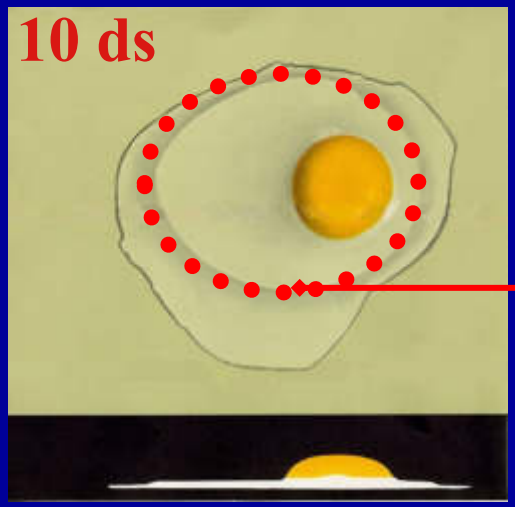


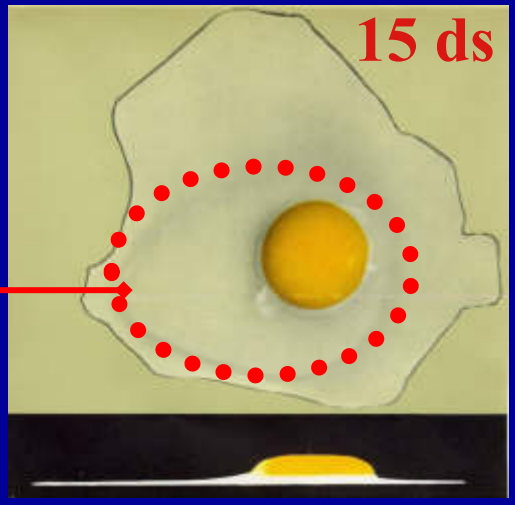
Hen egg albumen thinning



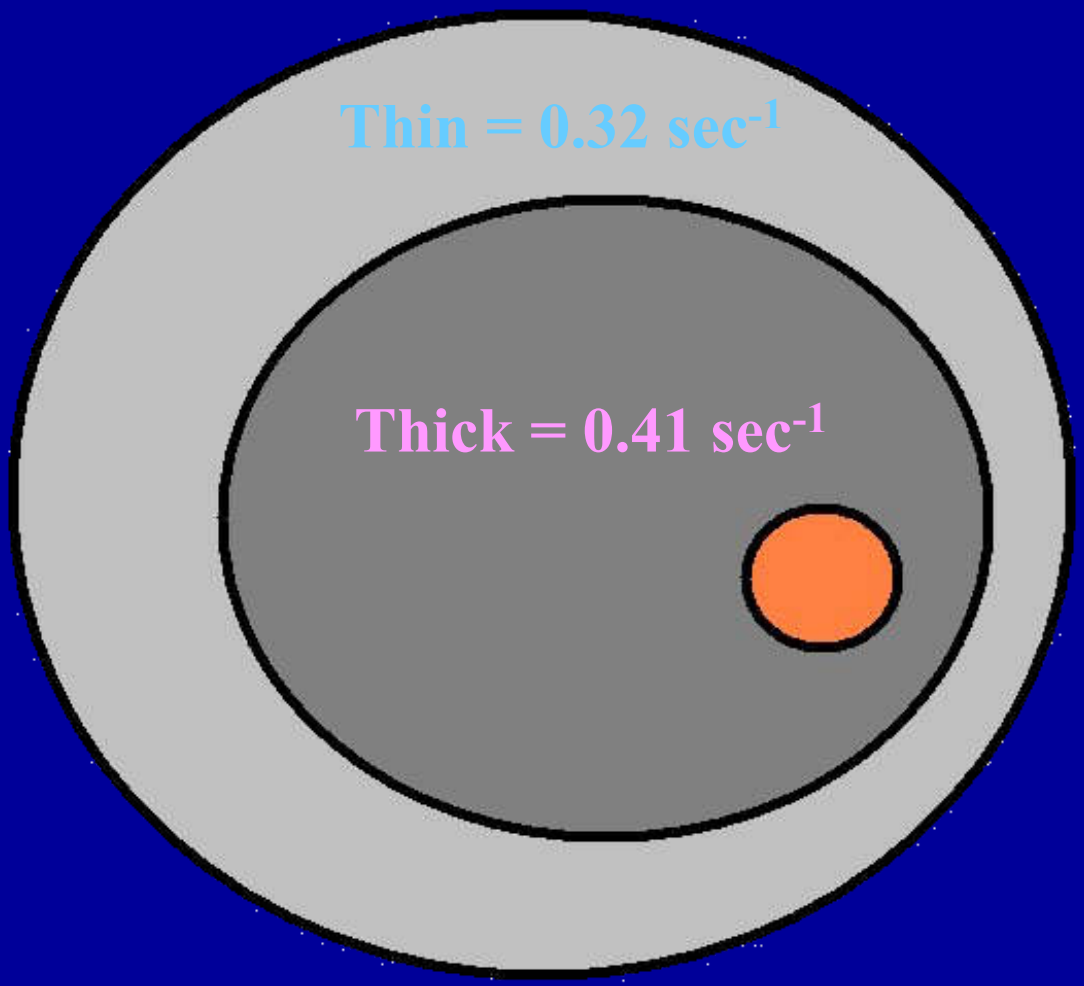


Thick layer





Water proton relaxation rate in egg albumen



JMR Journal of Magnetic Resonance
ACADEMIC PRESS

Journal of Magnetic Resonance
Vol. 138, No. 2, June 1999
ISSN: 1090-7807

SELECT: All Issues Go

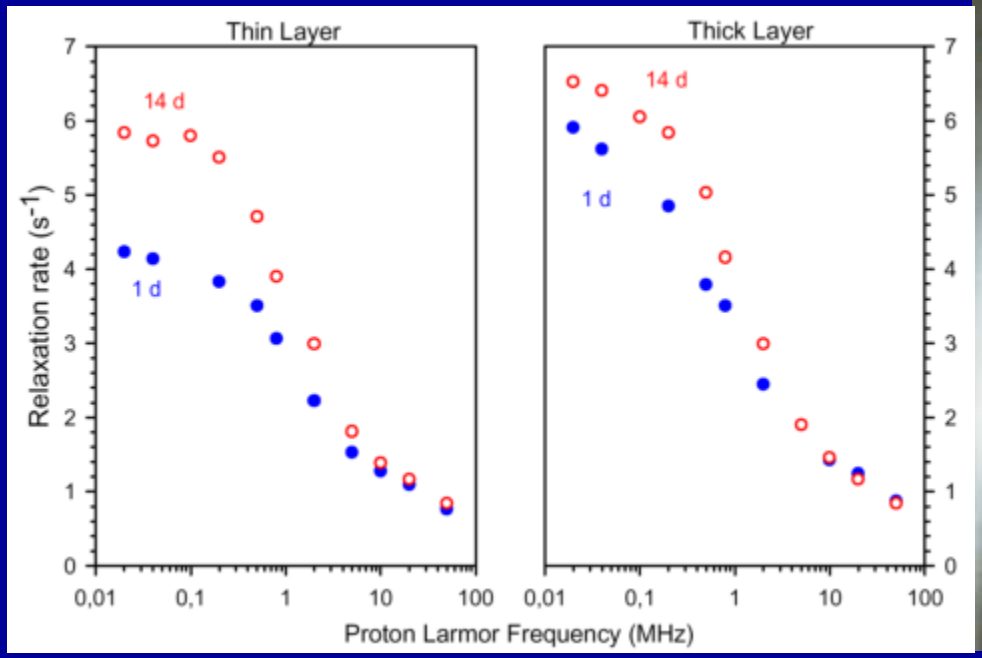
Academic Press
keeping you up-to-date
eAlert

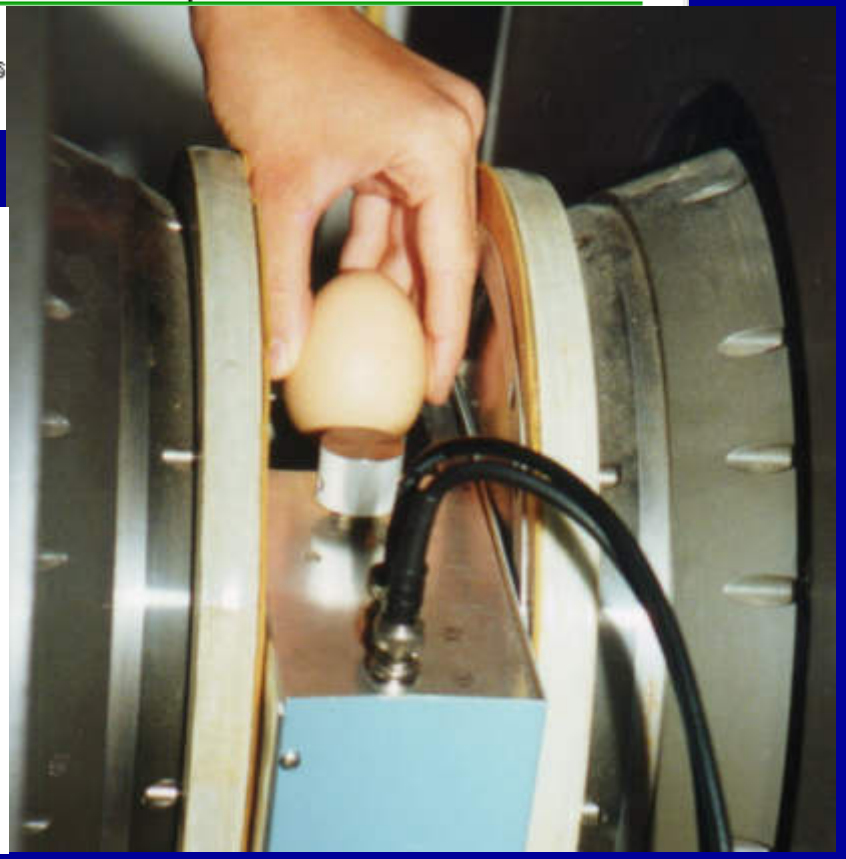
Table of Contents • Article(PDF) • References

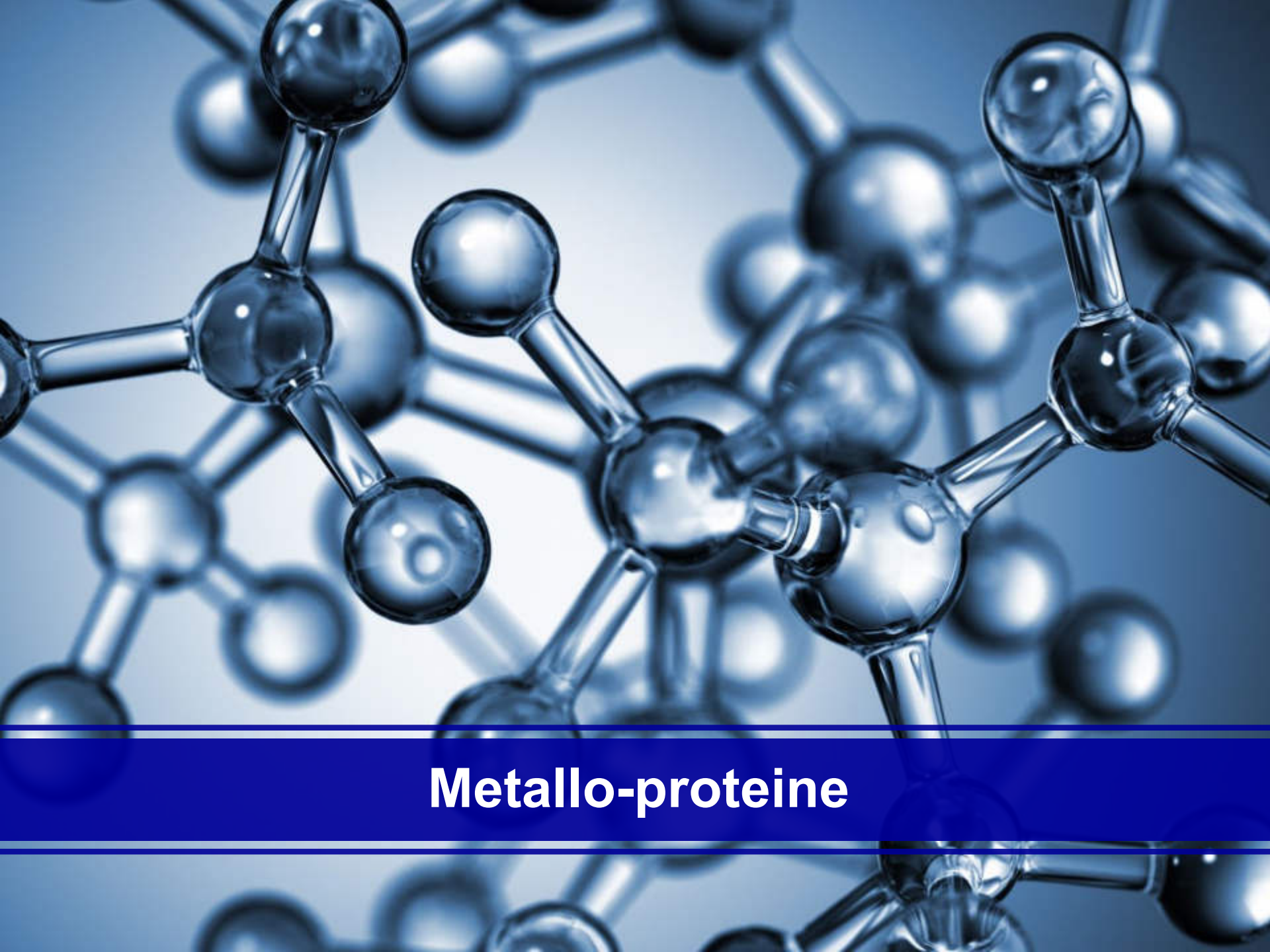
A Low Frequency ^{1}H-NMR External Unit for the Analysis of Large Foodstuff Samples

pp. 277-280 (doi:10.1006/jmre.1999.1729)

Francesco Capozzi*, Mauro A. Cremonini†, Claudio Luchinat‡, Giuseppe Placucci§







Metallo-proteine

Metalli necessari alla vita

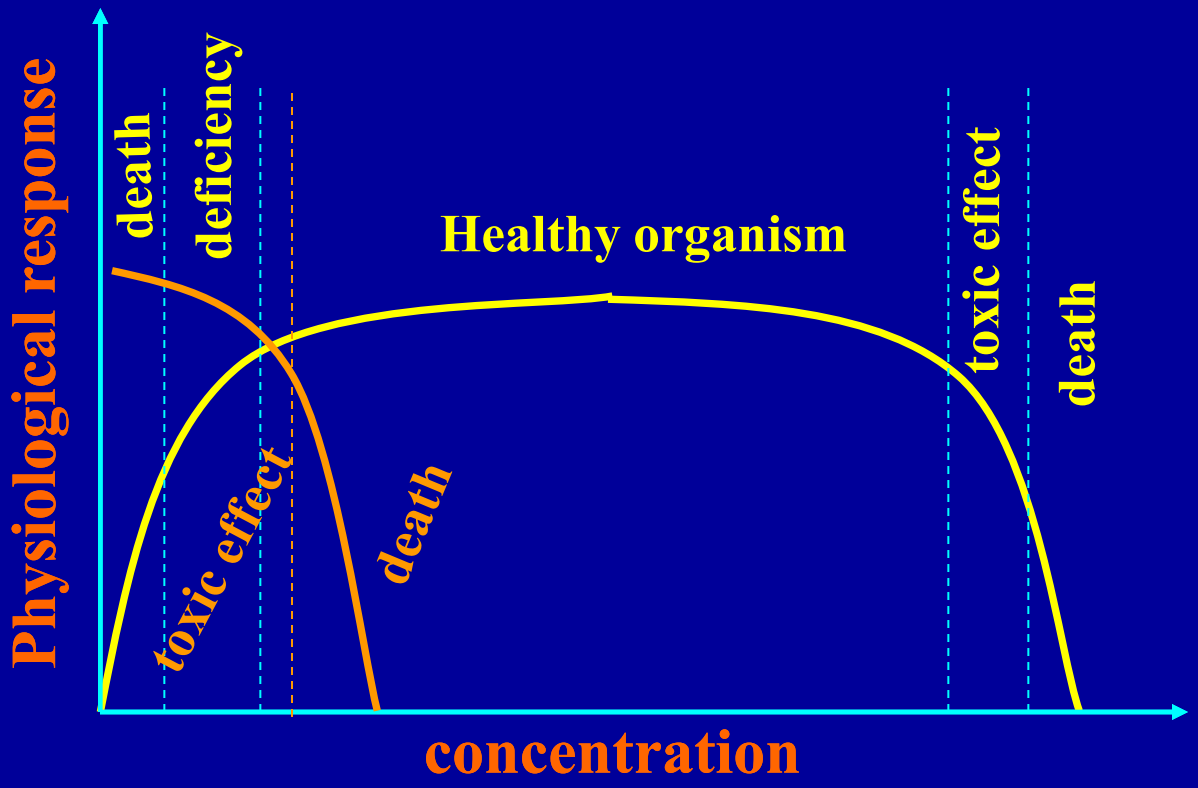
“Molti fra gli elementi più sconosciuti sono in realtà più comuni di altri ben più famosi. Per esempio, sulla Terra c’è più cerio che rame, più neodimio e lantanio che cobalto o azoto. Lo stagno si colloca, a stento, tra i primi cinquanta, eclissato, quanto ad abbondanza, da illustri sconosciuti quali il praseodimio, il samario, il gadolinio e il disprosio. L’abbondanza ha poco a che fare anche con il fatto che la presenza di un elemento nell’ambiente sia più o meno facile da rilevare.”

da “Breve storia di (quasi) tutto” di “Bill Bryson”

Metalli necessari alla vita

- Elemento essenziale per l'uomo
- Elemento supposto essenziale per l'uomo
- Elemento supposto avere funzione biologica
- Elemento inquinante dell'ambiente

Effetti degli ioni metallici dipendenti dalla dose



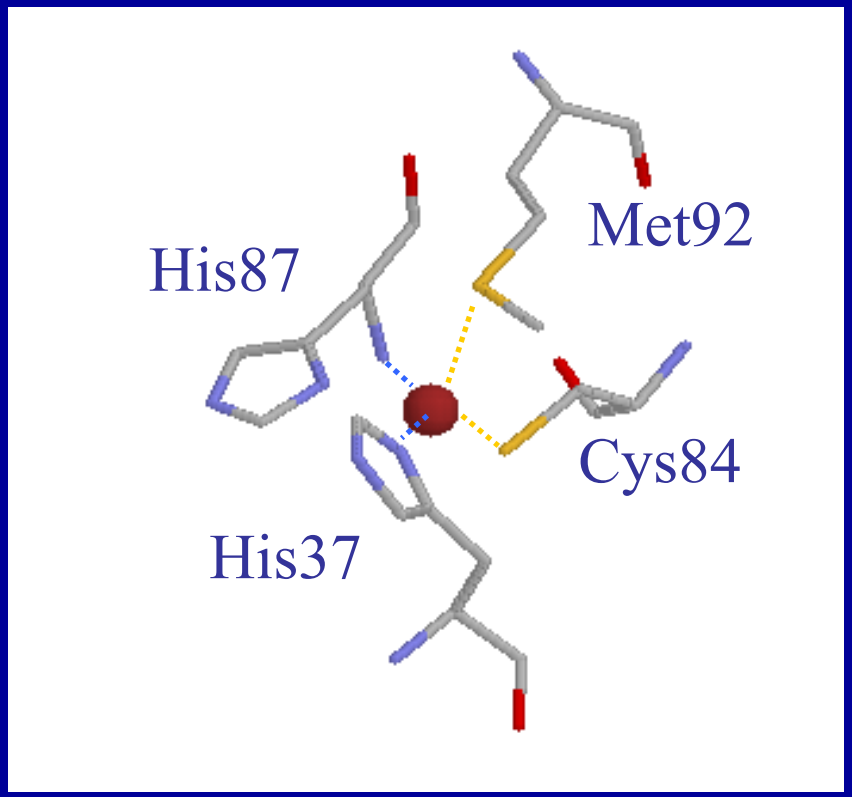
beneficial

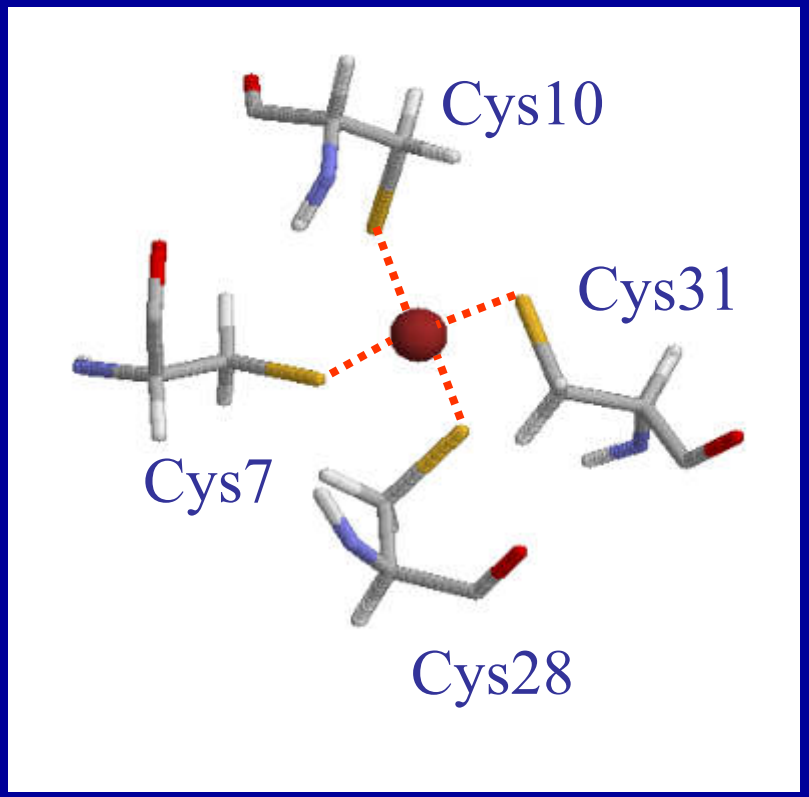
toxic

Il cofattore metallico

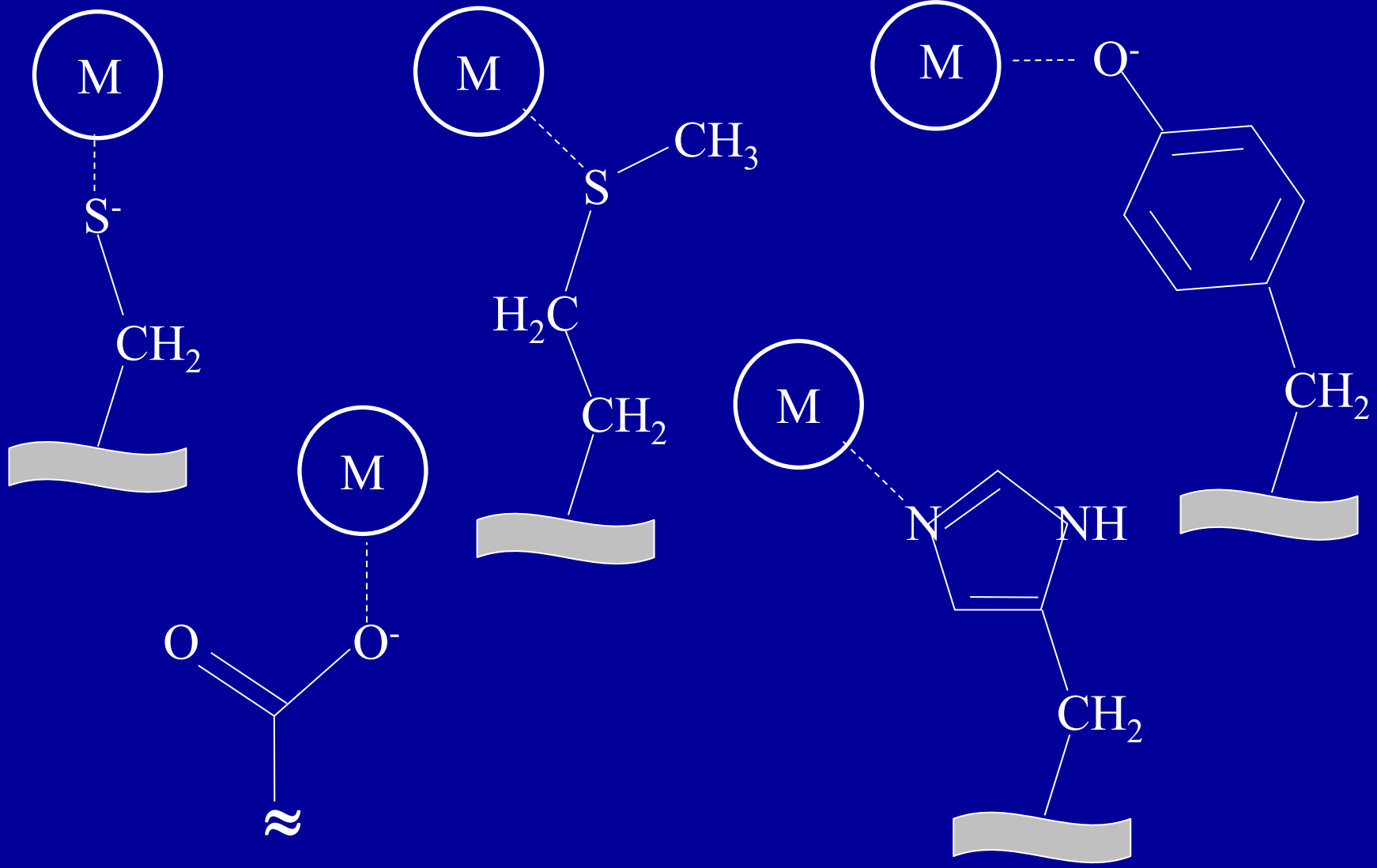
Metal ion(s) associated to polypeptides.

Typically, metal ions are cofactors that are used to catalyze reactions or to help providing the required geometry to proteins.

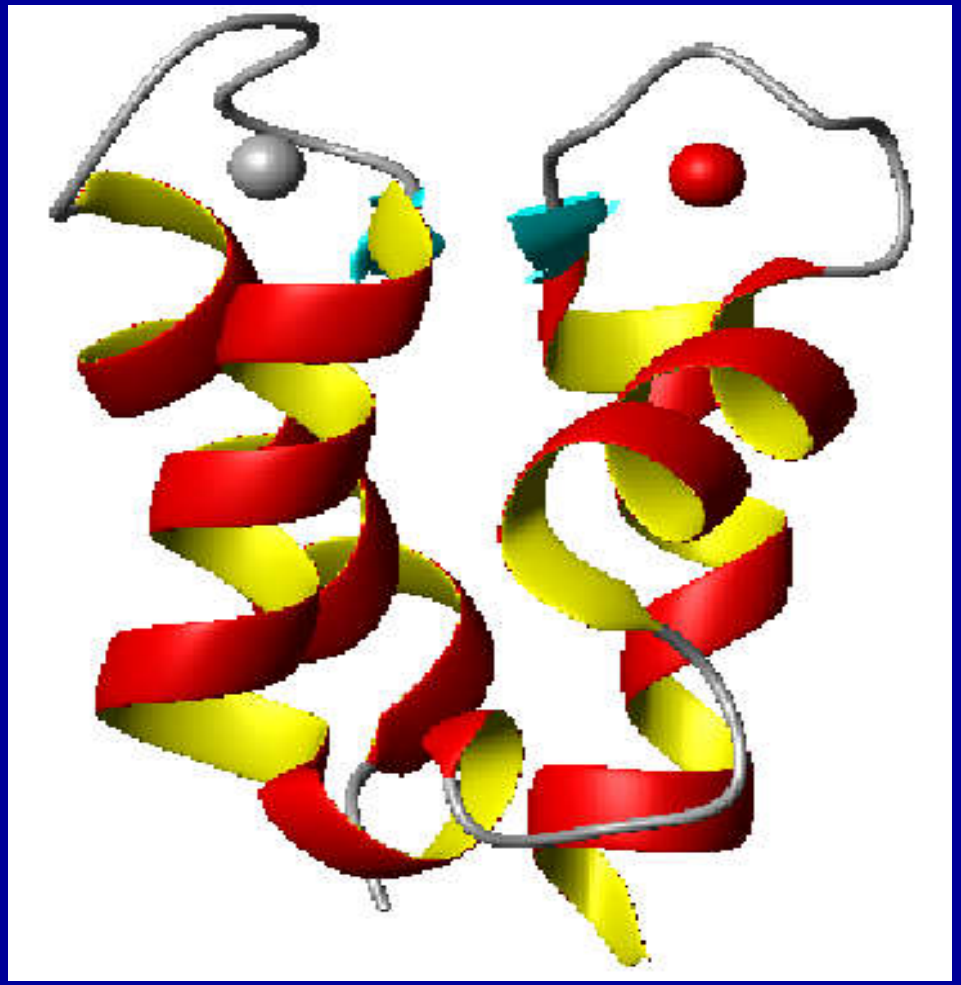




Le catene laterali degli amminoacidi come leganti



Metalloproteine



Calbindina

PM ca. 9000 Da
75 aa
2 Ca^{2+}

Costanti di affinità di leganti dei metalli alcalino-terrosi

BASIC FACTS ABOUT CALCIUM: ITS COMPOUNDS AND REACTIONS

Ca^{2+} and Mg^{2+} (where available) stability constants (log *K*) for different organic and biochemical ligands. Most values are at ionic strength 0.1 and 25°C.

Ligand	Ca^{2+}	Mg^{2+}
Acetate	0.5	0.5
Lactate	1.1	0.9
Malonate	1.5	2.1
Aspartate	1.6	2.4
Citrate	3.5	3.4
Nitrilotriacetate	6.4	5.5
EGTA[a]	10.9	5.3
EDTA[b]	10.6	8.8
Glycine (Gly)	1.4	3.4
γ-Carboxyglutamic acid (Gla)	1.3	
Gly-Gly dipeptide	1.2	
Gla-Gla dipeptide	3.2	
Macrobicyclic amino cryptate [2.2.2]	4.5	
Fluo-3	6.2	2.0
Fura-2	6.9	2.0
BAPTA	7.0	1.8
Quin-2	7.1	2.7
Phospholipase A2	3.6	
Thrombin fragment 1	3.7	3.0
Trypsinogen	3.8	
Chymotrypsinogen	3.9	
Chymotrypsin	4.1	
Calmodulin, N-terminal	4.5	3.3
Trypsin	4.6	
Calmodulin, C-terminal	5.3	
Protein kinase C	~7	
α-Lactalbumin	~7	
Rabbit skeletal muscle		
Troponin C, Ca^{2+}/Mg^{2+} sites	7.3	3.6
Carp parvalbumin	~8.5	4.2
Bovine calbindin D_{9k}	8.8	~4.3

[a] EGTA: ethylenebis(oxyethylenenitrilo)tetraacetate
[b] EDTA: ethylenedinitrilotetraacetate

Metalloproteine

$$M + P \rightarrow MP$$

$$K = [MP] / [M] [P]$$

where K is the equilibrium binding constant between the metal ion, M, and the polypeptide chain, P.

The thermodynamic stability of the metal-protein adduct in the case of **metalloproteins** is very high and K can have values higher than about 10^8 M^{-1}.

Metalli presenti nel corpo umano

Percentage Composition of Selected Elements in the Human Body

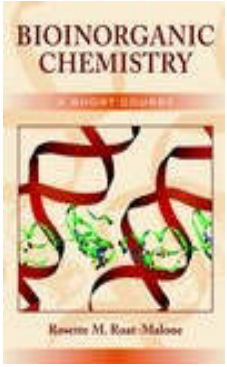
Element	Percentage (by weight)	Element	Percentage (by weight)
Oxygen	53.6	Silicon, Magnesium	0.04
Carbon	16.0	Iron, fluorine	0.005
Hydrogen	13.4	Zinc	0.003
Nitrogen	2.4	Copper, bromine	$2.\times10^{-4}$
Sodium, potassium, sulfur	0.10	Selenium, manganese, arsenic, nickel	$2.\times10^{-5}$
Chlorine	0.09	Lead, cobalt	$9.\times10^{-6}$

Concentrazione del Ca(II) nei fluidi e tessuti

Specimen	Units are mM if not otherwise stated
Sea water	10
Fresh water	0.02–2
Rain water	0.002–0.02
"Hard" tap water	1.5
"Good" beer	4
Adult human serum	2.45 ± 0.05
Serum of other vertebrates	1.5–5
Nematote body fluids	6
Molluscan serum—marine	9–15
—fresh water	1.5–7.8
—land	3.3–12.3
Milk	70
Bone	0.8–1.0
Mitochondria from rat liver	0.8 ± 0.1 mmol/kg
Endoplasmatic reticulum	8–10 mmol/kg
Cytoplasm of a resting mammalian cell	0.0001
Cytoplasm of *E. coli*	0.0001

Concentrations of transition metals and zinc in sea water and human plasma

Element	Sea Water (M) x 10^8	Human plasma (M) x 10^8
Fe	0.005-2	2230
Zn	8.0	1720
Cu	1.0	1650
Mo	10.0	1000
Co	0.7	0.0025
Cr	0.4	5.5
V	4.0	17.7
Mn	0.7	10.9
Ni	0.5	4.4



Un esempio…

Fe

- In un uomo di 75 kg sono presenti 4 g di Fe
- Nei Paesi occidentali ne assumiamo 15 mg/d

Table 1. Iron-Containing Compounds of the Human Body[a]

Compound	% of Total Body Iron[b]	Molecular Mass[c]	Iron Content per Molecule[c]	Heme or Nonheme	Function
Hemoglobin	60–85	6.4×10^4	4 Fe^{2+}	Heme	Oxygen transport
Myoglobin	10	1.7×10^4	1 Fe^{2+}	Heme	Oxygen transport
Ferritin	6–15	8.9×10^5	4500 Fe^{3+}	Nonheme	Iron storage
Hemosiderin	4–10	Ill defined	Ill defined	Nonheme	Iron storage
Transferrin	< 1	7.7×10^4	2 Fe^{3+}	Nonheme	Iron transport
Catalase	< 1	2.5×10^5	4 Fe^{3+}	Heme	Decompose H_2O_2
Peroxidase[d]	< 1	1.5×10^5	2 Fe^{3+}	Heme	Antimicrobial
Cytochromes[e]	< 1	1.3×10^4	1 Fe^{3+}	Heme	Electron transfer

Classificazione degli ioni metallici “bio”

- In base alla loro funzione:

  - ✓ Strutturale / Segnale
  - ✓ Trasporto
  - ✓Stoccaggio
  - ✓Trasporto di cariche
  - ✓ Catalitico
  - ✓Trasferimento elettronico

→ relazione struttura-funzione

Ruolo dei metalli (1)

Element	Role
Ca	Structure stabilization - Signaling - Enzyme activator
Co	Redox catalyst
Cu	ET - Redox catalyst - O_2 carrier
Fe	ET - Redox catalyst - O_2 carrier
Mg	Enzyme activator – DNA structure stabilization - Ribozymes
Mn	Redox catalist – Activator of hydrolases, ligases and transferases
Mo	Redox catalyst (nitrogenase, oxotransferases)
Ni	Hydrolytic activity – Redox catalyst

Ruolo dei metalli (2)

Element	Role
K	ATPases pumping - Structure stabilization – Electrostatic effects on redox potential of distant centers
Rb	Function similar to that of K in some plants
Se	Redox catalyst
Na	ATPases pumping - Structure stabilization – Electrostatic effects on redox potential of distant centers
Sr	Function similar to that of Ca in some plants
W	Replaces Mo in some hyperthermophilic archea
V	Peroxidase and nitrogenase activity in some algae and possibly fungi
Zn	Hydrolytic activity – Structure stabilization – Transcription factor

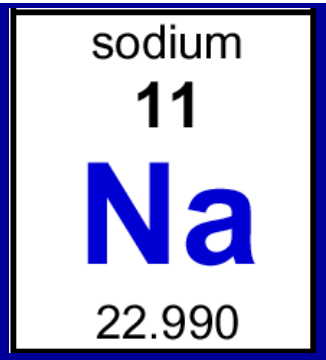
Metalli alcalini e alcalino terrosi

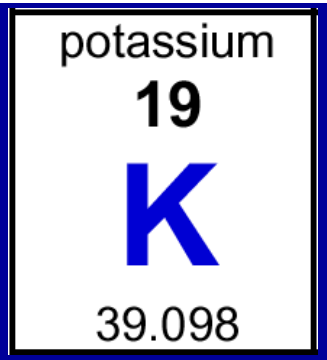
	Na^+	K^+	Mg^{2+}	Ca^{2+}
Ionic radius, Å	1.16	1.52	0.86	1.14
Charge/radius ratio	0.86	0.66	2.32	1.75
Coordination number	6	6-8	6	6-8
Preferred donors	O	O	N,O	O
Concentration (mmol/kg)				
Intracellular	11	92	2.5	0.1
Extracellular	152	5	1.5	2.5
Seawater	460	10	52	10

Ioni metallici per il trasporto di carica

Table 1.2 Metals in Biological Systems: Charge Carriers

Metal	Coordination Number, Geometry	Preferred Ligands	Functions and Examples
Sodium, Na^+	6, octahedral	*O*-Ether, hydroxyl, carboxylate	Charge carrier, osmotic balance, nerve impulses
Potassium, K^+	6–8, flexible	*O*-Ether, hydroxyl, carboxylate	Charge carrier, osmotic balance, nerve impulses





magnesium
12
Mg
24.305

calcium
20
Ca
40.078

zinc
30
Zn
65.39

manganese
25
Mn
54.938

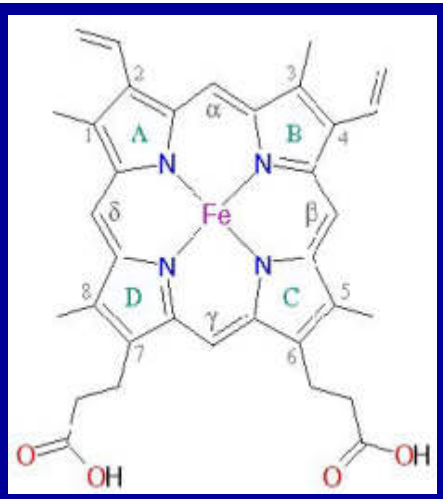
Table 1.3 Metals in Biological Systems: Structural, Triggers

Metal	Coordination Number, Geometry	Preferred Ligands	Functions and Examples
Magnesium, Mg^{2+}	6, octahedral	*O*-Carboxylate, phosphate	Structure in hydrolases, isomerases, phosphate transfer, trigger reactions
Calcium, Ca^{2+}	6–8, flexible	*O*-Carboxylate, carbonyl, phosphate	Structure, charge carrier, phosphate transfer, trigger reactions
Zinc, Zn^{2+} (d^{10})	4, tetrahedral	*O*-Carboxylate, carbonyl, *S*-thiolate, *N*-imidazole	Structure in zinc fingers, gene regulation, anhydrases, dehydrogenases
Zinc, Zn^{2+} (d^{10})	5, square pyramid	*O*-Carboxylate, carbonyl, *N*-imidazole	Structure in hydrolases, peptidases
Manganese, Mn^{2+} (d^5)	6, octahedral	*O*-Carboxylate, phosphate, *N*-imidazole	Structure in oxidases, photosynthesis
Manganese, Mn^{3+} (d^4)	6, tetragonal	*O*-Carboxylate, phosphate, hydroxide	Structure in oxidases, photosynthesis

Table 1.5 Metals in Biological Systems: Dioxygen Transport

Metal	Coordination Number, Geometry	Preferred Ligands	Functions and Examples
Copper, Cu^{2+} (d^9)	5, square pyramid 6, tetragonal	*O*-Carboxylate *N*-Imidazole	Type II copper oxidases, hydoxylases Type III copper hydroxylases, dioxygen transport in hemocyanin
Iron, Fe^{2+} (d^6)	6, octahedral	*N*-Imidazole, porphyrin	Dioxygen transport in hemoglobin and myoglobin

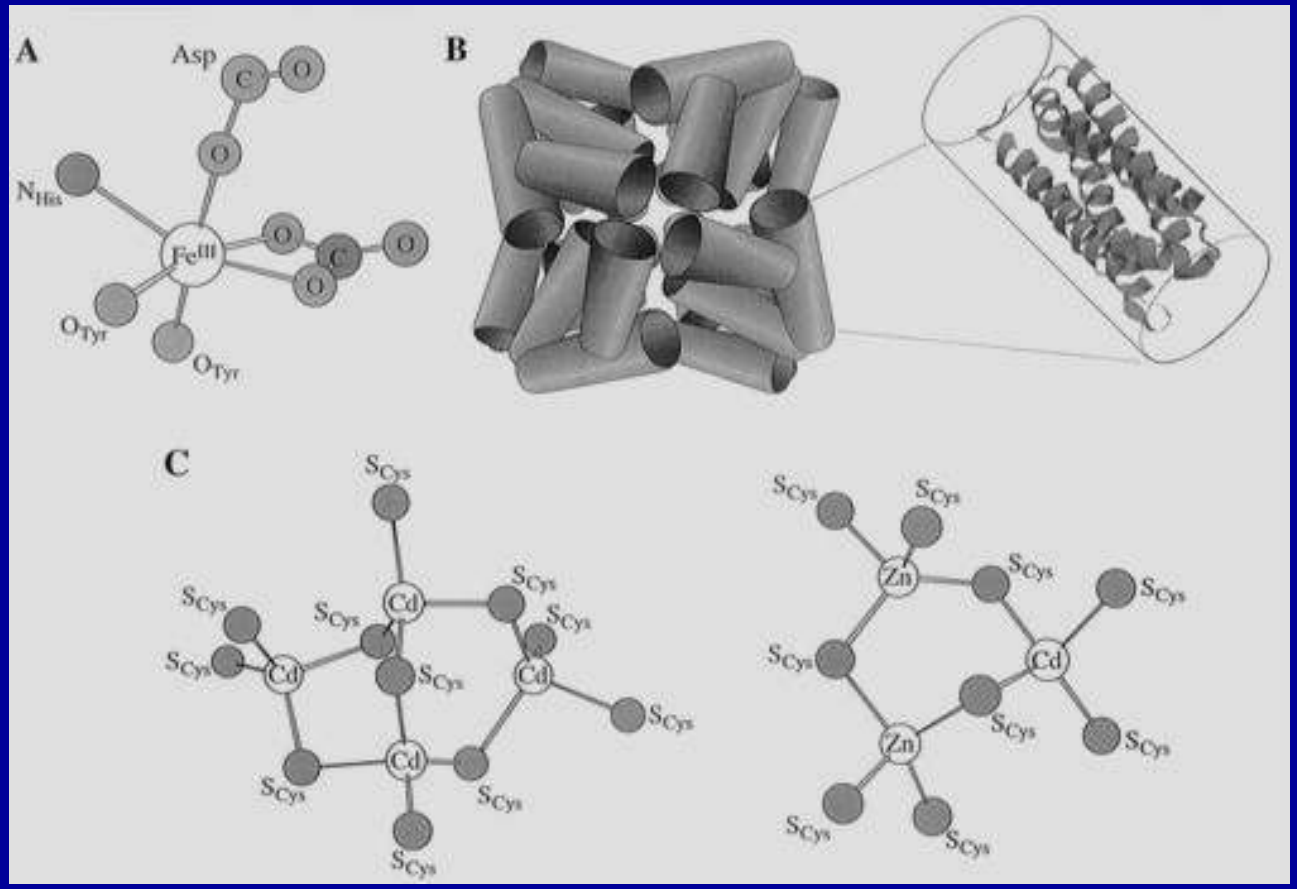
iron
26
Fe
55.845



copper
29
Cu
63.546

“Stoccaggio”

zinc
30
Zn
65.39



iron
26
Fe
55.845

Caratteristiche strutturali di proteine di *storage* e trasporto: A) il sito di legame della transferrina; B) struttura quaternaria della ferritina; C) strutture dei siti metallici della Cd_5Zn_2metallotioneina

Ioni metallici per la catalisi enzimatica

copper
29
Cu
63.546

cobalt
27
Co
58.933

nickel
28
Ni
58.693

molybdenum
42
Mo
95.94

Table 1.6 Metals in Biological Systems: Enzyme Catalysis

Metal	Coordination Number, Geometry	Preferred Ligands	Functions and Examples
Copper, Cu^{2+} (d^9)	4, square planar	*O*-Carboxylate, *N*-imidazole	Type II copper in oxidases
Cobalt, Co^{2+} (d^7)	4, tetrahedral	*S*-Thiolate, thioether, *N*-imidazole	Alkyl group transfer, oxidases
Cobalt, Co^{3+} (d^6)	6, octahedral	*O*-Carboxylate, *N*-imidazole	Alkyl group transfer in vitamin B_{12} (cyanocobalamin)
Cobalt, Co^{2+} (d^7)	6, octahedral	*O*-Carboxylate, *N*-imidazole	Alkyl group transfer in Vitamin B_{12r}
Cobalt, Co^{+} (d^8)	6, octahedral, usually missing the 6th ligand	*O*-Carboxylate, *N*-imidazole	Alkyl group transfer in vitamin B_{12s}
Nickel, Ni^{2+} (d^8)	4, square planar	*S*-Thiolate, thioether, *N*-imidazole, polypyrrole	Hydrogenases, hydrolases
Nickel, Ni^{2+} (d^8)	6, octahedral		Uncommon
Molybdenum, $Mo^{4+}(d^2)$, $Mo^{5+}(d^1)$, $Mo^{6+}(d^0)$	6, octahedral	*O*-Oxide, carboxylate, phenolate, *S*-sulfide, thiolate	Nitrogen fixation in nitrogenases, oxo transfer in oxidases

Ioni metallici per il trasferimento elettronico

iron
26
Fe
55.845

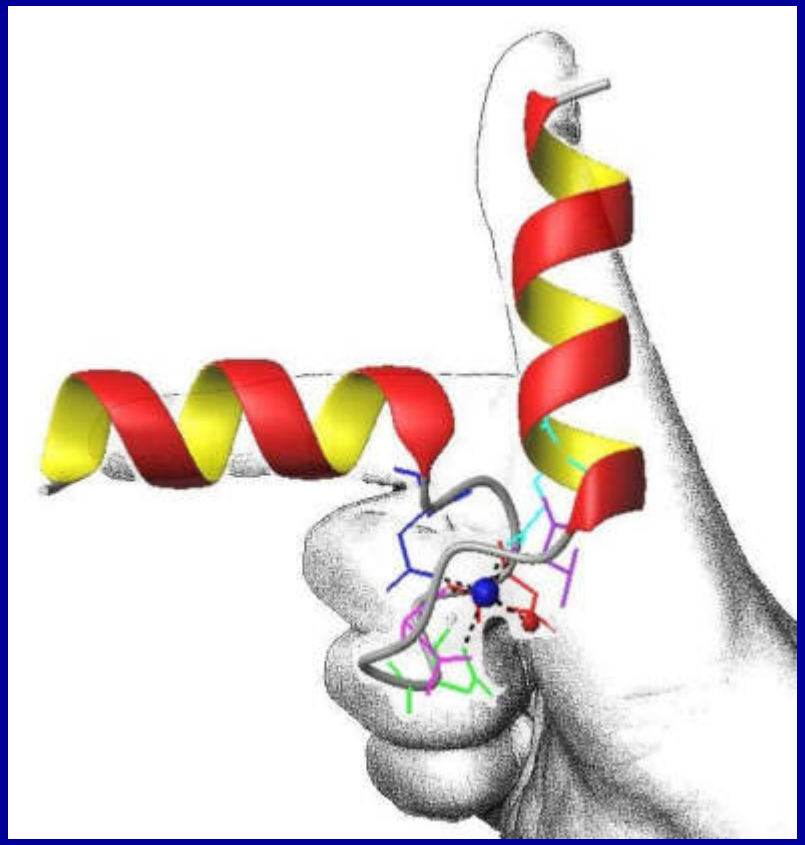
copper
29
Cu
63.546

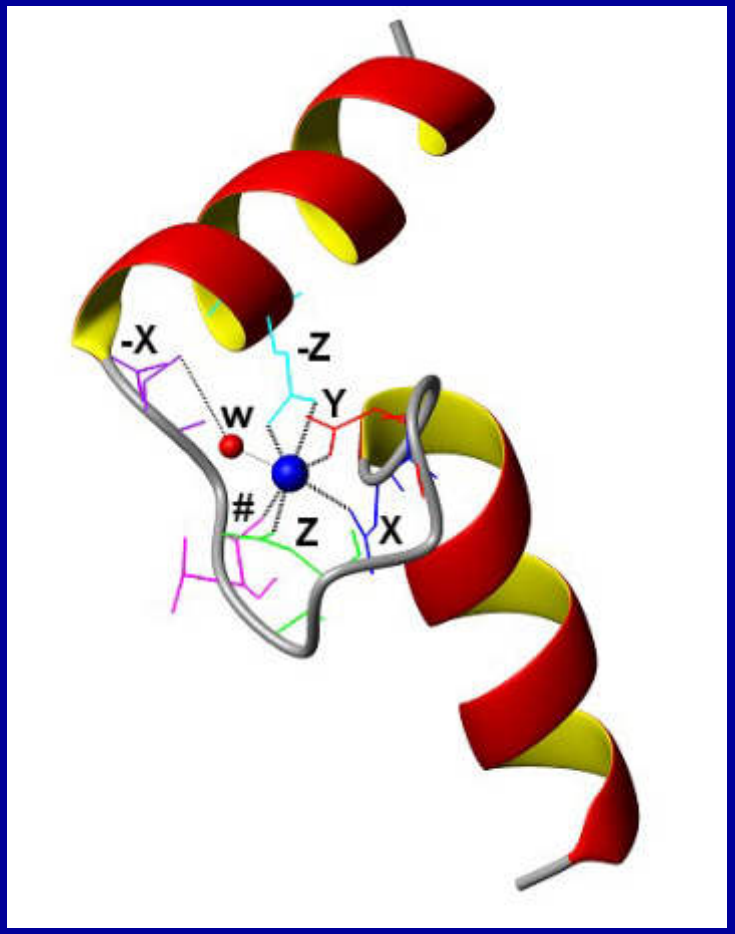
Table 1.4 Metals in Biological Systems: Electron Transfer

Metal	Coordination Number, Geometry	Preferred Ligands	Functions and Examples
Iron, Fe^{2+} (d^6)	4, tetrahedral	*S*-Thiolate	Electron transfer, nitrogen fixation in nitrogenases, electron transfer in oxidases
Iron, Fe^{2+} (d^6)	6, octahedral	*O*-Carboxylate, alkoxide, oxide, phenolate	
Iron, Fe^{3+}(d^5)	4, tetrahedral	*S*-Thiolate	Electron transfer, nitrogen fixation in nitrogenases, electron transfer in oxidases
Iron, Fe^{3+}(d^5)	6, octahedral	*O*-Carboxylate, alkoxide, oxide, phenolate	
Copper, Cu^{+}(d^{10}), Cu^{2+} (d^9)	4, tetrahedral	*S*-Thiolate, thioether, *N*-imidazole	Electron transfer in Type I blue copper proteins

Funzione strutturale

Il trasduttore universale: il motivo EF-hand





Function is changing structure: Apo CaM – 4Ca CaM

The CaM bite:
a wrap around movement

The calcium buffer S100G does not move much

Trasporto e stoccaggio di metalli

Solubilità del Ferro(III / II)

iron(II) carbonate	$FeCO_3$	3.2×10^{-11}
iron(II) hydroxide	$Fe(OH)_2$	8.0×10^{-16}
iron(II) sulfide	FeS	6×10^{-19}
iron(III) arsenate	$FeAsO_4$	5.7×10^{-21}
iron(III) ferrocyanide	$Fe_4[Fe(CN)_6]_3$	3.3×10^{-41}
iron(III) hydroxide	$Fe(OH)_3$	4×10^{-38}
iron(III) phosphate	$FePO_4$	1.3×10^{-22}

$K_{ps} = [Fe(III)]\,[OH^-]^3$

pH = 7 → [OH-] = 10^{-7}

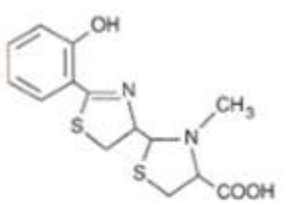
[Fe(III)]max = 10^{-38} / 10^{-21} = 10^{-17}
Nel plasma la conc di Fe(III) è $2 \cdot 10^{-5}$M

$K_{ps} = [Fe(II)]\,[OH^-]^2$

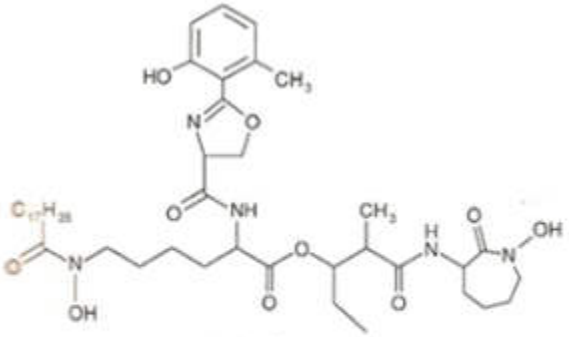
pH = 7 → [OH-] = 10^{-7}

[Fe(II)]max = 10^{-15} / 10^{-14} = 10^{-1}
Nel plasma la conc di Fe(III) è $2 \cdot 10^{-5}$M

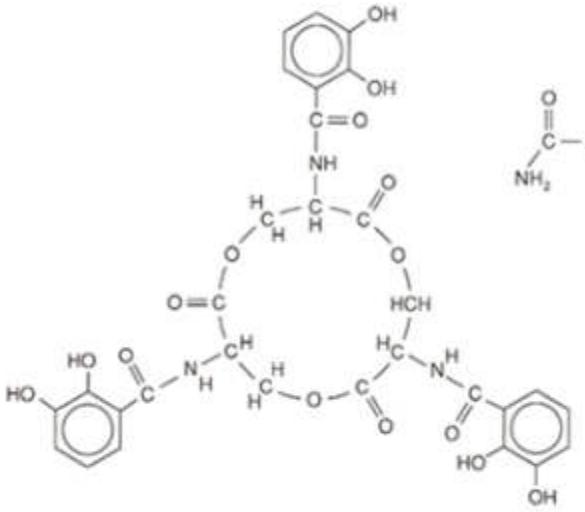
Siderofori: Enterobactine e analoghi sintetici



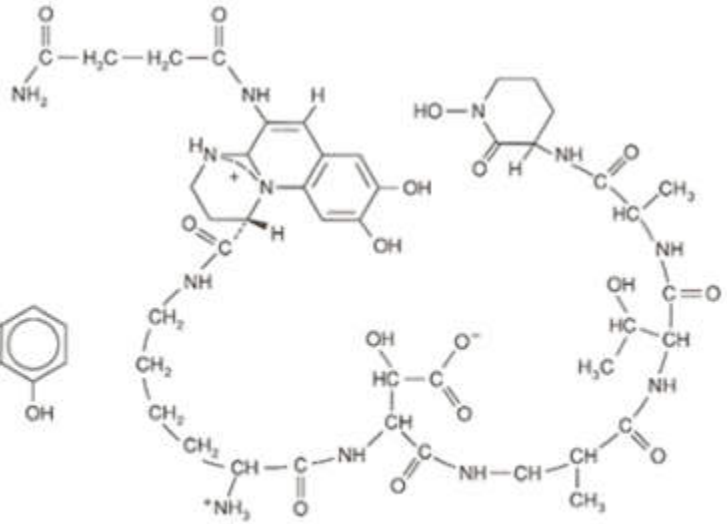
pyochelin



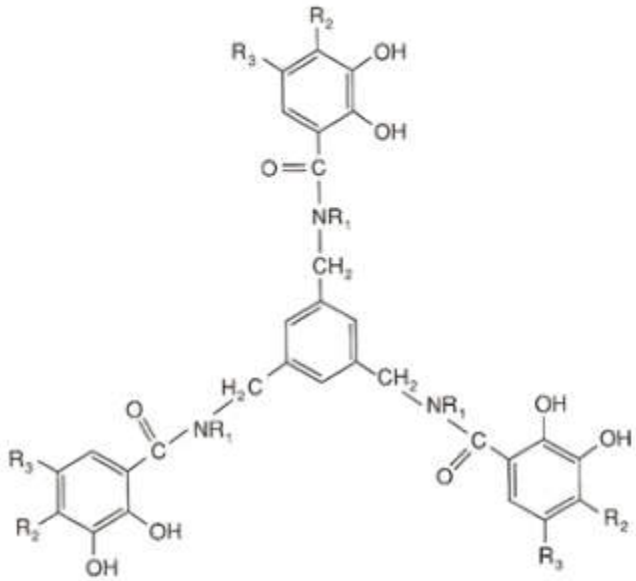
mycobactin P



enterobactin

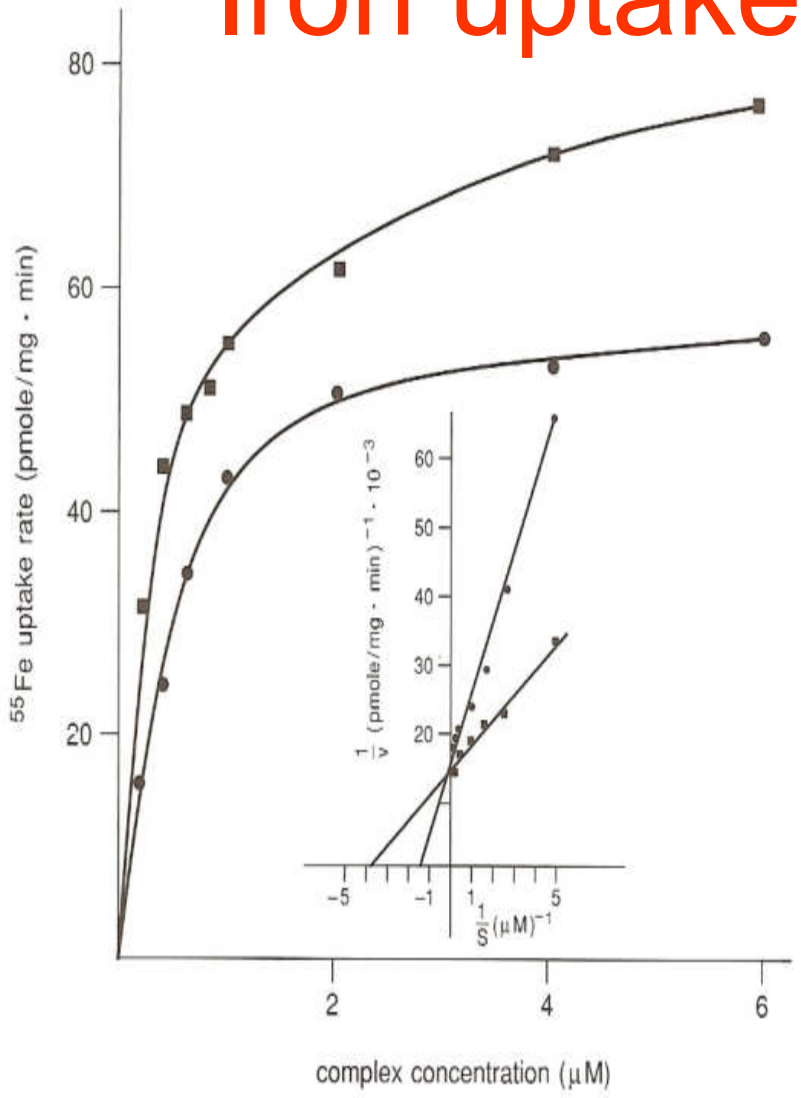


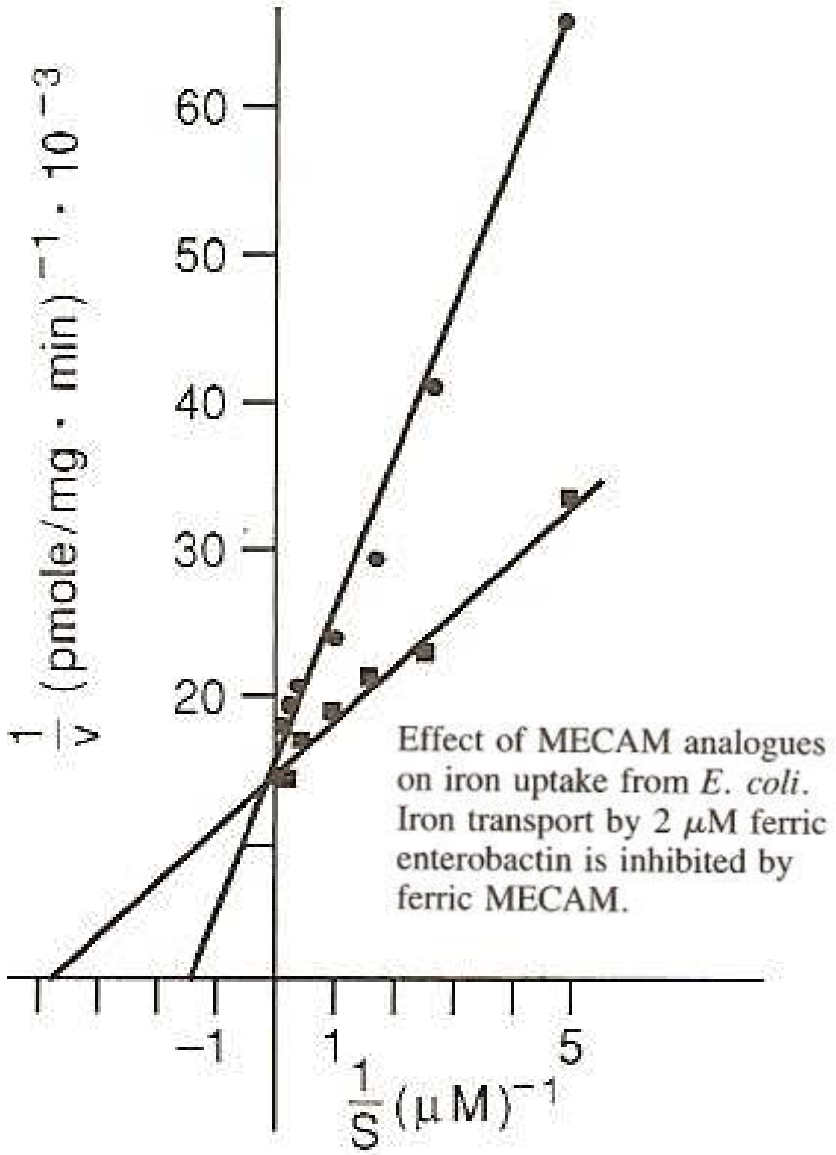
pseudobactin



MECAM ($R_1 = R_2 = R_3 = H$)
MECAM-Me ($R_1 = R_3 = H$, $R_2 = CH_3$)
MECAMS ($R_1 = R_2 = H$, $R_3 = SO_3^-$)
Me_3MECAM ($R_2 = R_3 = H$, $R_1 = CH_3$)

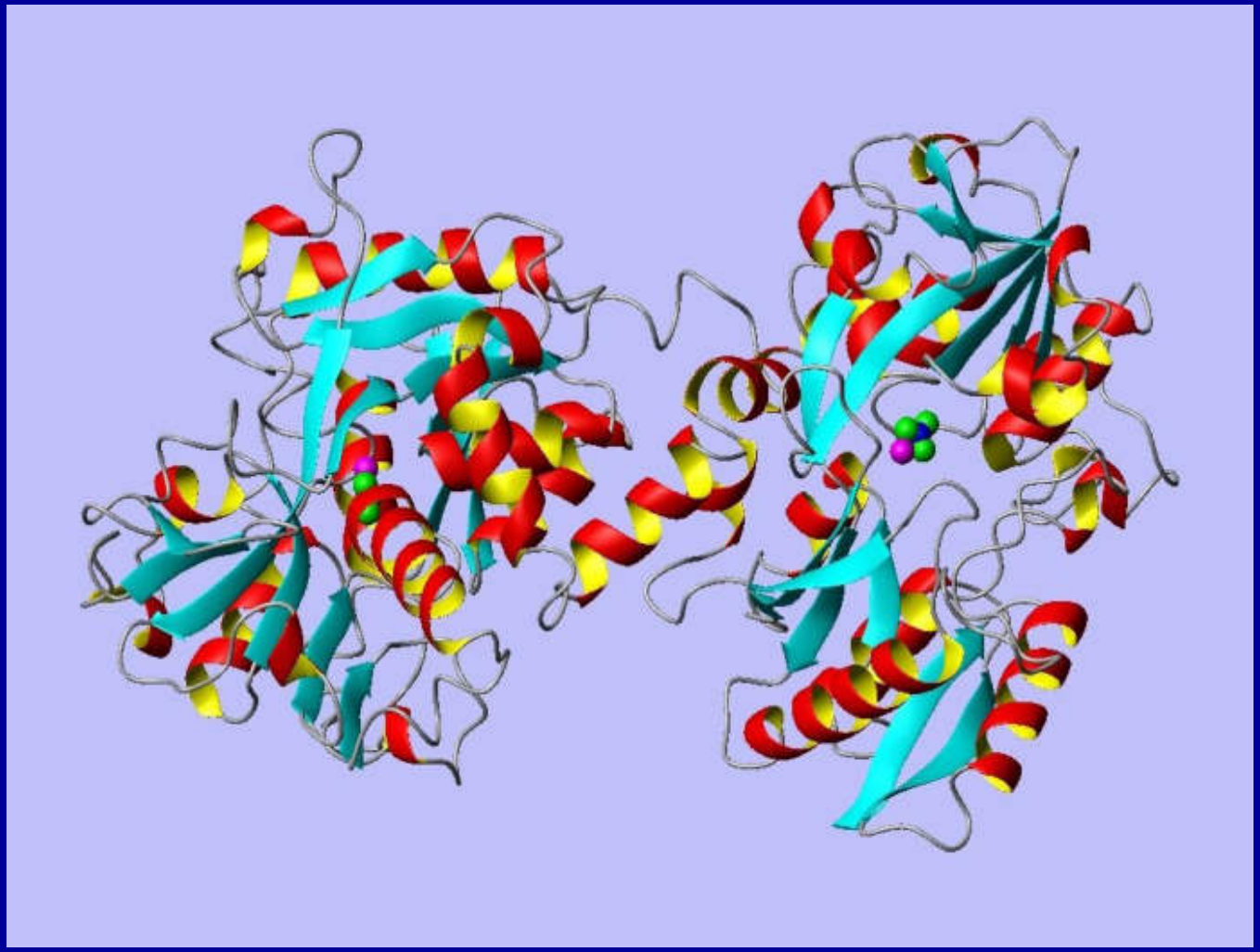
Iron uptake



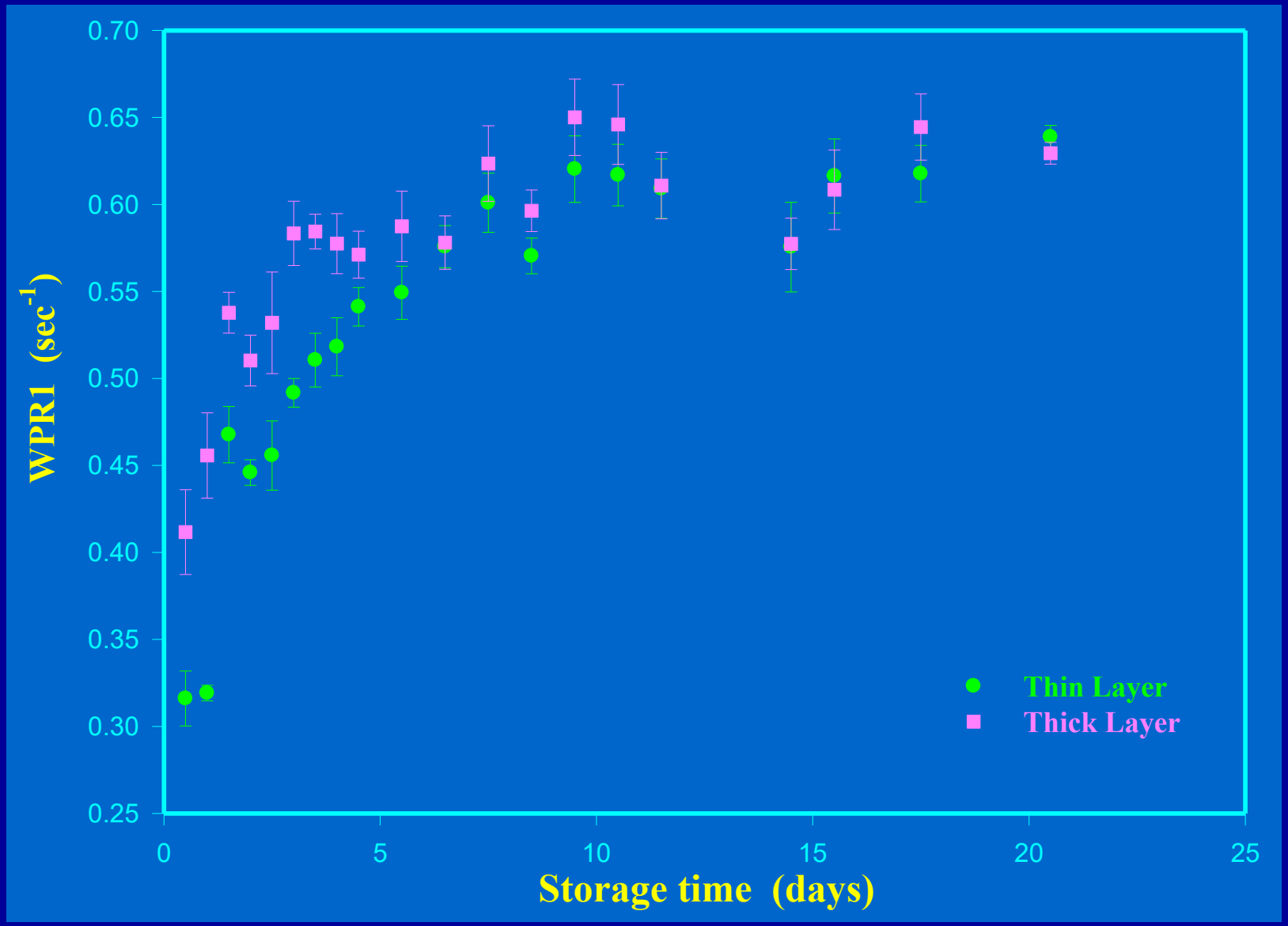


Effect of MECAM analogues on iron uptake from *E. coli*. Iron transport by 2 μM ferric enterobactin is inhibited by ferric MECAM.

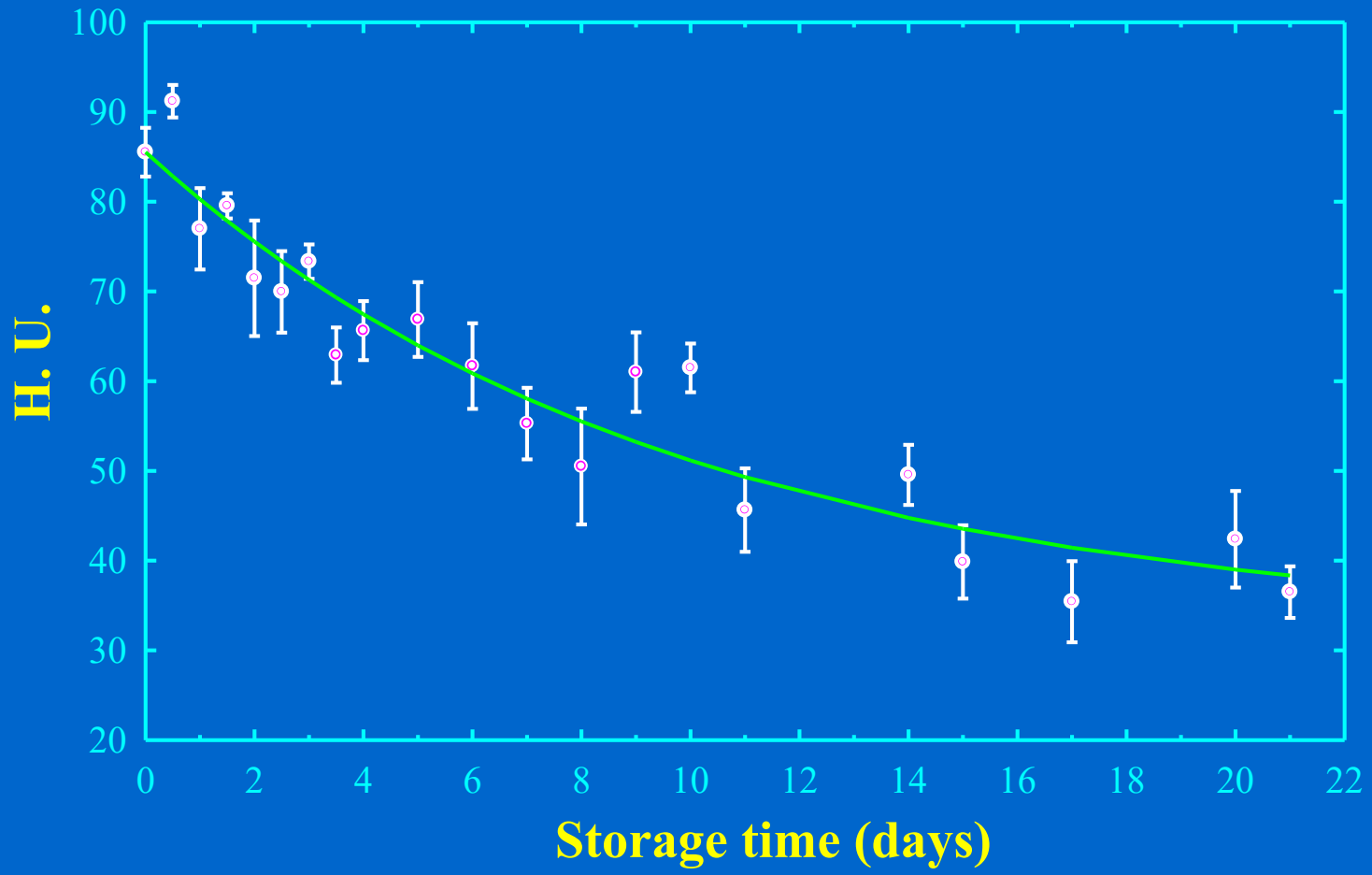
Transferrina



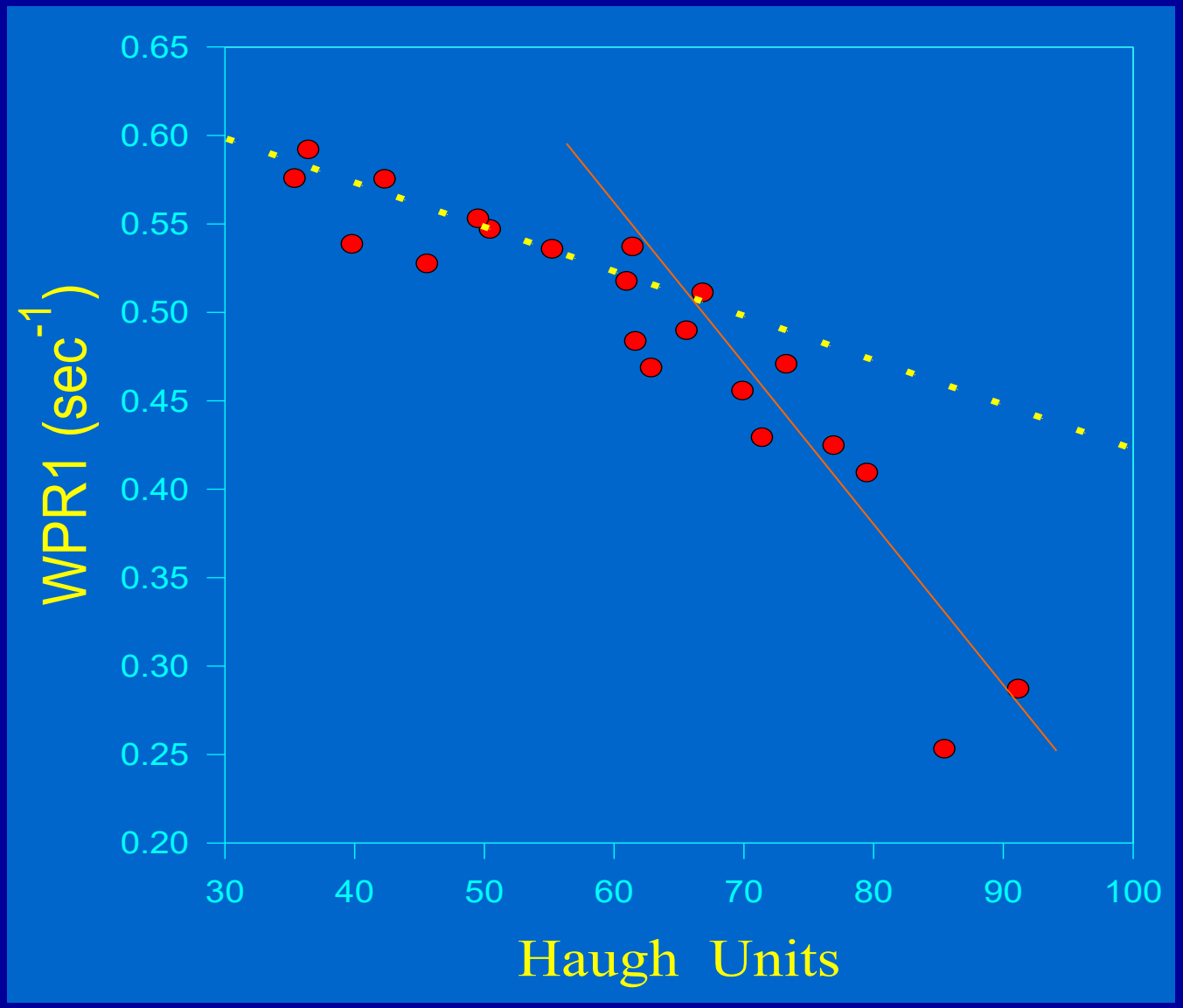
The storage effect on the water proton longitudinal relaxation rate



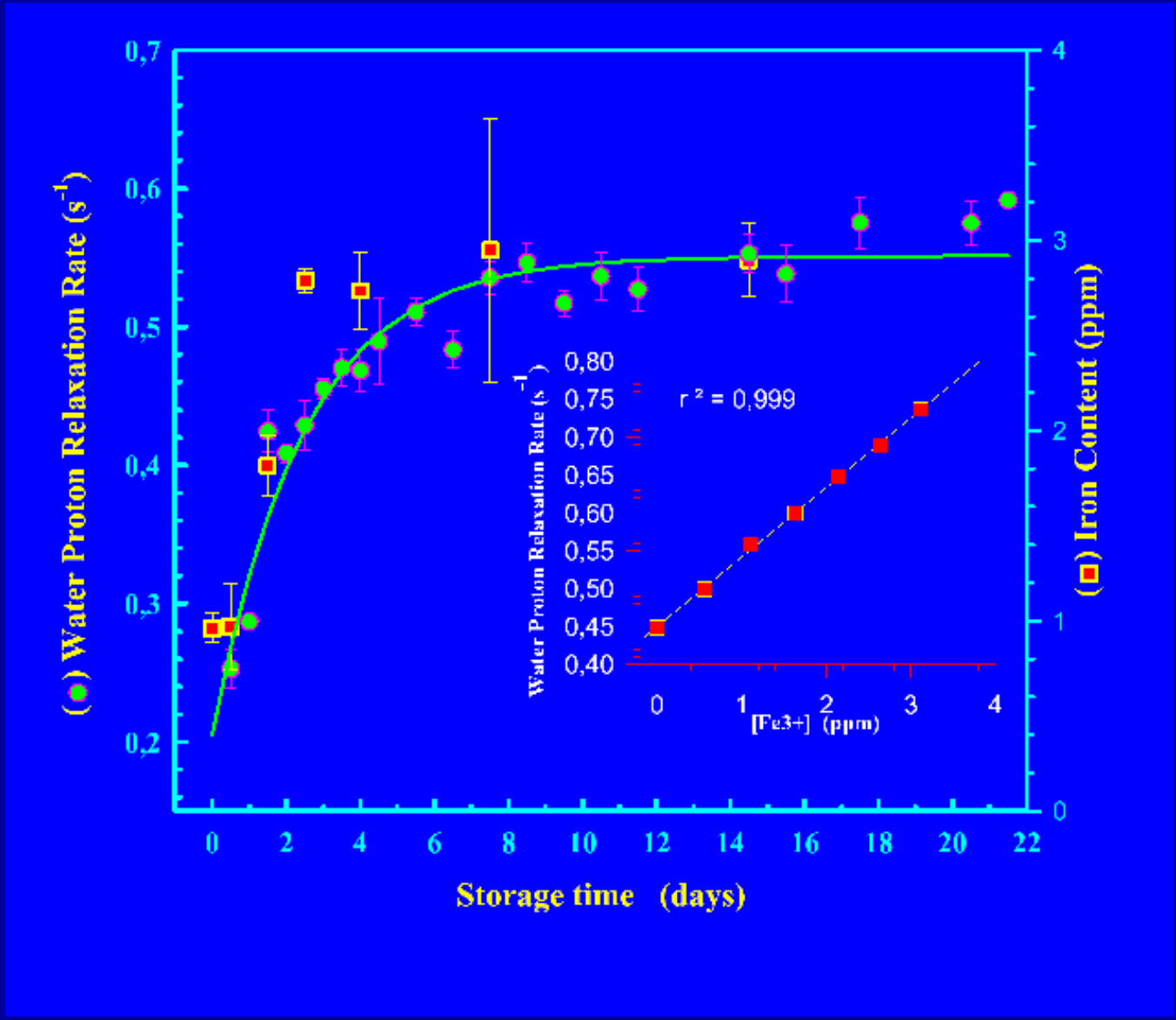
Variance of Haugh Units with storage time



NMR and Rheology do not look at the same phenomenon



The iron is readily captured by ovotransferrin



Funzione enzimatica

Enzimi idrolitici

Representative metallo-enzymes catalyzing hydrolytic and related reactions

Enzyme	Metal(s)	Function
Carboxypeptidase	Zn^{2+}	Hydrolysis of C-terminal peptide residues
Leucine aminopeptidases	Zn^{2+}	Hydrolysis of leucine N-terminal peptide residues
Dipeptidase	Zn^{2+}	Hydrolysis of dipeptides
Neutral protease	Zn^{2+}, Ca^{2+}	Hydrolysis of peptides
Collagenase	Zn^{2+}	Hydrolysis of collagen
Phospholipase C	Zn^{2+}	Hydrolysis of phospholipids
β-Lactamase II	Zn^{2+}	Hydrolysis of β-lactam ring
Thermolysin	Zn^{2+}, Ca^{2+}	Hydrolysis of peptides
Alkaline phosphatase	Zn^{2+}, Mg^{2+}	Hydrolysis of phosphate esters
Carbonic anhydrase	Zn^{2+}	Hydration of CO_2
α-Amilase	Ca^{2+}, Zn^{2+}	Hydrolysis of glucosides
Phospholipase A_2	Zn^{2+}	Hydrolysis of phospholipids
Inorganic pyrophosphatase	Mg^{2+}	Hydrolysis of pyrophosphate
ATPase	Mg^{2+}	Hydrolysis of ATP

Enzyme	Metal(s)	Function
Na^+ - K^+ - ATPase	Na^+, K^+	Hydrolysis of ATP with transport of cations
Mg^{2+} - Ca^{2+} - ATPase	Mg^{2+}, Ca^{2+}	Hydrolysis of ATP with transport of cations
Phosphatases	Mg^{2+}, Zn^{2+}	Hydrolysis of phosphate esters
Creatine kinase	M^{2+}	Phosphorylation of creatine
Pyruvate kinase	M^+, M^{2+}	Dephosphorylation of phosphoenolpyruvate
Phosphoglucomutase	Mg^{2+}	Phosphate transfer converting glucose-1-phosphate to G-6-P
DNA polymerase	Mg^{2+} (Mn^{2+})	Polymerization of DNA with formation of phosphate esters
Alcohol dehydrogenase	Zn^{2+}	Hydride transfer from alcohols to NAD^+

Acidità dell'acqua di coordinazione

The pK_a values of coordinated water in some metal complexes.

Complex	Note	Donor set	pK_a	Reference
$Ca(NO_3)_2(OH_2)_4^{2+}$		O_6	10.3	8
$Cr(OH_2)_6^{3+}$		O_6	4.2	8
$Cr(NH_3)_5OH_2^{3+}$		N_5O	5.1	8
$Mn(OH_2)_6^{2+}$		O_6	10.5	8
$Fe(OH_2)_6^{3+}$		O_6	1.4	8
$Co(OH_2)_6^{2+}$		O_6	9.8	8
$Co(dacoda)OH_2^{2+}$	(a)	N_2O_3	9.4	9
$Co(TPyMA)OH_2^{2+}$	(b)	N_4O	9.0	10
$Co(TMC)OH_2^{2+}$	(c)	N_4O	8.4	11
$Co(CR)OH_2^{2+}$	(d)	N_4O	8.0	12
$Co(NH_3)_5OH_2^{3+}$		N_5O	6.2	8
$Ni(OH_2)_6^{2+}$		O_6	10.0	8
$Cu(OH_2)_6^{2+}$		O_6	7.3	8
$Cu(DMAM\text{-}PMHD)OH_2^{2+}$	(e)	N_3O_2	7.1	13
$Cu(C\text{-}PMHD)OH_2^{+}$	(f)	N_2O_3	6.6	14
$Zn(OH_2)_6^{2+}$		O_6	9.0	8
$Zn(DMAM\text{-}PMHD)OH_2^{2+}$	(e)	N_3O	9.2	13
$Zn(C\text{-}PMHD)OH_2^{+}$	(f)	N_3O_2	7.1	14
$Zn(CR)OH_2^{2+}$	(d)	N_4O	8.7	12
$Zn([12]aneN_3)OH_2^{2+}$	(g)	N_3O	7.3	15,16
$Zn(HP[12]aneN_3)OH_2^{+}$	(h)	N_3O_2	10.7	16
$Zn(TImMP)OH_2^{2+}$	(i)	N_3O	<7	17
$Co(TImMP)OH_2^{2+}$	(i)	N_3O	7.8	17

(a) dacoda = 1,4-diaza-cyclooctane-1,4-diacetate. (b) TPyMA = tris(3,5-dimethyl-1-pyrazolylmethyl)amine. (c) TMC = 1,4,8,11-tetramethyl-1,4,8,11-tetraaza-cyclotetradecane. (d) CR = Schiff base between 2,6-diacetylpyridine and bis(3-aminopropyl)amine. (e) DMAM-PMHD = 1-[(6(dimethylamino)methyl)-2-pyridyl)methyl]hexahydro-1,4-diazepin-5-one.

(f) C-PMHD = 1[(6-carboxy)-2-pyridyl)methyl]hexa-hydro-1,4-diazepin-5-one. (g) [12]aneN$_3$ = 1,5,9-triaza-cyclododecane. (h) HP[12]aneN$_3$ = 2-(2-hydroxyphenylate)-1,5,9-triaza-cyclododecane. (i)TImMP = tris(4,5-dimethyl-2-imidazolylmethyl)phosphinoxide.

Sito catalitico dell'anidrasi carbonica

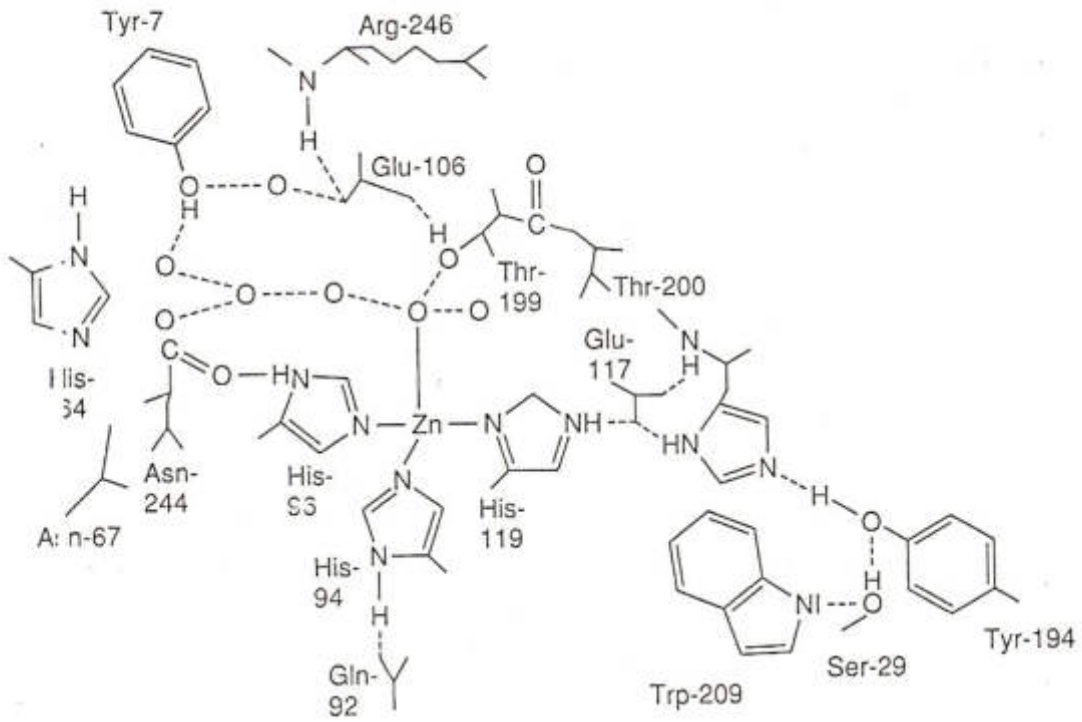
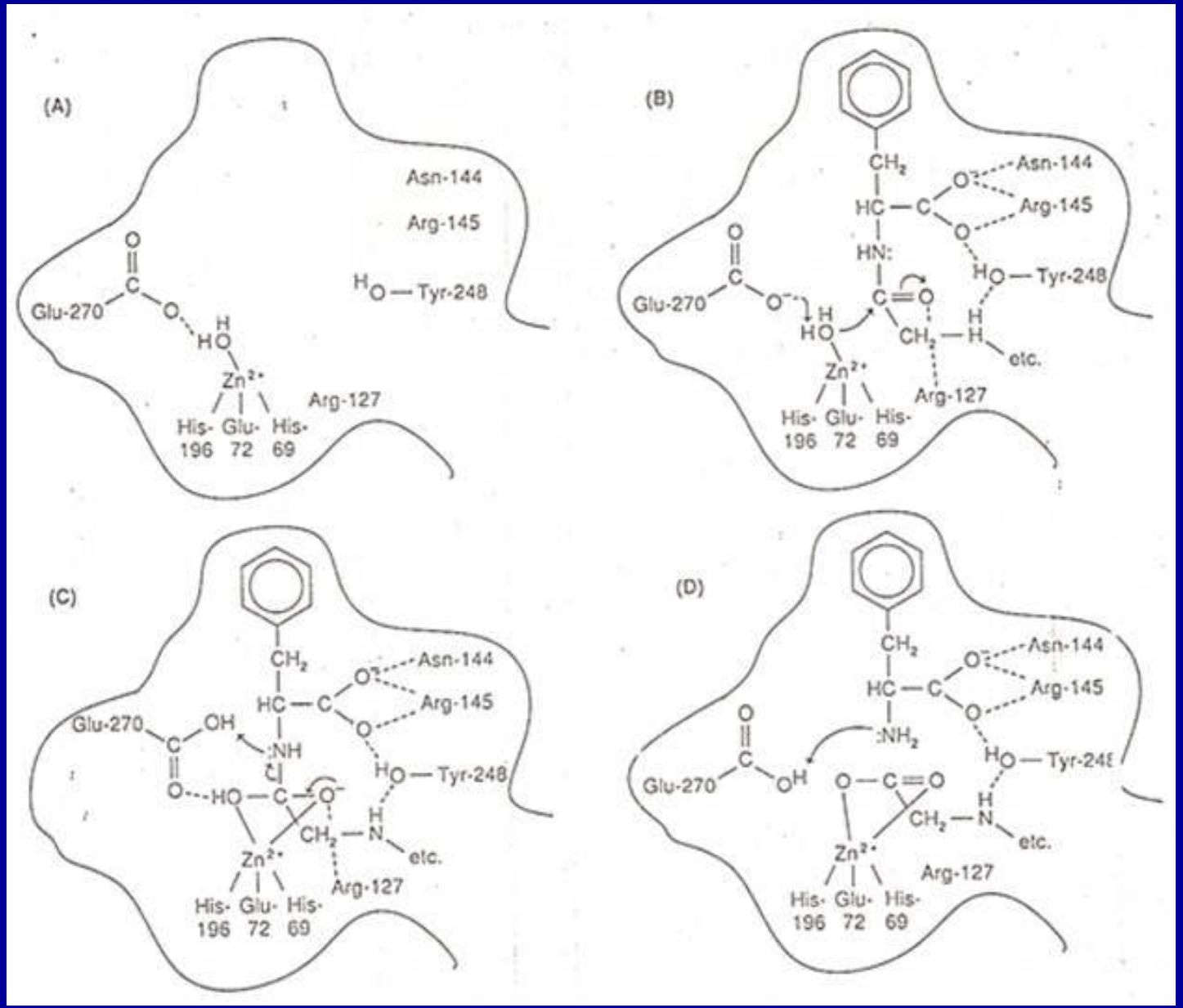
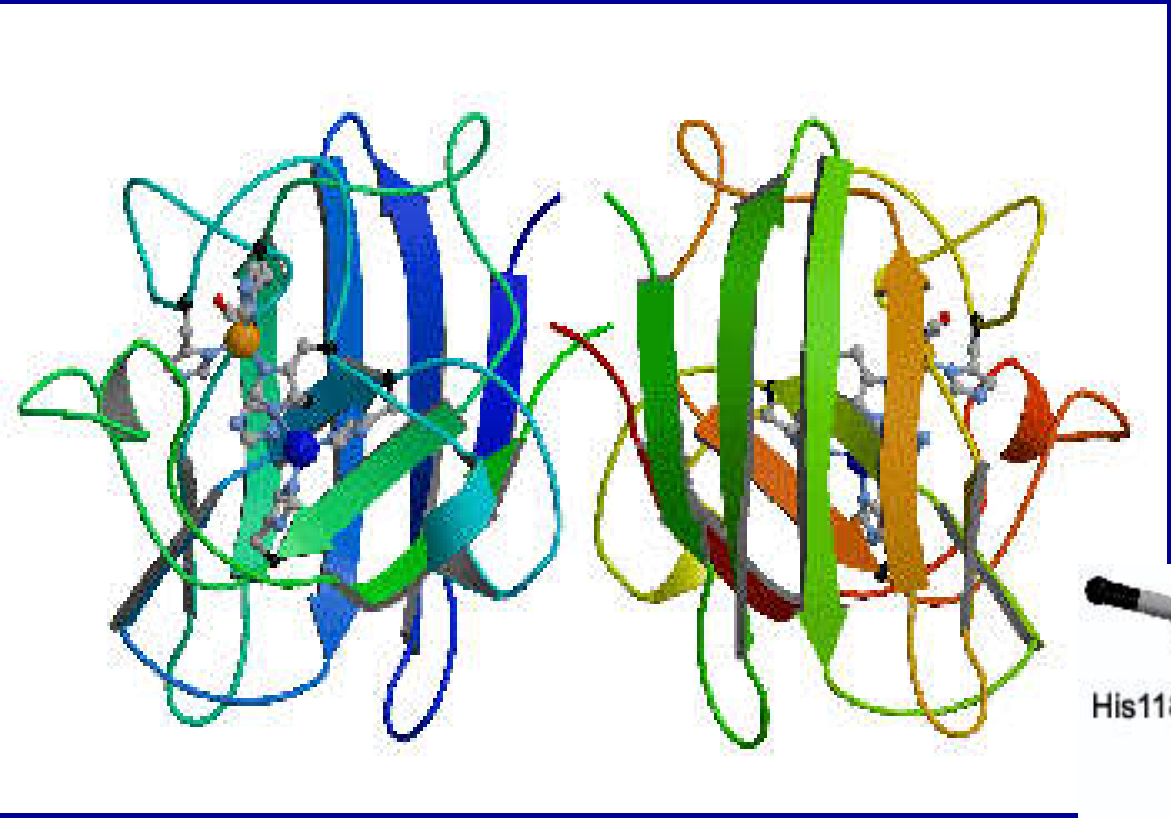


Figure 2.4
Schematic representation of the active site of human carbonic anhydrase II. Hydrogen bonds (---) and ordered water molecules (o) are indicated.[41]

Ciclo catalitico della carbossipeptidasi

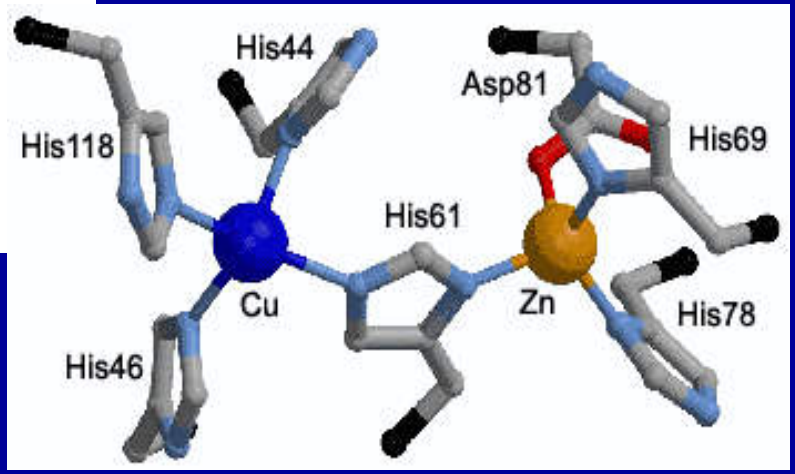


Cu, Zn Superossido dismutasi (SOD)



La SOD è un dimero costituito da due subunità identiche

Il monomero è inattivo



Superoxide ion dismutation

The predominant species present in solution at physiological pH is the unprotonated superoxide anion itself.

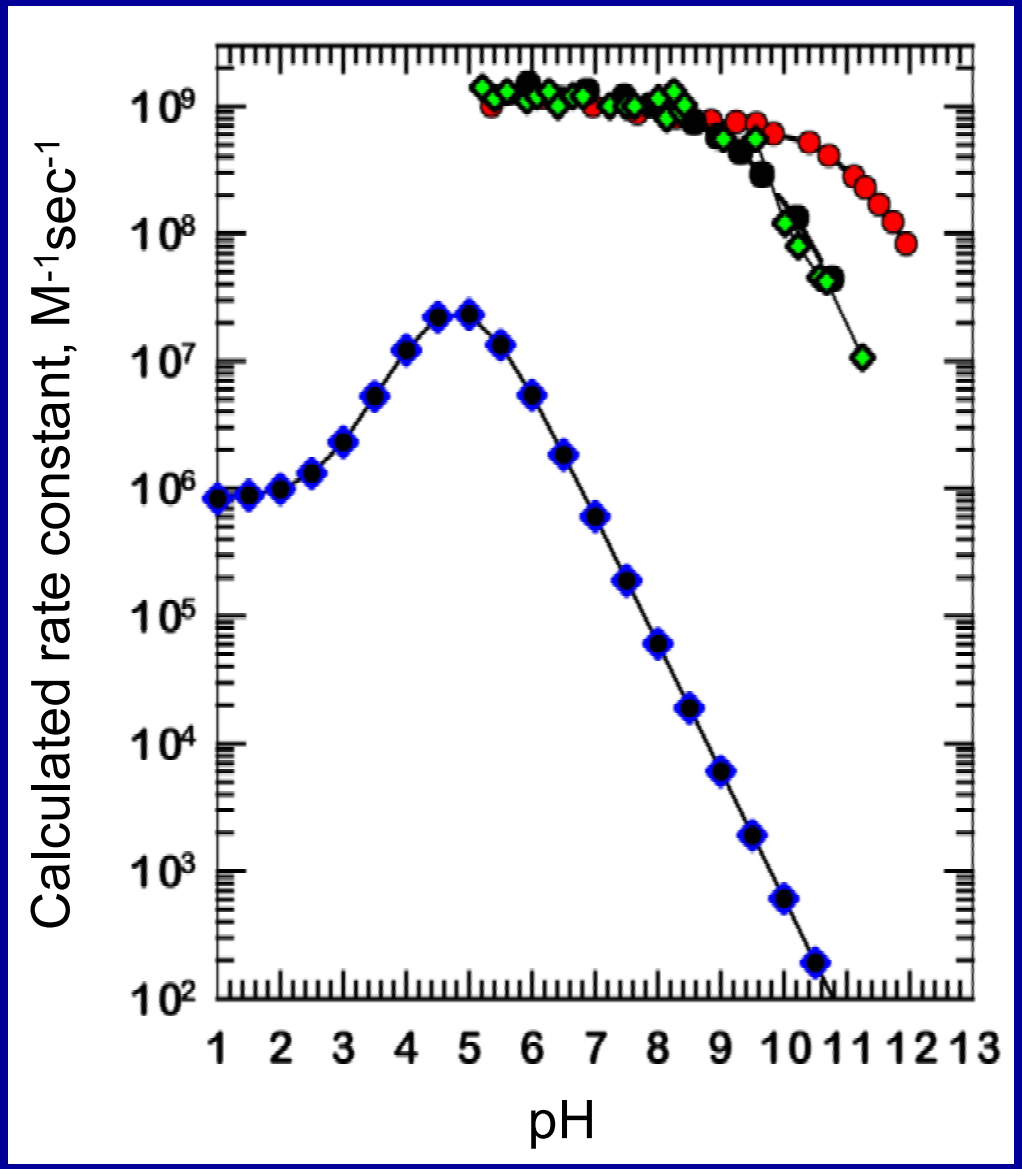
$HO_2 \leftrightarrows O_2^- + H^+$ $\quad Ka = 1.6 \times 10^{-5}$ M

$HO_2 + HO_2 \rightarrow H_2O_2 + O_2$ $\quad k = 8.3 \times 10^5\ M^{-1}s^{-1}$

$HO_2 + O_2^- + H^+ \rightarrow H_2O_2 + O_2$ $\quad k = 9.7 \times 10^7\ M^{-1}s^{-1}$

$O_2^- + O_2^- \rightarrow$ no reaction

Rate constants for superoxide dismutation



blue, no catalyst;
red, CuZnSOD;
green, E. coli MnSOD;
black, NiSOD.

Rate constants for the SODs are per concentration of Cu, Mn, and Ni, respectively

Dismutazione enzimatica del superossido

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$$

$$M^{n+} + O_2^- \rightarrow M^{(n-1)+} + O_2$$

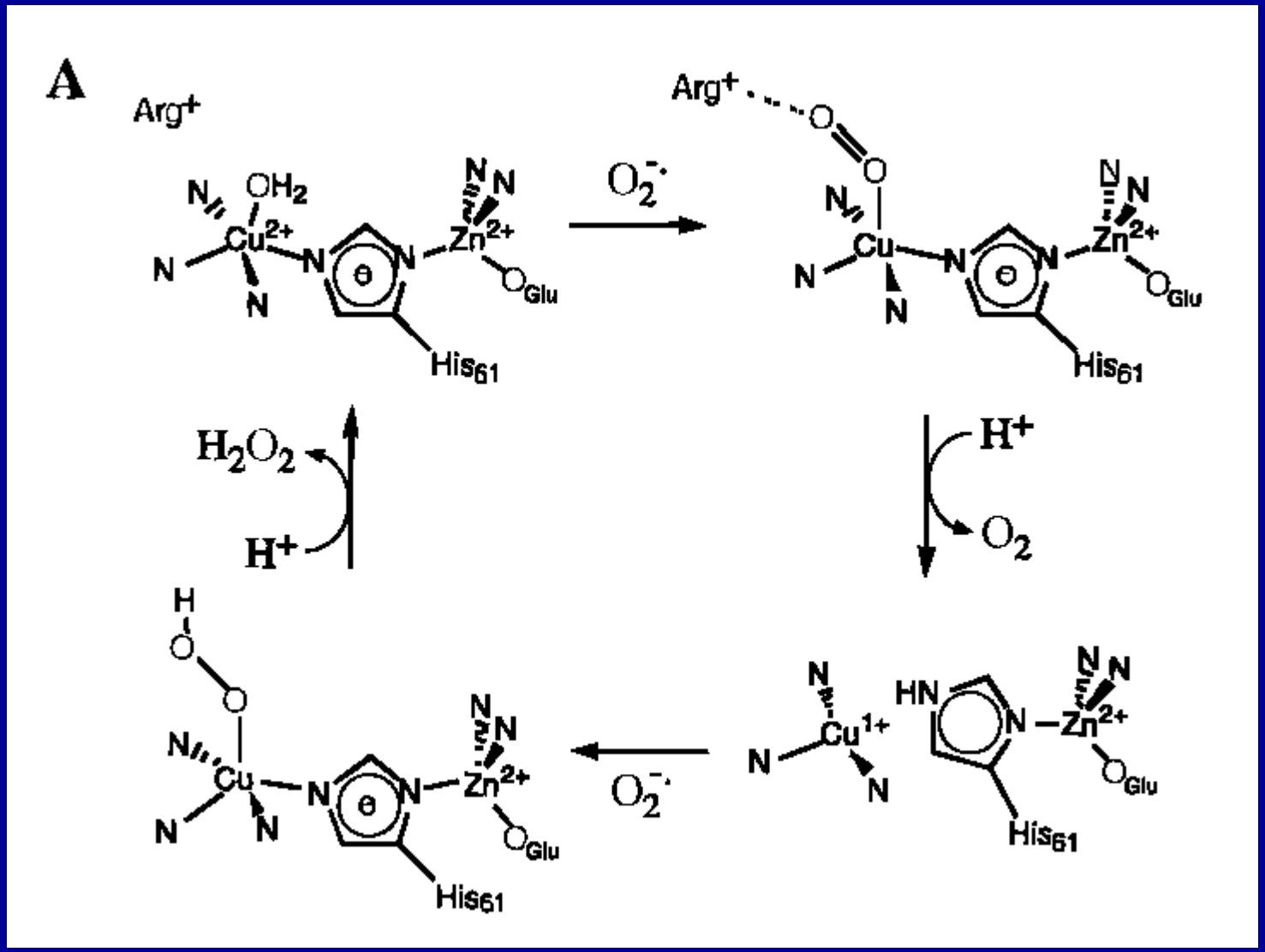
$$M^{(n-1)+} + O_2^- + 2H^+ \rightarrow M^{n+} + H_2O_2$$

✓Ogni metallo redox con potenziale di riduzione compreso tra quello della coppia O_2/O_2^- e della coppia O_2^-/H_2O_2 può catalizzare la reazione di dismutazione

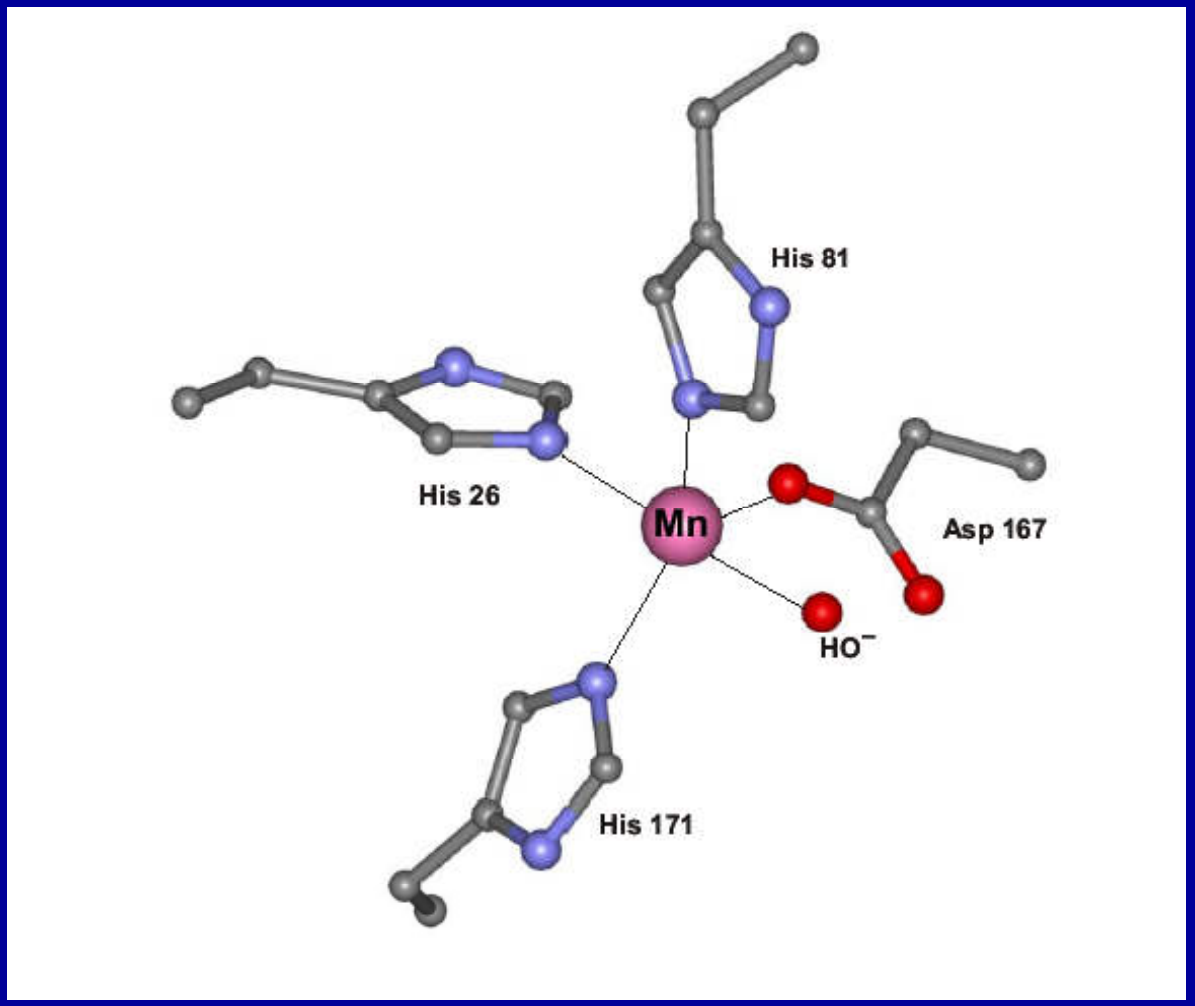
$$SOD\text{-}Cu_2^{2+} + 2O_2^- \rightarrow SOD\text{-}Cu_2^{+} + 2O_2$$

$$SOD\text{-}Cu_2^{+} + 2O_2^- + 4H^+ \rightarrow SOD\text{-}Cu_2^{2+} + 2H_2O_2$$

Il meccanismo di reazione



Metal site in E.coli Mn SOD (PDB code:1VEW)



Three equatorial ligands (His81, Asp167 and His 171) and two axial ligands (His26 and a solvent molecule, modeled as hydroxide ion) form a trigonal bipyramidal arrangement

Funzione di trasporto di ossigeno

Il trasporto dell'ossigeno

- Nel sangue la solubilità dell'O_2 è molto maggiore (30 volte) che in H_2O
  - In H_2O la solubilità è 6.59 cm^3 in 1 dm^3 a 293 K e 1 atm → 3×10^{-4} M
  - Nel sangue, nelle stesse condizioni di T e P, la solubilità sale a 200 cm^3 → 9×10^{-3} M

Il trasporto dell'ossigeno

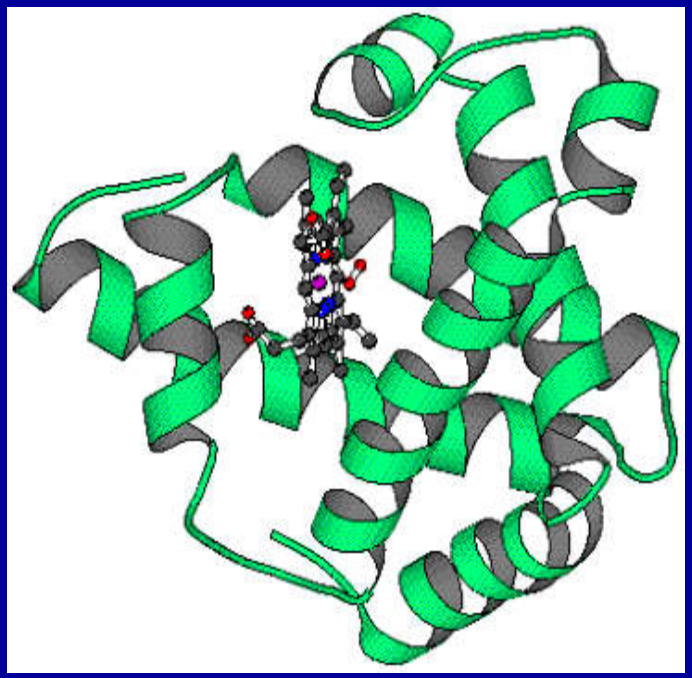
O_2 è un ossidante con un potenziale tale da poter ossidare Fe(II) a Fe(III)

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

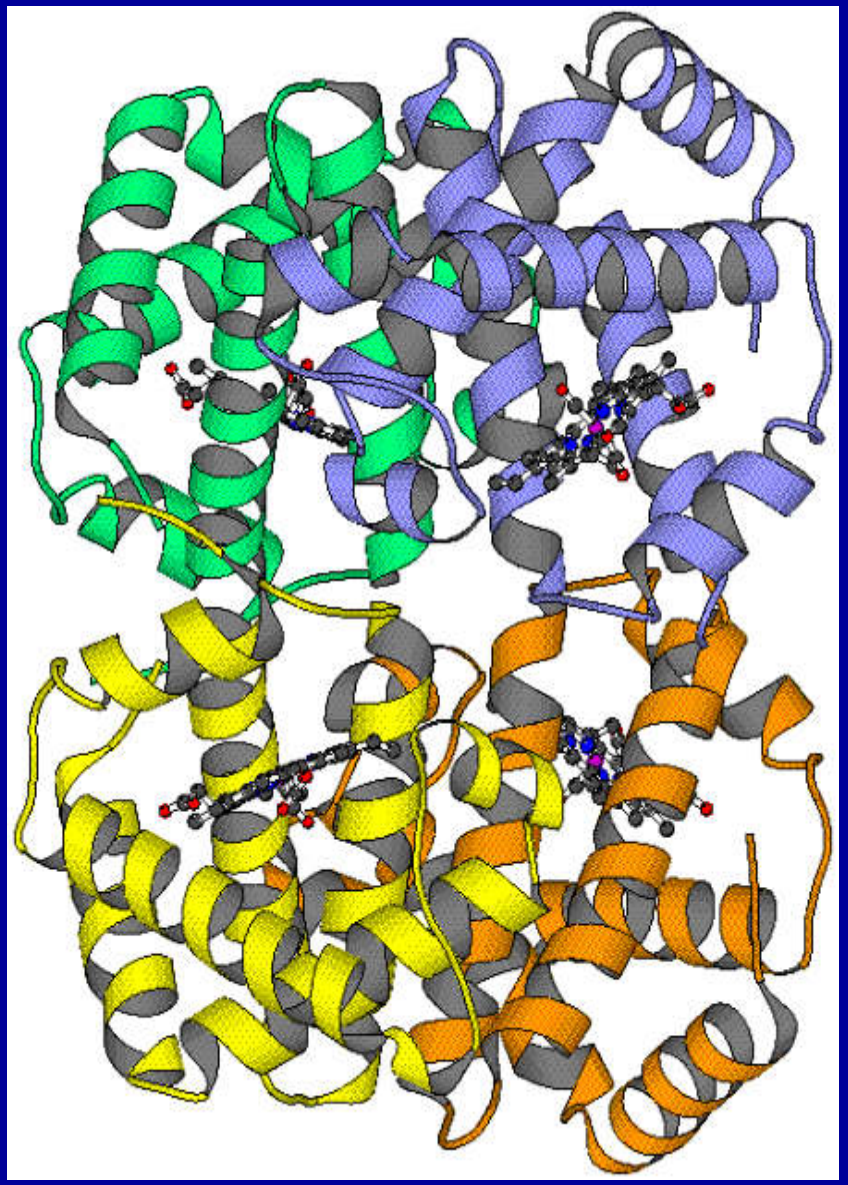
Fe(II) lega O_2

Fe(III) non lega O_2

Le globine

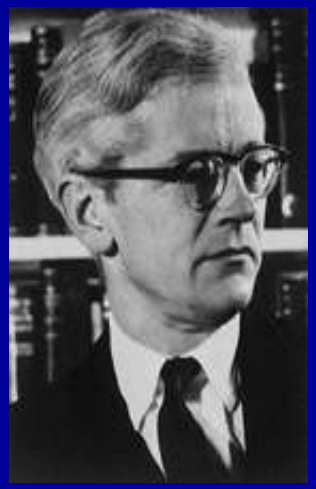


mioglobina



emoglobina

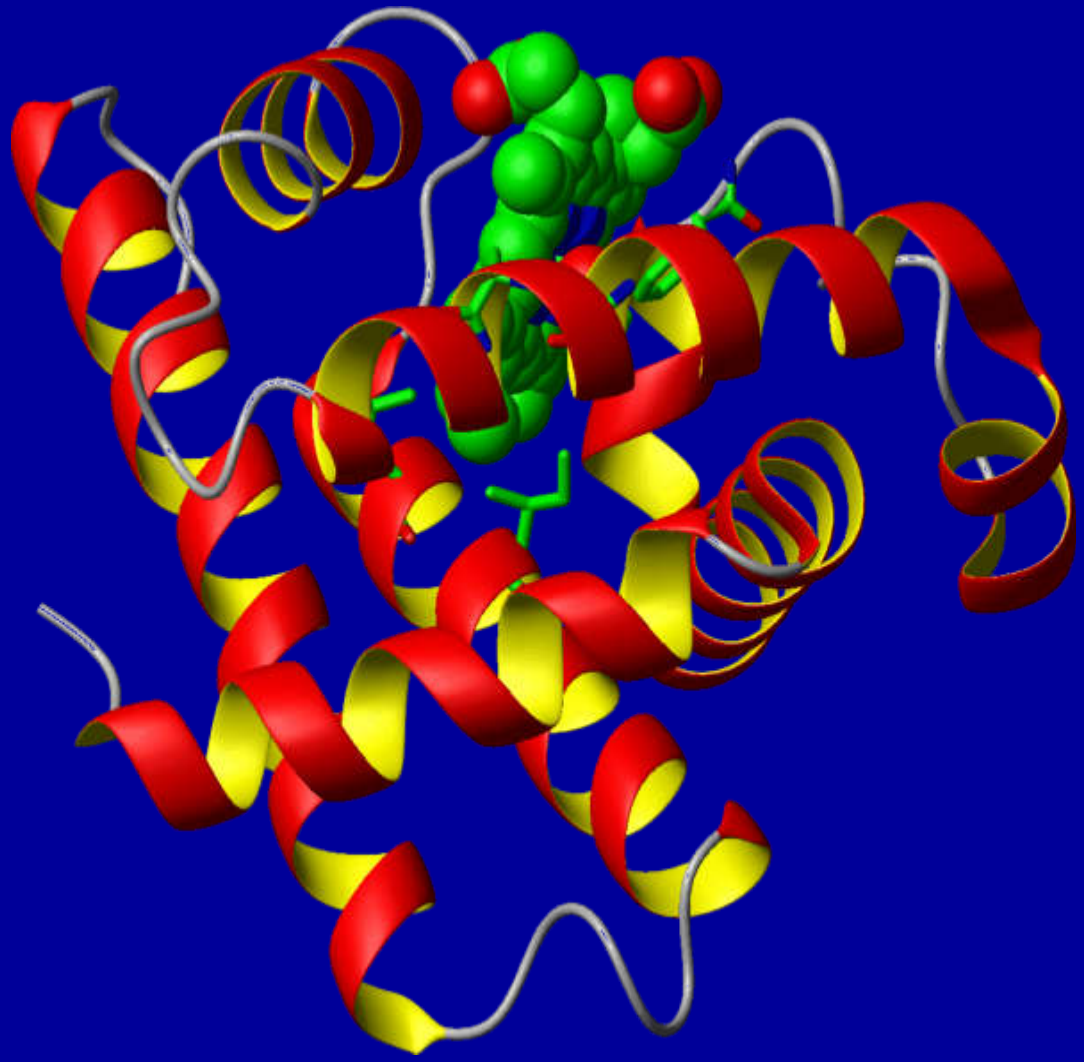
La struttura della mioglobina



John Cowdery Kendrew

MRC, Cambridge, UK

Nobel Prize in 1962

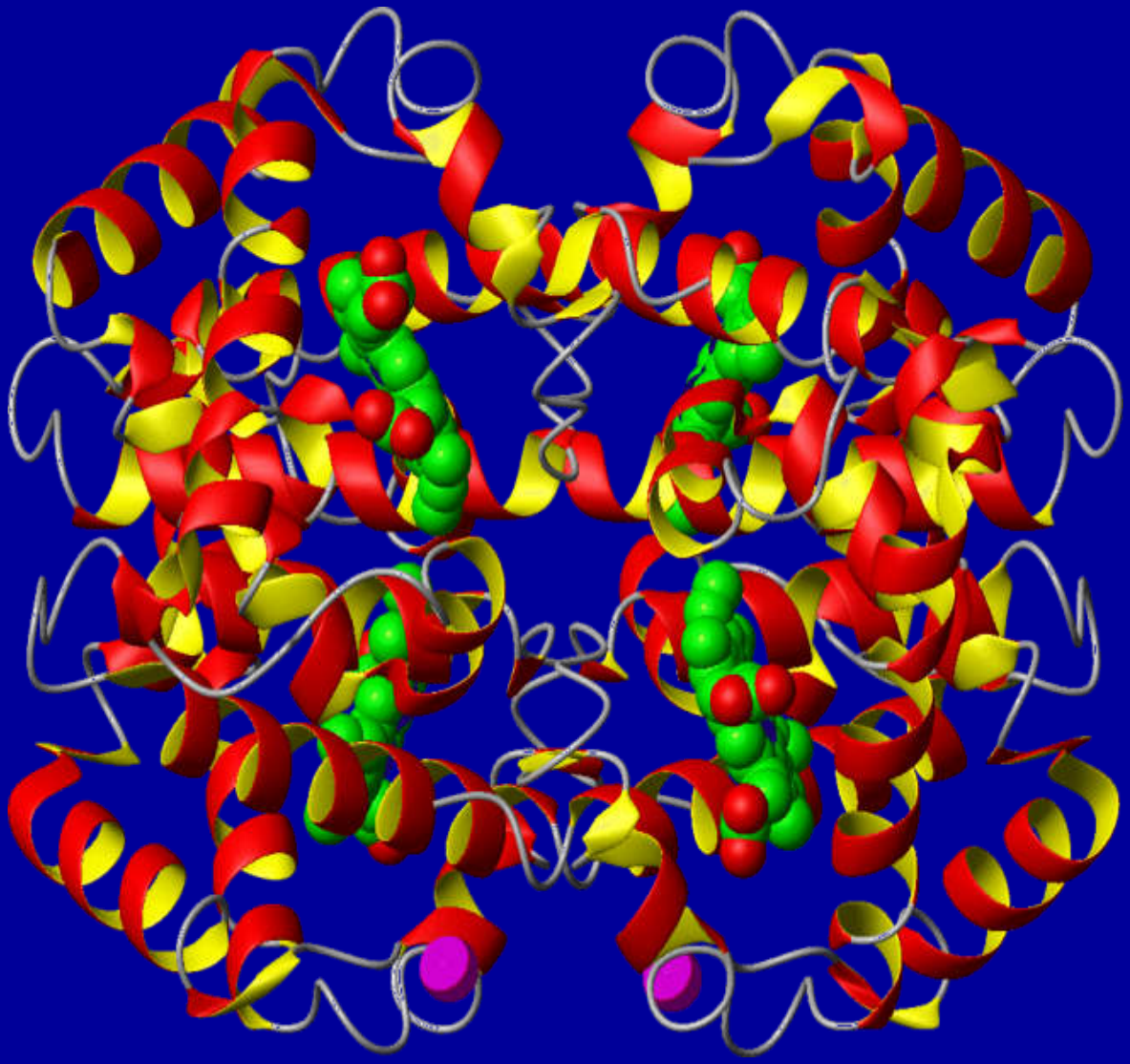


La struttura della emoglobina

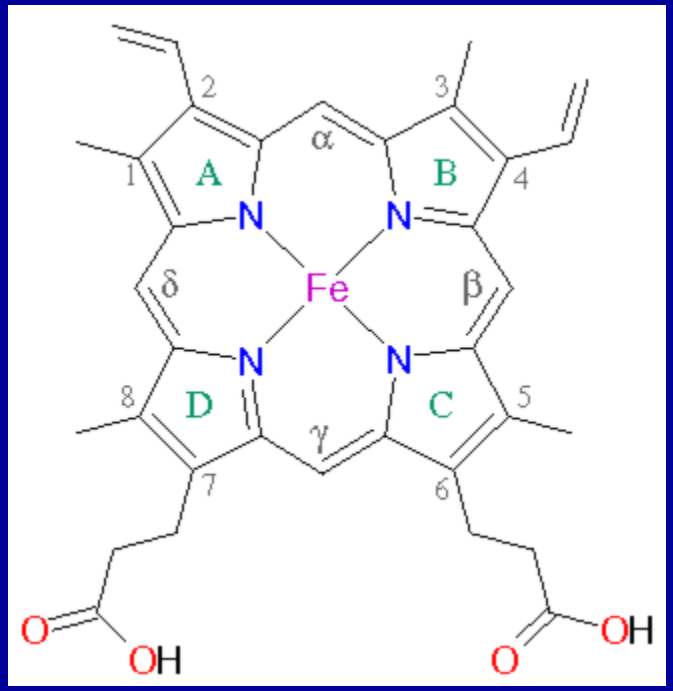
Max Ferdinand Perutz

MRC, Cambridge, UK

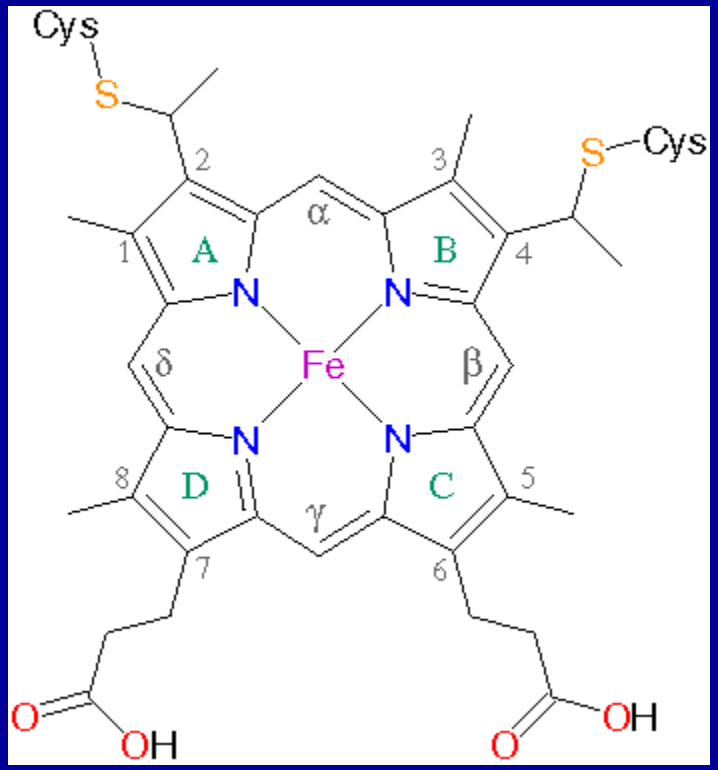
Nobel Prize in 1962



Protoporphyrin IX



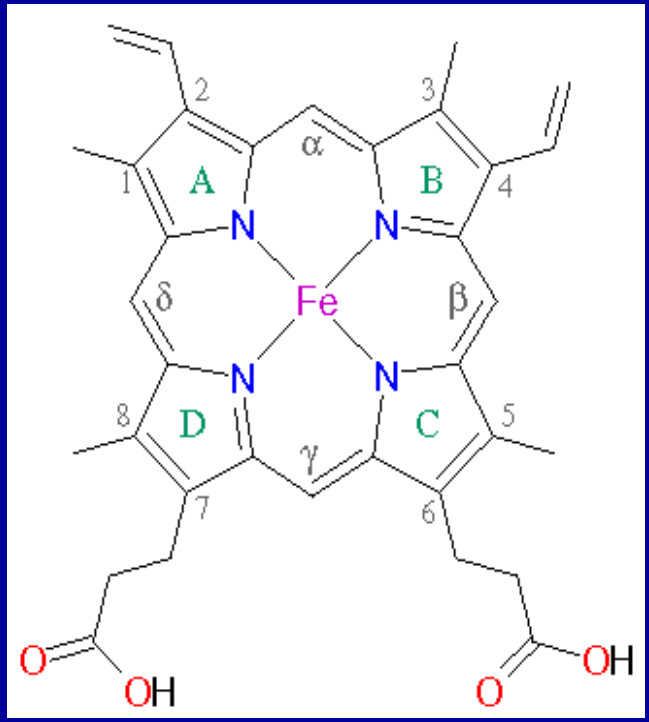
Heme b



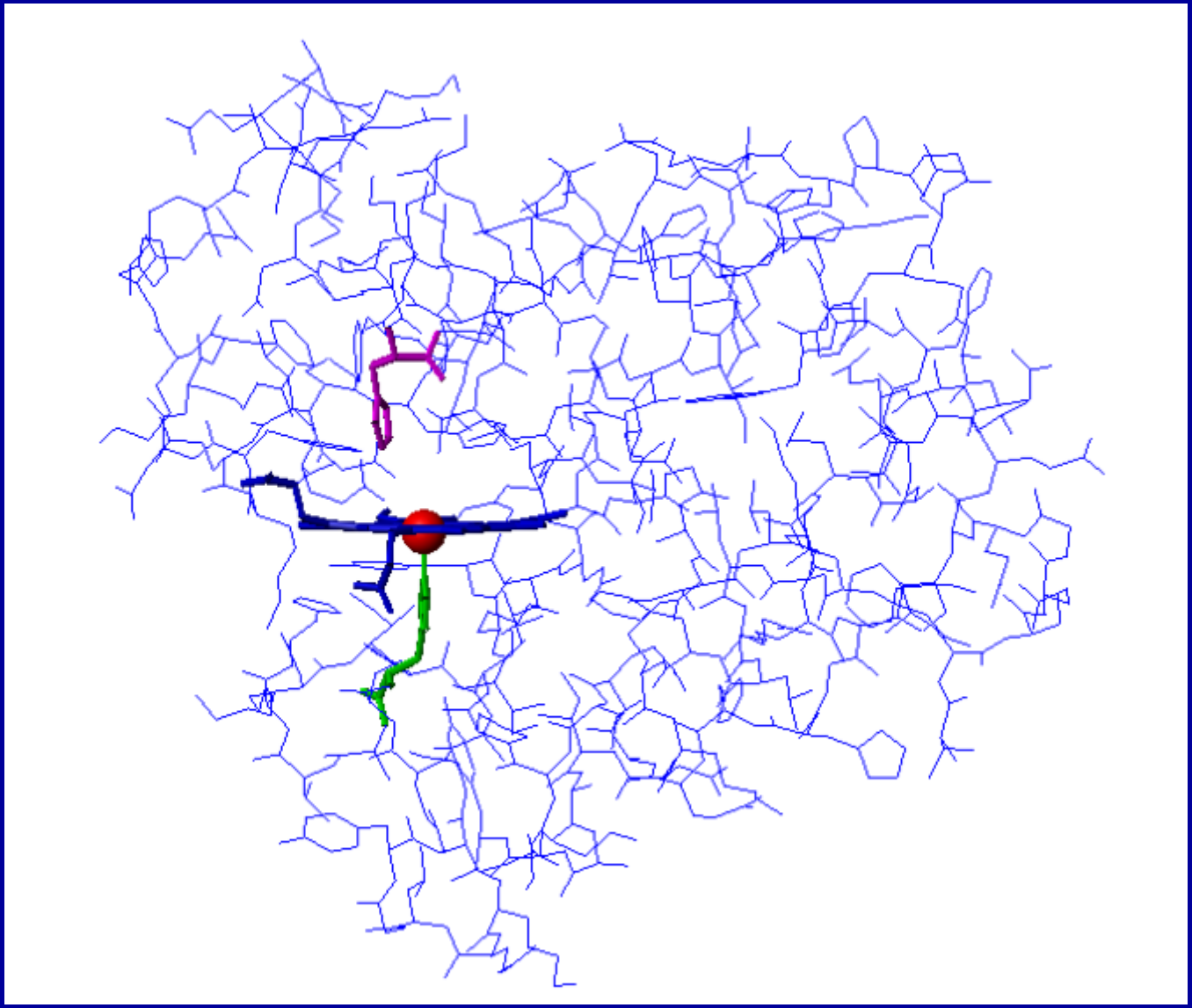
Heme c

Il gruppo eme

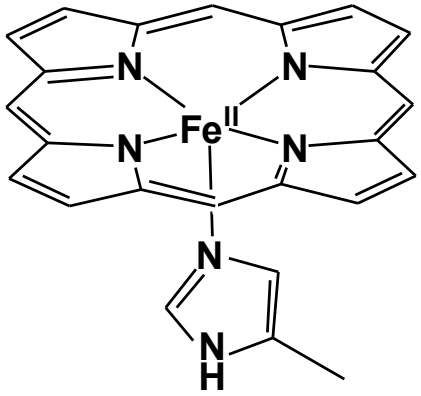
- Contengono un eme b
- Nella forma ridotta, il Fe(II), lega stabilmente O_2
- Il Fe(II) passa da alto a basso spin
- Si distinguono le forme:
  - Ossi
  - Deossi
  - Met



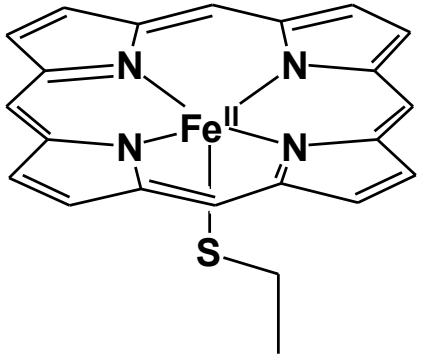
MolMol e Mioglobina



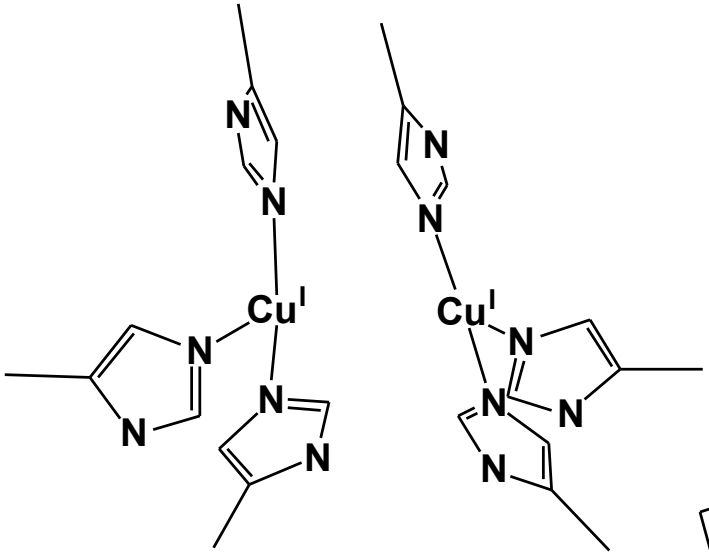
Active sites of the three known dioxygen carriers and their counterparts in oxygen activation



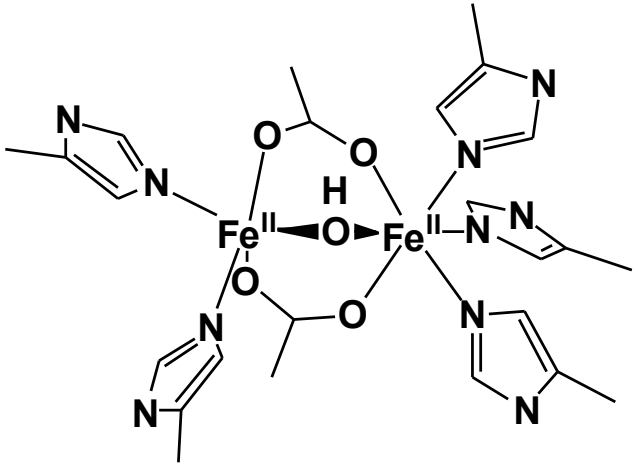
deoxyhemoglobin



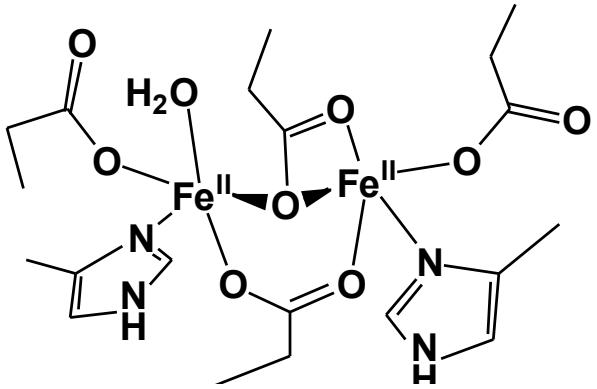
reduced P450



deoxyhemocyanin
and
reduced tyrosinase
(proposed)

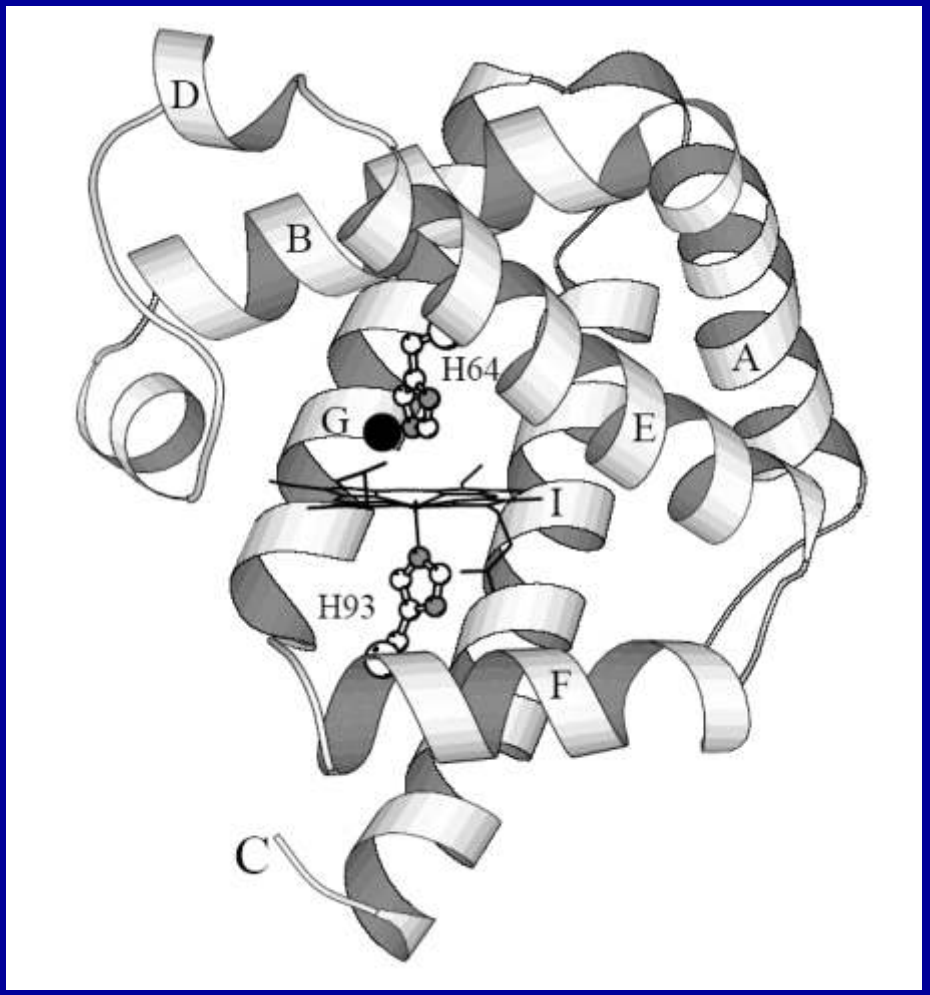


deoxyhemerythrin

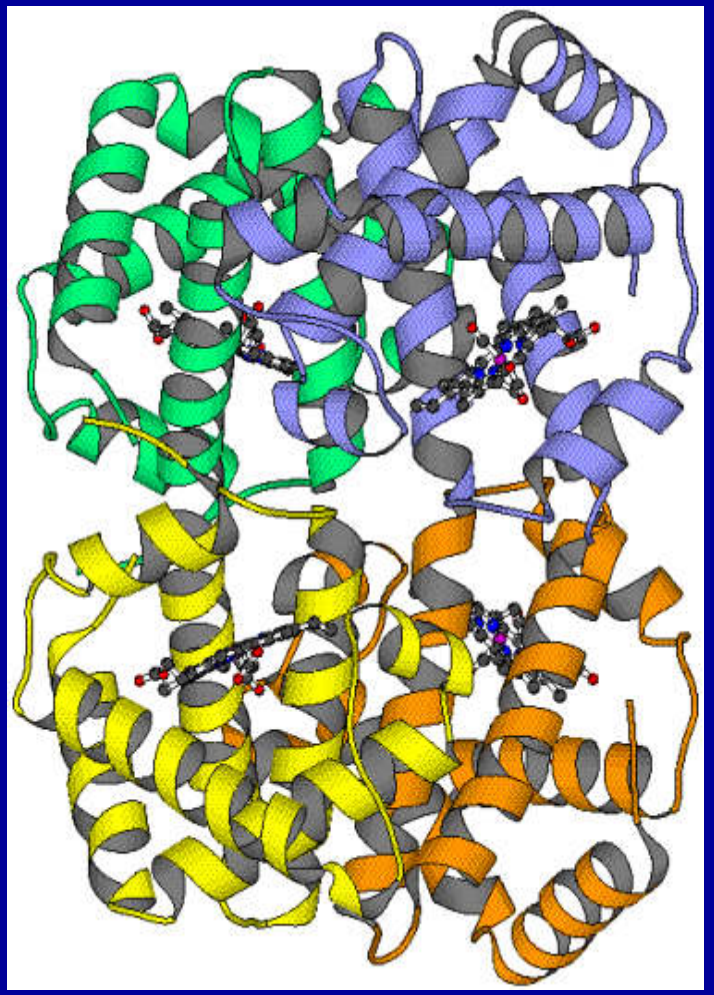


reduced methane monooxygenase

Trasporto e stoccaggio di ossigeno nei mammiferi ed altri animali superiori

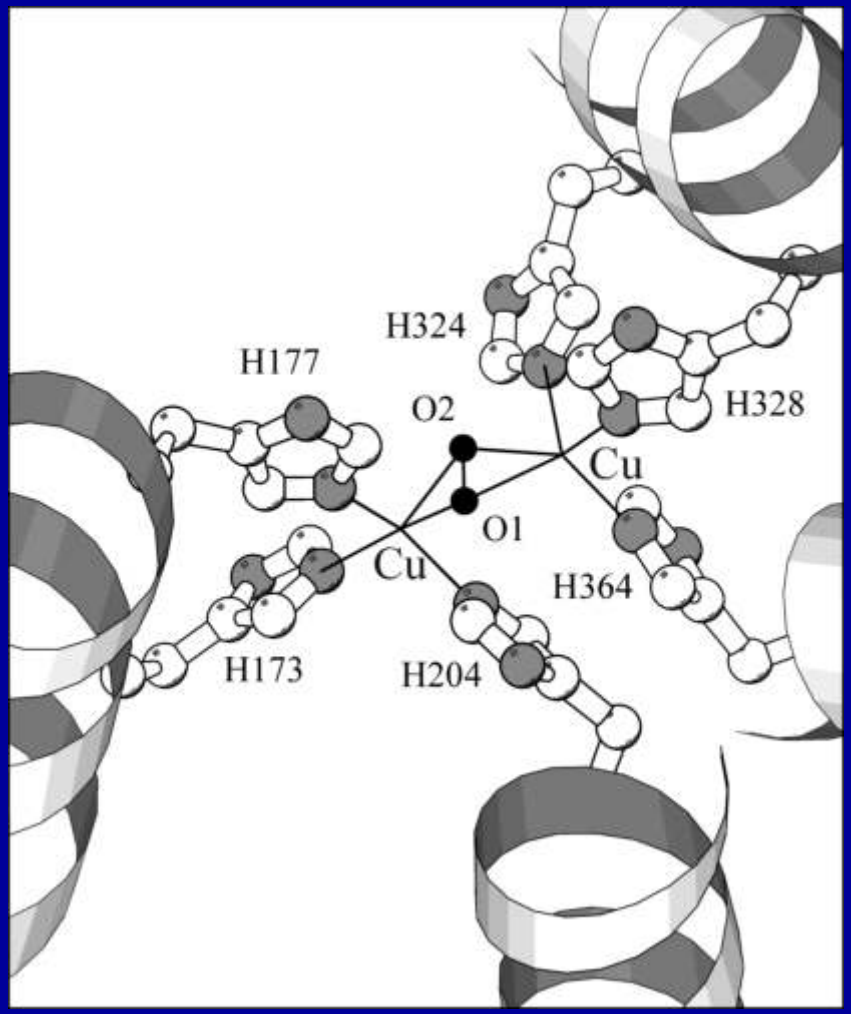


Myoglobin

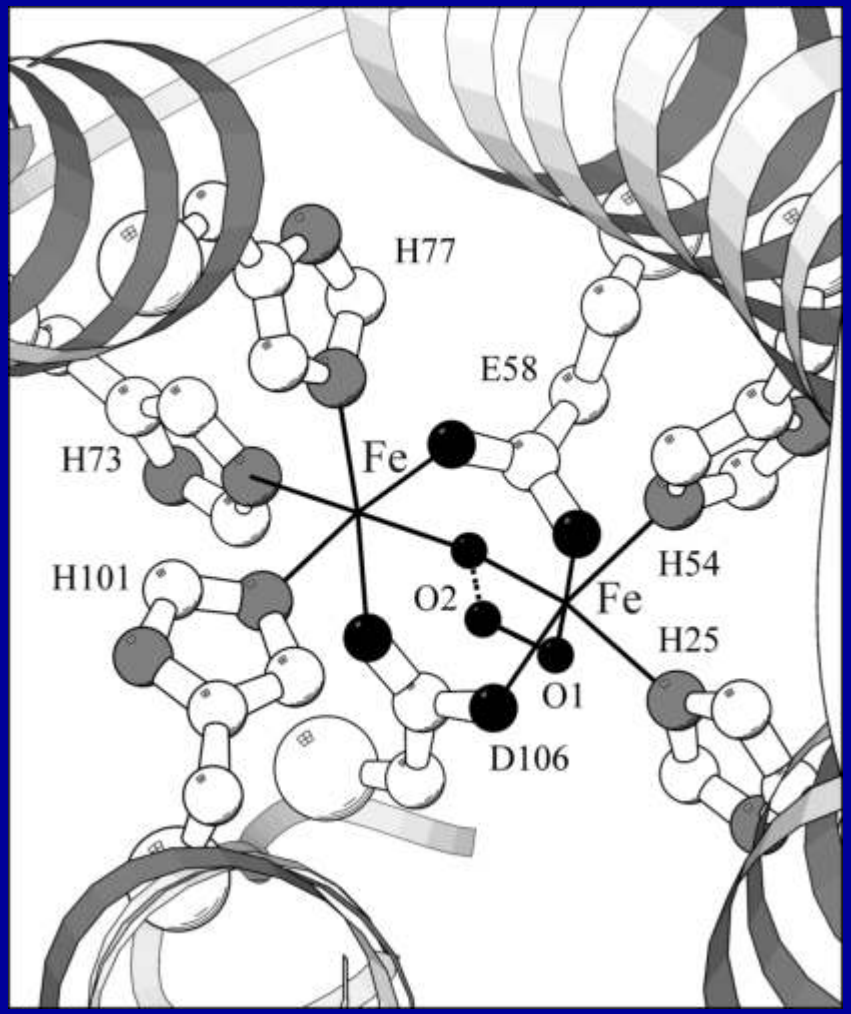


Hemoglobin

Trasporto di ossigeno in molluschi, artropodi e vermi



Hemocyanin



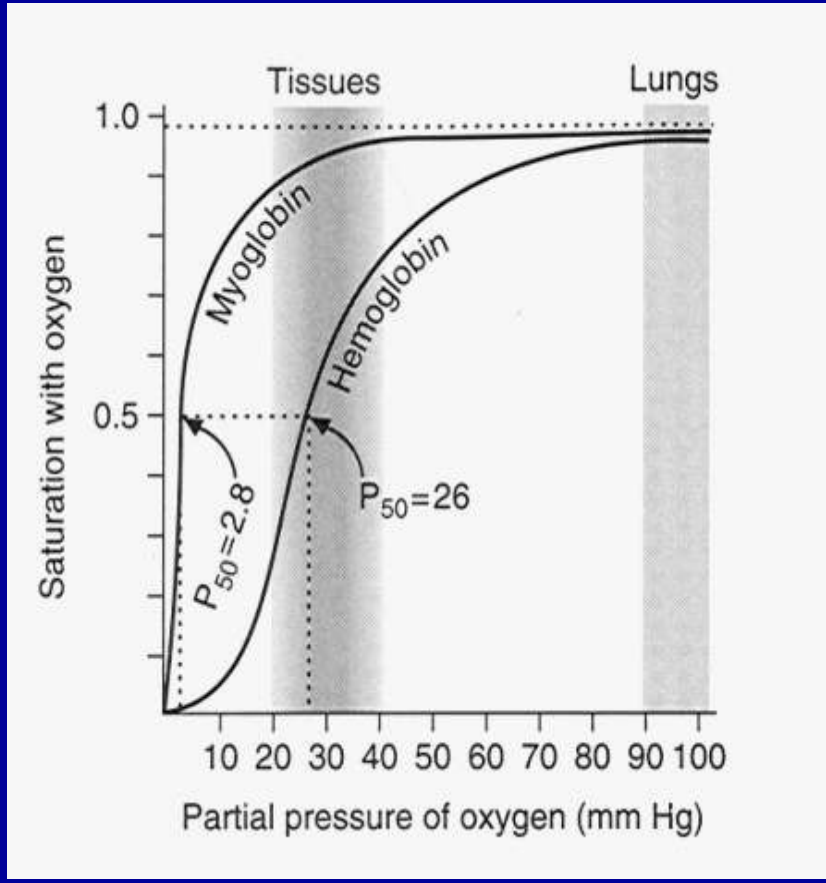
Hemerythrin

Proprietà di legame verso O_2

$Mb + O_2 \leftrightarrows MbO_2$

$Hb + 4O_2 \leftrightarrows Hb(O_2)_4$

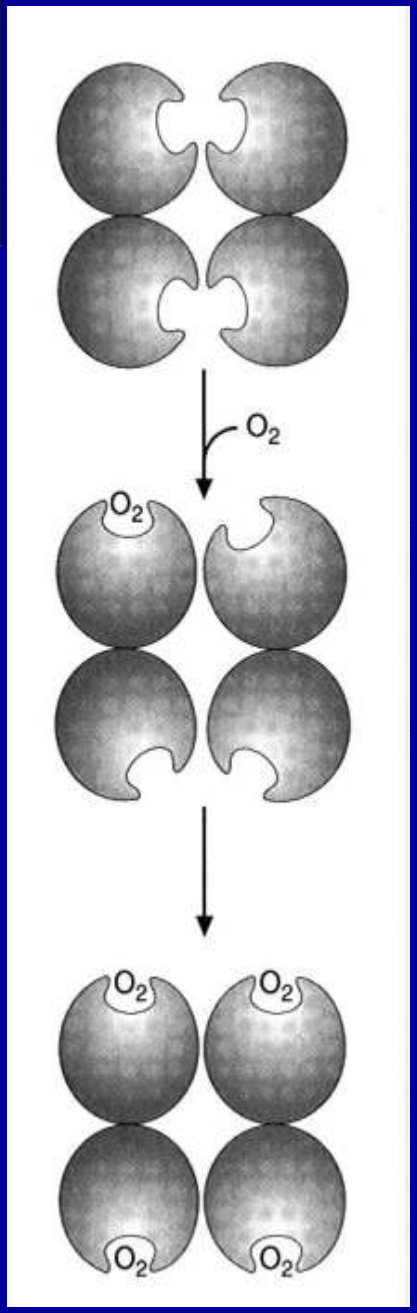
- La solubilità di un gas in un liquido dipende dalla pressione parziale del gas sovrastante la soluzione
- Nei polmoni la P_{O_2} è 3 volte maggiore che nei tessuti
- In presenza di basse pressioni di O_2 Hb è meno efficiente di Mb.
- Pertanto, il trasferimento di O_2 da ossi-Hb a ossi-Mb è favorito dal punto di vista termodinamico.



Legame cooperativo

- La forma deossi-Hb è strutturalmente diversa alla forma ossi-Hb

- Il legame di O_2 ad una subunità favorisce il legame di altro O_2 alla stessa molecola



Confronto delle strutture a raggi-X

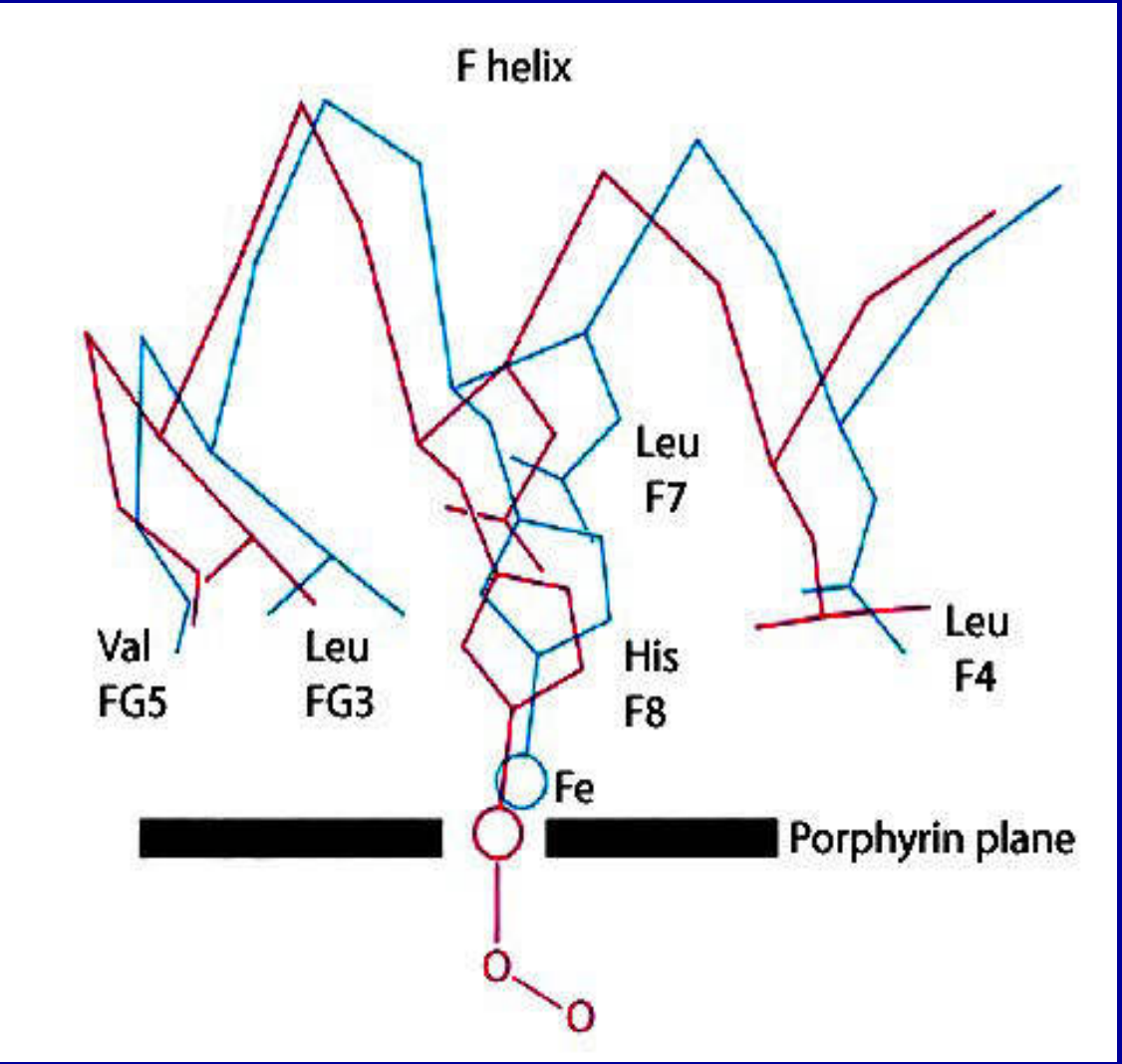
R & T

L'emoglobina può esistere in due stati conformazionali differenti:

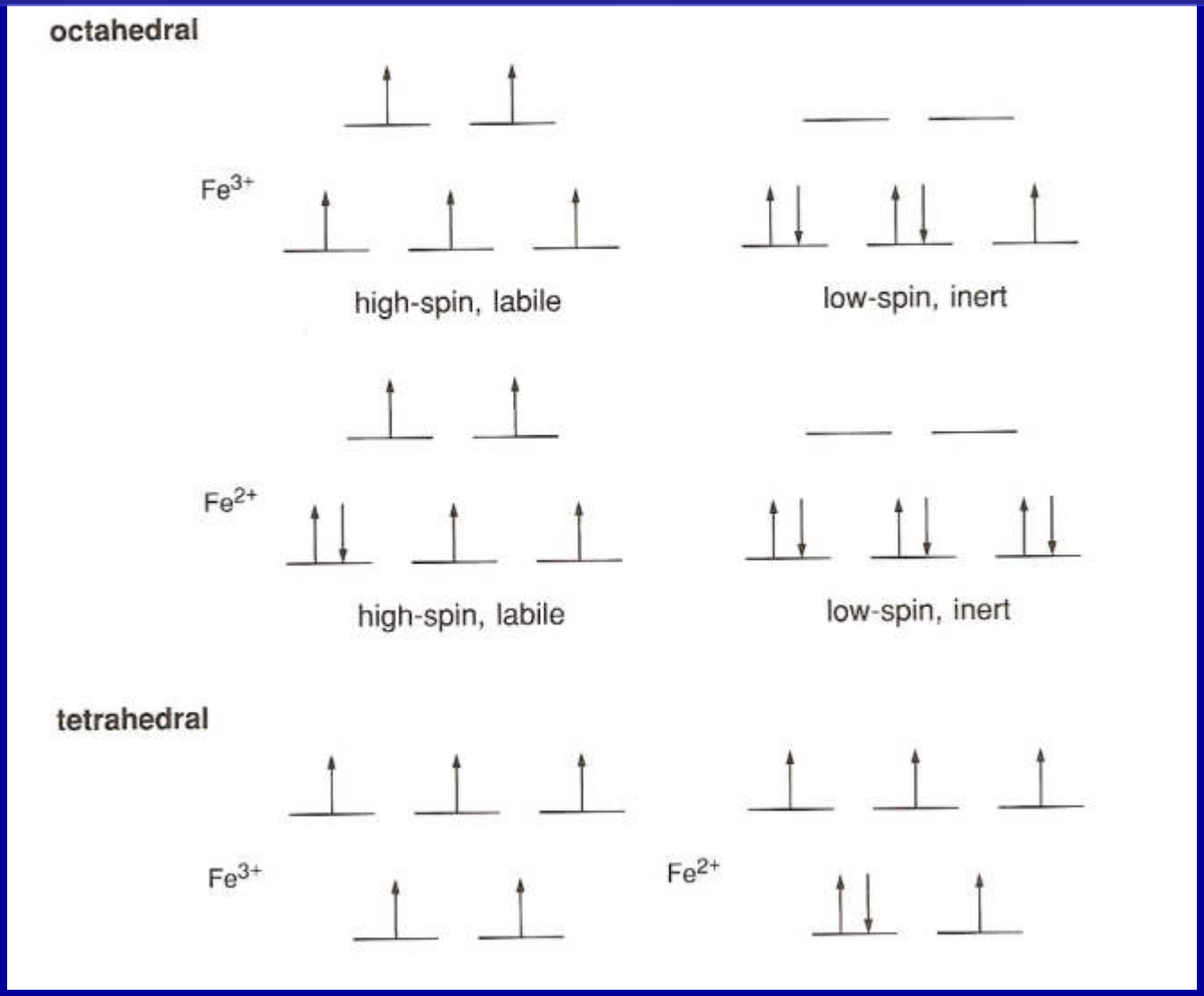
T (tesa) e R (rilassata).

Lo stato R ha una maggior affinità per l'ossigeno e quindi è la conformazione dell'ossiemoglobina.

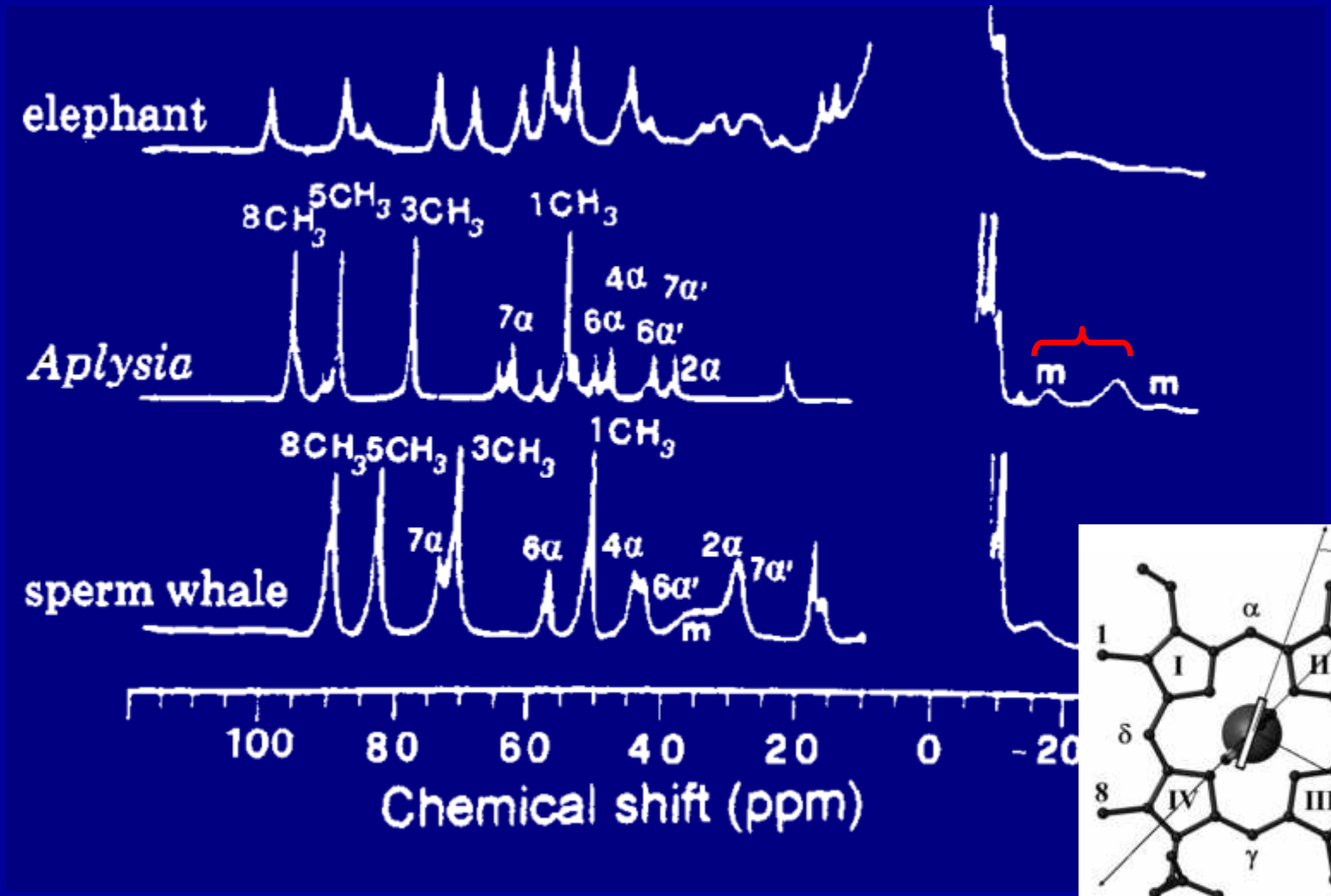
Lo stato T è la conformazione della deossiemoglobina



Geometria di coordinazione dello ione ferro e stato di spin

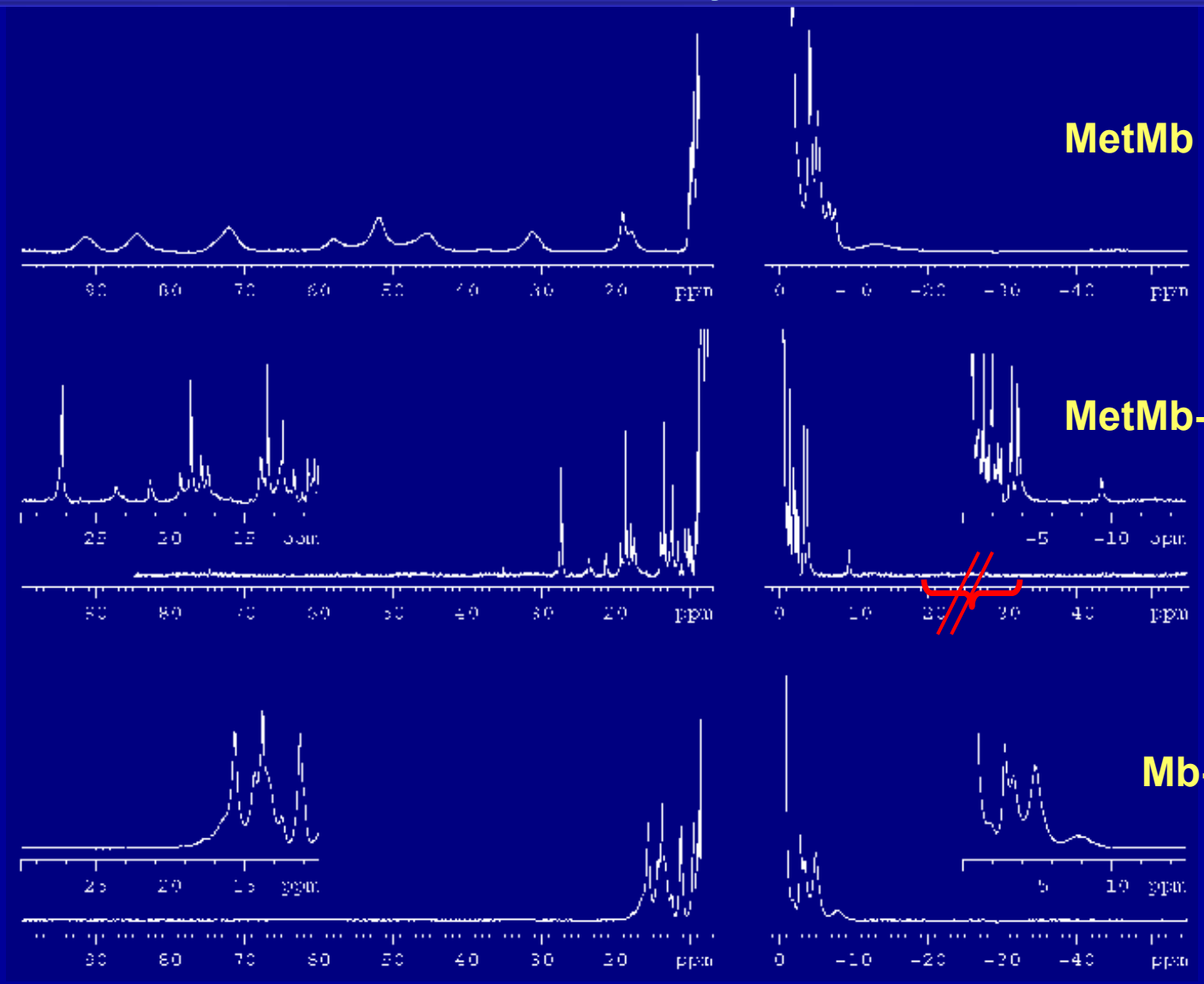


Cyanide adducts of Met-Myoglobin from different species

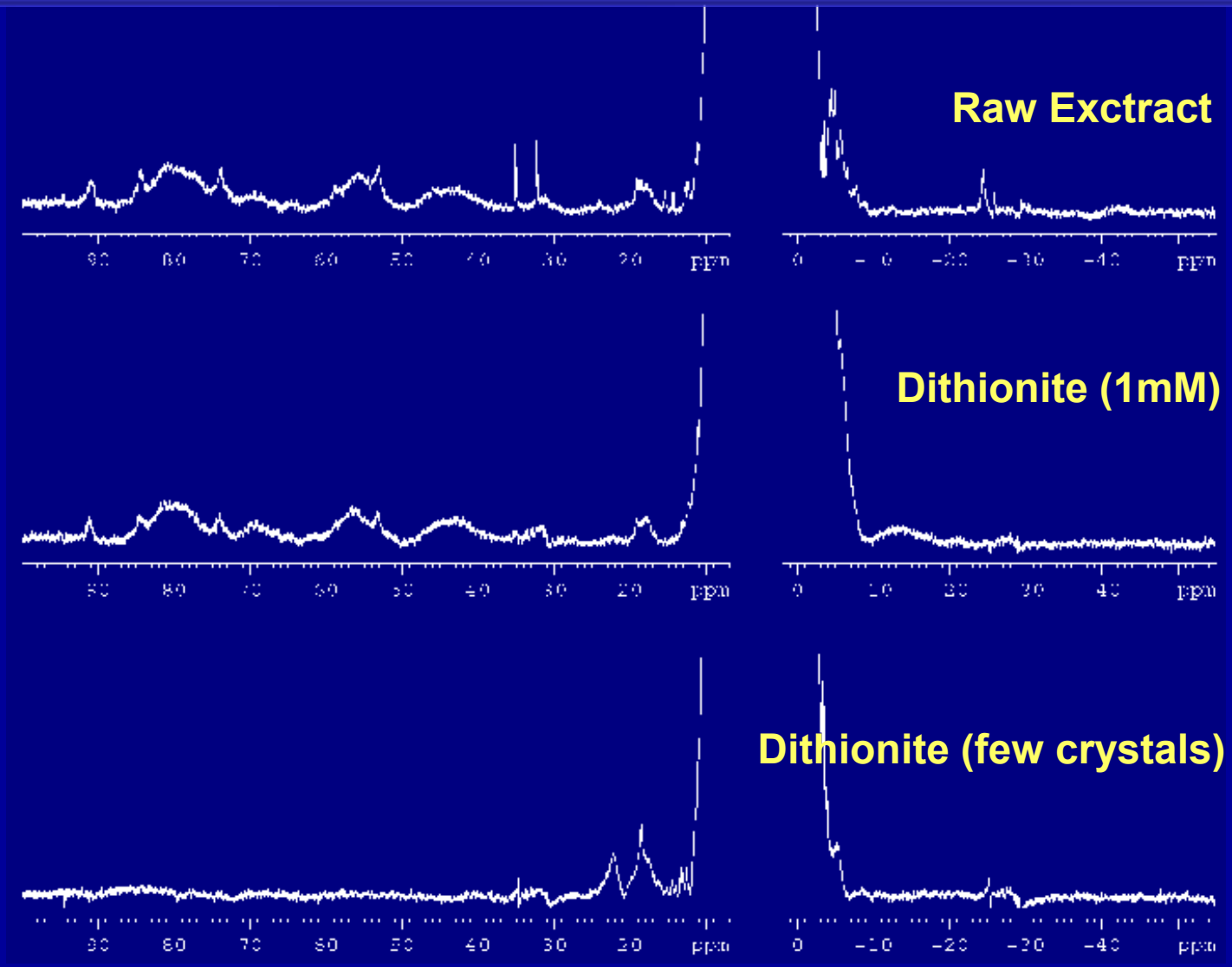


$\delta_i = 18.4 \sin2(\theta_i - \Phi) - 0.8 \cos2(\theta_i + \Phi) + 6.1$

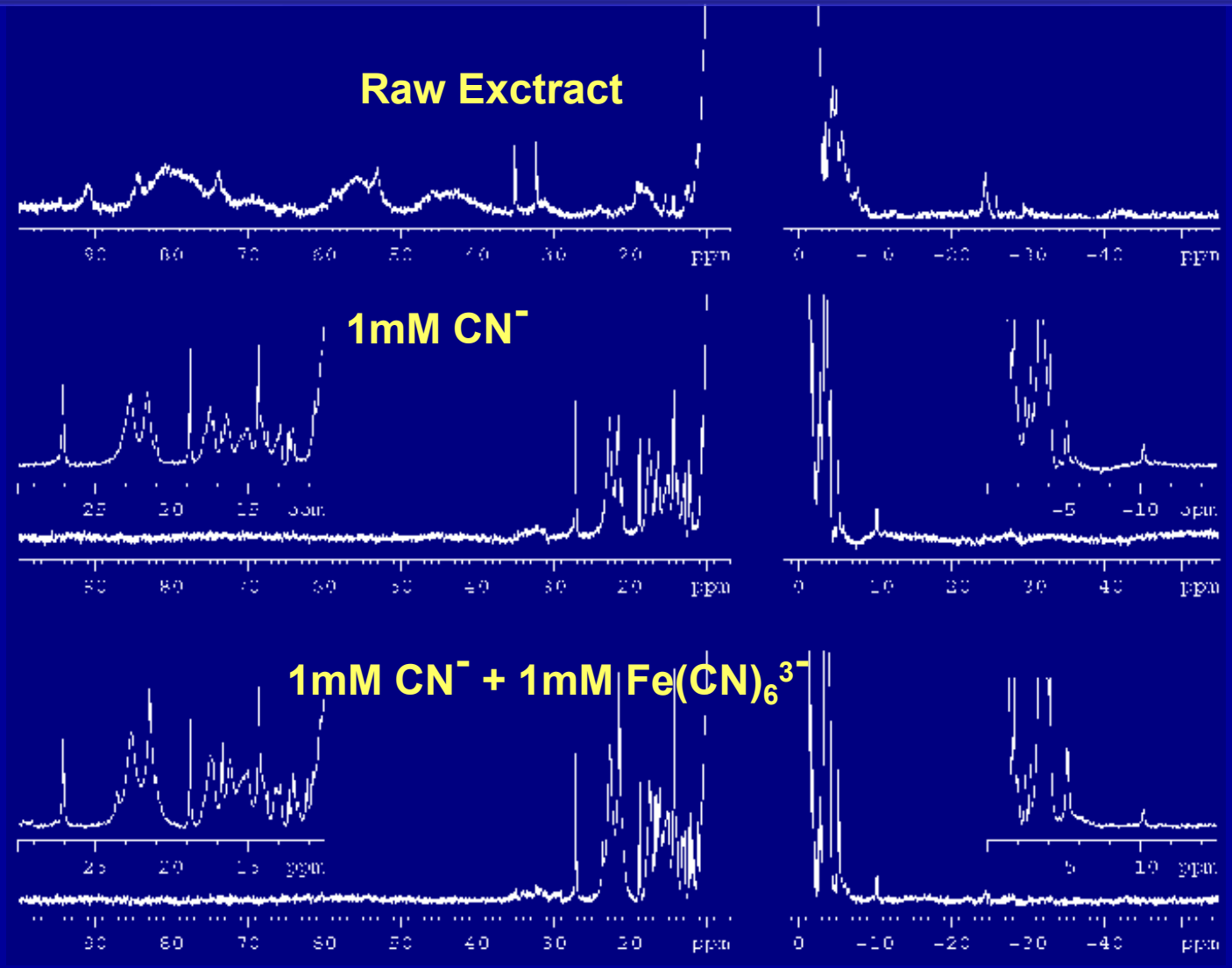
Horse heart Myoglobin in D_2O @ 400 MHz, 298K



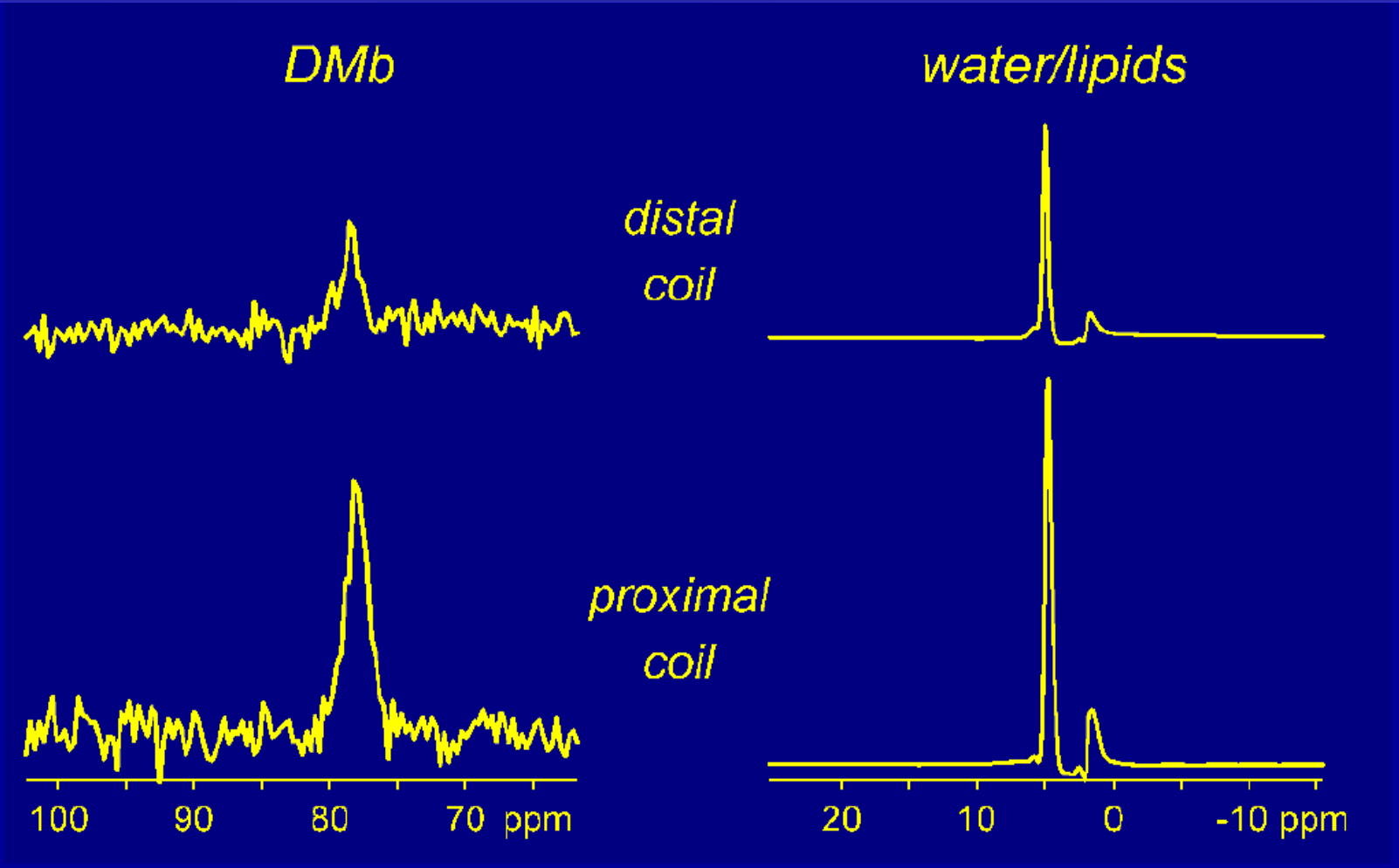
Turkey meat (thigh) extract in H_2O @ 400 MHz, 298K



Turkey meat (thigh) extract in H_2O @ 400 MHz, 298K

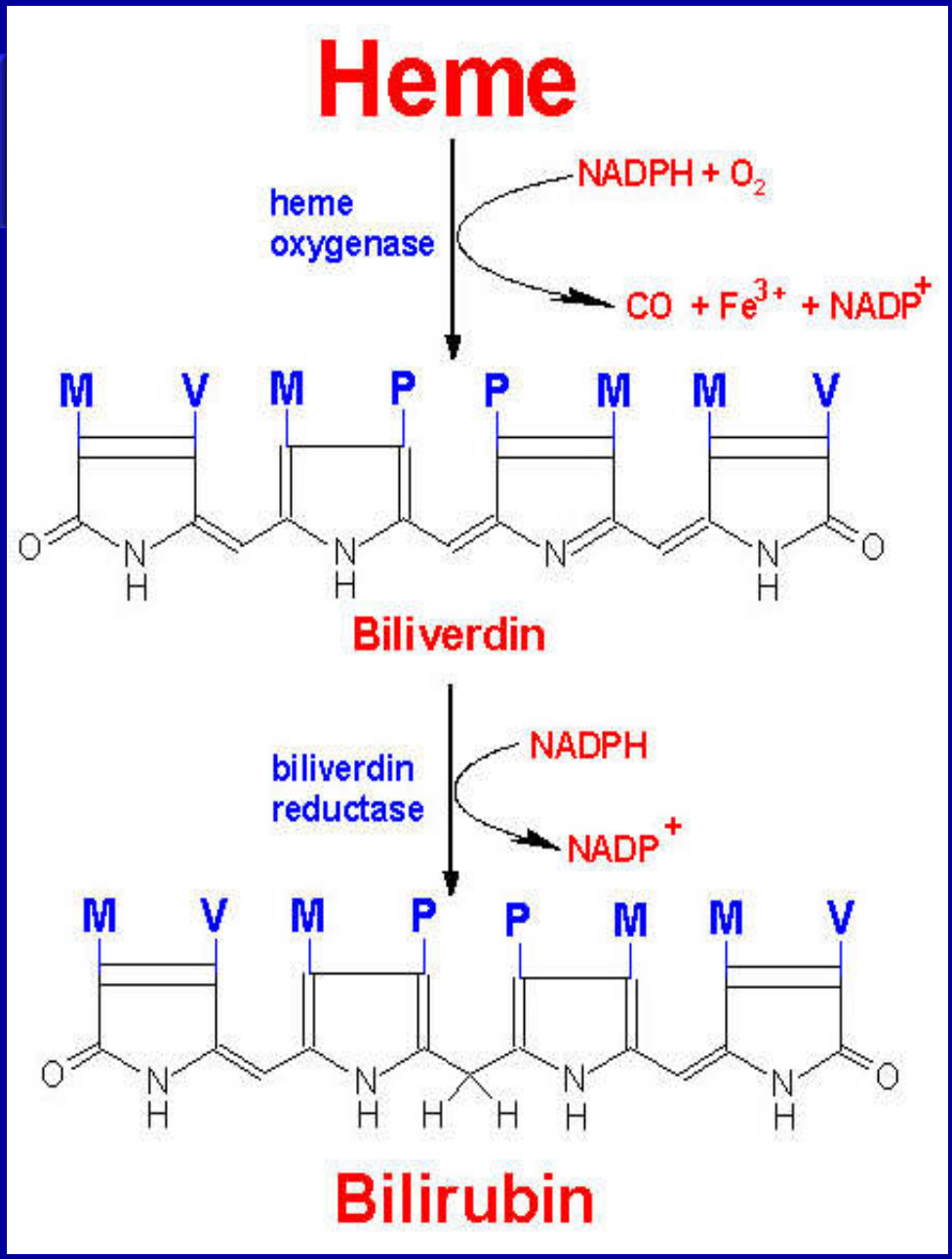


In vivo paramagnetic NMR spectroscopy: the anoxic human muscle



Il problema del CO

- Nel metabolismo dell'emoglobina si ha sempre la formazione di CO
- CO ha un elevata affinità per il Fe



Il ruolo del residuo distale

- CO si lega a Fe(II) 25000 volte più stabilmente di O_2
- Nella Mb questo fattore scende a 200

■ La presenza dell'istidina distale "forza" il CO a legarsi con una geometria meno favorita

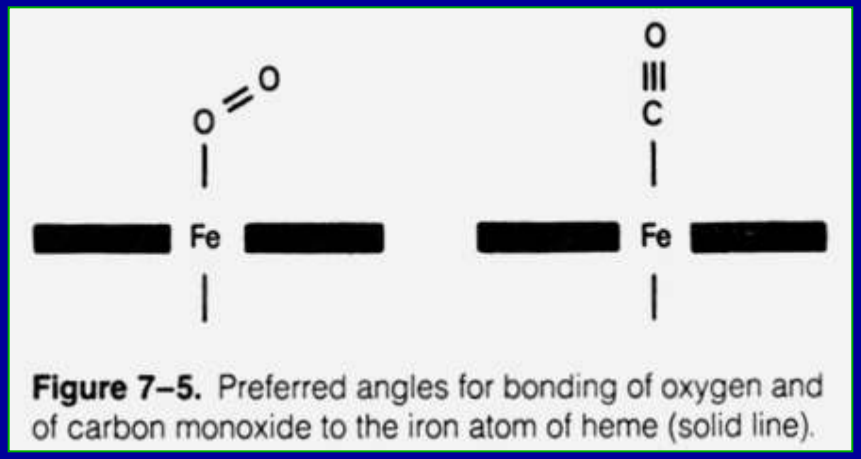
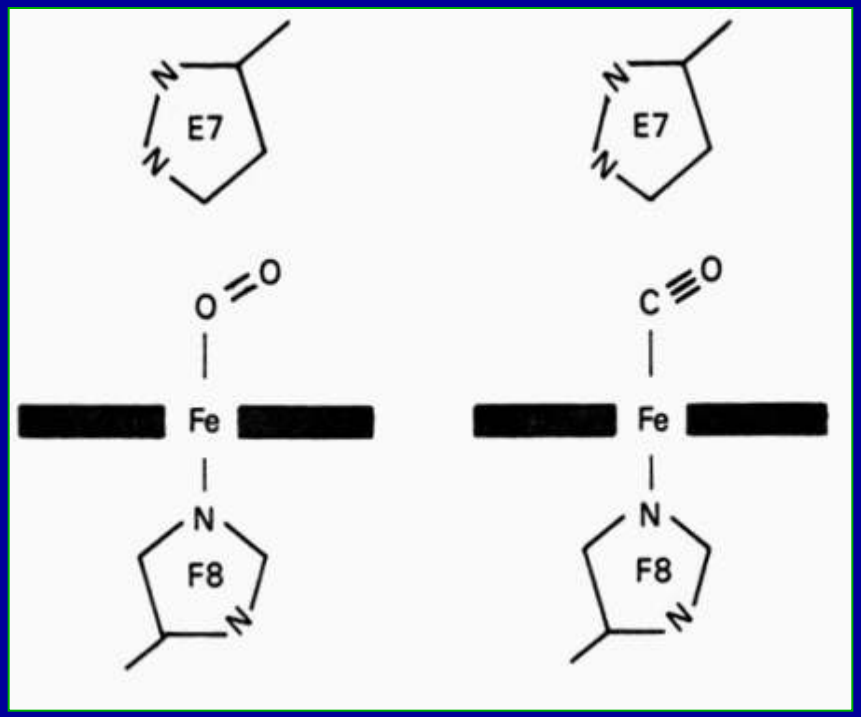
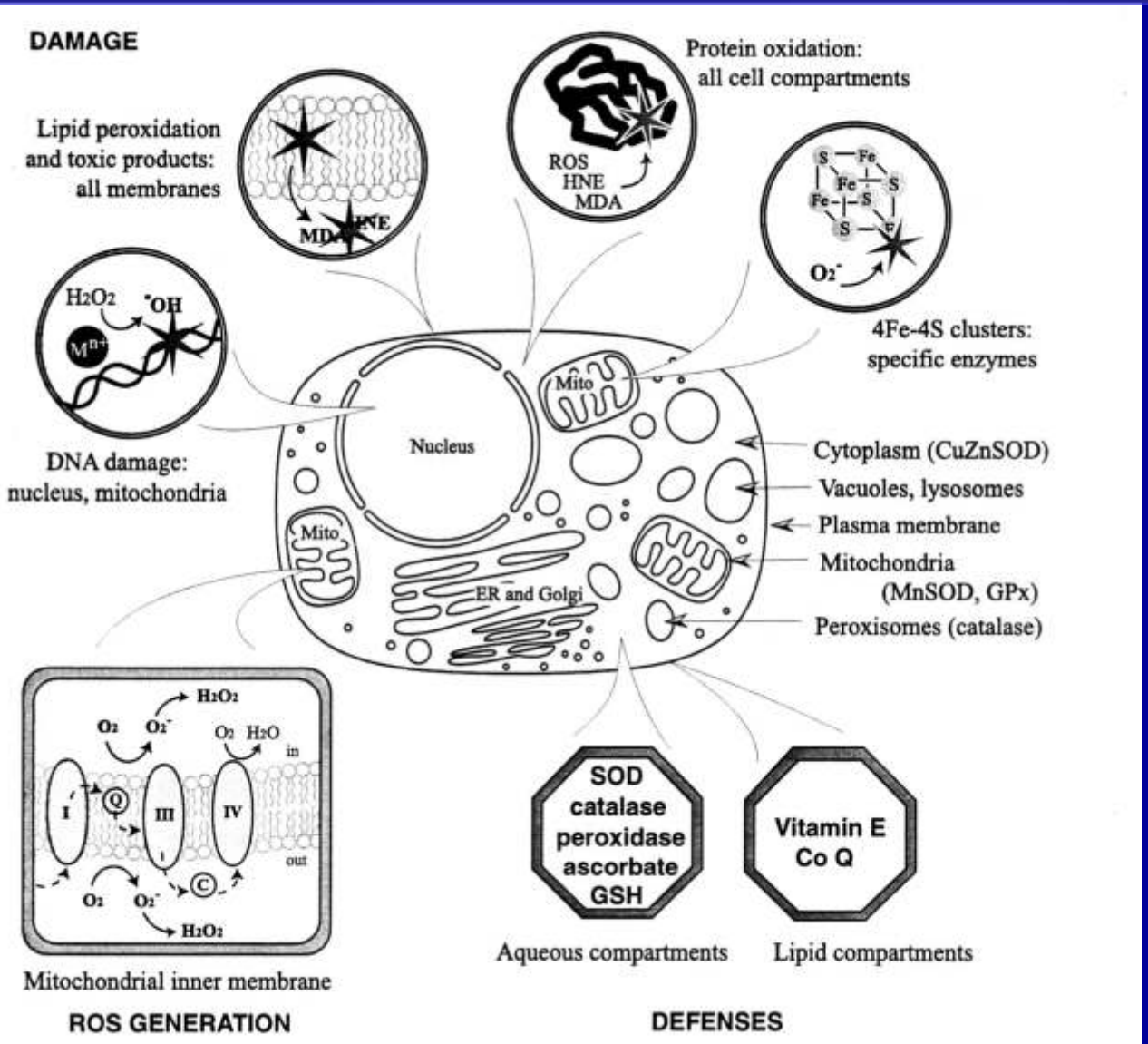


Figure 7–5. Preferred angles for bonding of oxygen and of carbon monoxide to the iron atom of heme (solid line).



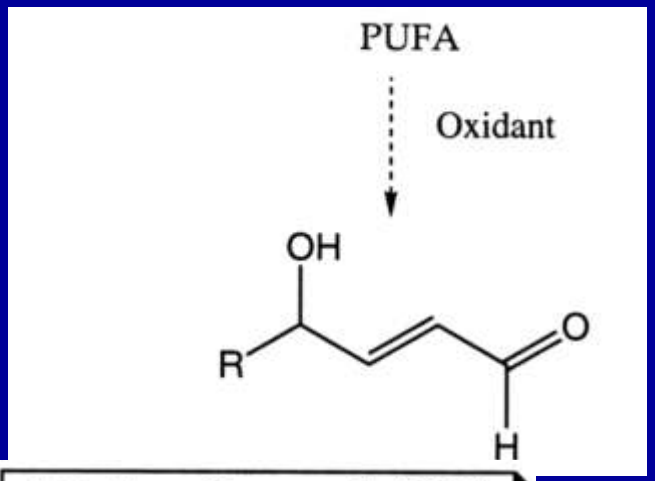
Pathways leading to oxidative stress

MDA (malondialdehyde) and HNE (4-hydroxy-2-nonenal) are toxic products from lipid peroxidation

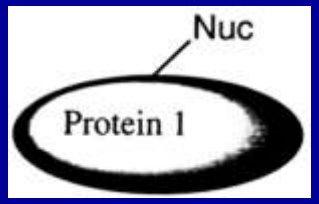


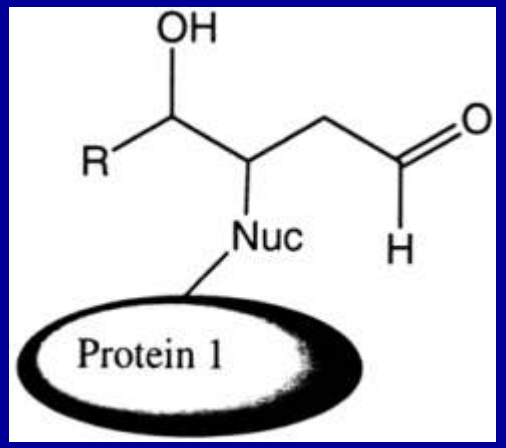
The major source of ROS (Reactive Oxygen Species) is the cell-leakage of electrons from the electron transport chain in Mitochondria

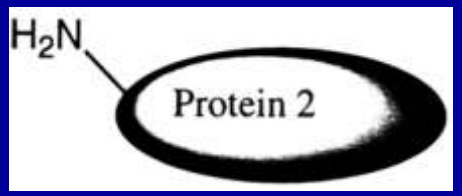
Reactions of nucleophilic protein side chains with HNE

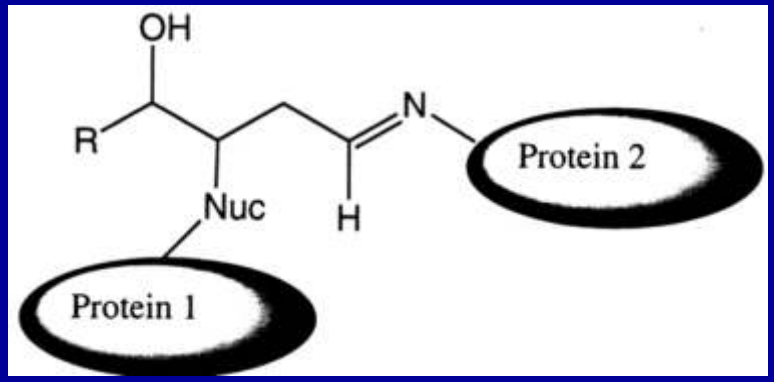


4-Hydroxy-2-nonenal, (HNE): PUFA oxidation product





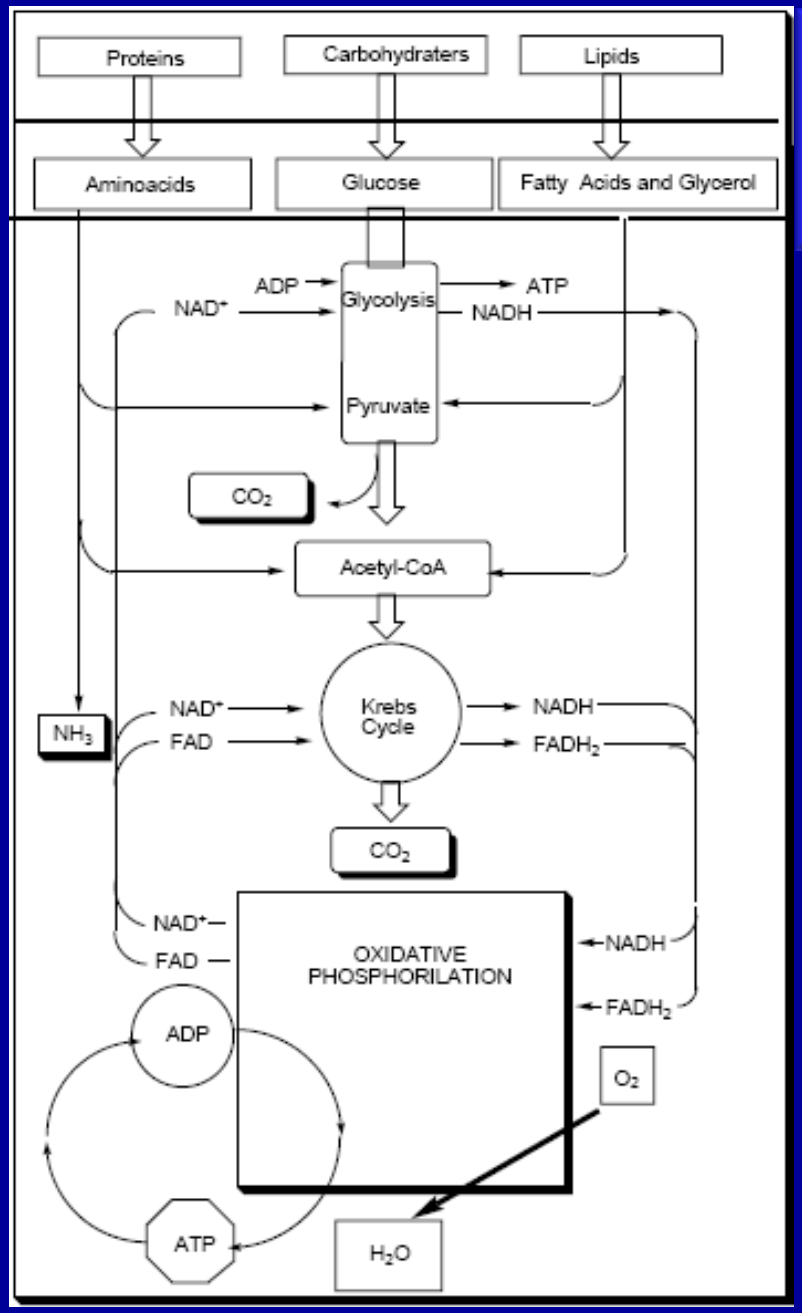




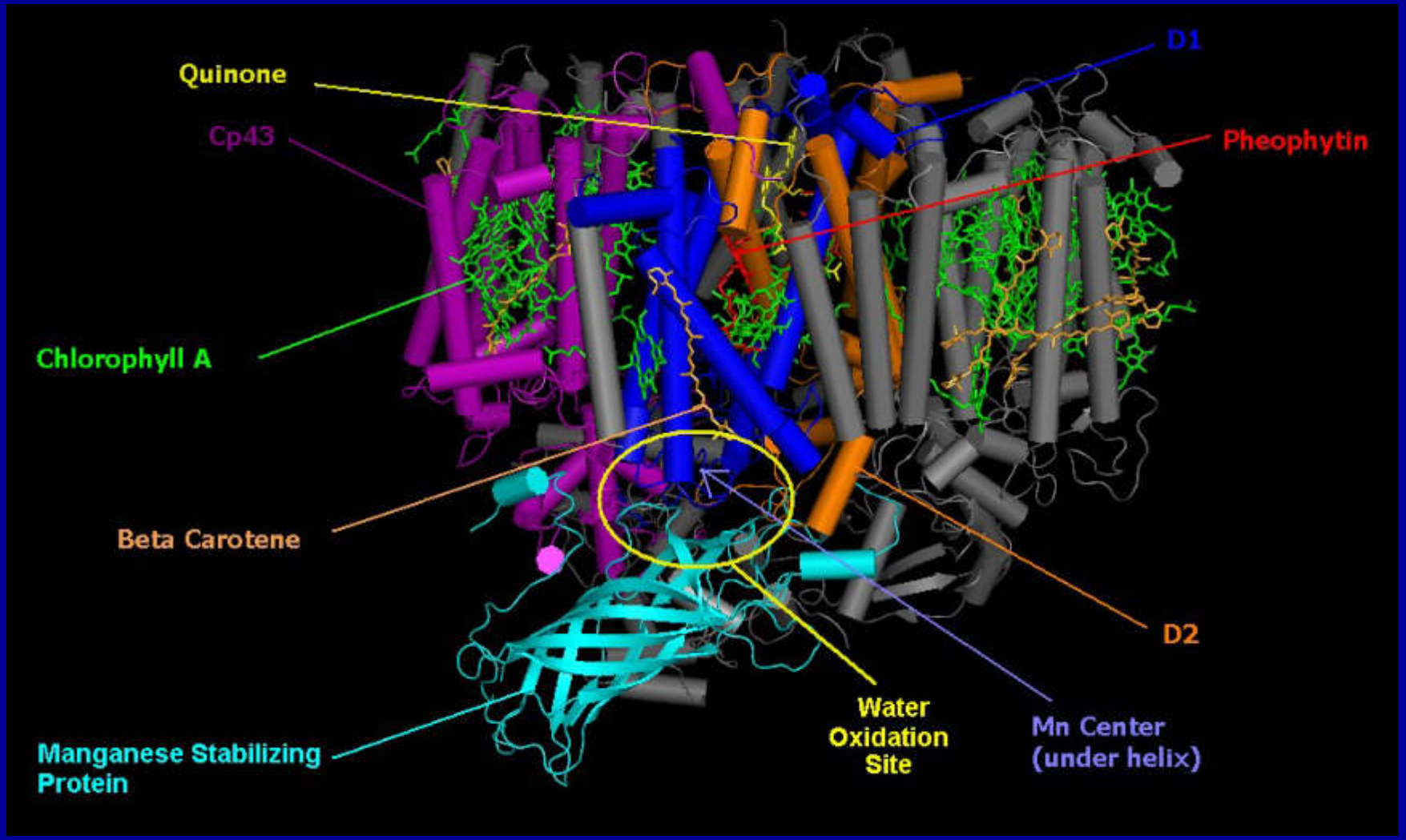
Nuc= ε-amino group of lysine, imidazole moiety of histidine, sulfhydryl group of cysteine.

Funzione di trasporto elettronico

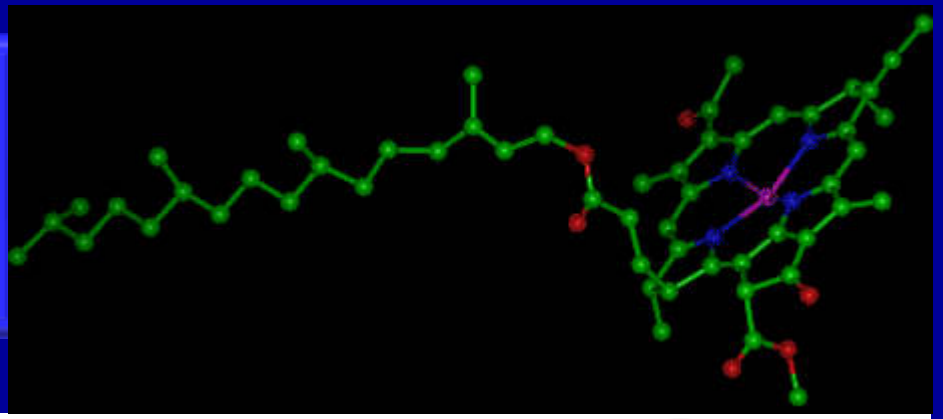
Metabolismo cellulare

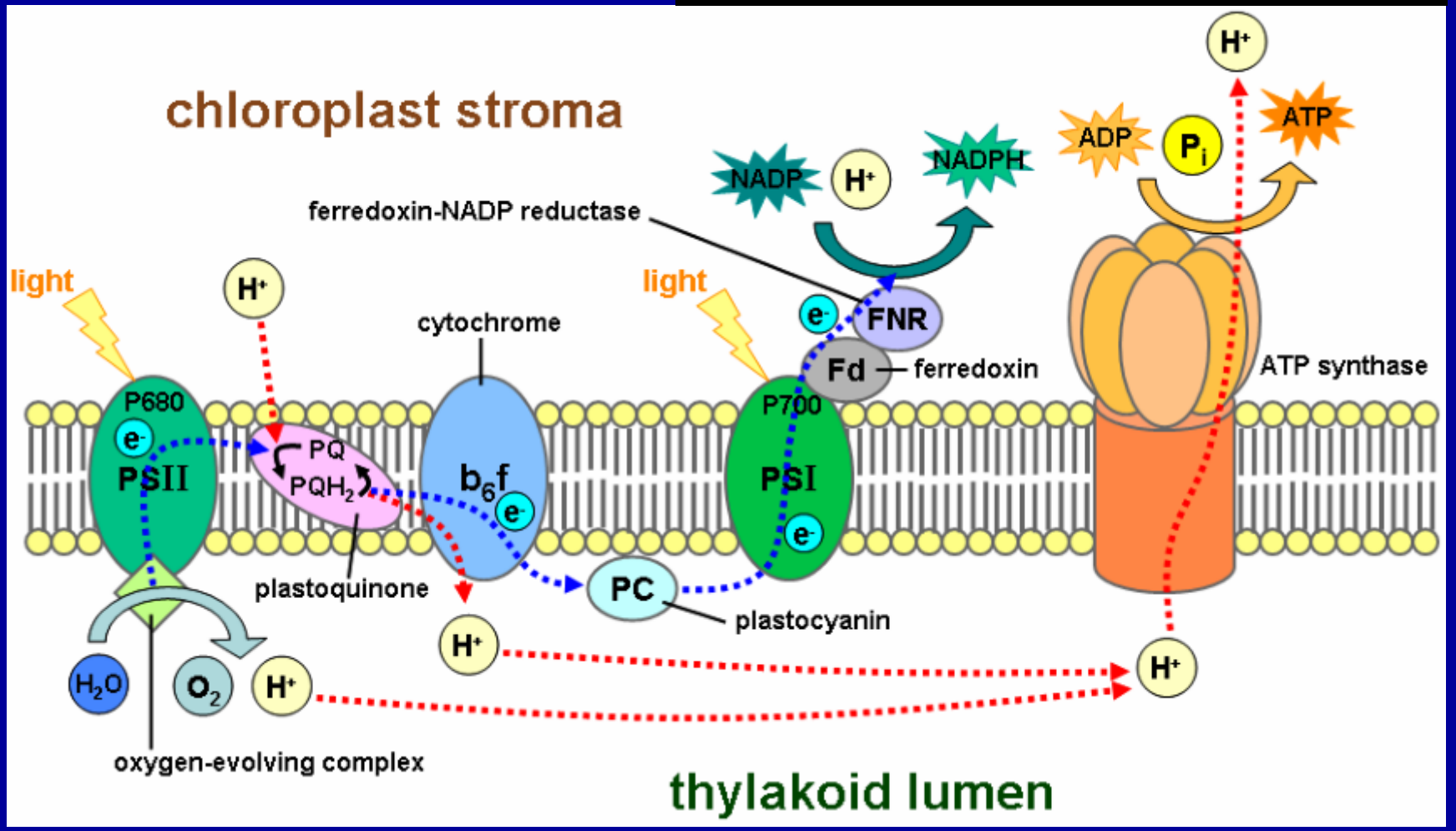


Photosystem II

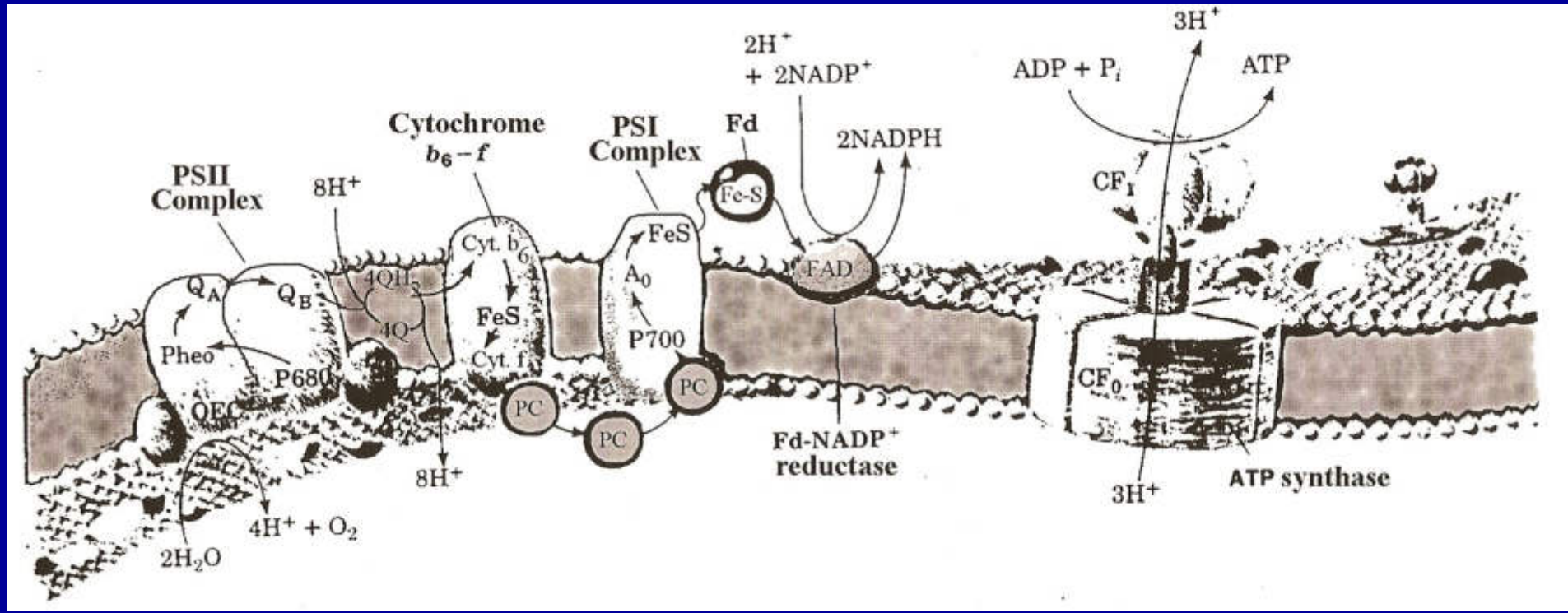


Specie coinvolte nella fotosintesi

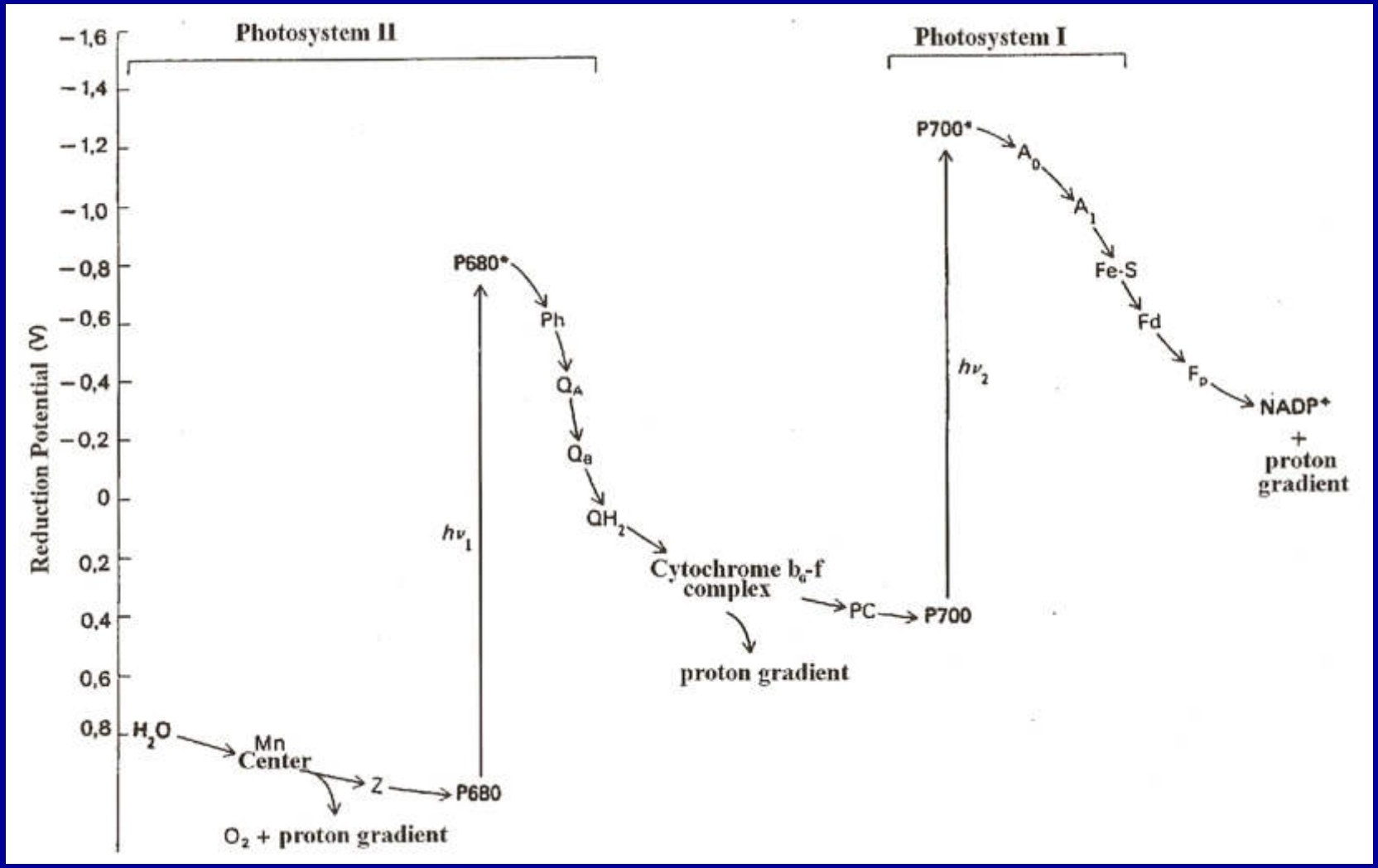




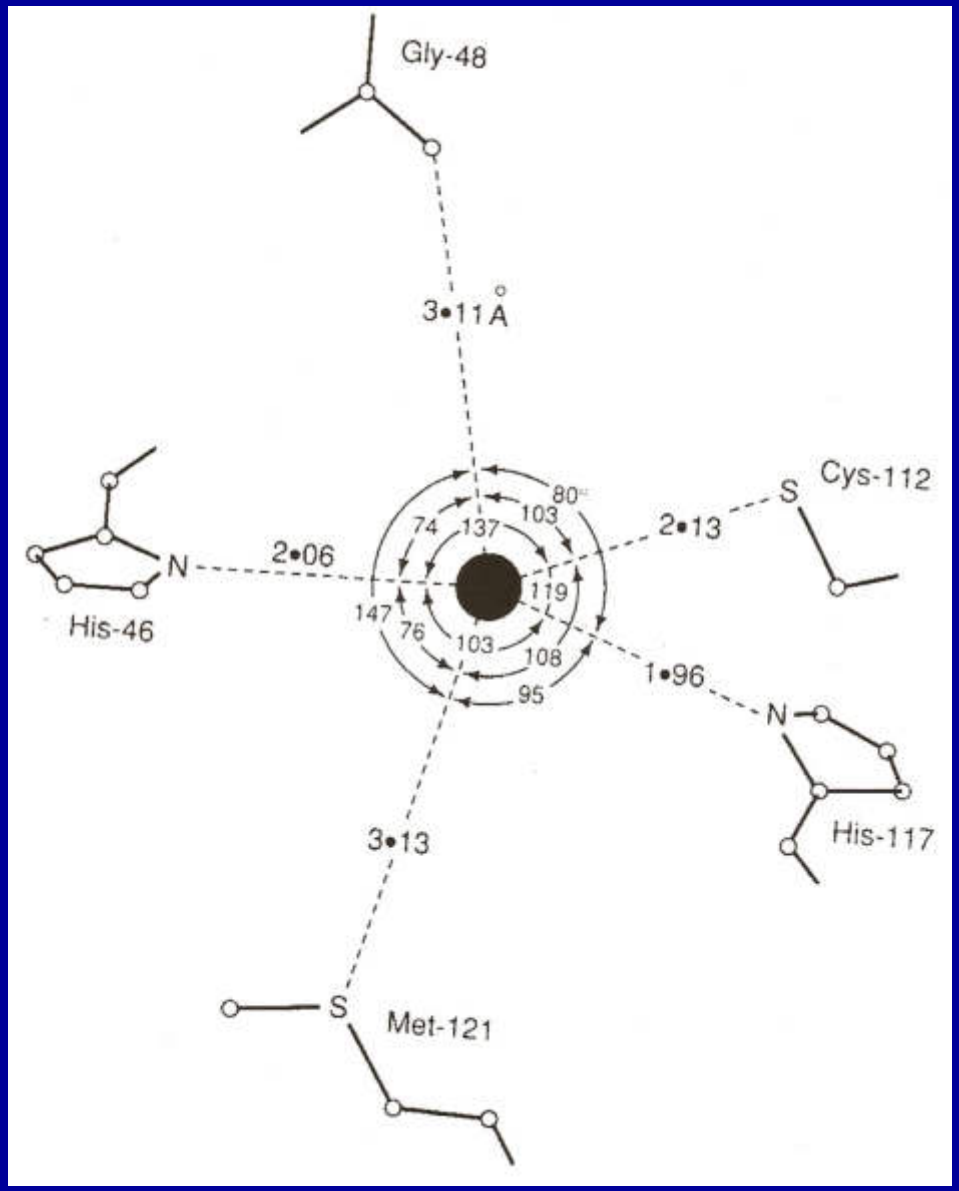
Lo schema Z


PSII Complex
8H+
Cytochrome b_6-f
PSI Complex
Fd
2H+ + 2NADP+
2NADPH
ADP + Pi
ATP
3H+
CF1
Fe-S
FeS
Cyt. b6
FAD
A0
4QH2
4Q
QA
QB
Pheo
P680
OEC
FeS
Cyt. f
P700
PC
PC
PC
8H+
Fd-NADP+ reductase
CF0
3H+
ATP synthase
4H+ + O2
2H2O

La catena elettronica



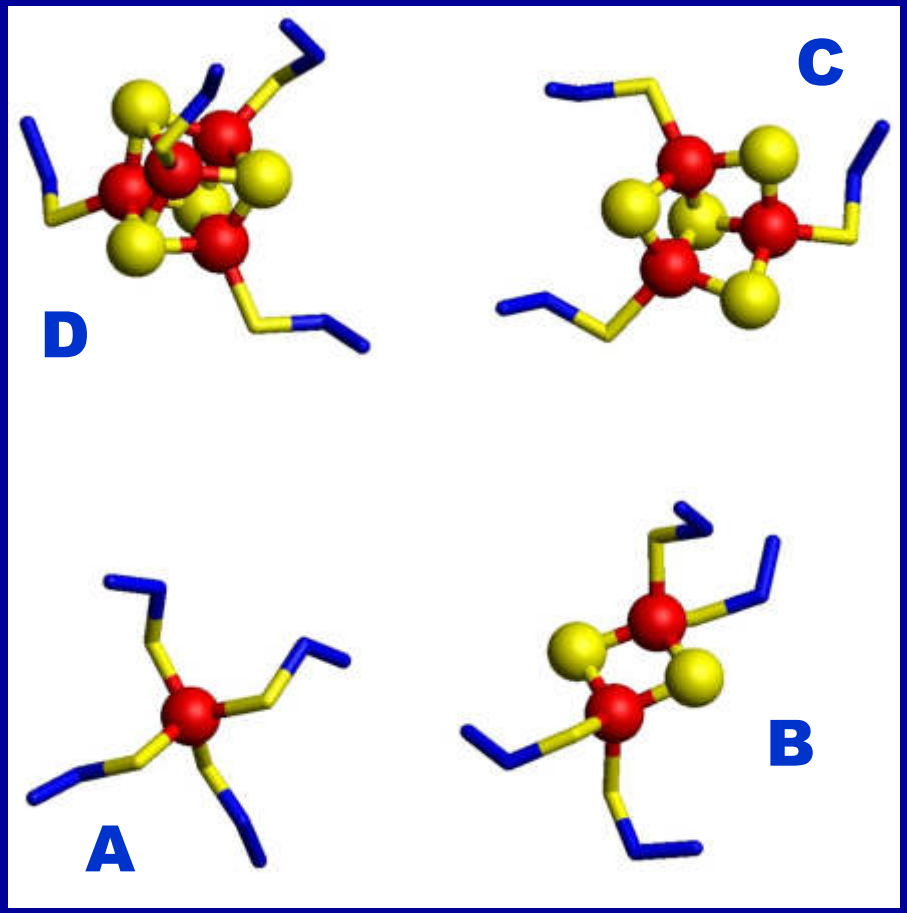
Modulazione del potenziale redox del Cu in Azzurrina mediante distorsioni della geometria di coordinazione



Potenziale redox di ioni Fe coordinati

Complex	Coordination number	Coordination type	Fe^{3+}/Fe^{2+} $E°'$(mV)
$Fe(OH_2)_6^{3+}$	6	aquo complex	770
HIPIPs	4	$Fe_4S_4(SR)_4^-$	460 / 90
Cytochrome *a3*	6	heme	390
Cytochrome *c*	6	heme	250
Rubredoxin	4	$Fe(SR)_4^-$	+20 / -60
Rieske Proteins	4	$Fe_2S_2(RS)_2N_2$	+300 / -155
[2Fe-2S] Ferredoxins	4	$Fe_2S_2(SR)_4^{2-}$	- 240 / -460
[4Fe-4S] Ferredoxins	4	$Fe_4S_4(SR)_4^{2-}$	-50 / -420

Reduction Potentials of Synthetic Iron-Sulphur Clusters

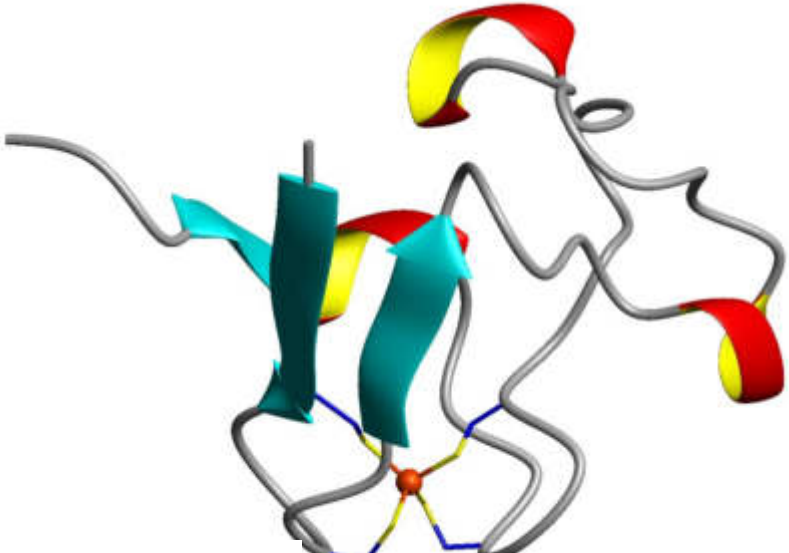


A: $[Fe(S_2\text{-o-xylyl})_2]^{1-/2-}$
-1030 mV in DMF

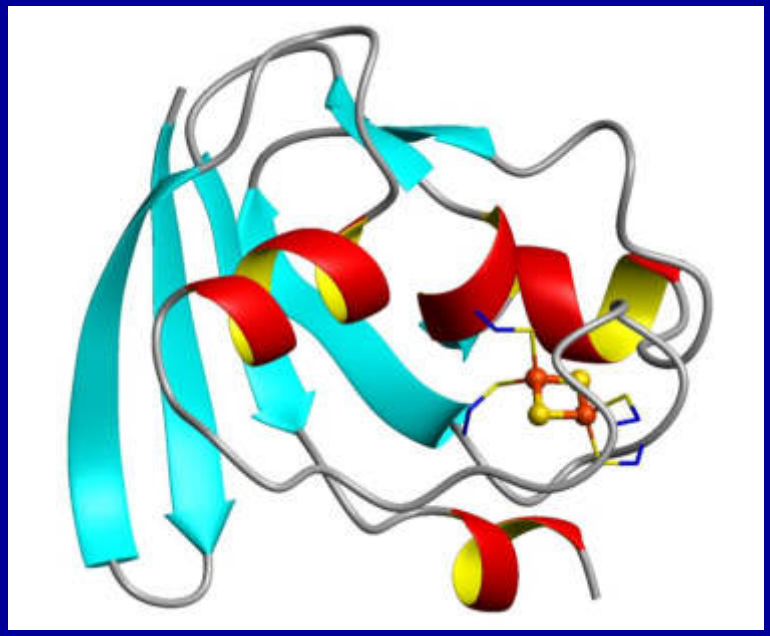
B: $[Fe_2S_2(S_2\text{-o-xylyl})_2]^{1-/2-}$
-1500 mM in DMF

C: $[Fe_3S_4(LS_3]^{2-/3-}$
-1060 mV in MeCN

D: $[Fe_4S_4(S\text{-}2,4,6(i\text{-}Pr)_3C_6H_4)_4]^{1-/2-}$
-150 mV in MeCN
$[Fe_4S_4(SCH_2Ph)_4]^{2-/3-}$
-1200 mV in MeCN

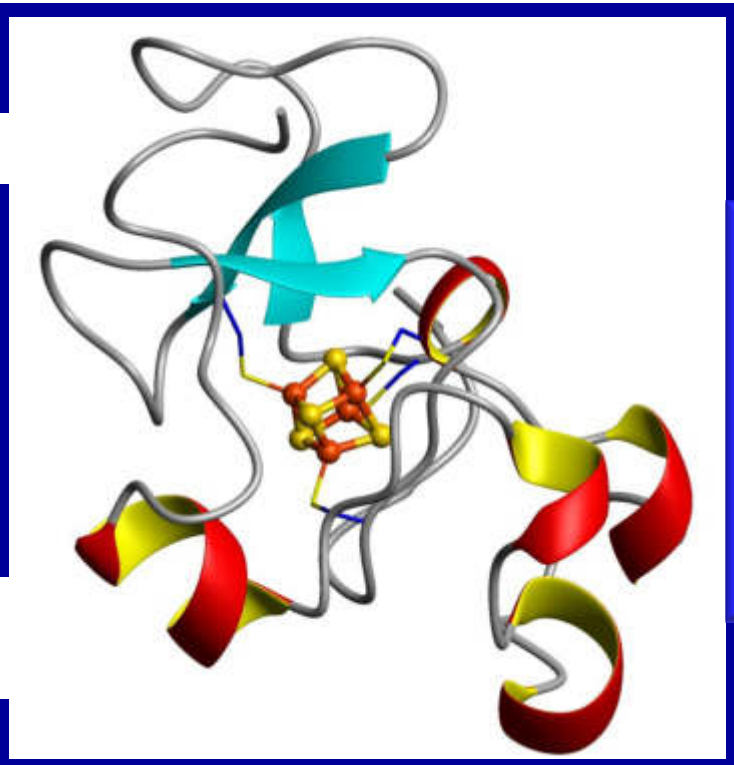


Rubredoxins



Plant-type Ferredoxins

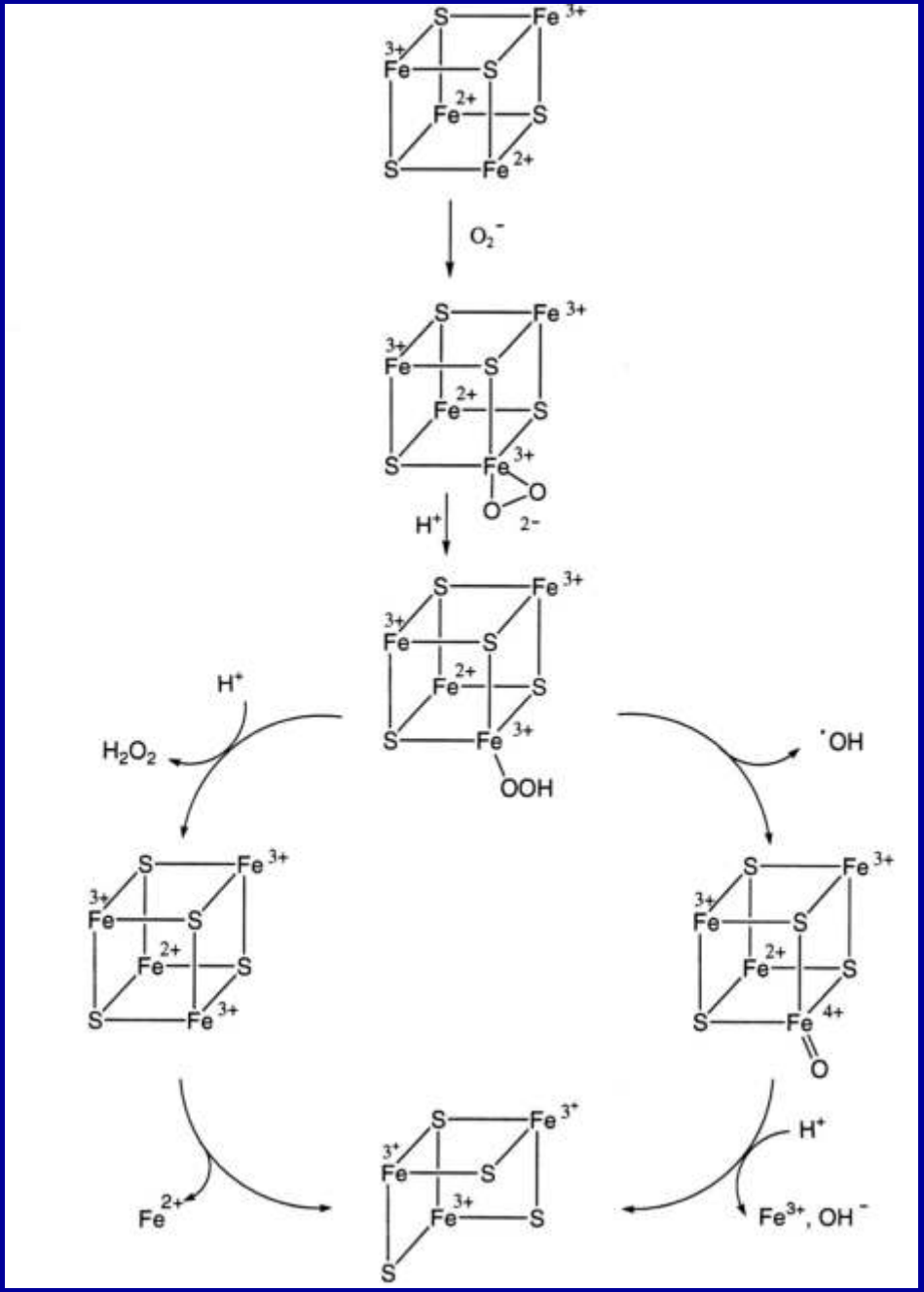
HiPIPs



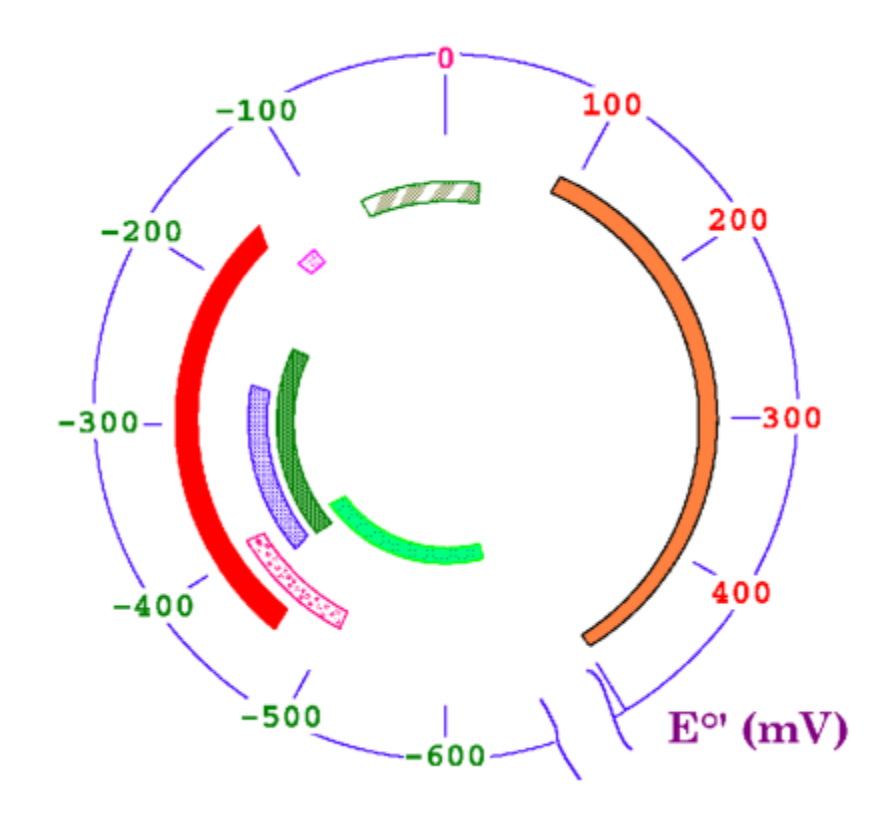
Bacterial Ferredoxins

Representative Iron-Sulphur Proteins

Oxidative decomposition of Iron-Sulfur Cluster

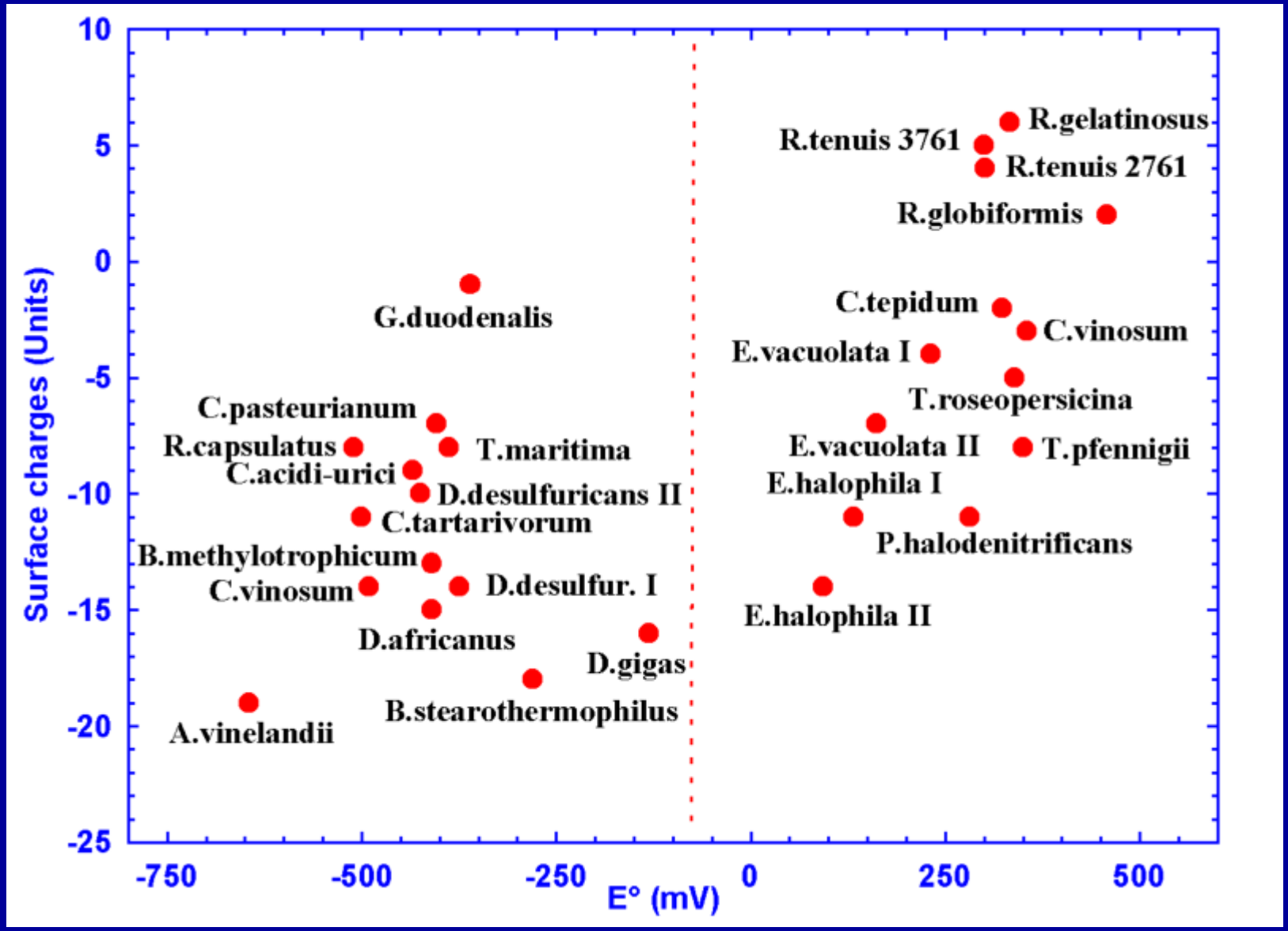


Experimental Reduction Potential observed in Iron-Sulphur Clusters in Proteins

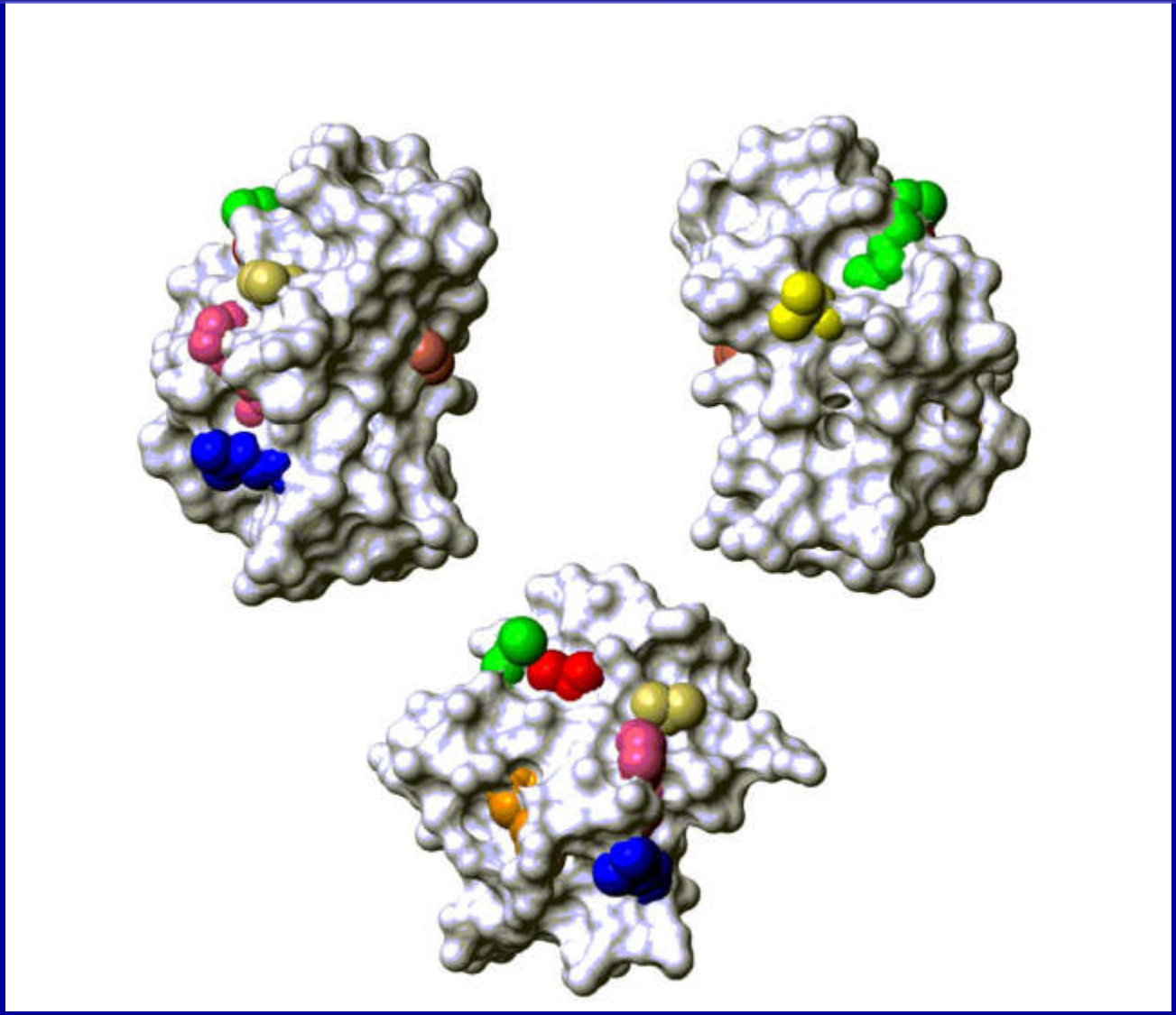


- Fe_3S_4 in 7Fe-8S Fd
- Fe_2S_2 in 2Fe-2S Fd
- Fe_4S_4 in 4Fe-4S Fd
- Fe_4S_4 in 7Fe-8S Fd
- Fe_3S_4 in 3Fe-4S Fd
- $Fe(SCys)_4$ in Rd
- Fe_4S_4 in 8Fe-8S Fd
- Fe_4S_4 in HiPIP

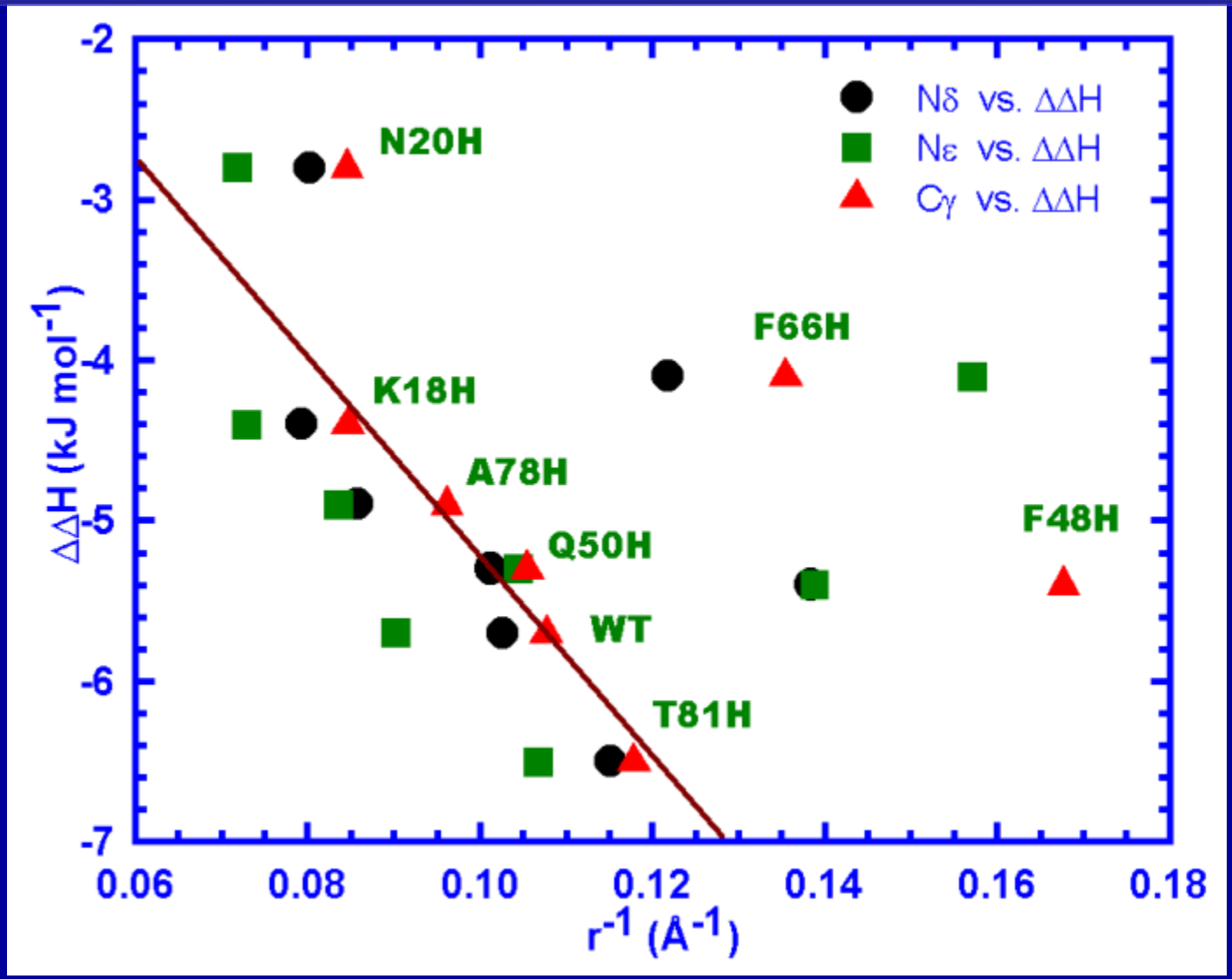
Total Surface Charge of Proteins and Reduction Potential



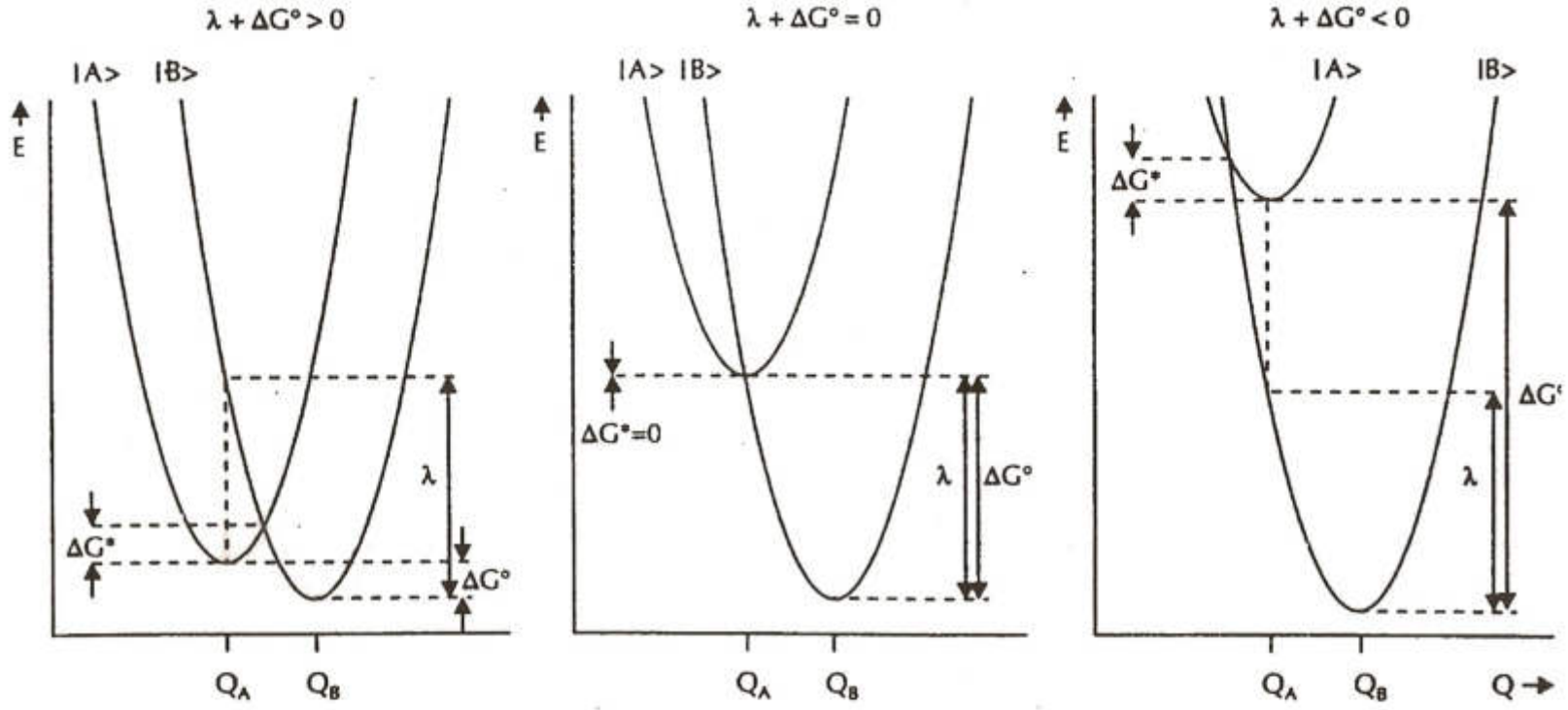
Collocare una carica sulla superficie dell'HiPIP da Chromatium vinosum



Protonation of a single HIS residue modifies the Enthalpic Contribution of HiPIPs Reduction



Marcus e la teoria dei piccoli passi



$$k_{ET} = \nu_n k_e \exp(-\Delta G^{\neq} / RT)$$

$$k_e = k_e^0 \exp(-\beta(d - d_0))$$

$$\Delta G^{\neq} = (\Delta G^0 + \lambda)^2 / 4\lambda$$