Introduction to Bioinorganic Chemistry

Part 1: Principles of Bioinorganic Chemistry

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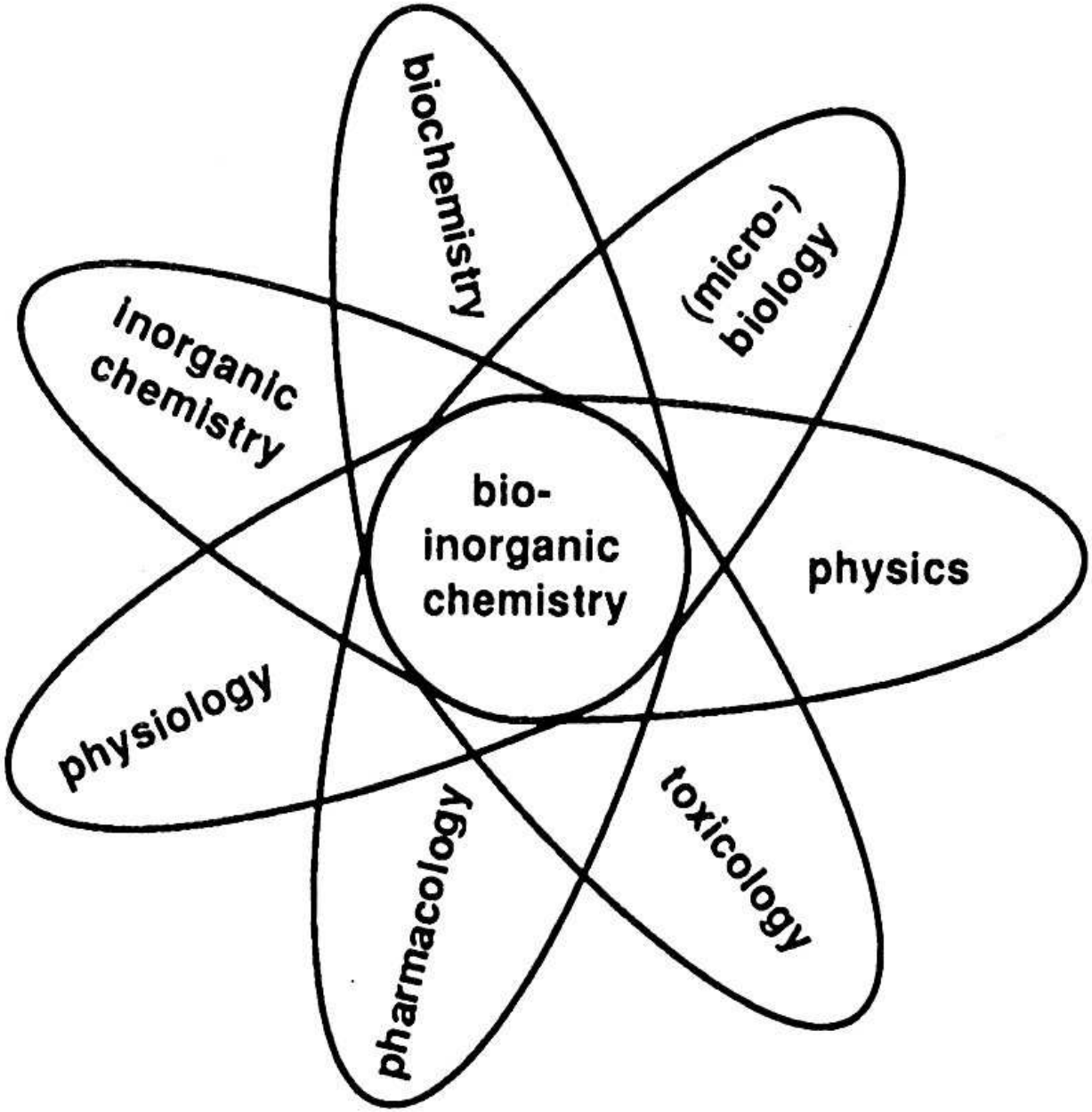
A multi-disciplinary area



A lively scientific area: main international meetings:

International Conference on Biological Inorganic Chemistry
ICBIC
International meeting. Odd years. # 1000 - 1200 participants

Euro-conference on Biological Inorganic Chemistry
EUROBIC
European meeting. Even years. # 600 - 800 participants

Useful references from which this course was setup

Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life
W. Kaim, B. Schwederski.
Wiley, 1994
ISBN: 0471 94368 1

Biological Chemistry of the Elements: The inorganic Chemistry of Life
J. J. R. Frausto de Silva, R. J. P. Williams.
Clarendon Press, Oxford, 1991 *(available from UdS)*
ISBN: 0-19-855598-9

Principles of Bioinorganic Chemistry
S. J. Lippard, J. M. Berg.
University Science Books, 1994 *(availabale from UdS)*
ISBN: 0-935702-72-5

Bioinorganic Chemistry: A Short Course
R. M. Moat-Malone
Wiley, 2002
ISBN: 0-471-15976-X

Biochemistry
L. Stryer
Freeman and Co. 1988
ISBN: 0-7167-1843-X

(available from UdS)

Inorganic Chemistry, 2nd edition
D. Shriver, P. Atkins, C. H. Langford.
Freeman and Co. 1994
ISBN: O-7167-2398-0

(available from UdS)

Physical Methods for Chemists, 2nd edition
R. S. Drago
Saunders College Publishing, 1992
ISBN: 0-03-075176-4

(available from UdS)

NMR of Paramagnetic Molecules in Biological Systems
I. Bertini, C. Luchinat.
Physical Bioinorganic Series – Current Methods in Inorganic Chemsitry Vol. 2
Benjamin Cummings Publishing Company 2001
ISBN: 0-8053-0780-X

Metals and biology:

From the point of view of simple curiosity…

(no life without metals!)

… to major economical outcomes!

(important chemical reactions)

A matter of curiosity:

No life without Tansition Metals!

Only small amounts are required

Well-known
Essential functions:

Oxygen transport and storage, protection of organisms, metabolization...

Table 2.1 Average elemental composition of a human body (adult, 70 kg [1])

element and symbol		mass (g)	year of discovery as an essential element
oxygen	O	45500	
carbon	C	12600	
hydrogen	H	7000	
nitrogen	N	2100	
calcium	Ca	1050	
phosphorus	P	700	
sulfur	S	175	
potassium	K	140	
chlorine	Cl	105	
sodium	Na	105	
magnesium	Mg	35	
iron	Fe	4.2	17th century
zinc	Zn	2.3	1896
silicon	Si	1.4	1972
rubidium[a]	Rb	1.1	
fluorine	F	0.8	1931
zirconium[a]	Zr	0.3	
bromine[b]	Br	0.2	
strontium[a]	Sr	0.14	
copper	Cu	0.11	1925
aluminum[a]	Al	0.10	
lead[b]	Pb	0.08	
antimony[a]	Sb	0.07	
cadmium[b]	Cd	0.03	(1977)
tin[b]	Sn	0.03	(1970)
iodine	I	0.03	1820
manganese	Mn	0.02	1931
vanadium[b]	V	0.02	(1971)
selenium	Se	0.02	1957
barium[a]	Ba	0.02	
arsenic[b]	As	0.01	1975
boron[b]	B	0.01	
nickel[b]	Ni	0.01	(1971)
chromium	Cr	0.005	1959
cobalt	Co	0.003	1935
molybdenum	Mo	<0.005	1953
lithium[b]	Li	0.002	

[a] Not essential. [b] Essentiality uncertain.

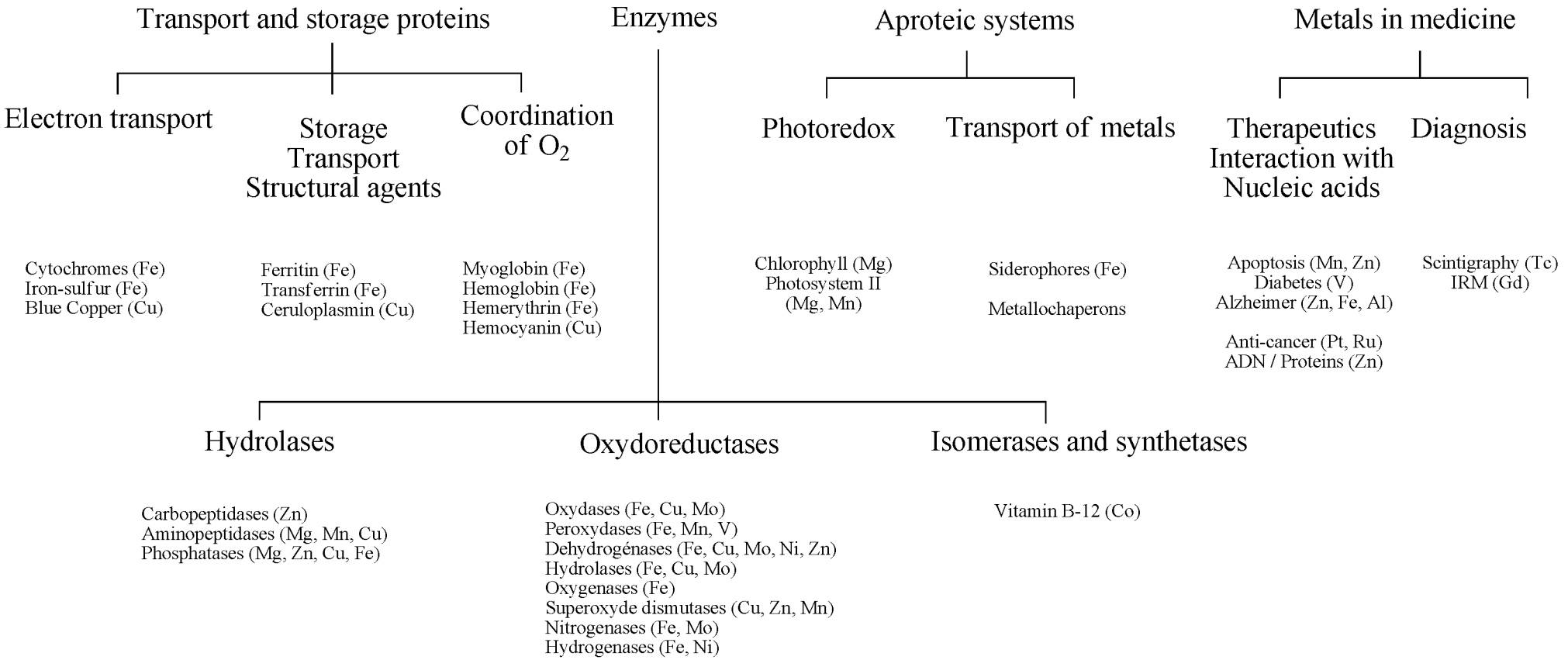
IA	IIA	IIIA	IVA	VA	VIA	VIIA	VIII	VIII	VIII	IB	IIB	IIIB	IVB	VB	VIB	VIIB	0
(H)																	He
Li	Be											B	C	N	O	F	Ne
(Na)	(Mg)											Al	Si	P	S	Cl	Ar
(K)	(Ca)	Sc	Ti	[V]	[Cr]	[Mn]	[Fe]	[Co]	[Ni]	[Cu]	[Zn]	Ga	Ge	[As]	[Se]	[Br]	Kr
Rb	Sr	Y	Zr	Nb	[Mo]	Tc	Ru	Rh	Pd	Ag	Cd	In	[Sn]	Sb	Te	[I]	Xe
Cs	Ba	Ln	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac	Th	Pa	U												

(○) Bulk biological elements

[ ] Trace elements believed to be essential for plants or animals

[ ] Possibly essential trace elements

Metals and biology: attempt for a partial classification according to the function



Major economical outcomes

3. Biological and biomimetic oxidation: another "hot" approach?

Enzymes that catalyze selective oxidation of one or more alkanes under ambient conditions—most notably the heme-based cytochromes P450 and the non-heme methane monooxygenases (MMO)—are well known. Furthermore, a very large number of metal complexes of varying complexity, ranging from close structural analogs of the enzymatic imple metal salts, can also effect some selectivity, although rarely if of the real enzymes. These latter termed "biomimetic", sometimes ut frequently require more reactive ides, hypochlorite, iodosylbenzene, They, too, frequently operate at or

s another "hot" approach? Because e majority of so-called biomimetic he biological ones as well, activate hat bears considerable mechanistic e high-temperature systems of the is, homolytic C–H bond cleavage is achieved by generating a "hot" H-abstracting species (i.e., a metaphorically, as opposed to literally, hot approach), under mild conditions.

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Selective alkane oxidation: hot and cold approaches to a hot problem

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Abstract

A large number of attempts at the selective oxidation of alkanes may be classified into three basic types: high-temperature heterogeneous catalysis, biological/biomimetic catalysis, and organometallic activation. In this essay I discuss mechanistic similarities and differences between the three approaches, and their implications for the best opportunities for achieving desired selective transformations.

Keywords: Alkane oxidation; Selectivity; Free radicals; Biomimetic oxidation; Organometallic activation

Industrial synthesis of Methanol

Lurgi and ICI process **from natural gas (CH_4)**.

Bond dissociation energy

H_3C-H 105 kcal/mol

Two steps

830°C, 18 bars

$CH_4 + H_2O \rightleftharpoons CO + 3\ H_2$

$CO + H_2O \rightleftharpoons CO_2 + H_2$

= Reforming: syngas

250°C, 15 to 100 bars

Copper-based catalyst

$CO + 2\ H_2 \rightleftharpoons CH_3OH$ $\Delta_r H° = -108$ kJ/mol

$CO_2 + 3\ H_2 \rightleftharpoons CH_3OH + H_2O$ $\Delta_r H° = -68$ kJ/mol

Exothermic reaction, cooling required;

Side products: ethanol, dimethylether, methyl formate

Biosynthesis of Methanol

from methanotrophic bacteries, **and biogas (CH_4)**.

Bond Dissociation Energy

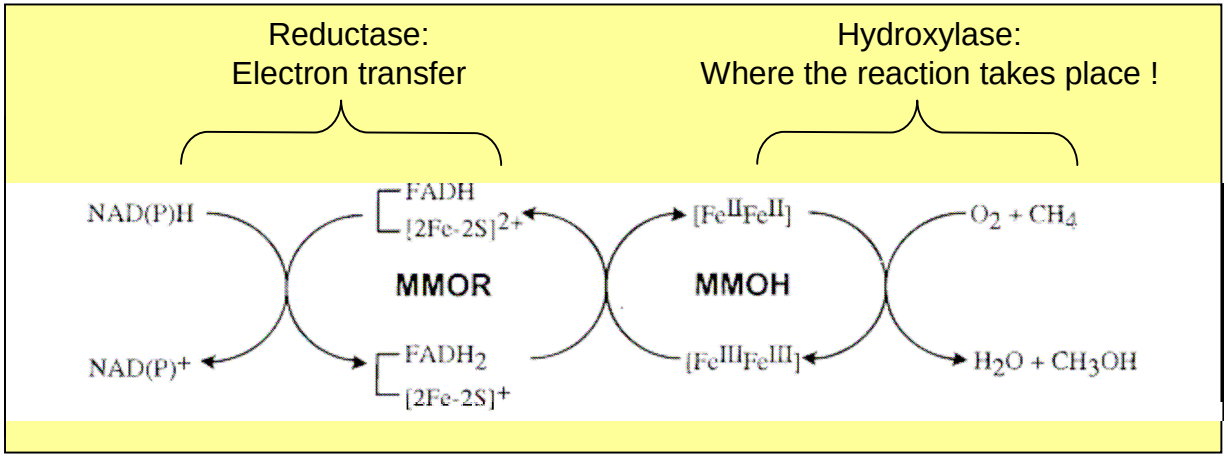
$H_3C—H$ 105 kcal/mol

One step at ambiant temperature and pressure !

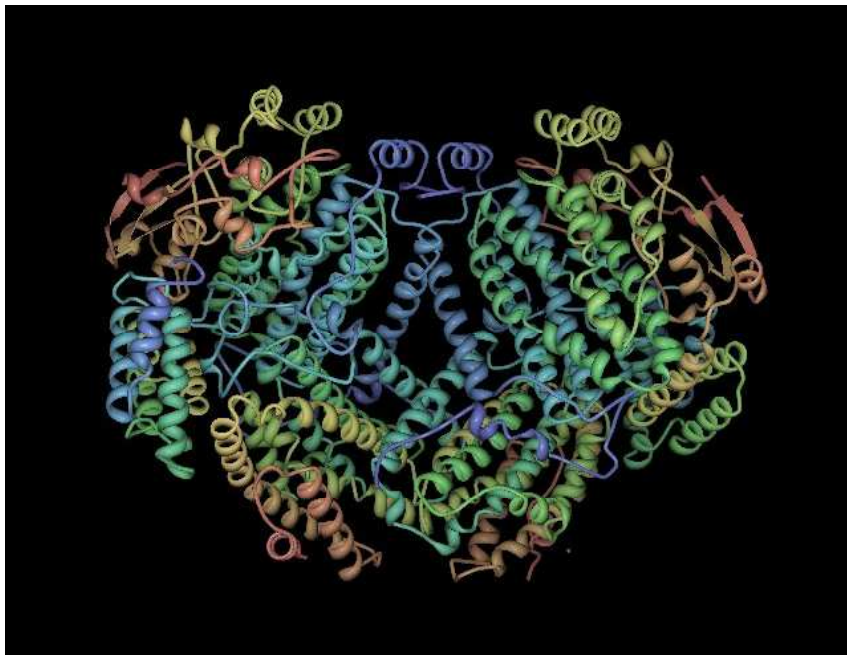
Iron- or copper-containing enzymes

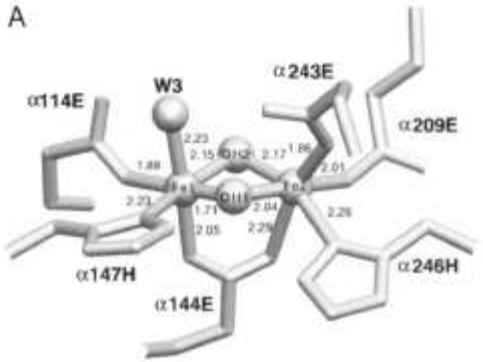
$$CH_4 + O_2 + 4H^+ + 2e^- \longrightarrow CH_3OH + H_2O$$

Iron-containing enzymes:

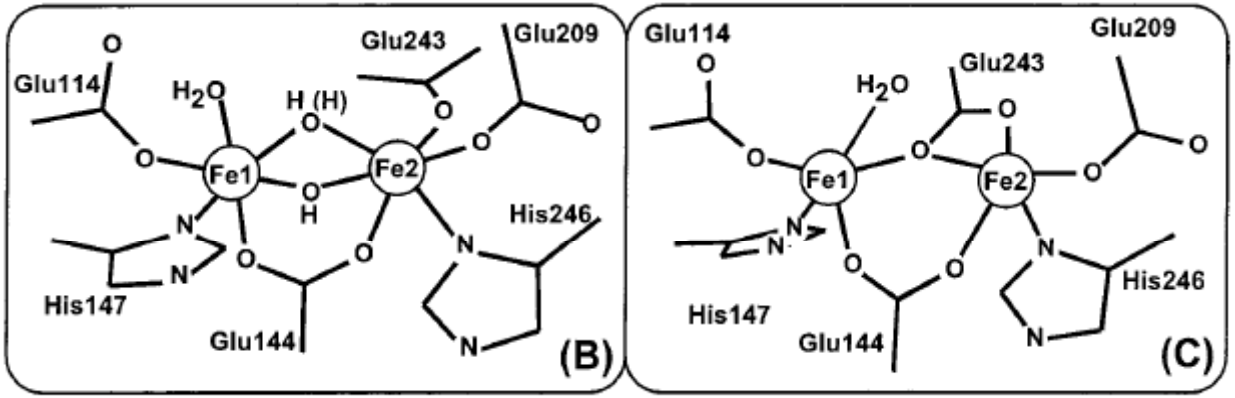


Hydroxylase component in Iron-containing Methane MonoOxygenases:





Dinuclear iron-containing active site:



Part 1: Principles of Bioinorganic Chemistry

Fundamental notions on protein chemistry:

- Peptides and amino acids
- Structure of proteins

The basics of coordination chemistry of transition metals

- Nature of a transition metal
- Geometrical and structural aspects

Coordination of proteins to transition metals

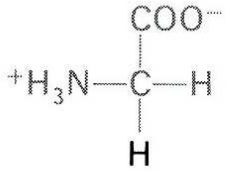
- Coordination modes
- Direct Coordination of amino acids
- Coordination *via* cofactors

Useful techniques in bioinorganic chemistry

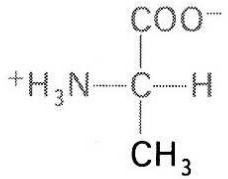
- UV-visible spectroscopy
- Vibrational spectroscopy (I.R., Raman)
- EXAFS
- EPR
- Paramagnetic NMR
- Mössbauer

Elemental components: amino acids - 1 -

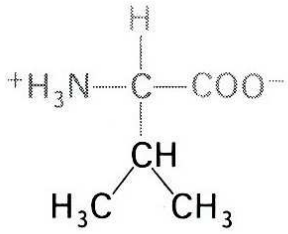
Aliphatic lateral chains



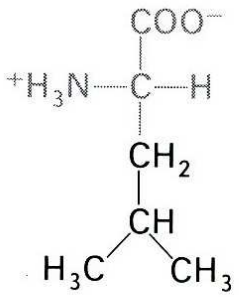
Glycine (Gly, G)



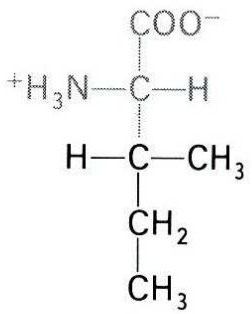
Alanine (Ala, A)



Valine (Val, V)

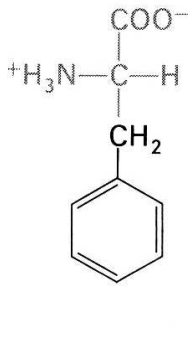


Leucine (Leu, L)

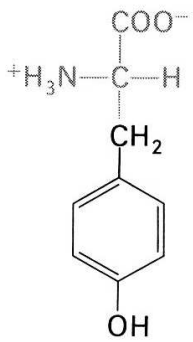


Isoleucine (Ile, I)

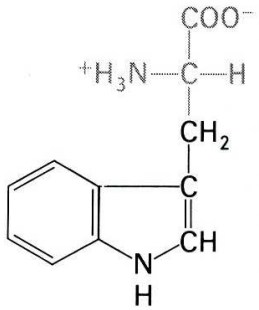
Aromatic lateral chains



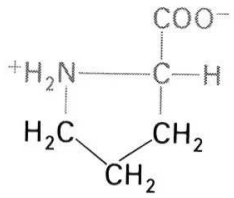
Phenylalanine (Phe, F)



Tyrosine (Tyr, Y)



Tryptophan (Trp, W)

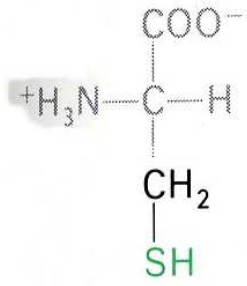


Proline (Pro, P)

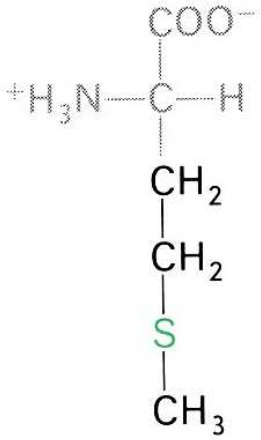
Proline contains a Secondary amine function

Elemental components: amino acids - 2 -

Sulfur-containing lateral chains

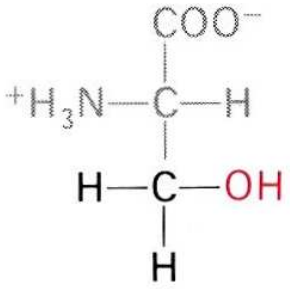


Cysteine
(Cys, C)

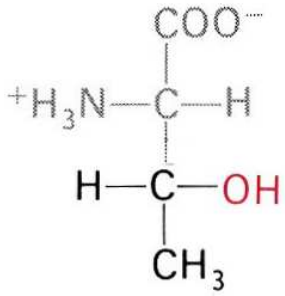


Methionine
(Met, M)

Hydroxylated aliphatic lateral chains



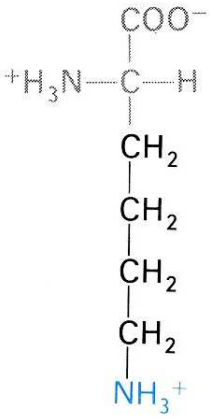
Serine
(Ser, S)



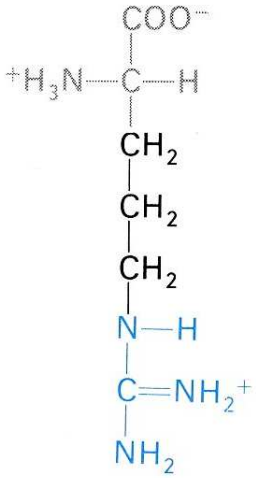
Threonine
(Thr, T)

Elemental components: amino acids - 3 -

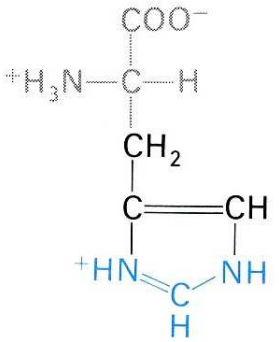
Lateral chains with basic character



Lysine (Lys, K)

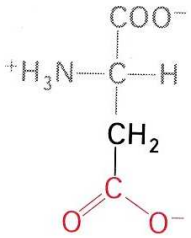


Arginine (Arg, R)

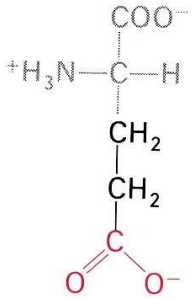


Histidine (His, H)

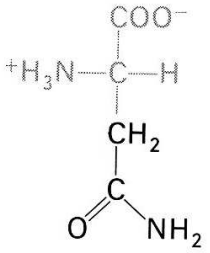
Lateral chains with acidic character. Presence of amides



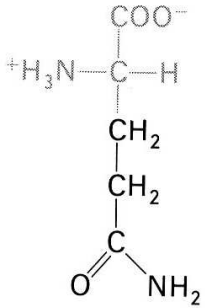
Aspartate (Asp, D)



Glutamate (Glu, E)



Asparagine (Asn, N)



Glutamine (Gln, Q)

Structure of proteins

Primary structure: sequence of amino acids and identification of disulfide bridges

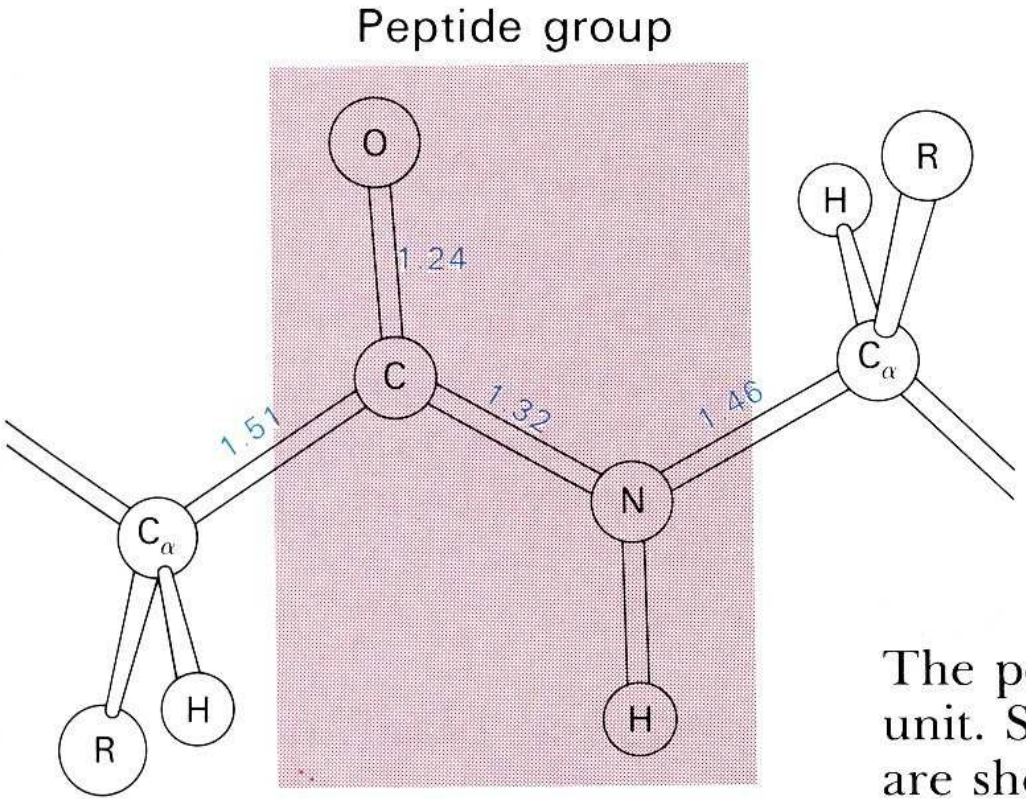
Secondary structure: through-space arrangement of near amino acid residues

Tertiary structure: through-space arrangement and tridimensional structure of pre-organized motives

Quaternary structure: through-space organization of subunits

Structure of proteins: secondary structure

The peptide group:

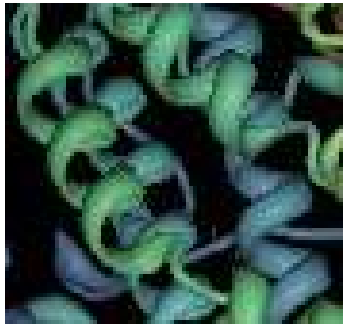


The peptide group is a rigid planar unit. Standard bond distances (in Å) are shown.

Structure of proteins: secondary structure

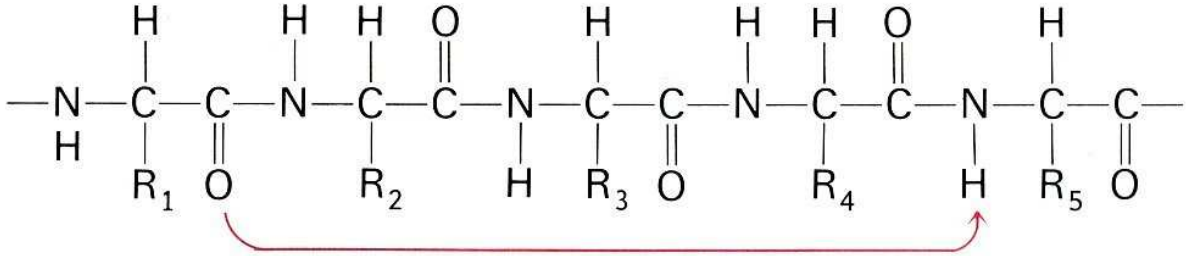
The through-space organization leads to two particular conformations

α helixes



β sheets

Structure of proteins: secondary structure: α helix



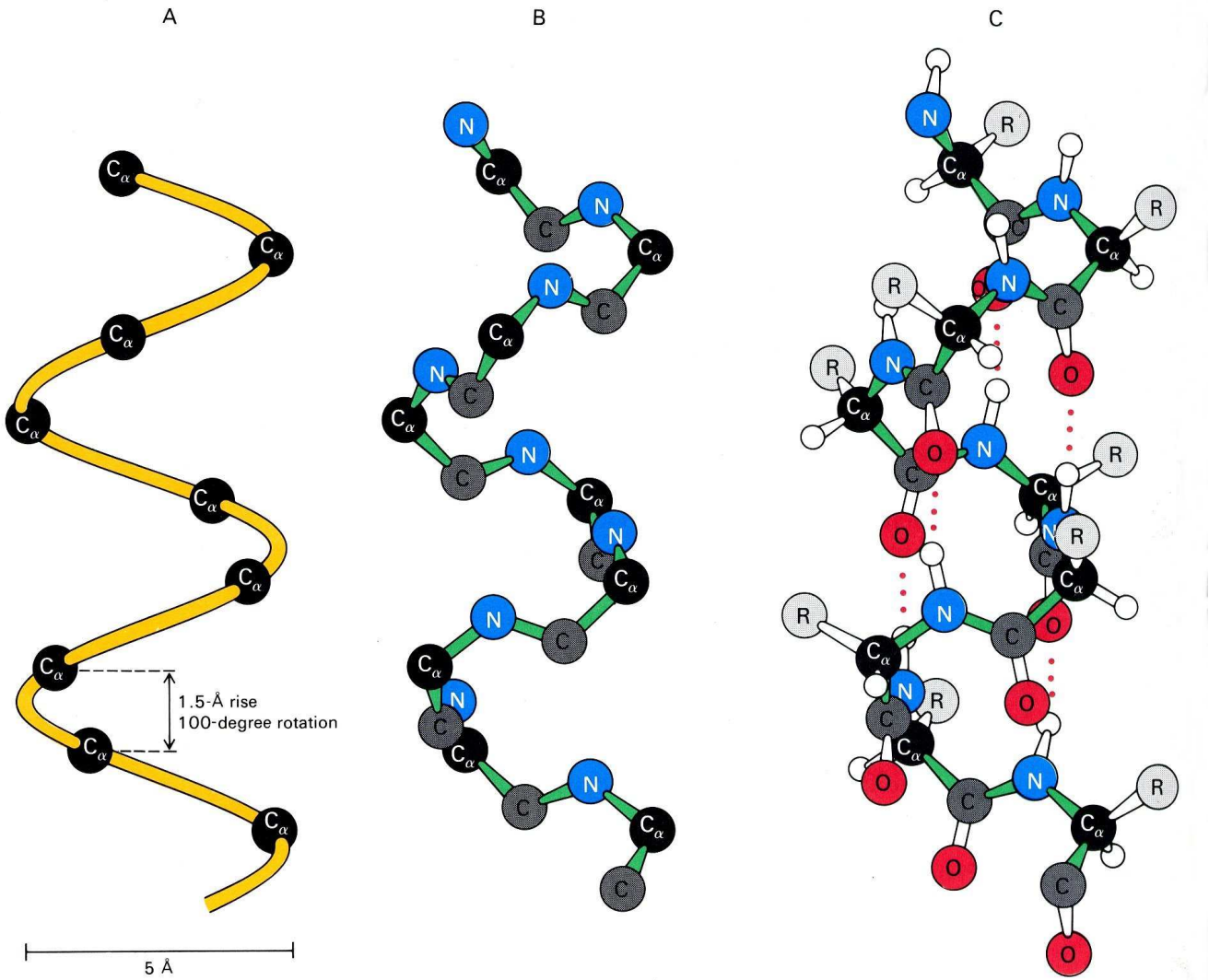
In the α helix, the CO group of residue n is hydrogen bonded to the NH group of residue $(n + 4)$.

Internal stabilisation

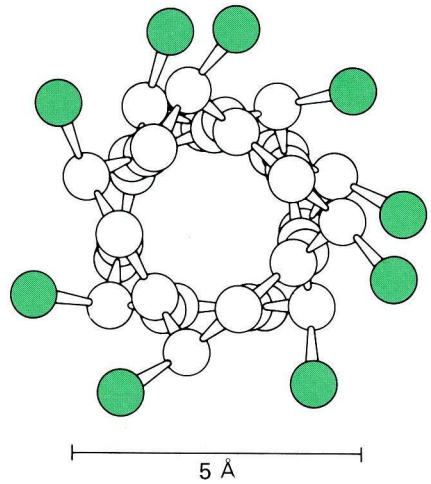
α helixes

The number of residues by helix step is # 3.6.
Helixes can be left- or right-handed (most of the cases)

Structure of proteins: secondary structure: α helix

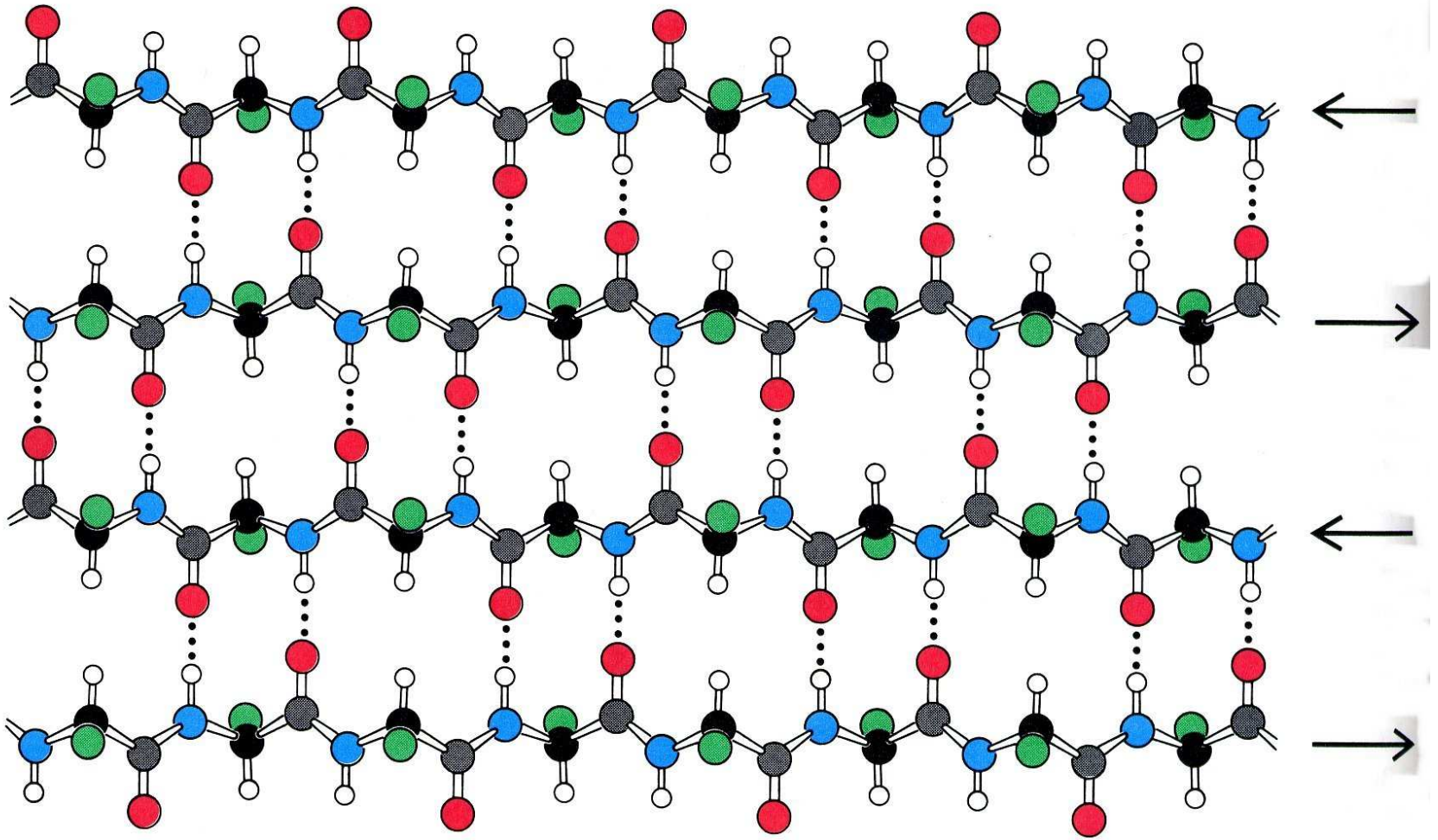


Models of a right-handed α helix: (A) only the α-carbon atoms are shown on a helical thread; (B) only the backbone nitrogen (N), α-carbon (C_α), and carbonyl carbon (C) atoms are shown; (C) entire helix. Hydrogen bonds (denoted in part C by red dots) between NH and CO groups stabilize the helix.



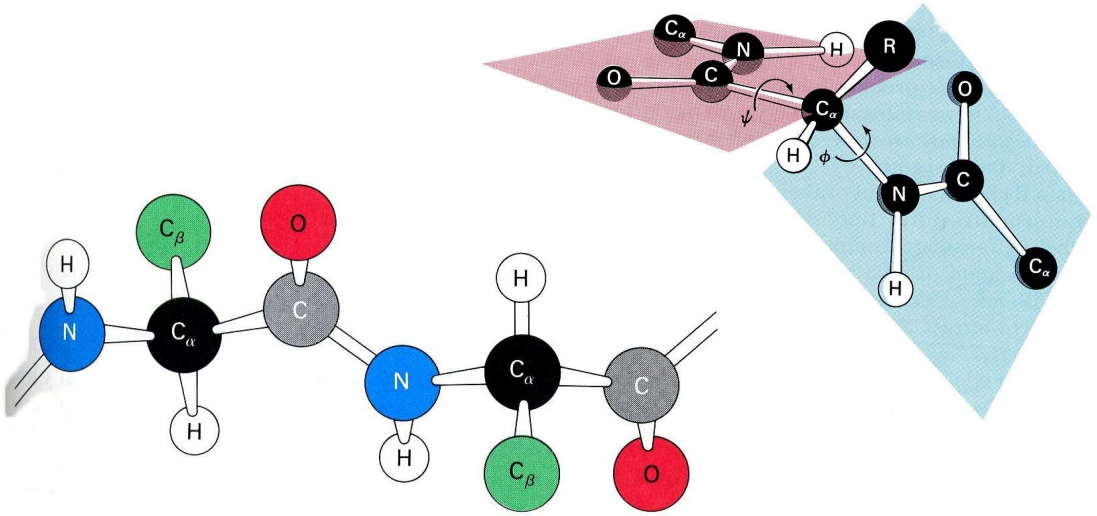
Cross-sectional view of an α helix. Note that the side chains (shown in green) are on the outside of the helix. The van der Waals radii of the atoms are larger than shown here; hence there is actually almost no free space inside the helix.

Structure of proteins: secondary structure: β sheet

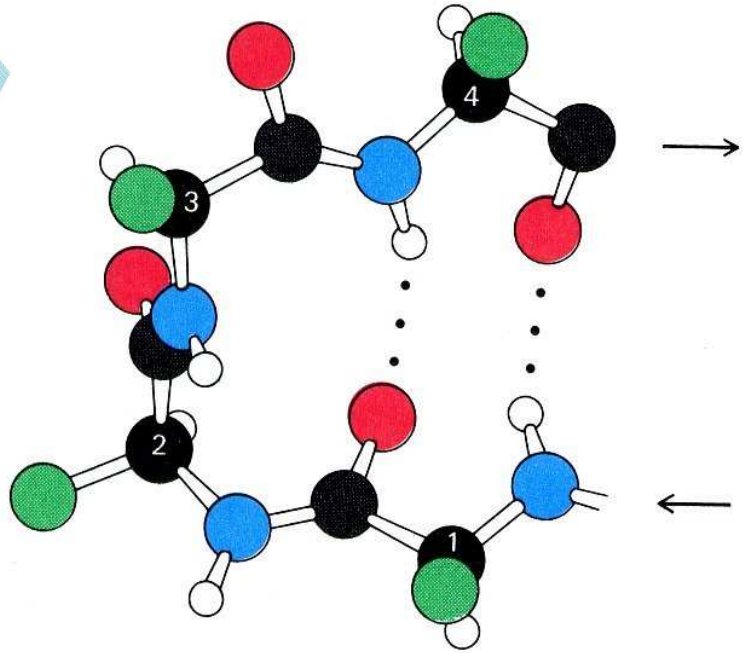


Antiparallel β pleated sheet. Adjacent strands run in opposite directions. Hydrogen bonds between NH and CO groups of adjacent strands stabilize the structure. The side chains (shown in green) are above and below the plane of the sheet.

Structure of proteins: secondary structure: β sheet



Conformation of a dipeptide unit in a β pleated sheet. The polypeptide chain is almost fully stretched out.

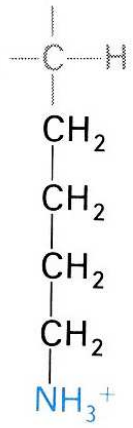


Structure of a β-turn. The CO group of residue 1 of the tetrapeptide shown here is hydrogen bonded to the NH group of residue 4, which results in a hairpin turn.

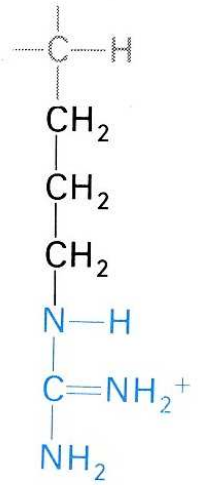
Proteins can be globular:
Occurrence of hairpin turns stabilized by H-bond

Structure of proteins: tertiary structure

>0 charges



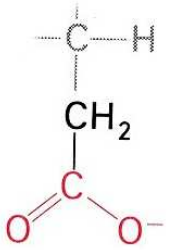
Lysine (Lys, K)



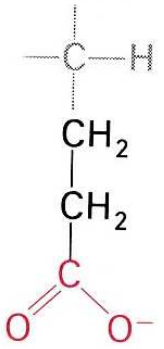
Arginine (Arg, R)

1. Ionic interactions: salt bridges

<0 charges



Aspartate (Asp, D)

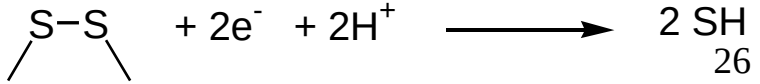


Glutamate (Glu, E)

2. H- bonds
3. Van der Waals interactions

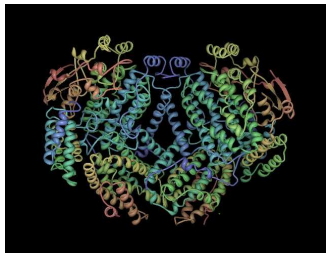
4. Others: disulfide bridges

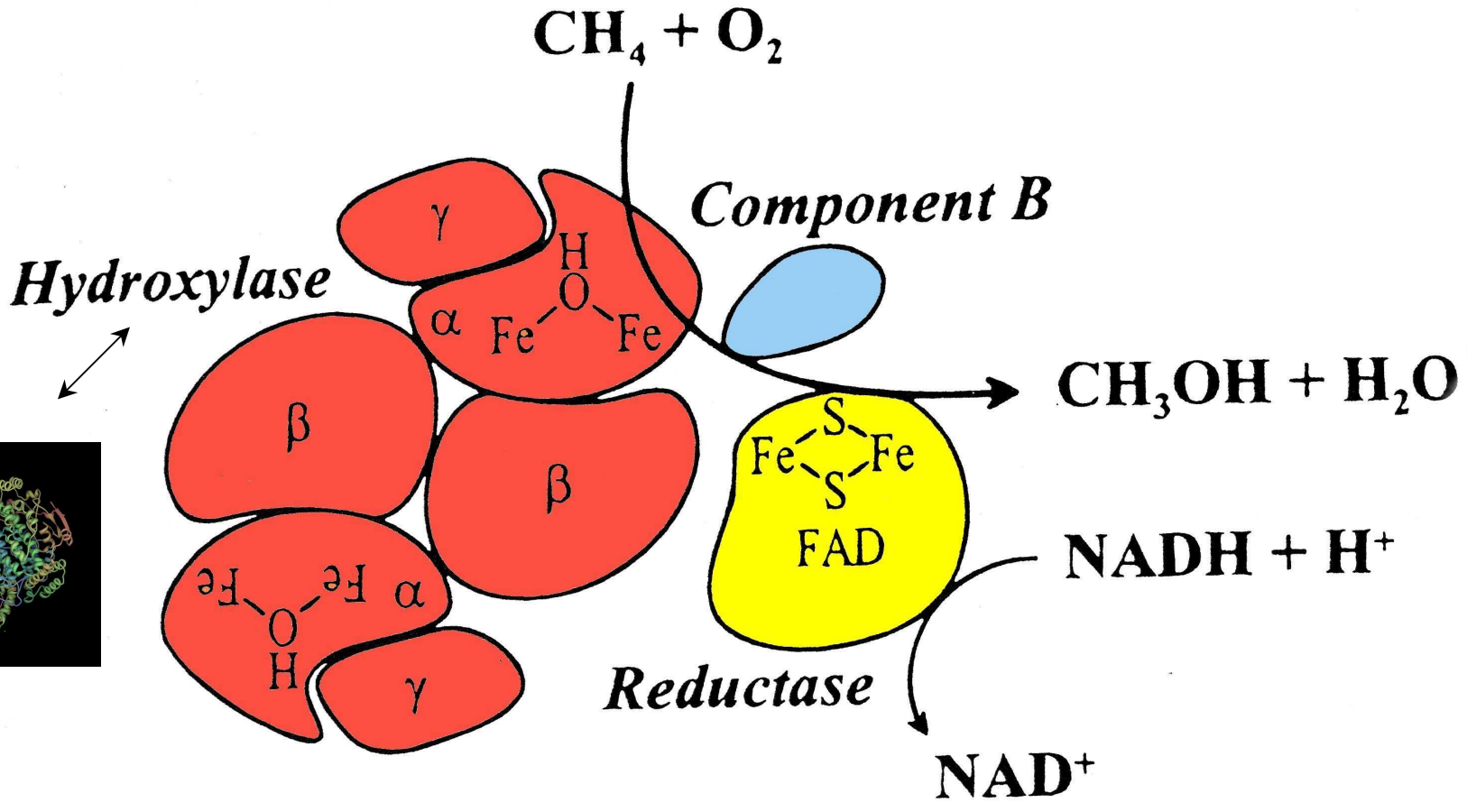
These bridges can be reduced leading to chain unfolding



Structure of proteins: quaternary structure

Assembly of subunits: the case of Methane Mono Oxygenase





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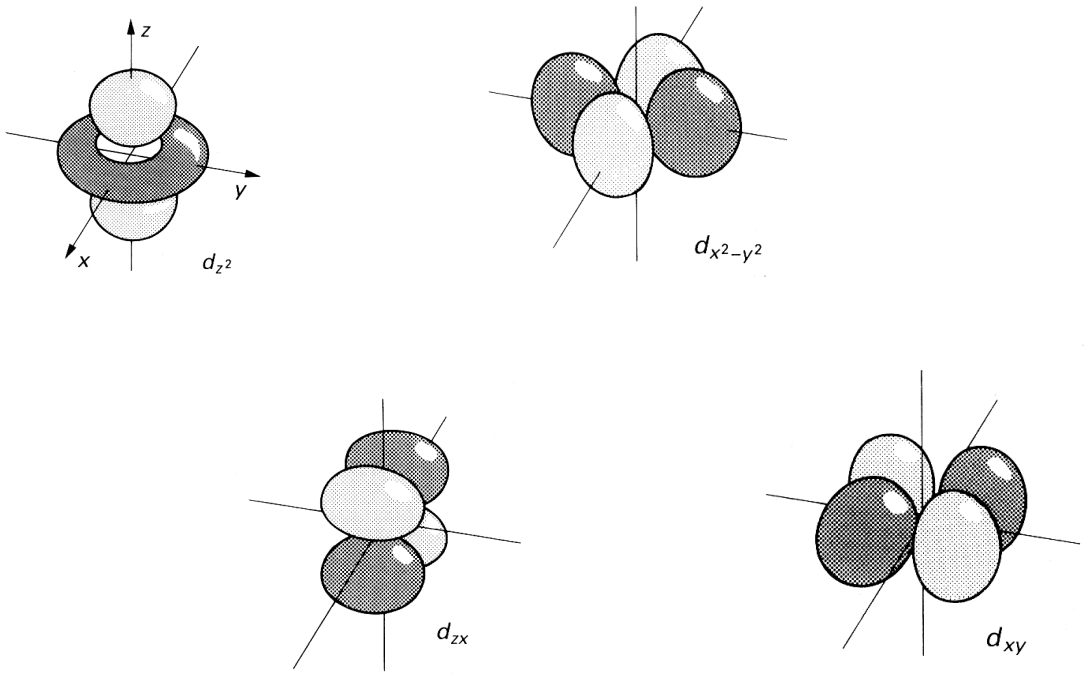
- Mössbauer

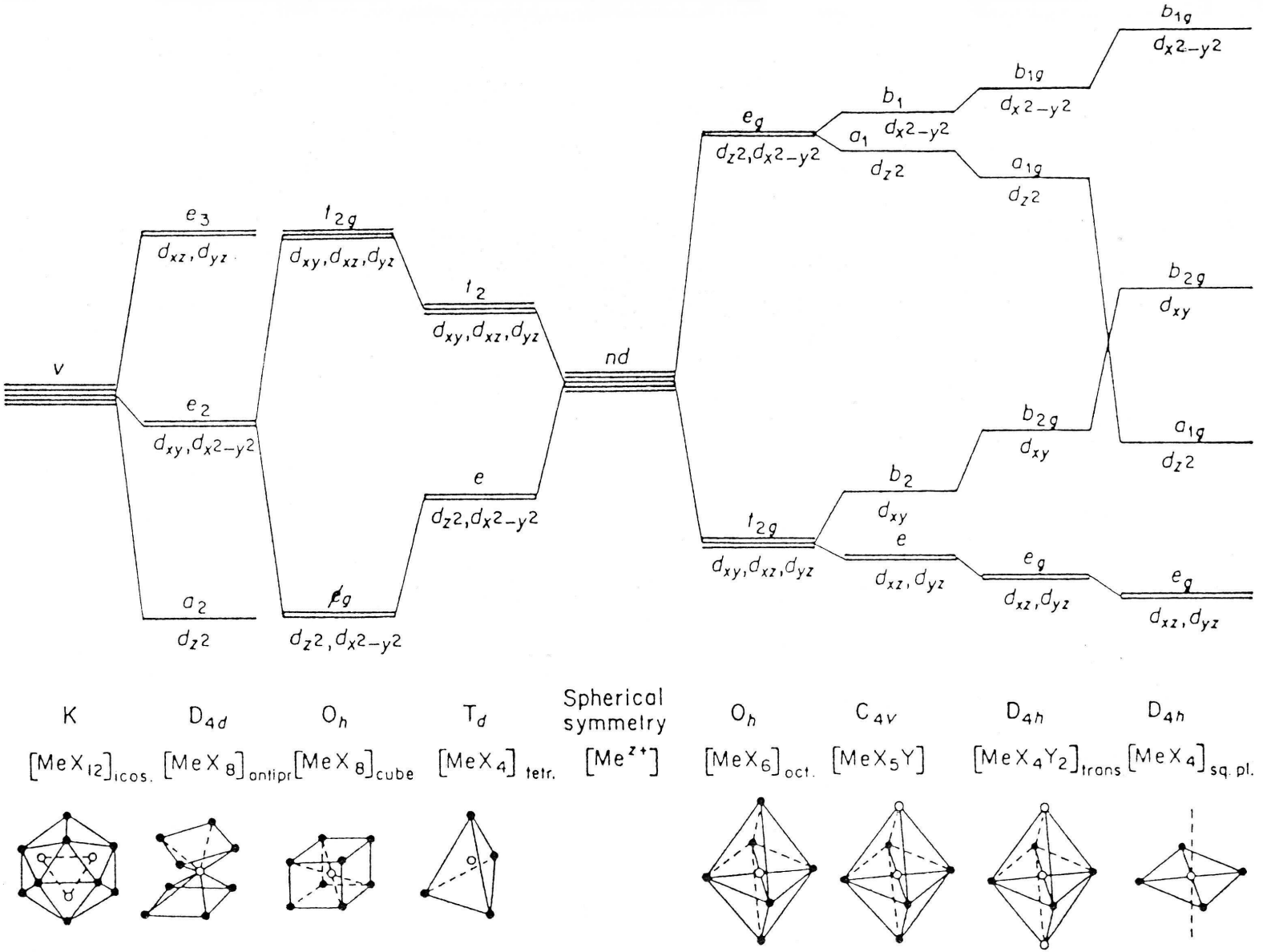
Transition metals

IIIA	IVA	VA	VIA	VIIA	VIII	VIII	VIII	IB	IIB
Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn
Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd

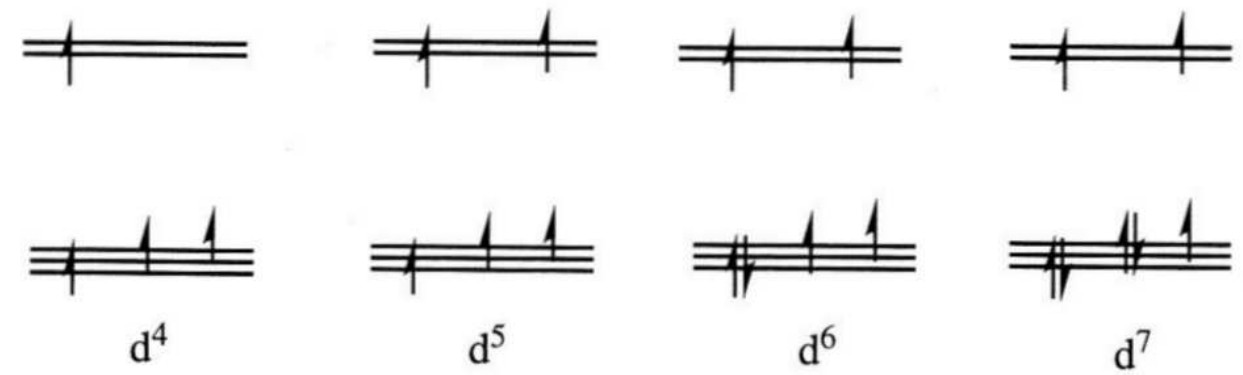


Representation of the five *d* orbitals along the *x*, *y* and z axis

Symmetry and Transition Metals

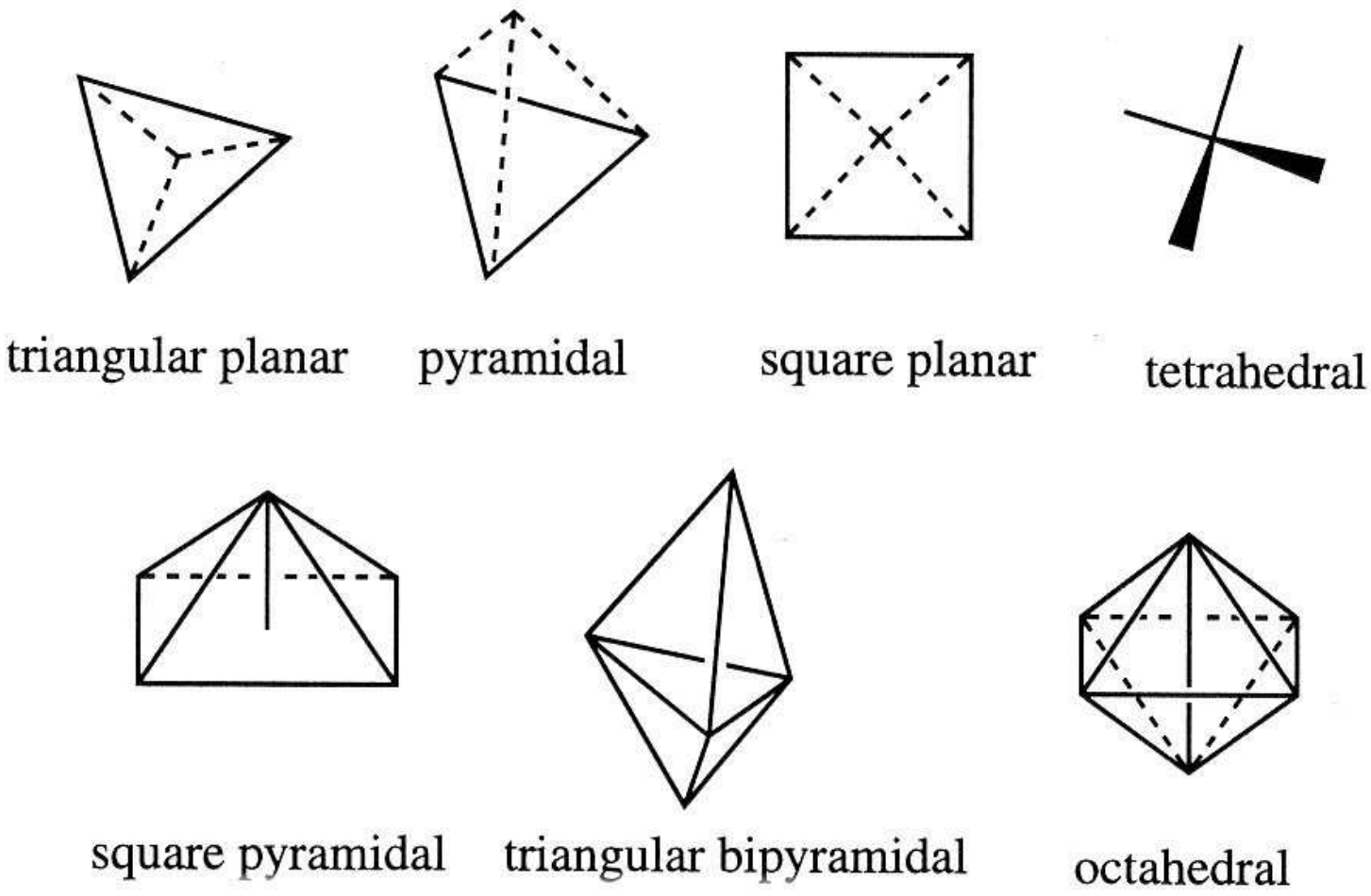


Magnetism and Transition Metals



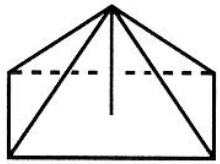
High-spin and low-spin *d*-electron configurations for the octahedral field.

Most frequently found geometry at active sites of metal-containing biomolecules

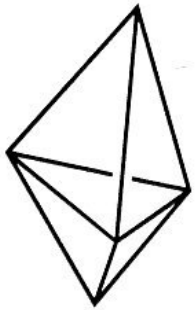


Flexibility – Role of exogenous ligands

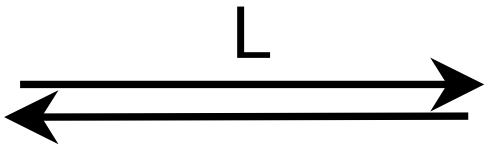
The properties differ from a given geometry to an other one



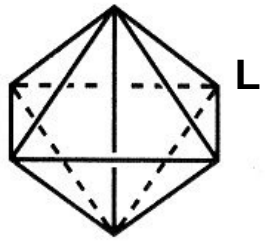
square pyramidal



triangular bipyramidal

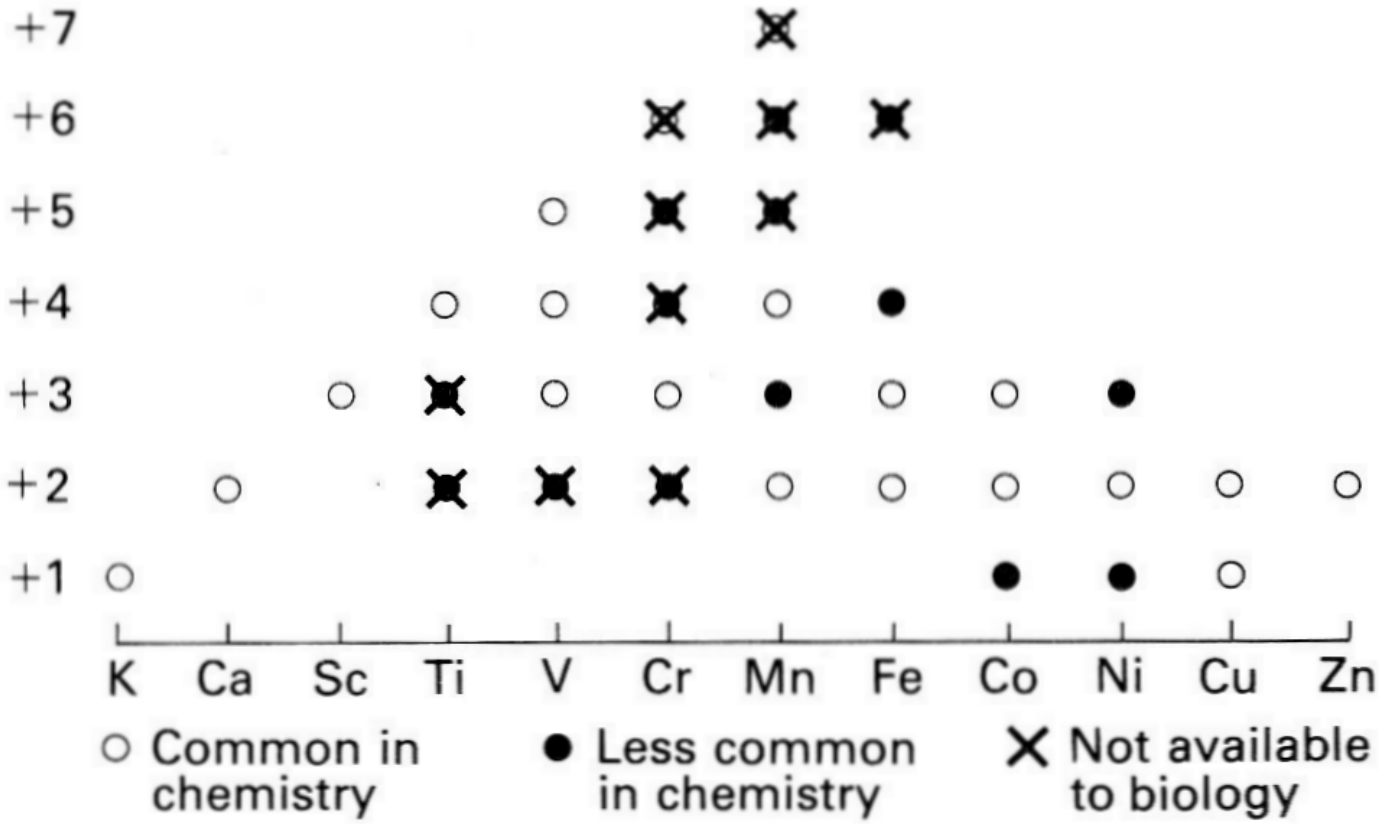


L: H_2O, OH^-, Substrate…

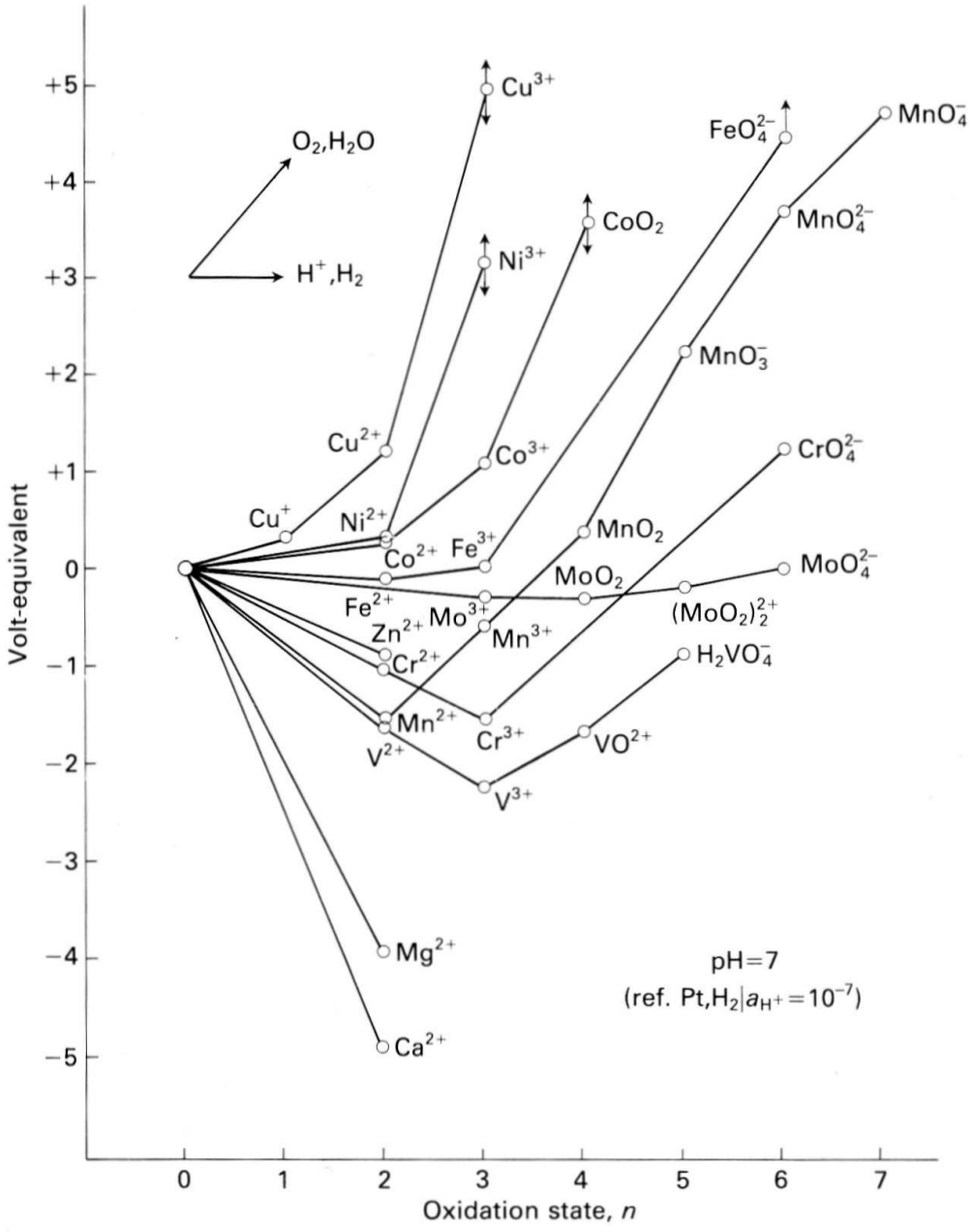


octahedral

Various oxidation states !



Oxidation states and stability



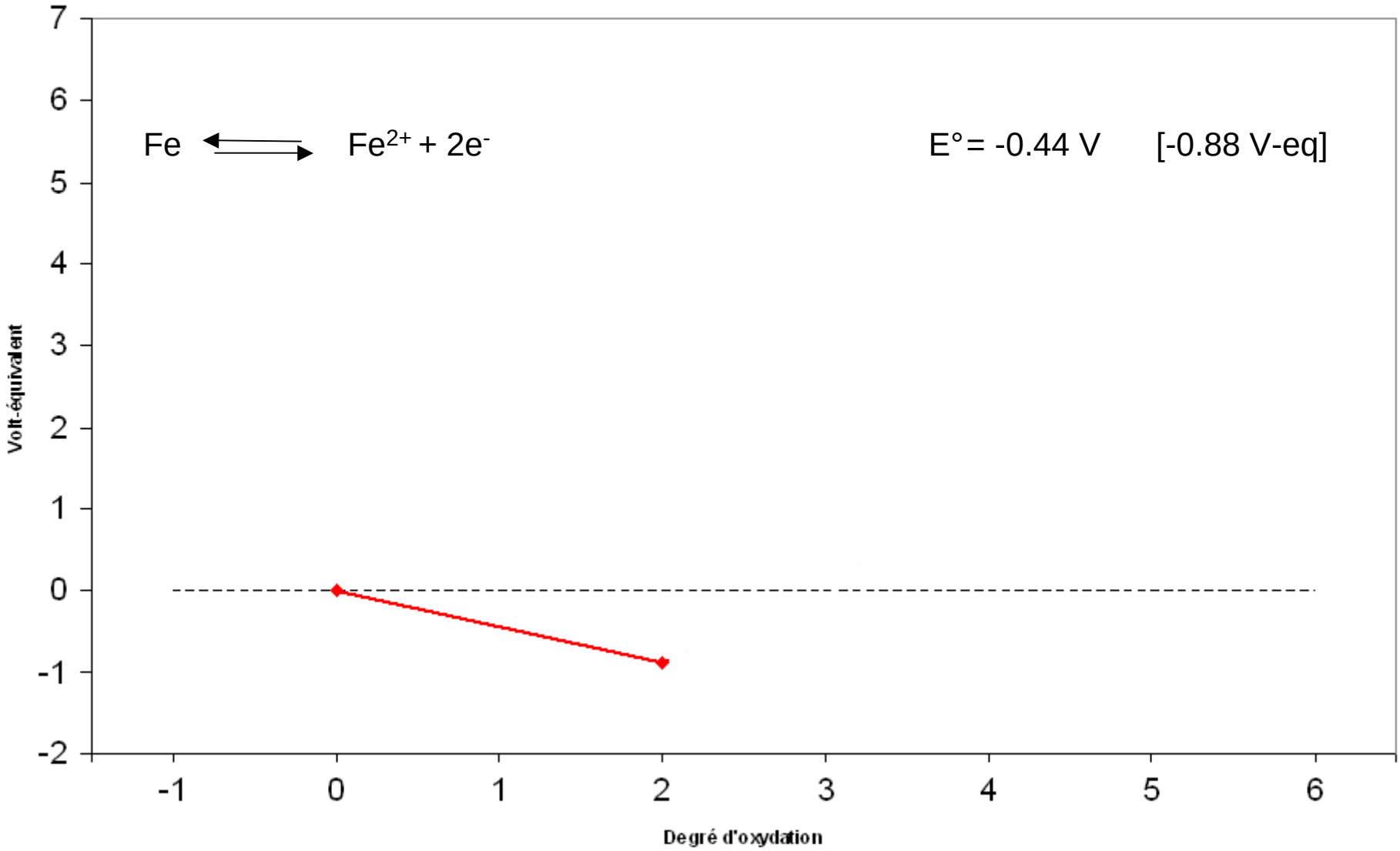
Building-up a Frost Diagram

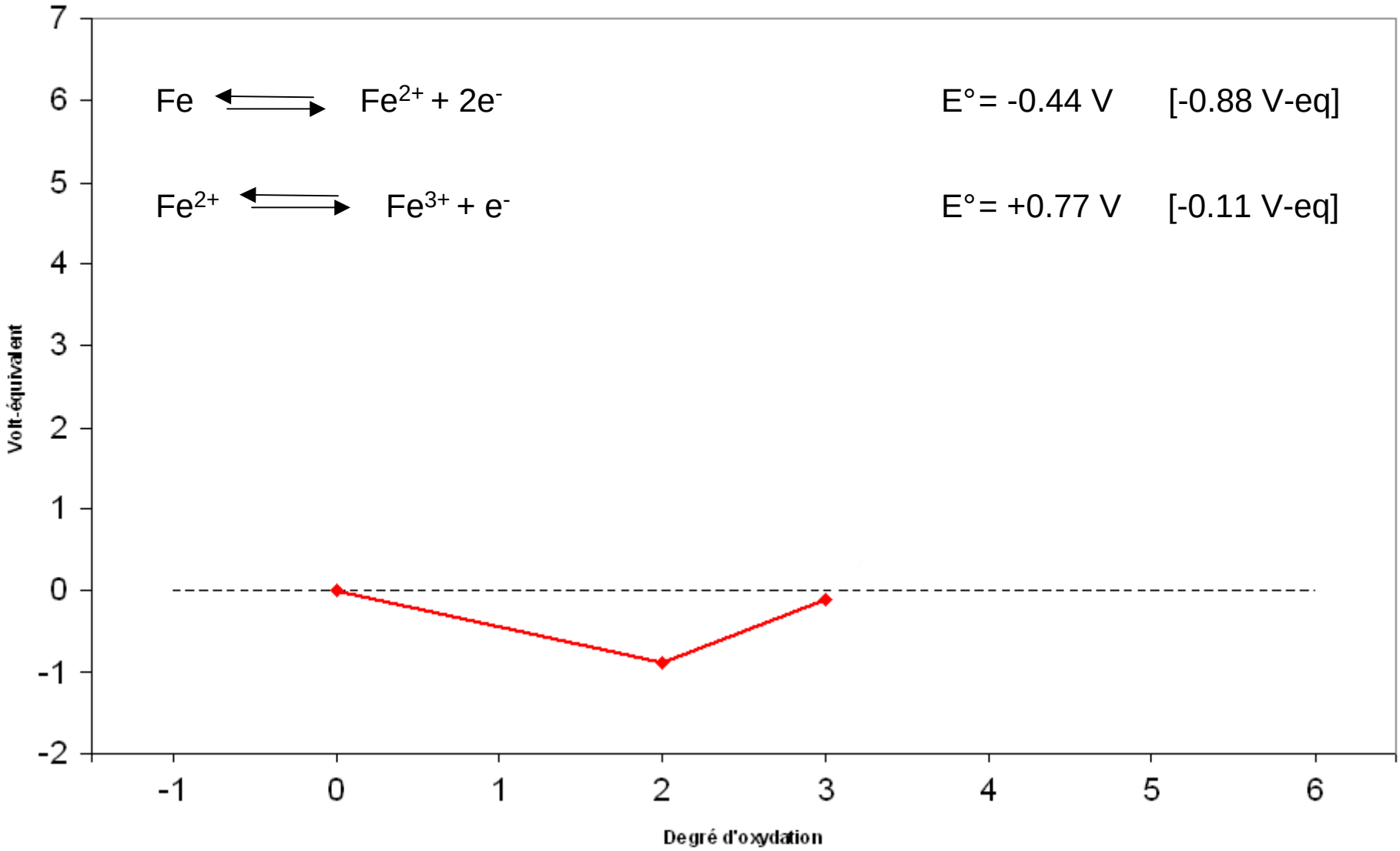
For iron in acidic conditions

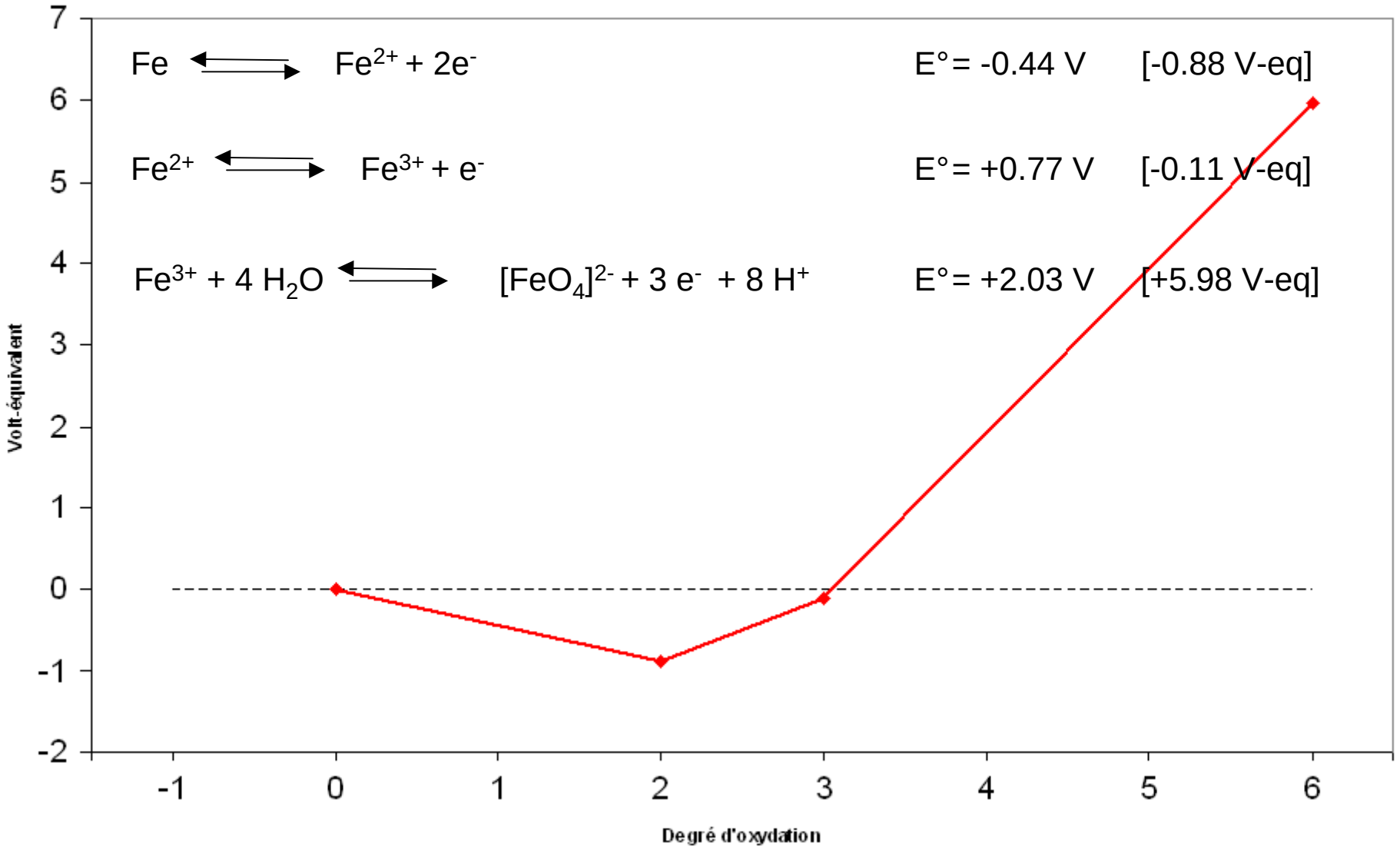
$Fe \rightleftharpoons Fe^{2+} + 2e^-$ $E° = -0.44$ V

$Fe^{2+} \rightleftharpoons Fe^{3+} + e^-$ $E° = +0.77$ V

$Fe^{3+} + 4\,H_2O \rightleftharpoons [FeO_4]^{2-} + 3\,e^- + 8\,H^+$ $E° = +2.03$ V


Fe ⇌ Fe2+ + 2e-
E° = -0.44 V [-0.88 V-eq]
Volt-équivalent
Degré d'oxydation
7
6
5
4
3
2
1
0
-1
-2
-1
0
1
2
3
4
5
6

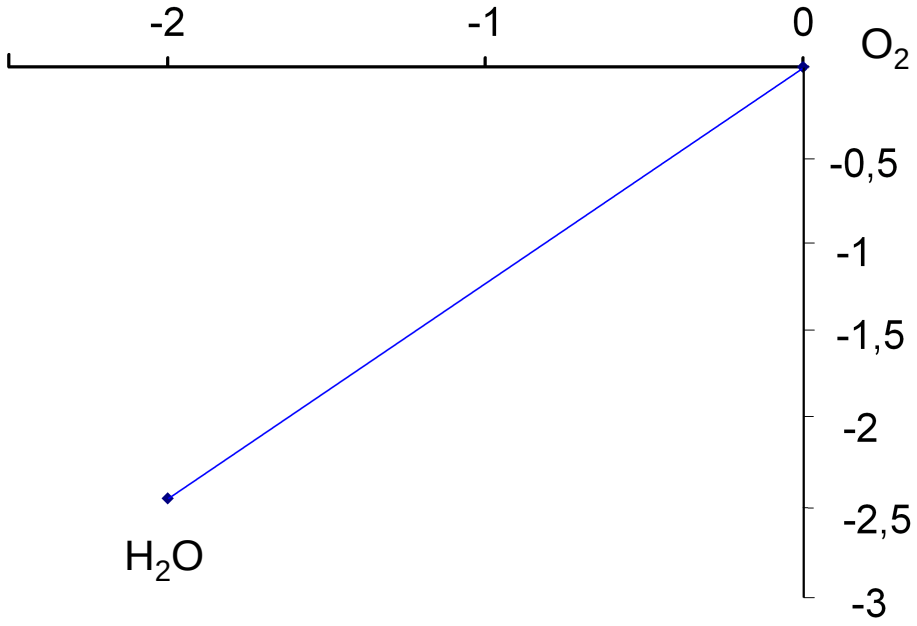

Fe ⇌ Fe2+ + 2e-
E°= -0.44 V [-0.88 V-eq]
Fe2+ ⇌ Fe3+ + e-
E°= +0.77 V [-0.11 V-eq]
Volt-équivalent
Degré d'oxydation
7
6
5
4
3
2
1
0
-1
-2
-1
0
1
2
3
4
5
6

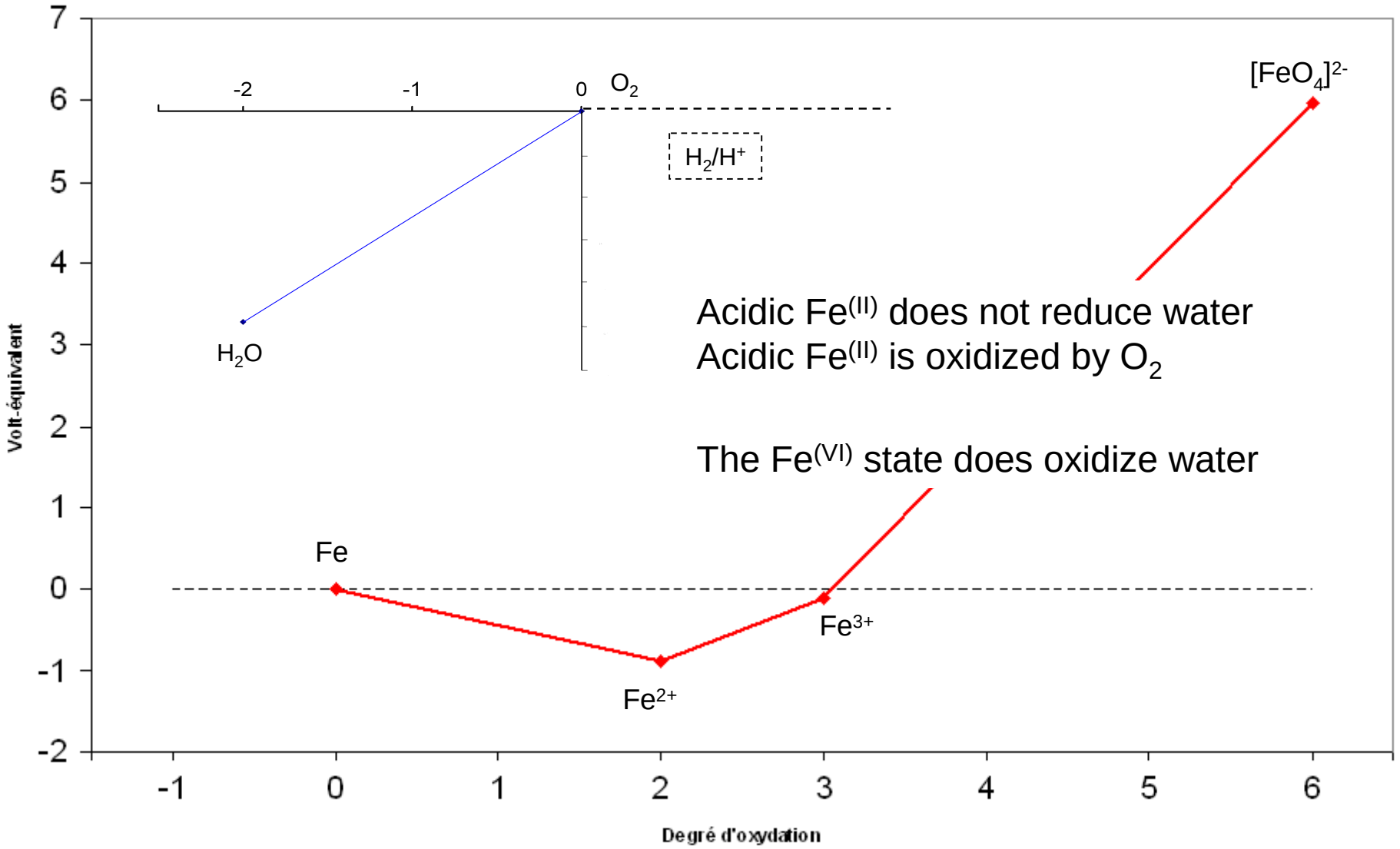

Fe ⇌ Fe2+ + 2e-
E° = -0.44 V
[-0.88 V-eq]
Fe2+ ⇌ Fe3+ + e-
E° = +0.77 V
[-0.11 V-eq]
Fe3+ + 4 H2O ⇌ [FeO4]2- + 3 e- + 8 H+
E° = +2.03 V
[+5.98 V-eq]
Volt-équivalent
Degré d'oxydation
7
6
5
4
3
2
1
0
-1
-2
-1
0
1
2
3
4
5
6

Building-up a Frost Diagram

For dioxygen in acidic conditions

$H_2O \rightleftharpoons \frac{1}{2} O_2 + 2H^+ + 2e^-$ $E° = +1.22$ V [-2.44 V-eq]



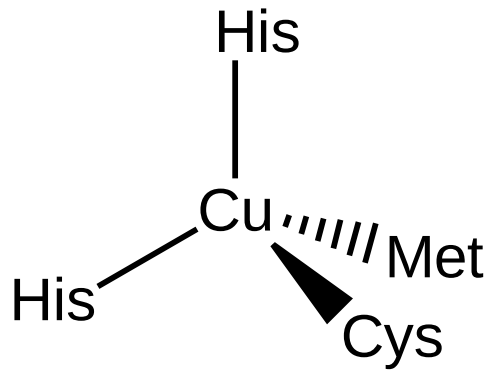

7
6
5
4
3
2
1
0
-1
-2
Volt-équivalent
-2
-1
0
O2
H2/H+
H2O
[FeO4]2-
Acidic Fe(II) does not reduce water
Acidic Fe(II) is oxidized by O2
The Fe(VI) state does oxidize water
Fe
Fe2+
Fe3+
-1
0
1
2
3
4
5
6
Degré d'oxydation

Redox potentials can be tuned by geometry

In electron transport systems, the conformational changes must be minor
to minimize the energy differences involved

If two oxidation states are presents, one of them being in a non-preferred geometry,
the redox potential can be dramatically shifted

$$Cu^{(I)} \rightleftharpoons Cu^{(II)} + e^-$$



The case of plastocyanin: a tetrahedral geometry is imposed to Cu(II):
=> the Cu(II)/Cu(I) potential is shifted to # 375 mV

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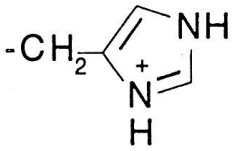
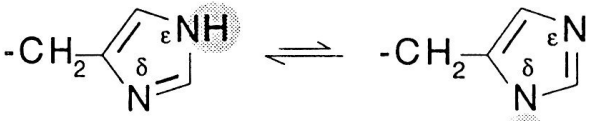
Amino acids bind transition metals - 1 -

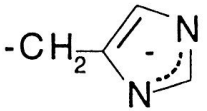
histidine (His)



$pK_a \approx 6.5$ + H^+ ⇅ - H^+



$pK_a \approx 14$ + H^+ ⇅ - H^+



methionine (Met) $-CH_2CH_2SCH_3$

cysteine (Cys) $-CH_2SH$

selenocysteine (SeCys) $-CH_2SeH$

tyrosine (Tyr)

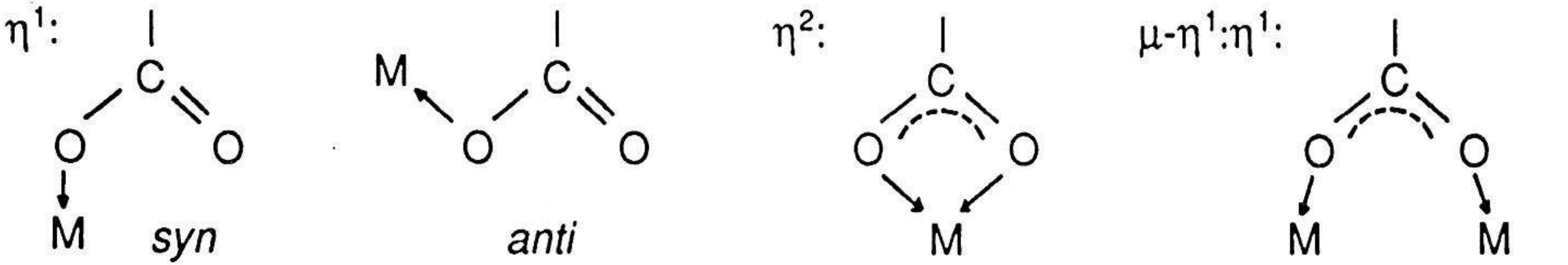
aspartic acid (Asp) $-CH_2COOH$

glutamic acid (Glu) $-CH_2CH_2COOH$

H : acidic protons which may be substituted by metal cations

Amino acids bind transition metals - 2 -

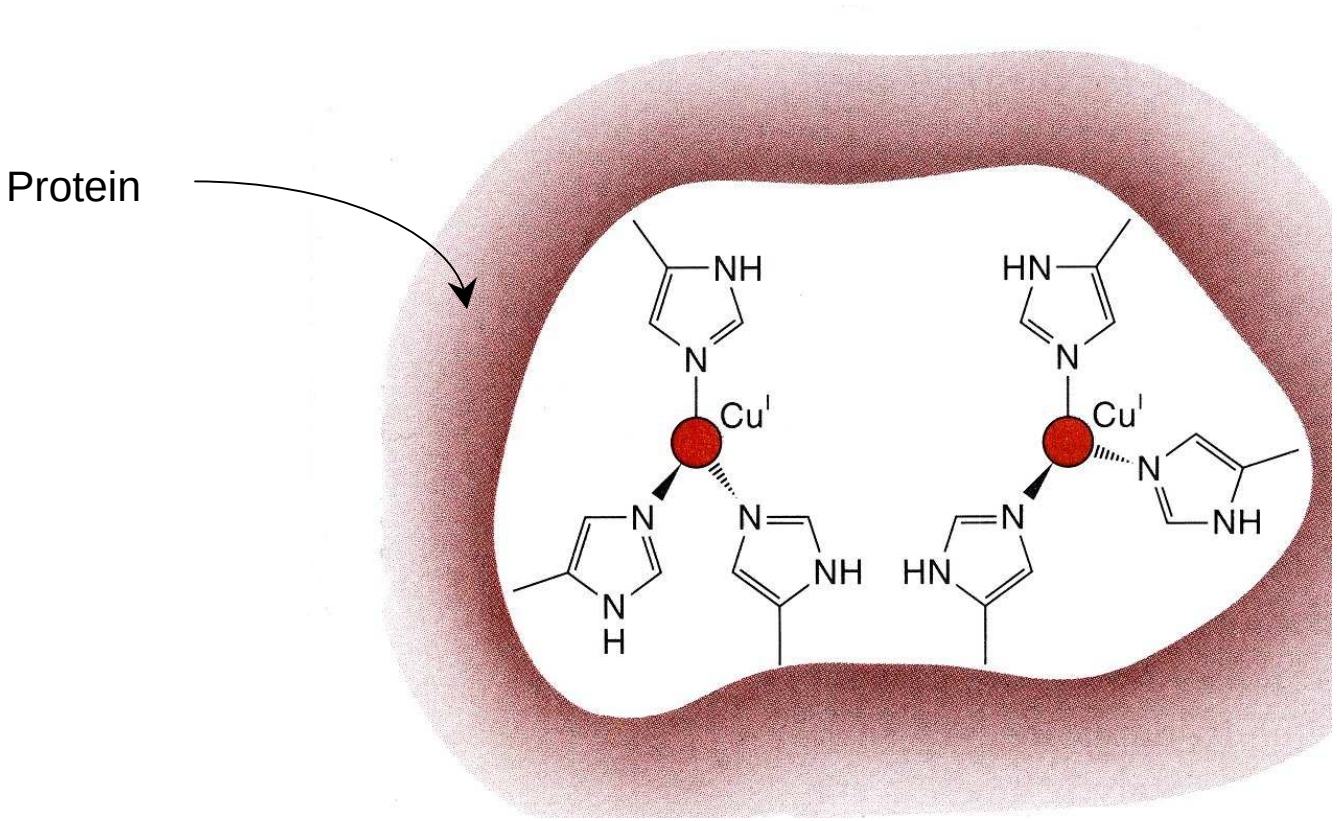
Aspartate (Asp) bind via the negatively charged carboxylate functions ($pK_a \approx 4.5$). Carboxylates can act as terminal (η^1), as chelating (η^2) or as bridging μ–$\eta^1 : \eta^1$ ligands (2.3; see also 7.14 [20]); a further distinction concerns the *syn* or *anti* positioning of the binding electron pairs.



(2.3)

Direct coordination to metals – Active site 1

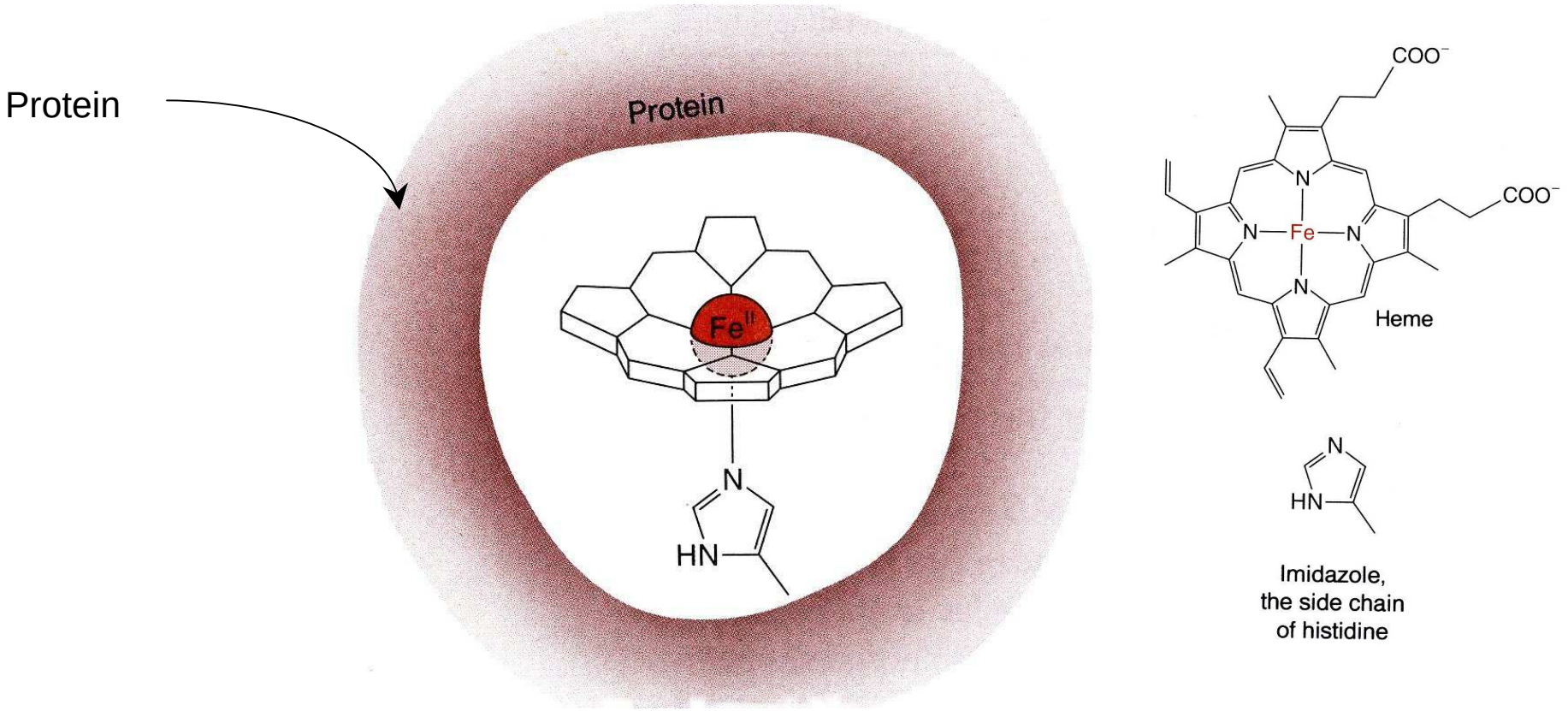
Using amino acid residues:



Histidins bind two copper atoms in deoxyhemocynanin

Indirect coordination: cofactors – Active site 2

Presence of a cofactor: HEME as example
(heme-containing proteins)



The heme can be bound to the protein by metal coordination via an amino acid residue (heme a, heme b), alternatively by covalent bond (disulfide bridges in heme c).

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Identification at the molecular level of a (metal-containing)protein

Best technique: **X-ray diffraction**

(X-ray generator or synchrotron beam)

This is not restricted to metal-containing proteins

Single crystals are required !

↓

The structure must be refined to acceptable resolution *(# one Ångström)*

This is a SOLID STATE technique

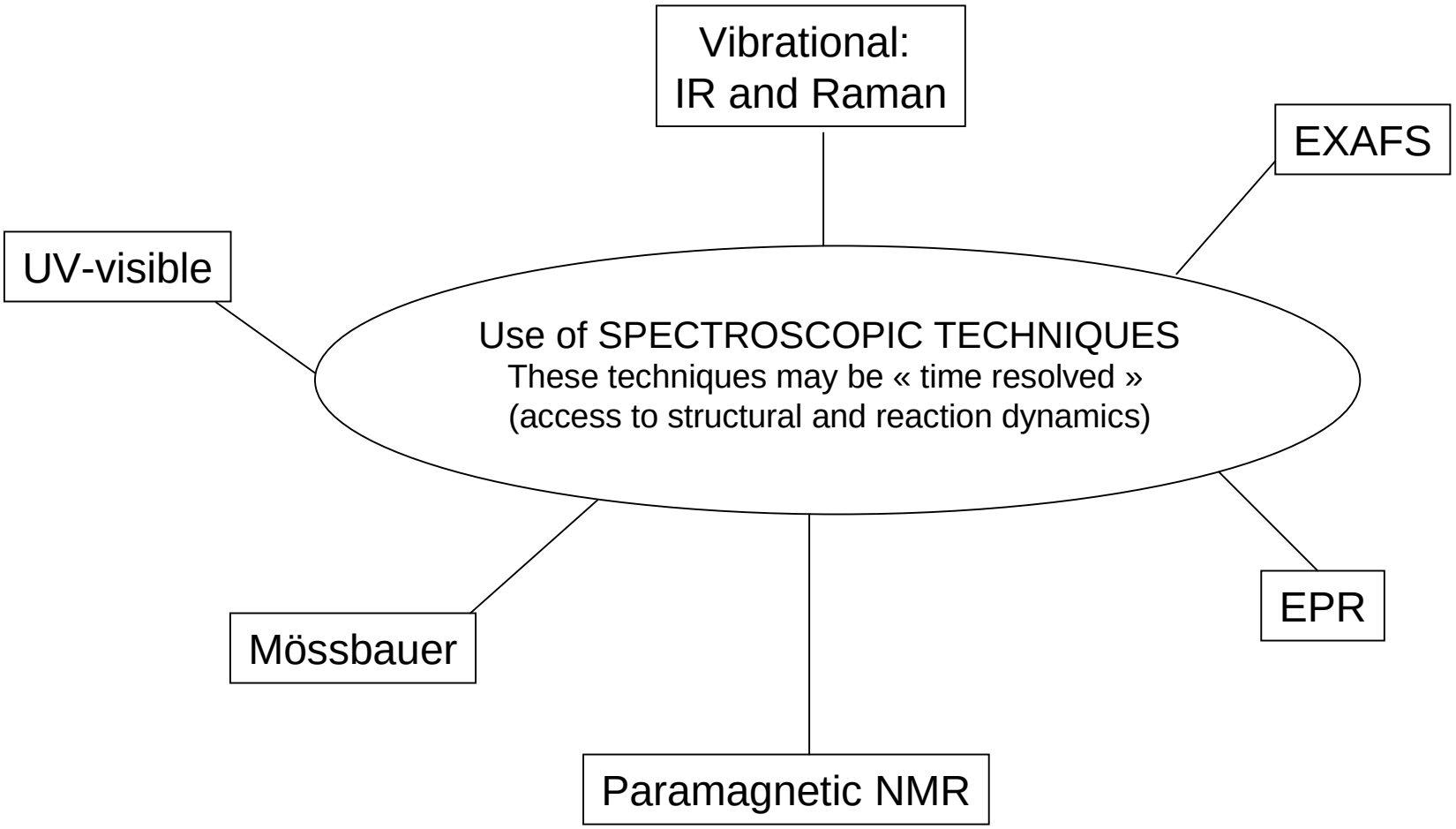
↓ ↑

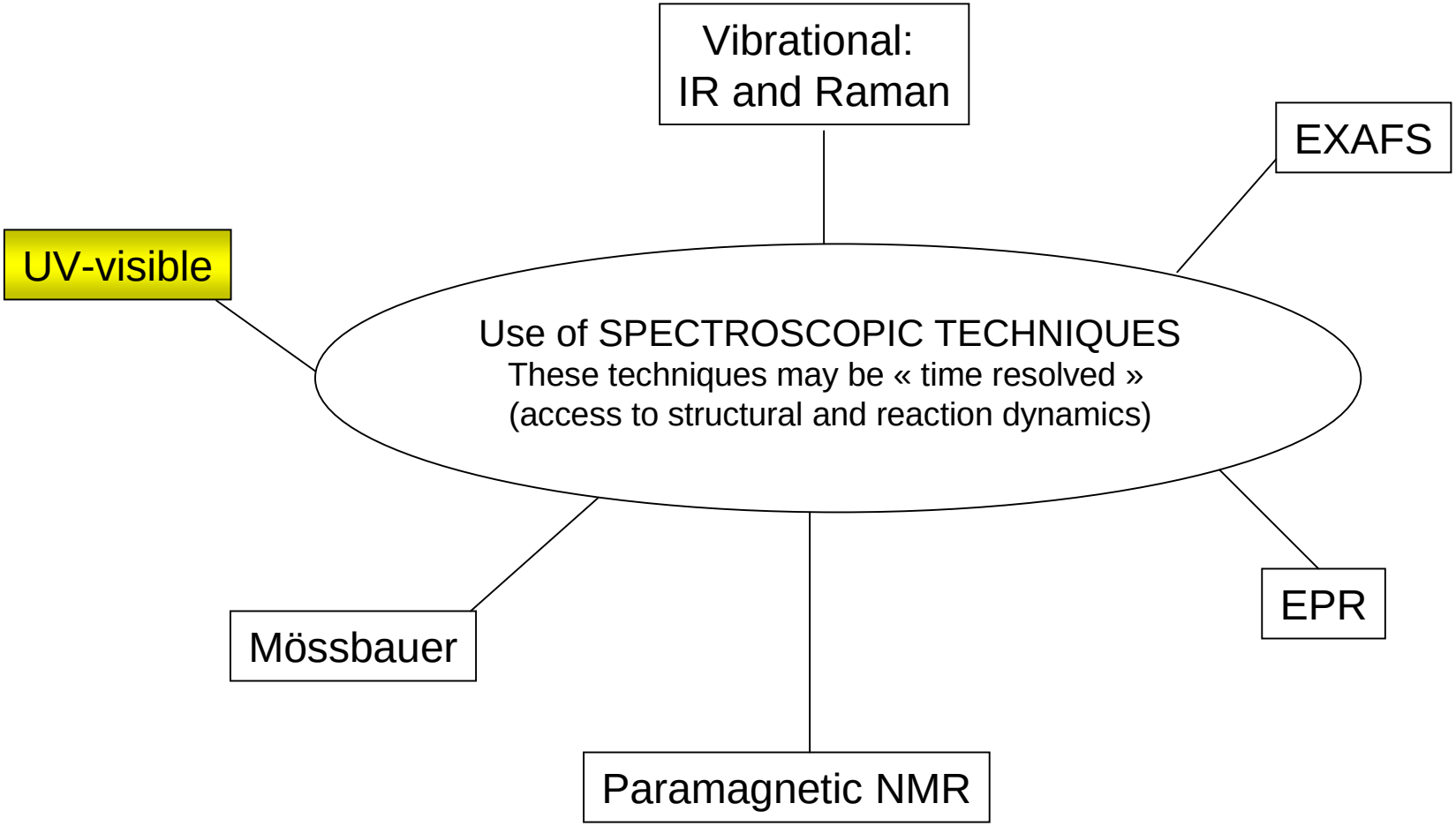
That must be completed by molecular dynamics studies.
Does not necessarily match the situation in solution

Evidence of the presence (detection) of metals

Very small amounts of metals in biological systems.

i.e. human hemoglobin uses 0.35 % only of available iron in body



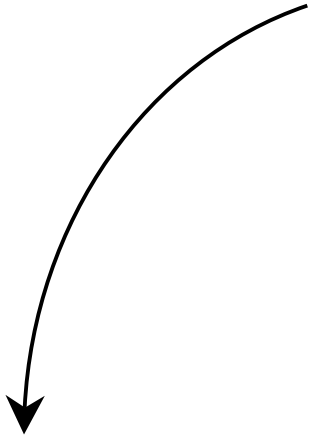

Vibrational:
IR and Raman
EXAFS
UV-visible
Use of SPECTROSCOPIC TECHNIQUES
These techniques may be « time resolved »
(access to structural and reaction dynamics)
Mössbauer
EPR
Paramagnetic NMR

UV-visible spectroscopy

Chromophore needed: d-d transitions : very weak intensity

LMCT – MLCT: useful

π -> π^* high intensity (authorized by Laporte's selection rule)



Iron/sulfur proteins (Fe/S)
Discovery in the 60's only

Weak exctinction coefficients

Oxidized state: Red color

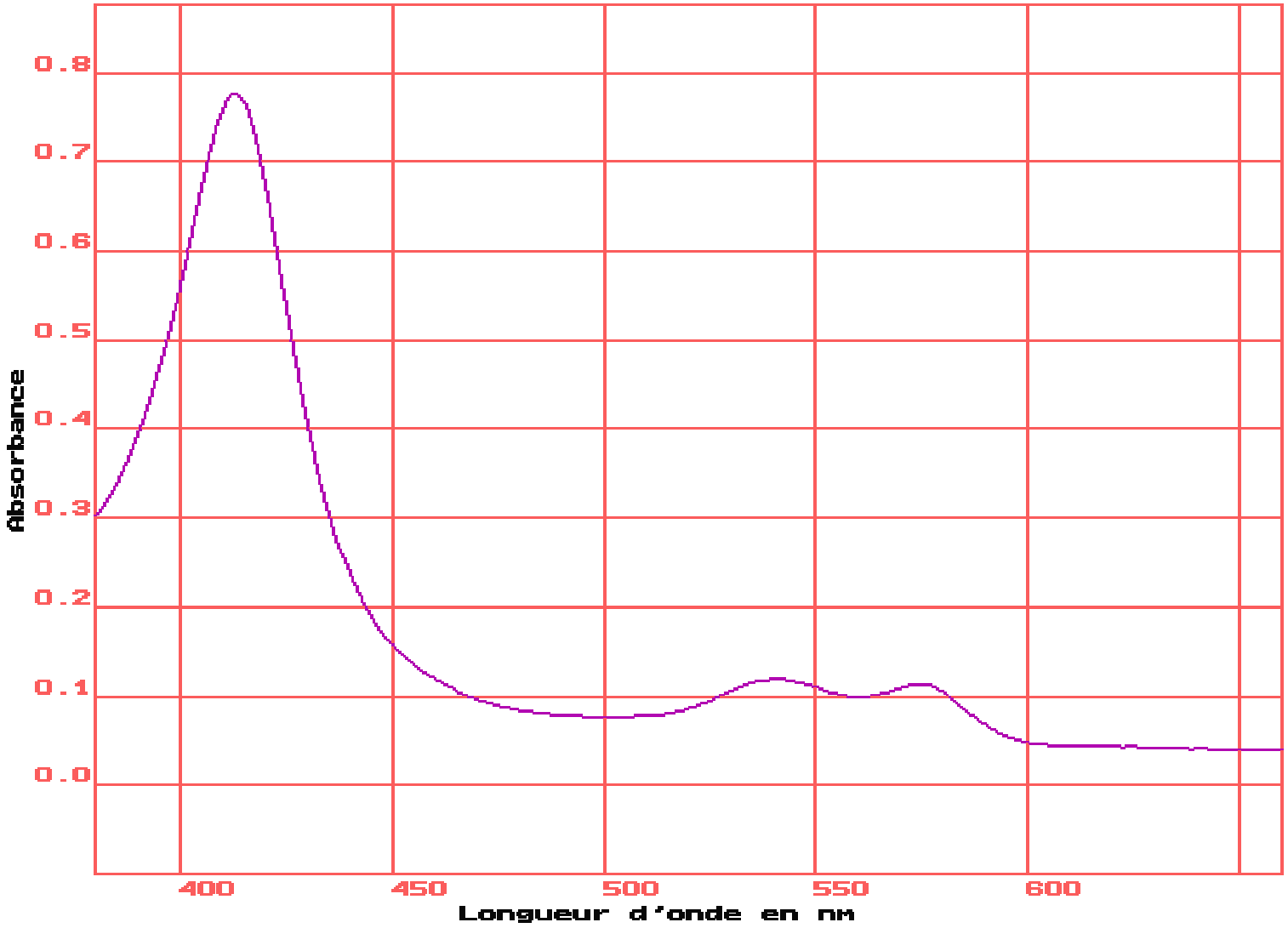
Iron: Heme-containing proteins
Discovery in the end of 19th century

Physiological function established in the 30's

Extremely intense absorptions
Purple-red (porphyrins)

Heme-containing porphyrins exhibit typical spectra: π -> π* transitions from the porphyrin macrocycle

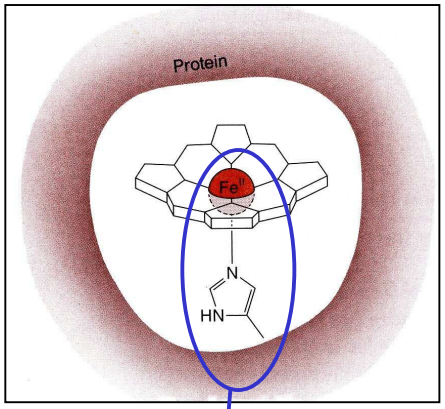
Horse deoxyhemoglobin



Heme-containing porphyrins exhibit typical spectra:
π -> π^* transitions from the porphyrin macrocycle

Weaker (less intense) π -> π^* contributions
Charge transfer spectroscopic zone

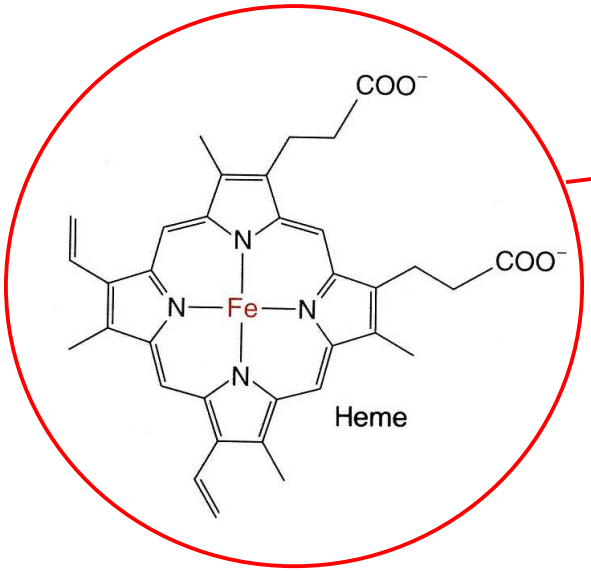
$500 < \lambda < 700$ nm

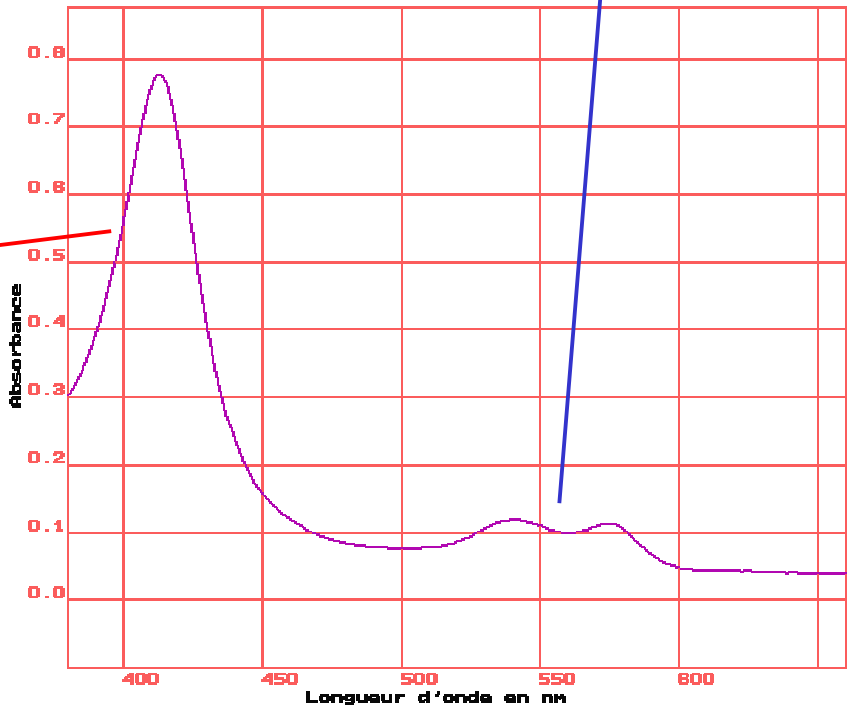


Fingerprint of an heme-containing protein

Very intense « Soret» band

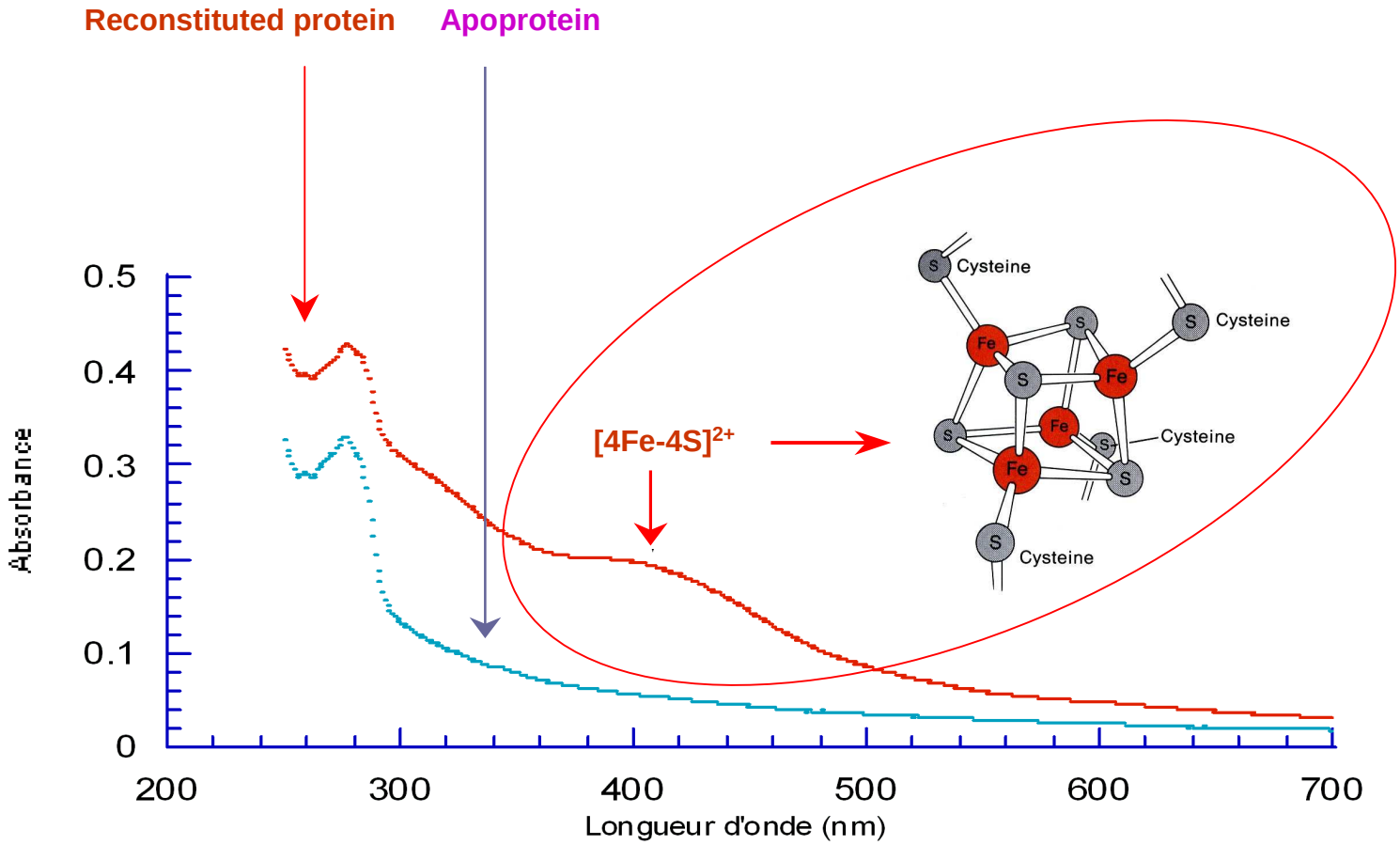
$350 < \lambda < 450$ nm,
ε # 20 – 30.10^3 mmol.cm^2

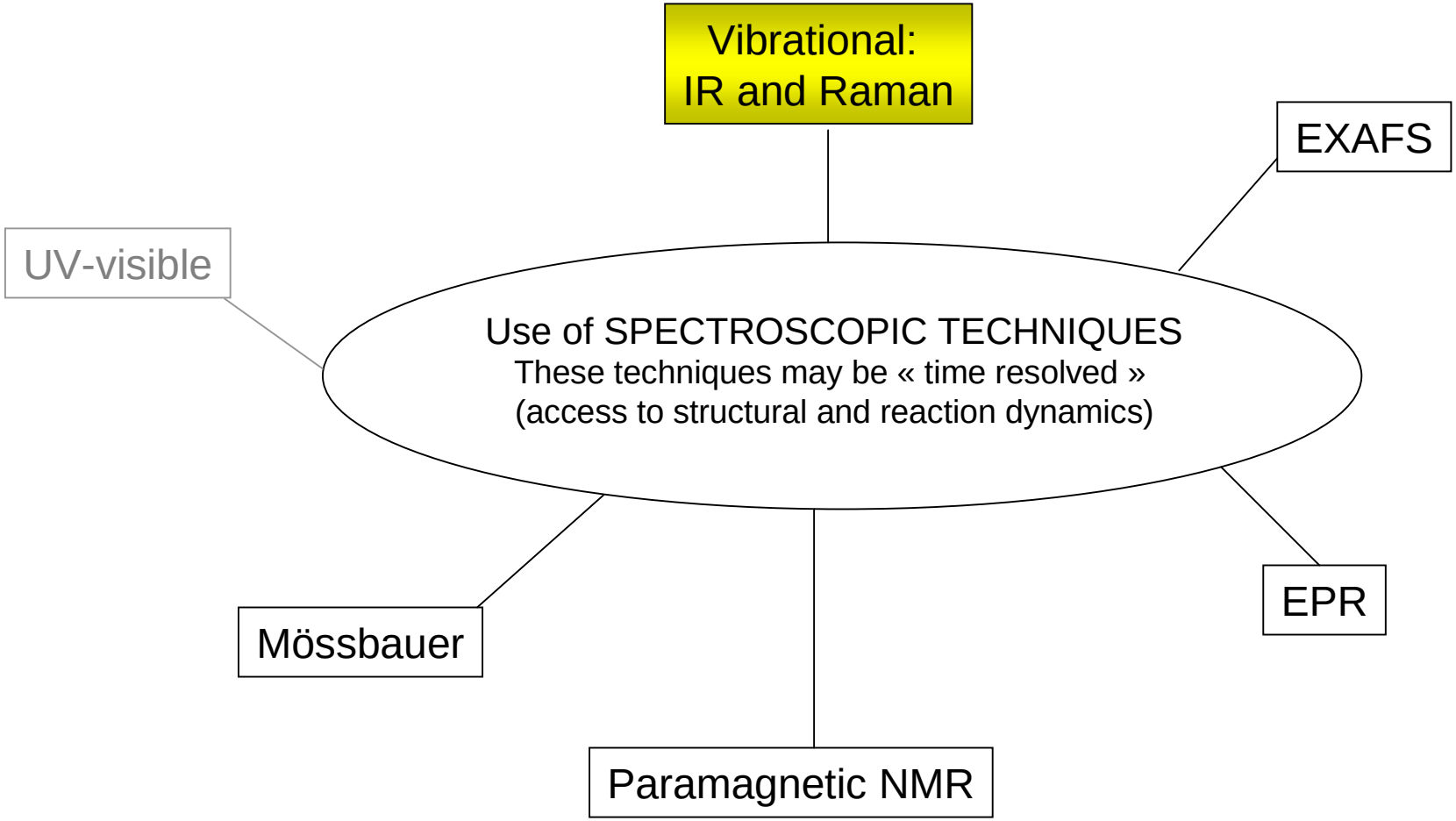




Iron / sulfur proteins are more difficult to detect by UV-visible

Chloroplasmic protein At-GcpE

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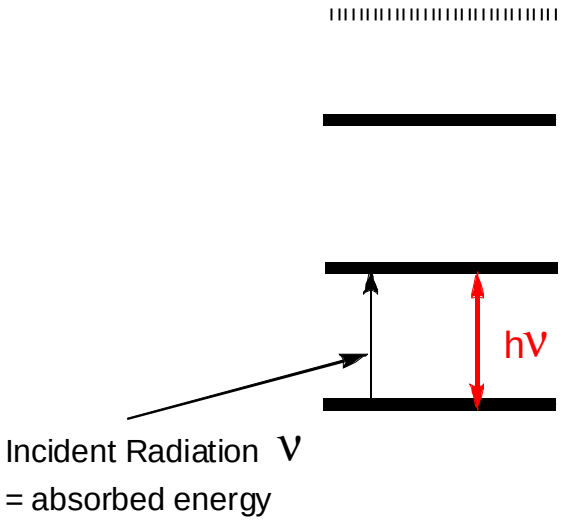
IR and Raman
EXAFS
UV-visible
Use of SPECTROSCOPIC TECHNIQUES
These techniques may be « time resolved »
(access to structural and reaction dynamics)
Mössbauer
EPR
Paramagnetic NMR

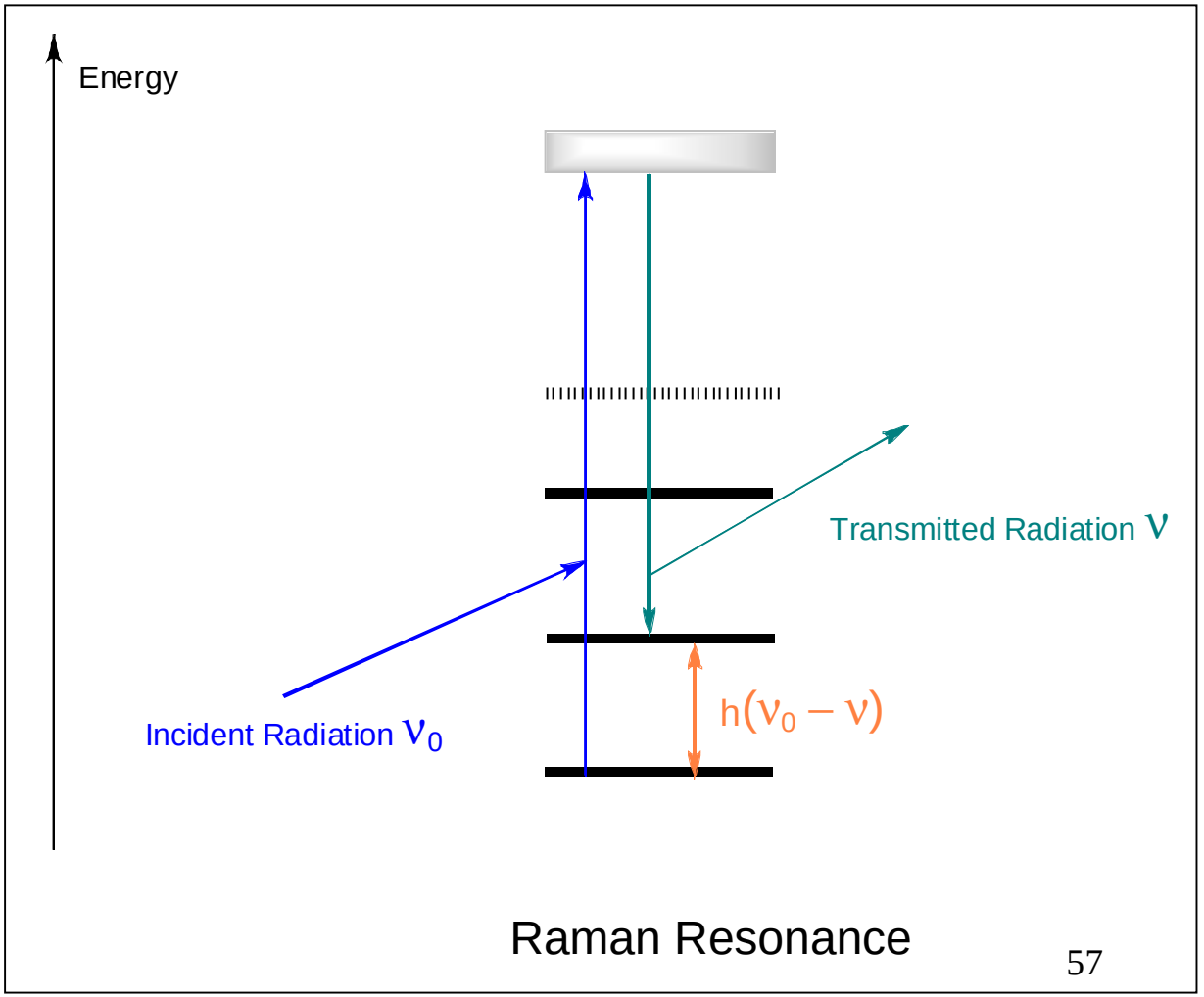
A vibrational spectroscopic technique: Raman Resonance

Allows measurements in water – possibility to use polarized light – IR/RR => involvement of symmetry

Irradiation of an already existing transition: need for a chromophore:

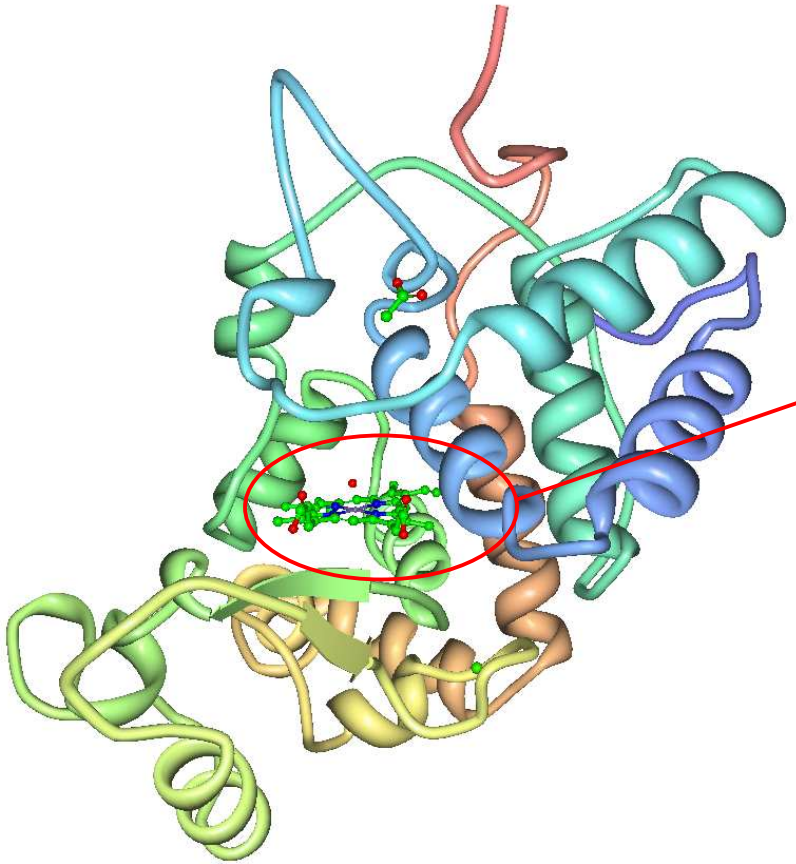


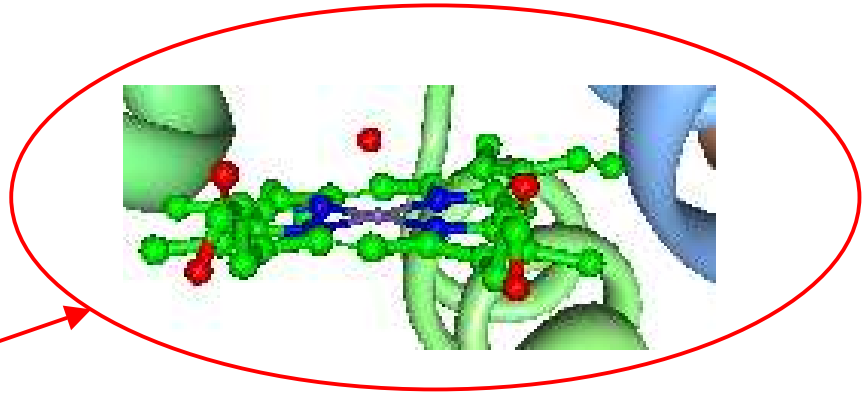
Infra-Red

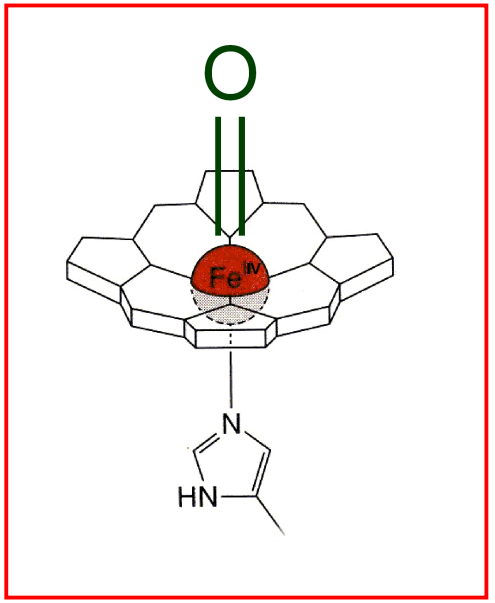


Raman Resonance

Identification of oxygen - iron bonds, Fe=O







?

Compound II of horseradish peroxidase
HRP II

Identification of Fe=O bonds by Resonance Raman

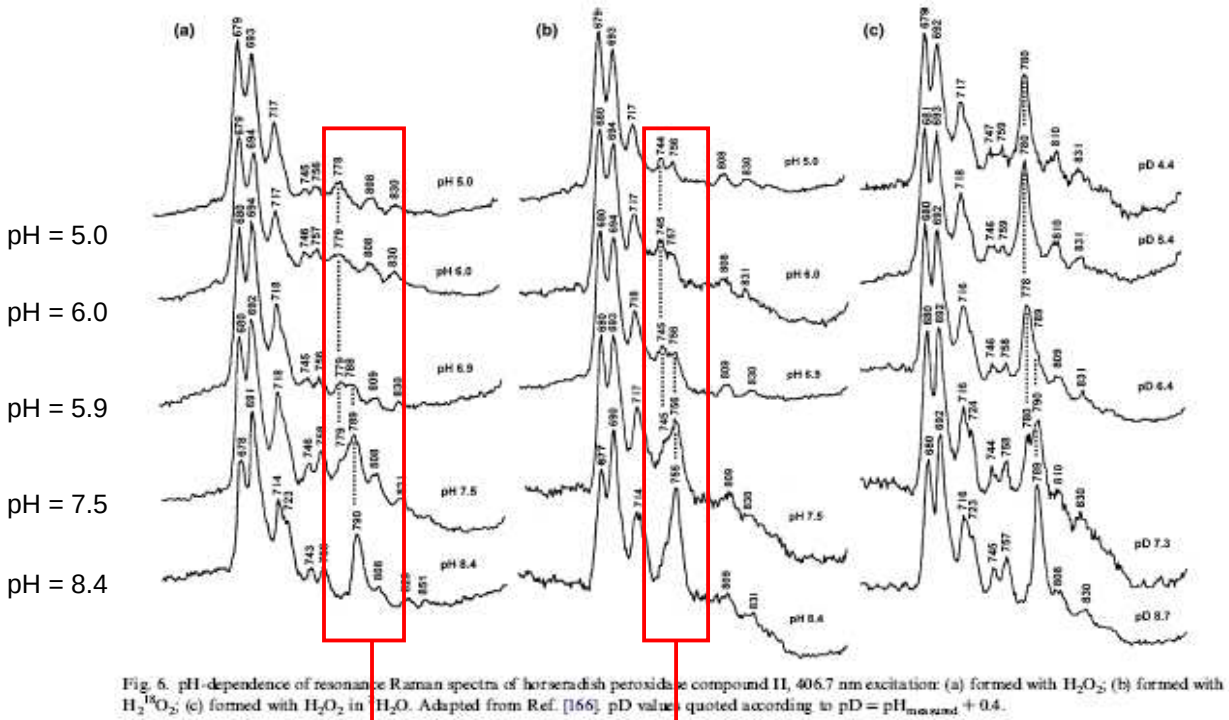
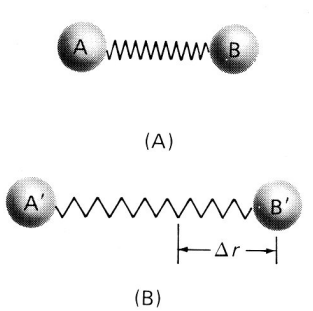


Fig. 6. pH-dependence of resonance Raman spectra of horseradish peroxidase compound II, 406.7 nm excitation: (a) formed with H_2O_2; (b) formed with $H_2{}^{18}O_2$; (c) formed with H_2O_2 in $^{18}H_2O$. Adapted from Ref. [166]. pD values quoted according to $pD = pH_{measured} + 0.4$.

$\nu_{Fe=O}$ = 779 cm^{-1} $\nu_{Fe=^{18}O}$ = 745 cm^{-1}

Compound II of horseradish peroxidase
HRP II



$$\Delta E = \left(\frac{h}{2\pi}\right)\left(\frac{k}{\mu}\right)^{1/2}$$

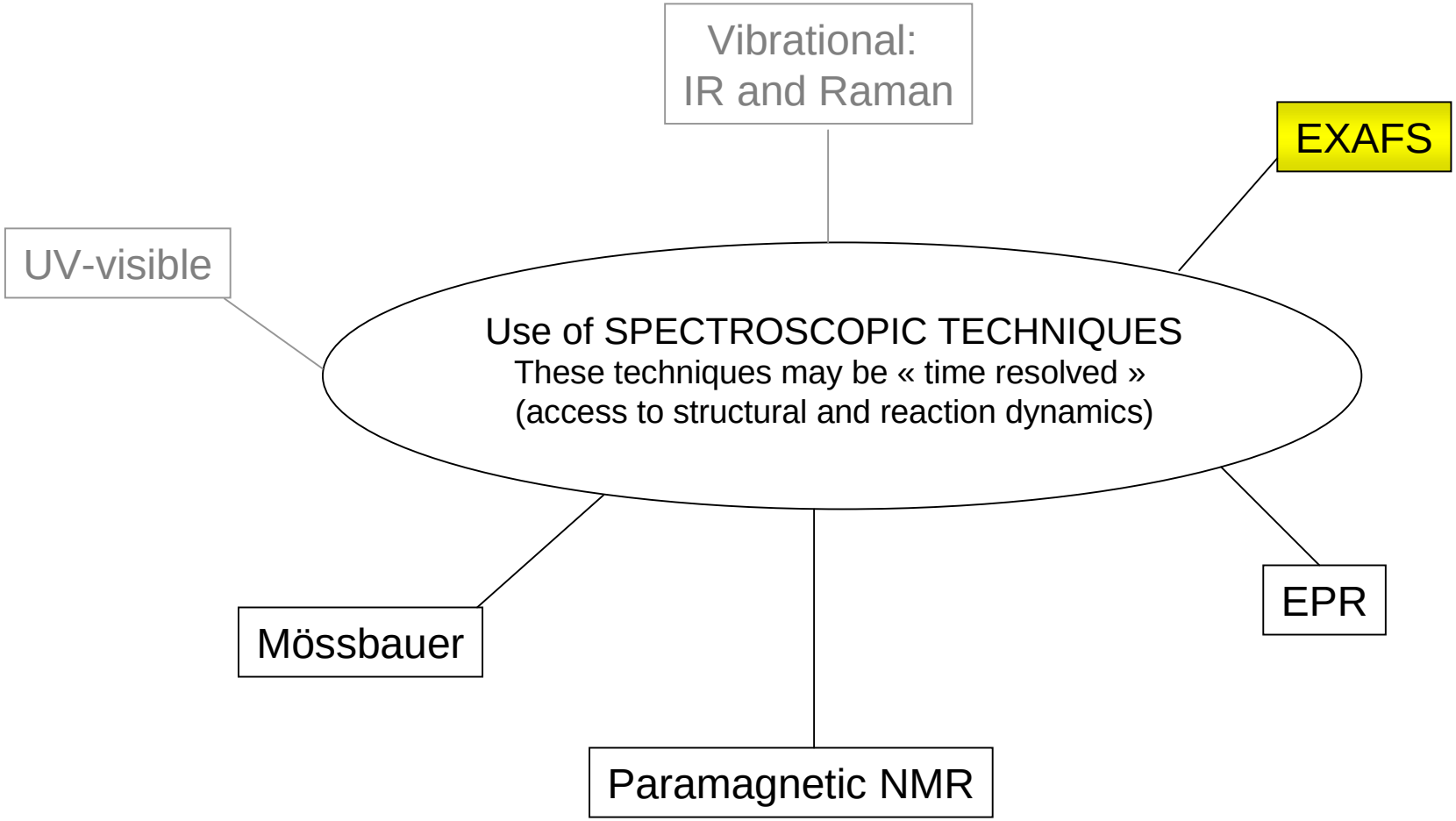
$$\mu = \frac{m_a m_b}{m_a + m_b}$$

$\Delta\nu_{Fe=^{16}O/Fe=^{18}O}$ = 34 cm^{-1}

Correct agreement with the predicted value:
(m_a= 56, m_{b1} = 16, m_{b2} = 18),

$\nu_1/\nu_2 = (\mu_2/\mu_1)^{1/2}$, = (56 x 18 x 72 / 74 x 16 x 56)$^{1/2}$

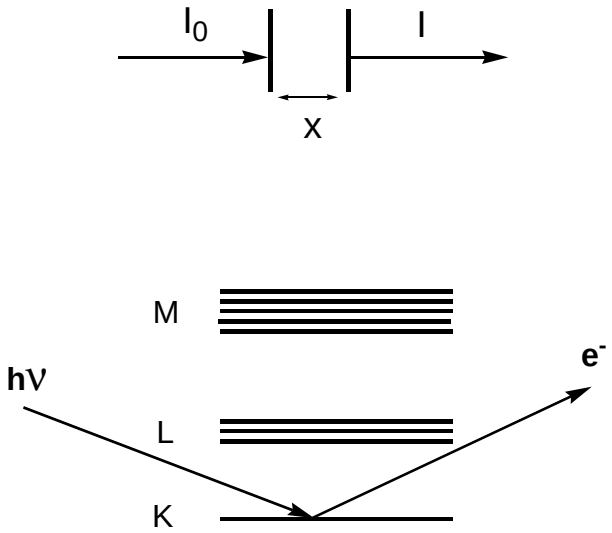
$\Rightarrow \nu_1 = 1.04\ \nu_2$

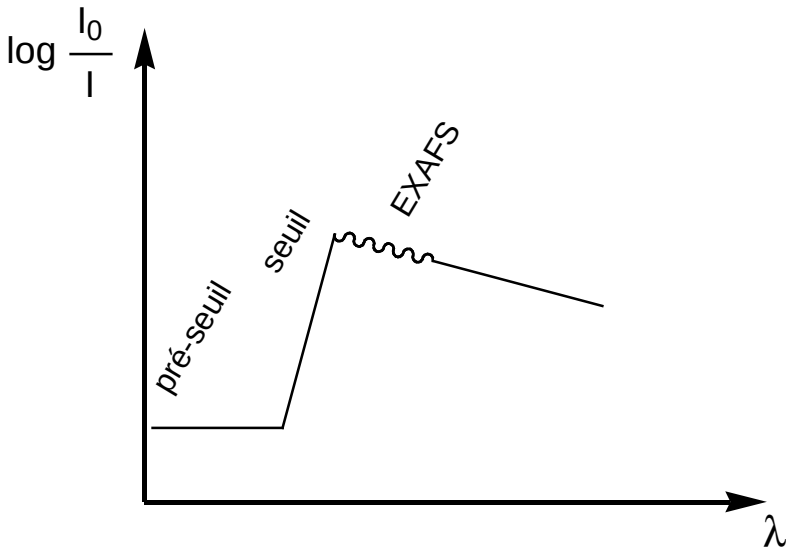

Vibrational:
IR and Raman
EXAFS
UV-visible
Use of SPECTROSCOPIC TECHNIQUES
These techniques may be « time resolved »
(access to structural and reaction dynamics)
Mössbauer
EPR
Paramagnetic NMR

X-rays absorption technique: EXAFS

Extended X-ray Absorption Fine Structure

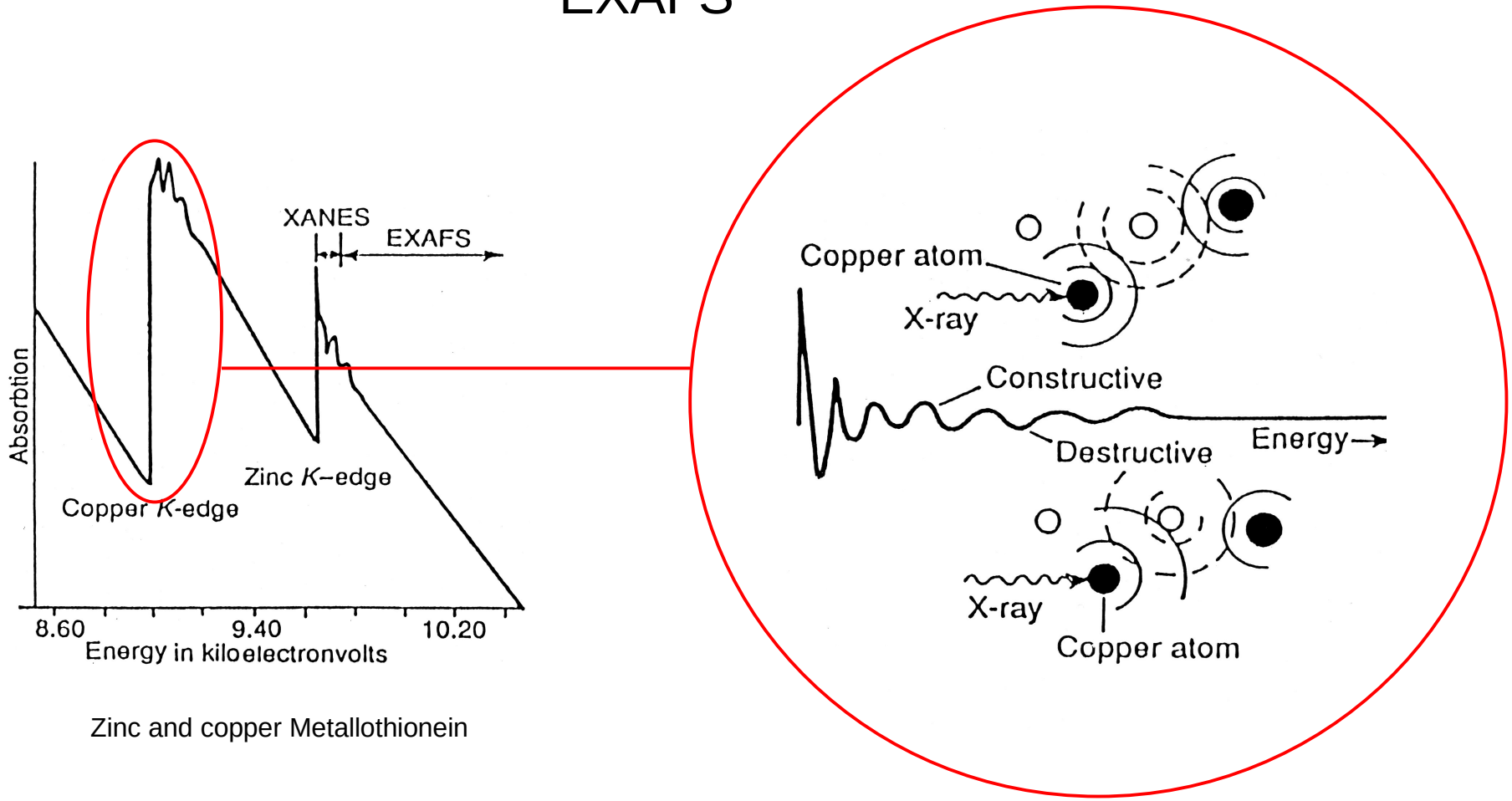
This technique is dedicated to **structural studies** in **case no single crystals** (for X-rays *diffraction*) can be obtained: no crystallization, or study of unstable transient. Samples in frozen solution, and detection in transmission or fluorescence.





It is a local method: the energy depends on the nucleus to be studied.
Need for an intense electronic beam: synchrotron

EXAFS



Zinc and copper Metallothionein

Irradiation yields so-called constructive and destructive oscillations, which patterns depend on the **oxidation state** (pre-edge energy), as well as the **environment** of the sudied nucleus.

EXAFS

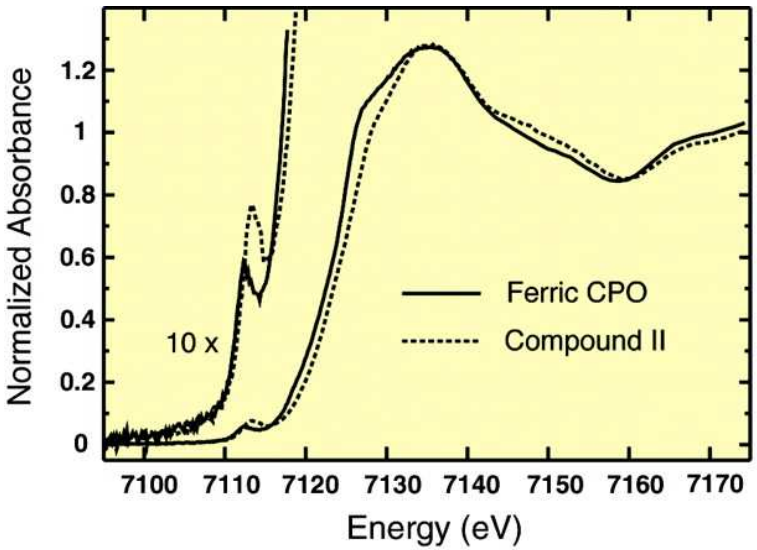
These oscillations yield an **interferogram** which can be simulated in order to get insight into the structural parameters corresponding to the first coordination sphere.

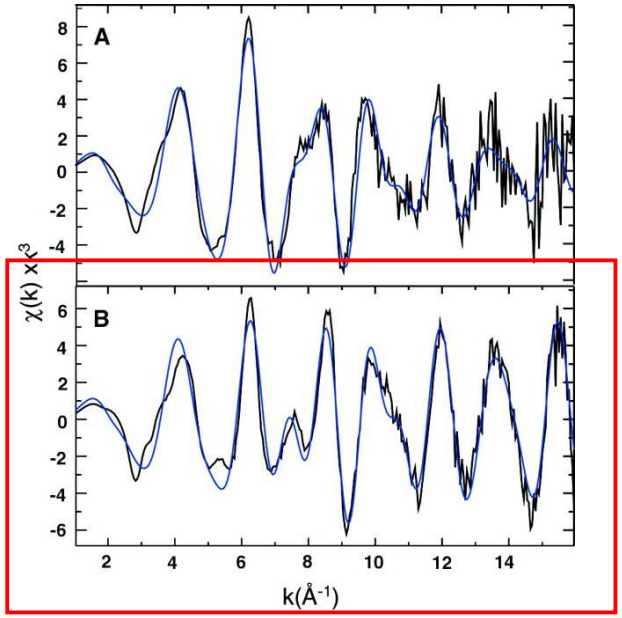
It is strongly recommended that these simulations use structural parameters obtained from perfectly characterized analogous compounds (or synthetic model analogues).

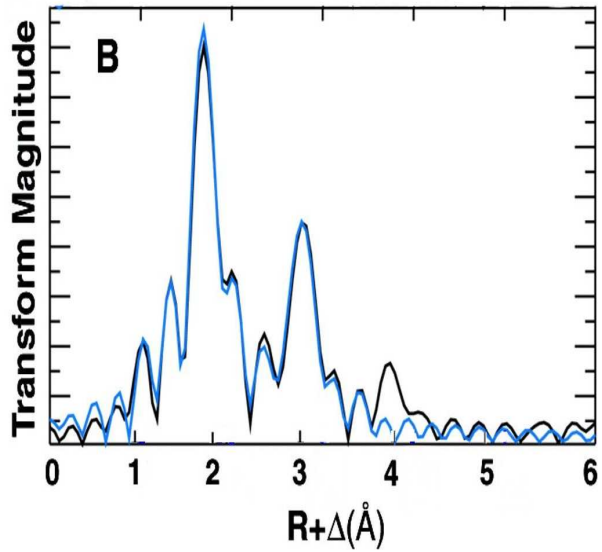
Number of ligands around the metal

Nucleus – ligand distance

Note: the oscillations parameters depend on the atomic number of the bound atom of the ligand, and the technique is not accurate enough to unequivocally distinguish nearby lying elements with similar distances, such as for instance, « N-metal» and « O-metal ».
Also, the angular data suffer from low accuracy.

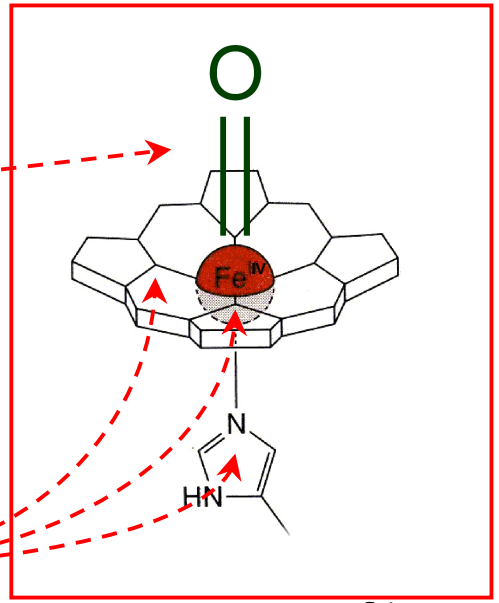


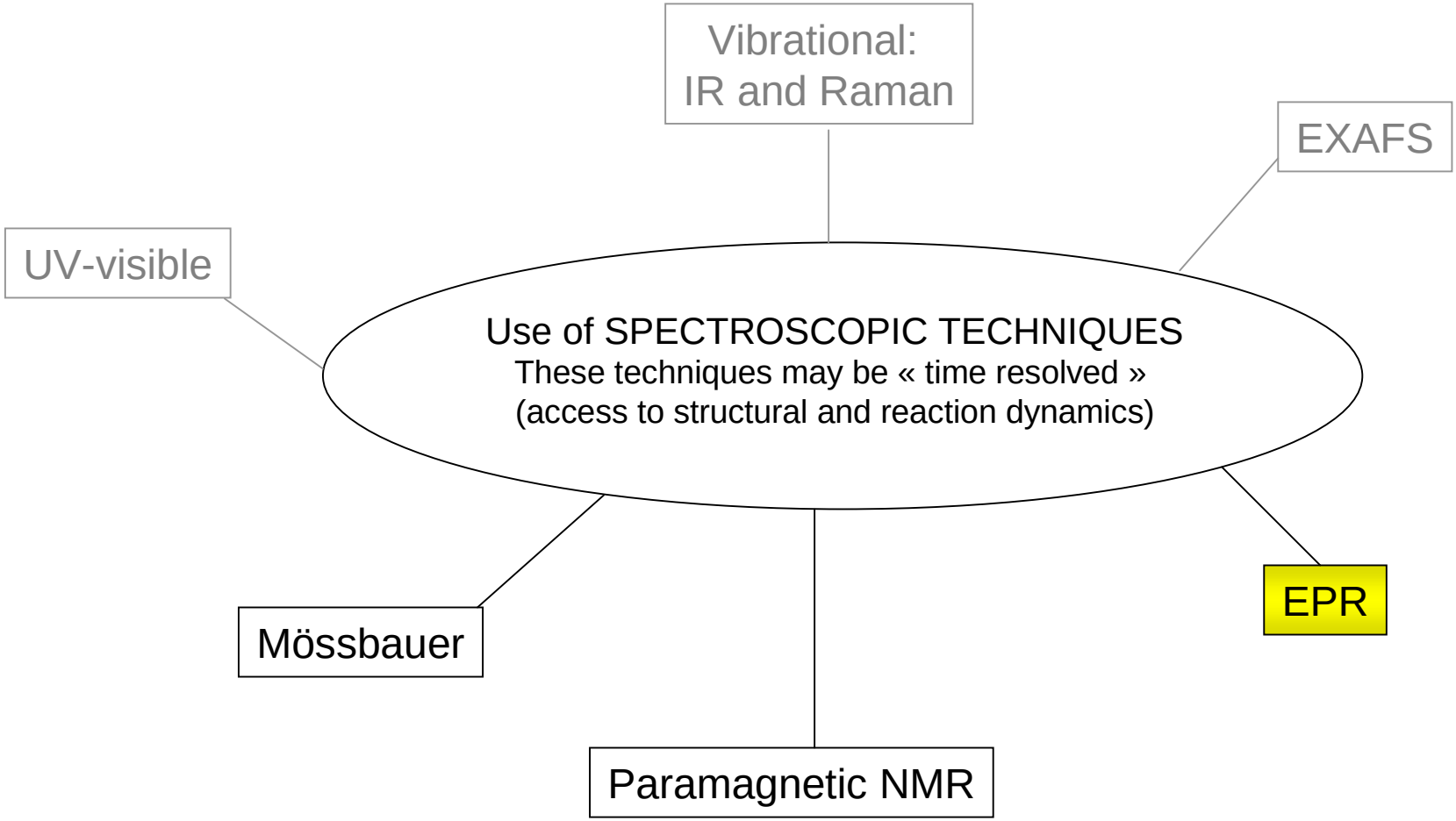




Fe-O		
N	*R*	σ^2
1	1.704 (10)	0.006 (1)
0		

Fe-N		
N	*R*	σ^2
5	1.991 (3)	0.002 (0)




Vibrational:
IR and Raman
EXAFS
UV-visible
Use of SPECTROSCOPIC TECHNIQUES
These techniques may be « time resolved »
(access to structural and reaction dynamics)
EPR
Mössbauer
Paramagnetic NMR

EPR: Electron Paramagnetic Resonance

or Electron Spin Resonance (ESR)

Detection of ***paramagnetic metal centres***

In *most of the cases*, an odd number of unpaired electrons is required

A sensitive technique: 10^{-6} à 10^{-7} molar solutions

Gas, powders, solutions, (single)crystals…

This technique is sensitive to the local symmetry around the metal

Principle of EPR

electronic spin / magnetic field *interaction*

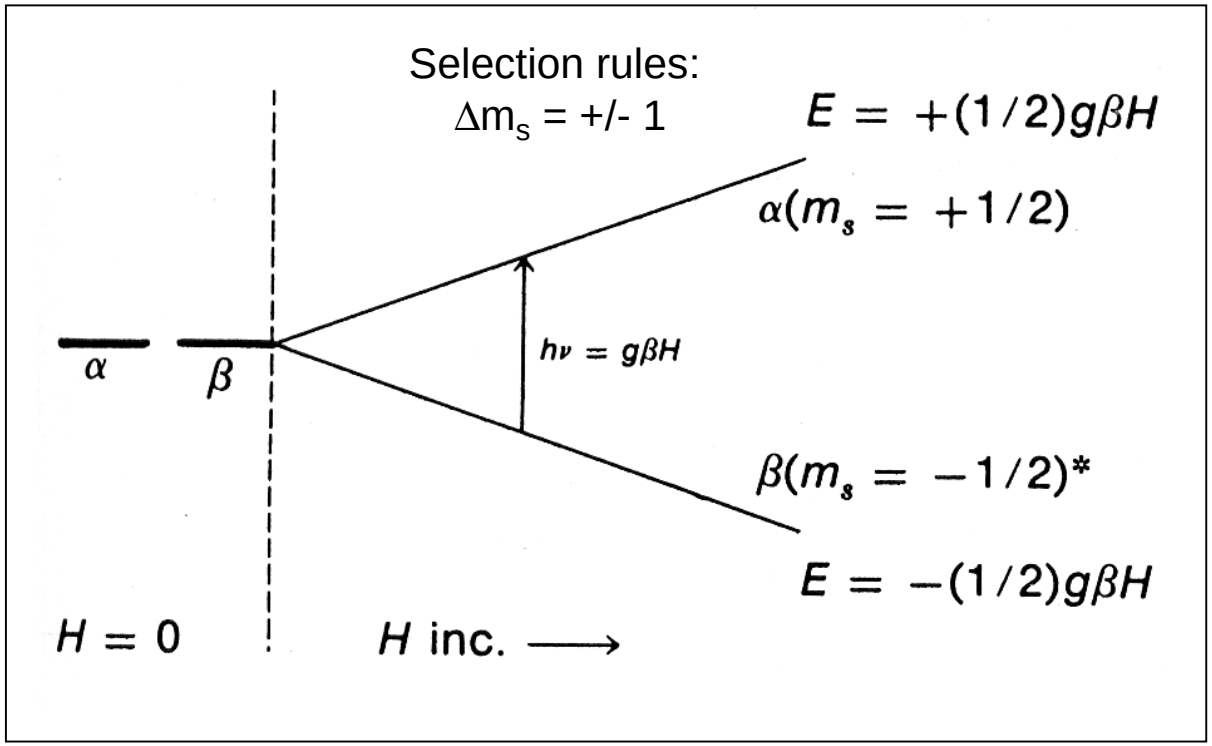
$$\hat{H} = g\mu_B H \hat{S}z$$

g = 2.0023193 (free electron)
β = μ_B = eħ/2m_ec = 9.274096 10^{-21} erg/G (Bohr magneton)
H = applied field
Ŝz = *spin operator*

$\hat{H}$ operates the electronic spin functions α and β corresponding to ms = + ½ et - ½

$$\Delta E = g\mu_B H$$

Electronic Zeeman effect:
degeneracy splitting of the α and β electronic spin states induced by a magnetic field

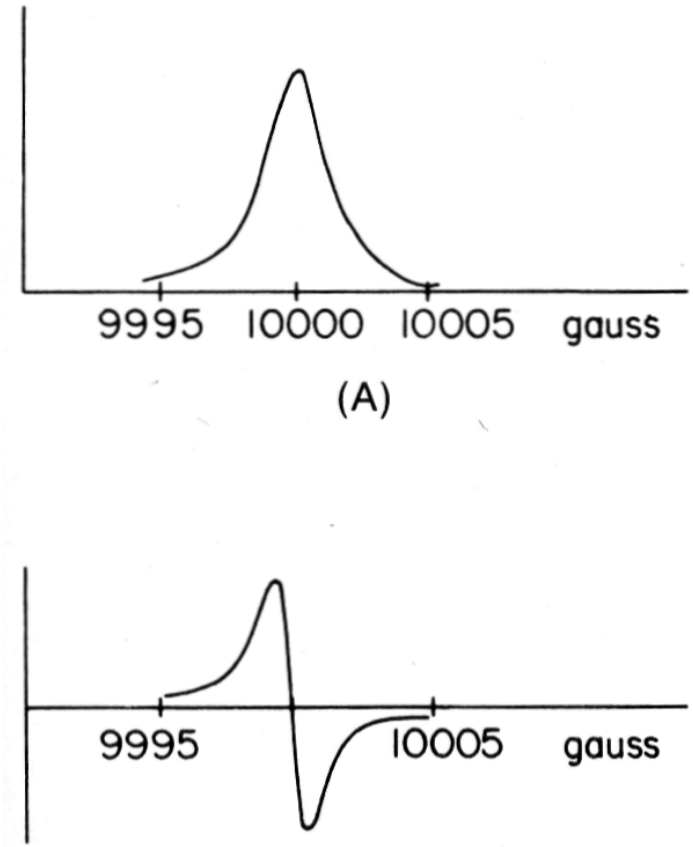


$$\nu_0 = \frac{g\beta H}{h}$$

$$H = \frac{h\nu_0}{g\beta}$$

$$g = \frac{h\nu_0}{H\beta}$$

For a given frequency ν, the field H is measured, yielding a **g value** different from that of the free electron, being in that case **characteristic of the studied system**.



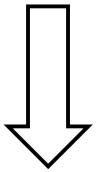
The spectrum is generally obtained as the derivated form of the absorbtion data

For a given frequency ν, the field H is measured, yielding a **g value** different from that of the free electron, being in that case **characteristic of the studied system**.

$$g = \frac{h\nu}{H\beta}$$

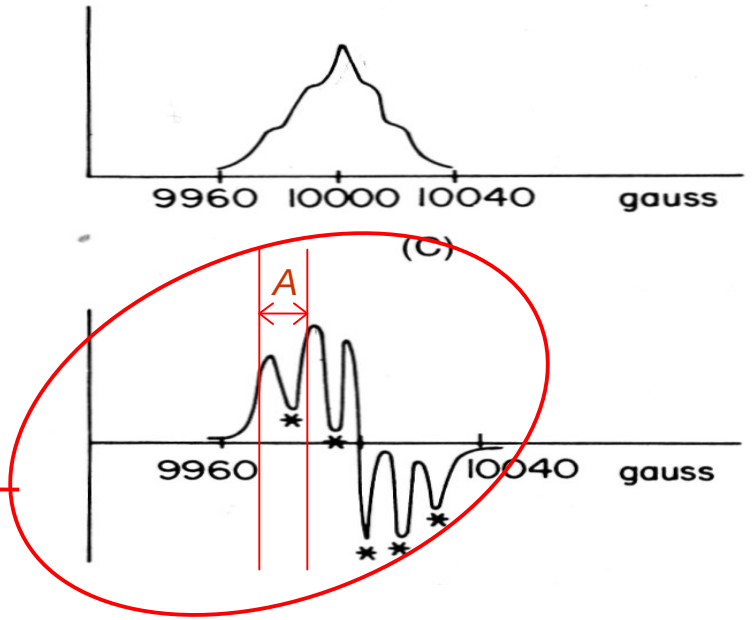
First subtility: there may be an interaction between the **electronic spin** and a **nuclear spin *I***: for ^{1}H, $I = ½$; for ^{14}N, $I = 1$; for $^{63,\ 65}Cu$, $I = 3/2$.

The nuclear spin I can adopt $2I + 1$ different orientations => $2I + 1$ different contributions

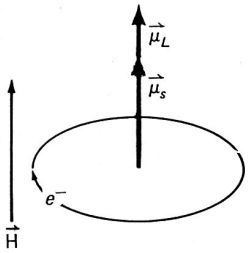


Hyperfine Structure, A = hyperfine coupling constant

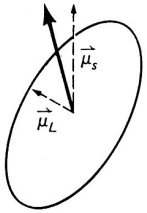
$H_3C^{\bullet}$ radical: 4 lines, N-R-N$^{\bullet}$: 5 lines, $Cu^{(II)}$: 4 lines



Second subtility: anisotropy. Affects both g values and the hyperfine coupling



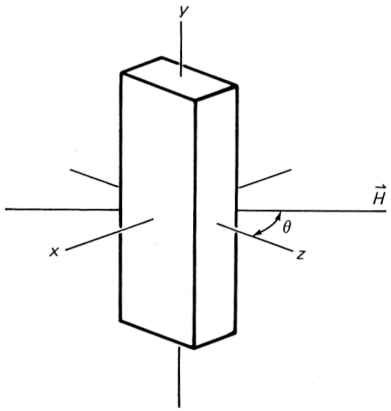
(A)

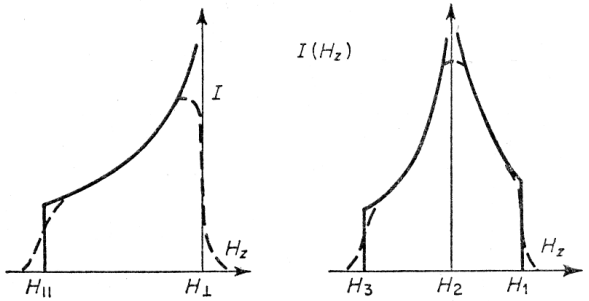


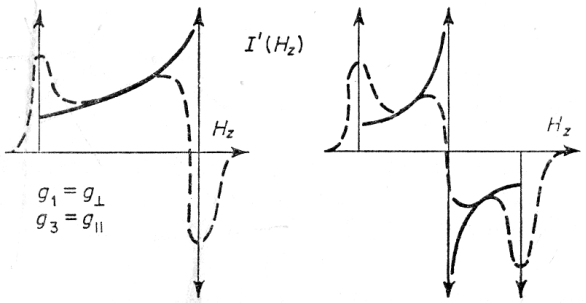
(B)

Coupling of the projections of the spin and orbital angular momentum for two different molecular orientations relative to the applied field *H*.

Mounting of a crystal for rotation in the *xz*-plane.

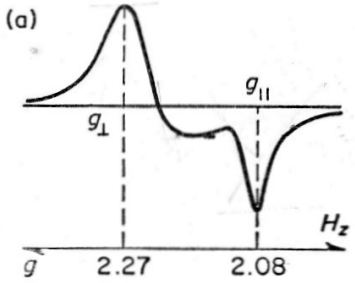


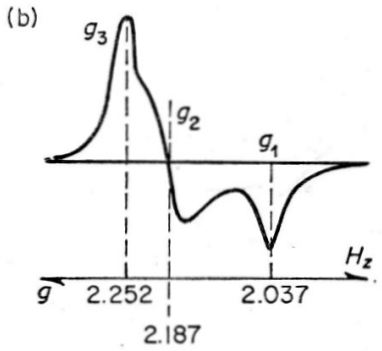




$g_1 = g_2 < g_3$

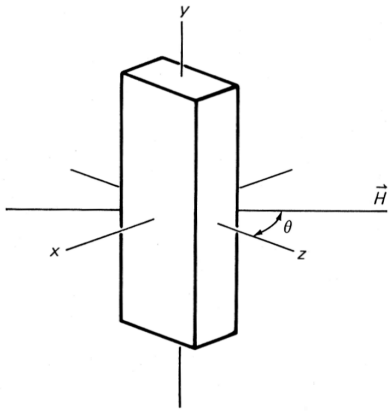
$g_1 < g_2 < g_3$

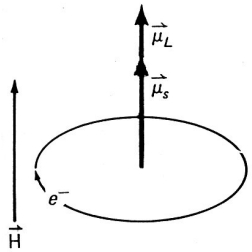




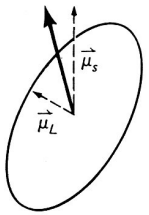
Second subtility: anisotropy. Affects both g values and the hyperfine coupling

Mounting of a crystal for rotation in the *xz*-plane.



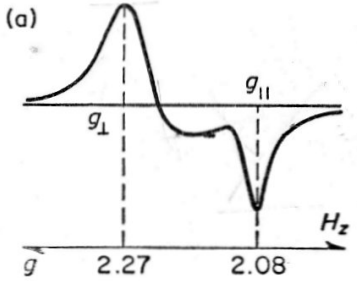


(A)

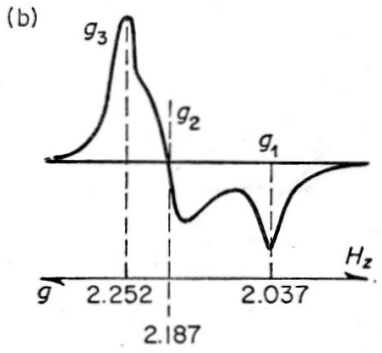


(B)

Coupling of the projections of the spin and orbital angular momentum for two different molecular orientations relative to the applied field *H*.

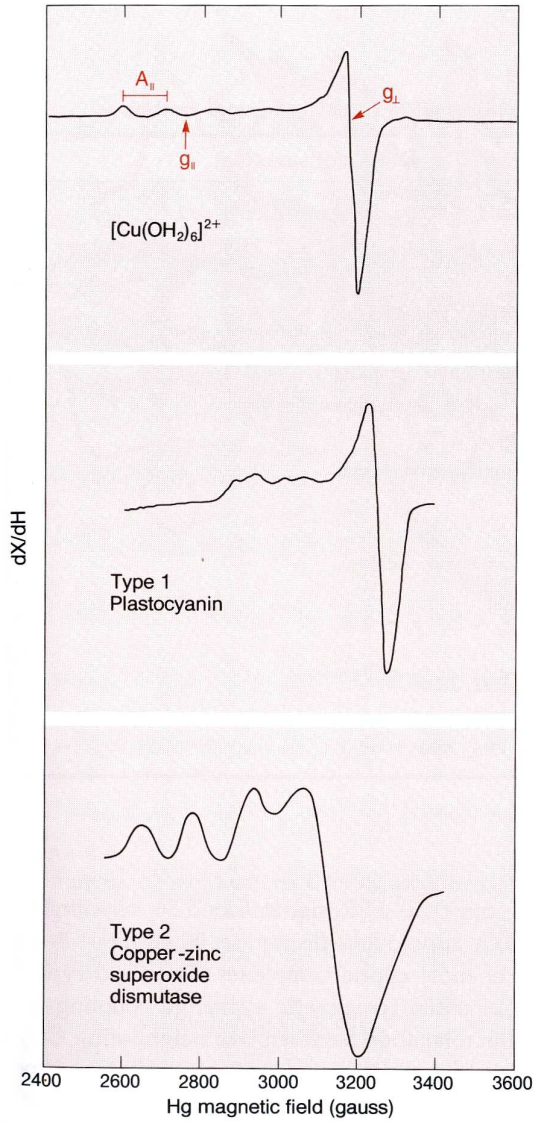


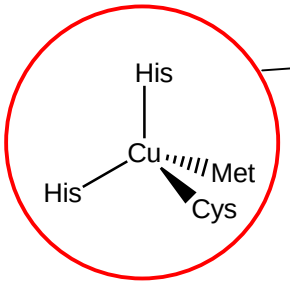
x = z ≠ y axial

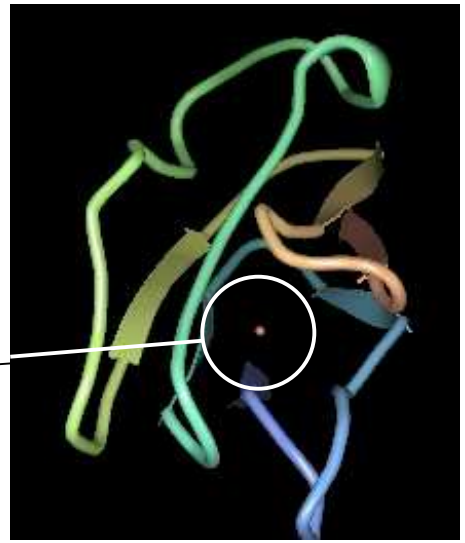


x ≠ z ≠ y rhombic

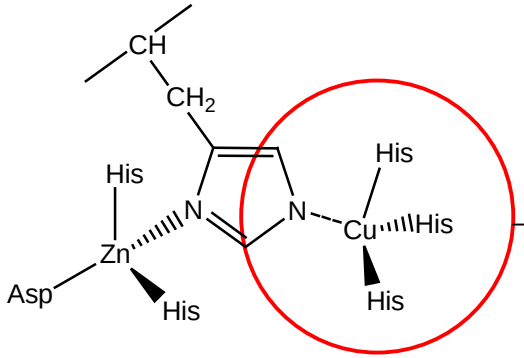
A suitable technique for copper-containing proteins

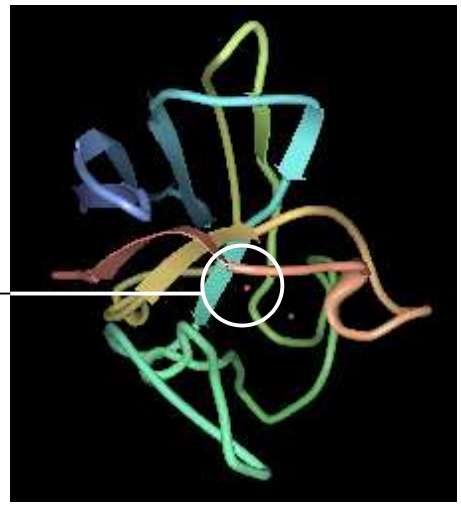




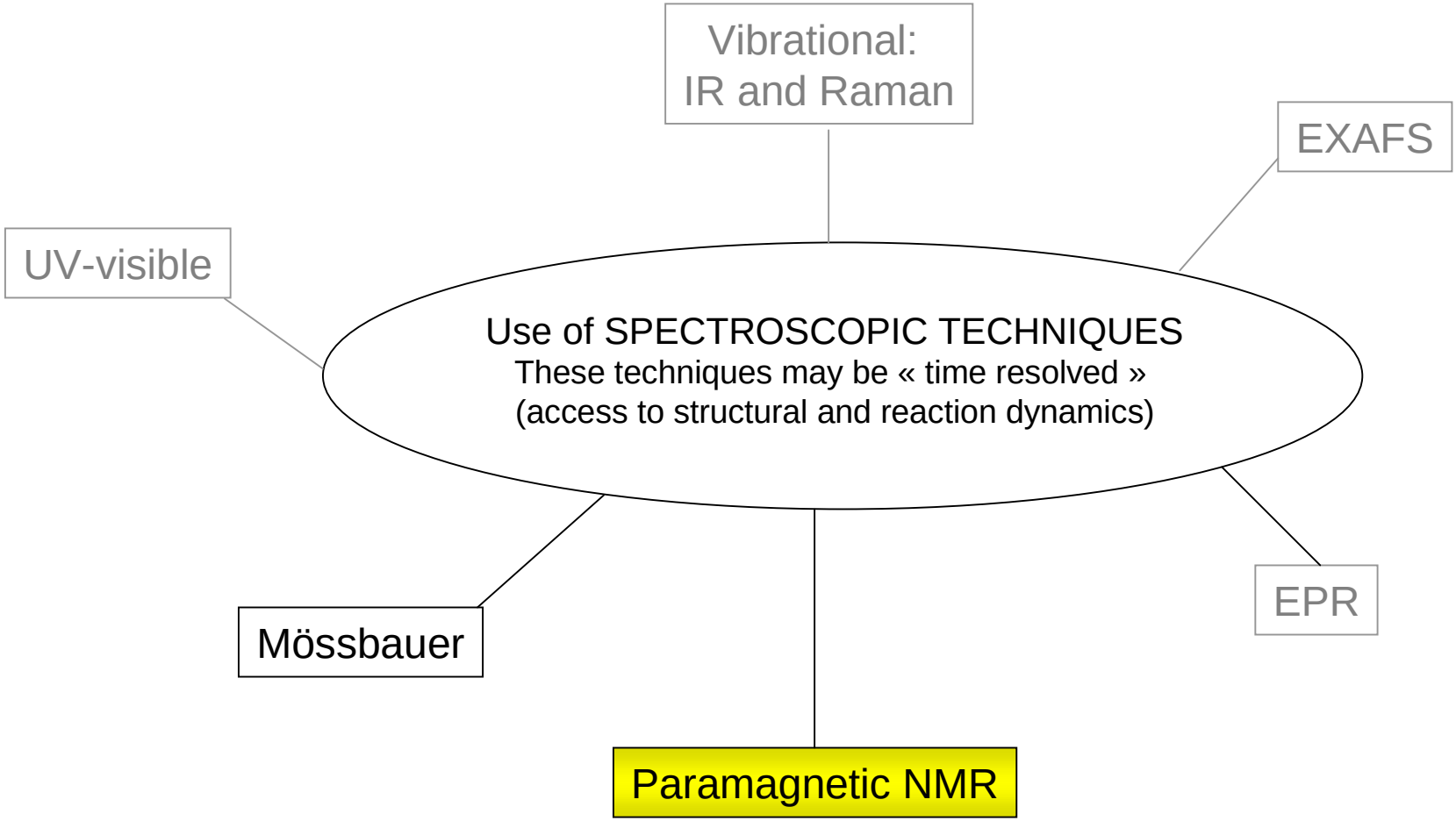


Plastocyanin





Superoxide
dismutase


Vibrational:
IR and Raman
EXAFS
UV-visible
Use of SPECTROSCOPIC TECHNIQUES
These techniques may be « time resolved »
(access to structural and reaction dynamics)
EPR
Mössbauer
Paramagnetic NMR

Paramagnetic NMR: nuclear spin / electronic spin interaction

Access to the close environment of the paramagnetic centre
Paramagnetic active sites

Electronic relaxation times must be short: $10^{10} < \tau_s^{-1} < 10^{12}$ s^{-1}
Depends on the metal ion

Technique perfectly suited to iron sites.
Well established for ^{1}H. Current development for ^{13}C and ^{15}N

Paramagnetic NMR

By comparison with conventional NMR, main differences in **chemical shifts** and **line width**

These differences are function of the « paramagnetism » of the molecule (HS / LS).
The magnetic susceptibililty in solution can be measured by ^{1}H NMR (Evans method)
=> Acces to the spin state of a paramagnetic metal ion.

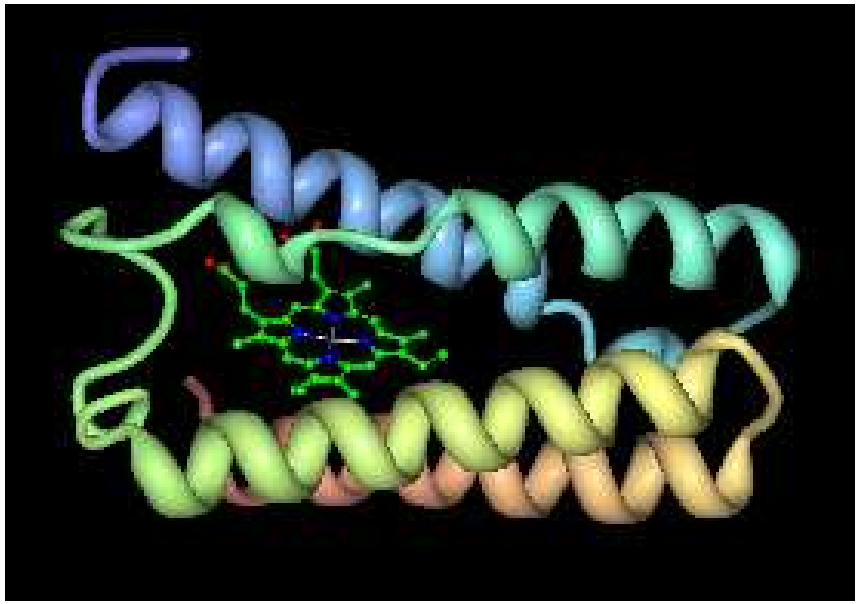
Once the magnetic properties of the metal centre are known, the measurement of the chemical shifts and relaxation times allows insights into the structural parameters of the active site, in particular the distance between the paramagnetic centre and the nucleus which signal is measured.

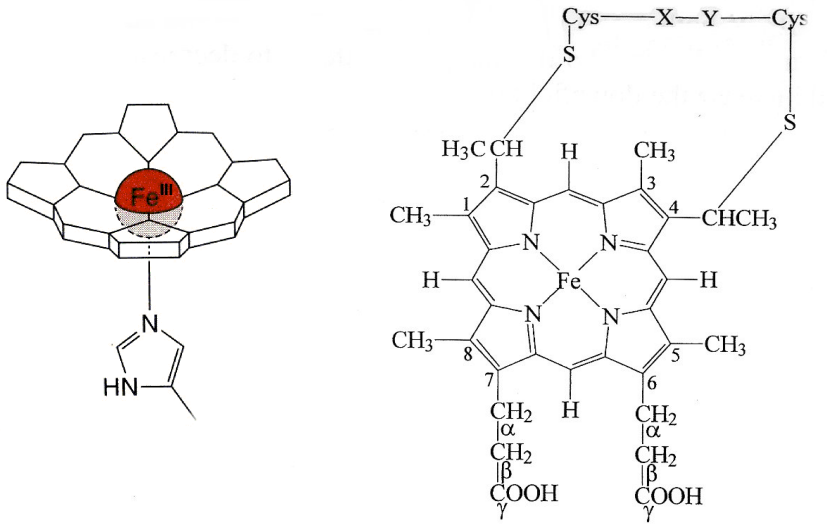
Contact interaction

Dipolar interaction

Paramagnetic ^{1}H NMR

Cytochrome c', Fe(III) S = 5/2. Five-coordinate, His. Broad lines





Schematic drawing of the heme moiety in cytochromes *c'*

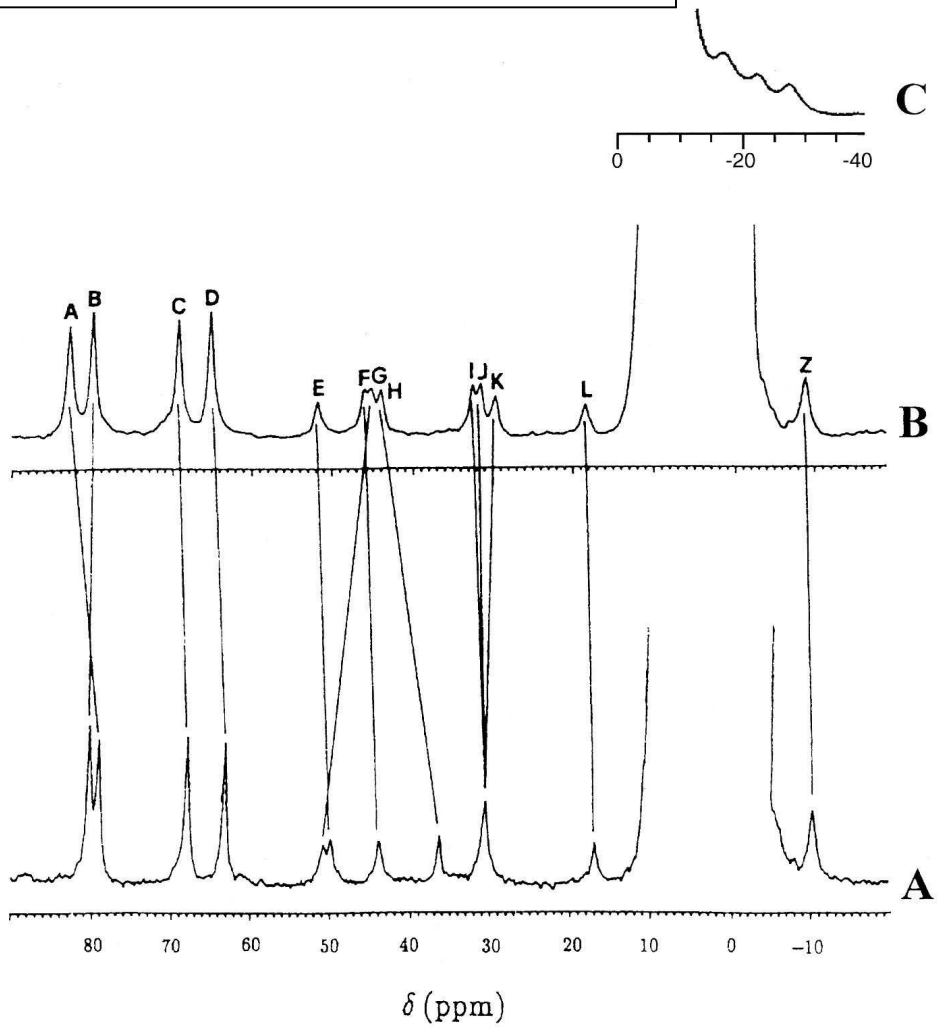


Fig. 5.12. 600 MHz ^{1}H NMR spectra of a five-coordinated iron(III) porphyrin, ferricytochrome *c'* from *R. gelatinosus* at 300 K in H_2O at (A) pH 7.4 and (B) pH 4.0 (adapted from [27]). Signals have been assigned: A, B, C, D, 1-, 8-, 5- 3-CH_3; E, 6-CHα′; F, 4-CHα; G, 7-CHα′; H, 7-CHα; I, 6-CHα; J, His β; K, His β′; L, 2-CHα. The upfield signals belonging to *meso* protons of cytochrome *c'* from *R. palustris*, at pH 5, are shown in (C) [23] (labeling as in Fig. 5.7B).

Paramagnetic ^{1}H NMR

Cytochrome c, Fe(III) S = ½. Six-coordinate, His + Met. Narrow lines

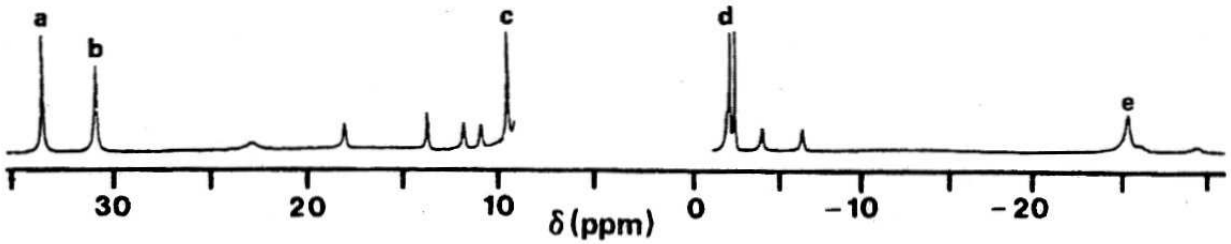
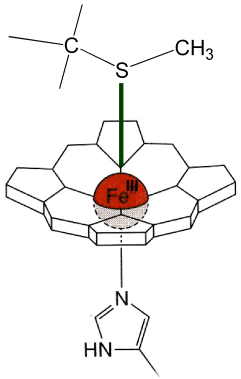
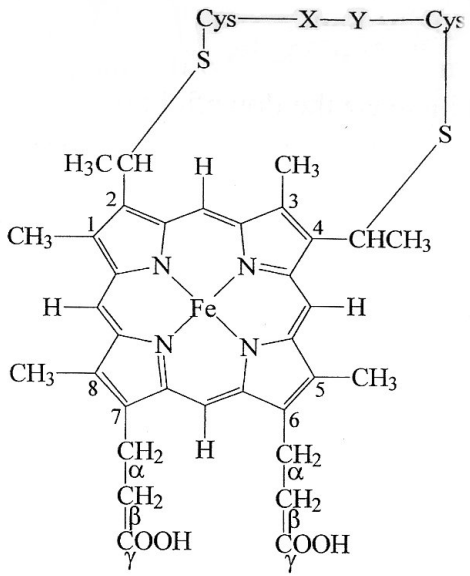
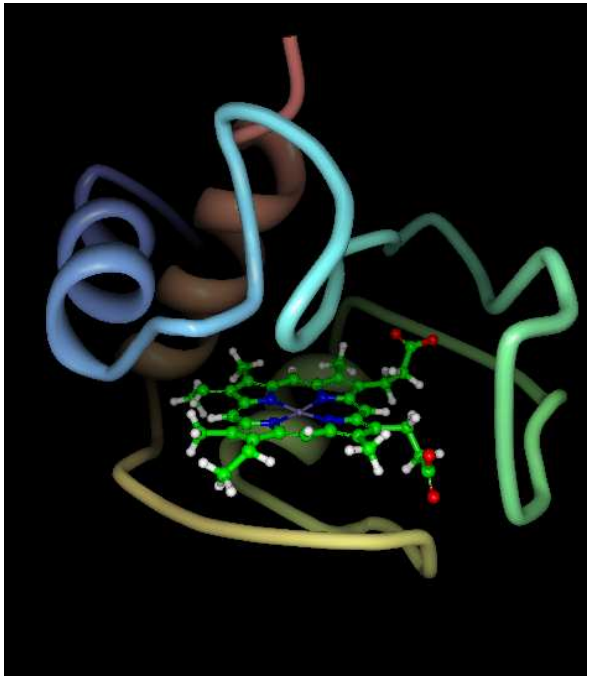
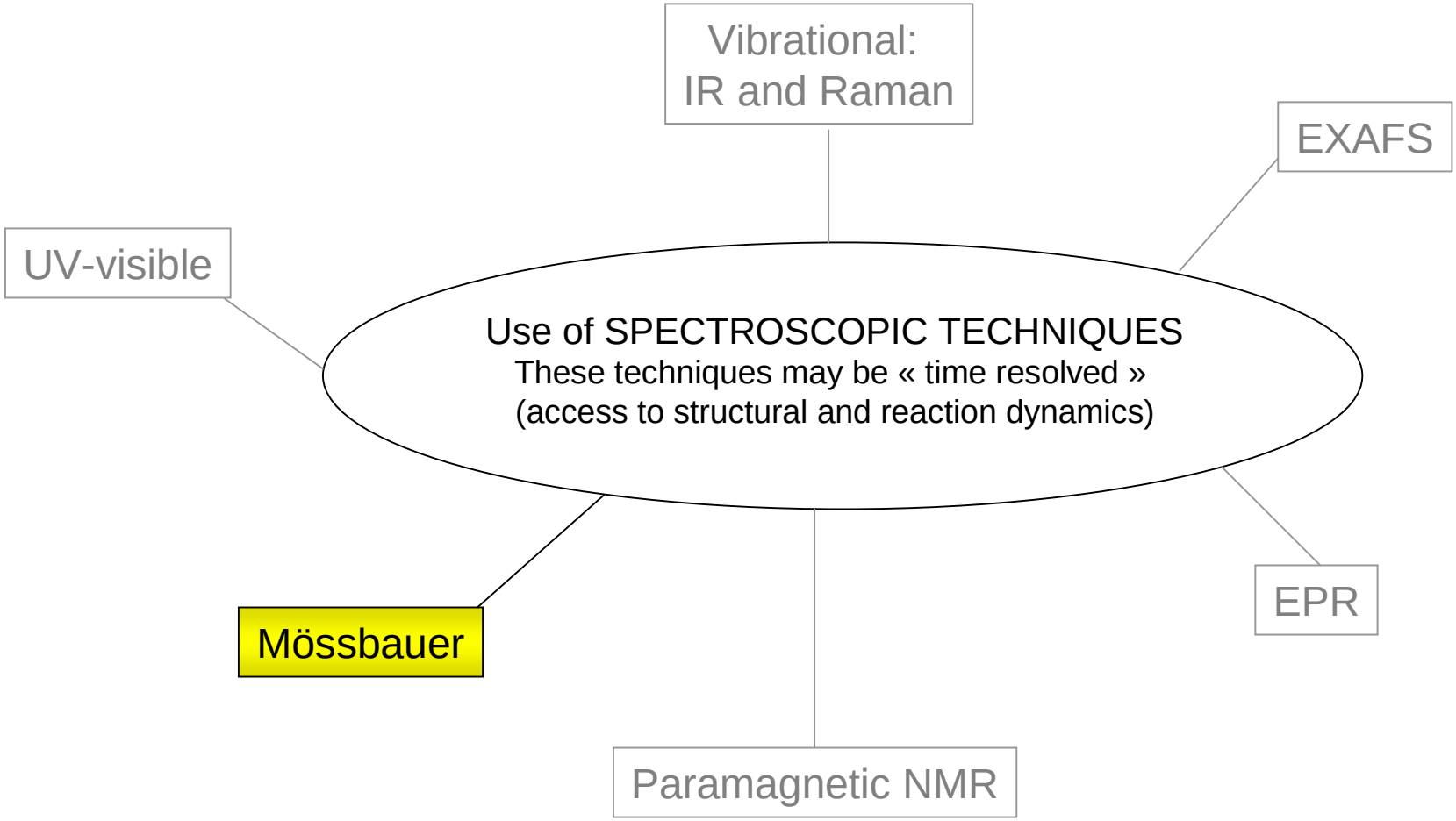


Fig. 5.18. 360 MHz ^{1}H NMR spectra of oxidized horse heart cytochrome *c*. The labeled signals are assigned to: a = 8-CH_3, b = 3-CH_3, c = 5-CH_3, d = thioether bridge 2-CH_3, e = axial methionine S-CH_3; the resonances at 7.4 ppm (1-CH_3) and 3.1 ppm (thioether bridge 4-CH_3) are not shown.







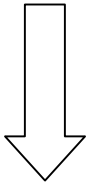

Vibrational:
IR and Raman
EXAFS
UV-visible
Use of SPECTROSCOPIC TECHNIQUES
These techniques may be « time resolved »
(access to structural and reaction dynamics)
Mössbauer
EPR
Paramagnetic NMR

Mössbauer Spectroscopy

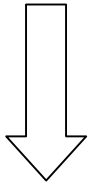
Recoiless absorption of γ rays by ^{57}Fe (also valid for 43 other elements of the periodic table)

Need for γ rays source, and enrichment with ^{57}Fe isotope
(Natural abundance of ^{57}Fe: 2.19%)
Reconstitution of a ^{57}Fe-containing protein from apoprotein and ^{57}Fe salts.
Isolation of microorganisms from a ^{57}Fe-enriched growing medium

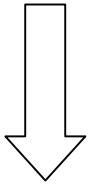
Access to the oxidation sate of the metal, electronic environment and magnetic properties



Isomer Shift

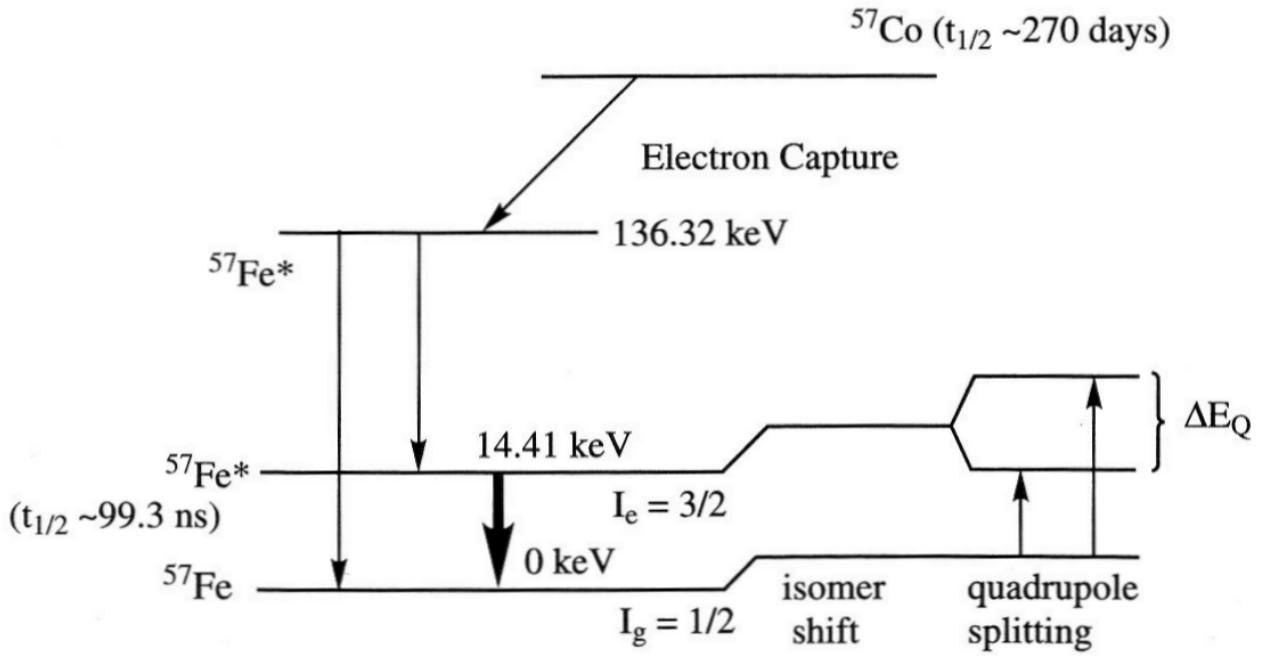


Quadrupole splitting



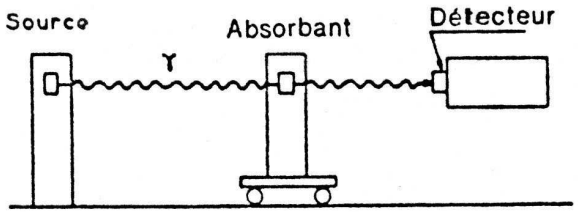
Magnetic properties

Iradiation of metal iron by γ rays => excited state.
Upon return to fundamental state, 14.4 KeV are released.



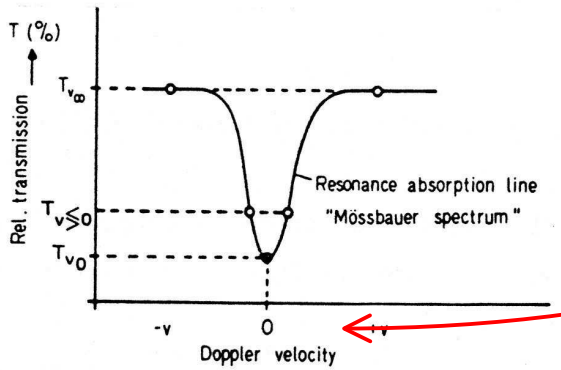
When iron is complexed, this energy vary, and depends on the oxidation state and coordination environment of the iron site.
=> Modulation of the γ rays energy. This is accomplished by Doppler effect

Historical setup



de 14 keV du $^{57}Fe^*$

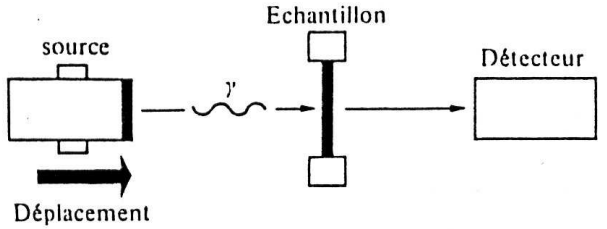
La source émet, par exemple, les rayons γ qui traversent un absorbant contenant le ^{57}Fe et les photons de 14 keV transmis par l'absorbant sont comptés à l'aide d'un détecteur de rayons X ou γ.
On déplace l'absorbant par rapport à la source et l'on enregistre les photons γ transmis en fonction de la vitesse Doppler. On obtient ainsi une raie de résonance [1]:



δ, mm.s^{-1}

Le spectre Mössbauer est représenté par la variation du taux de comptage en fonction d'une vitesse v.

Current setup



δ: electronic density at the nucleus site.

δ depends on the nucleus radius changes between fundamental and excites states.

$$\delta \propto \left(\frac{\Delta R}{R}\right)\left(\Delta\psi_{(0)}\right)^2$$

$\psi_{(0)}$

Zero coordinate: s electrons

$$\left(\frac{\Delta R}{R}\right) < 0$$

For Iron

Modification of d orbitals: induces modification of s orbitals
δ depends on the oxidation state, and spin state

ΔE_Q: present only for lower symmetries than cubic.
Electric Field Gradiant (EFG) at the nucleus site.
ΔE_Q depends on the electronic asymmetry around the nucleus.
=> Electrons involved between the ligands and the iron centre.

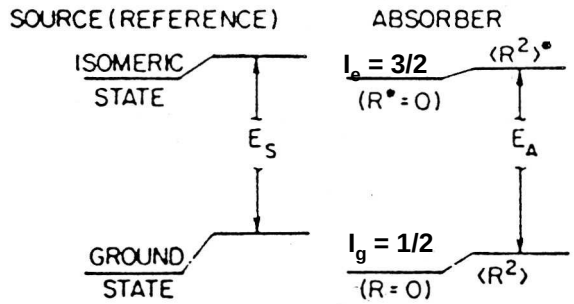
$$EFG \propto \frac{1}{r^3}$$

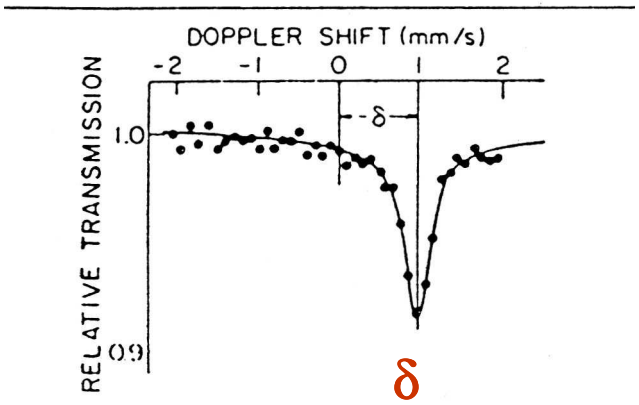
Isomer shift

δ

Energy released by Exc. => Fund.

Oxidation State





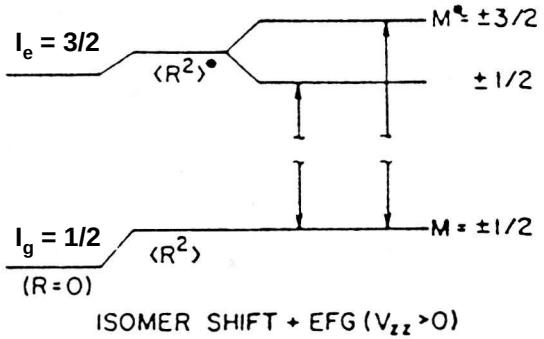
δ

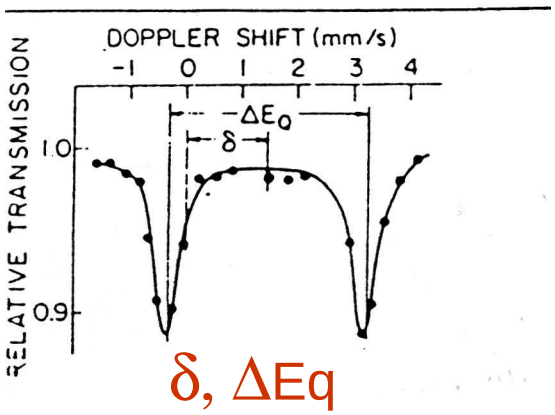
Quadrupole splitting

ΔEq

Electric Field Gradiant

Metal- ligand interaction

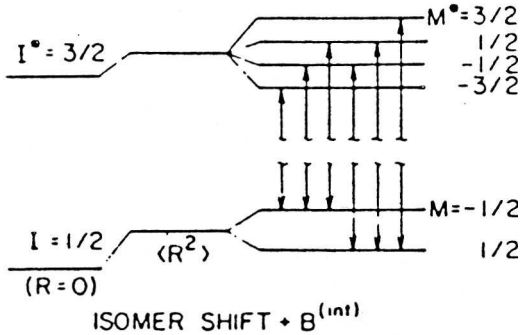


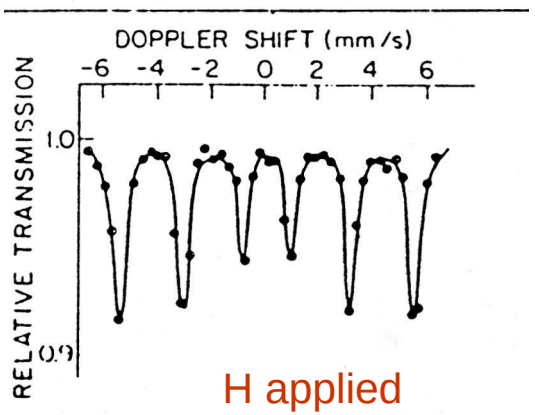


δ, ΔEq

Hyperfine Magnetic Interaction

Internal or external magnetic interaction

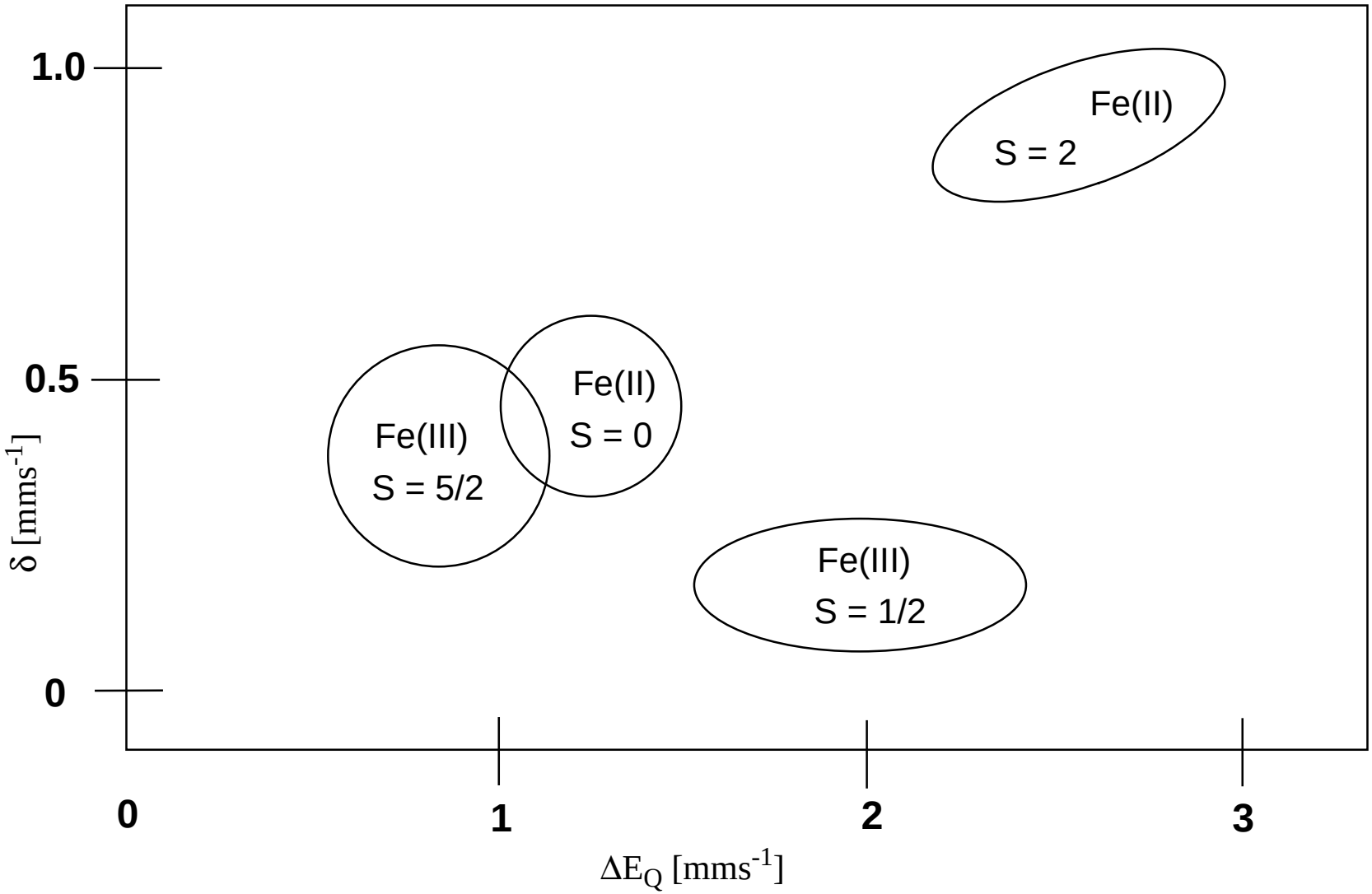




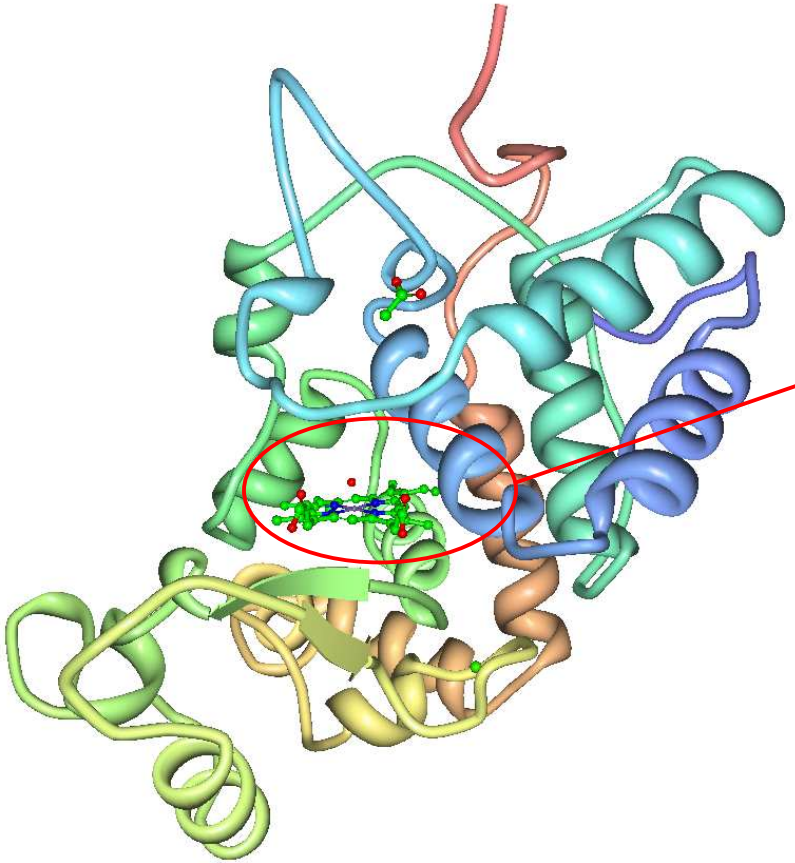
H applied

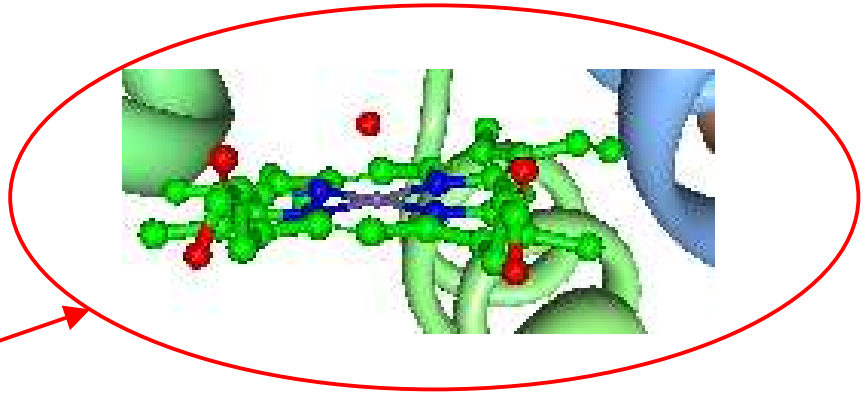
(ou B)

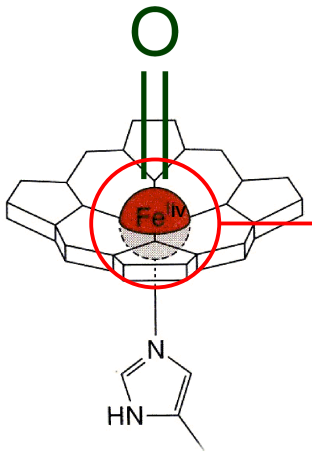
Tentative prediction of the oxidation and spin states of iron-containing centres



Oxidation state of iron: Fe – oxygen, Fe=O







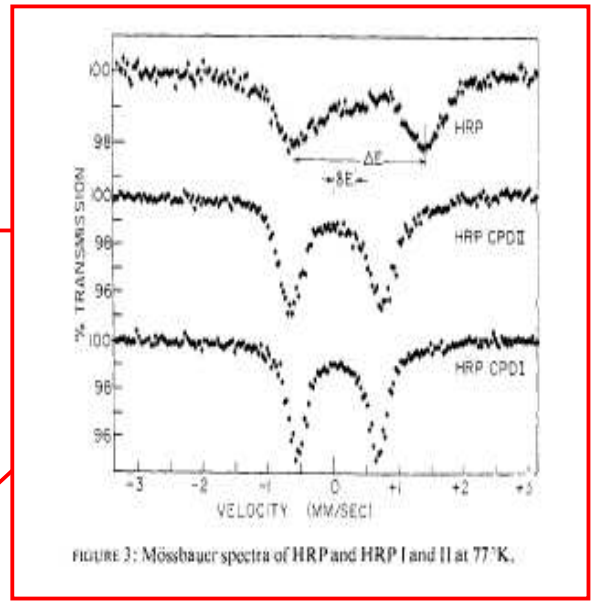


FIGURE 3: Mössbauer spectra of HRP and HRP I and II at 77°K.

Compound II of Horseradish peroxidase
HRP II

$\delta = 0.03$ mm.s^{-1}, $\Delta eq = 1.36$ mm.s^{-1} → $Fe^{(IV)}$: [Ar], $3d^4$

Evidence of the presence (detection) of metals

Very small amounts of metals in biological systems.

i.e. human hemoglobin uses 0.35 % only of available iron in body