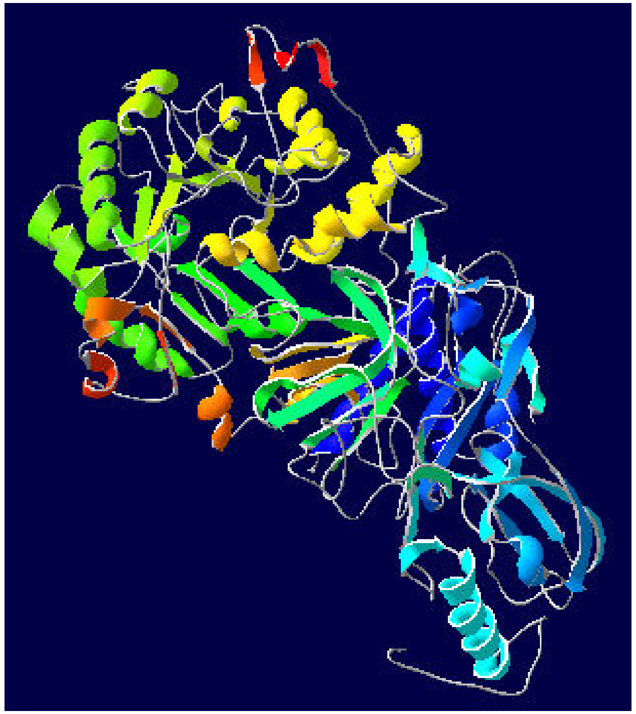
Chapter 2 – Techniques for Bioinorganic Studies

- Spectroscopic Methods
    - UV-vis
    - Mössbauer
    - EPR
- Methods which use DNA
    - DNA Interaction studies: Fluorescence, Viscosimetry, DNA *Melting* Temperature
    - DNA Cleavage: Electrophoresis, AFM
- Others: Cyclic Voltammetry

Techniques

- The structure of metal coordination environments has been determined mainly by X-ray diffraction (now mostly using a synchrotron, that is, high intensity X-rays) and sometimes by NMR (for low-PM proteins).
- Other methods (see later) are useful for identifying ligands, oxidation states,....



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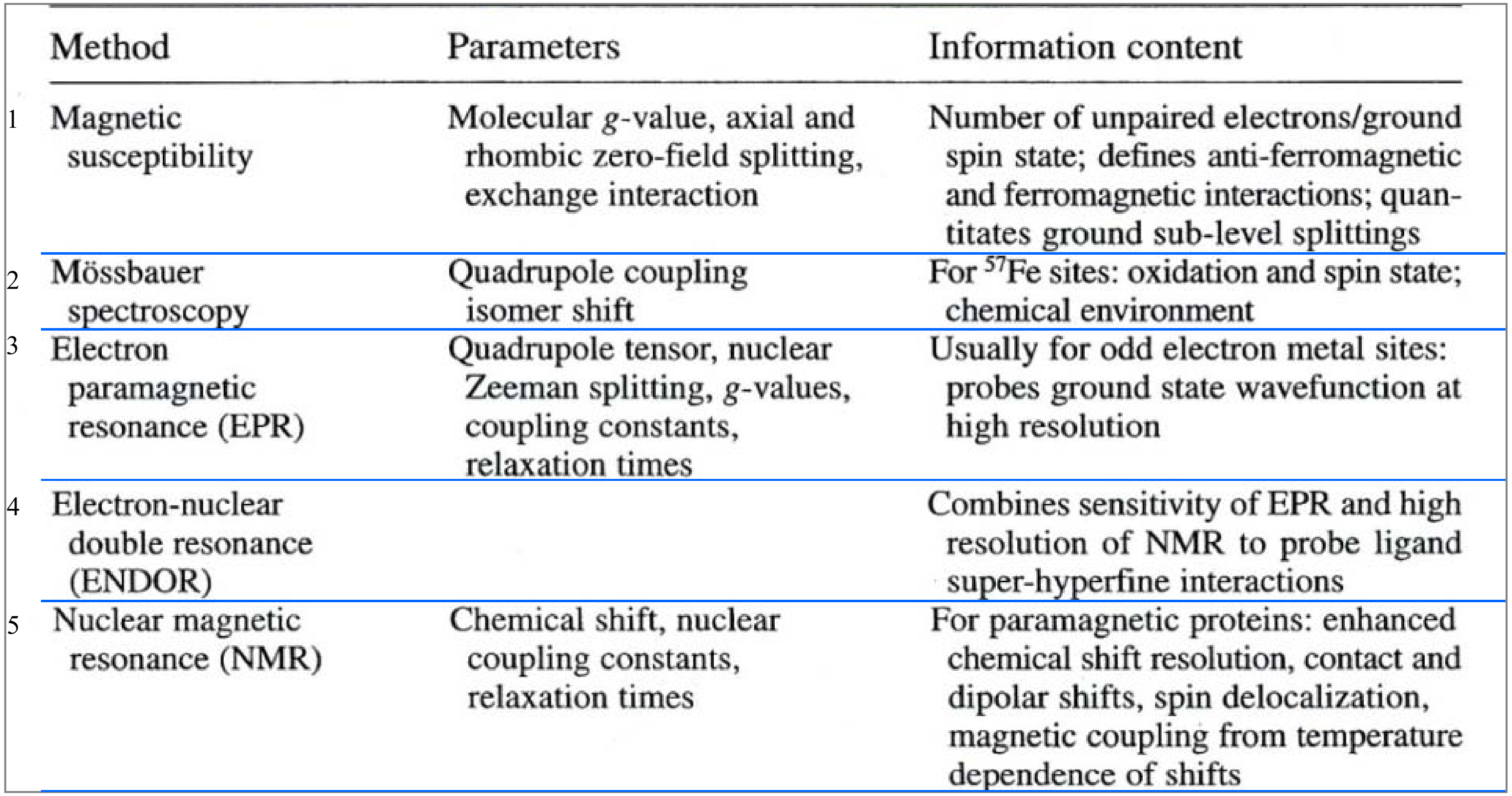
File:Urease-1E9Z.jpg

Crystal structure of

Helicobacter pylori **urease**

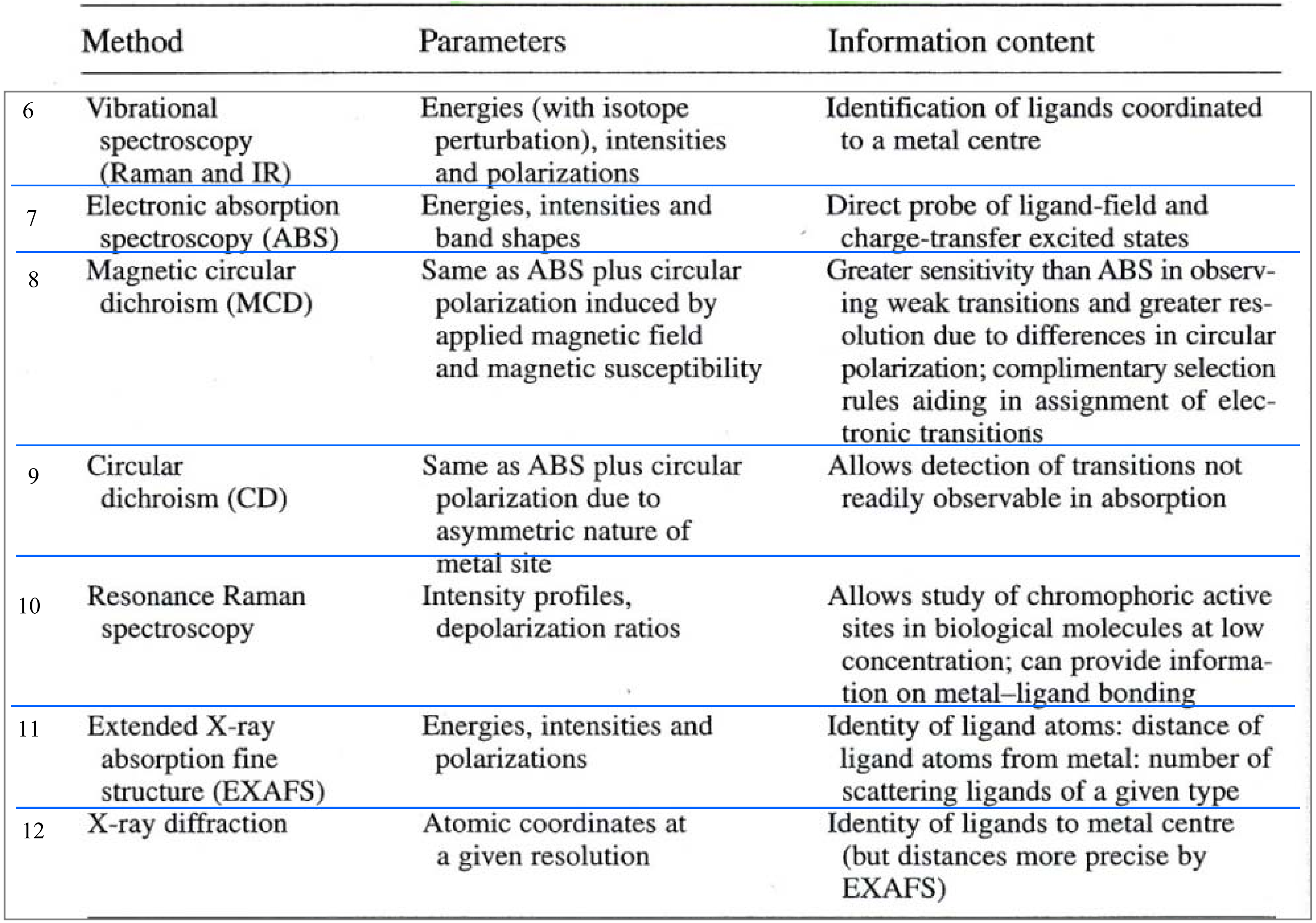
Spectroscopic Techniques (a)



Method	Parameters	Information content
1 Magnetic susceptibility	Molecular g-value, axial and rhombic zero-field splitting, exchange interaction	Number of unpaired electrons/ground spin state; defines anti-ferromagnetic and ferromagnetic interactions; quantitates ground sub-level splittings
2 Mössbauer spectroscopy	Quadrupole coupling isomer shift	For ^{57}Fe sites: oxidation and spin state; chemical environment
3 Electron paramagnetic resonance (EPR)	Quadrupole tensor, nuclear Zeeman splitting, g-values, coupling constants, relaxation times	Usually for odd electron metal sites: probes ground state wavefunction at high resolution
4 Electron-nuclear double resonance (ENDOR)		Combines sensitivity of EPR and high resolution of NMR to probe ligand super-hyperfine interactions
5 Nuclear magnetic resonance (NMR)	Chemical shift, nuclear coupling constants, relaxation times	For paramagnetic proteins: enhanced chemical shift resolution, contact and dipolar shifts, spin delocalization, magnetic coupling from temperature dependence of shifts

Ref. Crichton

Spectroscopic Techniques (b)

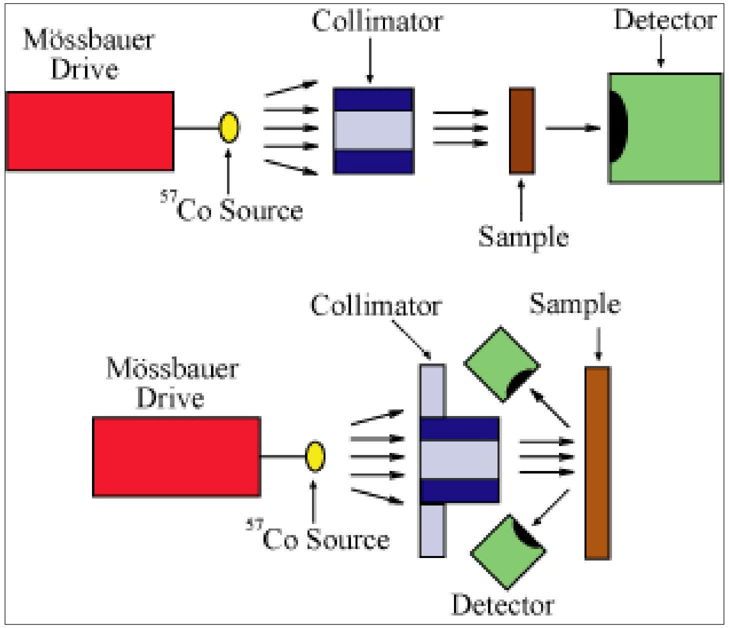


	Method	Parameters	Information content
6	Vibrational spectroscopy (Raman and IR)	Energies (with isotope perturbation), intensities and polarizations	Identification of ligands coordinated to a metal centre
7	Electronic absorption spectroscopy (ABS)	Energies, intensities and band shapes	Direct probe of ligand-field and charge-transfer excited states
8	Magnetic circular dichroism (MCD)	Same as ABS plus circular polarization induced by applied magnetic field and magnetic susceptibility	Greater sensitivity than ABS in observing weak transitions and greater resolution due to differences in circular polarization; complimentary selection rules aiding in assignment of electronic transitions
9	Circular dichroism (CD)	Same as ABS plus circular polarization due to asymmetric nature of metal site	Allows detection of transitions not readily observable in absorption
10	Resonance Raman spectroscopy	Intensity profiles, depolarization ratios	Allows study of chromophoric active sites in biological molecules at low concentration; can provide information on metal–ligand bonding
11	Extended X-ray absorption fine structure (EXAFS)	Energies, intensities and polarizations	Identity of ligand atoms: distance of ligand atoms from metal: number of scattering ligands of a given type
12	X-ray diffraction	Atomic coordinates at a given resolution	Identity of ligands to metal centre (but distances more precise by EXAFS)

Extracted from: R. R. Crichton, *Biological Inorganic Chemistry*, 1st Ed., 2007, Elsevier.

Mössbauer Spectroscopy

- Mössbauer spectroscopy is based on the emission and subsequent absorption (resonance) of γ radiation by the nuclei (in solid state) and takes advantage of the fact that nuclear energies are sensitive to the electronic environment.
- Mössbauer spectroscopy is similar to NMR in that it probes nuclear transitions and in that it is sensitive to similar electron-nucleus interactions as a cause of chemical displacement.
- ❖ The ^{57}Fe is the most studied element using the technique. It is possible to distinguish the states of oxidation Fe (II), Fe (III) and Fe (IV), high or low spin,... and obtain information on the symmetry of the molecule.



Mössbauer spectrometer

https://serc.carleton.edu/research_education/geochemsheets/techniques/mossbauer.html

Mössbauer spectrometer →

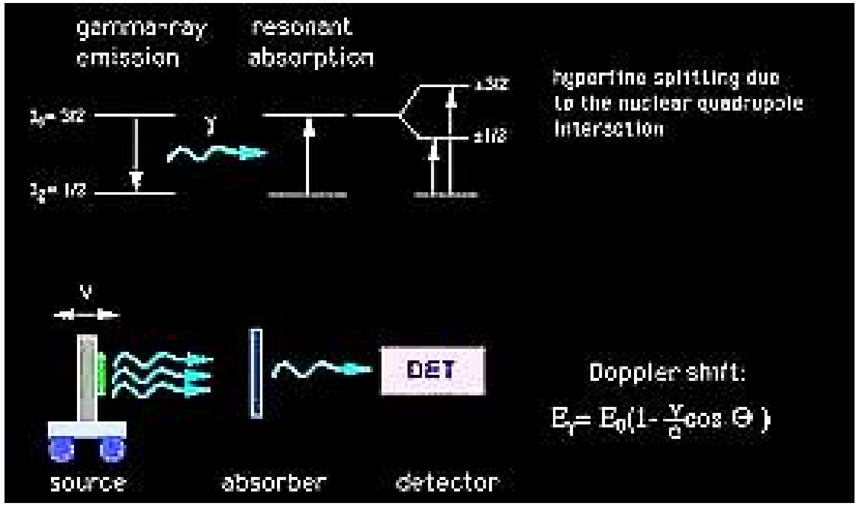
- Sample of Fe. ^{57}Fe: Isotope present in 2%.
- Source of ^{57}Co (radioactive father), decays and produces ^{57}Fe * *; This one, 57Fe *, and this one, ^{57}Fe.
- Box: Nuclear transitions emitting radiations γ.

Mössbauer

Based on:

- A significant fraction of the γ rays emitted will have approximately the appropriate energy to be absorbed by the atoms of the sample, being the only differences attributable to the chemical environment of the sample, which is what you want to observe. The power of source γ Ray varies by the Doppler effect when it accelerates within a range of velocities.

- The Mössbauer spectrum is a representation of the resonant absorption maxima that occur when the speed is modified. The number, positions and peak intensities provide information about the chemical environment of the absorbent nuclei and can be used to characterize the sample.

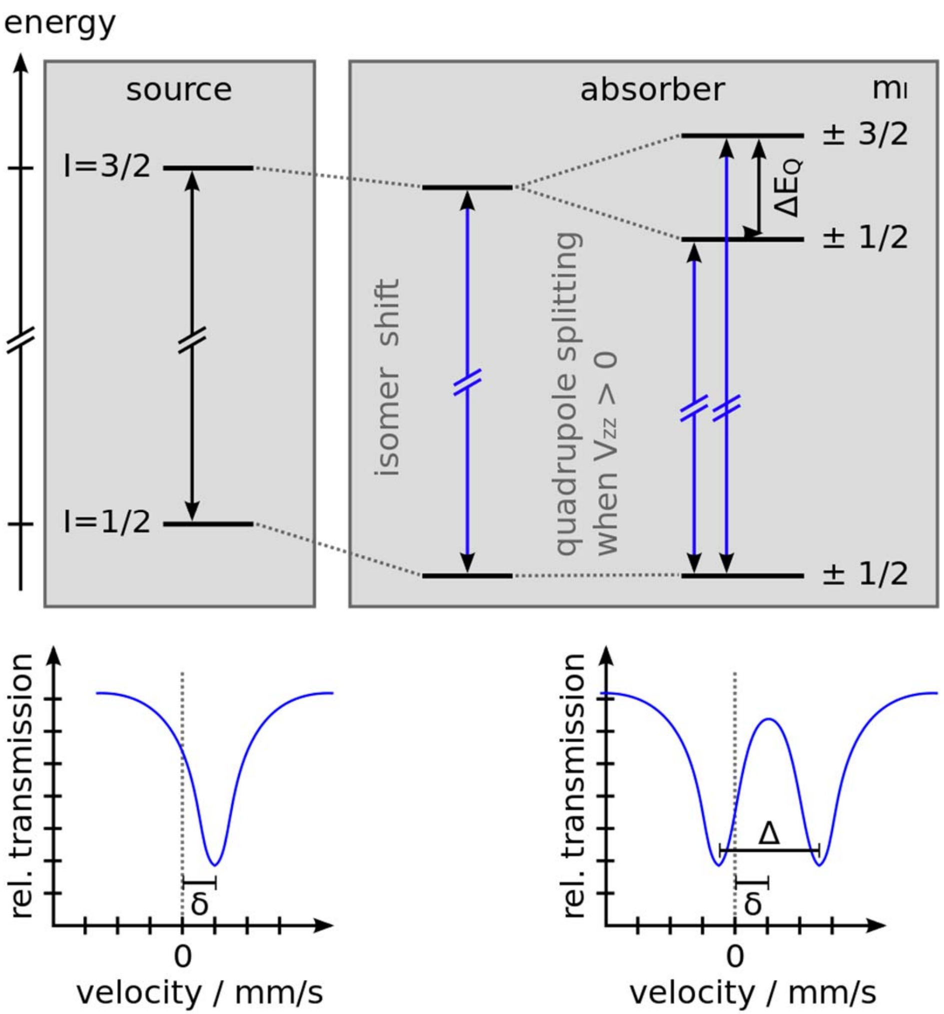


Mössbauer

Author: Joël Gubler

Parameters:

- The difference between ΔE of the sample and the metallic ^{57}Fe is called **isomeric displacement** and is expressed in terms of the V (mm s^{-1}) which is required to achieve resonance by Doppler displacement.
- In an environment not isotropic the spectrum is unfolded in two lines: **quadrupolar split**



Isomer Shift and Quadrupole Splitting with iron 57. Energy levels and schematic spectra.

EPR: Electronic paramagnetic resonance, or electronic spin resonance

- EPR spectroscopy is used to study compounds that possess unpaired electrons, in particular those of metals d^{n}.
- It is often the technique of choice to identify and study metals such as Fe and Cu in the active centers of the Metalloenzymes.

See figure in Weller *et al.*

Continuous wave spectrometer (OC) →

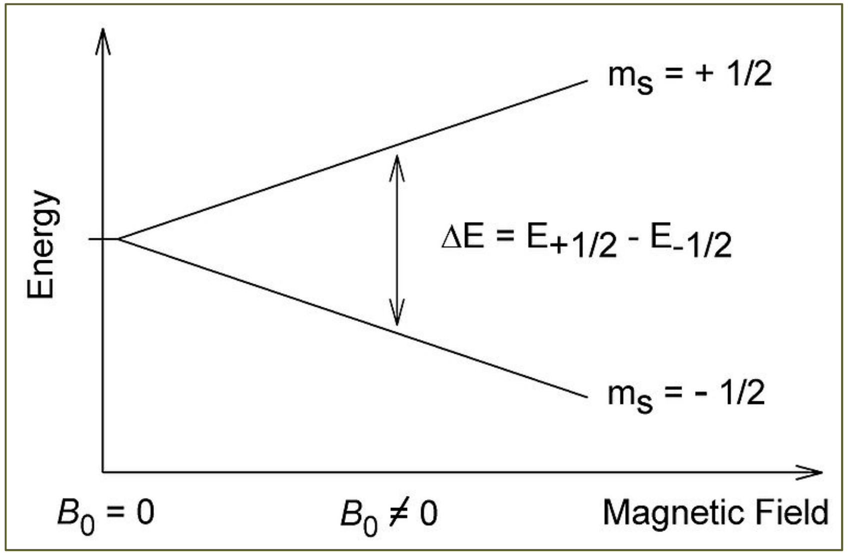
- The sample is radiated with constant microwave frequency (GHz: X-Band (9), Q-band (35), S-Band (3), W-Band (95)...)
- The magnetic field, B, is made vary

Continuous wave spectrometer (OC)

EPR

Based on:

- When a magnetic field is applied to an electron unpaired, its two orientations, α and β, have different energy.
- The resonance is reached when the energy separation is matched by the energy of the microwave photons incidents.



File:EPR splitting.svg. This work has been released into the public domain by its author, Astrochemist at English Wikipedia.

EPR

Parameters:

***A. g* value**

B. Hiperfine coupling

A. *g* = 2.0023 for the free electron

B. It changes with the anisotropy

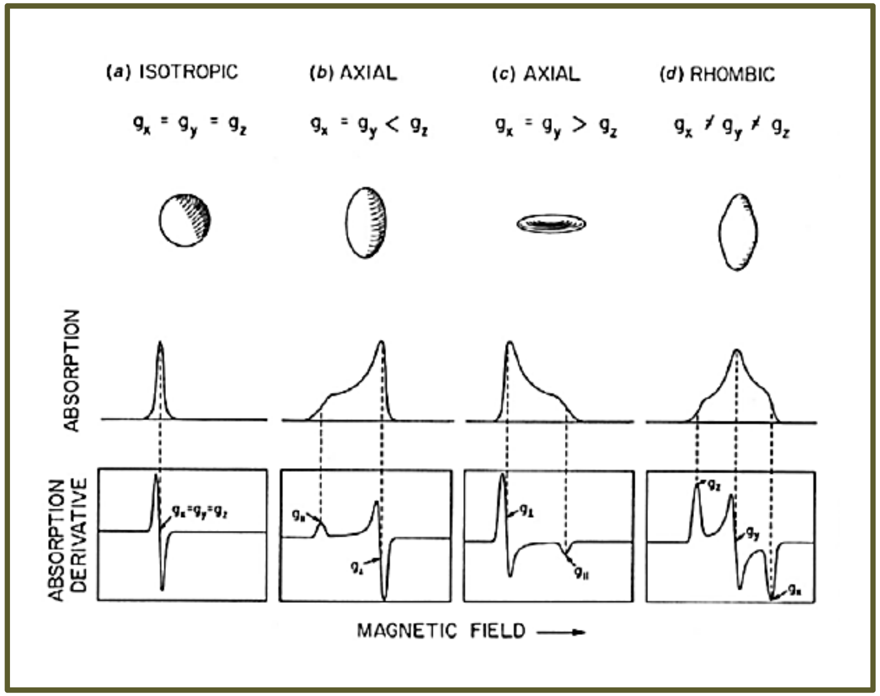


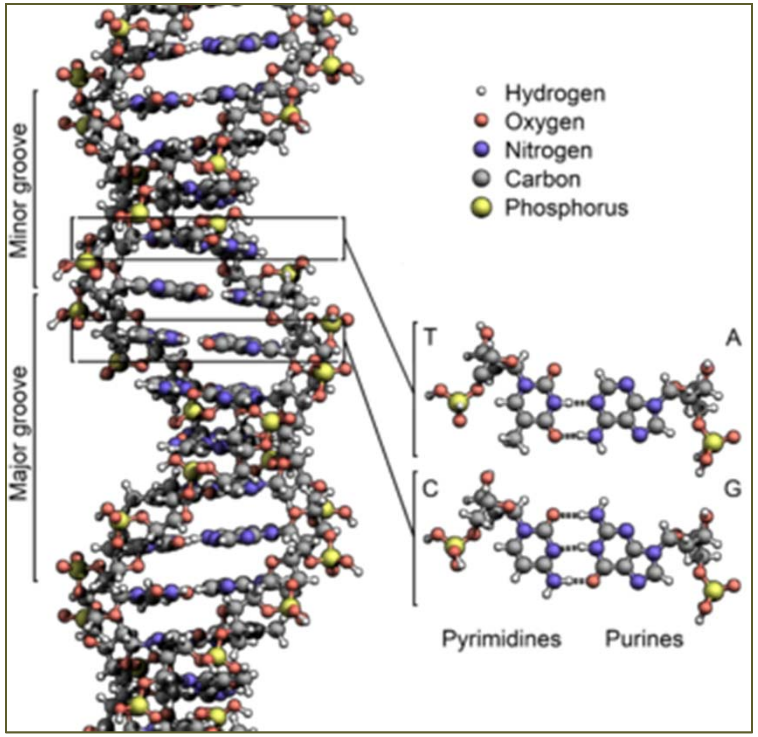
Fig.: Expected forms for the EPR spectra of samples in frozen solution:
(a) isotropic spin system
(b) (c) axial system
(d) rhombic system

Techniques for the study of DNA interaction

1. Fluorescence

2. Viscosimetry

3. ΔT_m



The structure of the DNA double helix

DNA interaction and cleavage

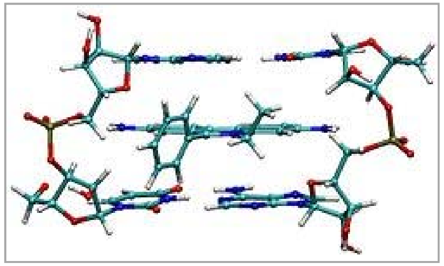
- ☐ Molecules of relatively small size can interact with nucleic acids:
  - by binding to grooves (major and/or minor):
  - by intercalation between base pairs;
  - by binding to the sugar-phosphate skeleton;
  - by covalent union or metal-base coordination.

- ☐ Nucleases (natural) are a class of enzymes that catalyze the hydrolytic degradation of the phosphodiester bond (P-O) of the DNA strand.

- ☐ Chemical (or synthetic) nucleases can produce DNA cutting through two mechanisms:
  - hydrolytic: the phosphodiester bond (P-O) is broken;
  - oxidative: the cleavage is produced by oxidation of the nucleobases and/or abstraction of the hydrogen atoms of the sugar.

- ➢ A research line in Bioinorganic is the development of low molecular weight metal complexes as mimetic agents of enzymatic nucleases.

1. Fluorescence spectroscopy



Wikipedia: Ethidium intercalated between two adenine-thymine base pairs.

Based on:

- The technique used here is based on the fluorescence emitted by the classic *intercalator* ethidium bromide (EB) when it is linked to the DNA.
- The fluorescence intensity of free EB is quenched by molecular oxygen in the aqueous environment and therefore unbound dye is only weakly fluorescent. However, the fluorescent quantum yield of the EB increases considerably on binding to the nucleic acid.
- **Competitive studies** with EB are based on the addition of a second non-fluorescent molecule (the complex to be tested) that competes with EB for binding to DNA.

Fluorescence spectroscopy

COMPETITIVE STUDIES

CT DNA
+
INTERCALATOR
(Ethidium bromide)

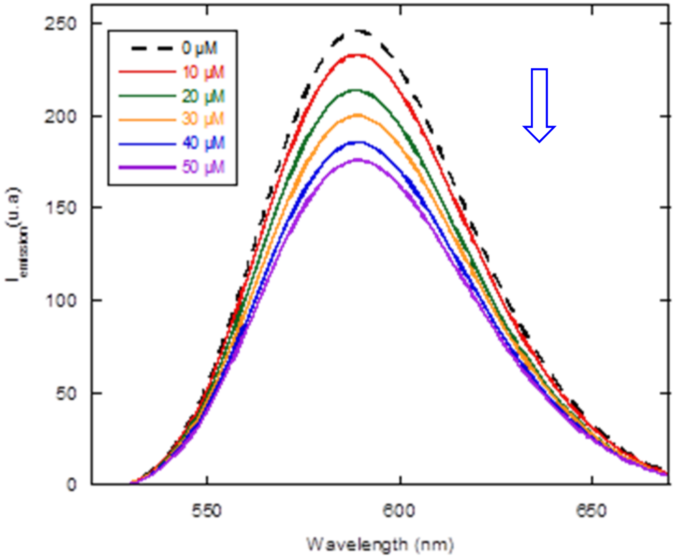
CT DNA
+
INTERCALATOR
(Ethidium bromide)
+
COMPLEX

⬇

Emission variation

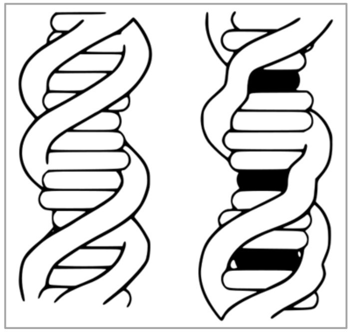
⇓

INTERACTION



- Typical (real) graphics of a **fluorescence assay**: DNA-EB emission spectrum in absence and in the presence of a compound.

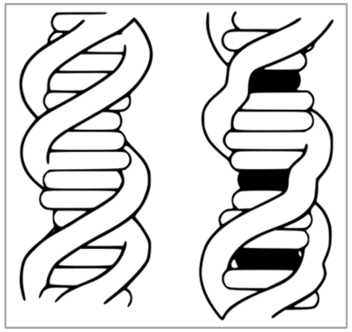
- The arrow shows the change in the emission intensity by increasing the concentration of the complex.



Wikipedia (public domain).
Left: unchanged DNA strand.
Right: DNA strand intercalated.

Experimental data taken from: PhD Thesis J. Hernández-Gil, University of València, 2014

2. Viscosimetry



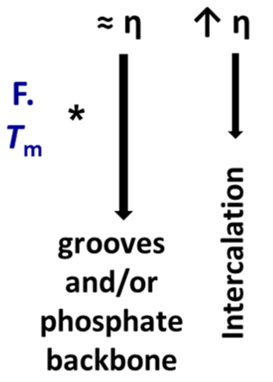
Based on:

- The separation between the base pairs of the DNA produced by an insert (of ca. 3.4 Å, which is the van der Waals thickness of an aromatic ring) produces an unwinding around the axis of the double helix.
- If the intercalation takes place in multiple places, an elongation of the double strand occurs and concomitantly an increase in viscosity is produced.
- A classic intercalation produces a significant *increase* in viscosity.
- In contrast, interactions through DNA grooves and/or through electrostatic attraction cause *a lesser variation* of viscosity or *no change* in it.

Viscosimetry

HIDRODYNAMIC STUDIES

- **Viscosity measurement**
- **at different [compound]/[DNA] ratios**

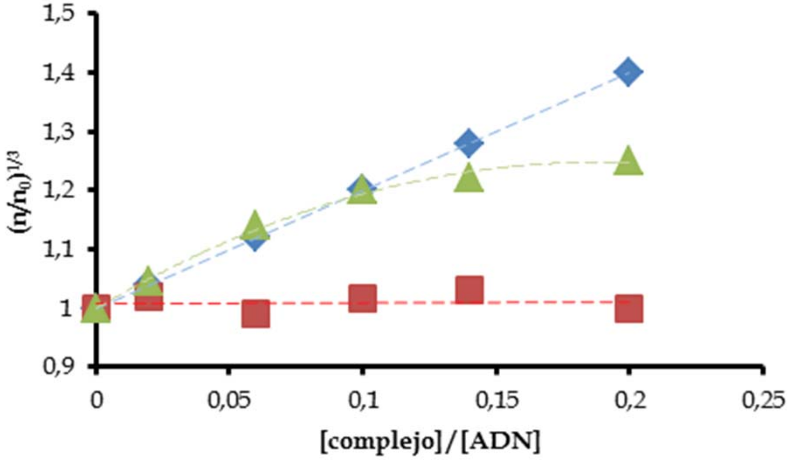


≈ η **↑ η**

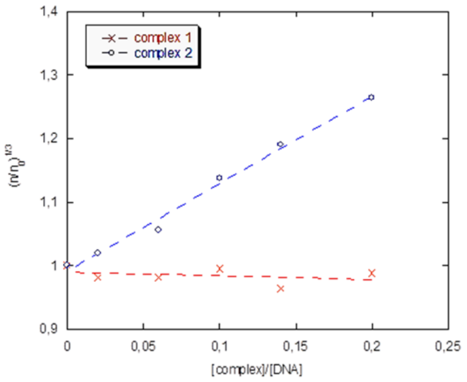
F.
***T*$_m$** *

grooves and/or phosphate backbone

Intercalation



"Ideal" representation **of the changes in viscosity of the DNA** produced by different modes of interaction:
(♦) classic intercalation,
(▲) partial intercalation, and
(■) interaction through grooves or electrostatic



Assay (real) **of viscosity**:
-compound 1: does not intercalate
-compound 2: intercalates

Experimental data taken from: PhD Thesis J. Hernández-Gil, University of València, 2014

3. Determination of DNA *melting* Temperature

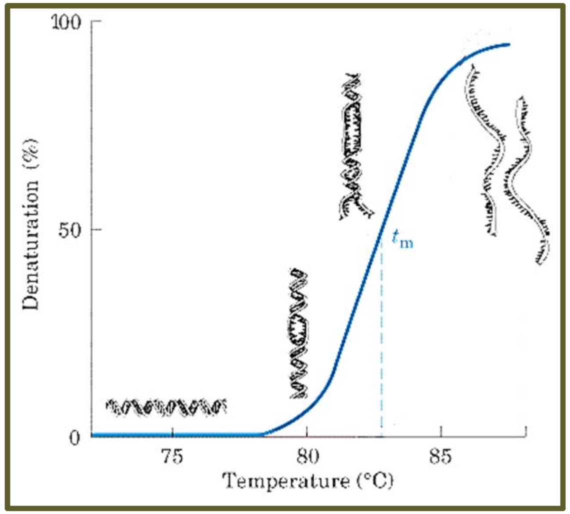
Based on:

- The double strand of DNA can be separated by an increase in temperature to give two nucleic acids of a single strand.
- This process is known as melting or thermal denaturation of the DNA.
- The temperature at which 50% of nucleic acid exists as helical state and 50% as single-stranded state is known as melting temperature or thermal denaturation temperature and it is designated as T_m.

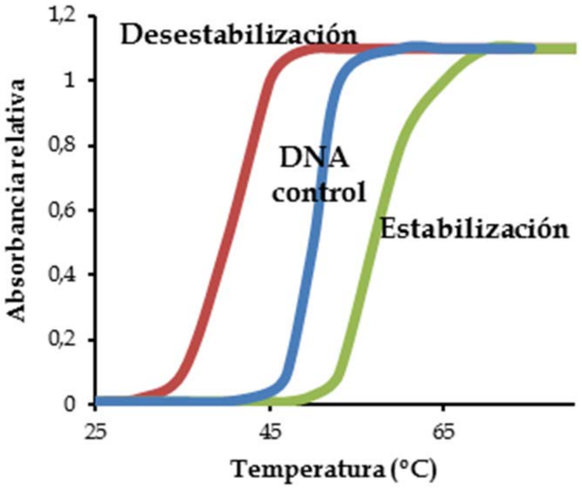
Determination of DNA *melting* Temperature

The process of determination of T_m by using UV spectroscopy is carried out by gradually rising temperature from 25 ° to 90 °C and measuring the A_{260} continuously.

DNA T_m profile



DNA + compound T_m profiles

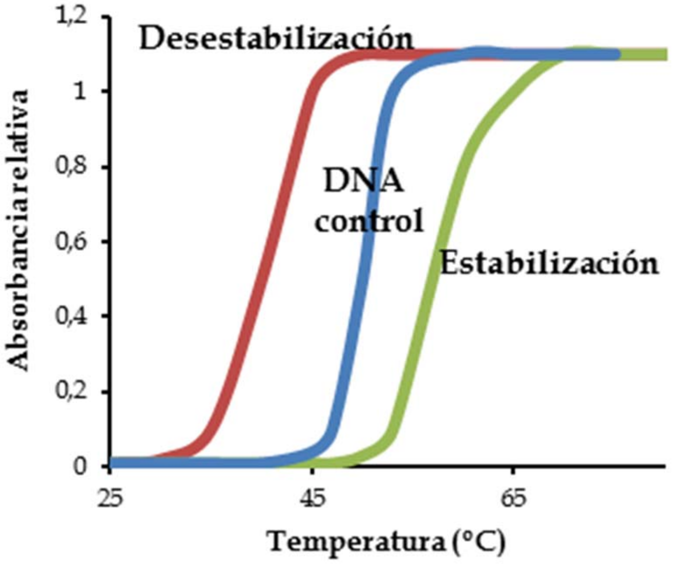


$\Delta T_m \Rightarrow$ Interaction

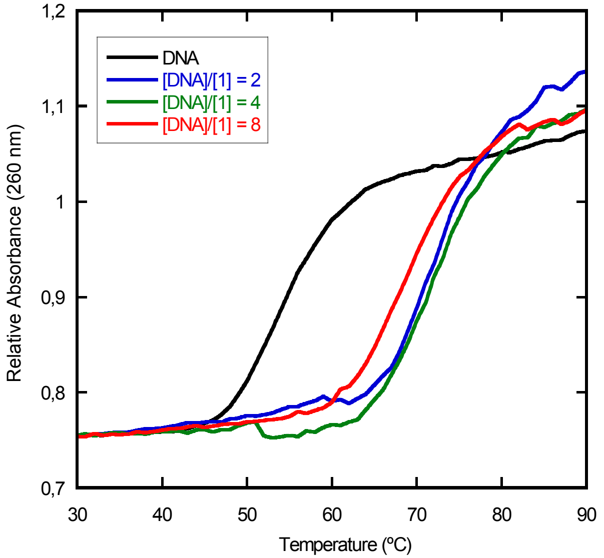
(a) DNA *melting* curve of a thermal denaturation experience

(b) Theoretical displacement of the DNA *melting* curve by a molecule that stabilizes or destabilizes the double strand of DNA

DNA *melting* Temperature



Theoretical displacement of the DNA *melting* curve by a molecule that stabilizes or destabilizes the double strand of DNA



Experimental curves of DNA *melting* temperature (CT-ADN) in absence and in presence of a Cu(II) compound at different ratios

Experimental data taken from: PhD Thesis J. Hernández-Gil, University of València, 2014

Literature

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