ATOMIC FORCE MICROSCOPY: IMAGING THE EFFECT OF METAL ION COMPLEXES ON DNA

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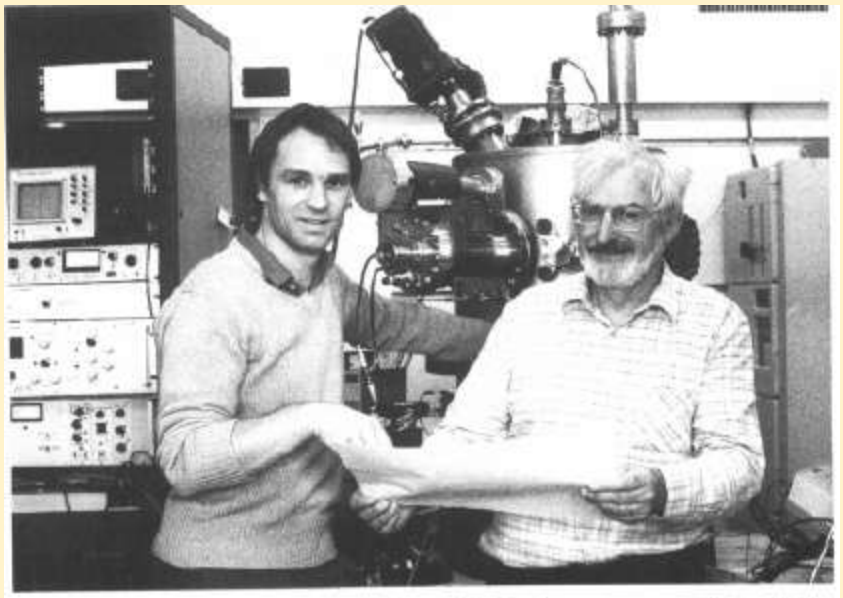
Introduction



Gerd Binnig (left) and Heinrich Rohrer (right) who were awarded the Nobel Prize for their invention of the scanning tunneling microscope.

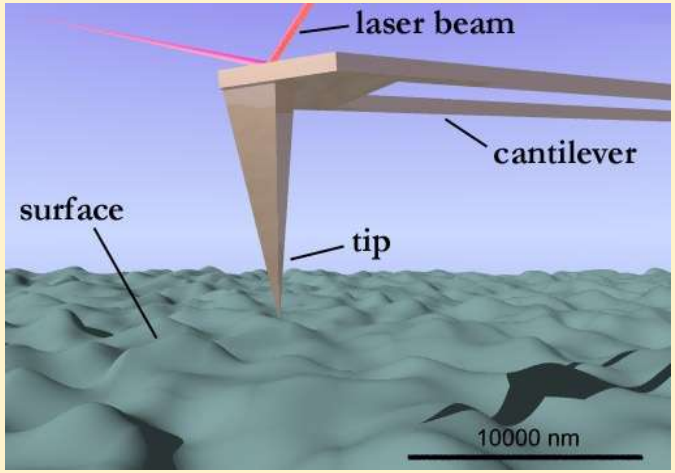
Atomic Force Microscope (AFM) is part of a large family of Scanning Probe Microscopes (SPM).

*** The First SPM was invented in 1981 by Gerd Binning and Heinrich Rohrer.**

*** Early SPM models acquired images by detecting the difference in electrical potential between two objects on the slide.**

*** AFM, developed in 1986, generates images based on the attraction and repulsion forces between the scanning tip and the objects on the slide.**

Principles of Atomic Force Microscopy

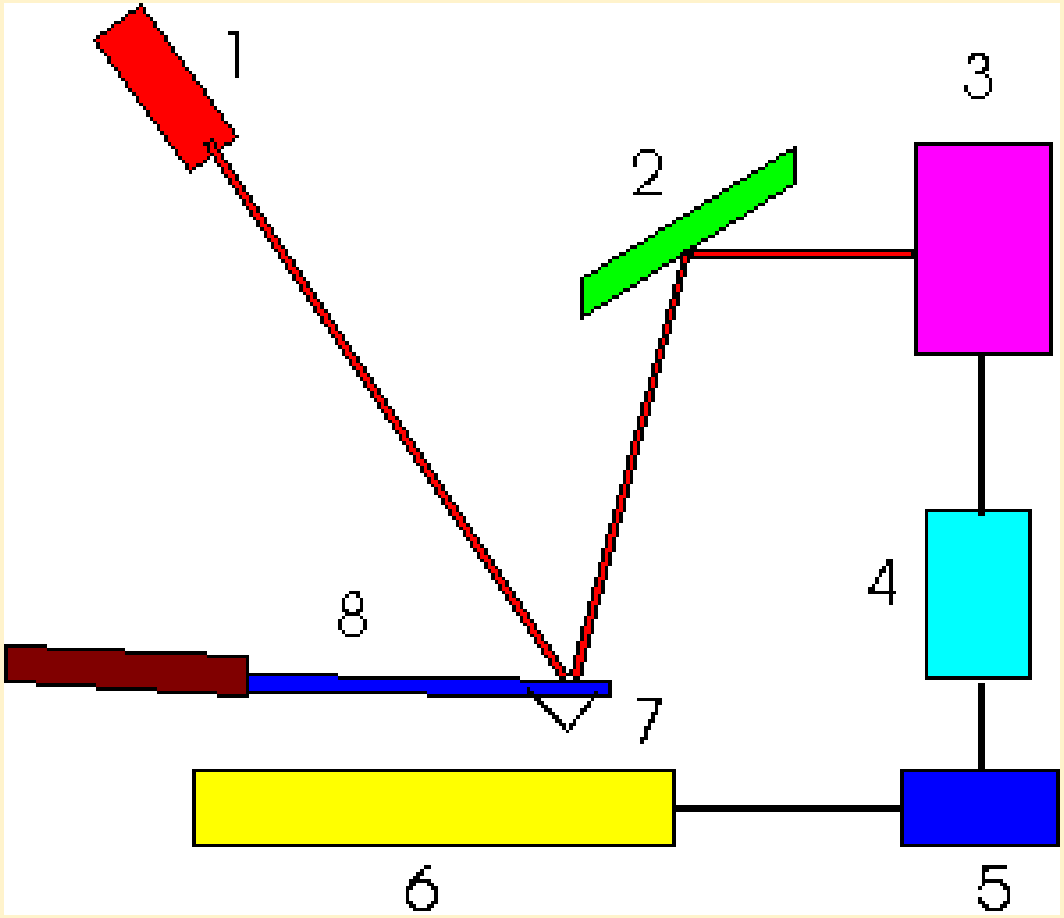


* Images in AFM are acquired by scanning the surface of the sample with a sharp tip.

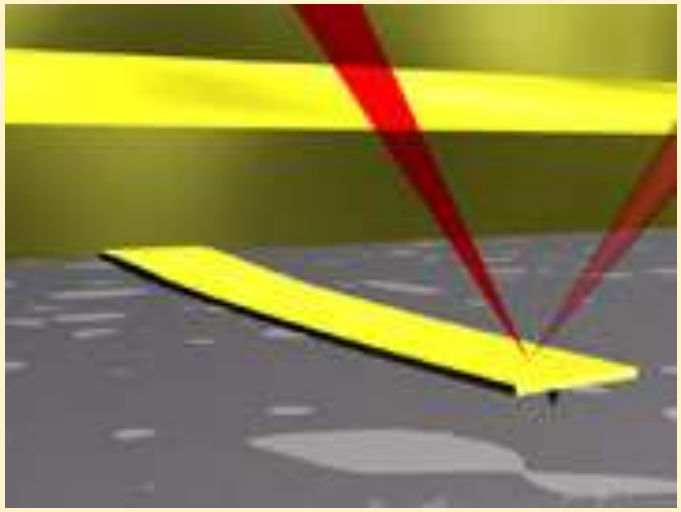
* The tip is located at the free end of a flexible cantilever.

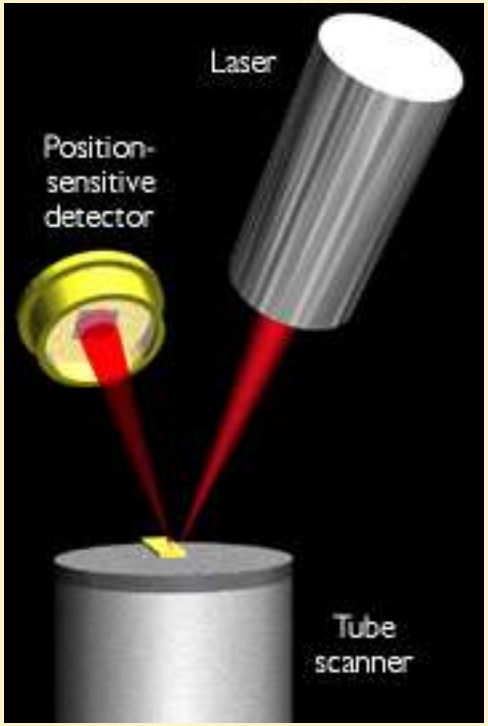
* The cantilever movements are detected by a laser beam that is reflected of the back of the cantilever to a photodiode.

AFM Scheme

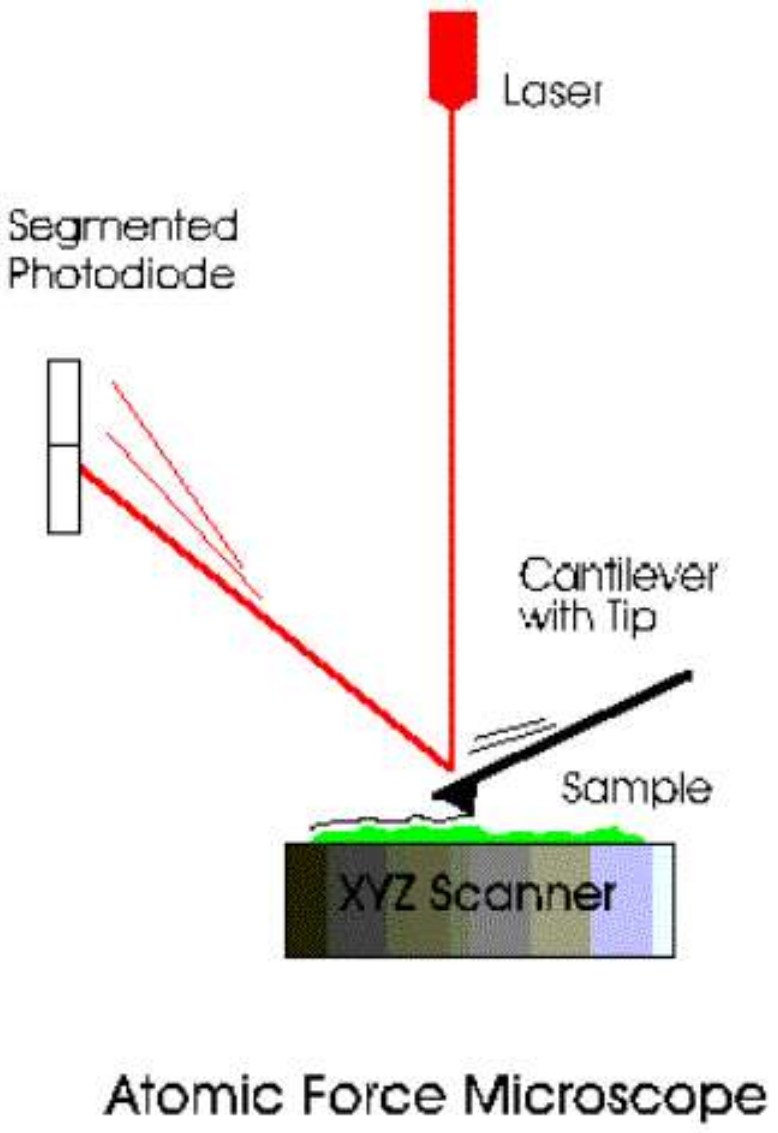


1. Laser
2. Mirror
3. Photodetector
4. Amplifier
5. Register
6. Sample
7. Probe
8. Cantilever





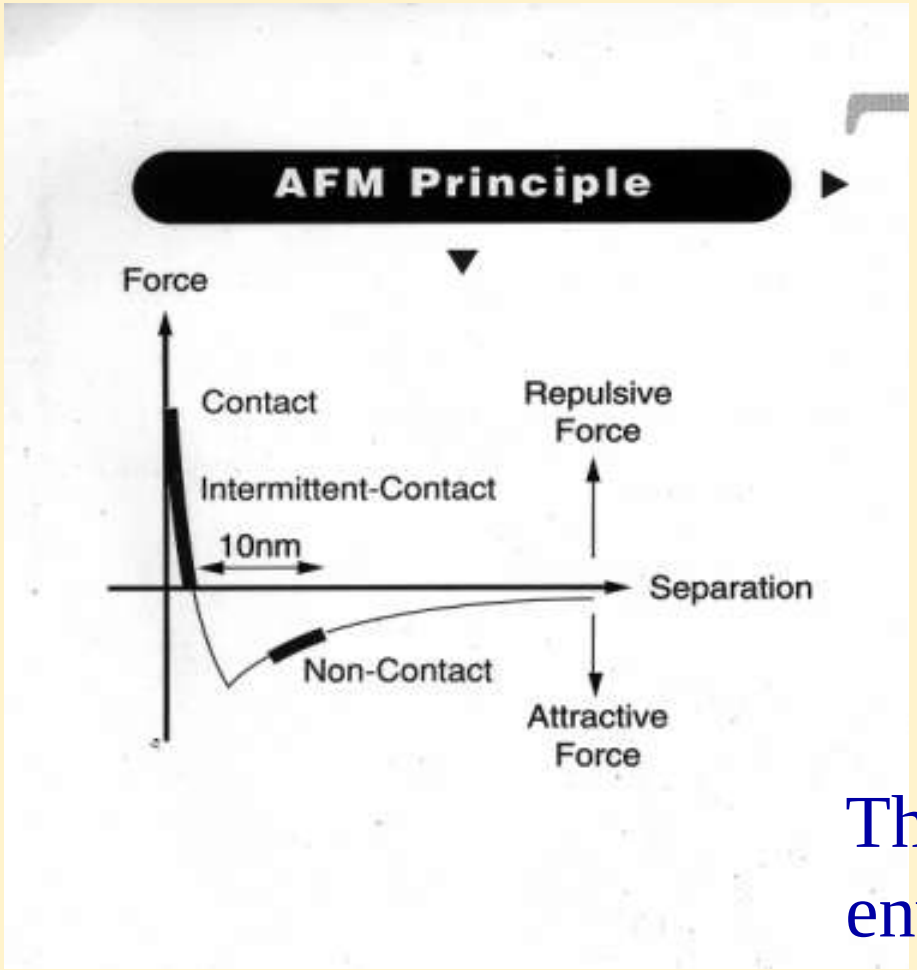
(Left) a cantilever touching a sample; (right) the optical lever. Scale drawing; the tube scanner measures 24 mm in diameter, while the cantilever is 100 µm long.



***Forces between the tip and the sample cause the cantilever to deflect**

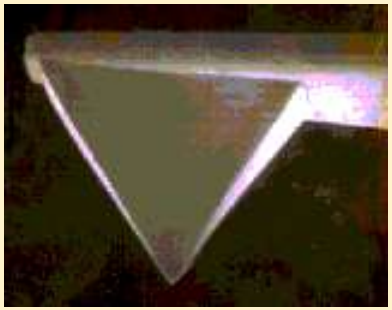
***The photodiode relays the information to the computer which in turn generates a topographical image of the sample**

AFM Operation

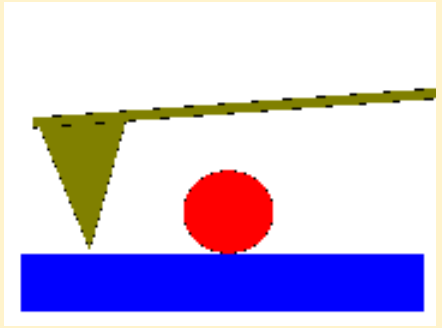


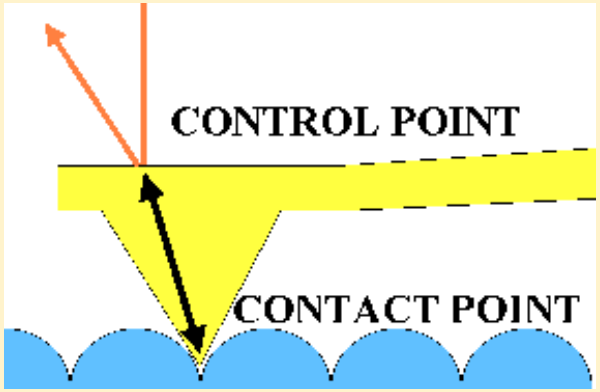
- Contact Mode
- Non Contact Mode
- Intermittent Contact Mode (Tapping)

The AFM can be used in a variety of environments: air, UHV (ultra high vacuum) and under liquids



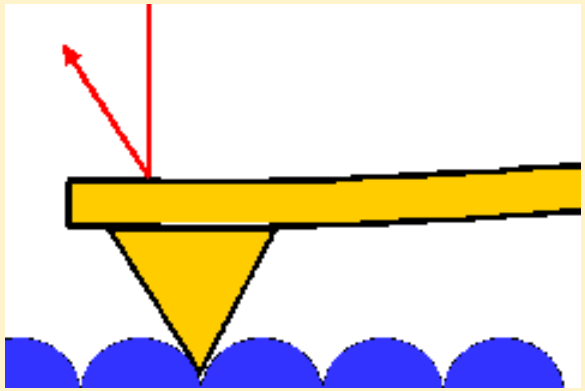
TIP

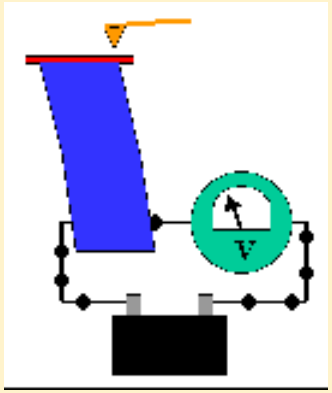


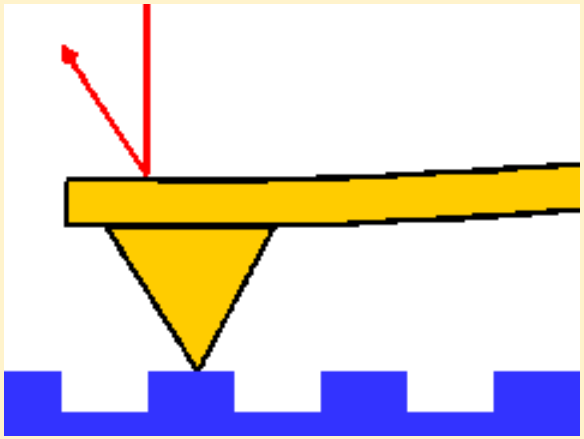


The sharp tip is dragged across a sample surface

Now, the sample is moving under the AFM tip







Resolution in AFM

- AFM does not use lenses to generate images.
- Resolution in AFM is dependent on:

The sharpness of the tip.

The distance between the objects to be resolved.

The height of the two objects to be resolved.

Sample Preparation

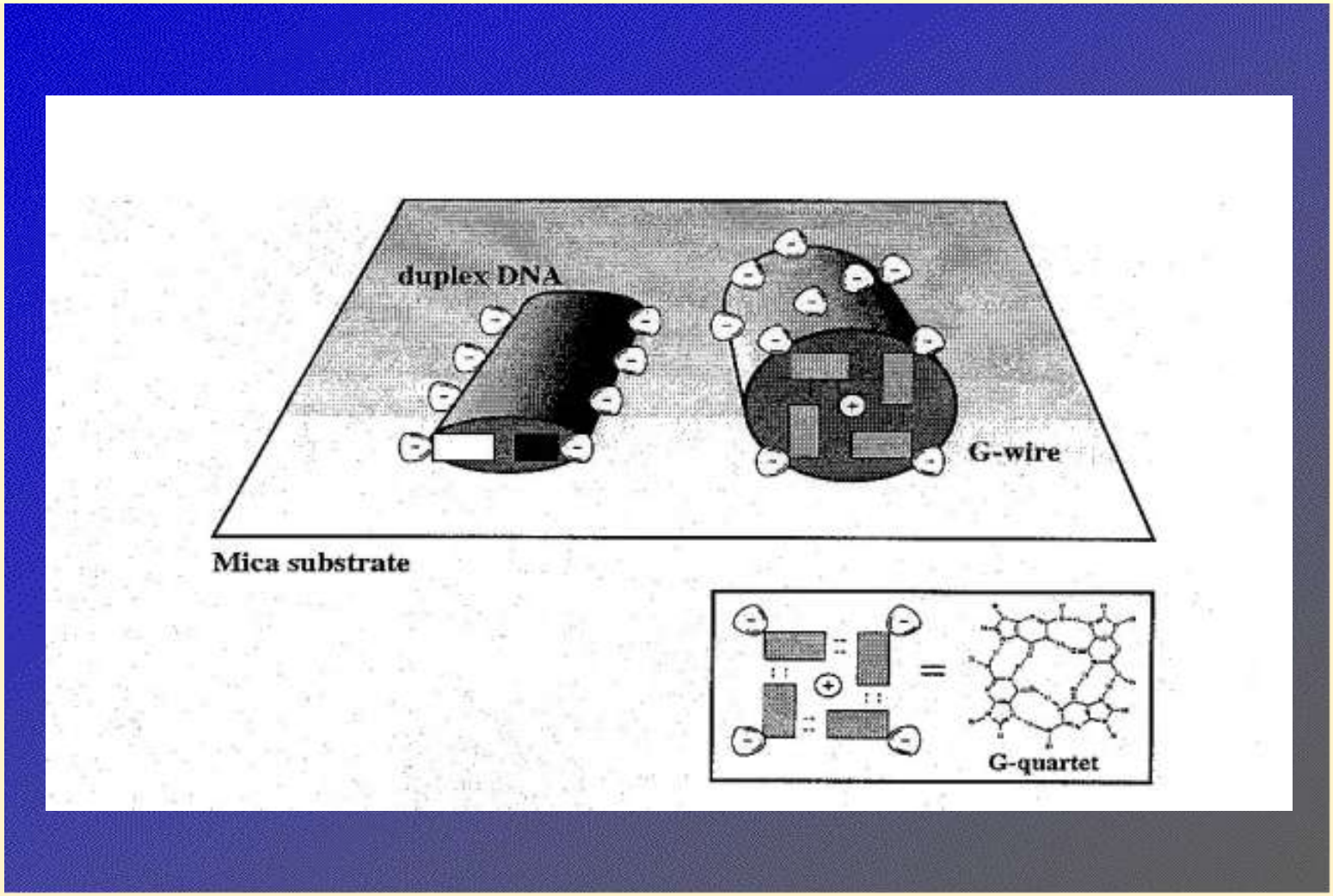
Deposition Buffer containing a divalent cation.

Proper concentration of reactants

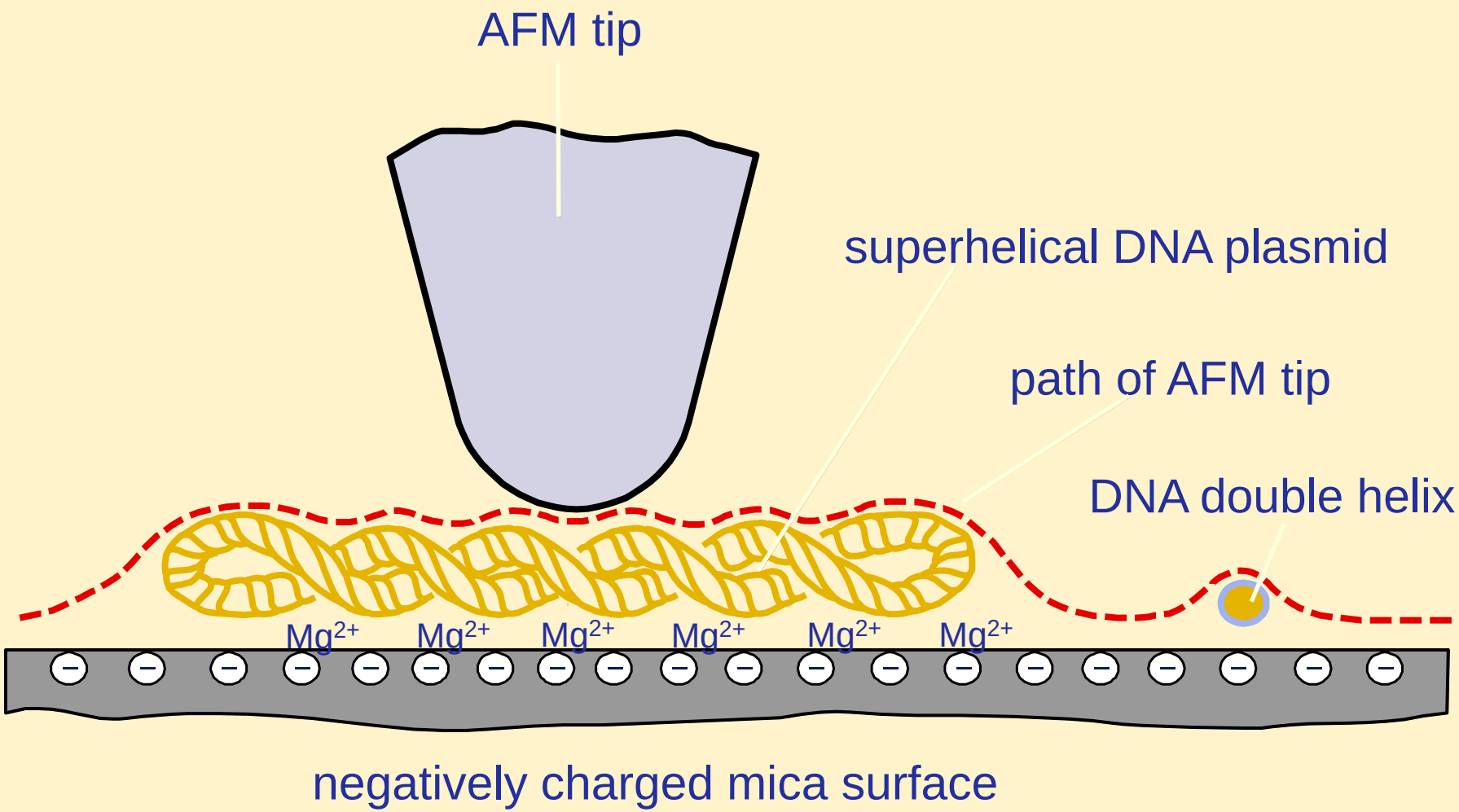
Flat substrate:

1. Plain mica
2. Aminopropyltrimethoxy saline (APTES) mica
3. Glow discharged mica

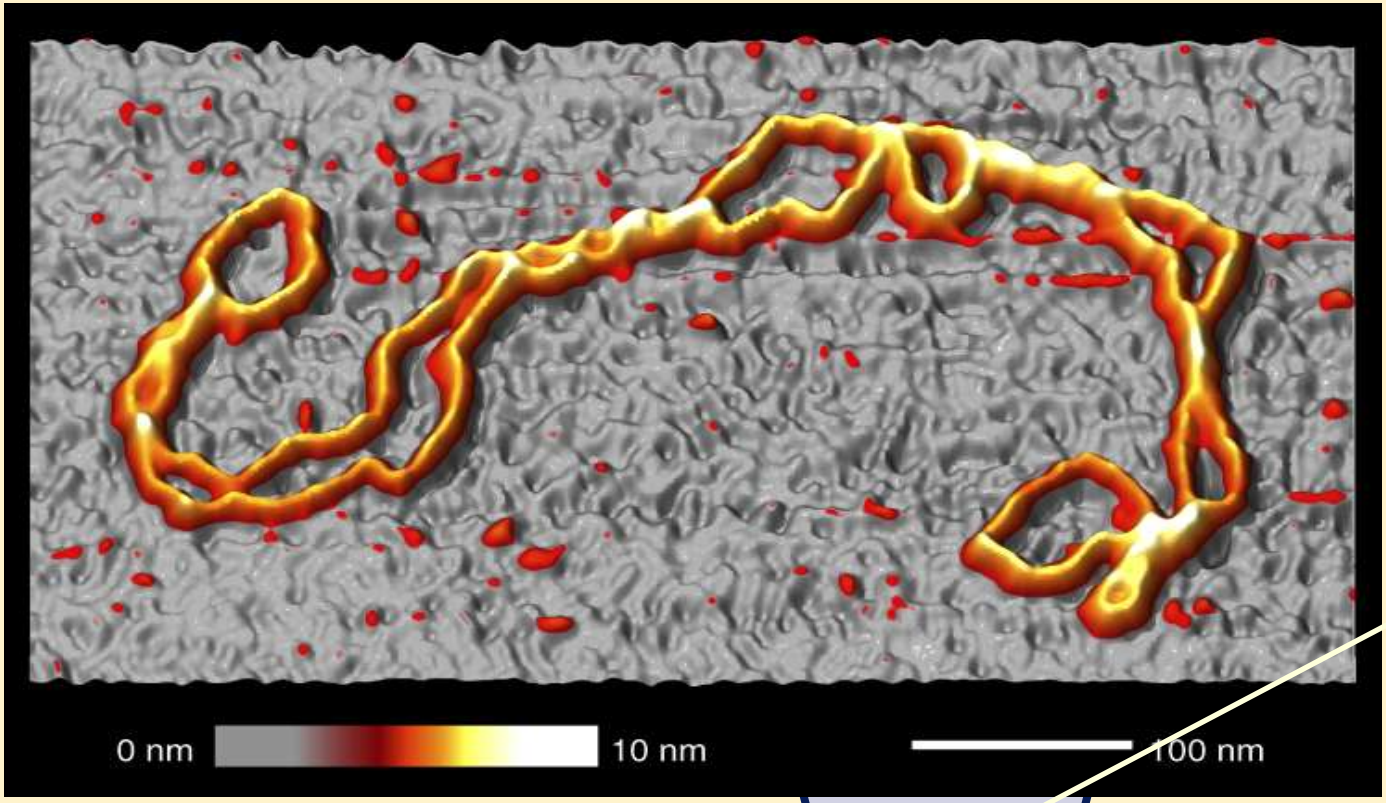
A drawing showing the attachment of the DNA molecules to the mica substrate.

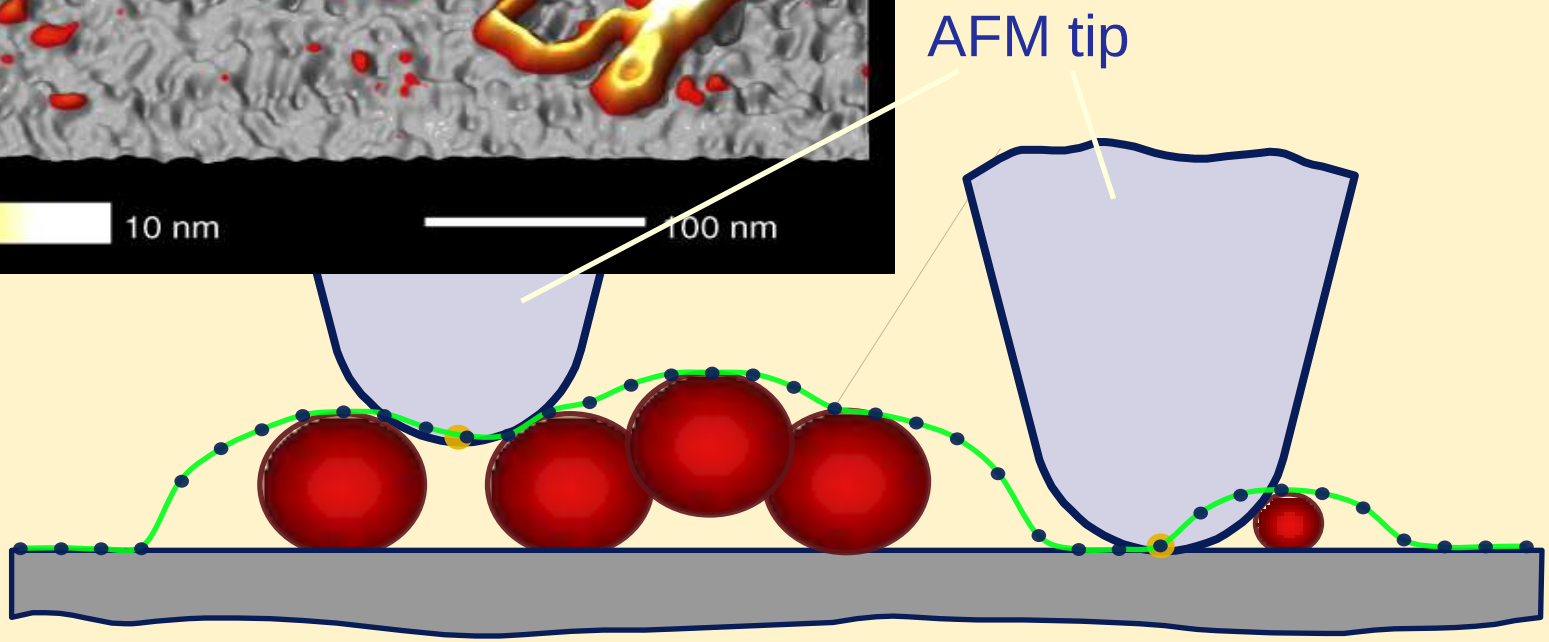


movement of the AFM tip along the sample



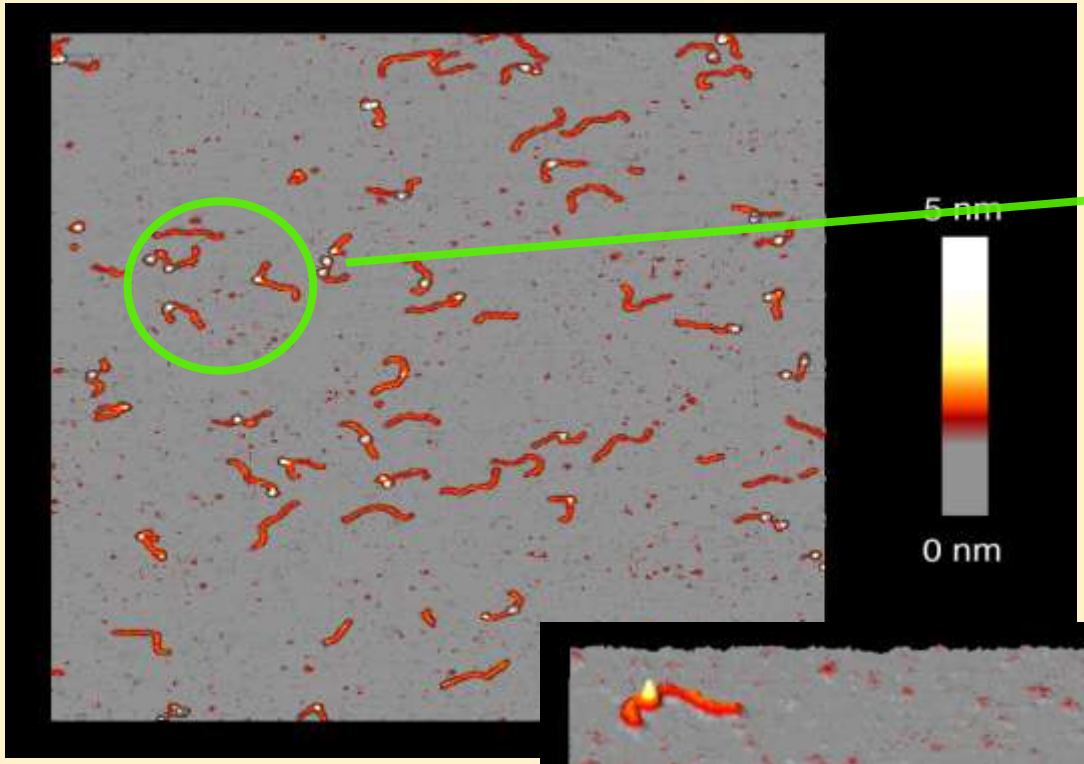
AFM image of a 6.8 kb superhelical plasmid

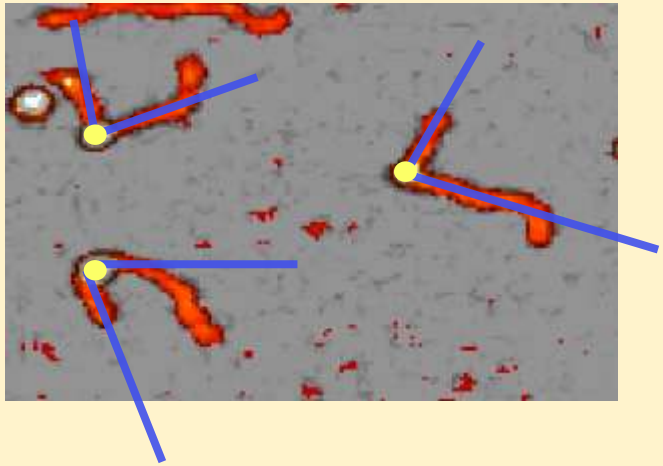




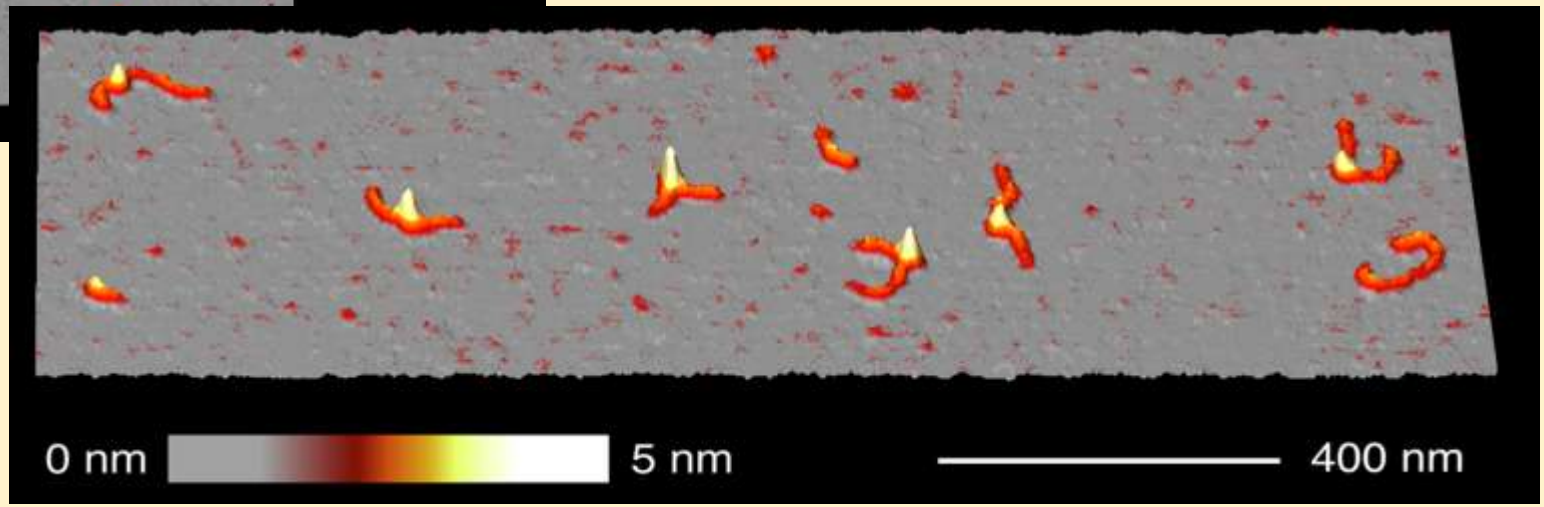
AFM image of a nucleosome on a 614 base pair DNA

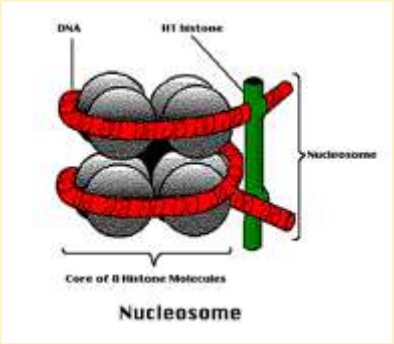
2 µm x 2 µm overview scan

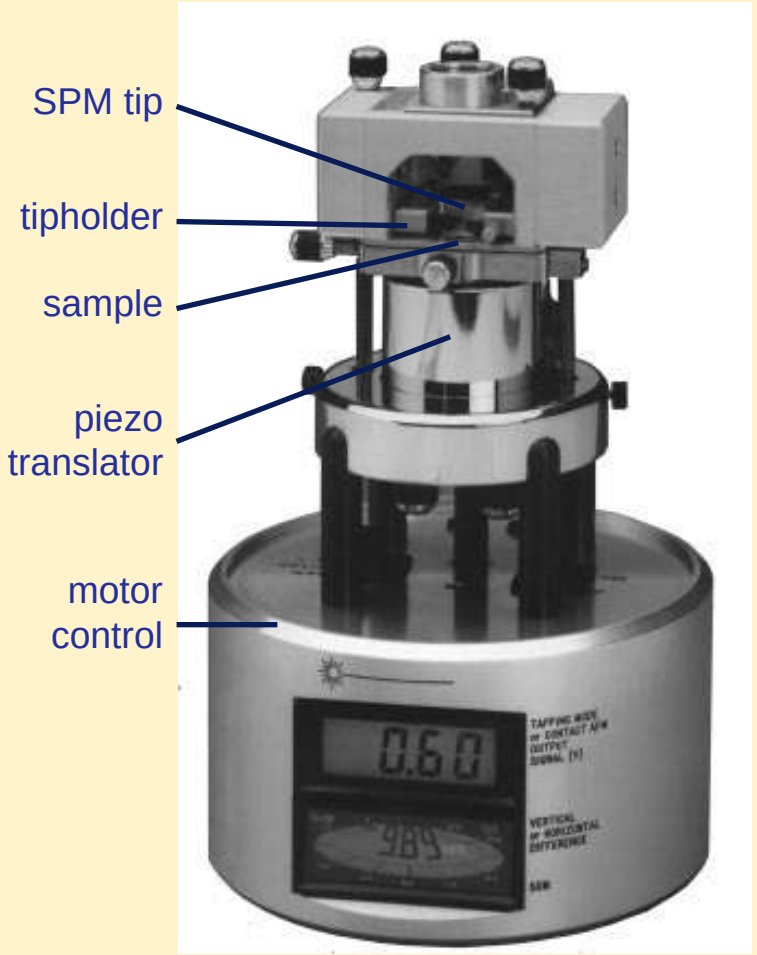




zoom of mononucleosome complexes



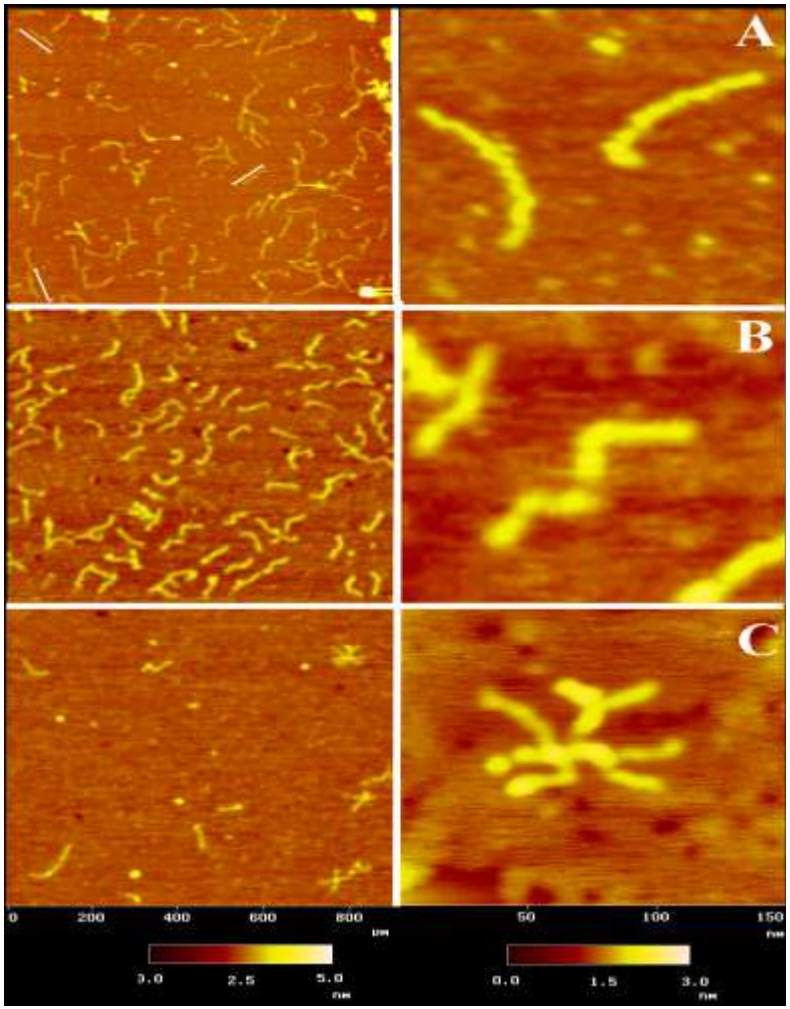




In the picture, one of the most recent SPM Microscope from Digital Instruments is shown. This is the Instrument available in the Laboratories of the Serveis Científics-Tècnics of our University.

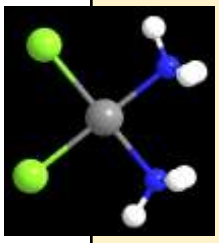
Probing the modifications in linear DNA and circular plasmid DNA caused by a variety of metal-complexes by Tapping Mode AFM.

The experiments were always done in air, on peeled mica and low humidity.

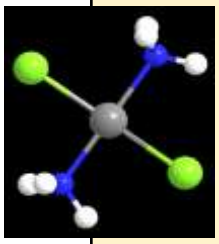


Linear DNA fragment
(*Hmly)*

Effect of cisplatin on *Hmly* DNA

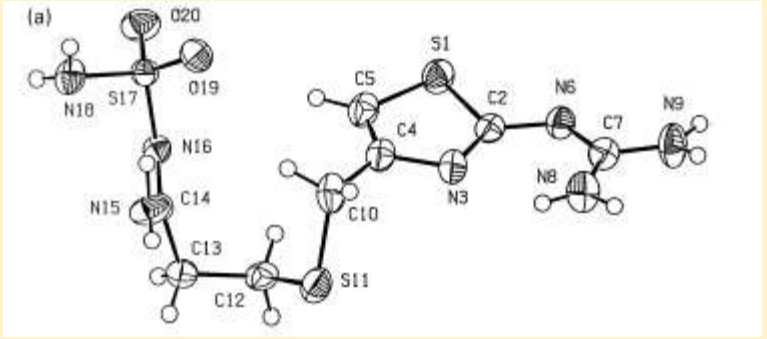


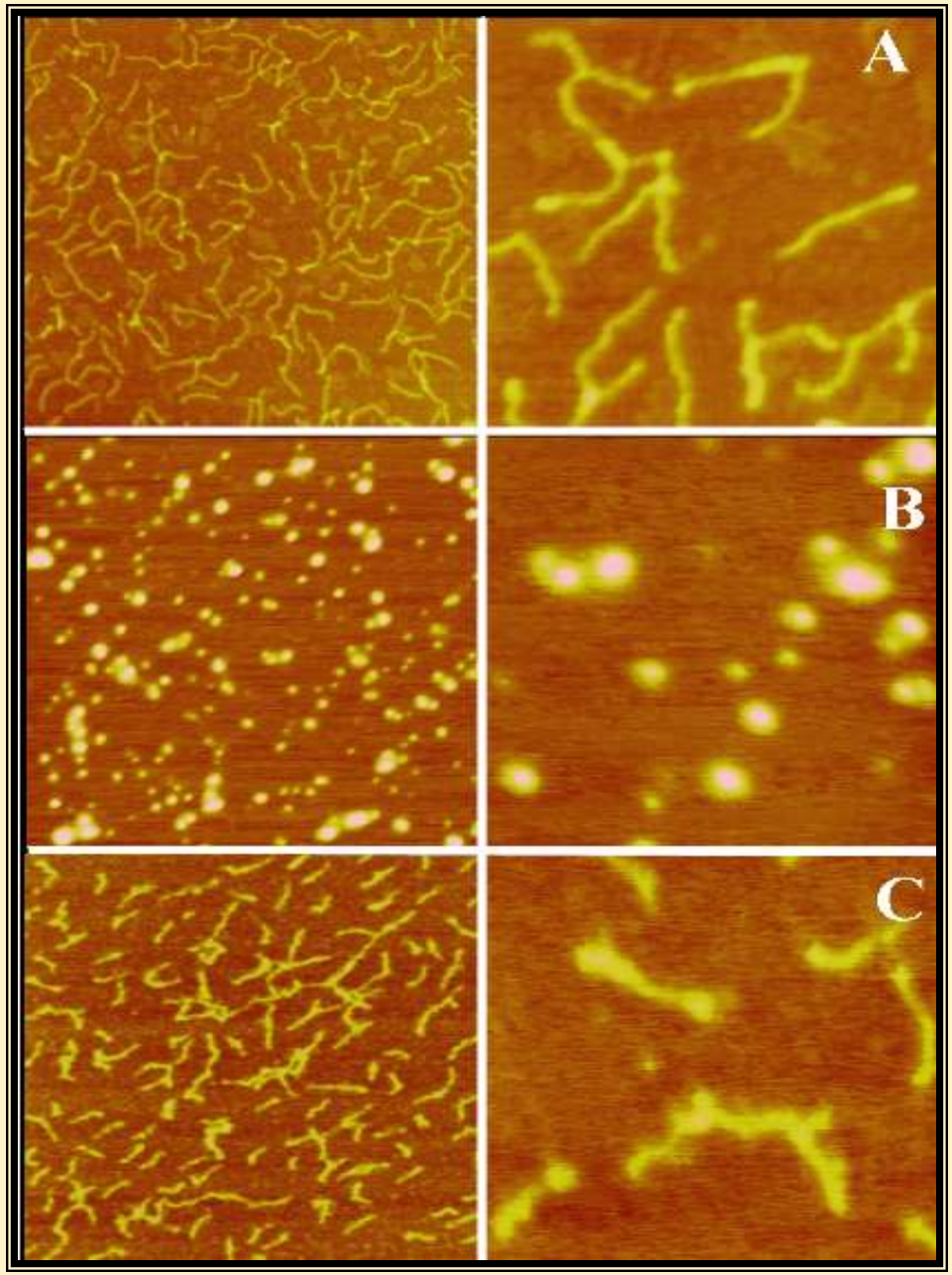
Effect of transplatin on *Hmly* DNA



Nucleic Acids Res. , 1998

Famotidine





Pd-Famotidine

Pt-Famotidine

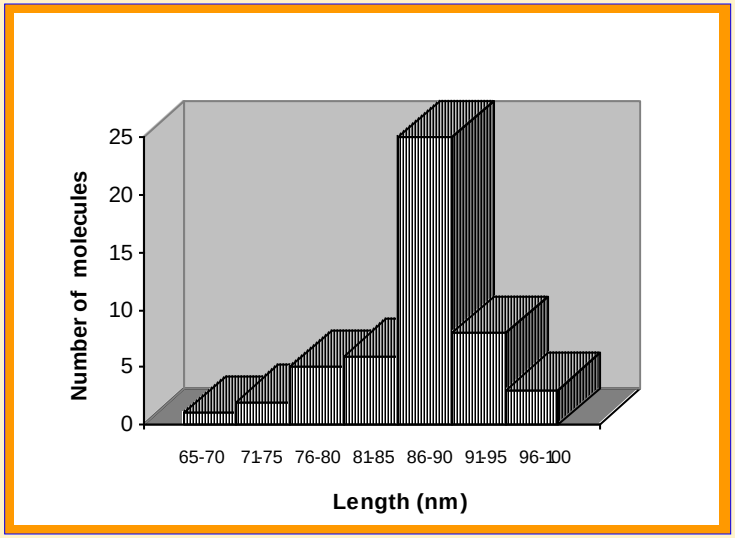
Effect of Famotidine and their Pd(II) and Pt(II) complexes on *Hmly*

Nucleic Acids Research, 1998

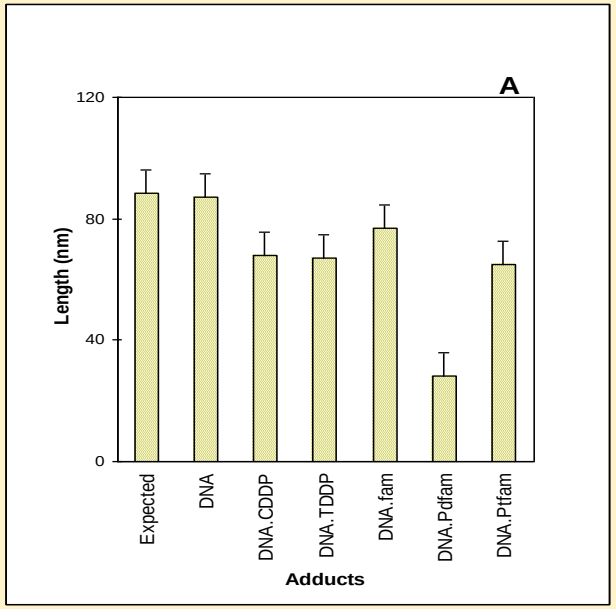
Dimensions of DNA and several DNA-complex adducts. (Standard deviation errors are ±1).

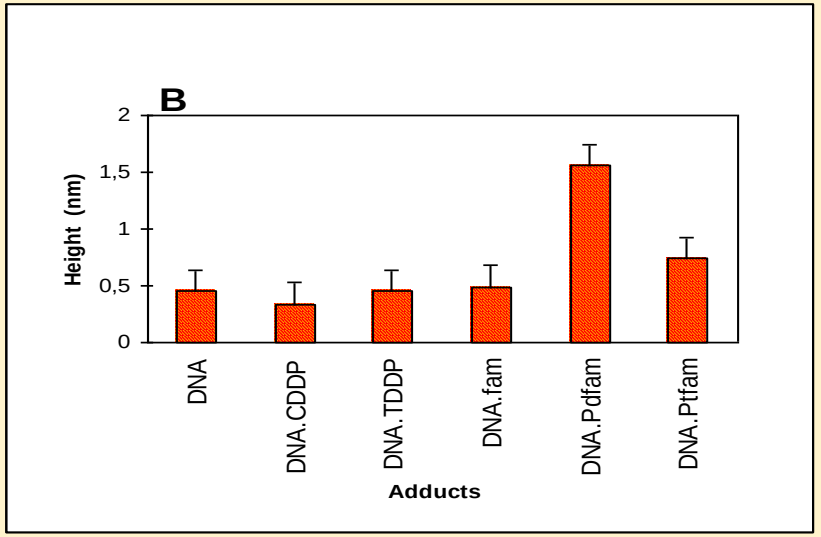
Adducts	Length (nm)	height (nm)	width(nm)
DNA	87±6.5	0.45±0.06	10.2±0.7
DNA.CDDP	68±9.0	0.35±0.15	13.1±0.6
DNA.TDDP	67±7.2	0.45±0.07	16.4±2.1
DNA.fam	77±7.9	0.50±0.07	14.6±0.6
DNA.Pdfam	28±20	1.57±0.38	**
DNA.Ptfam	64±12	0.74±0.16	22.2±3.5

** measurement of length and width are equivalents due to its oval shape.



Quantitative analysis of the TMAFM images. Histogram of the mean lengths of the free *hlyM* fragments. The apparent average length ± standard deviation was 87±6.5 nm for the 50% of the measured population.



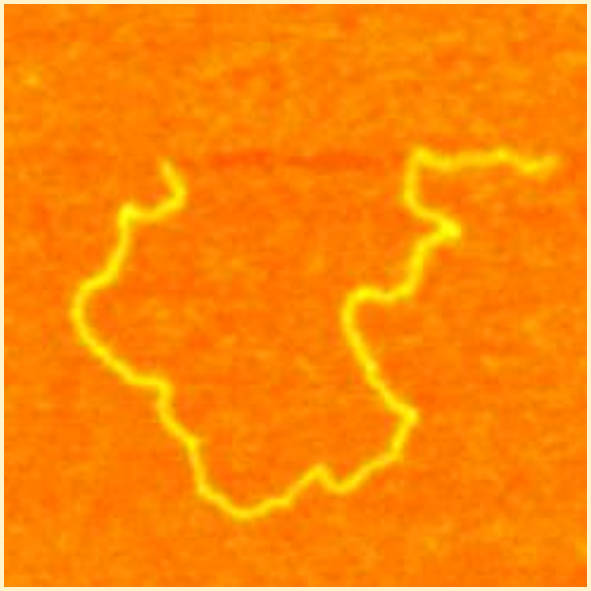


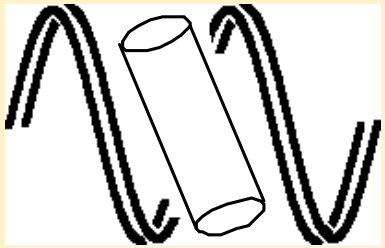
Graphical representation (error bars) of the standard deviation of the mean values for the different DNA-complexes adducts imaged.

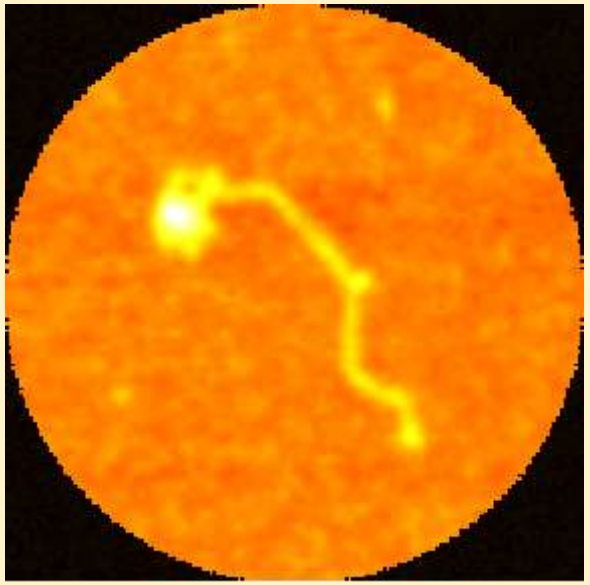
(A) Comparison between the different apparent mean values and the expected length for DNA.

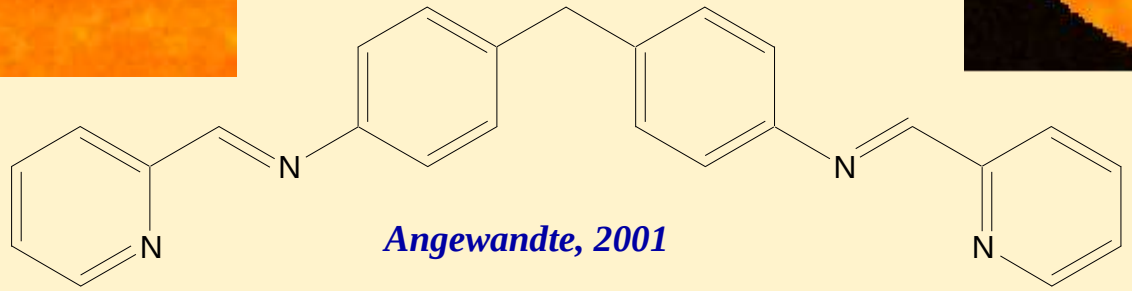
(B) Comparison between the apparent mean values and the expected height for DNA.

Binding of an iron-supramolecular cylinder to a linear fragment of plasmid DNA



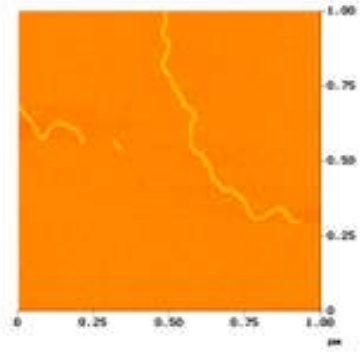




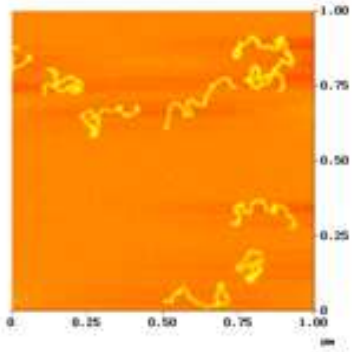


Angewandte, 2001

Linear fragment of pBR322

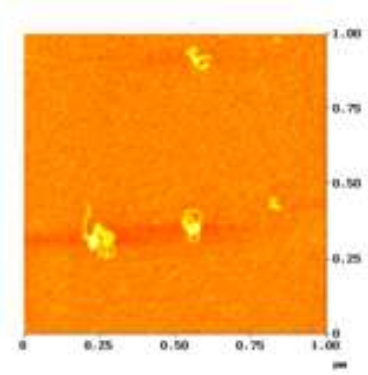


Iron complex

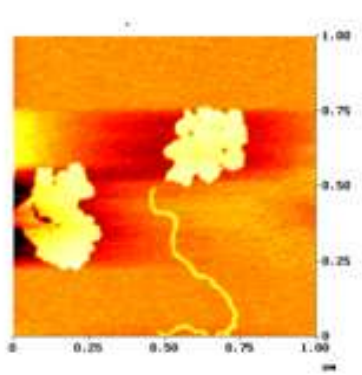


Iron complex

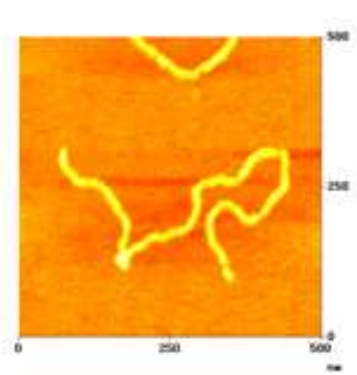
Medium concentration



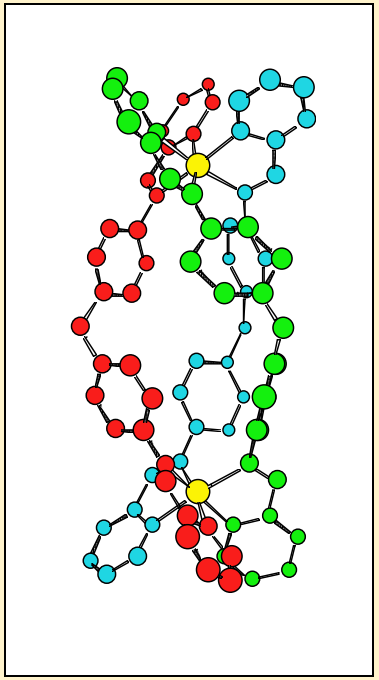
High concentration



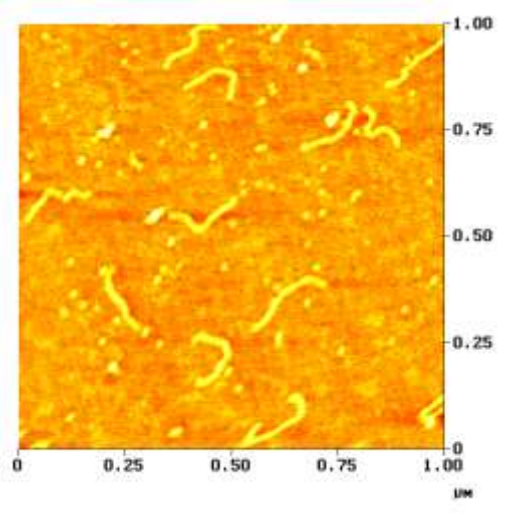
$Co(NH_3)_6Cl_3$



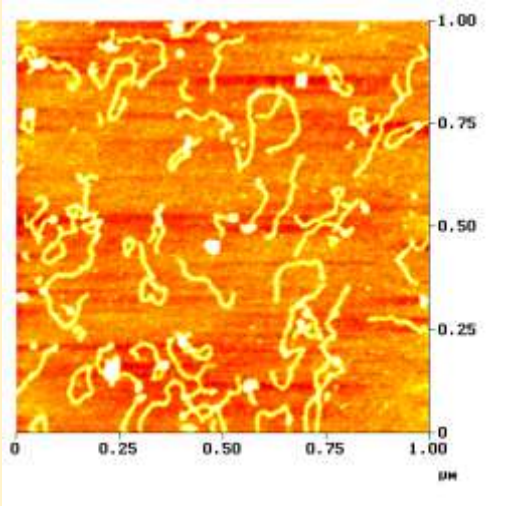
$Ru(phen)_3^{2+}$



Angewandte, 2001

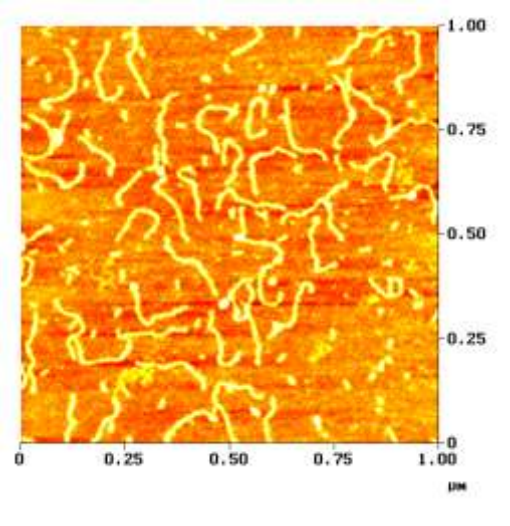


Linear DNA

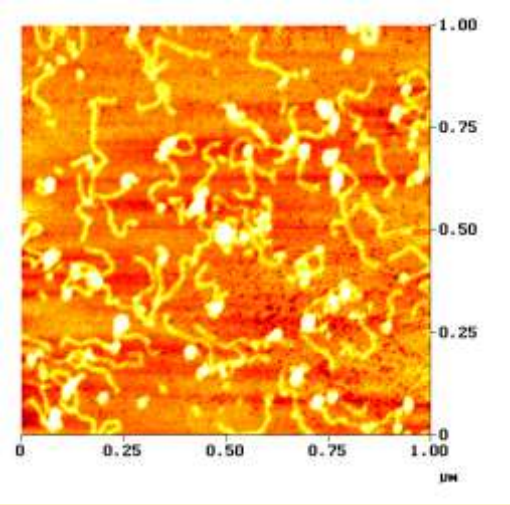


Effect of the racemic on the linear DNA

Effect of the P enantiomer on the linear DNA

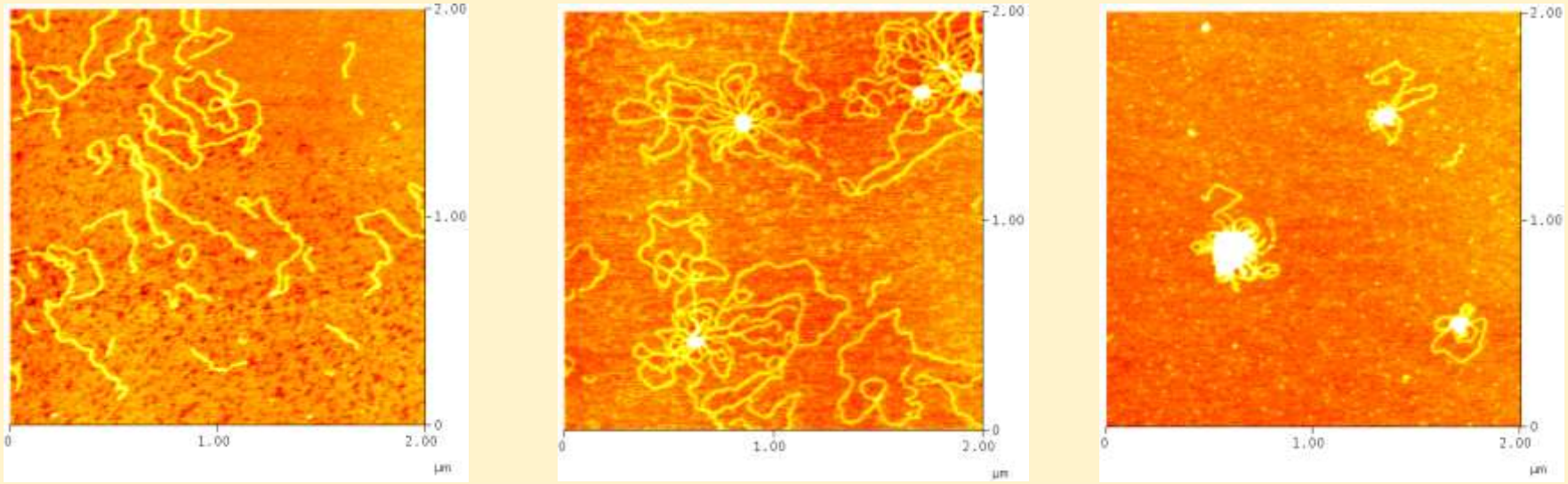


Effect of the M enantiomer on the linear DNA



PNAS, 2002

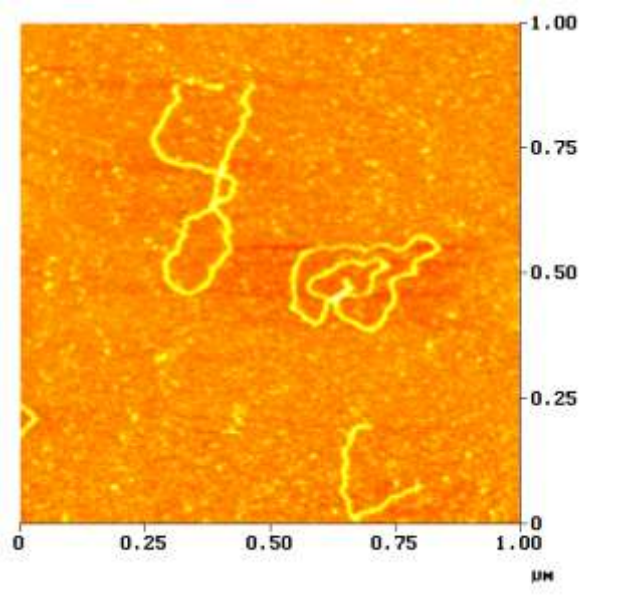
Calf Thymus DNA treated with Fe-cylinder at increasing relationships Fe:bp



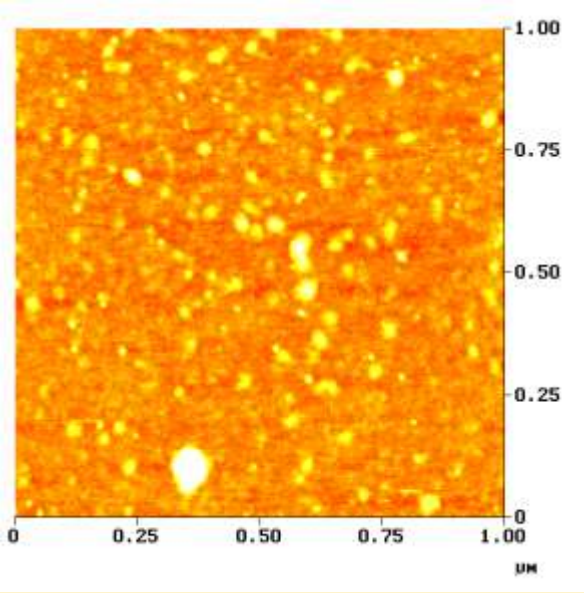
Fe:bp increasing

Nuclease activity of two copper complexes on pBR322 DNA

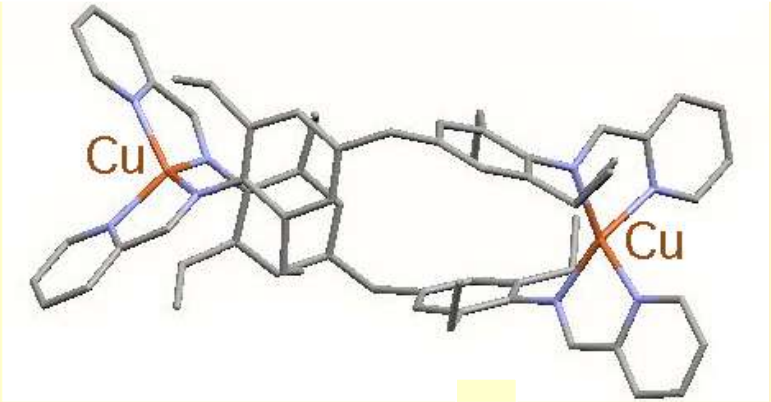
[Cu(L-val-gly)(phen)]

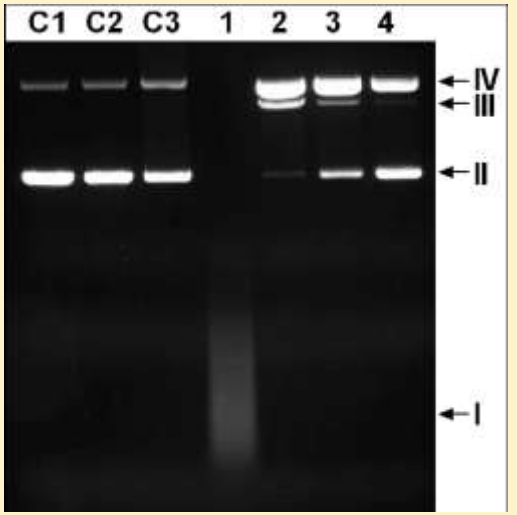


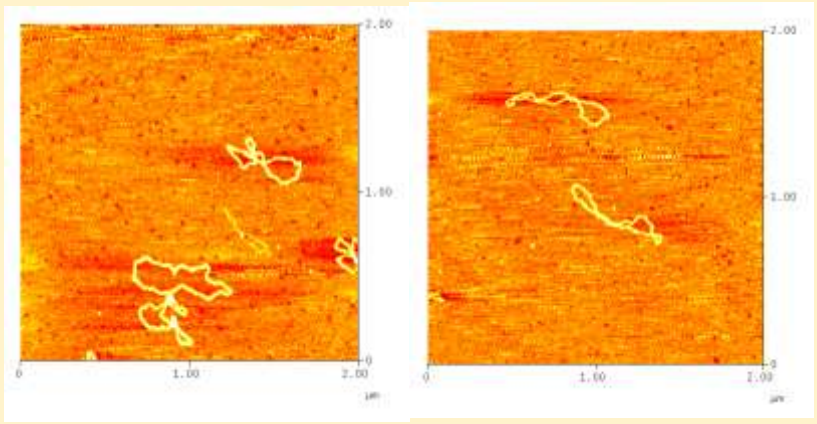
[Cu(gly-L-trp)(phen)]

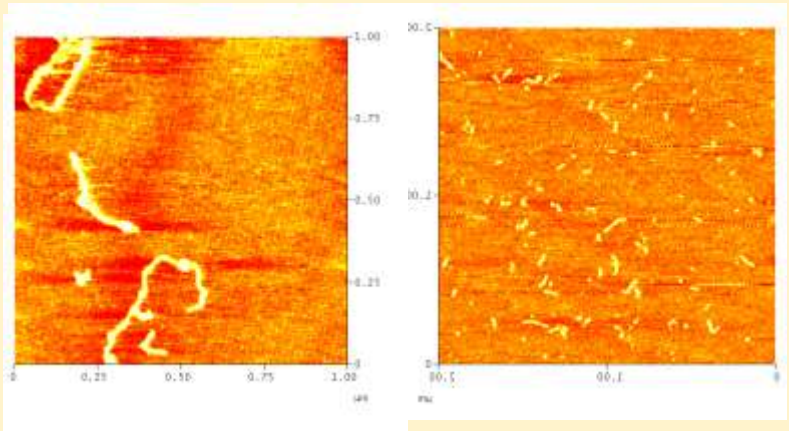


J.Inorg.Biochem., 2003







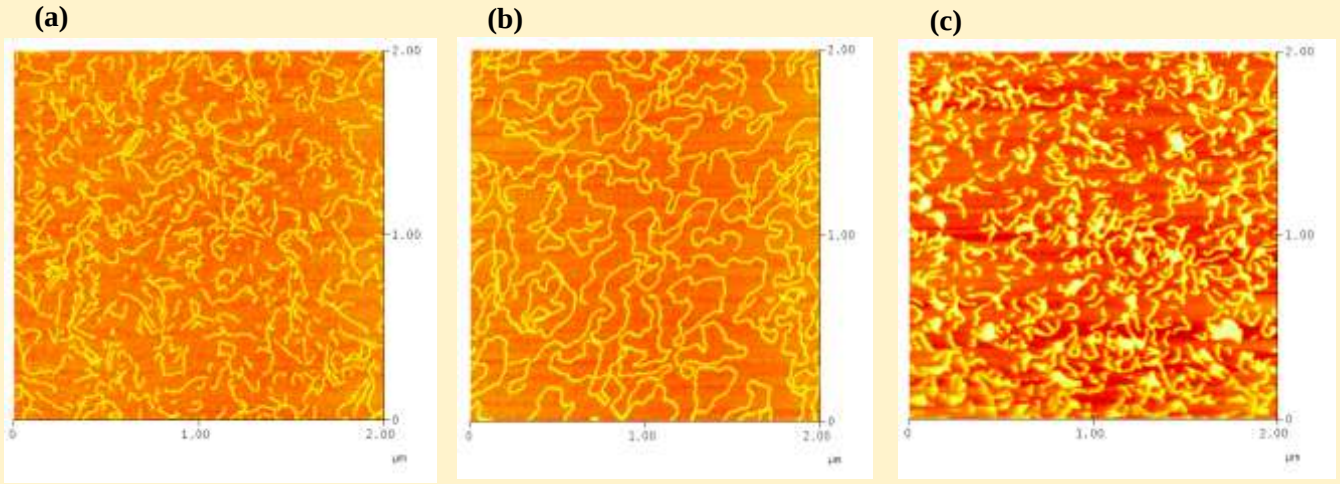


Electrophoresis in agarose gel: pUC19 incubated with $[Cu_2(L)_2]^{2+}$ and hydrogen peroxide (HP). Lane C1, free plasmid; lane C2, plasmid with HP. Lane C3, plasmid with $[Cu_2(L)_2]^{2+}$ at relationship 10:1 (bp : complex); lanes 1−4, with HP and modified with $[Cu_2(L)_2]^{2+}$ at respectively relationships 10:1, 20:1, 40:1 and 100:1. Band I, small fragments of DNA; band II, supercoiled plasmid; band III, linearised plasmid; band IV, relaxed plasmid. [HP] = 6.6 mM; [DNA] = 250 μM.

pBR322 DNA with (a) 6 μM Cu(I) cylinder and (b) 20 μM Cu(I) cylinder; (c) the same that a) with HP and (d) the same that b) with HP . All samples were incubated for 1 hour at 37 ºC.

Chemistry: A European J., 2006

Nuclease activity of copper casiopeinas



(a) Cas II-gly, [Cu(4,7-dimethyl-1,10-phenanthroline)(glycinate)]NO_3.

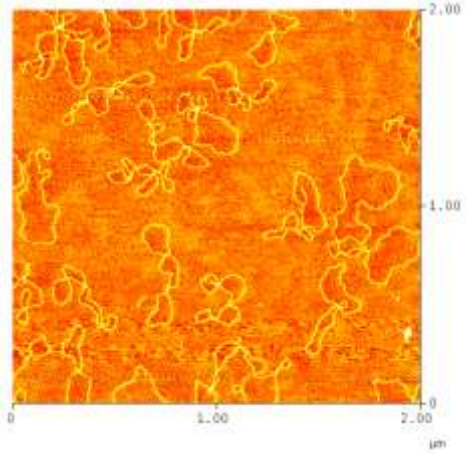
(b) Cas III-ia, [Cu(4,4'-dimethyl-2,2'-bipyridine)(acetylacetonate)] NO_3)

(c) Cas III-Ea, [Cu(4,7-dimethyl-1,10-phenanthroline)(acetylacetonate)]NO_3)

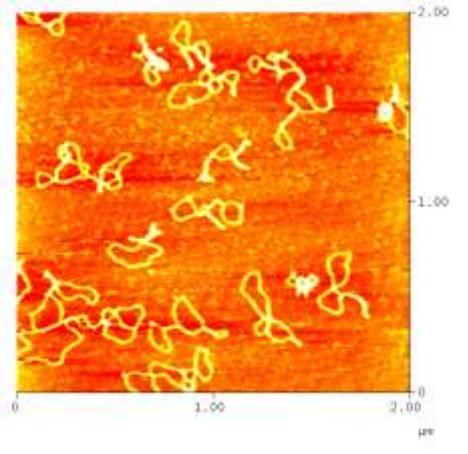
The three compounds were incubated with pBR322 DNA at 37 ºC for 20 h

L. Becco, M.E.Bravo, A. Rodríguez, M.J. Prieto, L. Ruiz-Azuara, B. Garat, V. Moreno, D. Gambino
New achievements on biological aspects of copper complexes Casiopeinas®: interaction with DNA and proteins and anti-Trypanosoma cruzi activity. Submmited

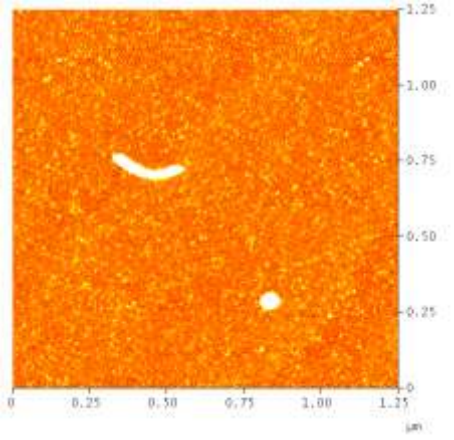
DNA pBR322



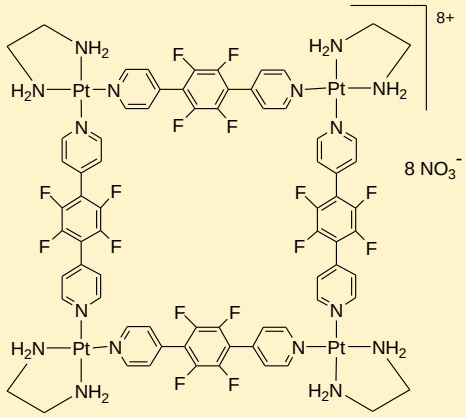
pBR322 + Pt square
(5 min incubation)



pBR322 + Pt square
(30 min incubation)

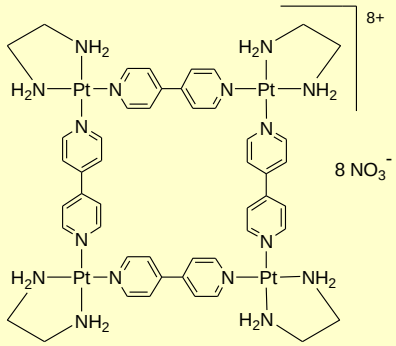


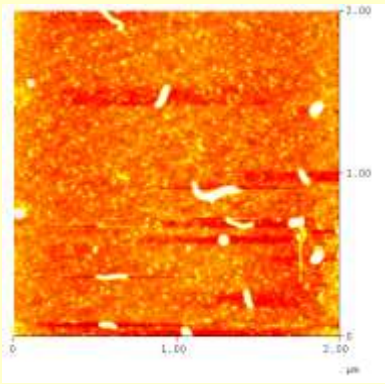
Effect of platinum supramolecular squares on plasmid DNA



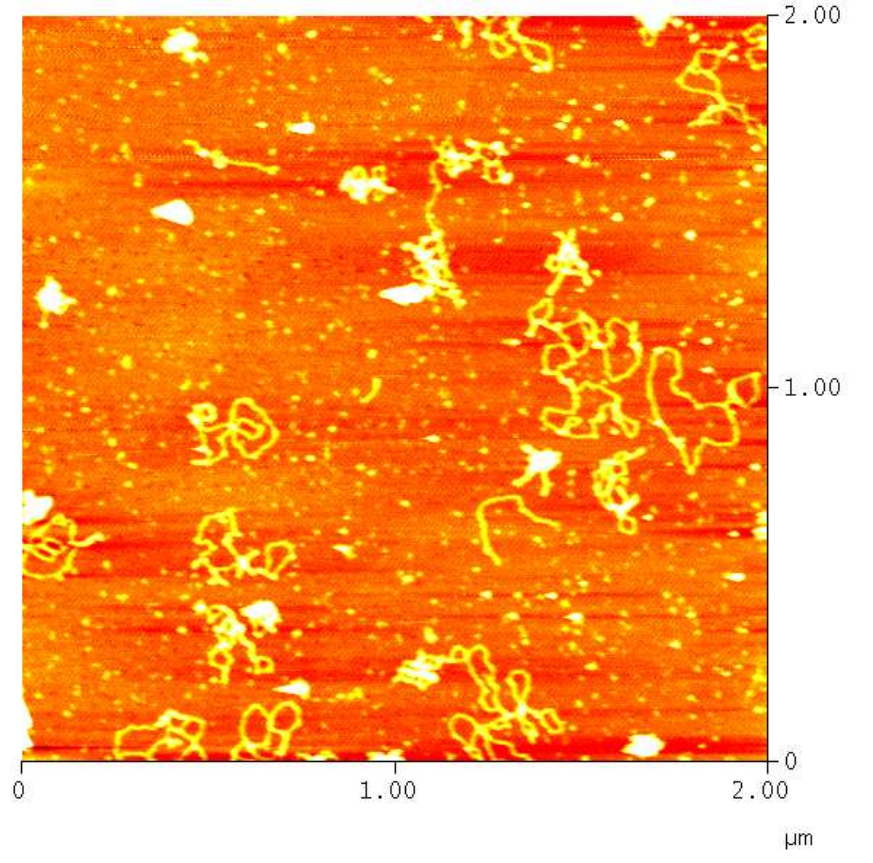
J.Inorg. Biochem. 2007

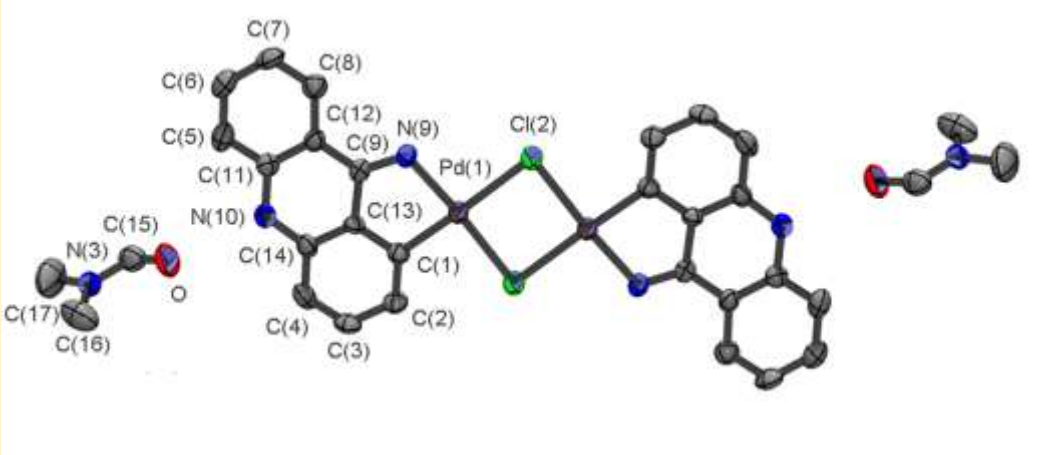
pBR322 + Pt square (same
concentration 5 min incubation)



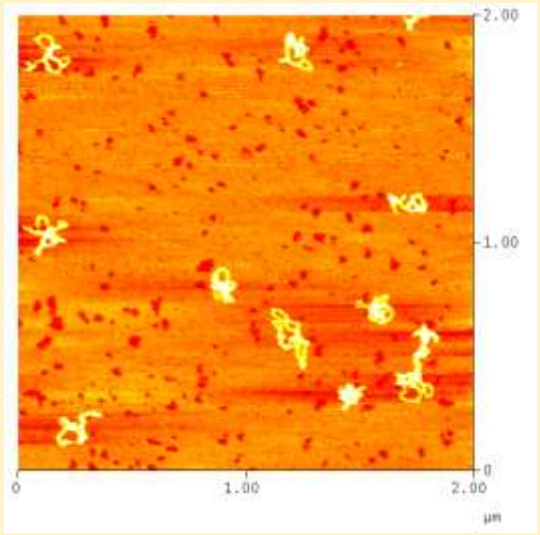


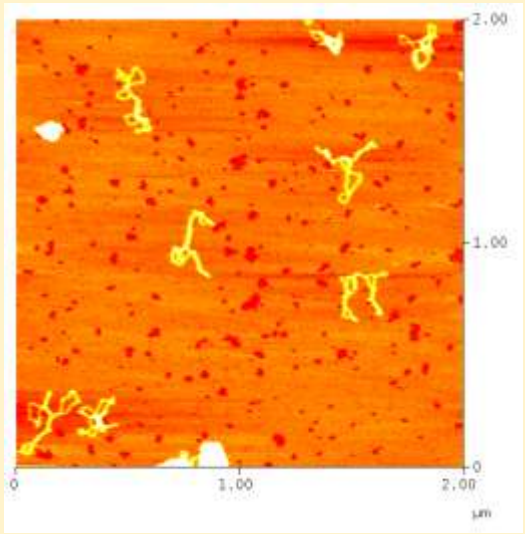
Effect of intercalation in pBR322 plasmid DNA of a di-palladium-9-aminoacridine planar complex





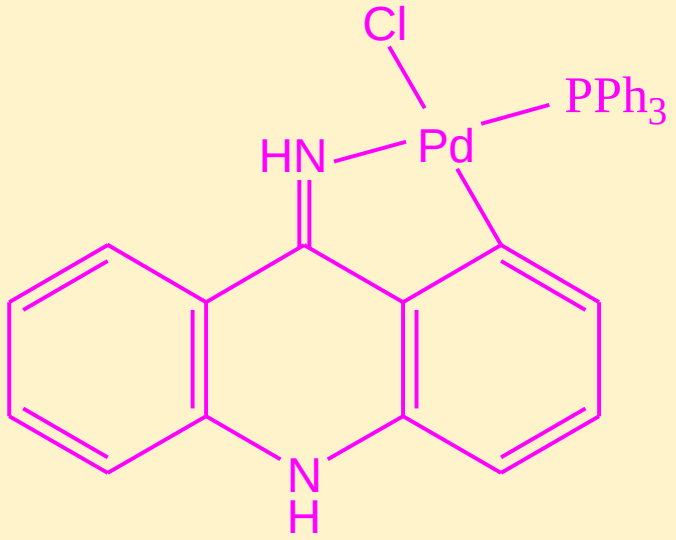
Bioinorganic Chemistry and Applications, 2007



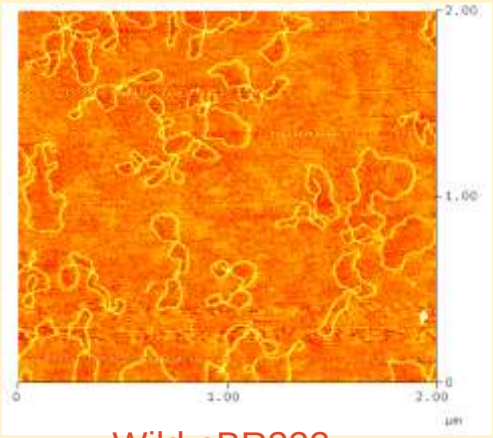


Strong effect on pBR DNA of the mononuclear complex

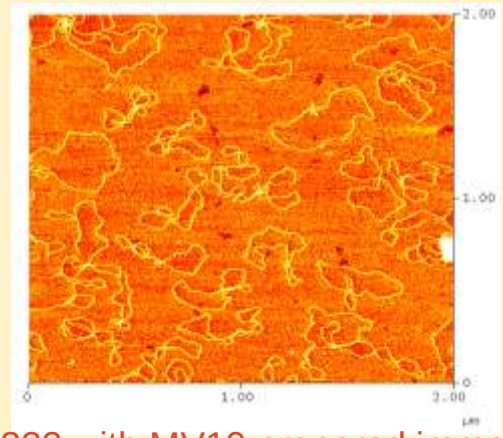
Palladium probably binds to the N of the bases the intercalation in addition to the intercalation of the acridine molecule



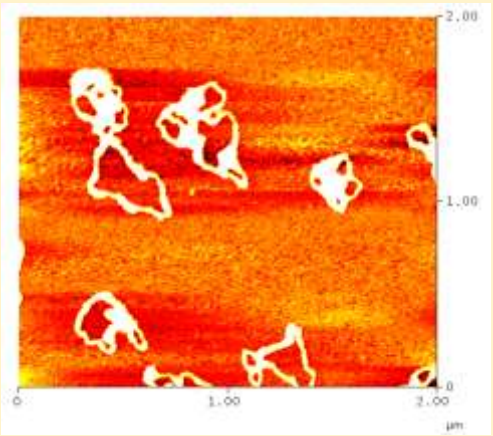
Bioinorganic Chemistry and Applications, 2007



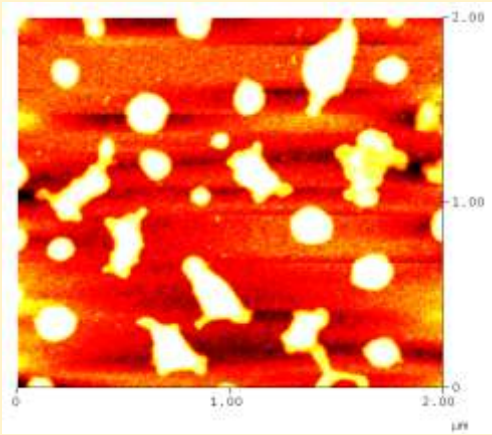
Wild pBR322



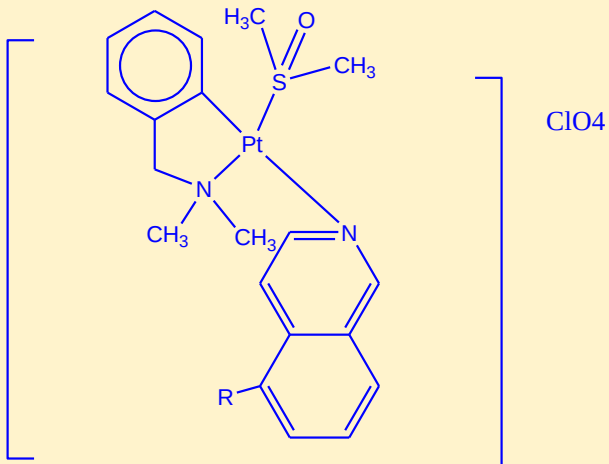
pBR322 with MV19 prepared immediately before imaged (25% diluted)



pBR322 with MV19 imaged after 1 h of incubation (25% diluted)



pBR322 with MV19 imaged after 1 h 30 min of incubation (25% diluted)



MV19

Spheres were observed using the usual relationship of compound : DNA. By dilution of 25% these images were obtained. Increasing the concentration of compound and/or the time of incubation, the strong interaction of the complex (probably formation of covalent bond by substitution of DMSO molecule by N7 of purine in DNA and intercalation of the planar ligands) produces aggregates forming spheres.

CONCLUSIONS

It is possible to image qualitative modifications caused by metal complexes on DNA

These modifications sometimes are due to formation of covalent bonds between metal ions and heterocycle nitrogen of the purine bases. In other cases non-covalent interactions (stacking, hydrogen bond, etc.) can be established between the ligands of the metal complexes and phosphate groups, ribose or bases

Metal ions with possibility of change their oxidation state can break the chains, acting as nucleases. This break can be observed by AFM

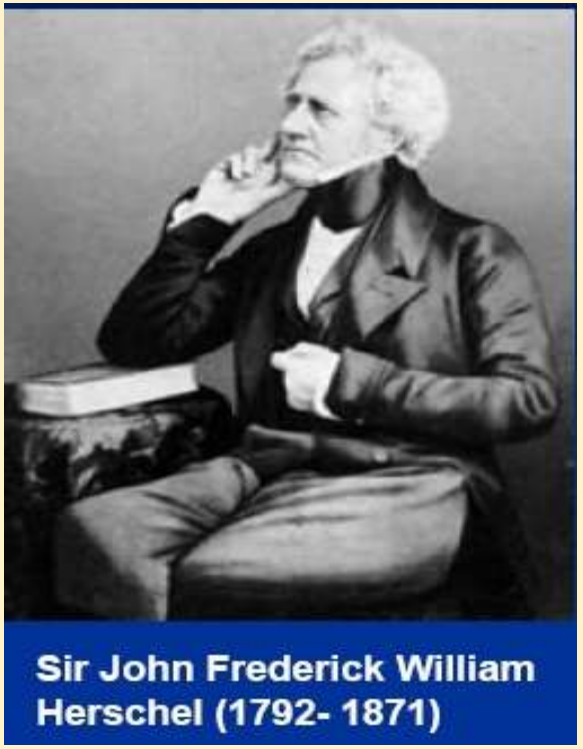
Quantitative analysis can be performed by statistical measurement of length, width and height of the forms observed.

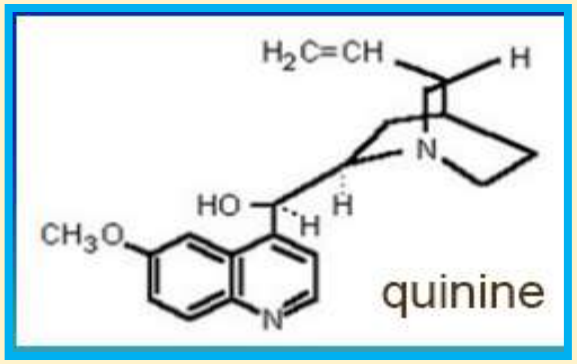
Useful information, complementary of other techniques can be obtained by the use of AFM

Molecular fluorescence

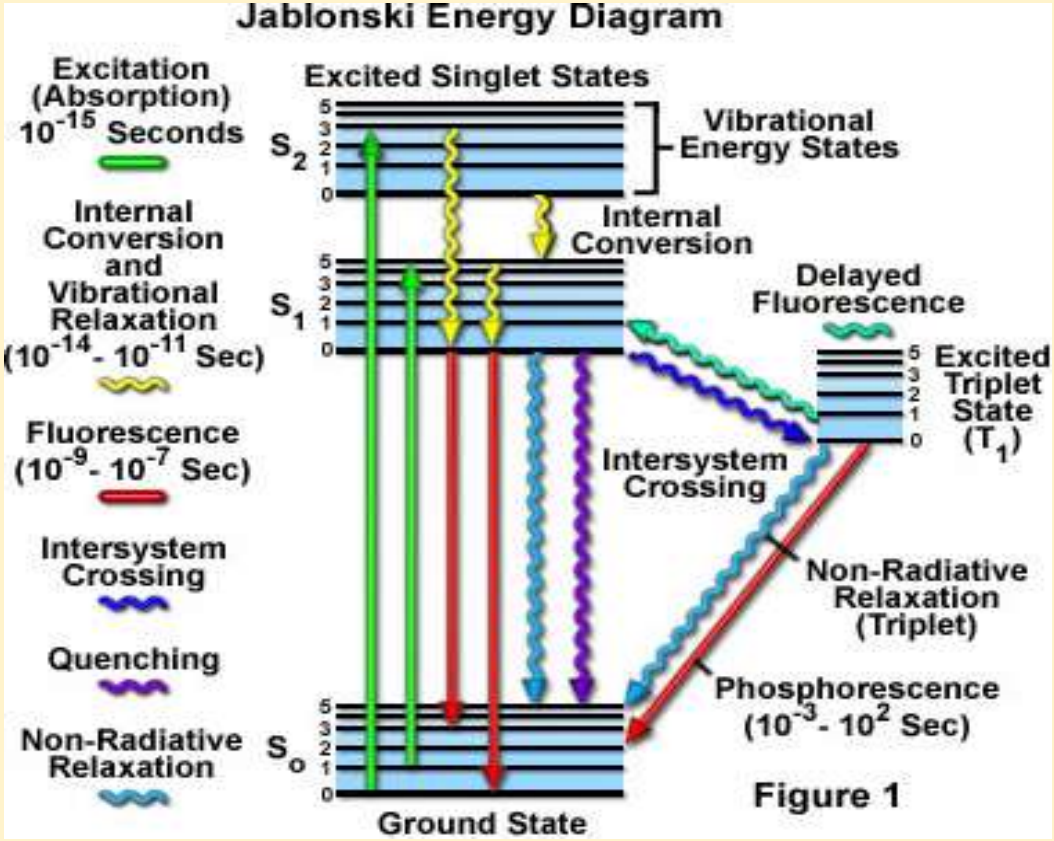
Looking for the binding mode of a metal ion compound to DNA

“The sulphate of quinine is well known to be of extremely sparing solubility in water. It is however, copiously soluble in tartaric acid. It is this solution which exhibits the optical phenomenon in question.
Though perfectly transparent and colorless, when held between the eye and the light, or white object, it yet exhibits in certain aspects, and under certain incidences of the light, an extremely vivid and celestial blue colour ….” (Sir John Frederick William Herschel)





Jablonski Energy Diagram

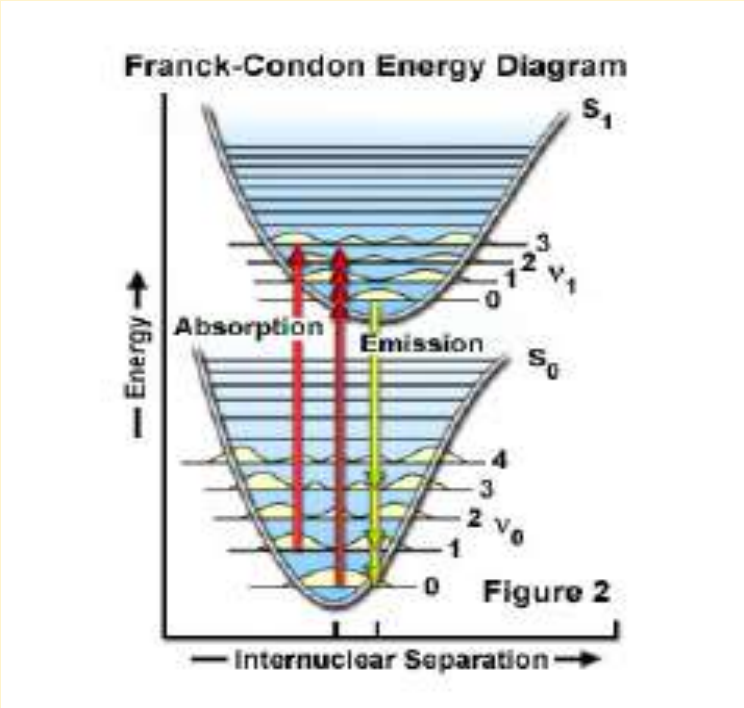


micro.magnet.fs.edu/.../jabintro/index.html

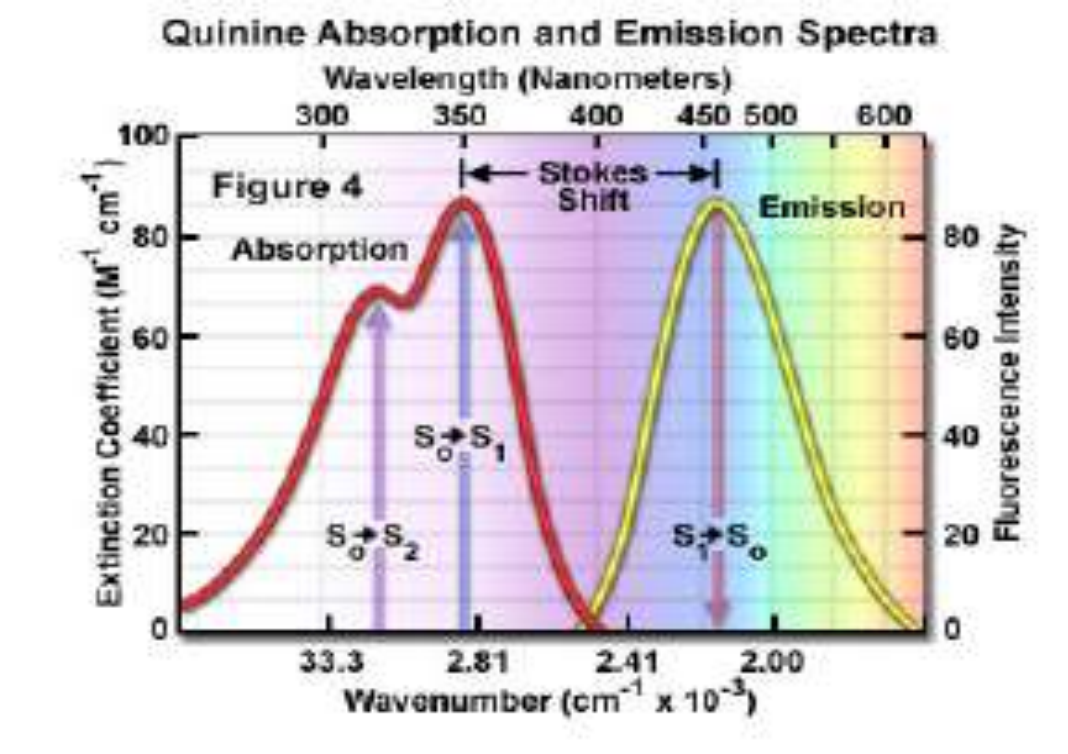
Four basic rules of fluorescence

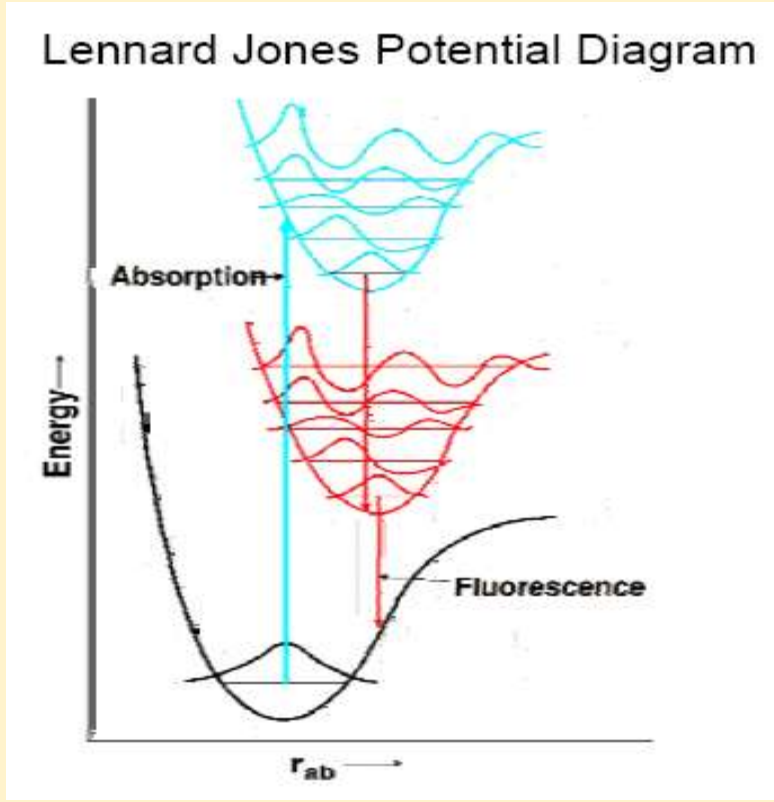
1. The Franck-Condon principle – the nuclei are stationary during electronic transitions, and so excitation occurs to vibrationally excited levels of the excited electronic state.

2. Emission occurs from the lowest vibrational level of the lowest excited singlet state because relaxation from the excited vibrational levels is much faster than emission.



3. The Stokes shift: emission is always of lower energy than absorption due to nuclear relaxation in the excited state.





4. The mirror image rule: emission spectra are mirror images of the lowest energy absorption band

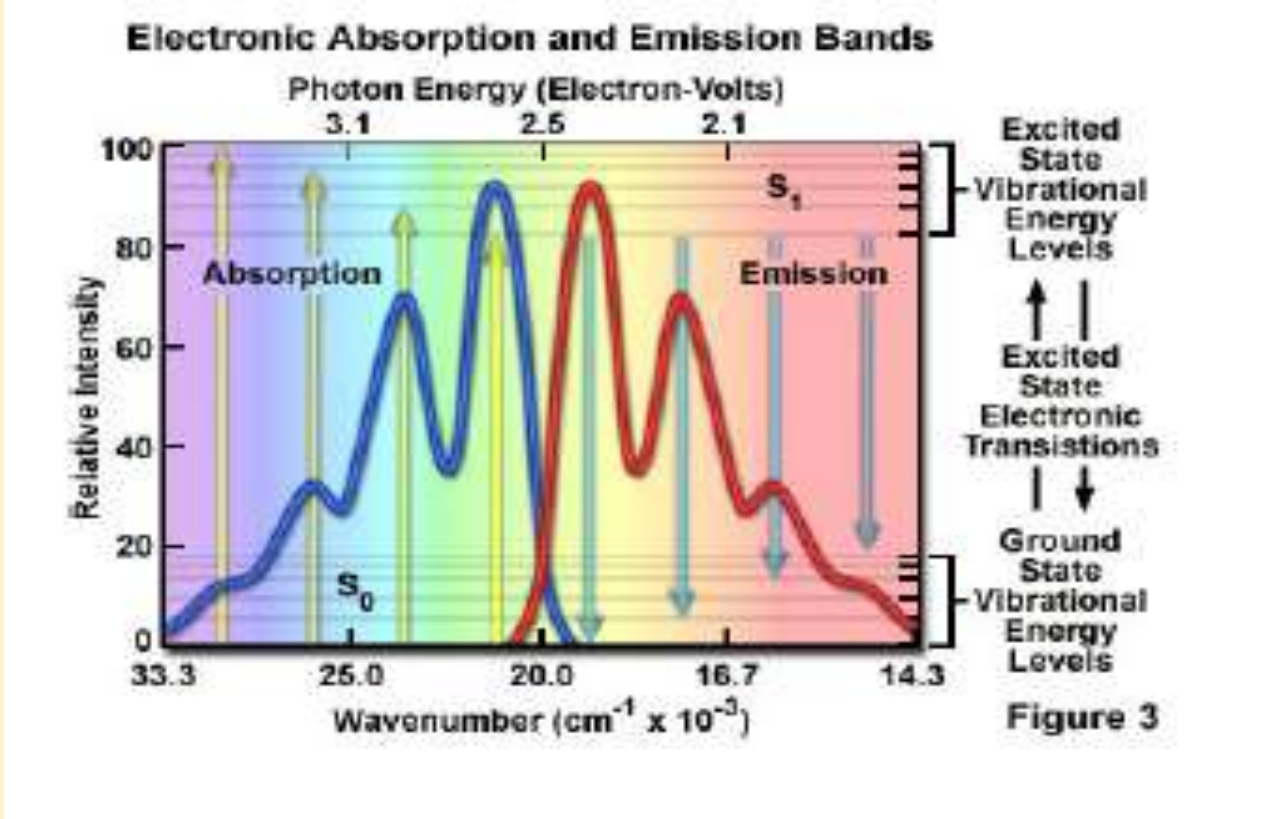


Figure 3

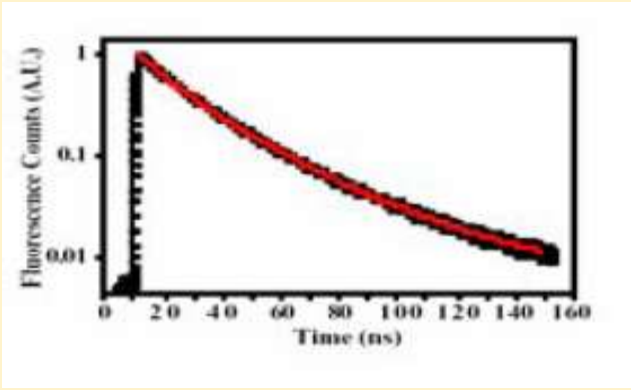
Fluorescence Lifetime

Question: how quickly do excited molecules relax back to the ground state?
Since emission is a spontaneous process, its rate is proportional to the concentration of molecules in the excited state [A*]:

$$\frac{d[A^*]}{dt} = k_f[A^*]$$

Exponential decay:

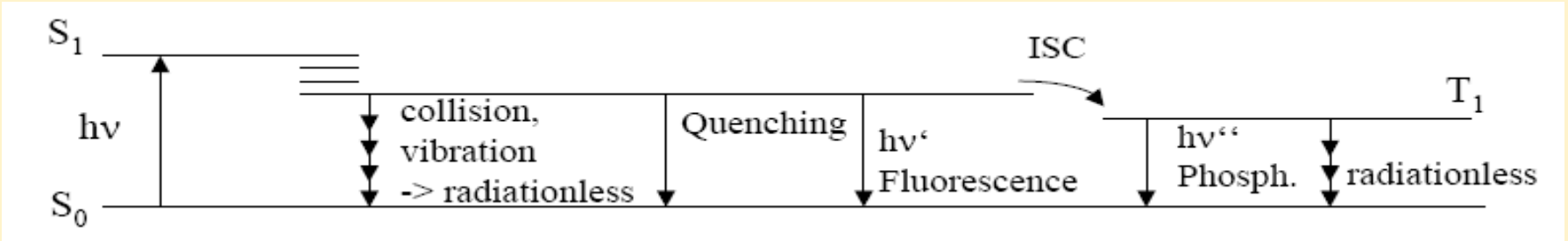
$$A^*(t) = A_0 * \exp(-t/\tau_f)$$



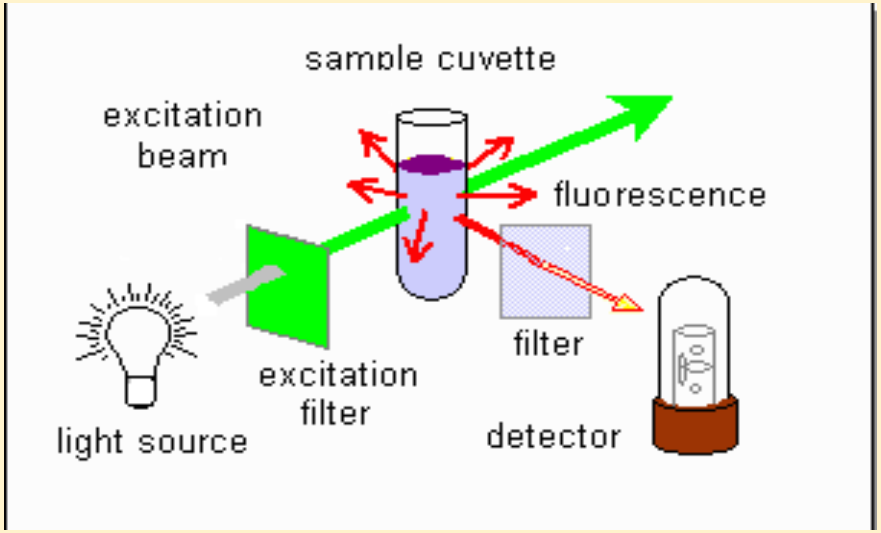
The experimentally determined excited state lifetime is always smaller than the theoretical one

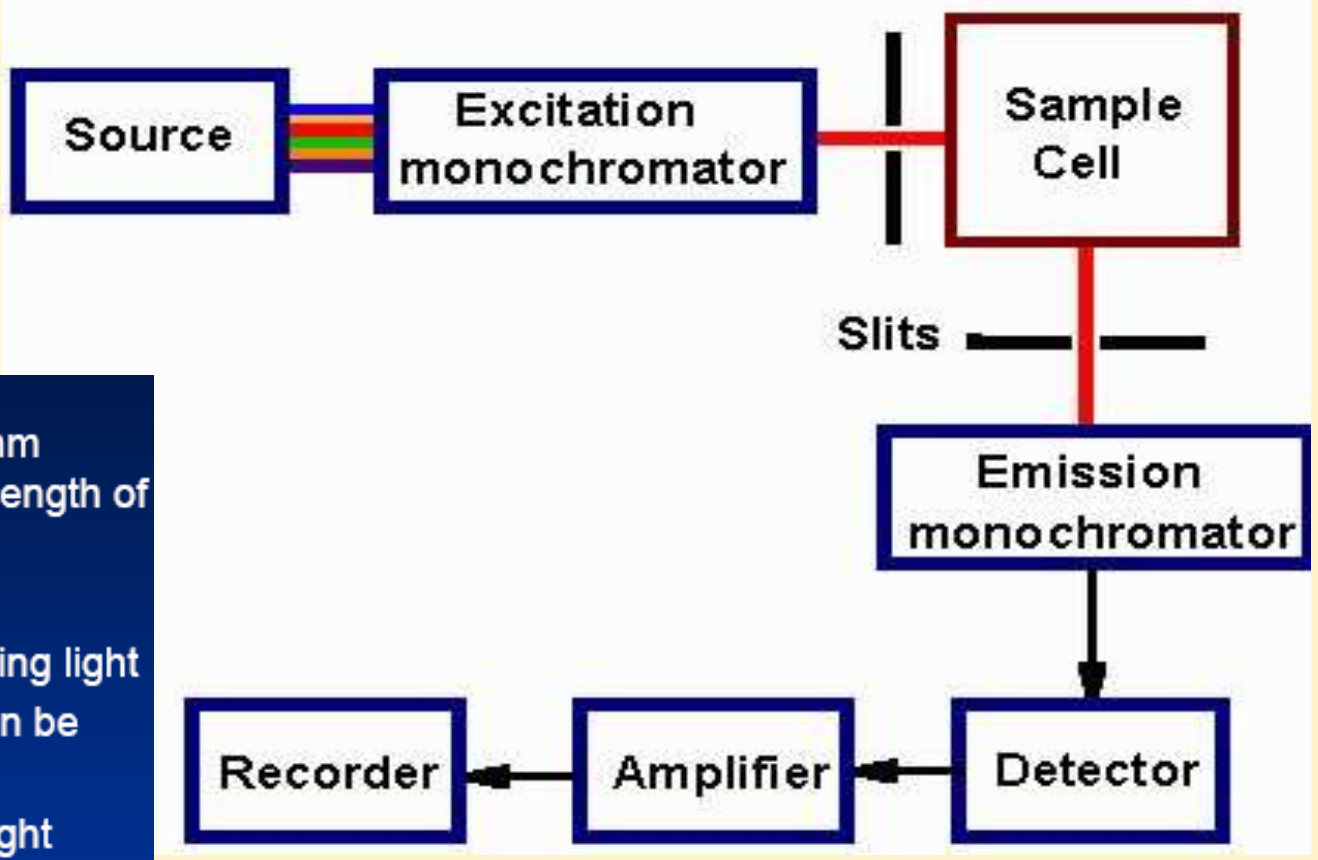
$$\tau_{ex} = \frac{1}{k_{fluor} + k_{nonfluor}} \Rightarrow \tau_f = \frac{\tau_{ex}}{q}$$

The larger the quantum yield, the longer τ_{ex}



The equipment….





-xenon lamp source (common)
-emits light at high intensity from 250 – 650 nm
-excitation monochromator selects the wavelength of the exciting light

-sample compartment

-emission monochromator to select the emitting light

-both monochromators are motorized and can be varied to scan a selected wavelength range

-photomultiplier tubes to detect the emitted light

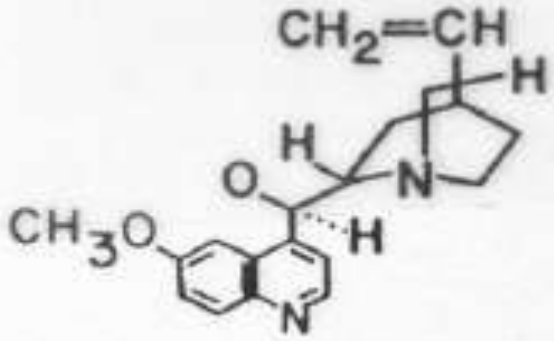
-interface to a computer

-the photon output (intensity) of the lamp source is not constant over the wavelength spectrum

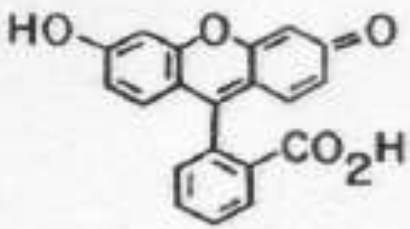
- -detector is also not equally efficient at detecting photons of all wavelengths
- -fluorescence spectra must be **corrected** for these factors

Monochromator.doc

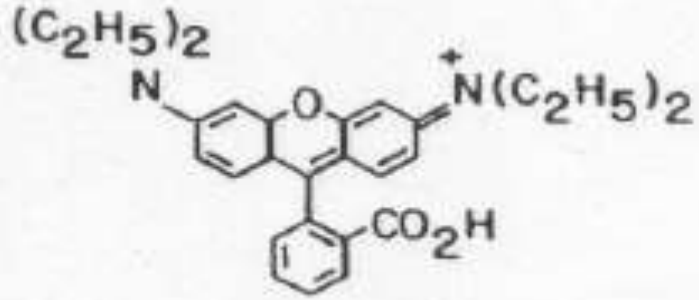
-fluorescent compounds are generally either aromatic, or have conjugated double bond systems



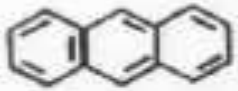
QUININE



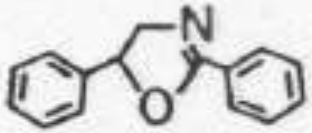
FLUORESCEIN



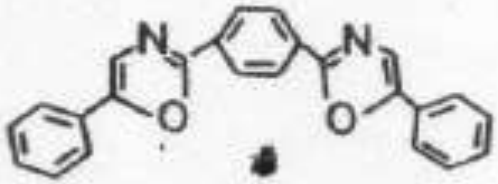
RHODAMINE B



ANTHRACENE

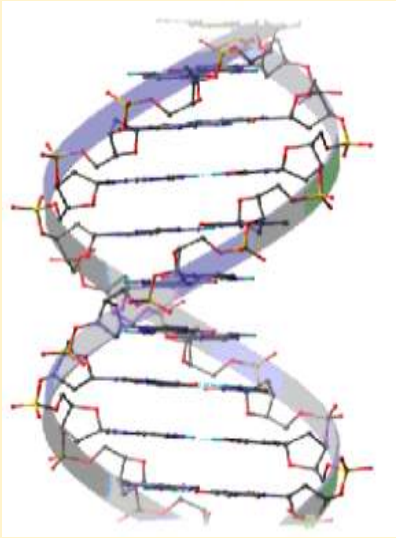


PPO

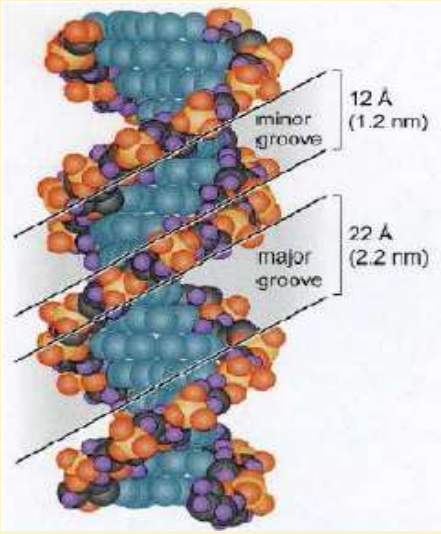


POPOP

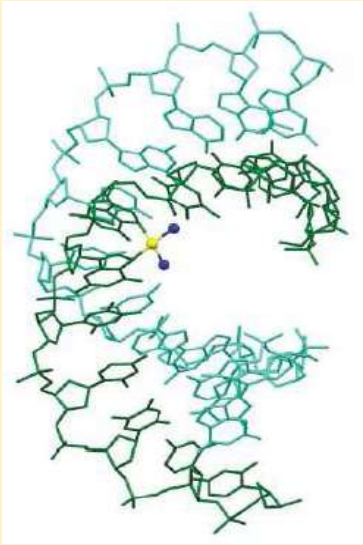
Application of fluorescence to determine the mode of binding of metal complexes to DNA



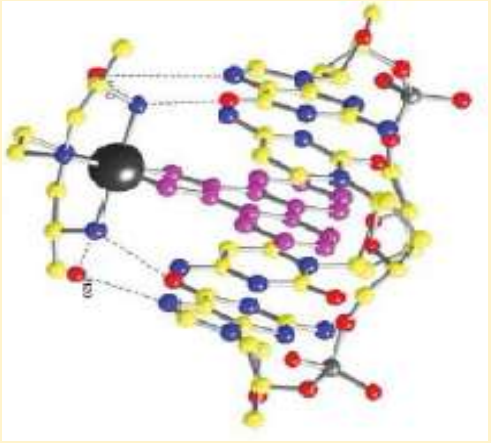
DNA-B

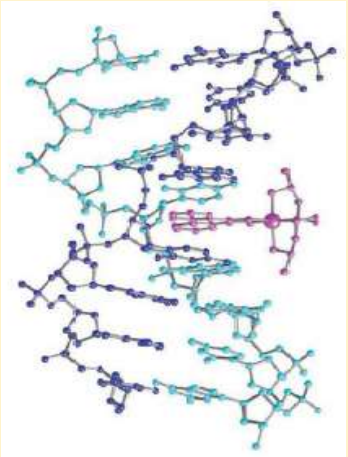


Structural data

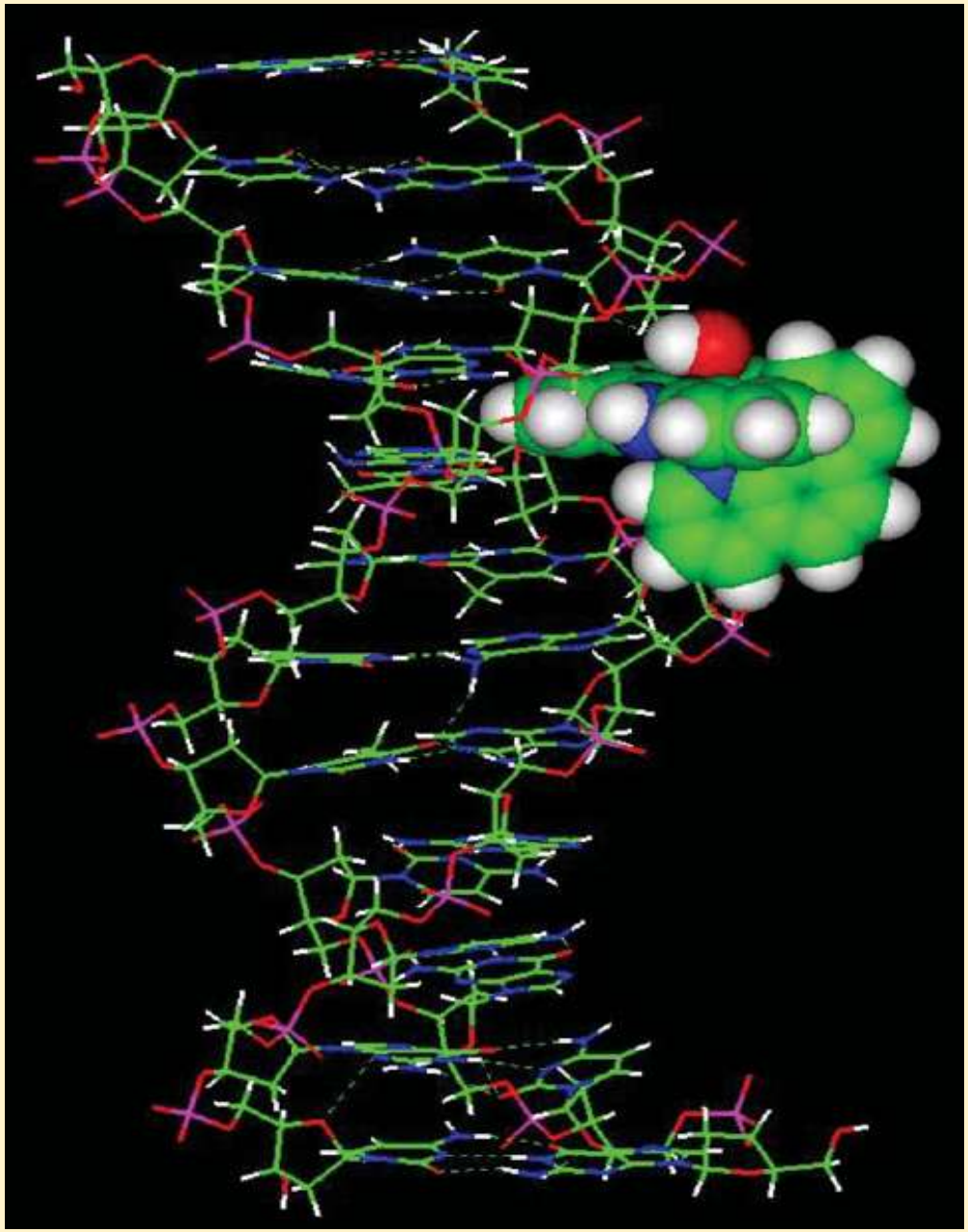


View of structural changes produced by covalent bifunctional binding

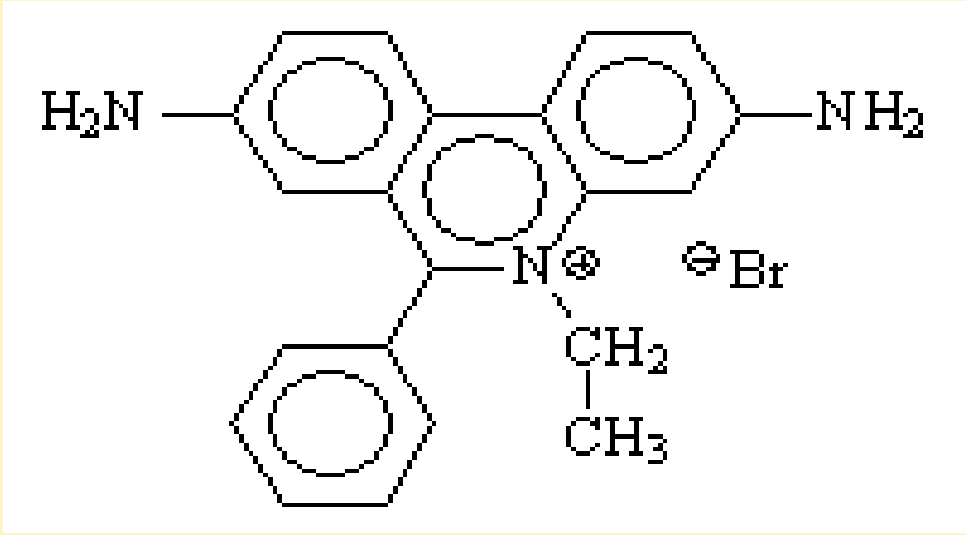


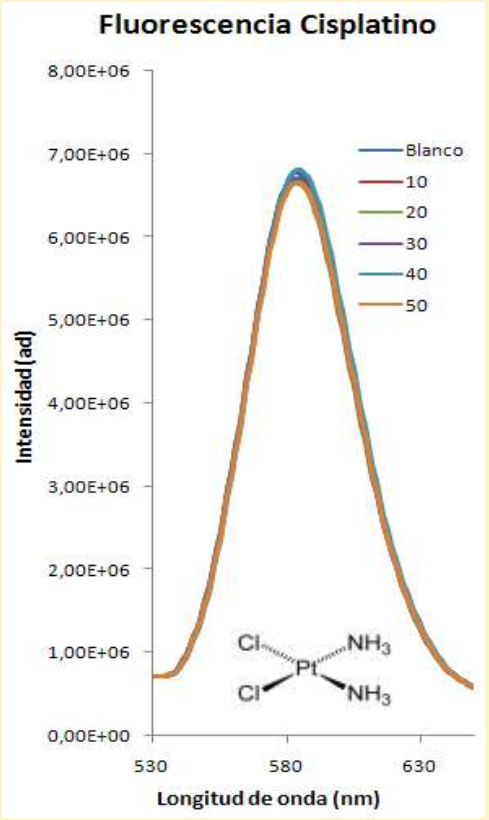


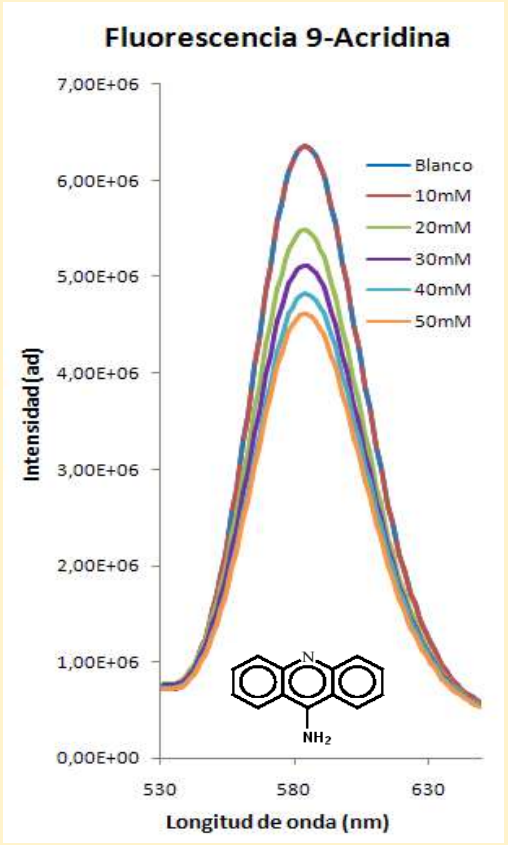
View of structural changes produced by intercalation

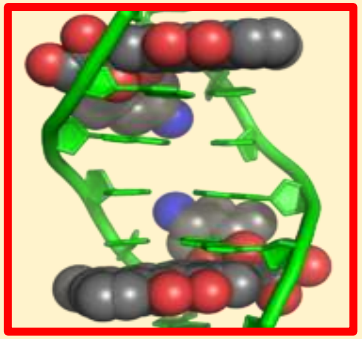


Ethidium bromide

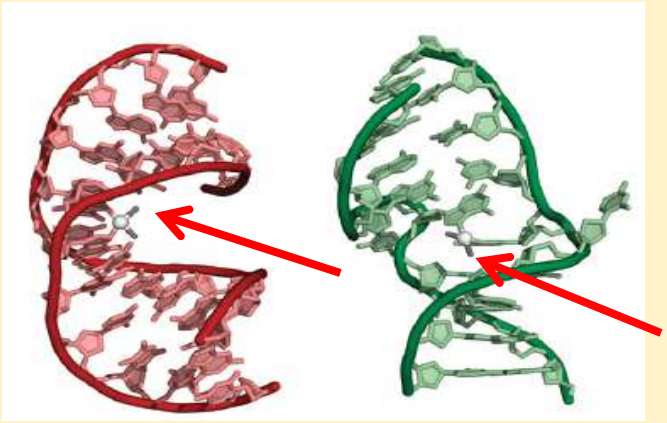




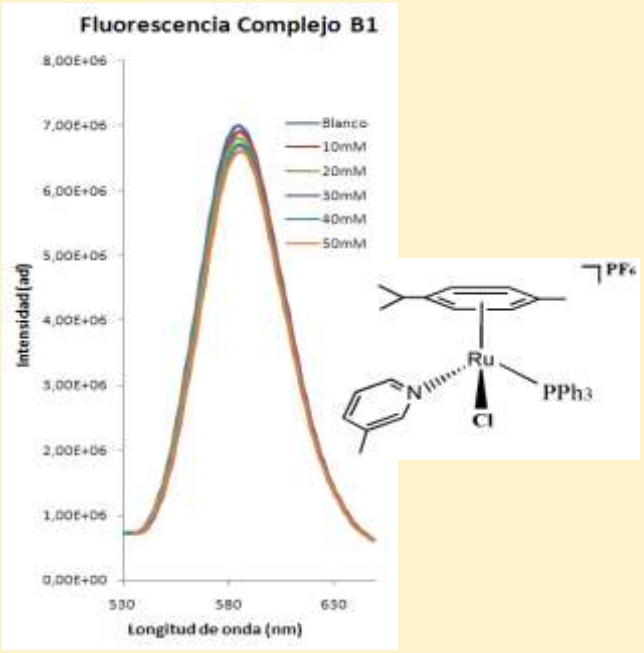


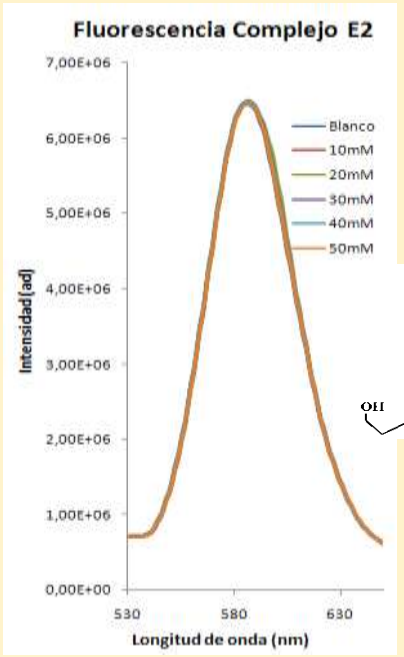


Intercalación

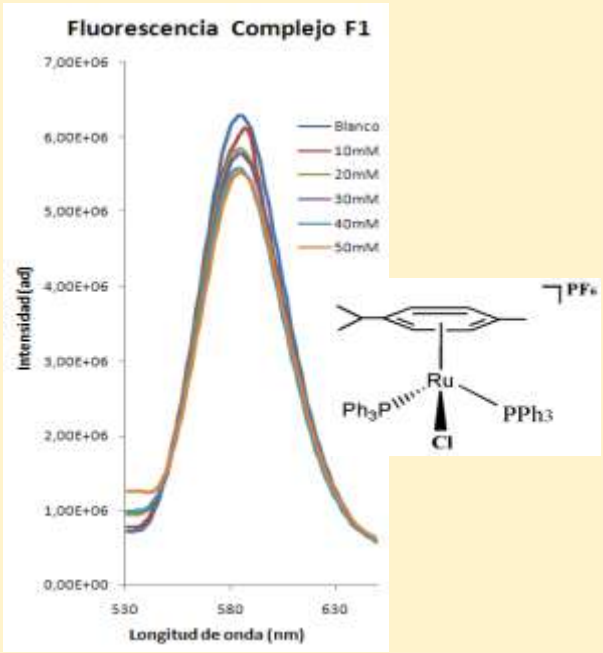


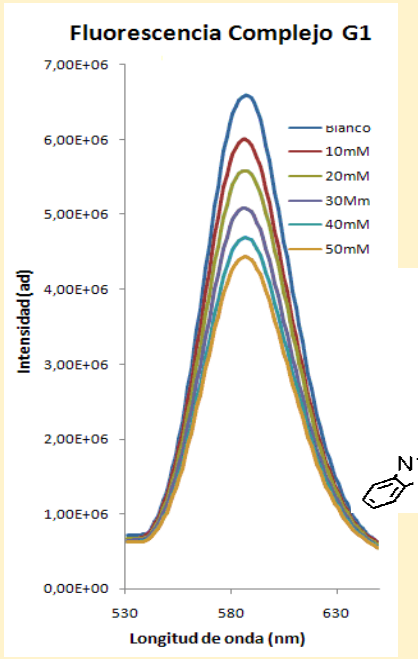
Interacción covalente

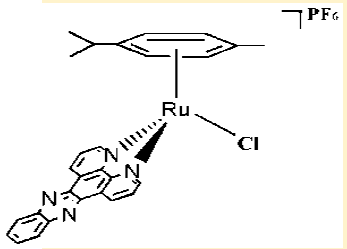




The complexes that bind DNA by covalent or other kind of interaction different than intercalation do not displaced ethidium bromide from DNA and the fluorescence remains.







Ethidium bromide is displaced by intercalator complexes, and the fluorescence decreases with the concentration

Sometimes a phenomenon can occure…

Fluorescense resonance energy transfer (FRET)

***Takes place in the excited state**
*** Occurs when the emission spectrum of one fluorophore (the donor) overlaps the absorption spectrum of another molecule (the acceptor)**
*** Does NOT involve emission and rebsorption of light; the donor and acceptor are coupled by a dipole-dipole interaction**
*** If the acceptor is also fluorescent we see a decrease in the emitted donor fluorescence (quenching of the donor) and emission of light by the acceptor at characteristic l em (ensitized emission by the acceptor)**

Fluorescense resonance energy transfer (FRET)

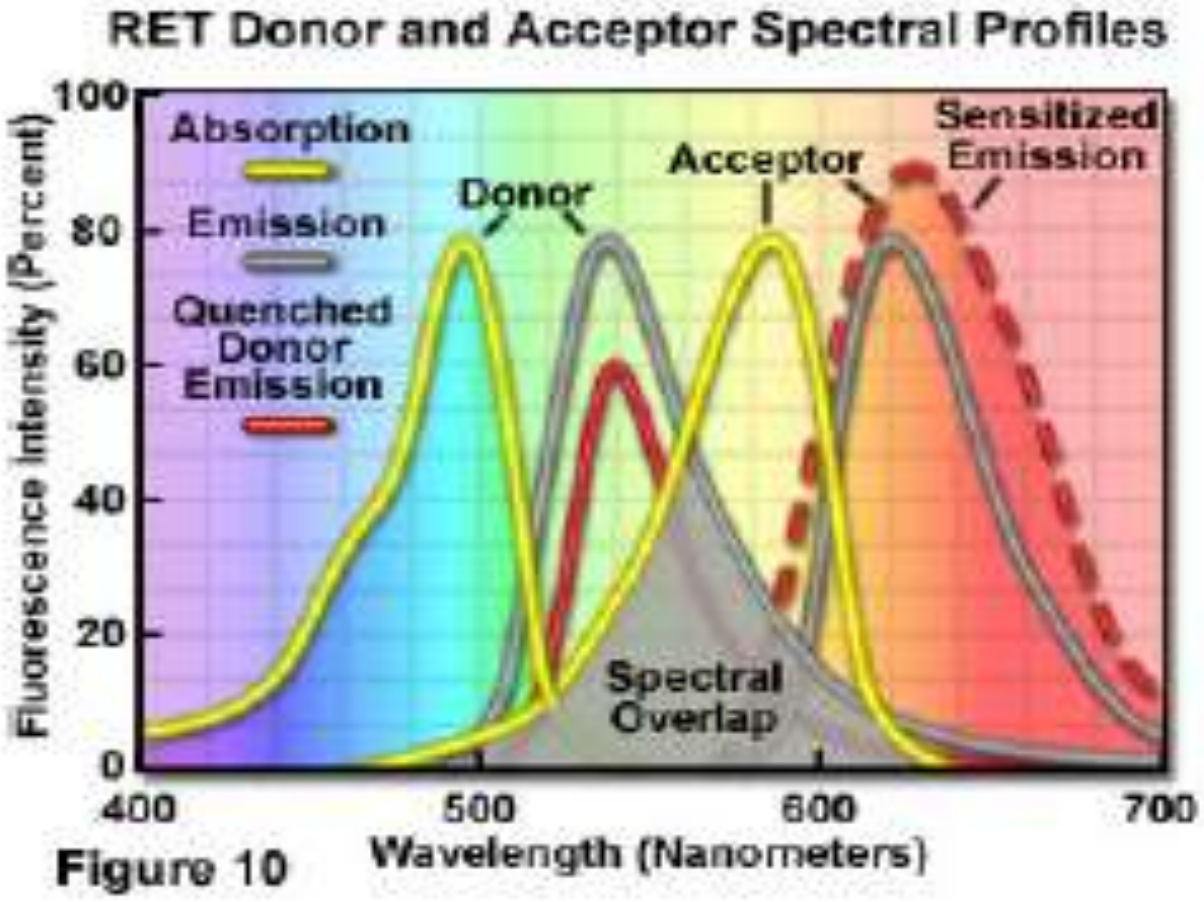
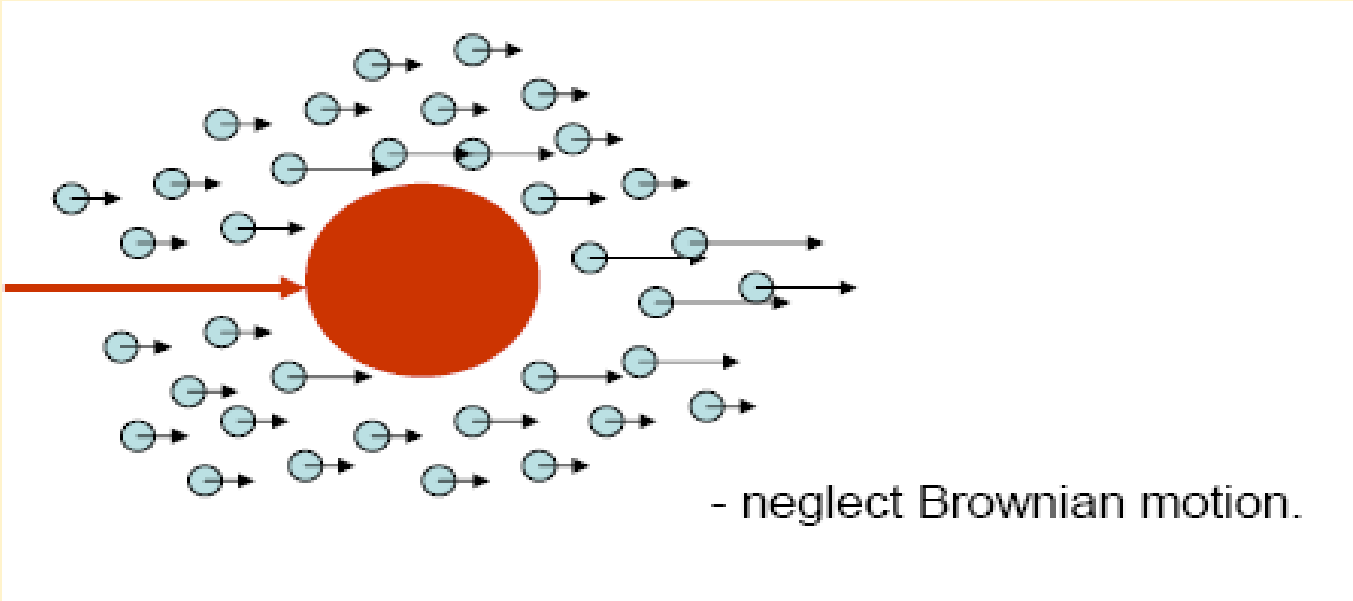


Figure 10

Viscosity Measurements

Viscometry

When a macromolecule moves in solution (e.g. of water), it induces net motions of the individual solvent molecules, i.e. the solvent molecules will feel a force.



Viscosity, then, which is also determined as a viscosity coefficient, is a measure of a fluid's resistance to flow.
It is the substance constant indicating the magnitude of the fluidity of a fluid. In general, viscosity is associated only with liquid.

When rotating a drum container filled with water on its vertical central axis, the water that was at rest in the beginning starts moving as it is dragged by the container's inside wall and then whirls completely together with the container as if it were a single rigid body.
This is caused by the force (resistance) generated in the direction of the flow (movement) on the surfaces of the water and the container's inside wall.

A fluid that generates this kind of force is regarded as having viscosity.

Viscous fluid is divided into two broad categories (constant temperature):

- **Newtonian fluid, subject to Newton's law of viscosity, viscosity is constant regardless of the flow (movement).**

- **Non-Newtonian fluid, which is not subject to Newton's law of viscosity, viscosity changes according to the flow (movement).**

Temperature is a very important factor for measuring viscosity. In fluids, as temperature goes up, viscosity goes down and *vice versa*. In the case of distilled water, if the temperature changes 1 centigrade, it produces a difference of 2 % to 3 % in viscosity.

As viscosity units, P (poise) , cP (centi-poise) – CGS unit system
Pa.s (pascal second), mPa.s (mili pascal second) – SI unit system

The viscosity of distilled water is 1.002 mPa.s at 20.00 centigrade at 1 atm

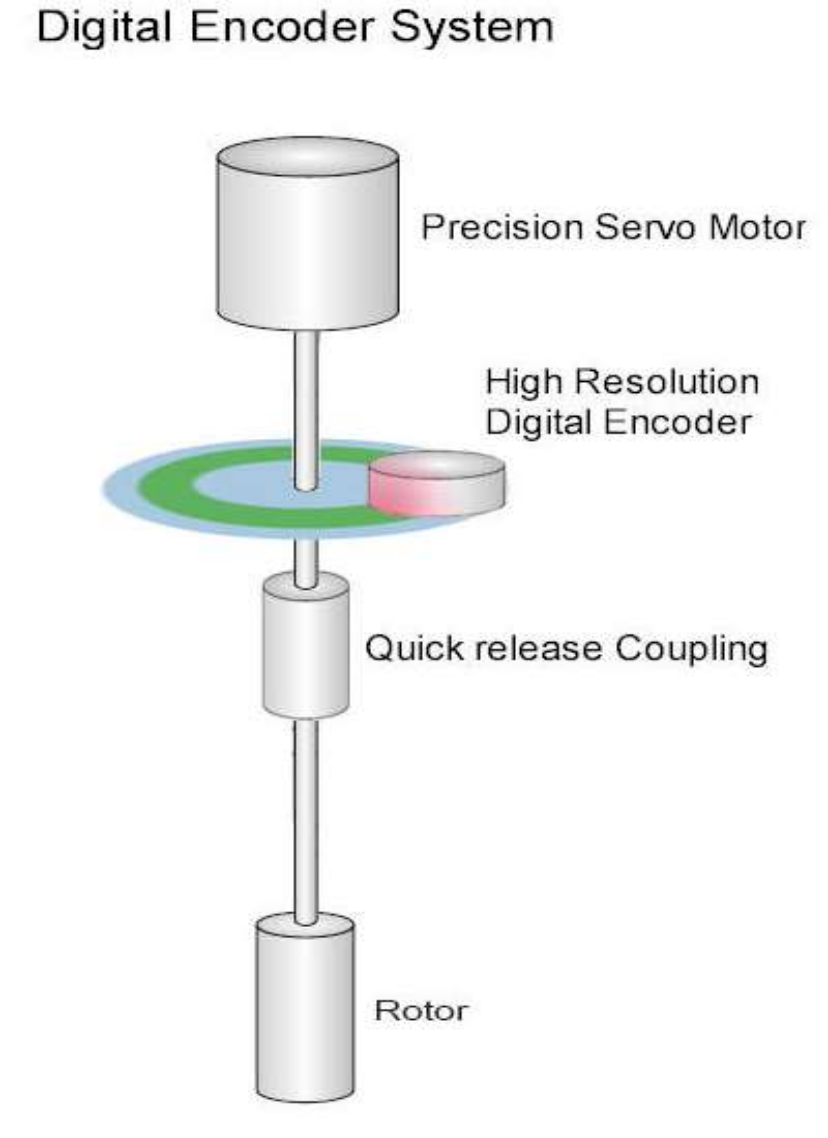
Finally, the Poiseuille's law allow to relate velocity of flow with different parameters and the viscosity η

$$\frac{dV}{dt} = \frac{\pi P a^4}{8 \eta l}$$ Poiseuille's law

MEASURING VISCOSITY

Several types of viscometers exist and are classified into five types by their measurement principles.

1. Vibro Viscometer: Measures viscosity by controlling the amplitude of the transducer immersed in a sample and measuring the electric current that drives the transducer

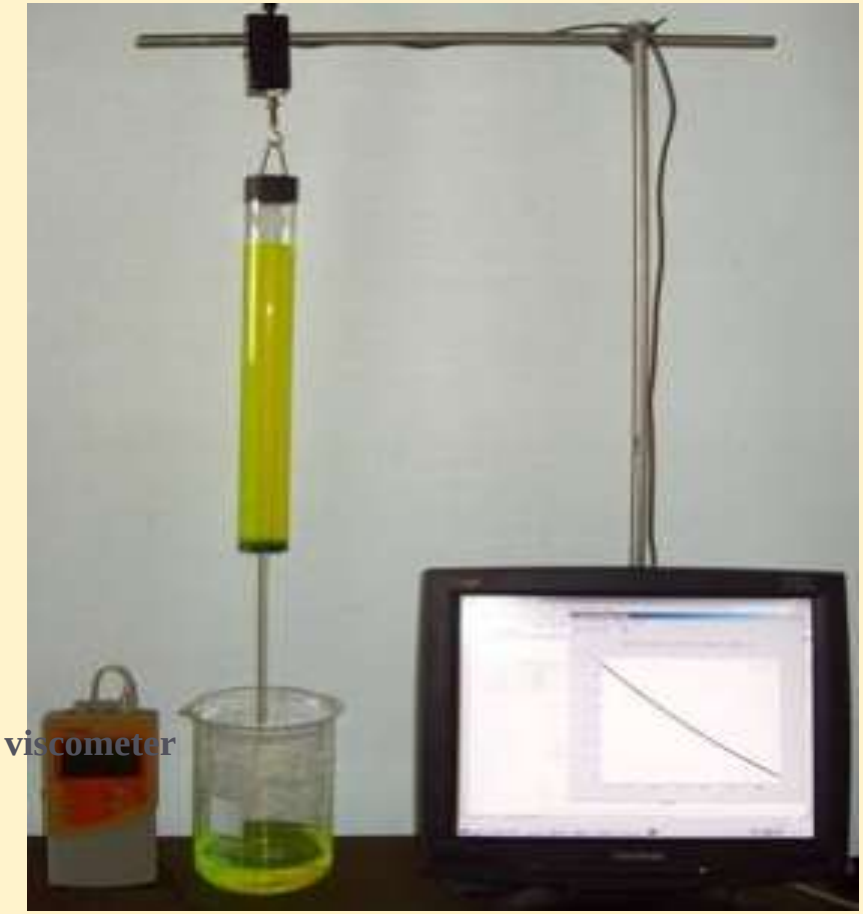


2. ***Rotational Viscometer:*** ***Measures viscosity by measuring the running torque of the cylindrical rotors immersed in a sample.***

Wilhelm Ostwald
(1853-1932)

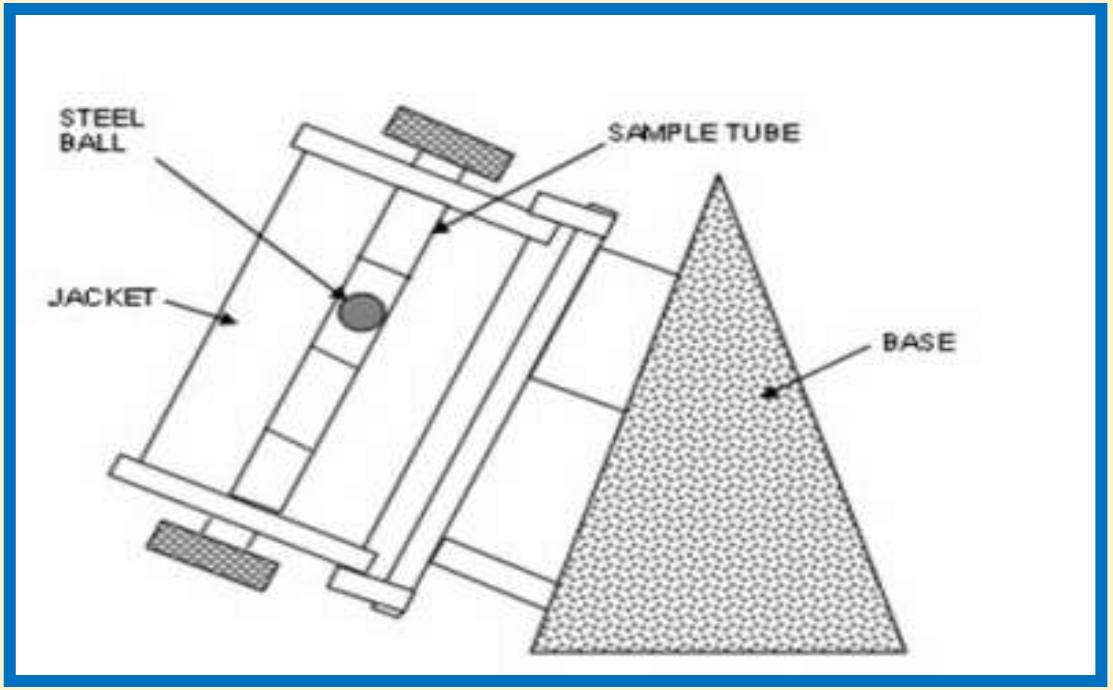
Nobel Laureate 1909
(for his work in catalysis, chemical balance, and Reaction rates)

Ostwald viscometer



Simple capillary viscometer

3. Capillary Viscometer: Obtains viscosity by letting a sample flow inside the capillary and measuring the difference in pressures between both ends of the capillary.



4. Falling-Ball Viscometer: Obtains viscosity by measuring the time it takes for a cylindrical or spherical object to fall through a sample over a specific distance.

5. Cup-Type Viscometer: Obtains viscosity by measuring the time it takes a sample to flow out of the orifice of the sample container.

Measuring DNA viscosity and its variation

The DNA helix lengthens, as the base pairs are separated to accommodate the bound ligand for the intercalation of the molecule, leading to increase in DNA viscosity. Viscosity measurement is thus a suitable method to detect such changes and, in the absence of crystallographic structural data, it is an essential evidence to support an intercalation model.

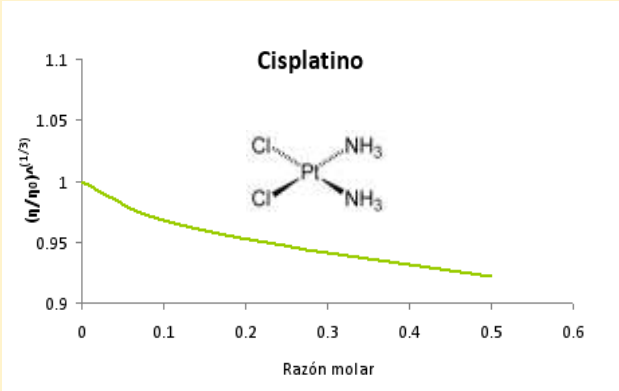
In contrast, nonclassical intercalation of ligands could bend (or kink) the DNA helix, reduce its effective length and in turn, its viscosity.

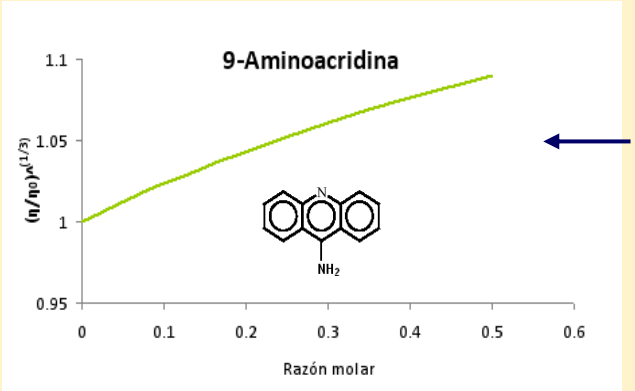
Two examples of covalent bond (cisplatin) to DNA and intercalation (9-aminoacridine) between DNA base pairs

Viscosity decreases

Viscosity increases

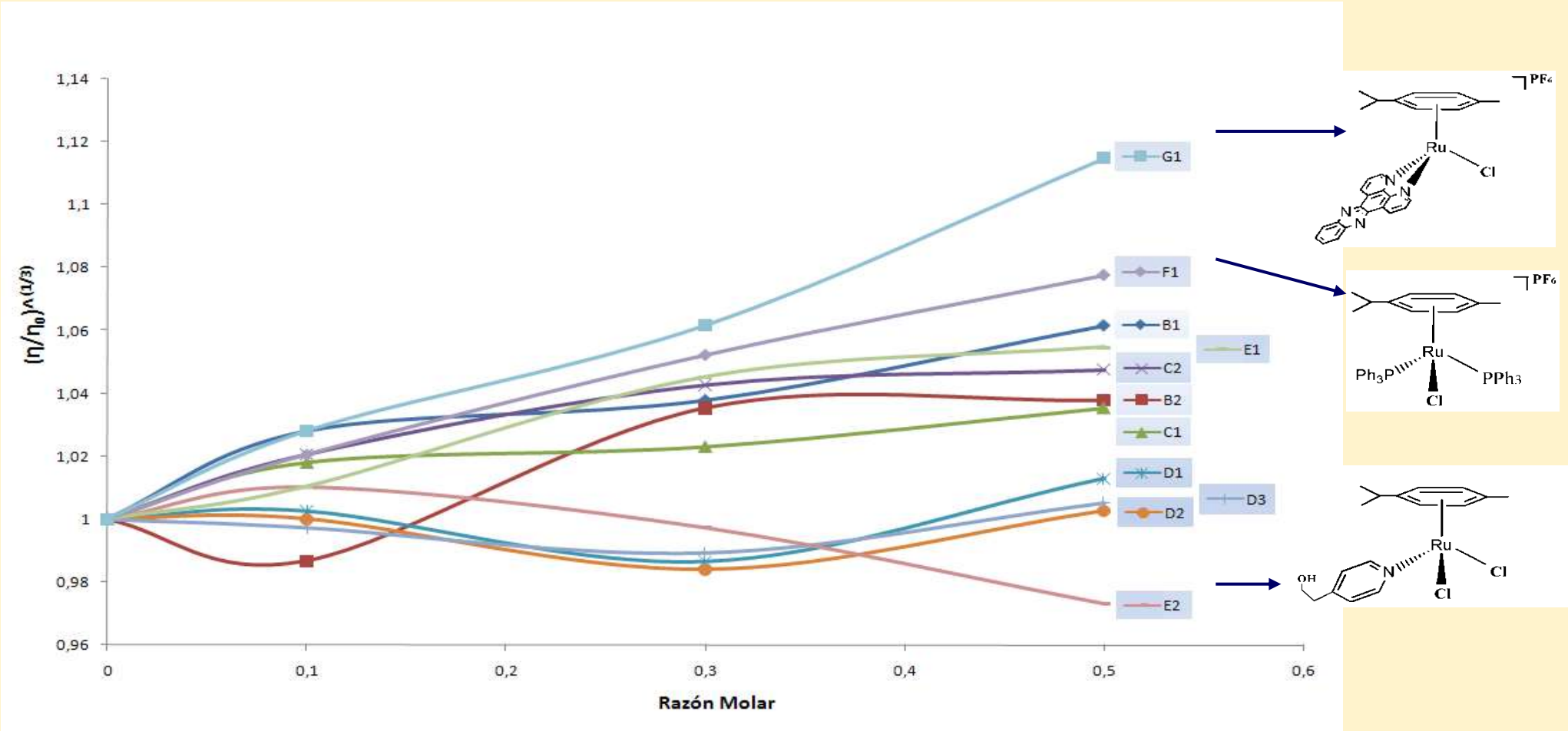
Covalent →



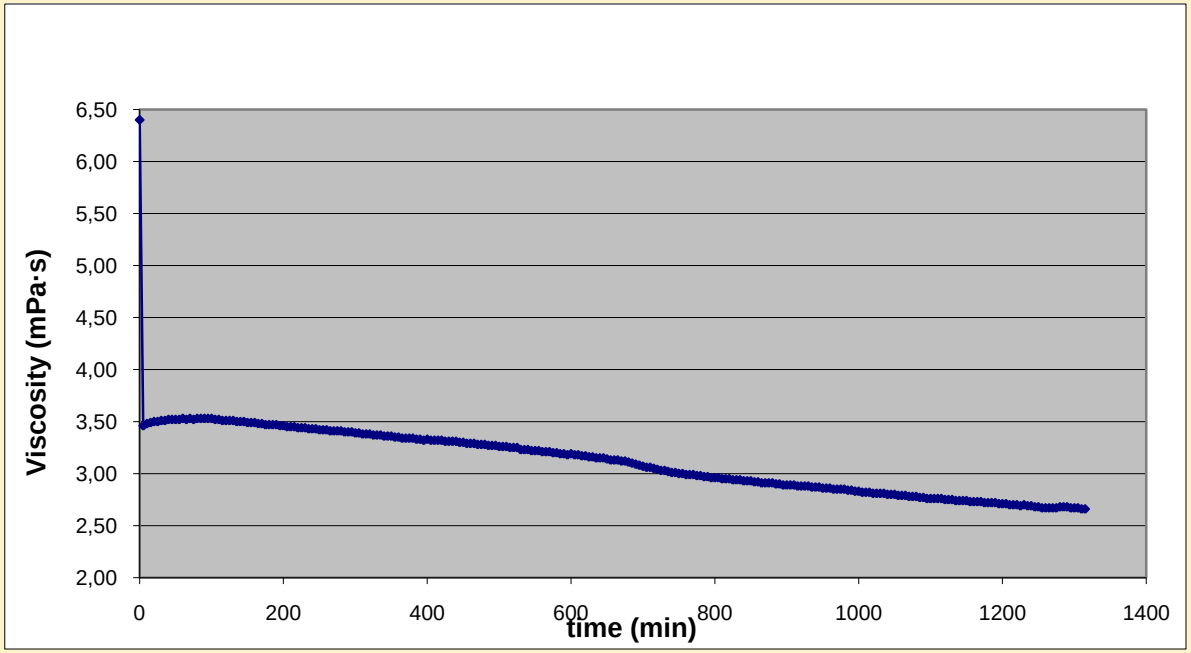


← **Intercalator**

Variation of viscosity of Calf Thymus DNA incubated with several ruthenium complexes (R_i=0.5) for 54 h at 25 ^oC.



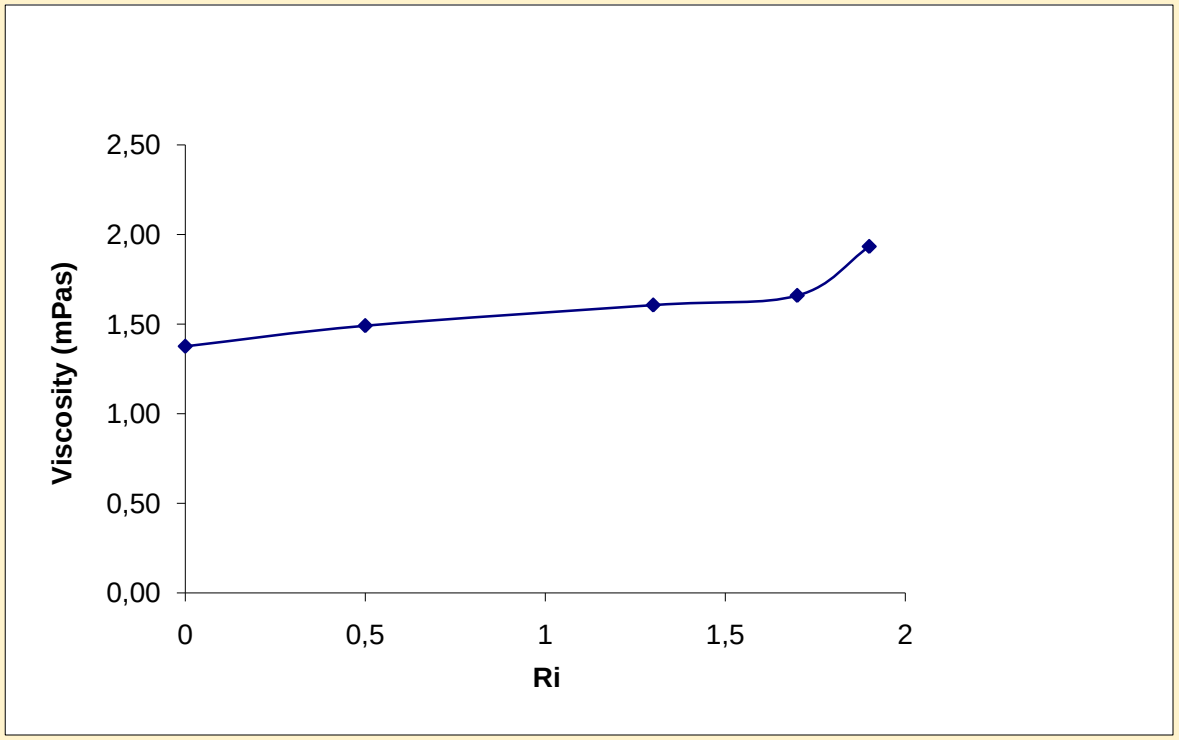
The viscosity decreases along time for a compound of ruthenium incubated with Calf Thymus DNA

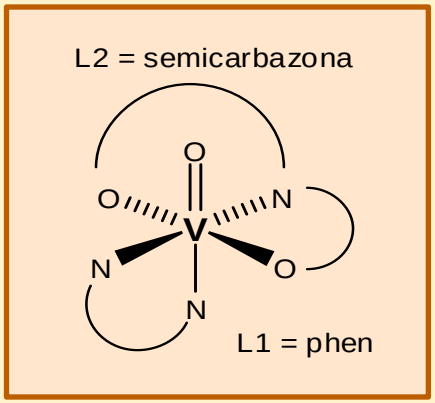


Example for weak interactions of the ligands with DNA or covalent bond by hydrolysis of carboxylate bond of picolinic acid with time

$[Ru(pic)(bipy)(dppb)]PF_6$

The viscosity increases when the molar ratio increases for a compound of vanadium incubated with Calf Thymus DNA





Example for Intercalation

CONCLUSION:

Measurement of viscosity is a very simple, available, complementary, cheap and excellent method to know the mode of binding of metal complexes (ruthenium, platinum, vanadium, gallium, iron drugs…) to DNA

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