Molecular fluorescence

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

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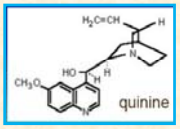
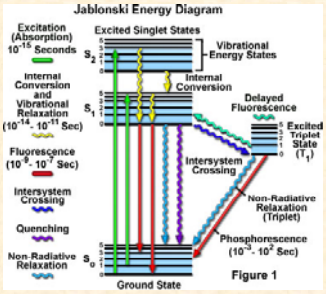
"The sulphate of quinine is well known to be of extremely sparing solubility in water. It is however, copiously soluble in tartaric acid. It is this solution which exhibits the optical phenomenon in question.
Though perfectly transparent and colorless, when held between the eye and the light, or white 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

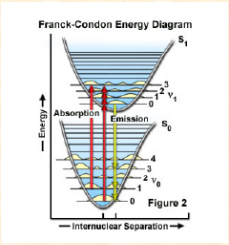


micro.magnet.fs.edu/.../jabintro/index.html

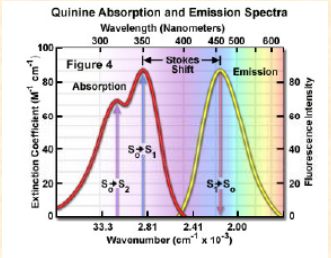
Four basic rules of fluorescence

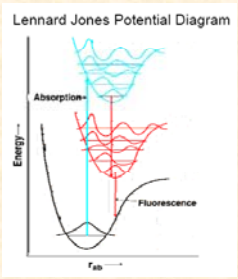
1. The Franck-Condon principle – the nuclei are stationary during electronic transitions, and so excitation occurs to vibrationally excited levels of the excited electronic state.

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.

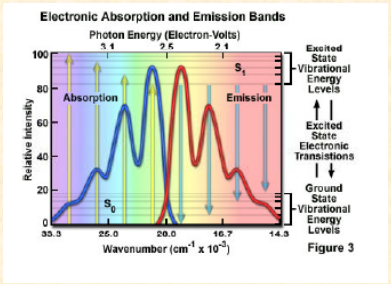


3. The Stokes shift: emission is always of lower energy than absorption due to nuclear relaxation in the excited state.





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



Fluorescence Lifetime

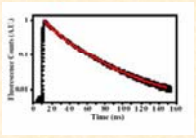
Question: how quickly do excited molecules relax back to the ground state?

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:

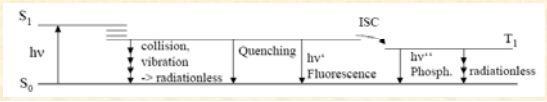
$$A^*(t) = A_0^* \exp(-t/\tau_f)$$



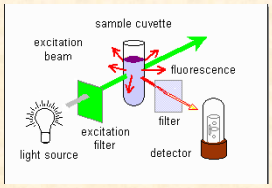
The experimentally determined excited state lifetime is always smaller than the theoretical one

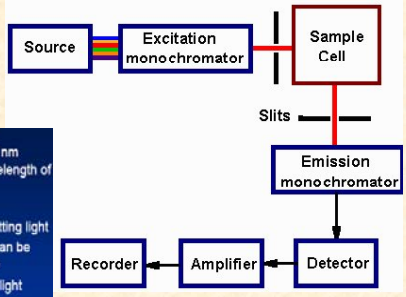
$$\tau_{ex} = \frac{1}{k_{fluor} + k_{nonfluor}} \Rightarrow \tau_f = \frac{\tau_{ex}}{q}$$

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



The equipment….

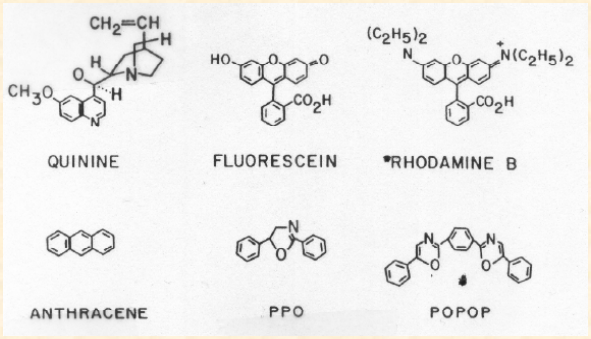




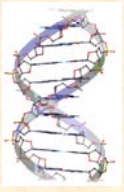
-xenon lamp source (common)
-emits light at high intensity from 250 – 650 nm
-excitation monochromator selects the wavelength of the exciting light
-sample compartment
-emission monochromator to select the emitting light
-both monochromators are motorized and can be varied to scan a selected wavelength 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

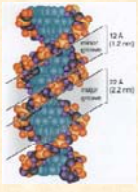
-fluorescent compounds are generally either aromatic, or have conjugated double bond systems



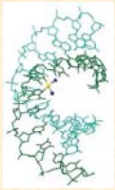
Application of fluorescence to determine the mode of binding of metal complexes to DNA



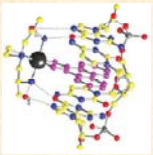
DNA-B



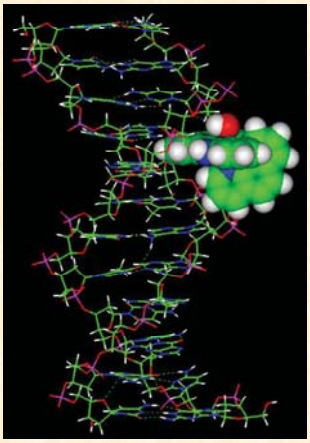
Structural data



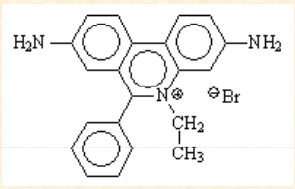
View of structural changes produced by covalent bifunctional binding

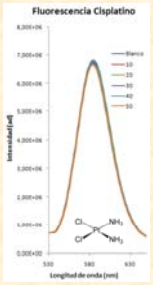


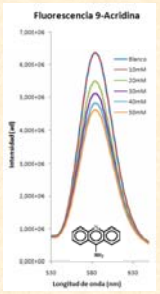
View of structural changes produced by intercalation

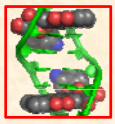


Ethidium bromide



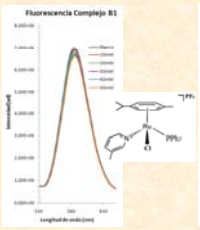


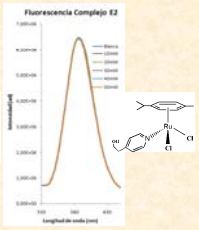




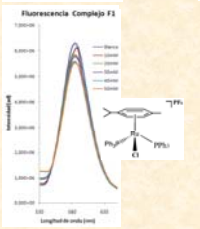
Intercalación

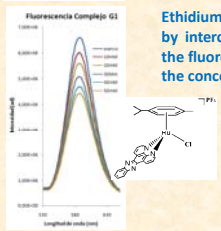
Interacción covalente





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)

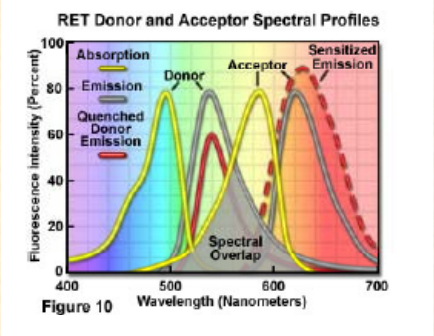


Figure 10

CONCLUSION:

1. This is a very simple method to know the possible intercalation in DNA, in the case that the ligands are not fluorescent molecules.

2. As a consequence of possible interactions donor-aceptor between the ethidium bromide and fluorescent ligands of the complexes, the interpretation of the emission spectra is not evident. This is a possible limit of this method.