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SECOND SUMMER COLLEGE IN BIOPHYSICS

30 July - 7 September 1984

Lecture 1: **Excited states of nucleic acids.**

Lecture 2: **Fluorescence and phosphorescence of DNA bases and DNA.**

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Lecture I

Excited States of Nucleic Acids

1. Interaction of light with matter.

transitions probabilities for absorption and emission processes – Einstein coefficients –

Spectroscopic relations between dipole strenght, transition dipoles and absorption spectrum.

Beer-Lambert law and exctiction coefficient

Franck Condon and shape of absorption band.

see: Biophysical Chemistry – Part II (1980)
Eds: C.R. Cantor and P.P. Schimmel – (Freeman)

2. Absorption of nucleic acids.

$\pi \to \pi^*$ and $n \to \pi^*$ transitions in nucleicacids bases.

Absorption spectra of DNA bases in solution.

Polarizations of the absorption bands (crystal studies)

Absorption coefficient for bases, nucleosides, nucleotides

Hypocromicity of nucleic acids

see: Physical Chemistry of Nucleic Acids (1974)
Eds: V.A Bloomfield, D.M. Crothers, I. Tinoco Jr – (Harper-Row)

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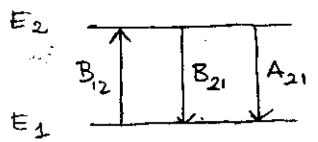
1. Interaction of light with matter

Einstein's coefficients



B_{12} induced absorption coeff.
B_{21} induced emission coeff.
A_{21} spontaneous emission coeff.

$$A_{21} = 8\pi h c \tilde{\nu}^3 B_{12}$$

$$B_{12} = \frac{2303}{h\tilde{\nu}N} \int \varepsilon(\tilde{\nu})\, d\tilde{\nu}$$

$\varepsilon(\tilde{\nu})$ = molar extinction coefficient

Transition probabilities between states 1 and 2 described by wave function ψ_1 and ψ_2 is proportional to the transition dipole moment:

$$\mu_{12} = \int \psi_2^* \left(\sum_j e x_j\right) \psi_1 \, d\tau$$

$\sum_j e \underset{\sim}{x_j}$ = sum of electric dipole operators $= \underset{\sim}{\mu}$

If $\psi_1 \equiv$ ground state $\Rightarrow$ μ_{11} = dipole moment of the molecule in ground state –

Dipole strength of transition $1 \to 2$

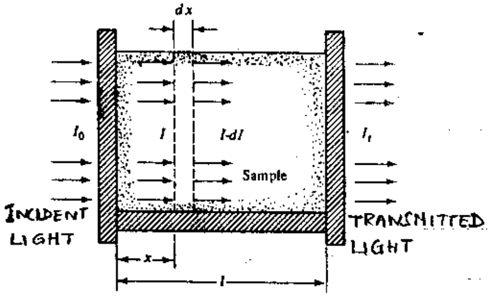
$$D_{12} = |\mu_{12}|^2$$

Oscillator strength or f-number of the transition

$$f = 4.319 \times 10^{-9} \int \varepsilon(\tilde{\nu})\, d\tilde{\nu} \qquad \tilde{\nu} \text{ in cm}^{-1}.$$

$$= \frac{8\pi^2 m c}{3 h e^2} \tilde{\nu} \cdot D_{12}$$

Beer-Lambert law



Fractional change in light intensity across dx

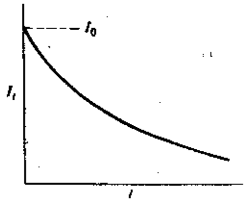
$$-\frac{dI}{I} = \alpha \cdot c \cdot dx$$

c = concentration of absorber

α = constant of proporzionality

$$-\int_{I_0}^{I_t} \frac{dI}{I} = \alpha \cdot c \int_0^{\ell} dx$$

$$\ln \frac{I_0}{I} = \alpha \cdot c \cdot \ell$$



$$I_t = I_0 \, e^{-\alpha \cdot c \cdot \ell}$$

I_t decreases exponentially with increacing path length ℓ for ℓ = const., decreases exp. with increacing c

Def: ABSORBANCE or OPTICAL DENSITY $A = \log \frac{I_0}{I_t} = \varepsilon \, c \, \ell$

$$\varepsilon = \frac{\alpha}{2.303}$$

ε = molar extinction coefficient in $\ell \, mol^{-1} cm^{-1}$
c = concentration of absorbing solute in $mol \cdot \ell^{-1}$

Selection rules

Spin forbidden transitions : transitions between states of different multiplicities are spin forbidden :

$$\langle \psi_i s_i | \mu | \psi_f s_f \rangle = \langle \psi_i | \mu | \psi_f \rangle \cdot \langle s_i | s_f \rangle$$

$$\langle s_i | s_f \rangle = 0 \quad \text{if } s_i \neq s_f$$

$$\langle s_i | s_f \rangle \neq 0 \quad s_i = s_f$$

Symmetry-forbidden transitions : transition is allowed if one of the components : $\langle \psi_i | \mu_x | \psi_j \rangle$, $\langle \psi_i | \mu_y | \psi_j \rangle$ or $\langle \psi_i | \mu_z | \psi_j \rangle \neq 0$. This indicate also the direction of polarization of the transition.

$\pi \to \pi^*$ polarized in the plane of the aromatic π electron system.
$n \to \pi^*$ $\perp$ " "

Overlap requirement : if the transition is allowed by symmetry and by spin, then it is necessary that $\langle \psi_i | \psi_f \rangle \neq 0$, i.e that the spatial overlap between the two wavefunctions is not negligible. (some $n \to \pi^*$)

Born-Oppenheimer aproximation

Wave function of the system = product of
ψ = electronic wave function
v - vibrational wave function

$$\psi_{tot} = \psi \cdot v$$

Transition probabilities between state $\psi_i v_a$ and $\psi_f v_b$

$$\mu_{if} = \langle \psi_i v_a | \mu | \psi_f v_b \rangle = \langle \psi_i | \mu | \psi_f \rangle \langle v_a | v_b \rangle$$

(μ = depends only on electronic coordinates)

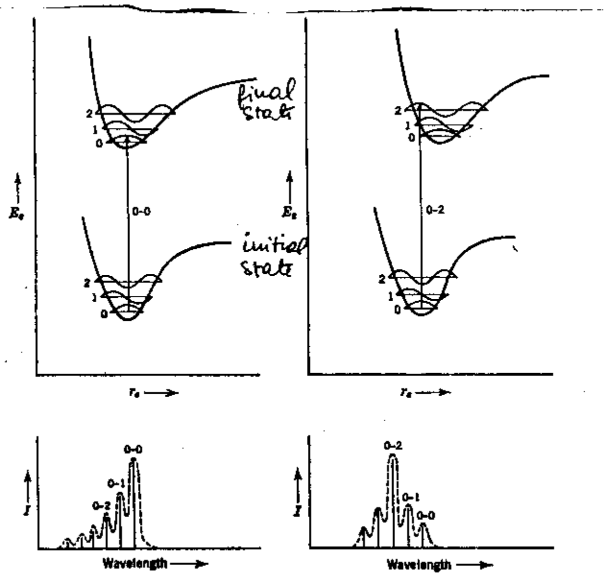


Figure 7 Franck-Condon principle and shape of absorption bands. The general shape of curves expected is indicated for vapor (———) and solution (– – – –).

Relative intensities of vibrational components of the electronic transition i→f.

$$I_{v_2 \leftarrow v_1} = \frac{8\pi^3}{3hc} I_0 l N_{v_1} \bar{R}_e^2 \left(\int \psi^i_{v_1} \psi^f_{v_2} \, d\tau \right)^2$$

where I_0 is the intensity of the incident radiation, l the path length, and N_{v_1} is the number of molecules in the v_1 vibrational state of a particular electronic state (normally, the ground state).

here $\bar{R}_e^2 = |\mu_{if}|^2$.

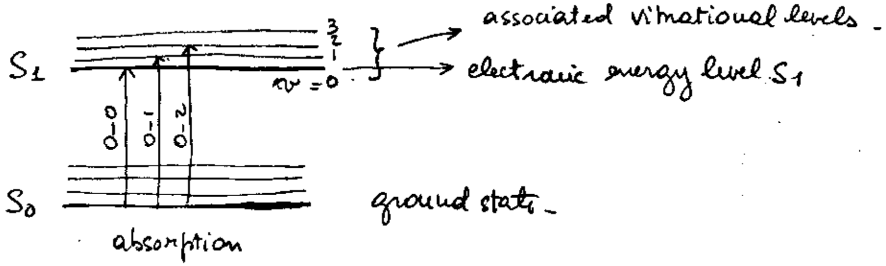
Since the speed of electronic motion ($\sim 10^{-15}$ sec) is much greater than that of nuclear motion ($10^{-10} - 10^{-12}$ sec) there are no changes in nuclear coordinates during the electronic transition = Franck Condon principle (vertical transition) The shape of the absorption spectrum depend upon the relative position of the minima of the Morse potential energy curves of the two electronic states.

0-0 transition = pure electronic transition

0-1, 0-2 etc " = vibronic transition whose intensities are proportional to $|\langle v_a | v_b \rangle|^2$

(vibrational wave functions ≈ harmonic oscillators)

Molecules in their ground state are in 0th vibrational level:



Since the number of vibrational states of a molecule is large, vibronic states are close to one another. Absorption bands are broad bands, unless very narrow (10^{-2} Å) excitation is performed.

2. Absorption spectra of nucleic acids

The absorption spectrum of nucleic acids in the near UV ($180\,nm < \lambda < 300\,nm$) is due to $\pi \rightarrow \pi^*$ electronic transitions of the aromatic bases.

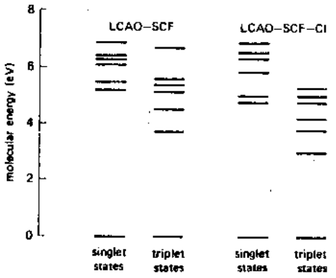


FIGURE 2-15 Calculated energies for π electron excited states of adenine. Linear combination of atomic orbitals self-consistent field calculations (LCAO-SCF) from H. Berthod, C. Giessner-Prettre, and A. Pullman, *Theoret. Chim. Acta*, 5, 53 (1966); LCAO-SCF-configuration interaction calculations from the same authors, *Inter. J. Quantum Chem.*, 1, 123 (1967).

Transitions involving σ electrons occur at much higher energies (for $\lambda < 180\,nm$, spectral region not accessible to absorption measurements in solution)

Transitions of $n \rightarrow \pi^*$ type are expected from:

- ring N atoms of bases ($n \rightarrow \pi^* \sim sp^2 \rightarrow p$) allowed, but not observed in solution
- carbonyl O atoms ($n \rightarrow \pi^* \sim p_x \rightarrow p_y$) forbidden

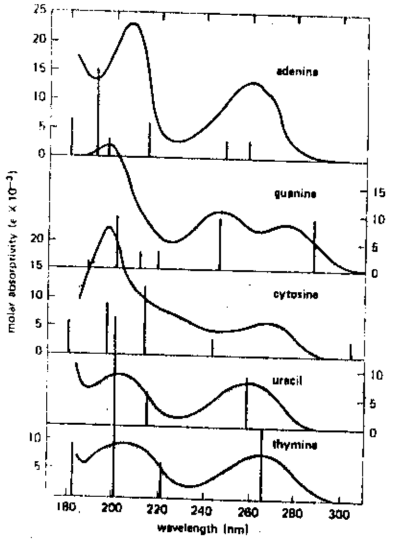


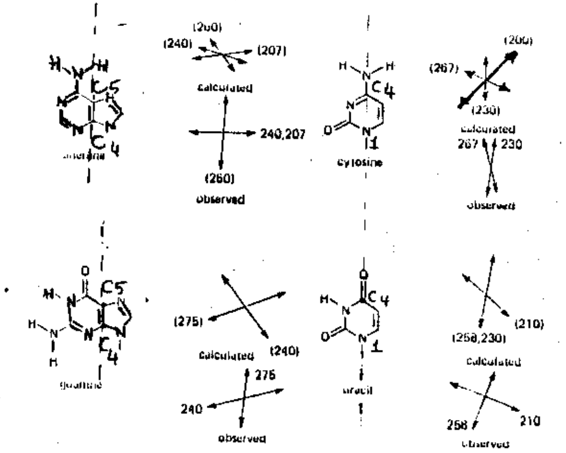
FIGURE 2-16 Comparison of experimental spectra with calculated transitions and magnitudes of their oscillator strengths. The length of each bar is proportional to the calculated oscillator strength. The experimental spectra are from D. Voet et al., *Biopolymers*, 1, 193 (1963); the calculated values are from H. Berthod, C. Giessner-Prettre, and A. Pullman, *Inter. J. Quantum Chem.*, 1, 123 (1967).

TABLE 2-3 THE SPECTRA OF NUCLEOSIDES IN NEUTRAL AQUEOUS SOLUTION[a,b]

	λ max[b]	ε max	λ max	ε max	λ max	ε max
Adenosine	259.5	14,900	206.3	21,200	190	19,800
Guanosine	276	9,000	252.5	13,700	188.3	26,800
Cytidine	271	9,100	230	8,200	198	23,200
Uridine	261	10,100	205	9,800		
Thymidine	267	9,700	206.5	9,800		

[a] D. Voet, W. B. Gratzer, R. A. Cox, and P. Doty, *Biopolymers*, 1, 193 (1963).

[b] The maximum wavelength (λ max) is given in nanometers and the maximum absorptivity (ε max) is given in liters per mole centimeter.



. Comparison of experimental and calculated directions of transition electric dipole moments. References for the experimental data are given in Table 2-7; the calculated directions are from H. Berthod, C. Giessner-Prettre, and A. Pullman, *Inter. J. Quantum Chem.*, 1, 123 (1967).

Polarization of the absorption bands of the base at their maximum absorption wavelenght. The experimental values are obtained by absorption of linearly polarised light on oriented single crystal samples. (see next table)

The direction of transition dipole moments can be also evaluated, relative to the direction of the permanent electric dipole, from electric dichroisme and birefringence measurements.

EXPERIMENTAL DIRECTIONS OF NUCLEIC ACID BASE TRANSITION MOMENTS

Base	λ(nm)	Direction[a]	Comments
Adenine	260 240 207	−3° ± 3° −90° −90°	The direction of the 260-nm band comes from studies of crystal 9-methyladenine (Refs. 1, 2).[b] The other directions are from polari fluorescence studies (Ref. 3).
Guanine	275 248	−6° ± 4° −75° ± 5°	L. B. Clark, U. California, San Diego, Personal communication and Ref. 6.
Cytosine	267 230	+12° ± 3° −5° ± 3°	The directions of the bands come from studies of crystalline cyto monohydrate and 1-methylcytosine (Ref. 4). Fluorescence studies 5-methylcytosine show the two bands are parallel to each other (Ref. 5).
Uracil and thymine	270 210	0° or +7° for uracil; −19° for thymine perpendicular to first band	The direction of the 270-nm band comes from studies of crystal 1-methylthymine (Ref. 1) and 1-methyluracil (Ref. 6). Polarized fluoresce and absorption shows the next band is perpendicular to the first (Refs. 4–6)

[a] The angle is measured relative to the C4–C5 axis in purines, or the N1–C4 axis in pyrimidines. Positive angles are measured counterclockwise w the bases are oriented as shown in Fig 2-1.

[b] References:

[1] R. F. Stewart and N. Davidson, *J. Chem. Phys.*, 39, 255 (1963).

[2] R. F. Stewart and L. H. Jensen, *J. Chem. Phys.*, 40, 2071 (1964).

[3] P. R. Callis, E. J. Rosa, and W. T. Simpson, *J. Amer. Chem. Soc.*, 86, 2292 (1964).

[4] P. R. Callis and W. T. Simpson, *J. Amer. Chem. Soc.*, 92, 3593 (1970); T. P. Lewis and W. A. Eaton, *J. Amer. Chem. Soc.*, 93, 2054 (1971).

[5] P. R. Callis, Ph.D. Thesis, University of Washington (1965).

[6] W. A. Eaton and T. P. Lewis, *J. Chem. Phys.*, 53, 2164 (1970).

[7] P. R. Callis, B. Fanconi and W. T. Simpson, *J. Amer. Chem. Soc.*, 93, 6679 (1971).

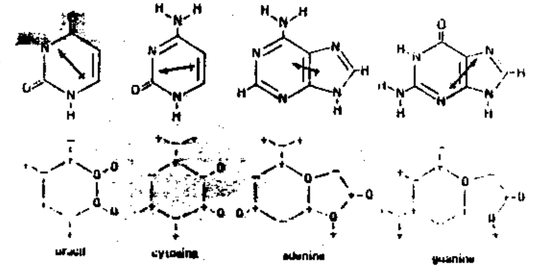


FIGURE 2-7 The calculated permanent dipole moments and net charges on each nucleus for the nucleic acid bases. [From B. Pullman and A. Pullman, *Prog. Nucleic Acid Res. Mol. Biol.*, 9, 328 (1969).]

MEASURED DIPOLE MOMENTS OF NUCLEIC ACID BASES

Compound	Solvent	Dipole moment (Debye)	Ref.
Pyrimidine	Dioxane	2.4	*a*
9-Butylpurine	Dioxane	4.3	*b*
9-Butyladenine	CCl_4	3.0	*b*
1,3-Dimethyluracil	Dioxane	3.9	*b*
1-Methyl-5-bromouracil	Dioxane	4.5	*b*

[a] C. P. Smyth, *Dielectric Behavior and Structure*, McGraw-Hill, New York (1955).
[b] H. DeVoe and I. Tinoco, Jr., *J. Mol. Biol.*, 4, 500 (1962).

CALCULATED[a] DIPOLE MOMENTS OF NUCLEIC ACID BASES

Compound	Total dipole moment (Debye)	Direction of μ[b]
Adenine	2.6	84° } (°)
Guanine	6.9	−34°
Cytosine	6.4	99° } (°°)
Thymine	3.3	32°

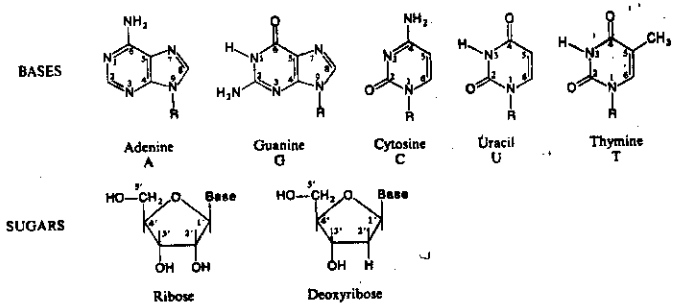
[a] E. Clementi, J. M. Andre, M. C. Andre, D. Klint, and D. Hahn, *Acta Phys.*, 27, 493 (1969).

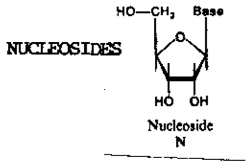
(°) $\mu, \widehat{C_4 - C_5}$ (°°) $\mu, \widehat{N_1 - C_4}$

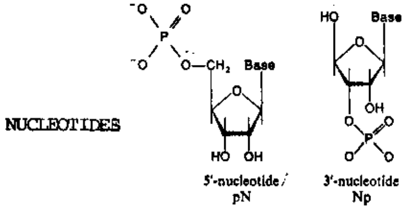
Permanent electric dipole of the bases due to the total electron distribution. Relevant parameter for the interaction between DNA bases ~~and other~~ molecules: aromatic a.a., intercalators

Molar extinction coefficient : ε at 260.5 nm

For adenine = base	13.4×10^3
adenosine = base + sugar	14.9×10^3
adenosine-5'-phosphate = base + sugar + phosph	15.4×10^3







Absorption properties of nucleotides*

	λ_{max} (nm)	$10^{-3}\varepsilon_{max}$ (M^{-1} cm^{-1})
Ribonucleotides		
Adenosine-5'-phosphate	259	15.4
Cytidine-5'-phosphate	271	9.1
Guanosine-5'-phosphate	252	13.7
Uridine-5'-phosphate	262	10.0
Deoxyribonucleotides		
Deoxyadenosine-5'-phosphate	—	15.3
Deoxycytidine-5'-phosphate	271	9.3
Deoxyguanosine-5'-phosphate	—	—
Thymidine-5'-phosphate	267	10.2

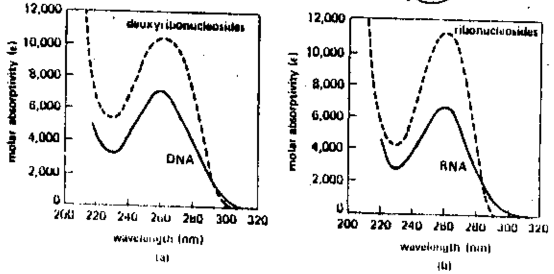
* Wavelengths of maxima and molar absorptivities of nucleotides at pH 7.
SOURCE: *Handbook of Biochemistry and Molecular Biology*, 3rd ed., CRC Press, Inc., Cleveland, Ohio, 1976.

1) Different bases display different absorption, however minor difference occurr for the same base attached to ribose or deoxyribose sugar.

2) Molar extinction coefficient (per phosphate)

$$\text{adenosine-5'-phosphate} \quad \varepsilon_{260} = 15.4 \times 10^{3}$$
$$\text{tetranucleotide pApApApA} \quad = 11.3 \times 10^{3}$$

$$\text{Hypercromicity ratio} = \frac{\text{adenosine} \overset{\text{(hydrolized monomer)}}{-5'}\text{-phosphate}}{\text{pApApA}\ \underset{\curvearrowleft}{\text{pA}}\ \text{(polymer)}} = \frac{15.4}{11.3} = 1.36$$



$$\frac{\text{monophosphate}}{\text{native DNA}} \approx 1.4$$

depend upon DNA base composition!

FIGURE 4-14 The molar absorptivity of double-stranded DNA and double-stranded RNA compared with their component mononucleosides. [*M. lysodeikticus* DNA data from Felsenfeld and Hirschman, *J. Mol. Biol.*, 13, 407 (1965). Rice dwarf virus RNA data from Miura et al., *Virology*, 28, 571 (1966). Nucleoside spectra calculated from the base composition (72% G + C for the DNA; 44% G + C for the RNA) and the absorption data of Voet et al., *Biopolymers*, 1, 193 (1963).]

Hypochromisme of the nucleic acids

Due to the interaction between the bases, the extinction coefficient of the dimer (dinucleotides) and of the polymer (polynucleotides single and double strands) is smaller than the sum of the extinction coefficients of the constituents (nucleotides). This effect is observed for all the nucleic acids and can be also used as a test for the secondary structure of them, since the hypochromisme is higher for the double stranded helix than the single stranded. This effect is due to the modification of the transition probabilities of each base in the field generated by the neighboring ones. It will depend therefore on the intrinsic transitions moments of the bases and on their relative orientation. This is different for the native double helix (ordered stacking of bases) than for the random coiled single strand. Upon thermal denaturation an hypochromicity of ~40% is observed.

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Lecture 2

Fluorescence and Phosphorescence of DNA bases and DNA

1. Excited state properties

Dissipation of excitation energy: internal conversion, fluorescence, intersystem crossing, phosphorescence – Non radiative deactivations –

Characteristics of the excited state: energy, lifetime, quantum yelds, polarization

Some experimental setups for luminescence measurements (corrected excitation and emission spectra, fluorescence anisotropy, etc)

see: 1) "Practical Fluorescence" (1973)
ed: G. Guilbault (M. Dekker)

2) "Biophysical Chemistry" Part II (1980)
Eds: C.R. Cantor and P.P. Schimmel (Freeman)

3) "Theory and Interpretation of Fluorescence and Phosphorescence" Ed: R.J. Becker (1969) Wiley

2. Luminescence of DNA bases and DNA at R.T and 80°K

Fluorescence of DNA basis in water solution at R.T, and at 77°K in frozen glasses. Phosphorescence – Excitation and emission spectra –

Emission spectra of dinucleotides, polynucleotides and DNA at R.T and 80°K – Degree of polarization – Exciplexes and Excimers –

see: 1) Excited states of Nucleic Acids
by Gueron, Eisinger, Lamola
in "Basic Principles in Nucleic Acids Chemistry"
vol I. Ed: P.O. Tsó. Academic Press (1974)

2) "Excited States of Proteins and Nucleic Acids"
Eds: R.F. Steiner and I. Weinryb (1971)
McMillan

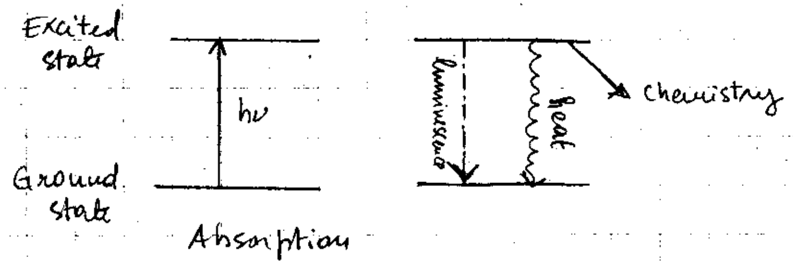
3) Recent Developments in the Fluorescence of DNA bases and DNA at 300 K.
in "Physico-chemical Properties of Nucleic Acids"
vol I, Ed J. Duchesne, Academic Press (1973)

The absorption of light by a molecule can result in different processes, depending how the excitation energy is dissipated:

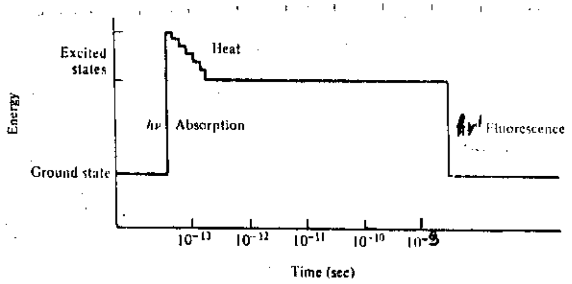
<u>heat</u>, with temperature rise in the sample)

<u>luminescence</u>, emission of a photon with the same or lower energy of the absorbed one)

<u>chemistry</u>, the absorbed energy is used for altering chemical structures.



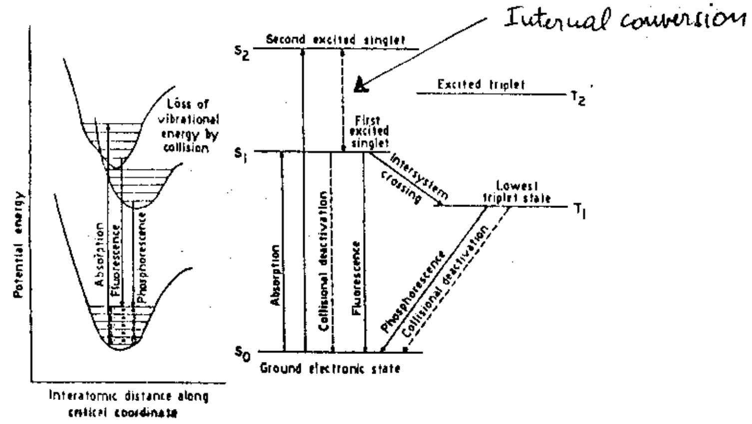
The absorption process is very rapid, 10^{-15} s.
The time dependence of the dissipative processes following absorption is ~



Internal conversion -

When a molecule is excited to an energy level higher then the lowest singlet state (i.e to an higher electronic state or to one of the vibronic states of the lowest singlet state) the molecule deactivates to the lowest singlet state in its 0th vibrational level (in about 10^{-11} - 10^{-14} sec) by releasing the difference in energy between the two states in form of vibrational energy.

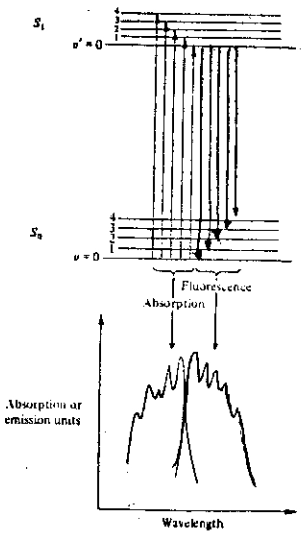
From this lowest excited state it is possible to observe: radiative (fluorescence) and/or unradiative transitions to the ground state, intersystem crossing to the lowest triplet state T_1, radiative (phosphorescence) and/or unradiative transition from the triplet to the ground state.

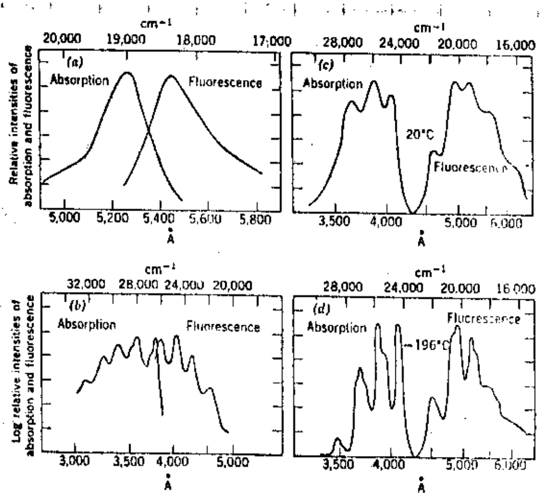


Fluorescence –

It is the radiative transition from the lowest excited singlet state S_1 to ground state S_0, this occours from the 0^{th} vibrational level of the excited states to a number of vibrational levels of the ground state. (10^{-7}-10^{-9} sec)
the energy of the 0-0 transition is the same in absorption and fluorescence; the fluorescence spectrum is instead at smaller energy (= higher wavelenghts)

If vibrational states are alike in ground and lowest excited state, the fluorescence spectrum is a "mirror" image of the absorption one. (the intersection of fluorescence emission and absorption correspond approximately to 0-0 transition energy).





Approximate mirror-image relation between absorption and fluorescence spectra: (*a*) fluorescein (E. L. Nichols and E. Merritt, *Phys. Rev.*, 31, 376 (1910); (*b*) anthracene in dioxane (G. Kortum and B. Finckh, *Z. Physik. Chem.*, B52, 263 (1942); (*c*) diphenyl-octatetraene in xylene at 20°C (K-W. Hausser, R. Kuhn, and E. Kuhn, *Z. Physik. Chem.*, B29, 417 (1935)); and (*d*) same as (*c*) at −196°C.

Fluorescence almost always occurs from the lowest singlet S_1 (Kesha rule), however a number of exceptions are known (azulene).

Intersystem crossing

It is the non radiative process (vibrational energy is dissipated) from the lowest excited singlet S_1 to the lowest triplet T_1. It is fast enough to compete with fluorescence (10^{-8} sec). It depends on the energy separation between S_1 and T_1 and on the probability of spin-orbit coupling (interaction between states of different multiplicity)

Phosphorescence

It is the radiative transition from lowest triplet T_1 to ground state. It has a low transition probability since it is spin forbidden. (Spin-orbit coupling then favors it) It occurs at longher wavelenghts (smaller energy) than the fluorescence. (10-10^{-3} sec)

Non radiative processes are dependent upon molecular vibrations and are then reduced by lowering the temperature. This correspond to an increase in the fluorescence and phosphorescence yelds-

Photochemical reactions can be observed for the molecules in the excited state (electron or proton transfers, isomerisation process, rupture of chemical bonds).

Rate constants for the deactivation processes -

They are first order process. For fluorescence of intensity F is:

$$F = -\frac{d[M^*]}{dt} = k\,[M^*]$$

$[M^*]$ = concentration of molecules in the excited state that undergo fluorescence -

k_r^F	rate constant	for radiative deactivation by fluorescence from S_1	
$k_{n.r}^F$	"	non radiative	"
k_{isc}	"	intersystem crossing to triplet	
k_r^P	"	radiative deactiv. by phosphoresc. from T_1	
k_r^P		non radiative	"

Kinetic equations for the molecules in the excited state S_1 and T_1:

$$\frac{dN_S}{dt} = \alpha I_0 - \left(k_r^F + k_{nr}^F + k_{isc}\right) N_S$$

$$\frac{dN_T}{dt} = k_{isc} N_S - \left(k_r^P + k_{nr}^P\right) N_T$$

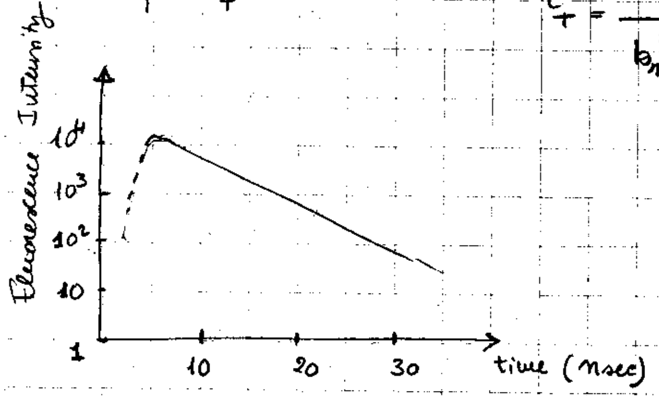
N_S, N_T = concentration of molecules in S_1 and T_1

αI_0 = fraction of light absorbed = N of molecules excited per second.

When the excitation is removed then the concentration of N_S and N_T and therefore the intensity of fluorescence and phosphorescence decay exponentialy with life time τ_S and τ_T

$$N_S = N_S^0\, e^{-t/\tau_S} \qquad \tau_S = \frac{1}{k_r^F + k_{nr}^F + k_{isc}}$$

$$N_T = N_T^0\, e^{-t/\tau_T} \qquad \tau_T = \frac{1}{k_r^P + k_{nr}^P}$$



Quantum yelds

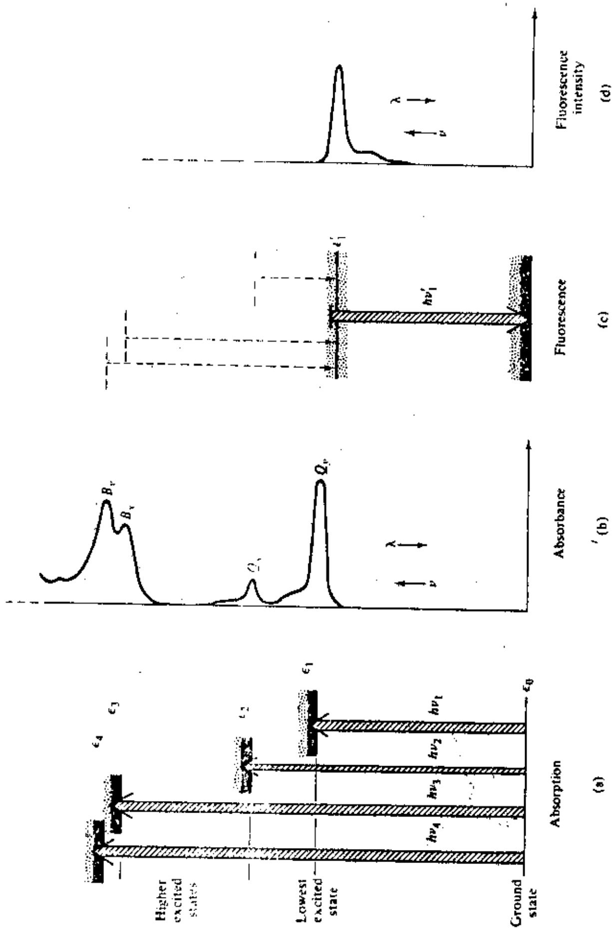
Fluorescence $$\phi_F = \frac{k_r^F}{k_r^F + k_{nr}^F + k_{isc}} = \frac{\tau_s}{\tau_0} \quad *$$

Intersystem Crossing $$\phi_{isc} = \frac{k_{isc}}{k_r^F + k_{nr}^F + k_{isc}}$$

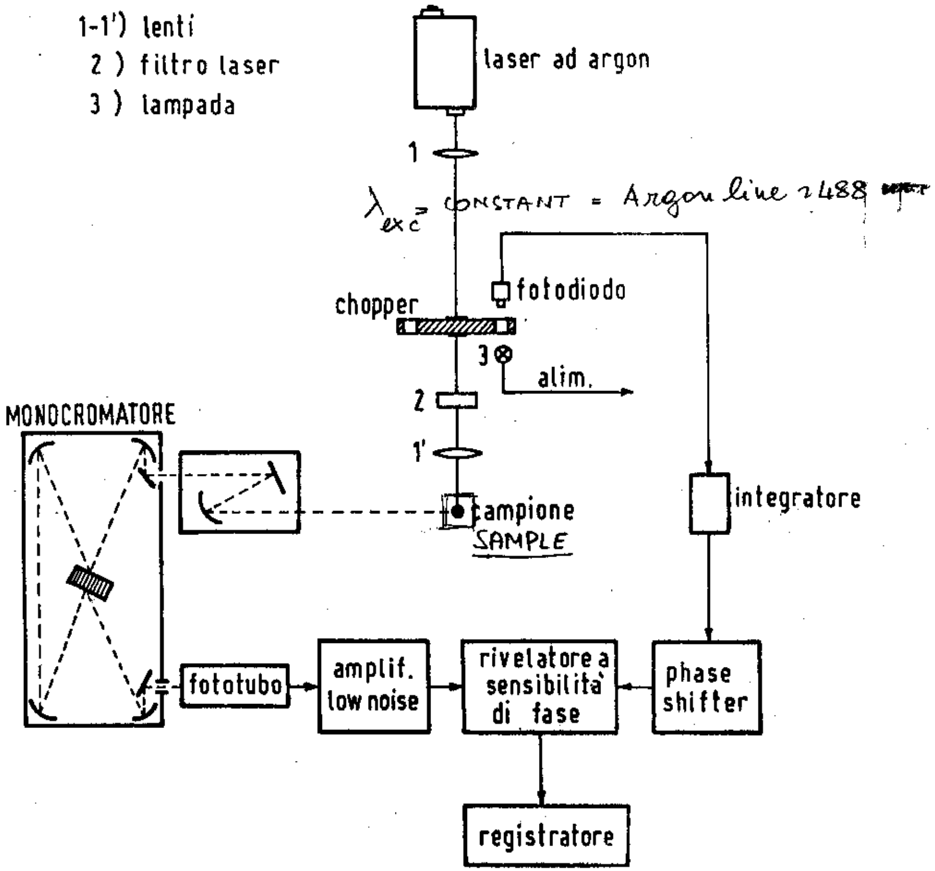
Phosphorescence $$\phi_P = \frac{k_r^P}{k_r^P + k_{nr}^P} \cdot \phi_{isc}$$

Fluorescence quantum yeld is then the percentage of molecules excited that decay radiatively from S_1 s (i.e. the number of photons emitted per photon absorbed)

* Natural lifetime of the excited state $\tau_0 = \frac{1}{k_r^F}$ (i.e. the life time of the excited state in the ideal case that every absorbed photon is reemitted) it can be obtained by the absorption spectrum since: $\frac{1}{\tau_0} = \text{const.}\ \bar{\nu}^2 \int \varepsilon(\tilde{\nu})\, d\tilde{\nu}$. Therephore from the measurements of the absorption and of quantum yeld it is possible to evaluate the life time of fluorescence $\tau_S = \phi_F \cdot \tau_0$



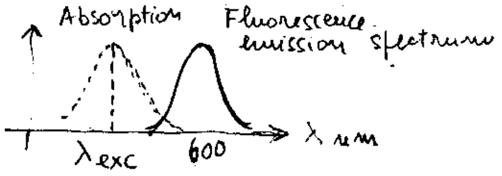
Absorption and fluorescence of bacteriochlorophyll. (a) Energy-level diagram, showing spectral transitions (vertical arrows). The energy levels are broadened (shading) by vibrational sublevels that are not usually resolved in solution spectra. (b) Absorption spectrum corresponding to energy levels of part (a). This spectrum is turned 90° from the usual orientation to show the relation to the energy levels. (c) Radiationless relaxation (dashed arrows) and fluorescence (shaded arrow). (d) Fluorescence emission spectrum corresponding to part (c). Note the red shift (shorter shaded arrow) of the fluorescence compared with the corresponding Q_Y absorption illustrated in parts (a) and (b). (From K. Sauer, in *Bioenergetics of Photosynthesis*, Govindjee (ed.), Academic Press, New York, 1975, pp. 115–181.)

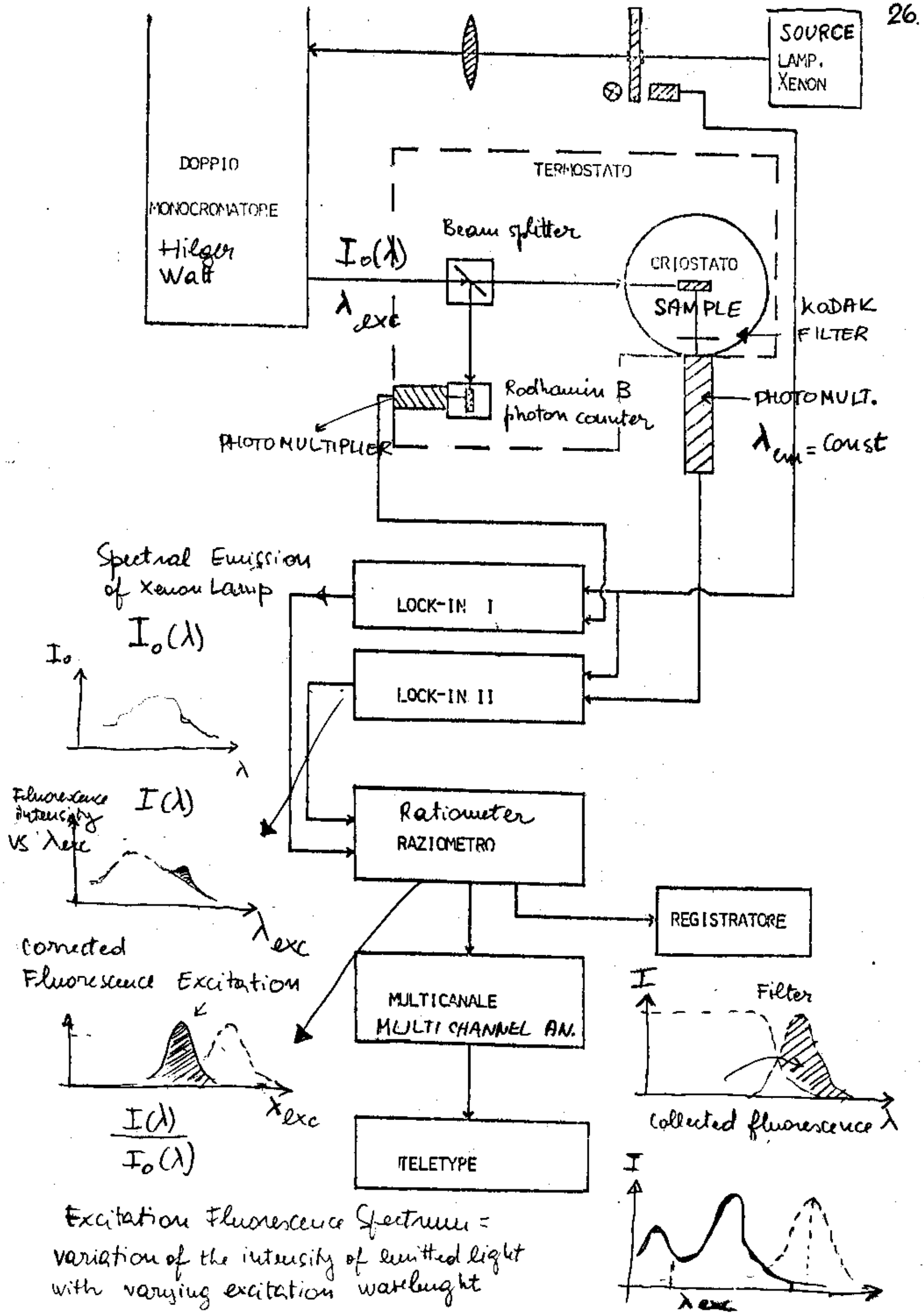


Experimental set up for emission spectra measurements:

$\lambda_{excitation}$ = constant = Argon laser lines (4880 Å, 5145 Å)

$\lambda_{emission}$ = analyzed through monochromator.





Excitation Fluorescence Spectrum = variation of the intensity of emitted light with varying excitation wavelength