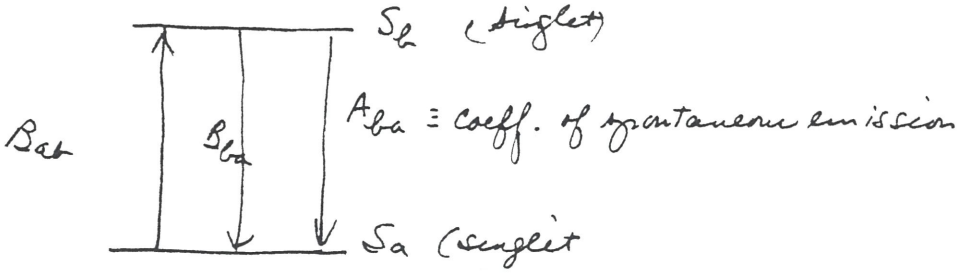
Biophysical Chemistry
for Life Scientists
NTU Fall 2000
Lecture 16
January 12, 2001

TS, Chapter 8, p 433 – 465

Emission of light from excited electronic state.

Absorption occurs ~ 10^{-15} s femto sec.
Emission ~ $10^{-12} - 10^{-9}$ s
ps nanosec.

Consider hypothetical situation



Light of radiation density $I(\nu)$ induces transition from $S_a \rightarrow S_b$ at a rate of B_{ab} per molecule per sec. $S_b \rightarrow S_a$
$= B_{ba}$

Einstein derived A_{ba} in terms of B_{ab} using the principle of detailed balance.

Suppose states b, a are at thermal equil.

$$\frac{n_b}{n_a} = e^{-(E_b - E_a)/k_B T}$$

If system is at thermal equil., it must be at thermal equil. with blackbody radiation; i.e., black body radiation induces $a \rightarrow b$, $b \rightarrow a$ transition at such a rate that it compensates for spontaneous emission.

i.e., the emission and absorption of radiation density must be equal.

Thus, we must have

$$n_a^{eq} \underset{\text{black body}}{I}(\nu_{ab})\, B_{ab} = n_b^{eq} \underset{\text{body}}{I_{\text{black}}}(\nu_{ab})\, B_{ba} + A_{ba}\, n_b^{eq}$$

$$\frac{n_a^{eq}}{n_b^{eq}} = \left[B_{ba}\, I_{bb}(\nu_{ab}) + A_{ba}\right] \Big/ B_{ab}\, I_{b\,body}(\nu_{ab})$$

$$= 1 + \frac{A_{ba}}{B_{ab}} \frac{1}{\underset{\text{radiation}}{I_{\text{thermal}}}(\nu_{ab})}$$

$$\underset{\text{thermal}}{I(\nu)} = \frac{8\pi h\nu^3}{c^3} \left(e^{+h\nu/k_BT} - 1\right)^{-1}$$

=

Substituting

$$\frac{n_a^{eq}}{n_b^{eq}} = 1 + \frac{A_{ba}}{B_{ab}} \left(e^{h\nu_{ab}/kT} - 1\right) \frac{c^3}{8\pi h \nu_{ab}^3} = e^{h\nu_{ab}/k_BT}$$

$$\therefore \frac{A_{ba}}{B_{ab}} \left(e^{h\nu_{ab}/k_BT} - 1\right) \frac{c^3}{8\pi h \nu_{ab}^3} = \left(e^{\frac{h\nu_{ab}}{k_BT}} - 1\right)$$

$$\therefore A_{ba} = B_{ab} \frac{8\pi h \nu_{ab}^3}{c^3}$$

$$= \left(\frac{32\pi^3\nu^3}{3c^3\hbar}\right) D_{ab}$$

Note ν^3 dependence
direct proportionality with D_{ab}

$\therefore \quad A_{ba} = 0 \quad$ if $\quad D_{ab} = 0$

spontaneous emission important for transitions in visible, uv, x-ray, δ-ray regions

Because D_{ab}, ν can usually be obtained from absorption spectrum, rate of spontaneous emission can be determined without performing an emission expt. In the absence of radiation or any other perturbations or interactions, the rate of deexcitation of molecules initially in state S_b will be

$$\frac{dn_b}{dt} = - A_{ba} n_b$$

$$n_b(t) = n_b(0)\, e^{-A_{ba} t} = n_b(0)\, e^{-t/\tau_R}$$

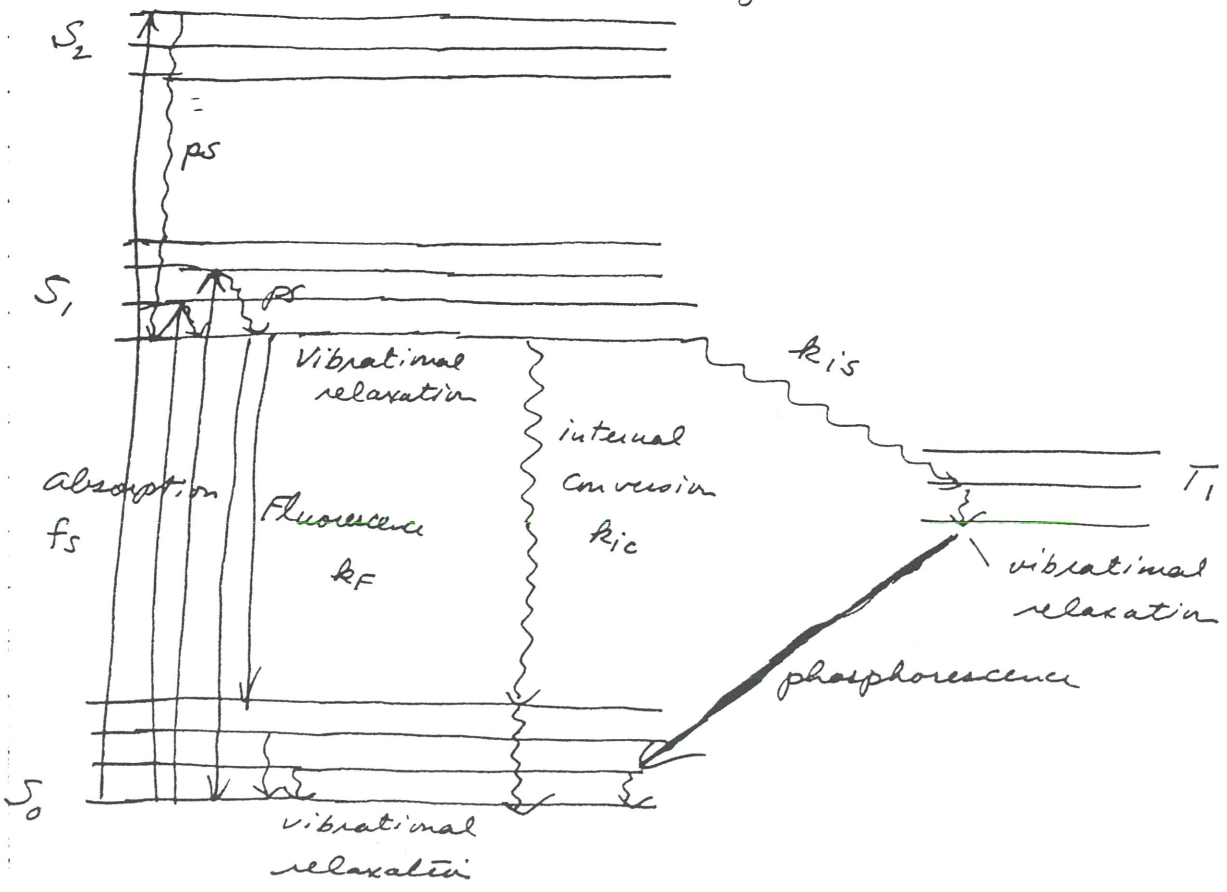
leads to definition of radiative lifetime of S_b as

$$\tau_R = \frac{1}{A_{ba}}$$

molecules

The analysis assumes so far that the same electronic state that absorbed the radiation is subsequently emitting it. This is not always the case. In reality, the problem is more complicated. To begin with, the excited state can lose its energy through many other processes besides direct emission of a photon, so that the actual observed lifetime of an excited singlet state is rarely as long as the radiative lifetime τ_R.

For real molecules, there are many pathways for production and de-excitation of an excited state.



$k_F \equiv$ intrinsic rate constant for fluorescence
$\equiv A_{ba} = 1/\tau_R$

$-\left(\frac{d[S_1]}{dt}\right)_F = k_F [S_1]$

} radiative process — negligible

non-radiative processes:

$k_{ic} \equiv$ rate constant internal conversion

$k_{is} =$ rate constant intersystem crossing

$k_Q \equiv$ rate constant for various deactivation processes induced by quenchers of various types

$$-\left(\frac{d[S_1]}{dt}\right)_T = -\left(\frac{d[S_1]}{dt}\right)_F + (-1)\left(\frac{d[S_1]}{dt}\right)_{ic} + (-1)\left(\frac{d[S_1]}{dt}\right)_{is} + (-)\left(\frac{d[S_1]}{dt}\right)_Q$$

$$= -\left(k_F + k_{ic} + k_{is} + k_q [Q]\right)[S_1]$$

Definition $\tau_F =$ observed fluorescence decay time

$$= \left(k_F + k_{ic} + k_{is} + k_q [Q]\right)^{-1}$$

$$[S_1(t)] = S_1(0)\, e^{-t/\tau_F}$$

measure of how fluorescence intensity decays

why?

$$\underset{\text{emission}}{E(t)} \propto \phi_F (-)\frac{d[S_1(t)]}{dt} = \phi_F \frac{1}{\tau_F} S_1(0)\, e^{-t/\tau_F}$$

ϕ_F: quantum yield

$$= k_F S_1(0)\, e^{-t/\tau_F}$$

τ_F: fluorescence decay time

ϕ_F = fluorescence quantum yield

= fraction of molecules deexcited thru fluorescence

$$= k_F / [k_F + k_{ic} + k_{IS} + k_q [Q]]$$

$$= \frac{\tau_F}{\tau_R}$$

Internal conversion

excitation energy in S_b lost by collision with solvent or dissipation thru internal vibration

(ps) or subnanosec

k_{ic} increases with T, $\therefore \phi_F$ decreases with increasing T

Quenching

deexcitation arising from collisions or complexation with solute molecules Q capable of quenching excited state

$$S_b + Q \xrightarrow{k_q} S_a + Q$$

Aromatic chromophores $\tau_R = 10^{-9} - 100 \times 10^{-9}$ sec

Therefore quenching processes need to be quite effective to compete.

Common quenchers O_2, I^-, Cs^+ deexcite essentially

every collision, so diffusion controlled.

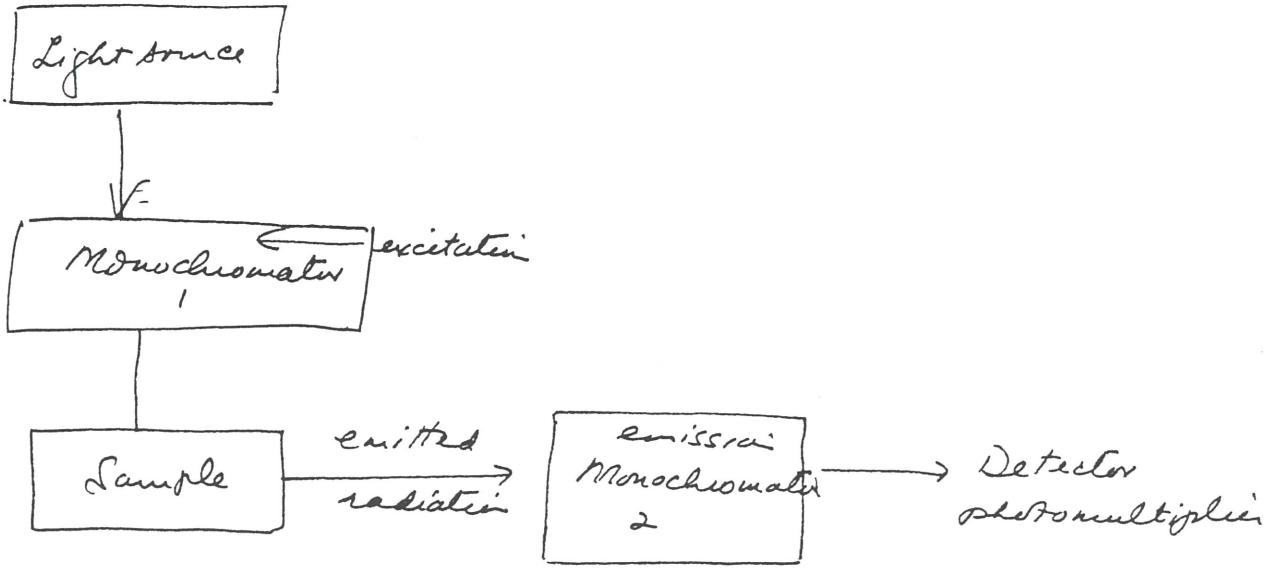
$10^{11}[Q] = 10^{+8}\ s^{-1}$ if $[Q]$ millimolar.

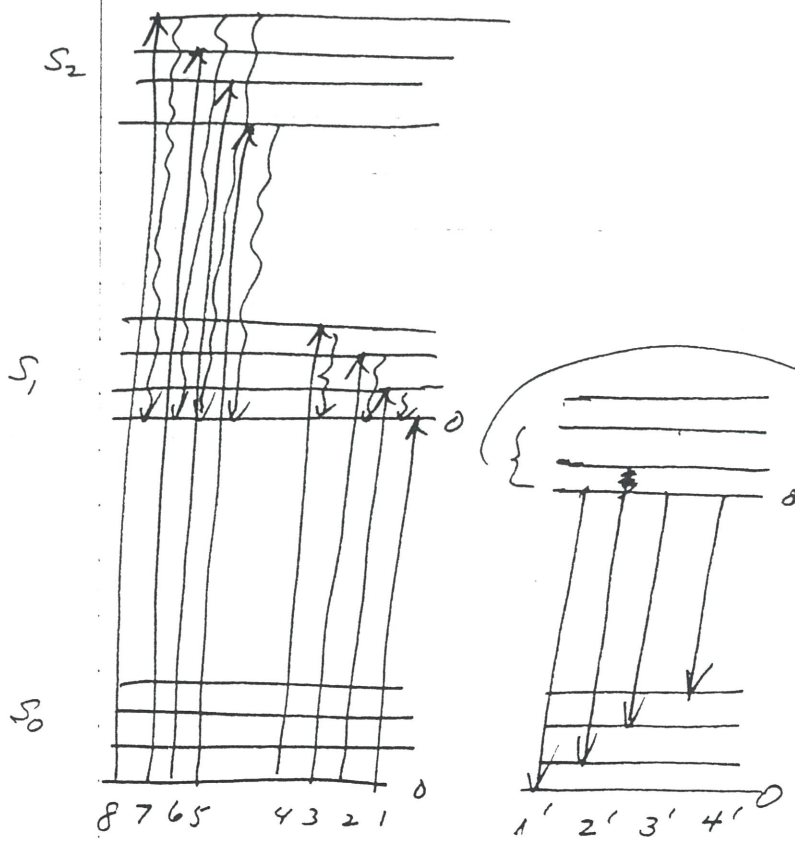
Intersystem crossing

crossing over from excited singlet into excited triplet manifold.

Spectrum

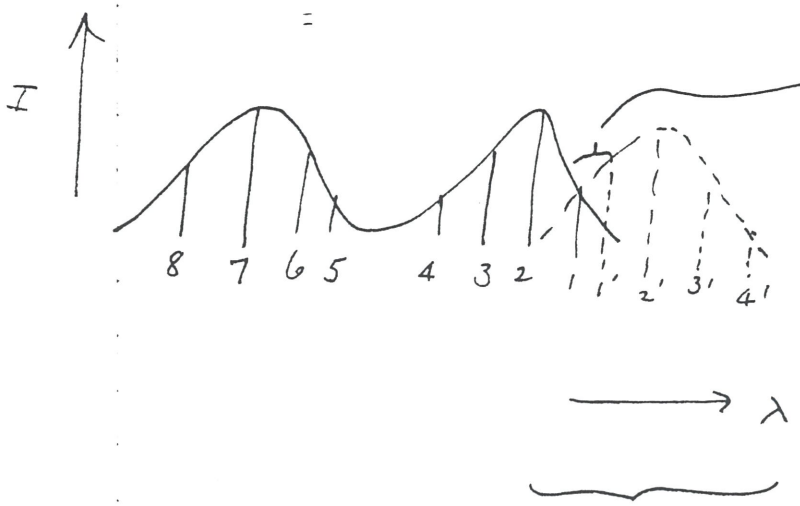
Emission Spectrum / Excitation Spectrum





solvent shift due to different orientation of solvents between ground & excited state

Excitation



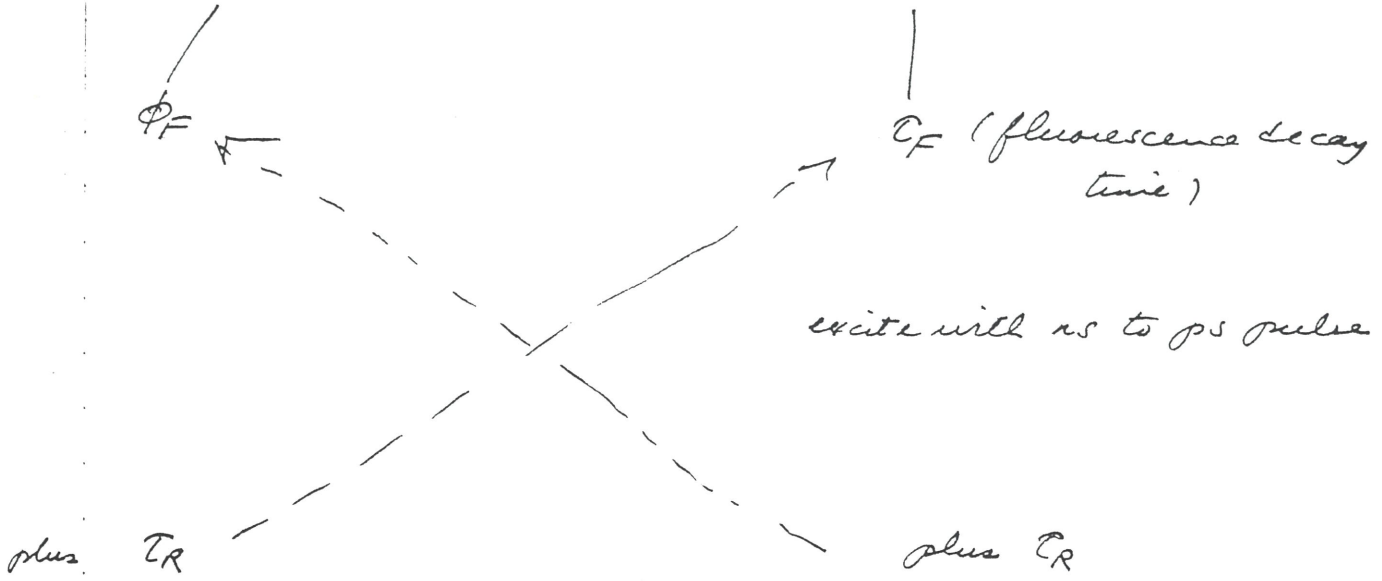
1 & 1' do not generally overlap

Excitation spectrum vary M1, fix M2

Fluorescence spectrum vary M2, fix M1

ϕ_F, τ_F

Steady-state measurements vs Time-resolved measurements



Applications October 26, 1987

Fluorescence λ_{max} & ϕ_F sensitive to environment of chromophore

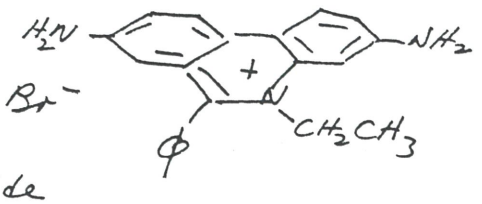
relatively long time molecule spends in excited state before deexcitation 10-100 ps to 100 ns

absorption ~ 10^{-15} sec.

→ ∴ fluorescence is a most effective technique for following binding of ligands, conformational changes, protein/denaturation,

→ some fluorescent molecules
in aqueous soln $\phi_F \to 0$ F strongly quenched
in nonpolar environment enhancement by 20

examples



Ethidium bromide

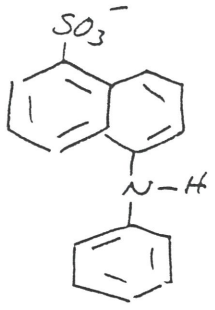
ϕ_F ~0 in aqueous sol'n (weak)

enhanced when bound to nucleic acids (intense)

ϕ_F ~1 $\quad$ 26.5 = τ_F ns

Dye ANS

8-anilinonaphthalene sulfonate



ϕ_F ~0 in aqueous sol'n

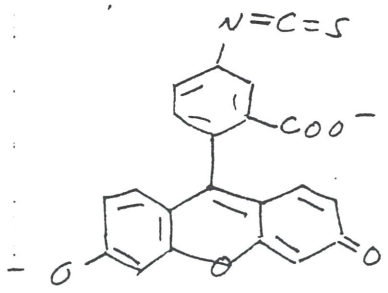
F. enhanced when [redacted] bound to hydrophobic regions of proteins membranes

λ_{max} = 454 nm

ϕ_F = 0.98

τ_F = 16 ns

Fluorescein isothiocyanate covalent attachment to lys



FITC

λ max

[redacted] $\quad$ λ_{max} $\quad$ 320 nm $\quad$ hydrophobic

Trp $\quad$ 340 nm $\quad$ aqueous (polar)

→ Fluorescence spectroscopy can be used to ascertain accessibility of fluorescent chromophore to collisional quenching by solute molecules

$$\frac{F_0}{F} = \frac{\phi_0}{\phi} = \frac{k_F + k_{ic} + k_{is} + k_q[Q]}{k_F + k_{ic} + k_{is}} = \left(\frac{\tau_Q}{\tau_0}\right)^{-1}$$

F_0: absence of quencher; F: in presence of quencher

$k_F + k_{ic} + k_{is} = \tau_0^{-1}$; $\tau_Q^{-1} = k_F + k_{ic} + k_{is} + k_q[Q]$

τ_0, τ_Q: time resolved expt

$$= 1 + k_q \tau_0 [Q]$$

(Steady state yields)

τ_0 = fluorescence decay time in absence of quencher

$$\phi_F = \frac{\tau_F}{\tau_R}$$

$\frac{F_0}{F}$ = $k_q\tau_0$ ← Stern Volmer

$k_q \sim 10^9 - 10^{10}\ M^{-1}s^{-1}$

[Q]

$$\therefore \frac{\phi_0}{\phi} = \frac{(\tau_F)_0}{(\tau_R)} \Big/ \frac{(\tau_F)}{\tau_R} = \frac{\tau_0}{\tau_Q}$$

$$\phi_0 = \frac{k_F}{k_F + k_{ic} + k_{is}}$$

$$\phi = \frac{k_F}{k_F + k_{ic} + k_{is} + k_q[Q]}$$