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Biophysical Chemistry for Life Scientists

National Tsing-Hua University

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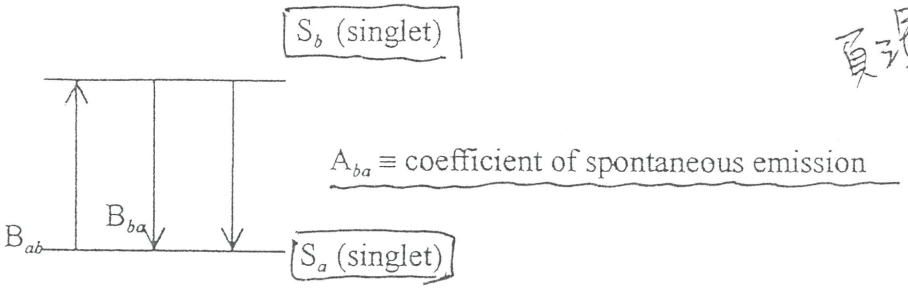
CH C-H 300 wavenumber

Emission of light from excited electronic states

- Absorption of a photon occurs in ~ 10^{-15} s or a femtosecond
- Emission occurs in ~10^{-12}–10^{-9} s, or a picosecond to a nanosecond

Relationship between absorption and emission

Consider the following hypothetical situation



負溫度效應.

Light of radiation density I(ν) induces transition from S_a®S_b, and from S_b®S_a at a rate of B_{ab} (= B_{ba}) per

$S_a \rightarrow S_b$ $S_b \rightarrow S_a$

molecule per sec.

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

Suppose states *b*, *a* are at thermal equilibrium. Then

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

If system is at thermal equilibrium, it must be at thermal equilibrium with blackbody radiation as well. That is, blackbody radiation induces $a \rightarrow b$, $b \rightarrow a$ transition at such a rate that it compensates for spontaneous emission; i.e., the rate of emission and absorption of radiation density must be equal.

Thus, we must have

$$n_a^{eq} \mathrm{I}_{blackbody}(\nu_{ab}) B_{ab} = n_b^{eq} \mathrm{I}_{blackbody}(\nu_{ab}) B_{ba} + A_{ba} n_b^{eq}$$

or

$$\frac{n_a^{eq}}{n_b^{eq}} = [B_{ba}\mathrm{I}_{bb}(\nu_{ab}) + A_{ba}]/B_{ab}\mathrm{I}_{bbody}(\nu_{ab}) = 1 + \frac{A_{ba}}{B_{ab}}\frac{1}{\mathrm{I}(\nu_{ab})_{thermalradiation}}$$

Now $$\mathrm{I}_{thermal}(\nu) = \frac{8\pi h\nu^3}{c^3}(e^{+h\nu/k_BT} - 1)^{-1}$$

Substituting, we have

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

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

or, $$A_{ba} = B_{ab}\frac{8\pi h\nu_{ab}{}^3}{c^3} = \left(\frac{32\pi^3\nu^3}{3c^3\hbar}\right)D_{ab}$$

Note ν^3 dependence as well as direct proportionality with D_{ab}. Thus,

- $A_{ba} = 0$ if $D_{ab} = 0$

and

- Spontaneous emission important for transitions in the visible, uv, x-ray, γ-ray regions.

Finally, because D_{ab} and ν_{ab} can usually be obtained from the absorption spectrum, the rate of spontaneous emission can be determined without

performing an emission experiment.

Of course, in the absence of radiation or any other perturbations or interactions, the rate of deexcitation of molecules initially in state S_b will be

$$\frac{dn_b}{dt} = -A_{ba} n_b \quad \text{so that}$$

$$-A_{ba}t = \frac{dn_b}{n_b} = \frac{-t}{\tau_R} \qquad \therefore \tau_R = \frac{1}{A_{ba}}$$

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

This leads to the definition of the radiative lifetime of S_b as

life time.

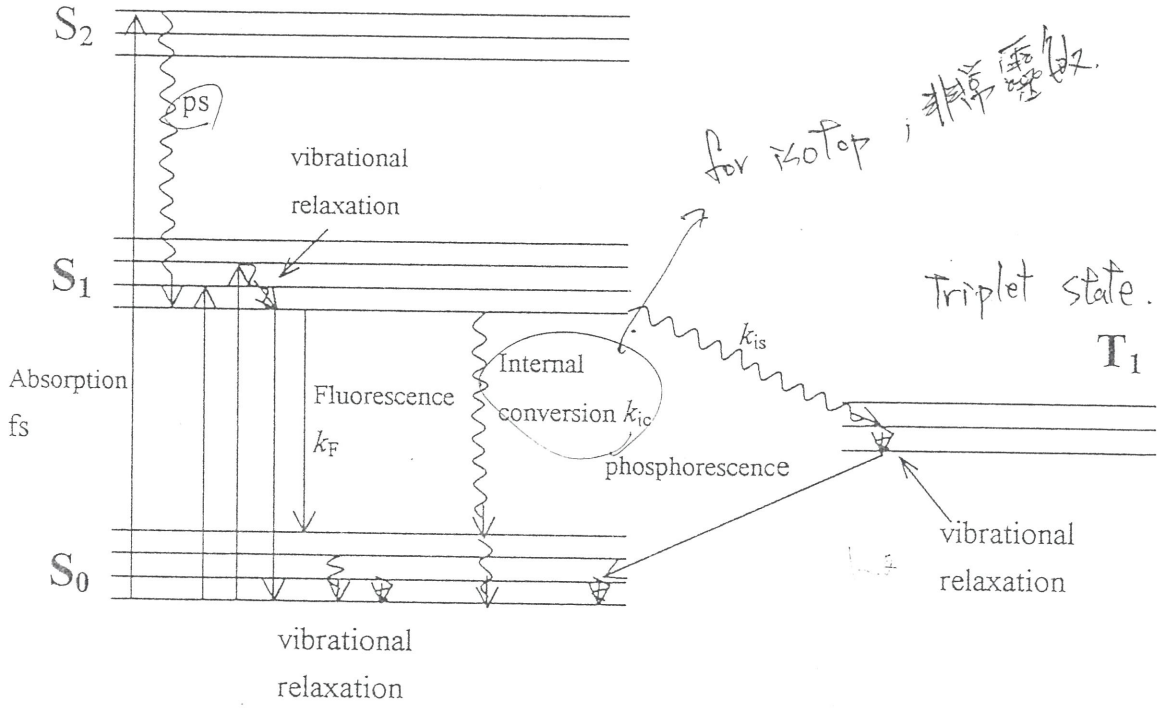
$$\tau_R = \frac{1}{A_{ba}} .$$

Real molecules

The above analysis assumes so far that the same electronic state that absorbed the radiation is subsequently emitting it. This is not always the case. In real molecules, 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 re-excitation of an excited state.



$k_F \equiv$ intrinsic rate constant for fluorescence

$\equiv A_{ba} = 1/\tau_R$

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

} radiative process

non-radiative processes {

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

$k_{is} \equiv$ rate constant for intersystem crossing

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

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

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

Fluorescence

Define τ_F = observed fluorescence decay time

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

$[S_1(t)] = S_1(0)e^{-t/\tau_F}$ measure of how fluorescence intensity decays

Why?

$$I(t) \propto \phi_F(-)\frac{d[S_1(+)]}{dt} = \phi_F \frac{1}{\tau_F} S_1(0)e^{-t/\tau_F} = k_F S_1(0)^{-t/\tau_F}$$

quantum yield — fluorescence decay

ϕ_F = fluorescence quantum yield = fraction of molecules deexcited through 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 through internal vibrations (ps) or sub-nanosecond; 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

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

For aromatic chromophores, $\tau_R = 10^{-9} - 100\times10^{-9}$ sec. Therefore quenching processes need to be quite effective to compete.

Common quenchers: O_2, I^-, Cs^+ deexcite essentially every collision, so process is diffusion

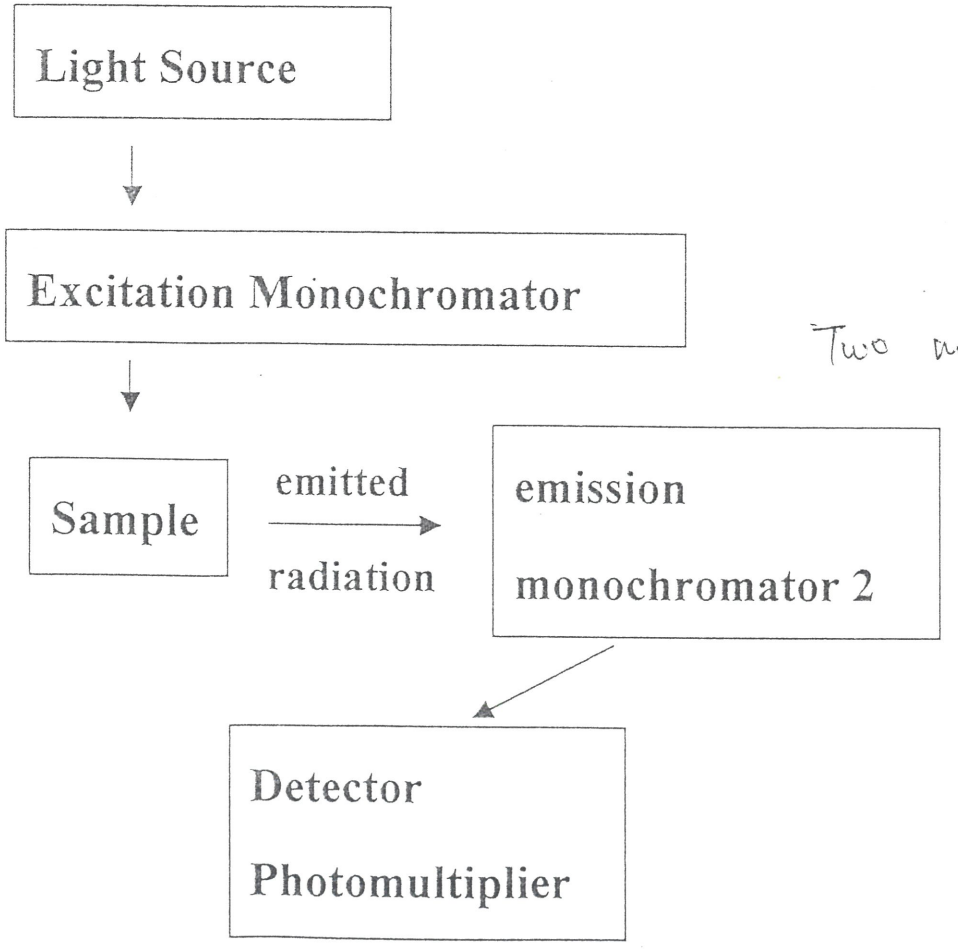
controlled.

$$10^{11}[Q] = 10^{+8}\ s^{-1} \quad \text{if} \quad [Q] \quad \text{is millimolar!}$$

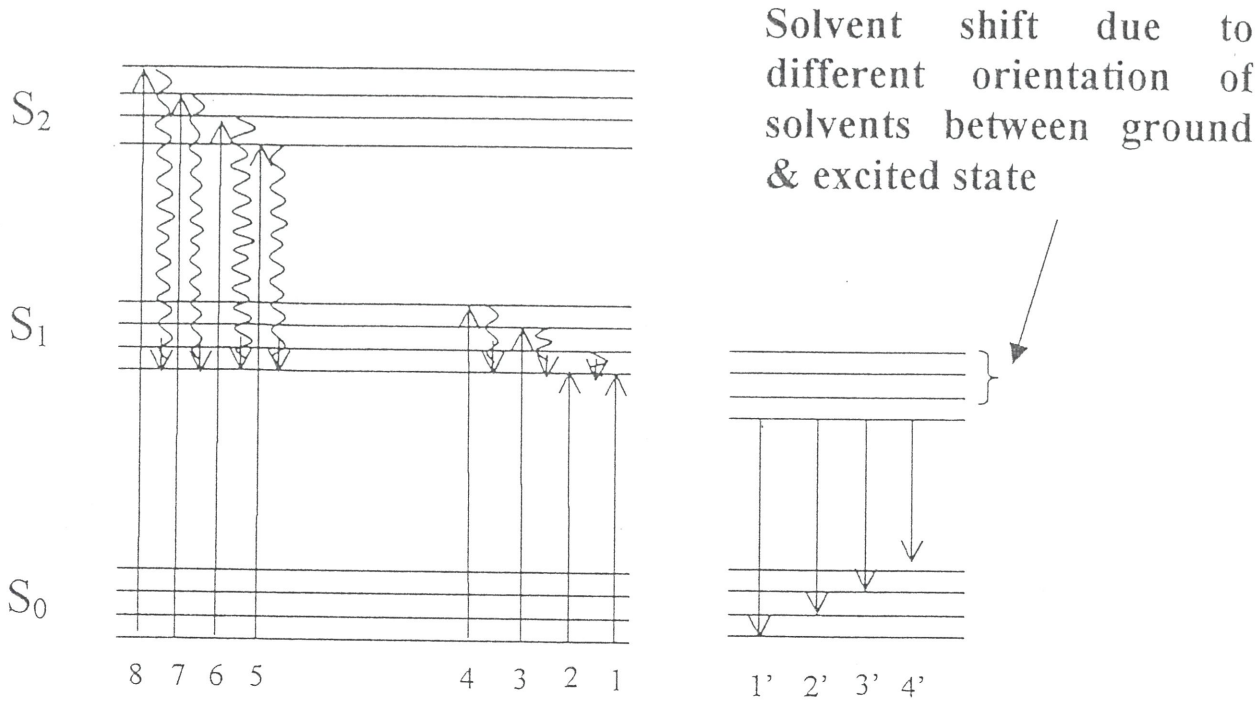
Intersystem crossing

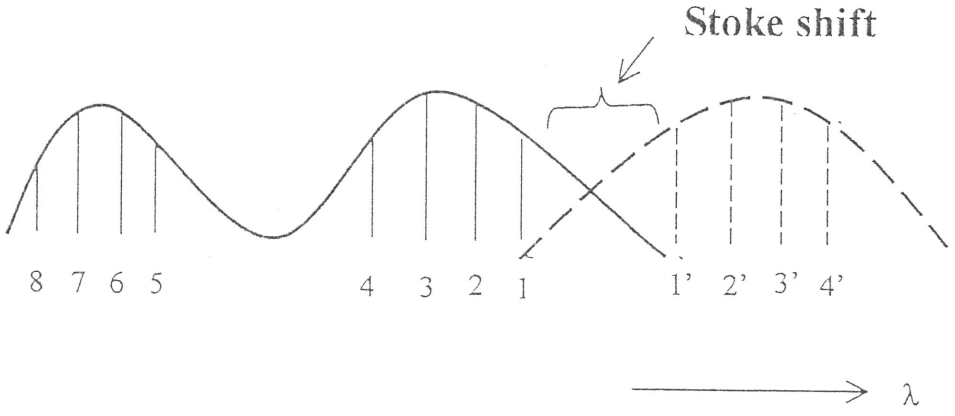
Crossing over from excited singlet into excited triplet manifold.

Spectroscopy: Emission / Excitation Spectum



Two monochromator.





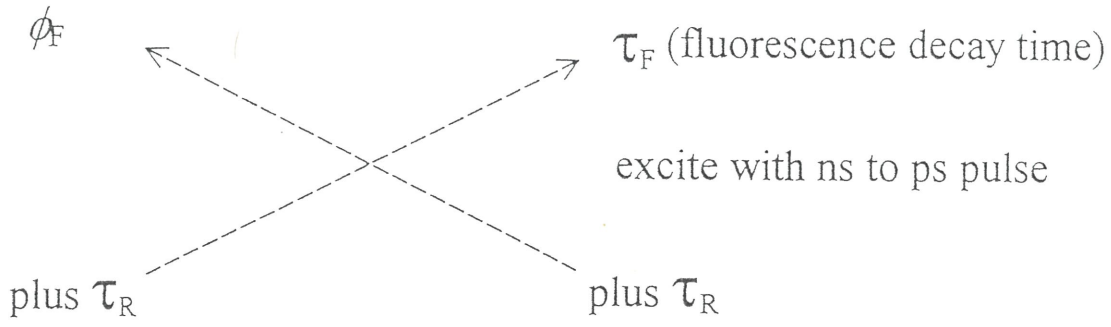
1 & 1' do not generally overlap

Excitation spectrum: vary M1, fix M2

Fluoresence spectrum: vary M2, fix M1

ϕ_F, τ_F 量子產率，半衰期.

Steady state vs Time-resolved measurements



Applications

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

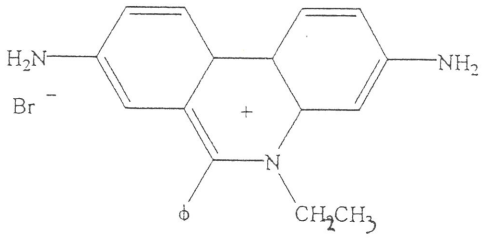
Relatively long time that molecule spends in excited state before deexcitation: 10-100 ps to 100 ns

compared to absorption: $\sim 10^{-15}$ sec

- **fluorescence is a most effective technique for following binding of ligands, conformational changes, protonation/deprotonation**

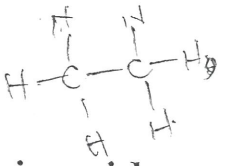
- some fluorescent molecules in aqueous solvent $\phi_F \rightarrow 0$; i.e., fluorescence is strongly quenched; in nonpolar environment, there is enhancement of the fluorescence by a factor of 20!

Examples



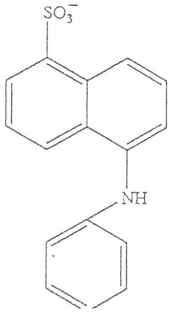
Ethidium bromide

$\phi_F \sim 0$ in aqueous solution (weak)



fluorescence is enhanced when bound to nucleic acids (intense) : $\phi_F \sim 1$, $\tau_F = 26.5$ ns

Dye ANS



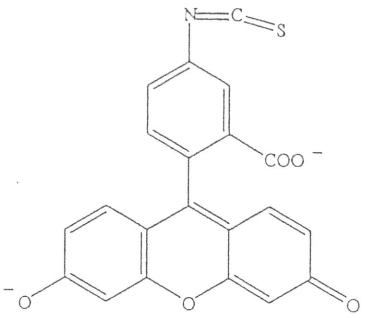
δ- anilinonaphthalene sulfonate

$\phi_F \sim 0$ in aqueous solution

Fluorescence is enhanced when bound to hydrophobic regions of proteins; and membranes

$\lambda_{max} = 454$ nm, $\phi_F \sim 0.98$, $\tau_F = 16$ ns

Fluorecein isothiocyanate (FITC) covalent attachment to lysine



Trytophan

λ_{max} 320 nm hydrophobic

340 nm aqueous (polar)

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

absence of quencher

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

τ_Q^{-1}

Steady state yields

τ_o^{-1}

Time resolved expt

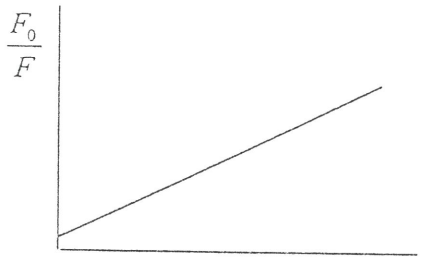
In presence of quencher

where τ_o = fluorescence decay time in absence of quencher

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

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

$$= \frac{\tau_Q}{\tau_o}$$



$k_q\tau_0$ ← Stern Volmer

10^9-10^{10} $M^{-1}s^{-1}$

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

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

October 30, 1987

Siglet-singlet energy transfer

quenching of fluorescence of a donor via migration of donor excitation to a suitable acceptor

Consider two chromophores

Donor ≡ D

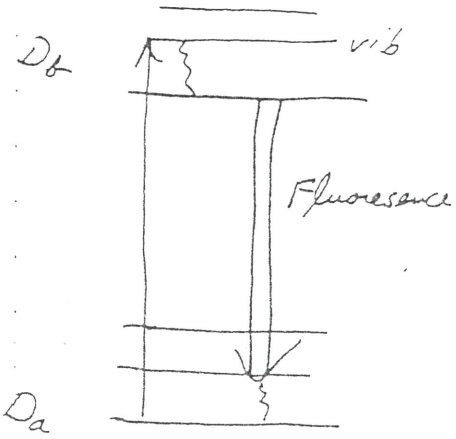
Acceptor ≡ A

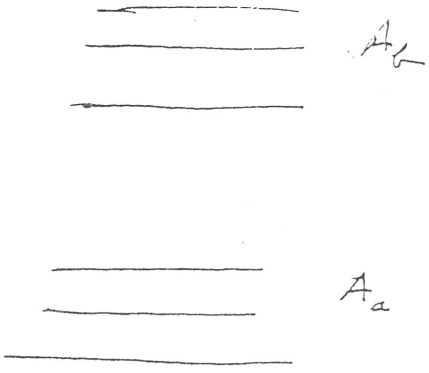
Donor

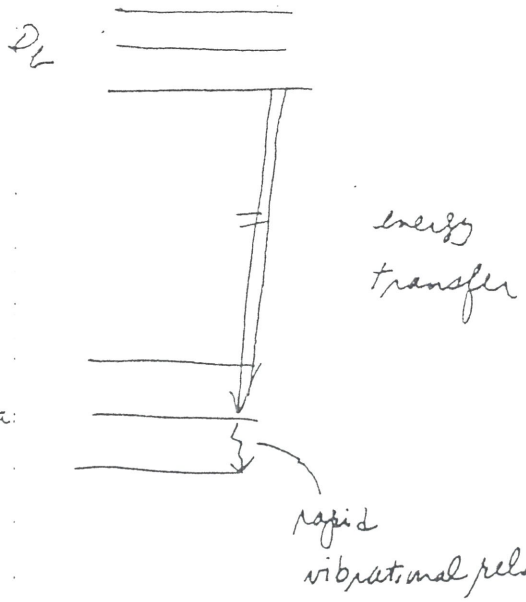
Acceptor

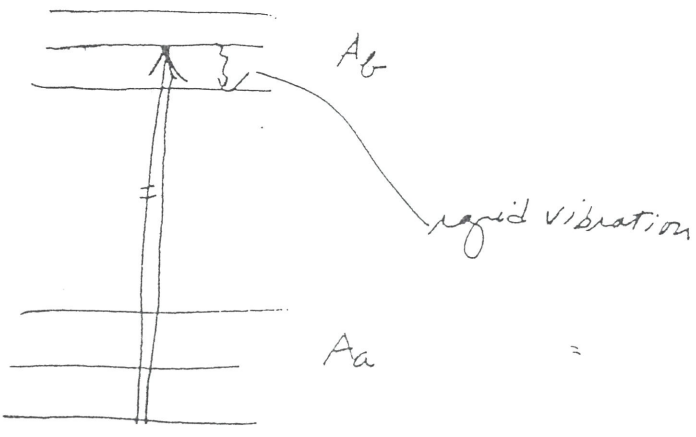
sufficiently far away so that there's no exciton interaction

or exciton interaction negligible









Donor de-excitation and acceptor excitation coupled in resonant interaction leading to energy transfer

$$D_b + A_a \underset{k_{-T}}{\overset{k_T}{\rightleftharpoons}} D_a + A_b$$

Vibrational relaxation rapidly converts resulting acceptor singlet (A_b) and donor singlet (D_a) to ground vibrational level.

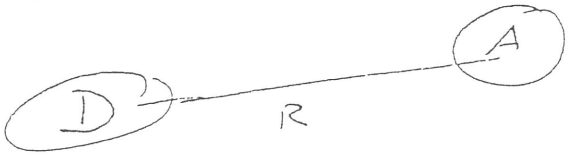
There $k_T >> k_{-T}$

Donor becomes quenched

Acceptor becomes excited and subsequently it can fluoresce

This process is called sensitized emission.

k_T = ?



$R > 10A^\circ$

$$\mathcal{H} = H_D + H_A + V(D,A)$$

Non-stationary state problem.
Non-stationary state problem Quantum

$$V(D,A) = \frac{\vec{\mu}_D \cdot \vec{\mu}_A}{R^3} - \frac{3(\vec{\mu}_D \cdot \vec{R})(\vec{R} \cdot \vec{\mu}_A)}{R^5}$$

dipole.

$t=0$: $\Psi(D,A)_{t=0} = \phi_b(D)\,\phi_a(A)\, e^{-i\varepsilon_b^D H_c} e^{-i\varepsilon_a^A(+)/\hbar}$

~~Error~~ : $= \phi_b(0)\,\phi_a(A)$

$t = t$: $\Psi(D,A,t) = c_{Db,Aa}\ \phi_b(D)\,\phi_a(A)\,e^{-i\epsilon_b^D t/\hbar}\,e^{-i\epsilon_a^A t/\hbar}$

$$+\ c_{Da,Ab}\ \phi_a(D)\,\phi_b(A)\,e^{-i\epsilon_a^D t/\hbar}\,e^{-i\epsilon_b^A t/\hbar}$$

$$k_T^{(\nu)} = \frac{1}{2\hbar^2}\left|\langle \phi_a(D)\phi_b(A)\,|\,\underset{\sim}{V}\,|\,\phi_b(D)\phi_a(A)\rangle\right|^2$$

$$\propto \left(\frac{\kappa}{R^3}\right)^2 \left|\langle \phi_a(D)\phi_b(A)\,|\,|\underset{\sim}{\mu}_D|\,|\underset{\sim}{\mu}_A|\,|\,\phi_b(D)\phi_a(A)\rangle\right|^2$$

$$V = \kappa\,|\underset{\sim}{\mu}_D|\,|\underset{\sim}{\mu}_A|/R^3$$

$$\propto \left(\frac{\kappa^2}{R^6}\right)\left|\langle \phi_a(D)|\vec{\mu}_D|\phi_b(D)\rangle\langle\phi_b(A)|\vec{\mu}_A|\phi_a(A)\rangle\right|^2$$

Now $D_{ab} = |\langle \phi_b|\mu|\phi_a\rangle|^2 \propto \int \frac{\epsilon}{\nu}\,d\nu$

$$\propto \frac{\kappa^2}{R^6}\ D_{ba}(D)\ D_{ab}(A)$$

$$\propto \frac{\kappa^2}{R^6}\ \frac{\epsilon_A(\nu)}{\nu}\ \frac{A_{ba}}{\nu^3}$$

absorption

$$\propto \frac{\kappa^2}{R^6}\ \frac{\epsilon_A(\nu)}{\nu}\ \frac{1}{\nu^3}\ \frac{1}{\tau_R}\ f_D(\nu)$$

$$\propto \frac{\kappa^2}{R^6}\ \frac{\epsilon_A(\nu)}{\nu^4}\left(\frac{\phi_D}{\tau_D}\right) f_D(\nu)$$

ϕ_D = quantum yield of fluorescence of Donor

τ_D = lifetime of donor in absence of acceptor

decay in

acceptor absorption and fluorescence of donor occurs over a band of frequencies. Let $f_D(\nu)$ be the fraction of donor fluorescence at frequency ν

Integrating over all frequencies

$$k_T \propto \left(\frac{\kappa^2}{R^6}\right)\left(\Phi_D/\tau_D\right)\underbrace{\int \epsilon_A(\nu) f_D(\nu)\, \nu^{-4}\, d\nu}_{J}$$

Förster

$$k_T = \left(\frac{1}{\tau_D}\right)\left(\frac{R_0}{R}\right)^6$$

τ_D = lifetime of donor in absence of the acceptor (fluorescence decay time)

$$R_0 = 9.7 \times 10^3 \left(J \kappa^2 n^{-4} \Phi_D\right)^{1/6} \text{ cm}$$

$\epsilon_{\text{dielectric constant}} = n^2$

$$V = \frac{1}{n^2}\left(\kappa^2 \langle \mu_D | \rangle \langle | \mu_A | \rangle\right)$$

↑ in fluid medium

a measure of spectral overlap between donor emission and acceptor absorption

κ^2 complex geometric factor that depends on orientation of donor & acceptor $\longrightarrow \frac{1}{3}$ if both donor and acceptor

are free to tumble rapidly and isotropically on the scale of fluorescence emission

$$R_0 = 8.79 \times 10^{-5} (J \kappa^2 n^{-4} \phi_D)^{1/6} \text{ Å}$$

where $J = \int \epsilon_A(\lambda) f_D(\lambda) \lambda^4 d\lambda$

Experiment

1) Measure quantum yield of fluorescence of Donor in presence and absence of acceptor

$$\phi_{D+A} = \frac{k_F^D}{k_F^D + k_{ic}^D + k_{is}^D + k_T}$$

$$\phi_D = \frac{k_F^D}{k_F^D + k_{ic}^D + k_{is}^D}$$

$$\therefore \phi_{D+A}/\phi_D = \frac{k_F^D + k_{ic}^D + k_{is}^D}{k_F^D + k_{ic}^D + k_{is}^D + k_T}$$

$$= 1 - E$$

$\phi_{D+A}/\phi_D = 0$ ⟹ $E = 1$

$\phi_{D+A}/\phi_D = 1$ ⟹ $E = 0$

where E = efficiency of transfer from D to A

$$= \frac{k_T^{D \to A}}{k_F^D + k_{ic}^D + k_{is}^D + k_T}$$

2) Measure fluorescence decay time of the donor

in presence ($\tau_{D,A}$) and absence (τ_D) of the acceptor

$$\left.\begin{aligned} \phi_{D+A} &= \frac{\tau_{D,A}}{\tau_R} \\ \phi_D &= \frac{\tau_D}{\tau_R} \end{aligned}\right\} \Rightarrow \quad 1-E = \frac{\tau_{D,A}}{\tau_D}$$

Measuring intrachromophore distances from energy-transfer efficiencies

$$k_T = \left(\frac{1}{\tau_D}\right)\left(\frac{R_0}{R}\right)^6$$ error in 8-54 CS

Now $$E = \frac{k_T}{k_T + k_F^D + k_{ic}^D + k_{is}^D}$$

$$\frac{1}{E} = \frac{k_T + k_F^D + k_{ic}^D + k_{is}^D}{k_T}$$

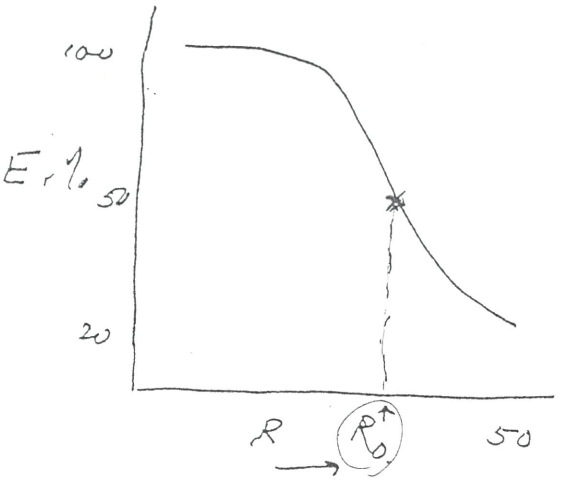
$$= 1 + 1/\tau_D k_T = \frac{1}{k_T}\left(k_T + \frac{1}{\tau_D}\right)$$

$$E = k_T \left(k_T + \frac{1}{\tau_D}\right)^{-1}$$

$$= \left(\frac{1}{\tau_D}\right)\left(\frac{R_0}{R}\right)^6 \left[\frac{1}{\tau_D}\left(\frac{R_0}{R}\right)^6 + \frac{1}{\tau_D}\right]^{-1}$$

$$= \left(\frac{R_0}{R}\right)^6 \left[1 + \left(\frac{R_0}{R}\right)^6\right]^{-1}$$

$$= (R_0)^6 / [R^6 + R_0^6]$$



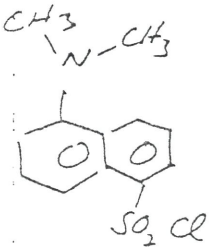
Donor — Acceptor

Dansyl-$(Pro)_n$-NH-NH-C(=O)-NH-Naphthyl

polyproline type II helix

n = 1 to 12

L. Stryer & Richard Haugland (1967) PNAS 98, 719 (1967)



		Absorption λ_{max}	ϵ_{max} $\times 10^{-3}$	Fluorescence λ_{max}	ϕ_F	τ_F
H_2O	Tyrosine	274	1.4	303	0.14	3.6
pH=7	Tryptophan	280	5.6	348	0.20	2.6
						ns

R_0 9 Å

∴ Tyrosine fluorescence usually quenched via singlet-singlet energy transfer to tryptophan