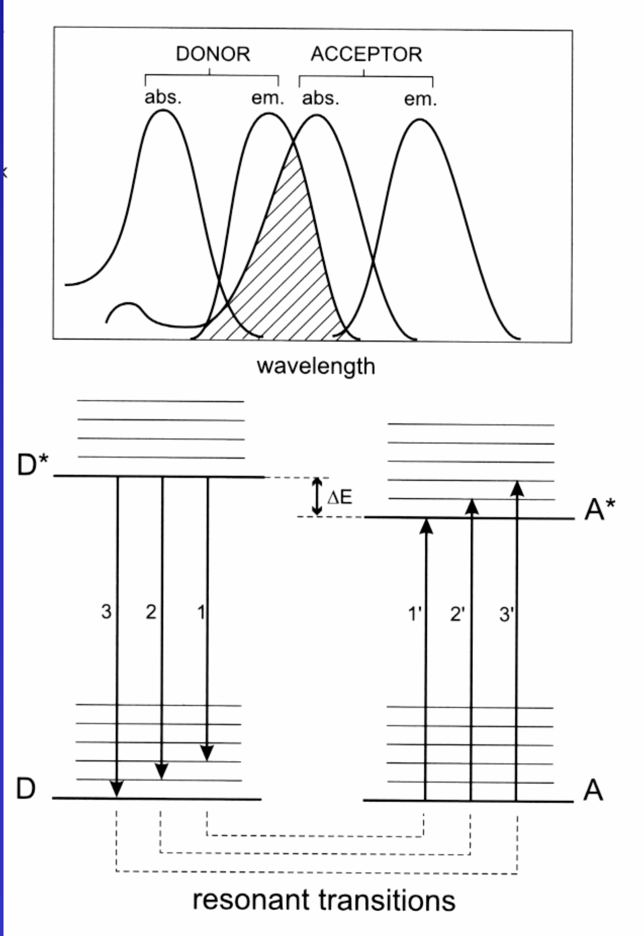
single-molecule fluorescence resonance energy transfer

(3) Förster theory of FRET

michael börsch

09/06/2004

fluorescence resonance energy transfer : FRET (1)



- non-radiative energy transfer
- electronic energy transfer
- coulomb interaction: very weak long-range dipole-dipole IA (Förster)

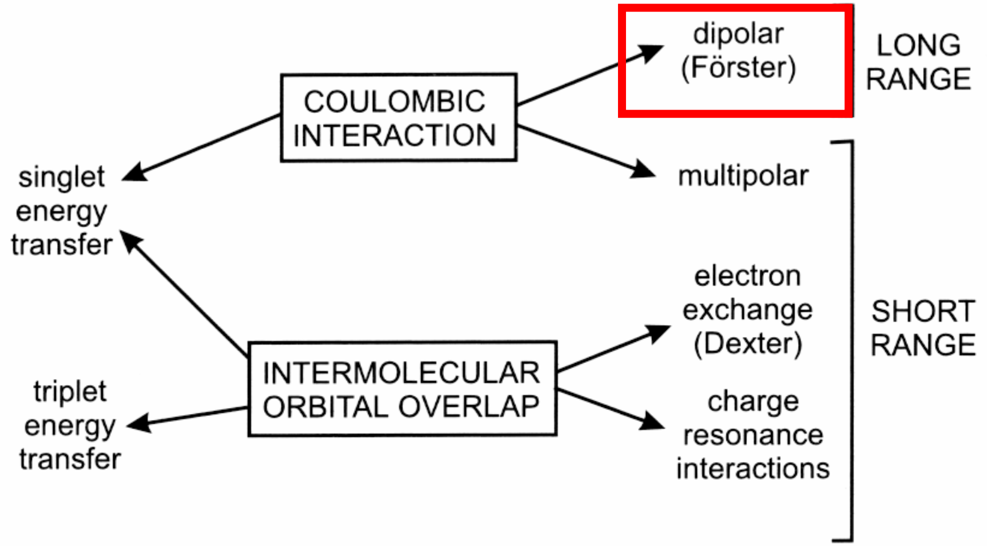


Fig. 4.13. Types of interactions involved in non-radiative transfer mechanisms.

fluorescence resonance energy transfer : FRET (2)

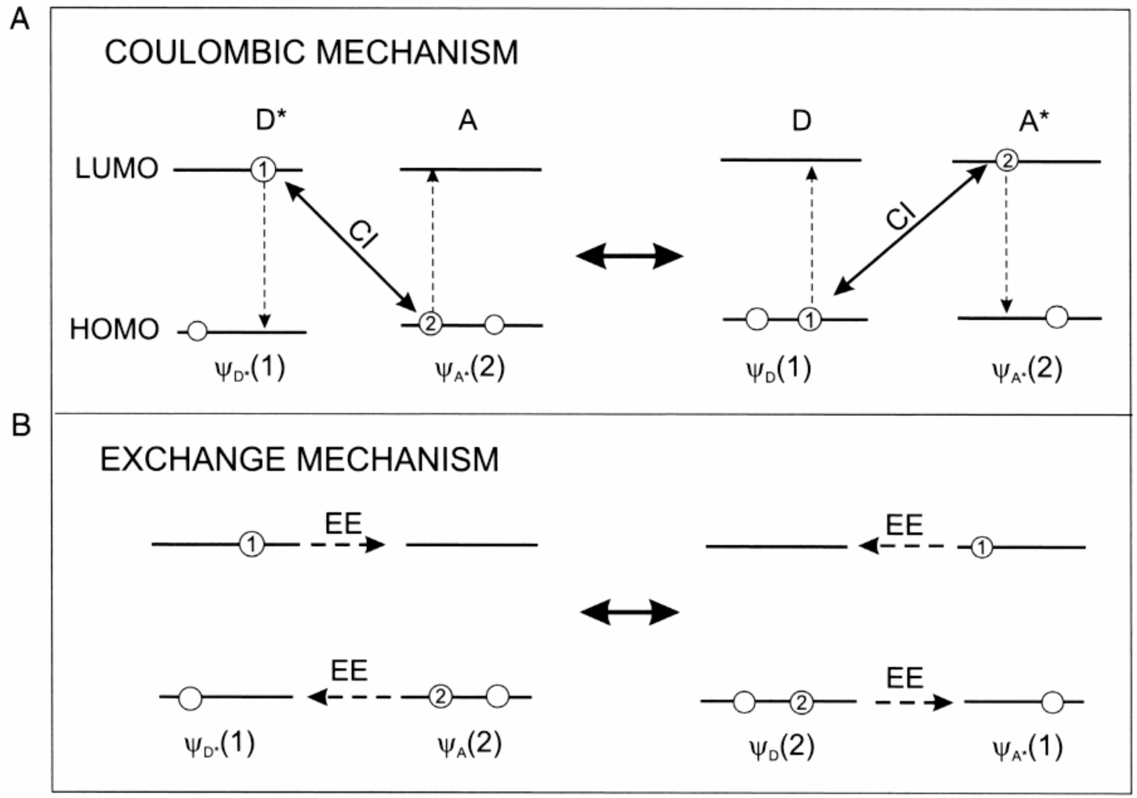


Fig. 4.14. Schematic representation of the (A) Coulombic and (B) exchange mechanisms of excitation energy transfer. CI: Coulombic interaction; EE: electron exchange.

intermolecular energy transfer

- RET
- reabsoprtion
- complex formation
- collisional quenching

The Förster theory of resonance energy transfer:

concentration dependent depolarization of fluorescence

- the polarization of fluorescein in glycerol is 0.4 for $c_{Fl} < 0.1$ mM, and decreases to 0.05 for $c_{Fl} = 10$ mM
- the quantum yields decreases for $c_{Fl} > 1$ mM (quenching)
- similar for trypaflavin: the polarization decreases for c > 0.3 mM, the lifetime decreases for c > 3 mM
- in a viscous solvent depolarization occurs by energy transfer between 'identical' fluorophores (homotransfer) with different transition dipole moments
- simple reabsorption depends on concentration <u>and</u> cuvette volume, which was not observed.
- J. Perrin (1924) theory with 'transfert d'activation' resulted in too large distances between the molecules

Förster theory:
the classical version (1951)

'Fluoreszenz Organischer Verbindungen'

(i) classical approach with one frequency
(ii) classical approach with a range of frequencies

(1) a donor and an acceptor in a medium with index of refraction n
(2) donor is excited and produces an radiating electromagnetic field
(3) this field depends on charge distribution and is described in an multipole expansion
monopole: vanishes because donor is electrically neutral
dipole: relevant for large distances > 10 Å, donor is a set of dipoles oszillating at different frequencies
multipoles: not important for large distances

Förster theory (2)

(4) donor dipole oszillates along a line (simplification) with unit vector d and frequency ν , or circular frequency $\omega = 2\pi\nu$

(5) dipole moment vector $\boldsymbol{p} = d\, p_0 \cos(\omega t)$ with maximum value p_o

(6) implications: absorption and emission spectrum are very narrow peaks

(7) electric dipole field E_D of the donor at a point at distance R

$$E_D = E_F + E_M + E_R$$

with 'Förster field' E_F depending on R^{-3} , E_M on R^{-2} , and the 'radiation field' E_R depending on R^{-1}

(8) defining distance b:

$$b = \frac{c}{\omega n} = \frac{\lambda}{2\pi n}$$

(9) than E_F dominates at small R and E_R at large R with respect to b

Förster theory (3)

(10) b is in the order of 1000 Å

(11) sphere for 0.1b 'Förster zone' or 'near field zone' from 10Å to 100Å

(12) donor charge distribution within zone D

(13) radiation zone >10b from 1µm

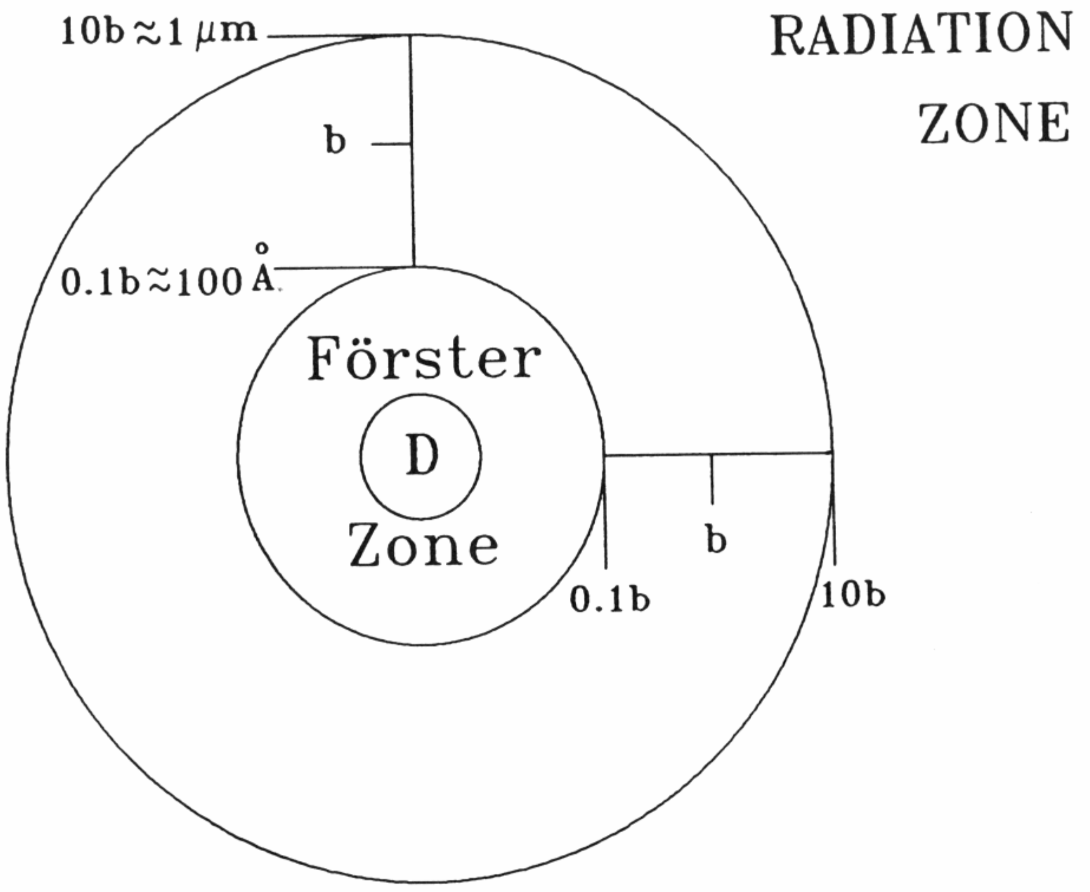


Figure 3.1. The Förster zone and the radiation zone, on a logarithmic scale, around the donor (D) at the origin. In the Förster zone the electric field caused by the donor dipole falls off with the third power of the inverse distance and gives rise to resonance energy transfer, in the radiation zone the donor electric field is proportional to the inverse of the distance and corresponds with radiation. The parameter b is equal to $\lambda/(2\pi n)$.

Förster theory (4)

(14) unit vectors d, r, θ

(15) defining:

$$e_D = d - 3r\cdot\cos\theta$$

(16) in cgs-units:

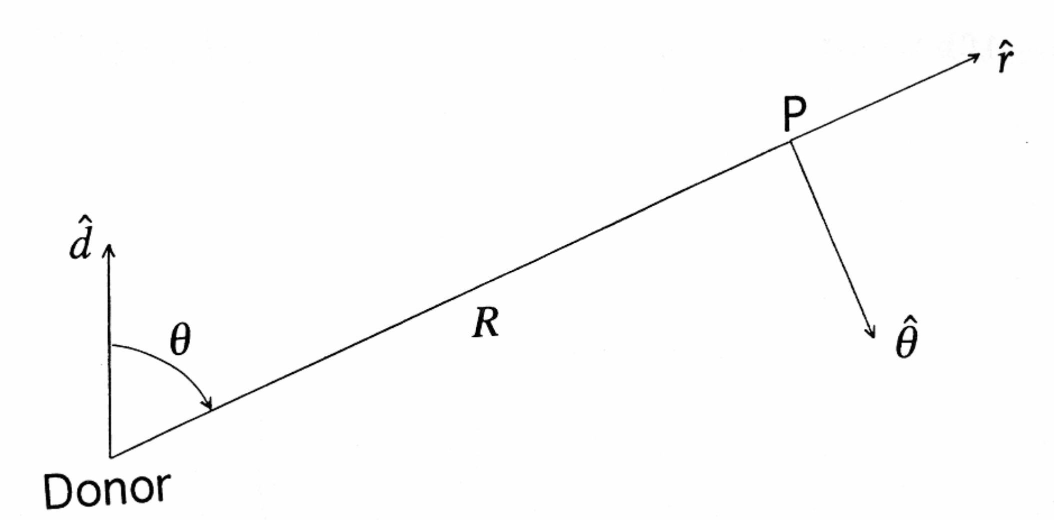


Figure 3.2. The donor dipole produces an electric field in point P at a distance R from the donor. This field depends on the angle θ, and the unit vectors $\hat{d}$, $\hat{r}$, and $\hat{\theta}$. $\hat{d}$ is along the donor dipole, $\hat{r}$ is in the direction in which R increases, and $\hat{\theta}$ is in the direction in which θ increases, θ is the angle between $\hat{r}$ and $\hat{d}$.

$$E_F = E_{F0}\cos(\omega t - R/b) \quad with \ E_{F0} = \frac{-p_0\, e_D}{n^2 R^3}$$

$$E_M = E_{M0}\sin(\omega t - R/b) \quad with \ E_{F0} = \frac{p_0\, e_D}{n^2\, b\, R^2}$$

$$E_R = E_{R0}\cos(\omega t - R/b) \quad with \ E_{F0} = \frac{-p_0\, \hat{\theta}\sin\theta}{n^2\, b^2 R^1}$$

Förster theory (5)

(17) the fluorescence intensity I_D emitted by the donor and detected in the radiation zone

$$I_D = \frac{nc}{8\pi} E_{R0}^2$$

with donor quantum yield 1

(18) the emitted power P_D is the integral of I_D over a sphere around the donor with radius R

$$P_D = \int_0^{2\pi} d\varphi \int_0^{\pi} d\theta \sin\theta \, R^2 \, I_D = \frac{np_0^2\omega^4}{3c^3}$$

Förster theory (6)

(19) the acceptor molecules in the Förster zone interact with the oszillating electric field E_F of the donor in the same way that they would absorb it from an electromagnetic wave

(20) calculating the power P_A absorbed by the acceptor

(21) layer of thickness l , surface area S_l , acceptor: concentration c_A extinction coefficient ε_A

(22) for $l << 1/ \varepsilon_A c_A$ the transmitted intensity I_T is

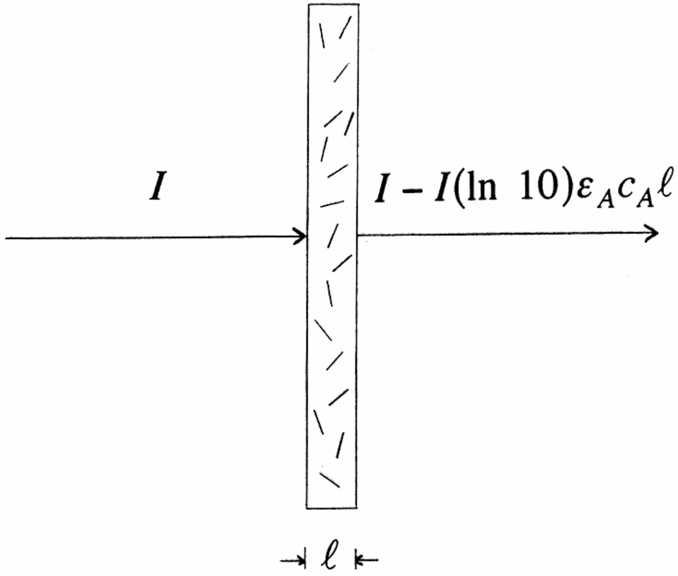


Figure 3.3. If the incident intensity is I, the acceptor concentration is C_A, and its extinction coefficient is ε_A, then the absorbed intensity is $I(\ln 10)\varepsilon_A c_A \ell$, if the thickness ℓ of the layer is sufficiently small.

$$I_T = I \bullet 10^{-\varepsilon_A c_A l} = I \bullet e^{-(\ln 10)\varepsilon_A c_A l} = I - I(\ln 10)\varepsilon_A c_A l$$

Förster theory (7)

(23) the absorbed power is

$$P_{ABSORBED} = S_l \, I \, (\ln 10) \varepsilon_A c_A l$$

(24) the number of acceptor molecules in the layer equals

$$Acceptor\ molecules = 1000 \, S_l \, c_A l \, N_A$$

(25) and the power absorbed by one molecule is

$$P_{Absorbed\ by\ one\ molecule} = \frac{1000 \, I \, (\ln 10) \varepsilon_A}{N_A}$$

with random direction between the electric field and the dipole moment of the acceptor with unit vector a

Förster theory (8)

(26) with *a* aligned in the direction of the electric field follows:

$$P_{Absorbed\,by\,one\,molecule}=\frac{3000\,I\,(\ln 10)\varepsilon_A}{N_A}=\frac{3000\,nc\,E_A^2(\ln 10)\varepsilon_A}{8\pi N_A}$$

with E_A *: amplitude of the component of the electric field along a*

$$E_A=a\bullet E_{F0}=\frac{-p_0\,\kappa}{n^2\,R^3}$$

$$with \qquad \kappa=a\bullet e_D=(a\bullet d)-3(a\bullet r)\cos\theta=(a\bullet d)-3(a\bullet r)(r\bullet d)$$

and substituted :

$$P_{Absorbed\,by\,one\,molecule}=\frac{3000\,(\ln 10)\,c\,p_0^2\,\kappa^2\varepsilon_A}{8\pi\,n^3N_A\,R^6}$$

Förster theory (9)

(27) the distance, where the radiated power, P_D, equals the power transferred to the acceptor in the near field, P_A, is the Förster distance, R_0 .

(28) with $P_D = P_A$

$$P_D = \frac{np_0^2\omega^4}{3c^3} = P_{Absorbed\,by\,one\,molecule} = \frac{3000\,(\ln 10)\,c\;p_0^2\;\kappa^2\varepsilon_A}{8\pi\,n^3 N_A\,R^6}$$

and $r = R_0$ follows for the special single-frequency case:

$$R^6_{0,(one\ frequency)} = \frac{9000\,(\ln 10)\,c^4\;\kappa^2\varepsilon_A}{128\pi^5\;N_A\,n^4\nu^4}$$

(29) $\kappa^2 = 2/3$, $c=3*10^{10}$ cm/s, $\varepsilon_A=10^5\ M^{-1}cm^{-1}$, $\nu=10^{15}s^{-1}$, $n=1.4$, N_A yields $R_0 \sim 50$Å

Förster theory (10)

(30) assuming the donor as a composite of several dipoles oszillating in a range of frequencies

(31) consider an electrical charge e with mass m, oscillating around an equillibrium position with eigenfrequency ν_0 and a spring konstant k

$$\nu_0 = \frac{\omega_0}{2\pi} = \frac{1}{2\pi}\sqrt{\frac{k}{m}}$$

(32) the oscillator is forced into oscillation along the x-axis by an external electric field

$$E = \hat{x}\,E_{0x}\cos\omega_0 t$$

(33) the equation of motion is

$$m\frac{d^2}{dt^2}x = -m\omega_0^2 x + eE_{0x}\cos\omega t$$

with x is the displacement of the oscillator.

Förster theory (11)

(34) for initial displacement and initial velocity are zero

$$x = -\frac{2eE_{0x}}{m(\omega^2 - \omega_0^2)} \sin\left(\frac{\omega - \omega_0}{2} t\right) \sin\left(\frac{\omega + \omega_0}{2} t\right)$$

which is large only at resonance, i.e. $\omega \sim \omega_0$

(35) with

$$\sin\left(\frac{\omega - \omega_0}{2} t\right) \approx \frac{\omega - \omega_0}{2} t \quad and \quad \frac{\omega + \omega_0}{2} = \omega_0$$

(36) results

$$x \approx -\frac{eE_{0x}}{2m\omega_0} t \sin \omega_0 t$$

(37) the potential energy W = 1/2 kx^2 of the oscillator is at times when ω_0t is a multitude of π/2 , and the kinetic energy is zero,

$$W = \frac{e^2 E_{0x}^2}{8m} t^2$$

which is true for

$$\omega_0 - \pi / t < \omega < \omega_0 + \pi / t$$

Förster theory (12)

(38) if the electric dipole is oriented along the x-axis and has same frequency as an external field (osz. sinusoidally) the energy W absorbed after time t is with e is the electron charge, E_{0x} is the amplitude of the electric field, m is the mass of the oscillator

$$W = \frac{e^2 E_{0x}^2}{8m} t^2$$

(39) the amplitude by one donor oscillator with κ is the orientational factor (26), **a** is the amplitude of the oscillator, R is the distance between donor and acceptor

$$E_{0x} = \frac{\kappa e a}{n^2 R^3}$$

(40) inserting (39) into (38) yields the energy absorbed by one acceptor, if the eigenfrequencies match exactly at resonance

$$W_A = \frac{\kappa^2 e^4 a^2}{8 n^4 m R^6} t^2$$

Förster theory (13)

(41) the energy of the donor dipole is $$W_D = \frac{1}{2} k\, a^2 = \frac{m}{2} \omega^2 a^2$$

(42) substituting a^2 yields
for perfect resonance

$$W_A = \frac{\kappa^2 e^4}{4 n^4 m^2 \omega^2 R^6} W_D \bullet t^2$$

(43) donor and acceptor consist of a distribution of oscillators, with different oscillator strengths, f_{eD} (ω) for donor emission, and f_{aA} (ω) for acceptor absorption
(44) Only the fraction of acceptor dipoles oscillating within the frequency interval between ω and $\omega+d\omega$ can pick up energy

(45) The timedependent width of the donor interval is $\Delta = 2\pi / t$ according to (37)

(46) multiplying (42) with f_{aA} $(\omega)d\omega$ and f_{eD} $(\omega)2\pi/t$
and integrating over ω :

Förster theory (14)

(46) multiplying (42) with f_{aA} (ω)dω and f_{eD} (ω)2π/t and integrating over ω :

$$W_A = \frac{\pi \kappa^2 e^4}{2n^4 m^2 \omega^2 R^6} W_D \bullet t \int_0^\infty f_{eD}(\omega) f_{aA}(\omega) \frac{d\omega}{\omega^2}$$

(47) the transferred power is the time derivative of this energy

$$P_{\text{taken up by acceptor}} = P_{\text{given off by donor}} = \frac{d}{dt} W_A = \frac{\pi \kappa^2 e^4}{2n^4 m^2 \omega^2 R^6} W_D \bullet \int_0^\infty f_{eD}(\omega) f_{aA}(\omega) \frac{d\omega}{\omega^2}$$

(48) rate of transfer, k_T, corresponds to the average number of transfers per time unit for a permanently excited emitter:

$$\frac{d}{dt} W_D = k_T W_D$$

Förster theory (15)

(49) combining (47) and (48) and replacing $\nu=2\pi/\omega$

$$k_T = \frac{\kappa^2 e^4}{16\pi^2 n^4 m^2 \omega^2 R^6} \cdot \int_0^\infty f_{eD}(\nu)\, f_{aA}(\nu)\, \frac{d\nu}{\nu^2}$$

$$with\ f(\omega) = f(\nu)/2\pi \quad and$$

$$f(\omega)d\omega = f(\nu)d\nu \quad by\,definition$$

(50) the oscillator strength f_{aA} (ω) is proportional to the molar acceptor extinction coefficient $\varepsilon_A(\nu)$

(51) the oscillator strength f_{eD} (ω) is proportional to the donor fluorescence spectrum $f_D(\nu)$ which is normalized, τ_D and ϕ_D are the lifetime and quantum yield of the donor on the absence of the acceptor

$$f_{aA}(\nu) = \frac{3000n(\ln 10)mc}{\pi N_A e^2}\,\varepsilon_A(\nu)$$

$$f_{eD}(\nu) = \frac{3mc^2\varphi_D}{8\pi^2 ne^2\tau_D}\,\frac{f_D(\nu)}{\nu^2} \qquad with \int f_D(\nu)d\nu = 1$$

Förster theory (16)

(52) substituting (51) into (50) yields the Förster equation

$$k_T = \frac{9000(\ln 10)\kappa^2 \varphi_D}{128\pi^5 n^4 N_A \tau_D R^6} J$$

with the overlap integral, *J*, in frequency form or in wavenumber form

$$J = c^4 \int_0^\infty \frac{f_D(\nu)\varepsilon_A(\nu)}{\nu^4} d\nu \quad \text{or} \quad J = \int_0^\infty \frac{f_D(\tilde{\nu})\varepsilon_A(\tilde{\nu})}{\tilde{\nu}^4} d\tilde{\nu}$$

(53) the Förster distance is the distance at which k_T equals $1/\tau_D$

$$R_0^6 = \frac{9000(\ln 10)\kappa^2 \varphi_D}{128\pi^5 n^4 N_A} J$$

literature

- B. Wieb van der Meer, George Coker III, S.-Y. Simon Chen, Resonance Energy Transfer; VCH 1994
- Th. Förster, 'Fluoreszenz Organischer Verbindungen; Vandenhoeck & Ruprecht, 1951 (reprint 1982)

next 08/07/2003

- www.m-boersch.org   slides
- email: m.boersch@physik.uni-stuttgart.de   literature