

# *Förster Energy Transfer - AKA - Fluorescence Resonance Energy Transfer*

1. Origins: Theory of Energy Transfer developed by T. Förster  
(Förster. **1948**. *Annalen der Physik*. **2**:55-75.)
2. Development of FRET as a Spectroscopic Ruler.  
(Stryer & Haugland. **1967**. *Proc. Natl. Acad. Sci.* **58**:719-726.)
3. Some Recent Applications:
  - a. Fluorogenic Energy Transfer Substrate  
(Hasegawa *et al.* 2003. *Proc. Natl. Acad. Sci USA* **100**:14892-14896)
  - b. FRET & Single Molecule Ribozyme Action  
(Zhaung *et al.* 2002. *Science* **296**:1473-1476)
  - c. Single Molecule Protein Folding  
(Lipman *et al.* 2003. *Science* **301**:1233-1235)

## Energy Transfer Efficiency depends on . . .

1. **Separation Distance ( $r^{-6}$ )**. QM dipole-dipole operator has an  $r^{-3}$  dependence, but the probability of transfer is proportional to the square of the expectation value; thus a  $r^{-6}$  dependence.
2. **Orientation**. Dipole-dipole interactions are orientation dependent.
3. **Spectral Overlap**. The emission band of the donor must overlap with the absorption band of the acceptor.
4. **Quantum Yield**. Fluorescence yields of donor and acceptor should be high for efficient transfer.
5. **Non-overlapping Absorption Bands, Non-overlapping emission bands**. Not a necessity, but simplifies life.

$$k_T = \frac{1}{\tau_D} \frac{R_0^6}{R^6}$$

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

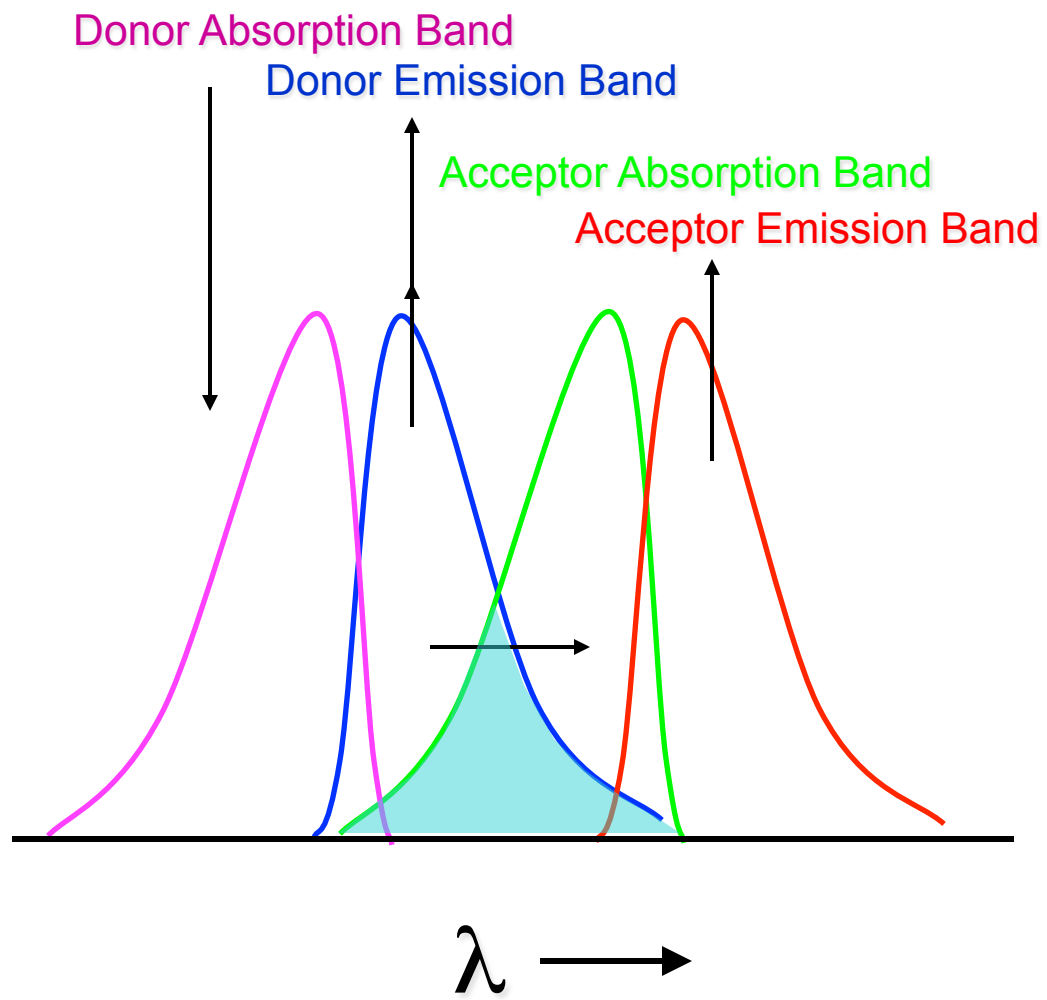
where

$\tau_D$  lifetime of the donor in the absence of acceptor

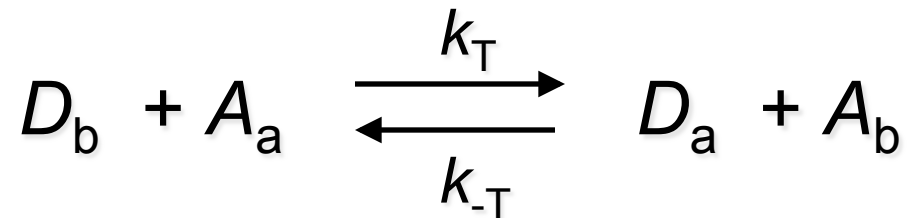
$J$  is the overlap integral

$\kappa^2$  is the orientation factor  $\rightarrow$  2/3 rapid tumbling limit

$\phi_D$  is quantum yield of the donor in the absence of acceptor



# Relaxation following $D \rightarrow A$ transfer lowers the probability of reverse transfer



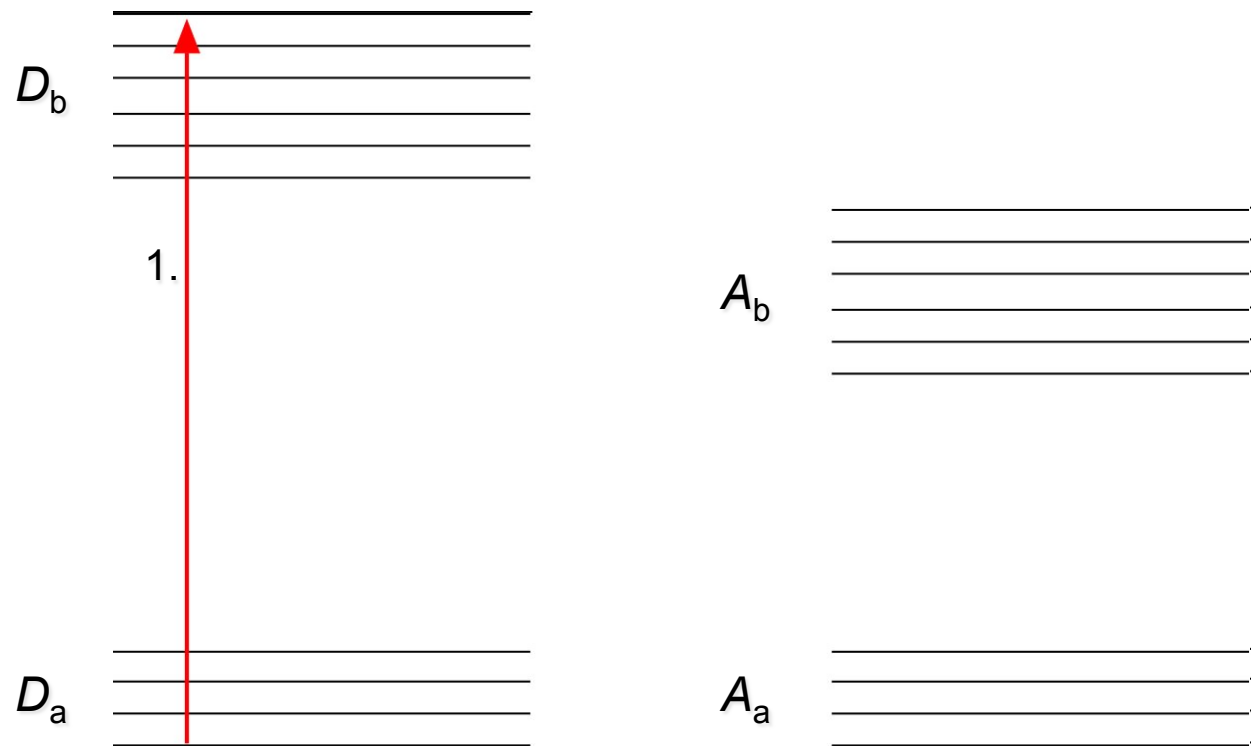
1. Absorption ( $10^{-15}$  s)

2. Vibronic Relaxation ( $10^{-12}$  s)

3. Energy Transfer (One Coupled Transition) ( $10^{-15}$  s)

4. Vibronic Relaxation ( $10^{-12}$  s)

5. Additional Coupled Transitions



## Isolated chromophore lifetime & quantum yield

$$\phi_F = \frac{k_F}{k_F + k_{ic} + k_{is} + k_q[Q]} \quad \text{Fluorescence Quantum Yield}$$

$$k_F = A_{ba} = \frac{1}{\tau_R} \quad \text{The intrinsic fluorescence rate constant is inversely proportional to the time constant for radiative decay, } \tau_R$$

$$\phi_F = \frac{\tau_F}{\tau_R} \quad \text{where} \quad \tau_F = \frac{1}{k_F + k_{ic} + k_{is} + k_q[Q]} \quad \text{The observed fluorescence time constant, } \tau_F, \text{ is the inverse of the observed rate constant.}$$

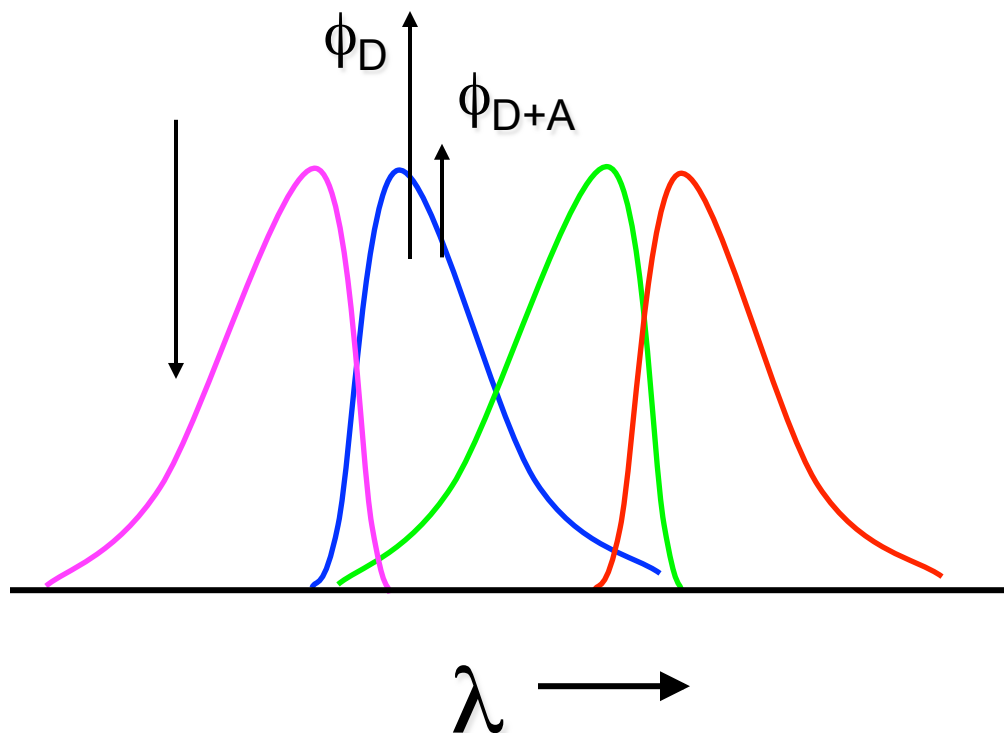
## Two chromophores – efficiency of transfer

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

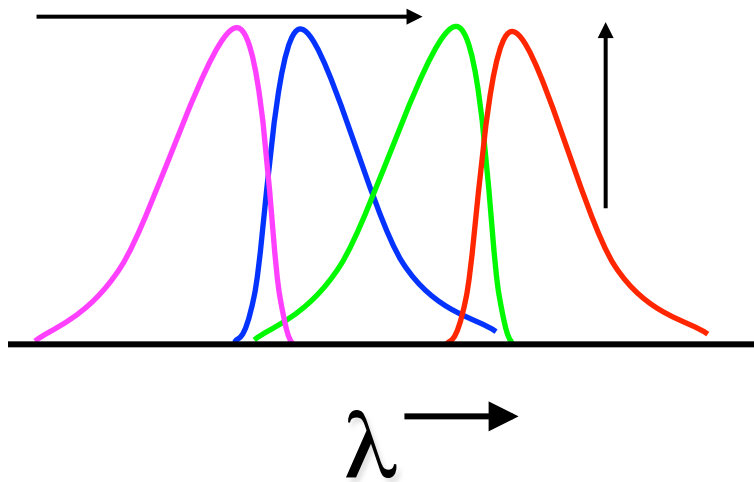
## Measurement of transfer efficiency (1)

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

Relative quantum yields of donor ( $\phi_D$ ) and donor in the presence of acceptor ( $\phi_{D+A}$ )



## Measurement of transfer efficiency (2)



A Donor/Acceptor excitation scan in the presence of the acceptor is affected by the presence of the donor

$$F_A \propto \varepsilon_A C_A \phi_A + \varepsilon_D C_D E \phi_A$$

$$\frac{F_A(D+A)}{F_A(A)} = 1 + \left( \varepsilon_D C_D / \varepsilon_A C_A \right) E$$

## Measurement of transfer efficiency (3)

$$\frac{\tau_{D(+A)}}{\tau_D} = 1 - E$$

Fluorescence lifetime of donor is shortened in the presence of acceptor

# What is $k_T$ ?

$$k_T(\nu) \propto \left| \left\langle \Psi_{Da} \Psi_{Ab} \left| \tilde{V} \right| \Psi_{Db} \Psi_{Aa} \right\rangle \right|^2$$

“ $k_T$  is proportional to the square of the expectation value for the interaction causing the excitation”

Where

$$\tilde{V} = \frac{(\hat{\mu}_D \cdot \hat{\mu}_A)}{R^3} - \frac{(\hat{\mu}_D \cdot \hat{R})(\hat{R} \cdot \mu_A)}{R^5}$$

$$\tilde{V} = \kappa \frac{|\hat{\mu}_D| |\hat{\mu}_A|}{R^3}$$

$$k_T(\nu) \propto \left| \left( \frac{\kappa}{R^3} \right) \left\langle \Psi_{Da} \Psi_{Ab} \left| \hat{\mu}_D \right| \hat{\mu}_A \right| \Psi_{Db} \Psi_{Aa} \right\rangle \right|^2$$

$$k_T(\nu) \propto \left( \frac{\kappa^2}{R^6} \right) \left| \left\langle \Psi_{Da} \left| \hat{\mu}_D \right| \Psi_{Db} \right\rangle \right|^2 \left| \left\langle \Psi_{Ab} \left| \hat{\mu}_A \right| \Psi_{Aa} \right\rangle \right|^2$$

The orientation and distance contributions to the dipole-dipole operator are separated, removed from the integral, which are then also factored



## Donor & Acceptor Contributions to the Overlap Integral

$$D_{ab} \equiv \left| \langle \Psi_{Ab} | \hat{\mu}_A | \Psi_{Aa} \rangle \right|^2 = 9.18 \times 10^{-3} \int \left( \frac{\epsilon_A}{\nu} \right) d\nu$$

$$\left| \langle \Psi_{Ab} | \mu_A | \Psi_{Aa} \rangle \right|^2 \propto \frac{\epsilon_A}{\nu}$$

### Acceptor

The square of the expectation value for the acceptor is Dipole Strength of the acceptor

..... at a single frequency

### Donor

The donor undergoes spontaneous emission (fluorescence). The Einstein coefficient for spontaneous emission is related to the Dipole Strength.

$$A_{ba} = \left( \frac{32\pi^3 \nu^3}{3c^3 \hbar} \right) D_{ab} \quad D_{ab} \propto \frac{A_{ba}}{\nu^3} \quad \boxed{D_{ab} \propto \frac{A_{ba}}{\tau_R \nu^3}} \quad \text{since } \tau_R = \frac{1}{A_{ba}}$$

$$\phi_F = \frac{k_F}{k_F + k_{ic} + k_{is} + k_q[Q]} = \frac{\tau_F}{\tau_R} \quad \rightarrow \quad \frac{1}{\tau_R} = \frac{\phi_D}{\tau_D}$$

$$\left| \langle \Psi_{Da} | \hat{\mu}_D | \Psi_{Db} \rangle \right|^2 \propto \frac{\phi_D}{\tau_D \nu^3}$$

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

$$\boxed{k_T(\nu) \propto \frac{\kappa^2 \phi_D}{R^6 \tau_D} \int_{band} \frac{\epsilon_A}{\nu^4} f_D(\nu) d\nu = \frac{\kappa^2 \phi_D}{R^6 \tau_D} J}$$

### Together

These contributions (integrated over the acceptor absorbance band) produce the overlap integral ( $J$ ) the orientation factor ( $\kappa^2$ ) and the dependence of the donor quantum yield and lifetime ( $\phi_D$ ,  $\tau_D$ ), as well as the  $R^{-6}$  distance dependence.



# Absorption and Emission Spectra of Donor and Acceptor

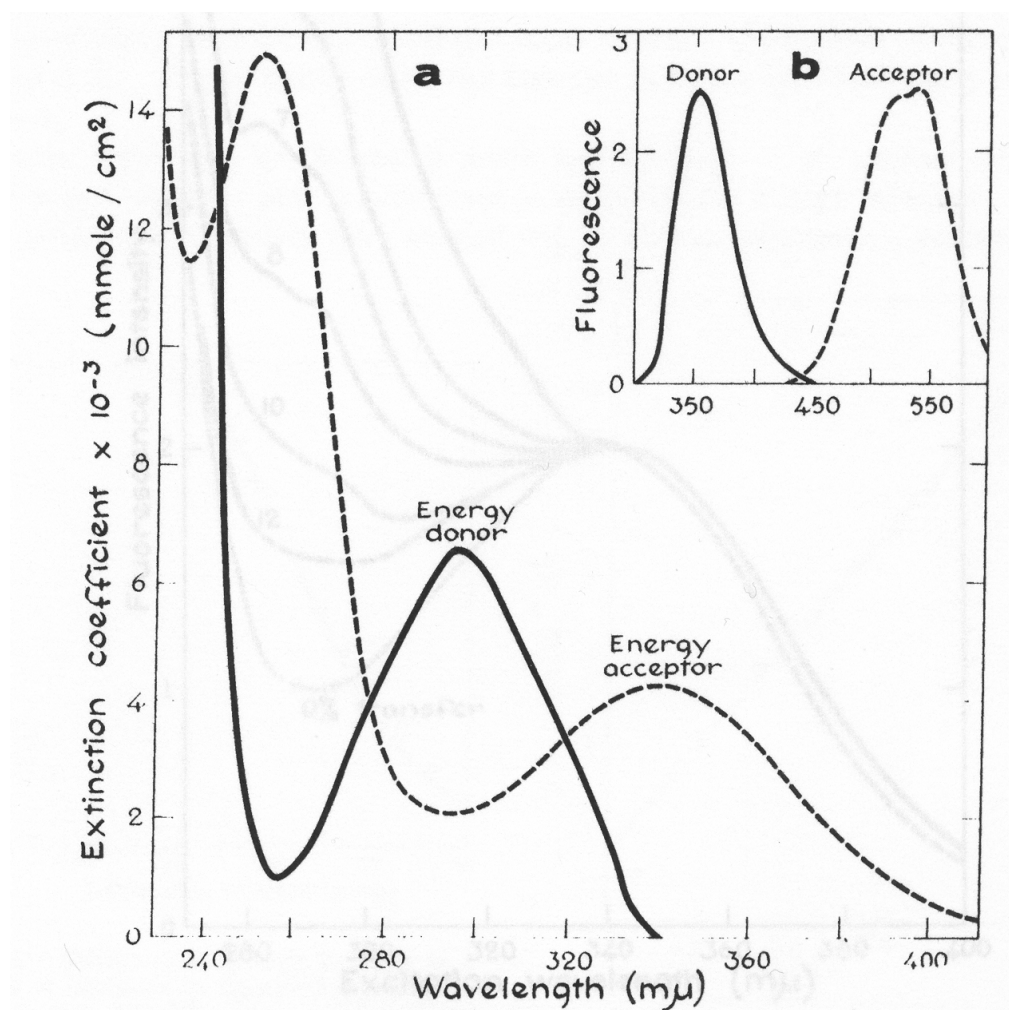


FIG. 2.—(a) Absorption spectrum of the energy donor (1-acetyl-4-(1-naphthyl) semicarbazide, —) and the energy acceptor (dansyl-L-prolyl-hydrazide, - - - -) in ethanol; (b) emission spectrum of the energy donor (—) and the energy acceptor (- - - -) in ethanol.

# Excitation Scans measure Transfer Efficiency

$$\frac{F_A(D+A)}{F_A(A)} = 1 + (\epsilon_D C_D / \epsilon_A C_A) E$$



$$E = \left( \frac{F_A(D+A)}{F_A(A)} - 1 \right) \left( \frac{\epsilon_A}{\epsilon_D} \right)$$

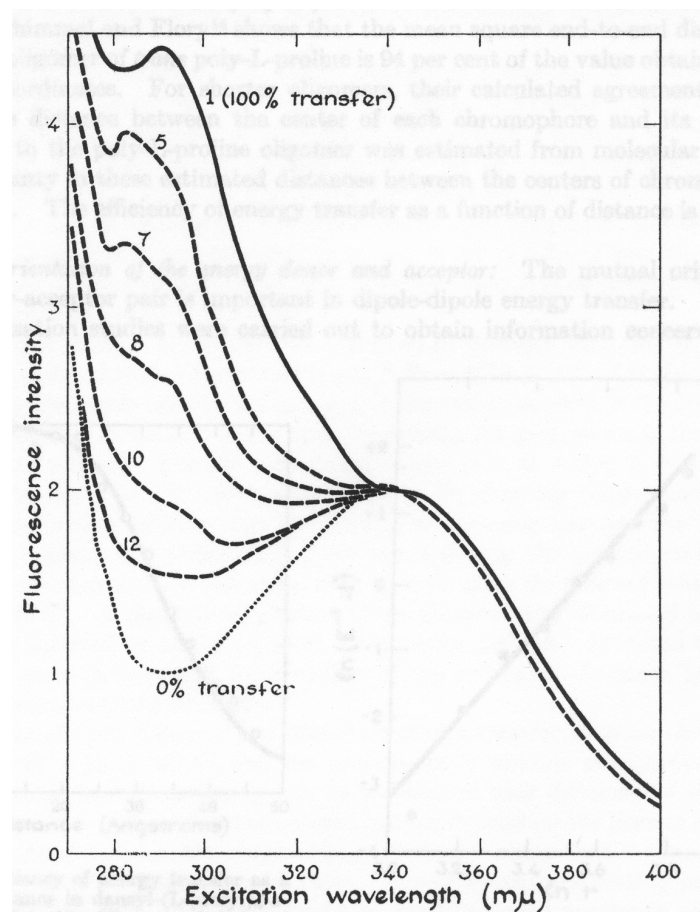


FIG. 3.—Excitation spectrum of dansyl-L-prolyl-hydrazone (....., 0% transfer), dansyl-L-prolyl- $\alpha$ -naphthyl (—, 100% transfer), and dansyl-(L-prolyl) $_n$ - $\alpha$ -naphthyl (----,  $n = 5, 7, 8, 10, 12$ ) in ethanol.

# $E$ has a $1/R^6$ dependence

$$E = \frac{R_0^6}{R_0^6 + R^6}$$

$$\downarrow$$

$$\frac{1}{E} = 1 + \frac{R^6}{R_0^6}$$

$$\downarrow$$

$$\ln\left(\frac{1}{E} - 1\right) = n \ln(R) - \ln(R_0^n)$$

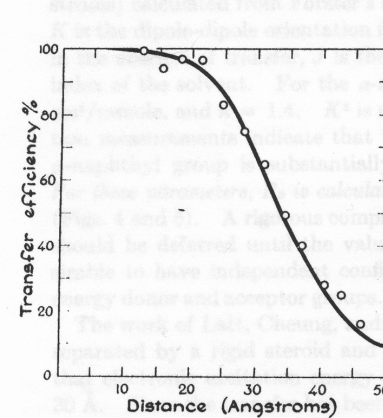


FIG. 4.—Efficiency of energy transfer as a function of distance in dansyl-(L-prolyl)<sub>n</sub>- $\alpha$ -naphthyl,  $n = 1$  to 12. The  $\alpha$ -naphthyl and dansyl groups were separated by defined distances ranging from 12 to 46 Å. The energy transfer is 50% efficient at 34.6 Å. The solid line corresponds to an  $r^{-6}$  distance dependence.

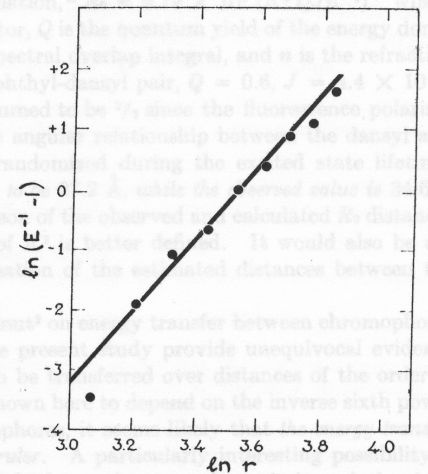
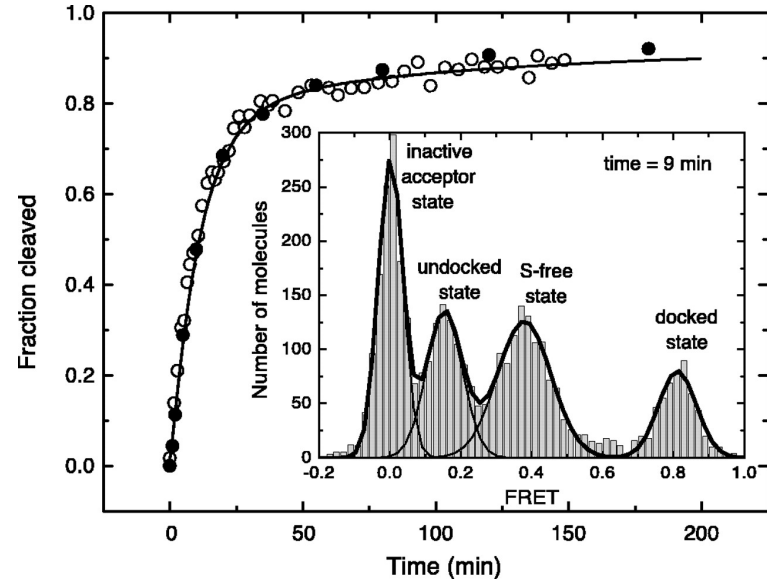
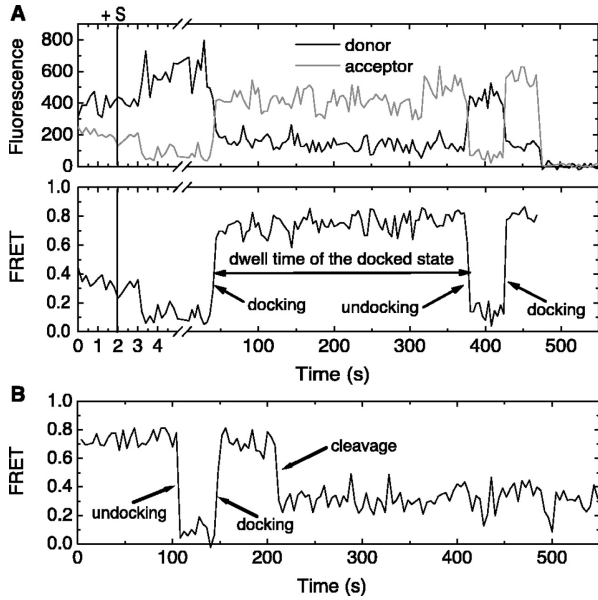
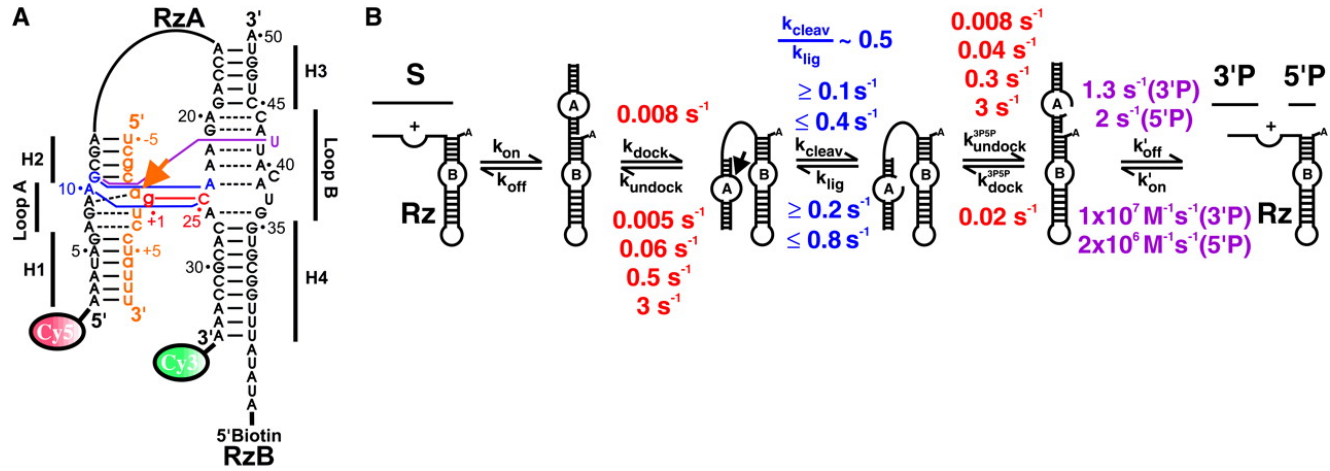


FIG. 5.—The dependence of the efficiency of energy transfer on distance is given by the slope in this plot of  $\ln(E^{-1} - 1)$  versus  $\ln r$ . The slope is 5.9, in excellent agreement with the  $r^{-6}$  dependence predicted by Förster.

# Single Molecule FRET (An Example, Zhaung et al.)



# In Vivo FRET (An Example, Hasegawa et al.)

