FRET: Fluorescence Resonance Energy Transfer

FRET works via proximity, there is an energy transfer if the energies match

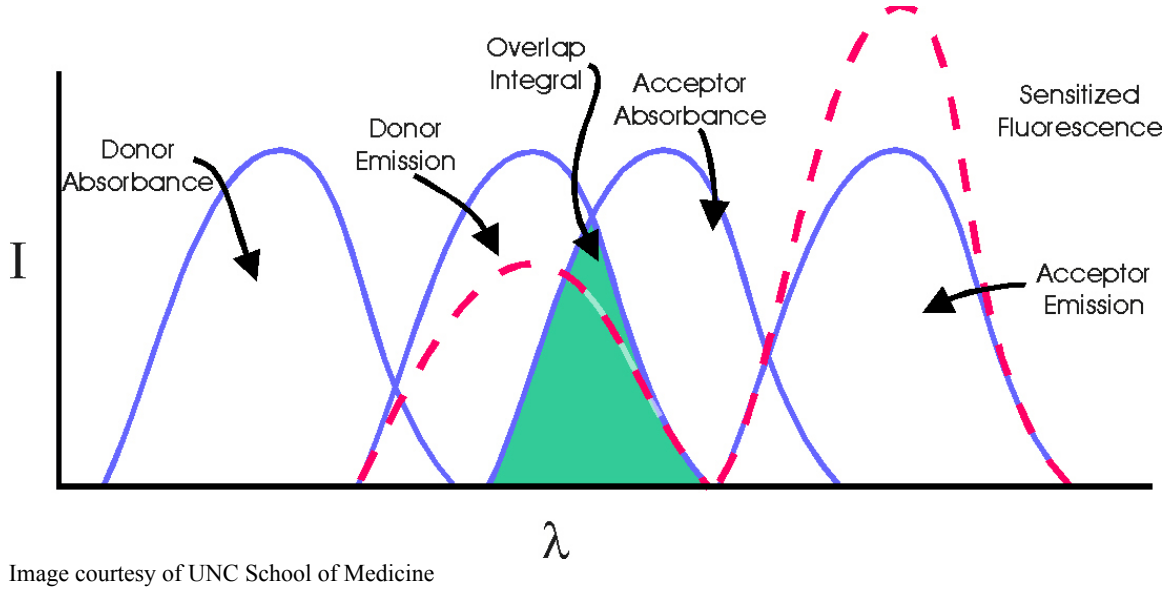
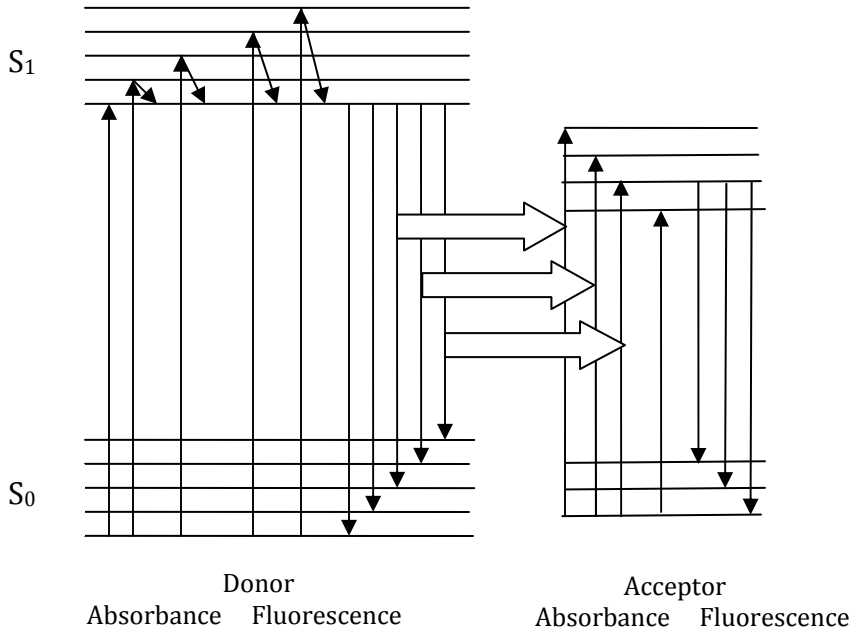


Image courtesy of UNC School of Medicine
http://www-cellbio.med.unc.edu/facilities/fret.htm

The donor will transfer energy to the acceptor if they are resonating at the same λ



The wide arrows represent the FRET. Note that FRET takes place at the lower energies (why?). Note that there is no FRET to the lowest absorbance band of the acceptor…why?

$h\upsilon + D \rightarrow D^* \rightarrow h\upsilon'$

$D^* + A \rightarrow D + A^* \rightarrow A + h\upsilon''$

$\Phi_D = k_f/k_d$ (the quantum yield of the donor) decreases when the acceptor is present because there is another mode of decay…k'_T the rate constant of transfer

The amount of fluorescence produced by the donor will be reduced because there is another reaction that can take place

τ_D change?
Yes: $\tau_D = 1/k_D$ and this does change

<u>The Efficiency of Transfer</u>

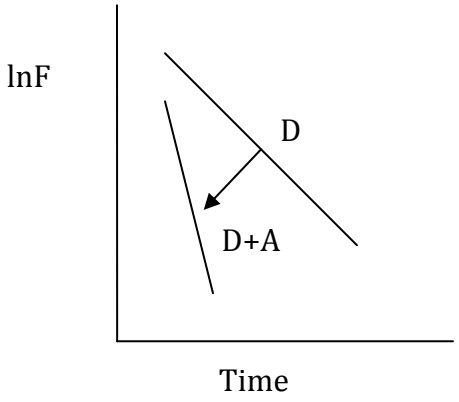
Efficiency = $k_T'\ [D^*][A]/k_T'[D^*][A] + k_D[D^*]$

$k_T = k_T'[A]$

Eff = $k_T/(k_T + k_D)$

$= 1 - \Phi_{D+A}/\Phi_D = 1 - \frac{\tau_{D+A}}{\tau_D}$

Can determine the time constants directly from the fluorescence decay:



Therefore can calculate the efficiency. How to go from efficiency to distance…

Distance r_0 defined when efficiency = 0.5
$r = r_0\ (1/Eff - 1)^{1/6}$

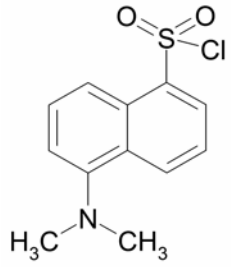
What determines the transfer rate….

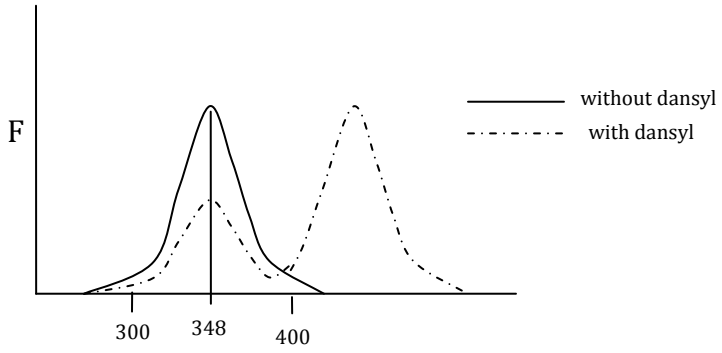
$k_T' = 8.7x10^{23} JK^2 n^{-4} k_f \Phi_D r^{-6}$

J= spectral overlap or degree of overlap between donor fluorescence spectrum and acceptor fluorescence spectrum
K= relative orientation of the excited state dipoles of the donor and acceptor $0 \leq K \leq 4$ ($\langle K^2 \rangle = 2/3$ for randomly oriented donors and acceptors)
n= refractive index of medium (assumed to be 1.4 for proteins)
r^{-6}= distance between donor and acceptor…very sensitive to distance
Φ_D = fluorescence quantum yield of the donor in the absence of acceptor

Example: ACTH…determine if the peptide hormone, ACTH, has a folded structure. …measure the distance between position 9 (tryptophan) and the lysine at position 21. The tryptophan has a natural fluorescence. The lysine can be labeled with a dansyl group.

Dansyl:





After Dansyl is bound to Lysine, there is a drop in the fluorescence of the tryptophan

Theoretical distance for a polypeptide with random structure: r = 2.9
Measured distances:

Solution	H_2O	Buffer 7.0	Urea 8M	70°C
r	2.0	2.5	2.6	2.5

The distance is insensitive to denaturation both by heat and urea. Therefore probably no structure.