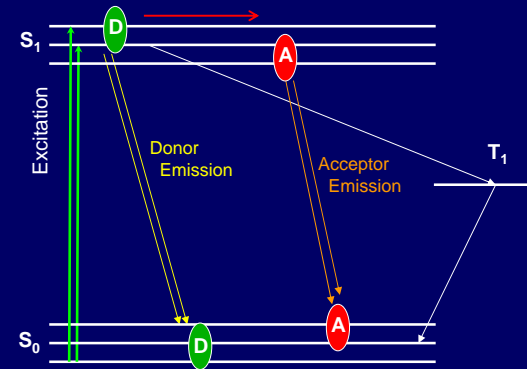


Lecture 9  
Fluorescence Resonance Energy Transfer

Ji-Xin Cheng

Fluorescence Resonance Energy Transfer (FRET)

RRET is a process of radiationless energy transfer from a donor to an acceptor molecule.



Columbic Interaction

$$H = H_a + H_b + V_{ab},$$

Dipole-Dipole

Dipole-Quadrupole

Quadrupole-Quadrupole

Two Limiting Cases of Dipole-Dipole Interaction

$$V_{ab} \ll \Delta$$

Forster Mechanism

$$V_{ab} \gg \Delta$$

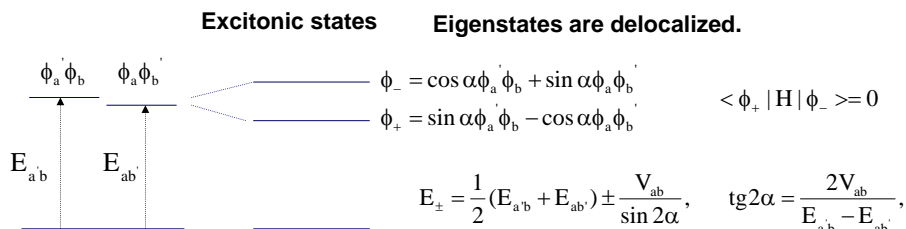
Excitonic splitting

$V_{ab}$  : Coupling strength

$\Delta$  : Total electronic band width

## Standard Exciton Theory for a Strongly Interacting Dimer

$$H = H_a + H_b + V_{ab}, \quad V_{ab} \approx \frac{K}{n^2 R_{ab}^3}$$



Transition dipoles of the two excitonic states

$$\mu_+ = \cos \alpha \mu_a + \sin \alpha \mu_b$$

$$\mu_- = \sin \alpha \mu_a - \cos \alpha \mu_b$$

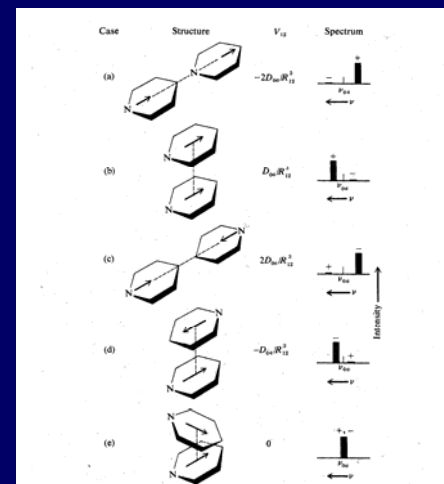


Figure 7-19

Monomer and dimer spectra for solutions of bacteriochlorophyll. A pronounced splitting of the longest-wavelength band in the dimer is visible. [After K. Sauer, J. R. L. Smith, and A. J. Schultz, *J. Am. Chem. Soc.* 88:2681 (1966).]

## Radiationless Transition Rate – Fermi Golden Rule

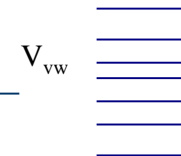
$$k_v = \frac{2\pi}{\hbar} \sum_w |V_{vw}|^2 \delta(E_{vD} - E_{wA})$$

Rate of radiationless transitions

$$k \propto V^2 F$$

F is the Franck-Condon overlap

$|v D\rangle$        $|w A\rangle$



Ground state

Sauer et al, JACS, 1966, 88: 2681.

Monomer-interaction energies, pair interaction potentials, and schematic spectra for six possible geometries of a dimer of pyridine. The potential  $V_{12}$  is expressed in terms of the dipole strength of the monomer ( $D_{0a} = |\langle \phi_0 | \mu | \phi_a \rangle|^2$ ). The interchromophore distance,  $R_{12}$ , is shown as a dashed line; transition dipole orientations are shown as arrows for the transition  $\Psi_0 \rightarrow \Psi_A$ . (if the transition  $\Psi_0 \rightarrow \Psi_{A-}$  were shown instead, one of the arrows would point toward the nitrogen instead of away from it). Note that, for a dimer, the intensity of a band can be computed by adding the two individual transition dipoles vectorially and computing the square of the length of the resulting vector.

TABLE I  
Intermolecular Nonradiative Processes in Condensed Phase

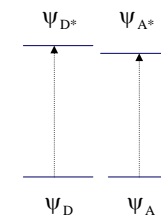
Process	Electronic states		$V$ electronic coupling
Electronic transfer in solids, liquids, and biological systems	$DA \rightarrow D^+A^-$ D = electron donor A = electron acceptor	J. Franck R. A. Marcus	Two-center Coulomb, and exchange
Small polaron	$A^-A \rightarrow AA^-$ A = neutral molecule $A^-$ = negative ion	T. Holstein	Two-center one-electron Coulomb, and exchange
Electron-hole recombination in semiconductors	$D^+ k\rangle \rightarrow D^+ b\rangle$ $ k\rangle$ = free electron $ b\rangle$ = electron bound to $D^+$ $D^+$ = positive ion	R. Kubo Y. Toyozawa	Nuclear momentum
Electronic energy transfer in solids, glasses, liquids, and biological systems	$D^*A \rightarrow DA^*$ D = energy donor A = energy acceptor	T. Förster D. Dexter	Intermolecular electrostatic interaction dipole-dipole monopole-monopole, also electron exchange
High-spin-low-spin interconversion in transition metal compounds	$M(S1) \rightarrow M(S2)$	M. Bixon	Spin-orbit
Group transfer in hemoglobin	$Fe(S=2) + CO \rightarrow Fe(S=0) \cdot CO$	H. Frauenfelder	Spin-orbit

## Energy Transfer

$$H = H_D + H_A + H_{int},$$

$$\Psi_i = \frac{1}{\sqrt{2}} (\Psi_{D^*}(1)\Psi_A(2) - \Psi_{D^*}(2)\Psi_A(1)),$$

$$\Psi_f = \frac{1}{\sqrt{2}} (\Psi_D(1)\Psi_{A^*}(2) - \Psi_D(2)\Psi_{A^*}(1)),$$



$$H_{if} = \langle \Psi_i | H_{int} | \Psi_f \rangle = \langle \Psi_{D^*}(1)\Psi_A(2) | H_{int} | \Psi_D(1)\Psi_{A^*}(2) \rangle - \langle \Psi_{D^*}(1)\Psi_A(2) | H_{int} | \Psi_D(2)\Psi_{A^*}(1) \rangle$$

Coulombic - Long range interaction

Exchange - Short range interaction

### Forster Mechanism

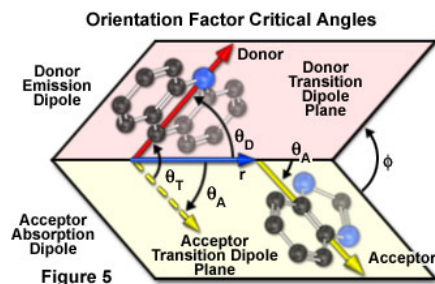
### Dexter Mechanism

Pure classical analog

Exponential distance (instead of  $r^6$ ) dependence  
Operational for dipole forbidden transitions

## Dipole-Dipole Interaction

$$V_{ab} \approx \frac{K}{n^2 r_{ab}^3}$$



Cubic dependence on distance

Orientation Factor:

$$K = \cos \theta_T - 3 \cos \theta_D \cos \theta_A$$

$\theta_T$  is the angle between the emission dipole of the donor and the absorption dipole of the acceptor

## Energy Transfer Rate

Transfer rate according to Fermi Golden Rule

$$k_{a \rightarrow b} = \frac{9\kappa^2 \ln 10}{128\pi^5 n^4 N \tau_a r_{ab}^6} \int f_a(\nu) \epsilon(\nu) d\nu / \nu^4,$$

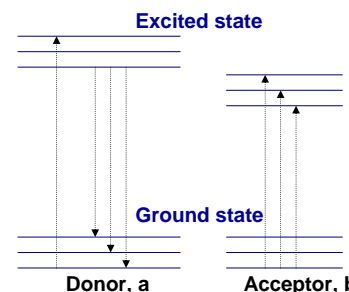
$N$  is the Avogadro No.

The energy transfer rate depends on (1) donor radiative life time  $\tau_a$ ,

(2) overlap of donor emission  $f_a(\nu)$  and acceptor absorption  $\epsilon(\nu)$  spectra,

(3) orientation factor, and

(4) donor -acceptor distance.



## FRET as a Molecular Ruler

### FRET rate

$$k_T(r) = \frac{1}{\tau_a} \left( \frac{R_0}{r_{ab}} \right)^6,$$

$r_{ab}$  is the distance between donor **a** and acceptor **b**

$\tau_a$  is the fluorescence lifetime of donor **a**

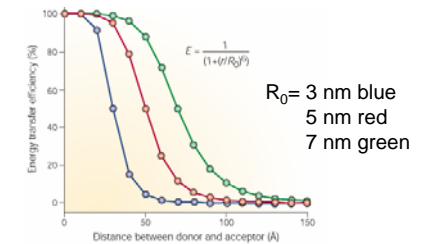
$R_0$  is the Förster radius at which  $k_T$  equals  $1/\tau_a$

One can use FRET to monitor intermolecular interaction by measuring the proximity of one component to another.

## FRET Efficiency

$$E_{FRET} = \frac{k_T}{k_T + 1/\tau_a} = \frac{1}{1 + \left( \frac{r_{ab}}{R_0} \right)^6}$$

FRET Efficiency as a function of donor-acceptor distance



## A FRET Pair

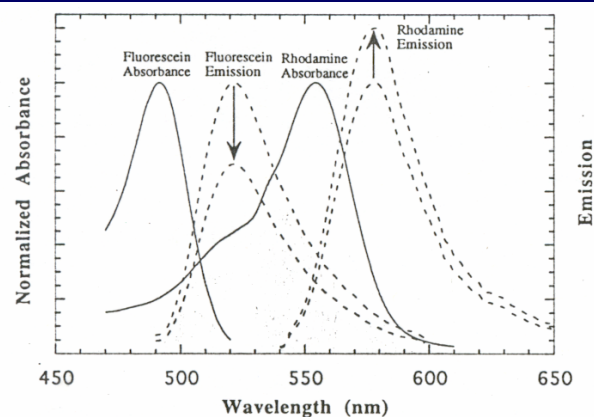
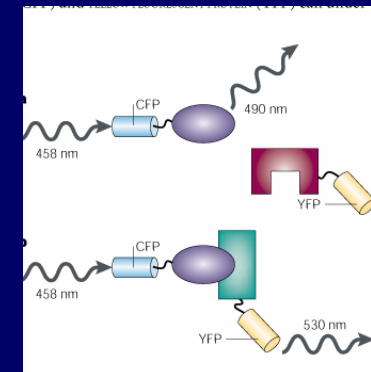


FIG. 1. Spectral characteristics and changes of the donor fluorescein and acceptor tetramethylrhodamine undergoing energy transfer. The donor intensity decreases and the acceptor is sensitized with energy transfer. The spectral overlap which makes energy transfer possible is shown in gray. The absorbance and emission intensities are normalized for display purposes. The  $R_0$  for the pair is approximately 45 Å.

## A FRET Pair : CFP / YFP

Excitation

Excitation

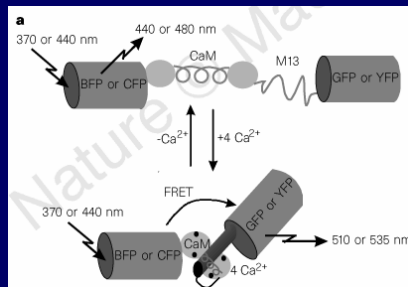


CFP: cyan mutant GFP  
 YFP: yellow mutant GFP

$R_0$  is about 50 Å

CFP / YFP is a FRET pair

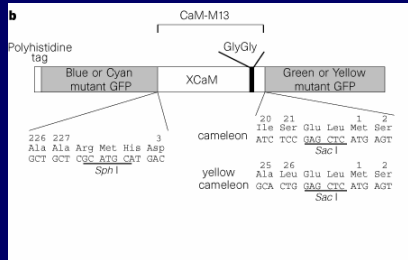
## A Chameleon Consisting of Tandem Fusion of CFP, Calmodulin, and YFP



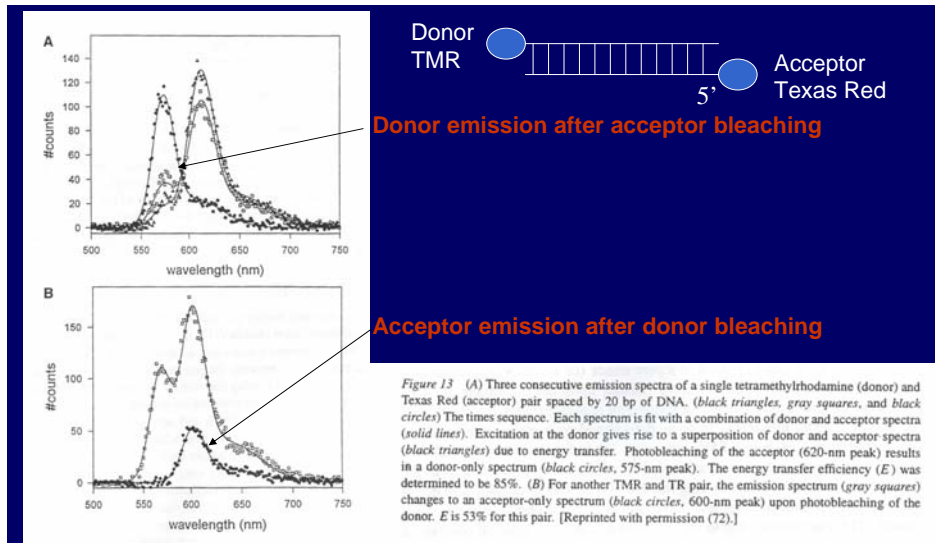
Calmodulin (CaM) is a ubiquitous Calcium-binding protein

Binding of Calcium makes calmodulin wrap around the M13 domain, increasing FRET between the flanking GFPs.

FRET-based Chameleons can measure  $\text{Ca}^{2+}$  concentration



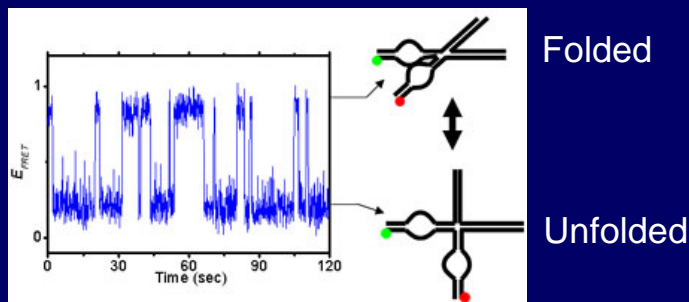
Miyawaki et al., Nature, 388:882 (1997)



## Single Molecule FRET Pair

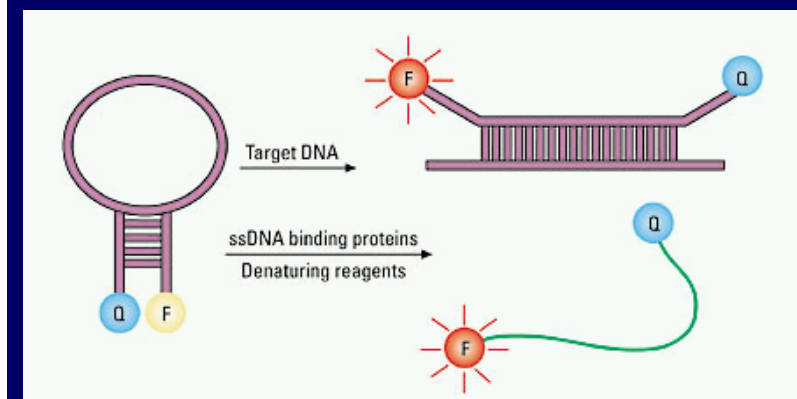
Ha, et al. Proc. Nat. Acad. Sci. USA, 93: 6264 (1996)

## FRET Measurement of RNA Folding



T Ha lab, UIUC

## Molecular Beacon Based on FRET



Q: quencher, F: fluorophore

