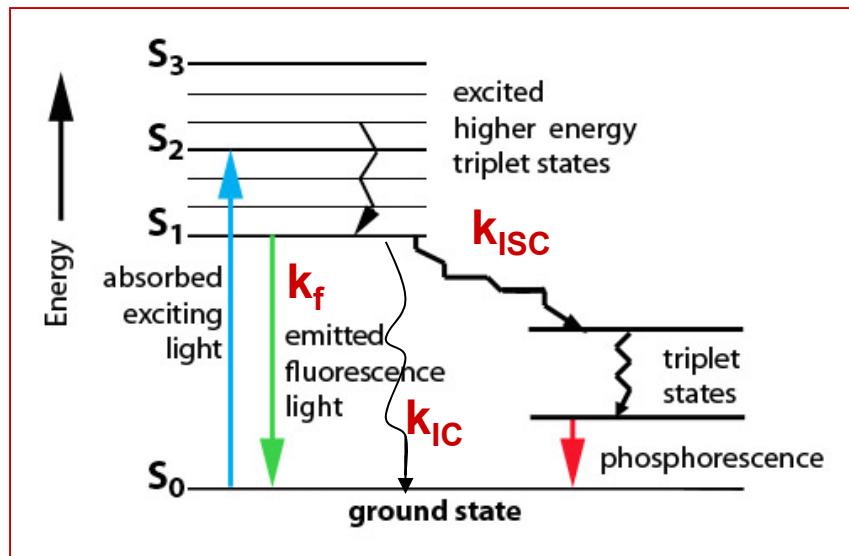


# **Molecular Photonics**

Lecture 4

# Emission behavior

Two processes diminish fluorescence:  
internal conversion and intersystem crossing



$$\Phi_f = k_f / (k_f + k_{ISC} + k_{IC})$$

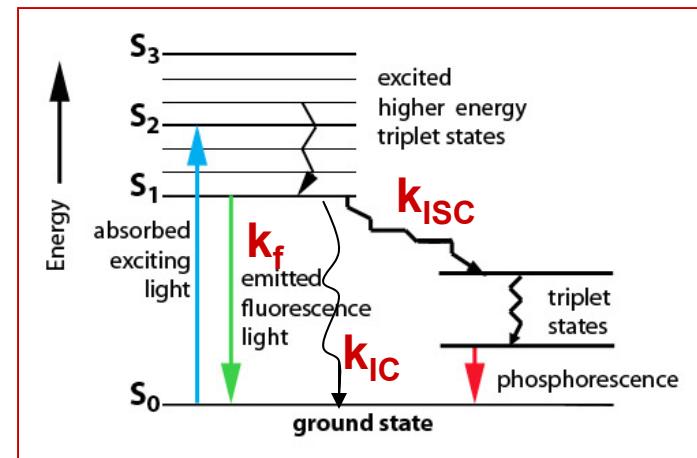
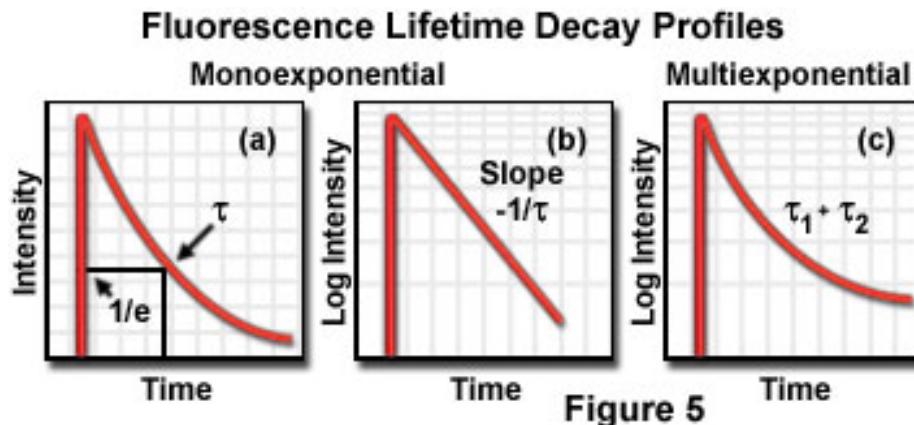
$$\tau_f = 1/k_f$$

$$\tau_{obs} = 1 / (k_f + k_{ISC} + k_{IC})$$

$$\Phi_f = k_f \tau_{obs}$$

$$\Phi_f = \tau_{obs} / \tau_f$$

# Lifetime



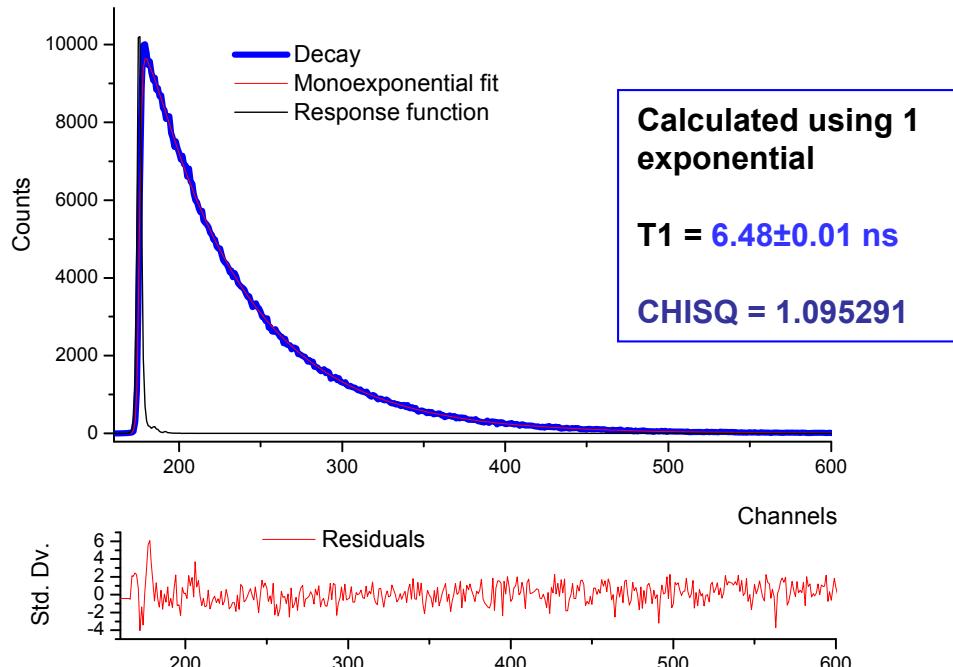
**Monoexponential – a particular species (one Jablonski diagram)**

**Multiexponential – more than one species (several emitters)**

**Experimental lifetime:**

$$\tau_{\text{obs}} = 1/(k_f + k_{\text{ISC}} + k_{\text{IC}})$$

$$\Phi_f = \tau_{\text{obs}} / \tau_f$$



# **The fate of the excited state**

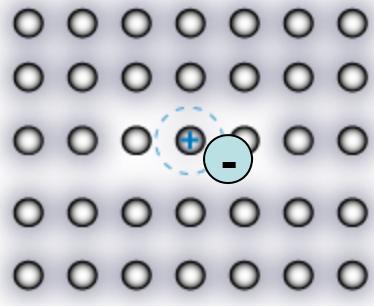
More than one chromophore

Excitons and beyond

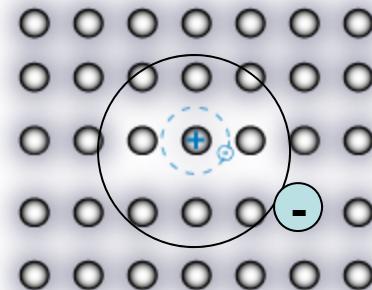
Energy transfer (exciton hopping)

## **Exciton** – a useful notion when looking at more than one chromophore

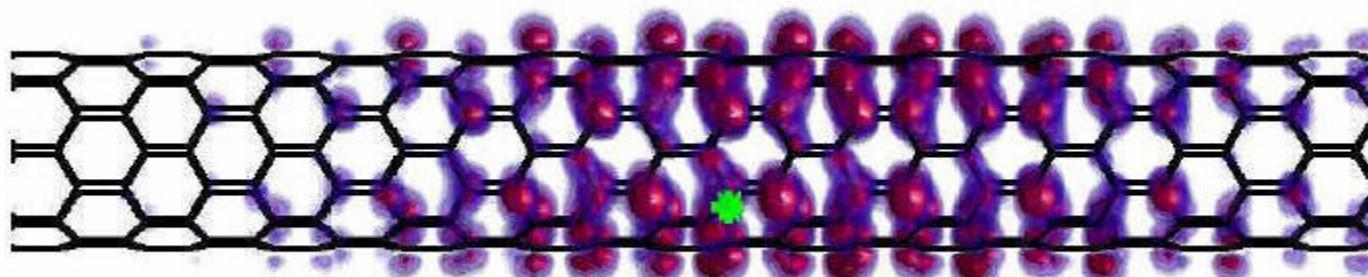
- An **exciton** is a bound state of an electron and a hole (exciton is a result of excitation with light!).
- Because the electron and the positive hole have equal but opposite electrical charges, the exciton as a whole has no net electrical charge (though it transports energy).
- Exciton binding energy
- Frenkel and Wannier-Mott excitons

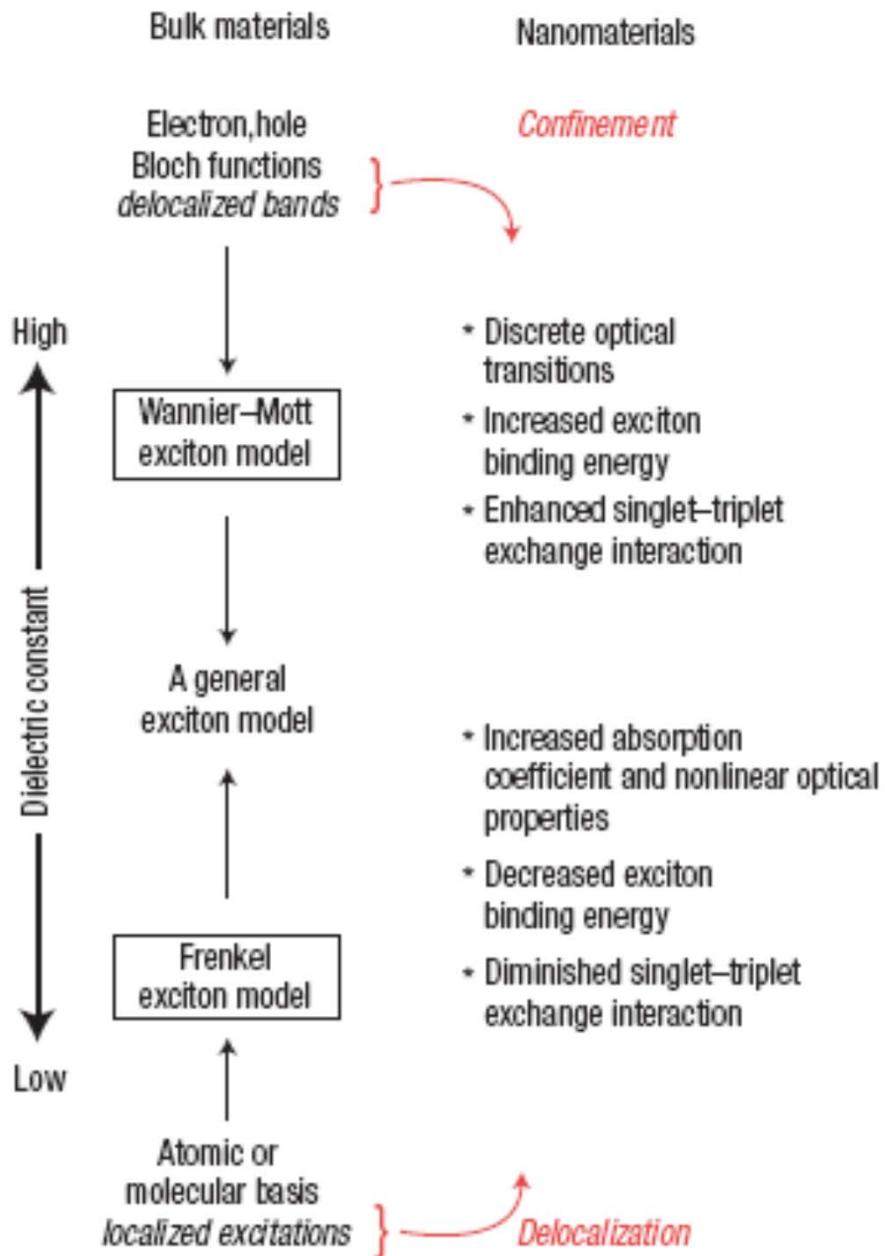


**Frenkel exciton**  
(strongly bound)

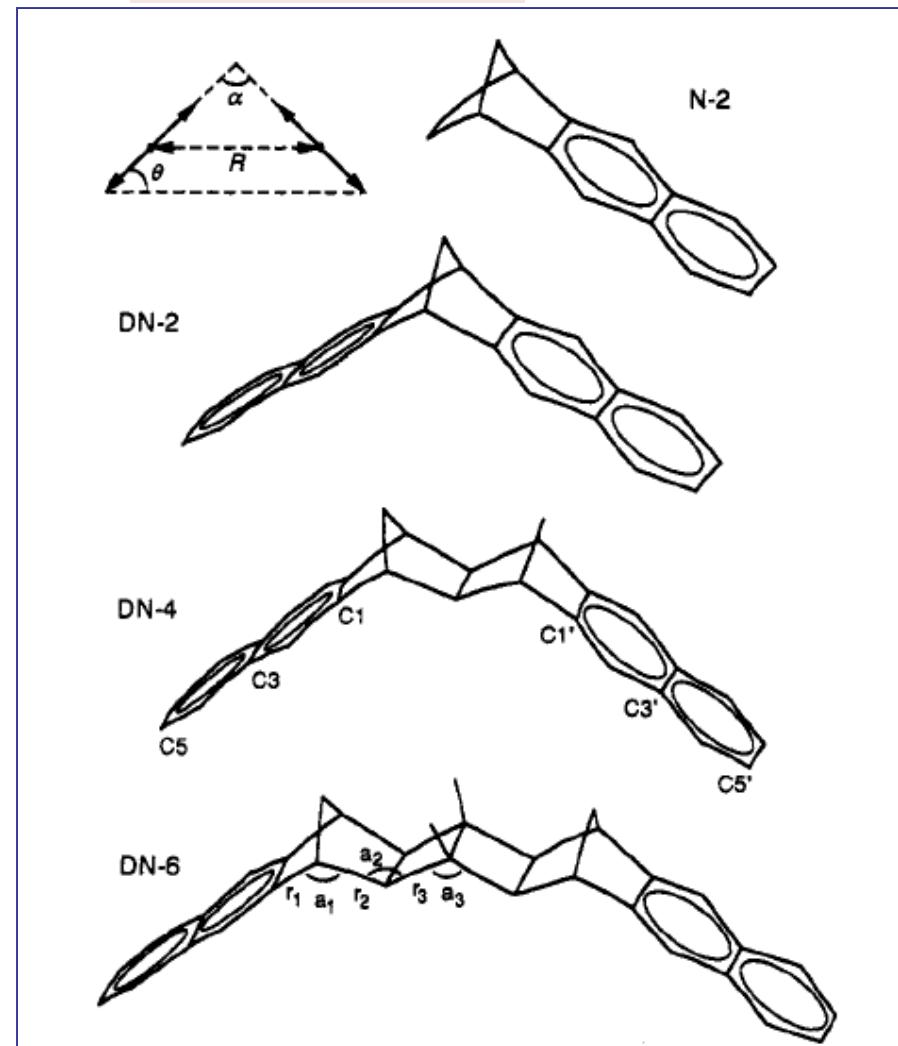
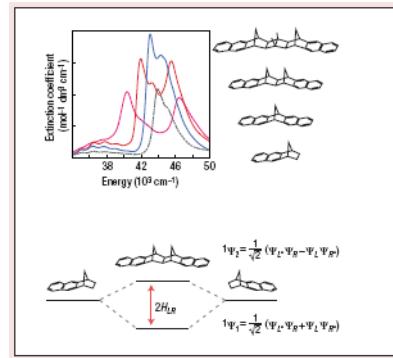
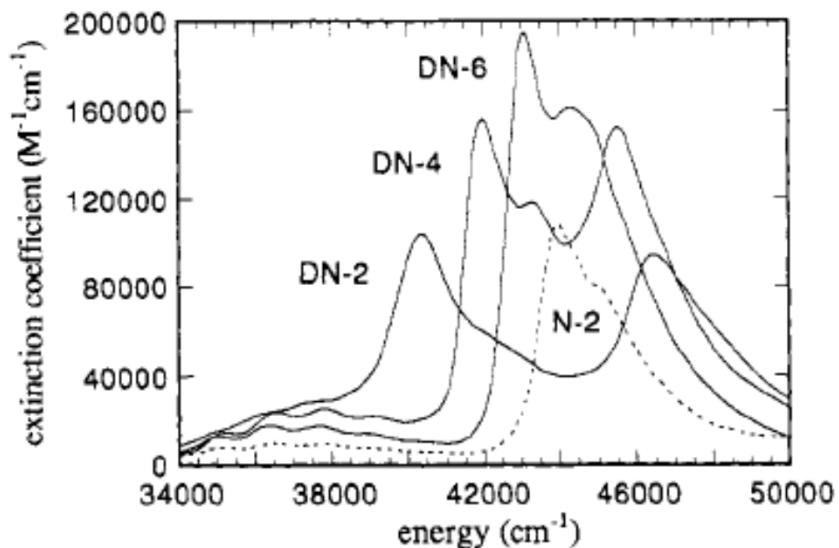


**Wannier-Mott exciton**  
(Loosely bound)





Absorption spectra of a series of naphthyl dimer molecules compared with a monomeric model chromophore (dashed line). The dimer spectra consist of two bands, with a splitting equal to the electronic coupling  $H_{LR}$  between the naphthyl groups on the left (L) and right (R), indicating that the excitation is shared between the two chromophores: a molecular exciton.



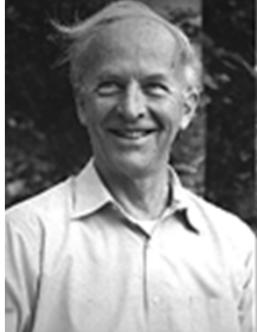
Scholes, Gregory D.; Ghiggino, Kenneth P.; Oliver, Anna M.; Paddon-Row, Michael N. **Through-space and through-bond effects on exciton interactions in rigidly linked dinaphthyl molecules.** *J. Am. Chem. Soc.* 1993, 115, 4345.

# Davydov Splitting/Kasha's theory: Exciton coupling

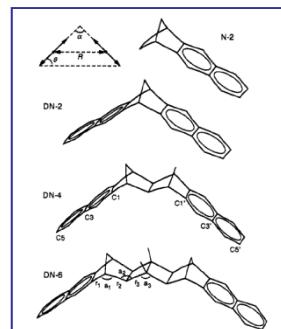
The rationale: we look at a dimer of interacting chromophores where the nature of the electronic transitions depends on the mutual orientation of the transition dipoles.



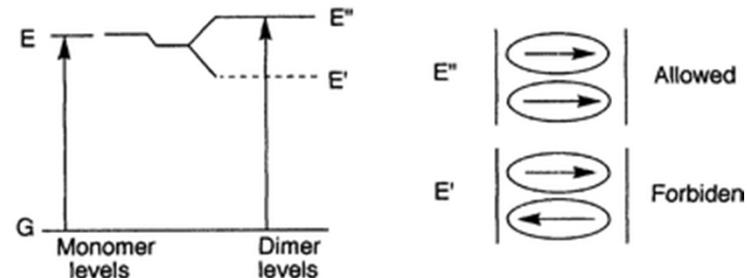
Alexander Davydov



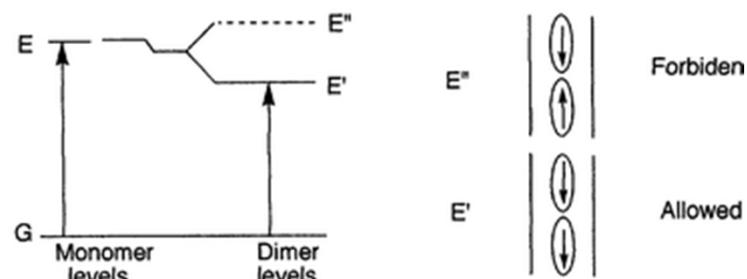
Michael Kasha



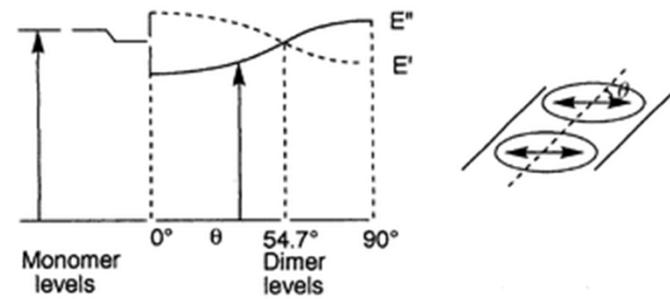
(a) Parallel(cofacial) transition dipoles: Blue-shift case



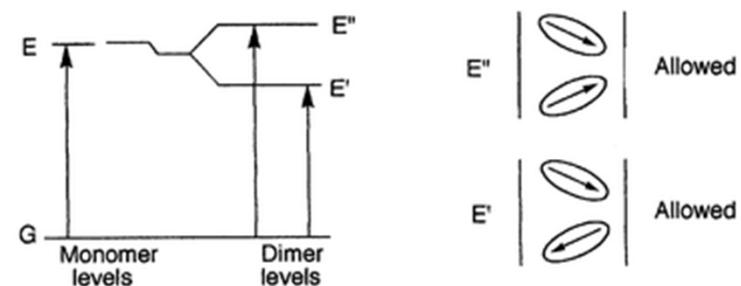
(b) In-line transition dipoles: Red-shift case



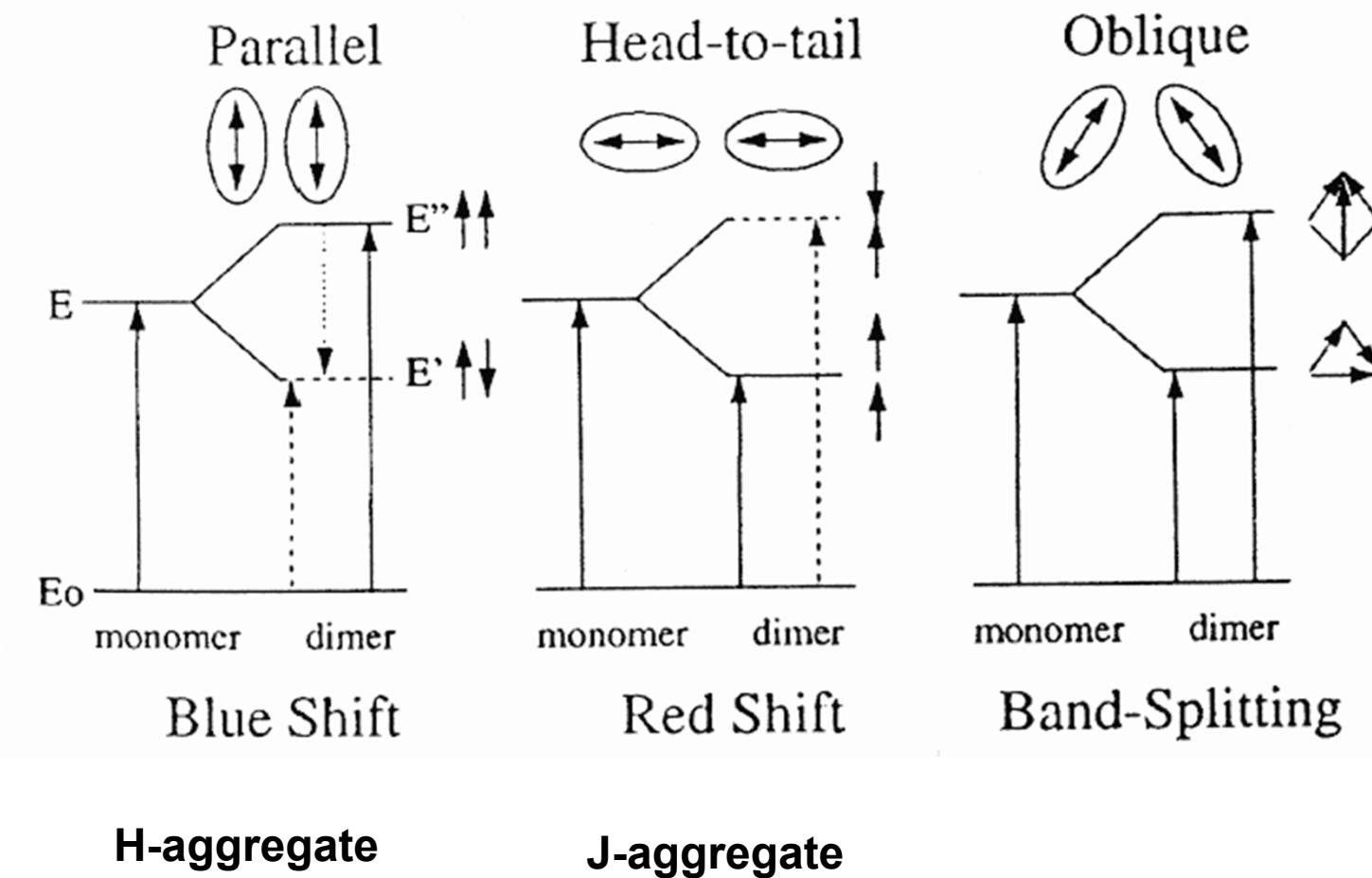
(c) Co-planar(slipped) transition dipoles: Shift depends on angle  $\theta$ .



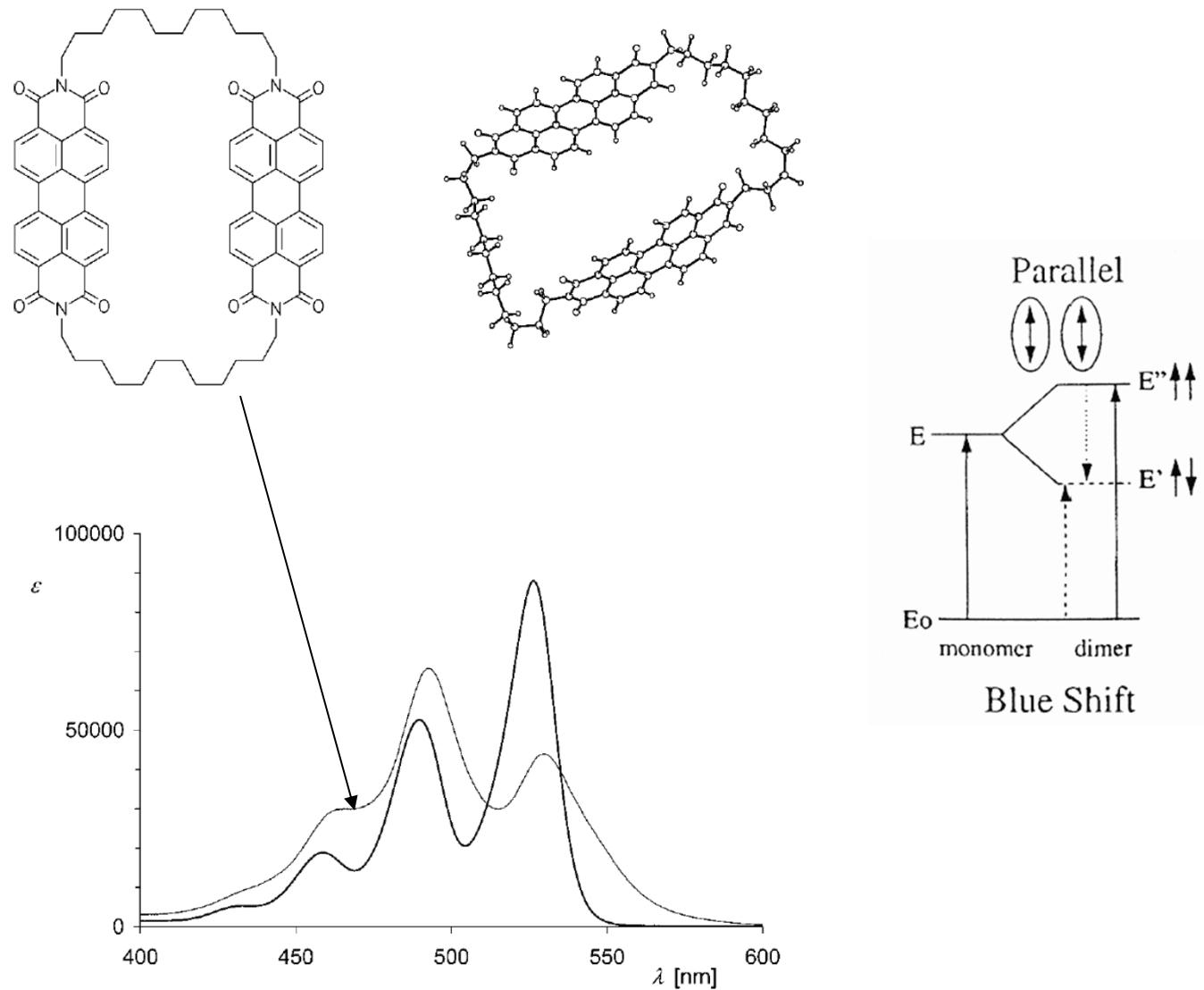
(d) Oblique(herringbone) transition dipoles: Band-splitting case



# Kasha theory

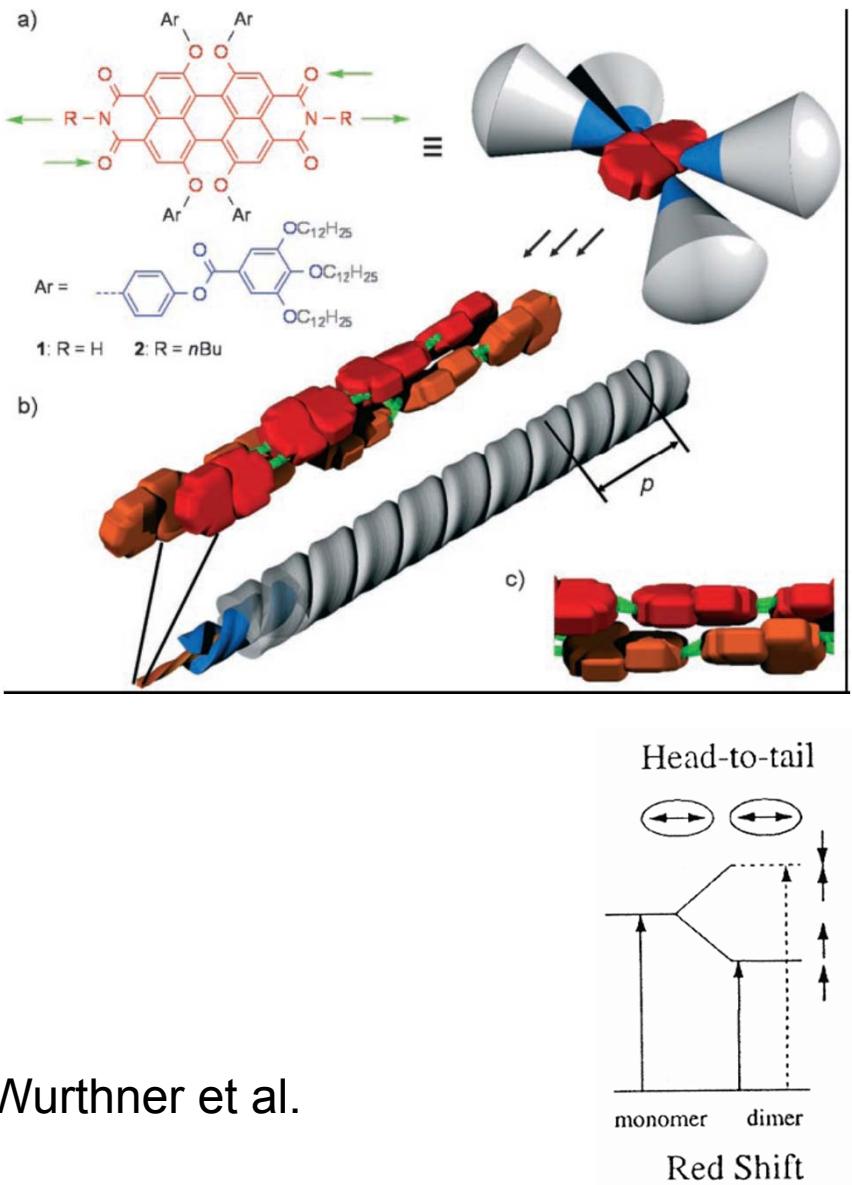


## PDI H-aggregate

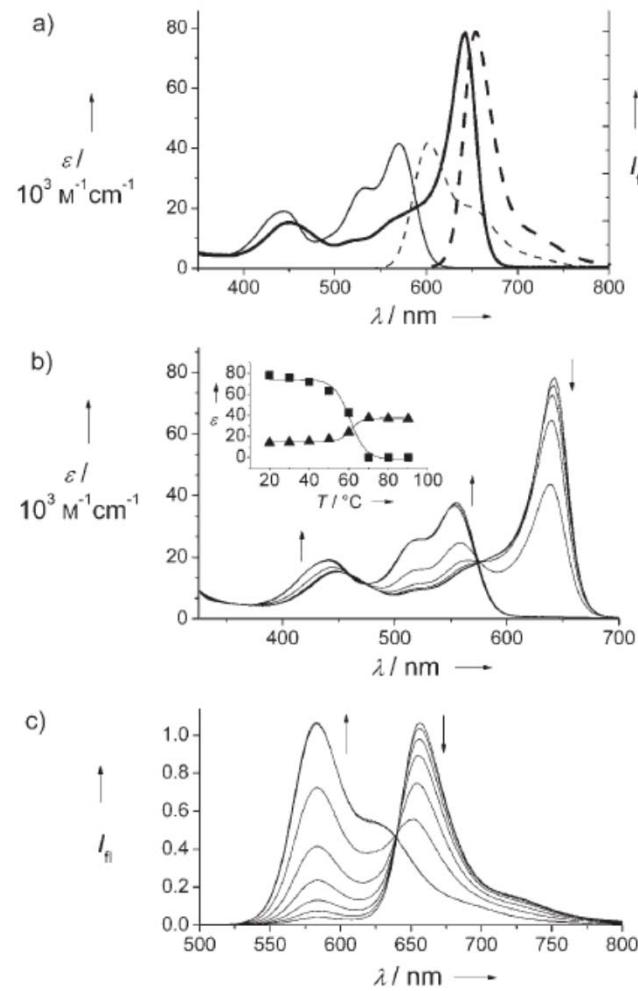


Langhals et al.

# PDI J-aggregate

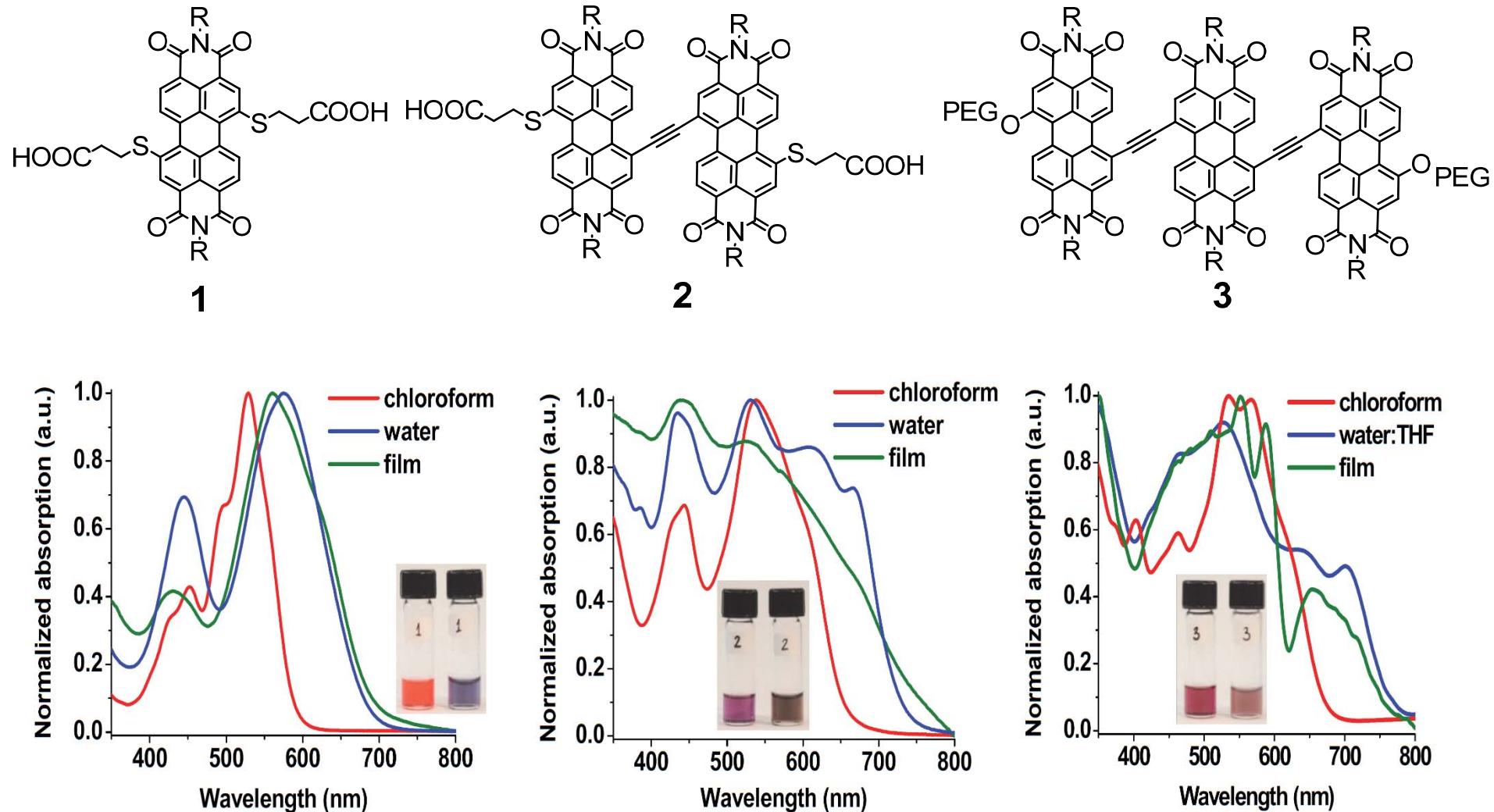


Wurthner et al.

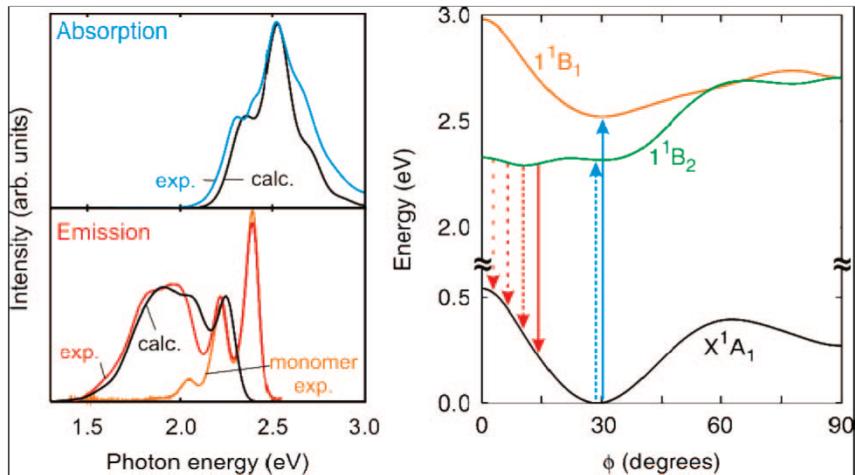


**Figure 2.** a) UV/Vis (solid lines) and fluorescence spectra (dashed lines) of **1** in  $\text{CH}_2\text{Cl}_2$  ( $10^{-5}$  M, thin line) and MCH ( $10^{-5}$  M, bold line). b) Temperature-dependent UV/Vis spectra of **1** in MCH ( $1.5 \times 10^{-5}$  M) at 20–90°C; arrows indicate the spectroscopic changes with increasing temperature. Inset: Changes in absorption at 642 nm (■) and 553 nm (▲) with increasing temperature; lines were calculated according to a sigmoidal fit. c) Temperature-dependent fluorescence spectra of **1** in MCH ( $6 \times 10^{-7}$  M,  $\lambda_{\text{ex}} = 476$  nm) at 15–50°C; arrows indicate the spectroscopic changes with increasing temperature.

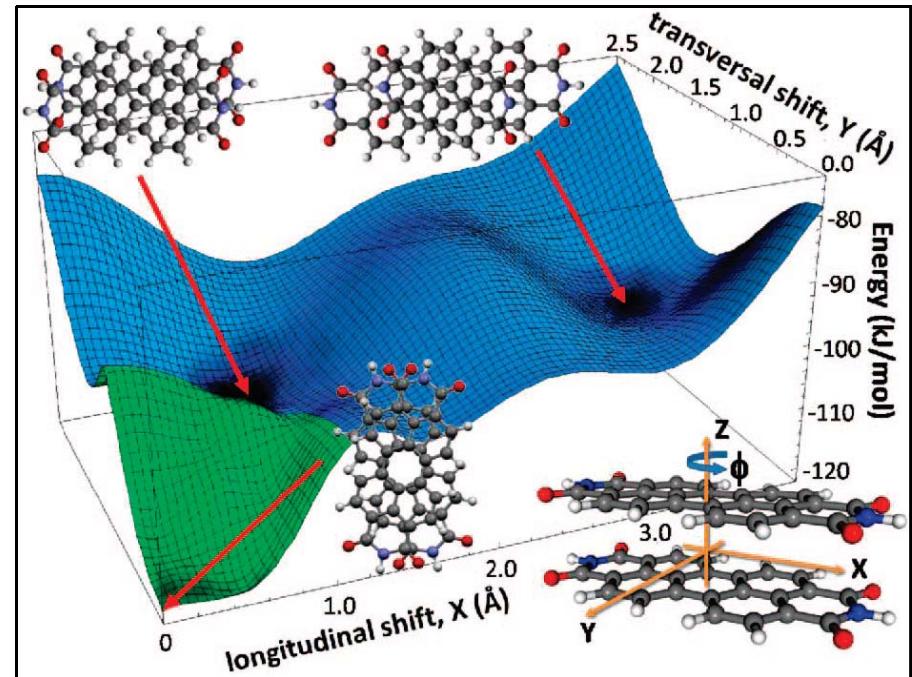
# Real life



# TDDFT



Left: Experimental UV/vis absorption and emission spectra for a mixture of PBI monomers and aggregates in methylcyclohexane and calculated spectra for monomers and aggregates. Right: Computed Potential Energy Surface of ground and excited states.



Computed potential energy surface of the ground state. The rotational angle  $\phi$  is indicated on the right-hand side.



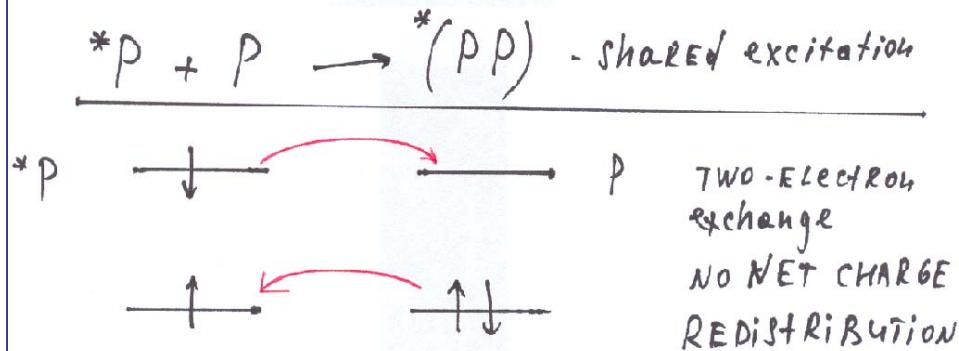
We looked at the absorption of multichromophore systems.  
What is about fluorescence?

# More than one molecule in excited state: Excimers and Exciplexes

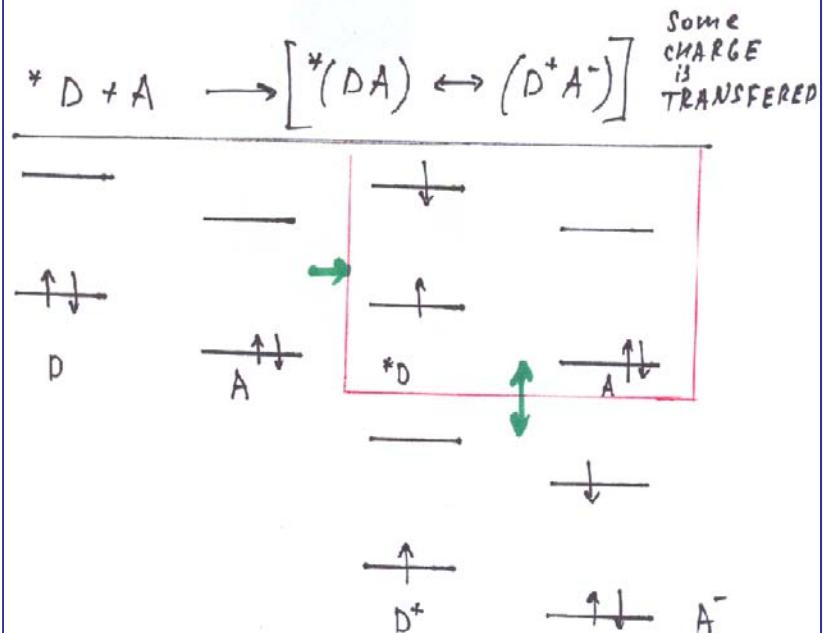
Excimers  
 ↓  
 excited state dimer

Exciplexes  
 ↓  
 excited state  
 donor-acceptor complex

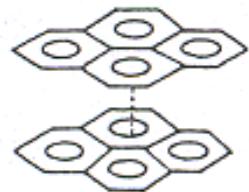
## I. Excimers



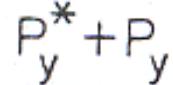
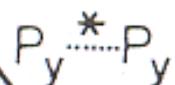
## Exciplexes



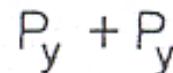
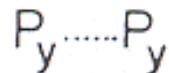
# Example: Excimers



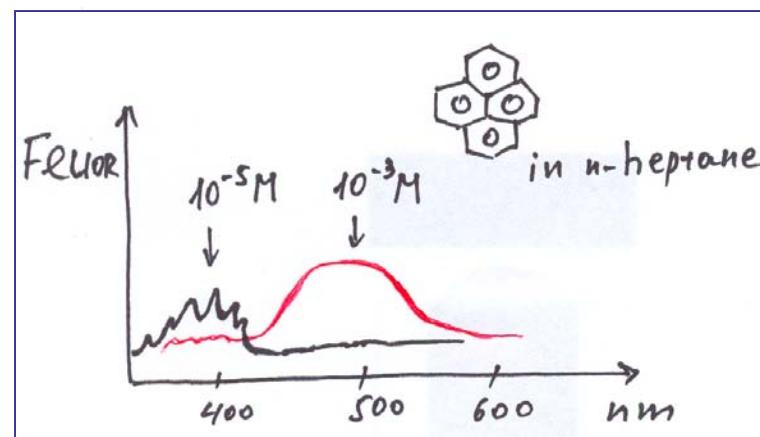
FACE TO FACE EXCIMER

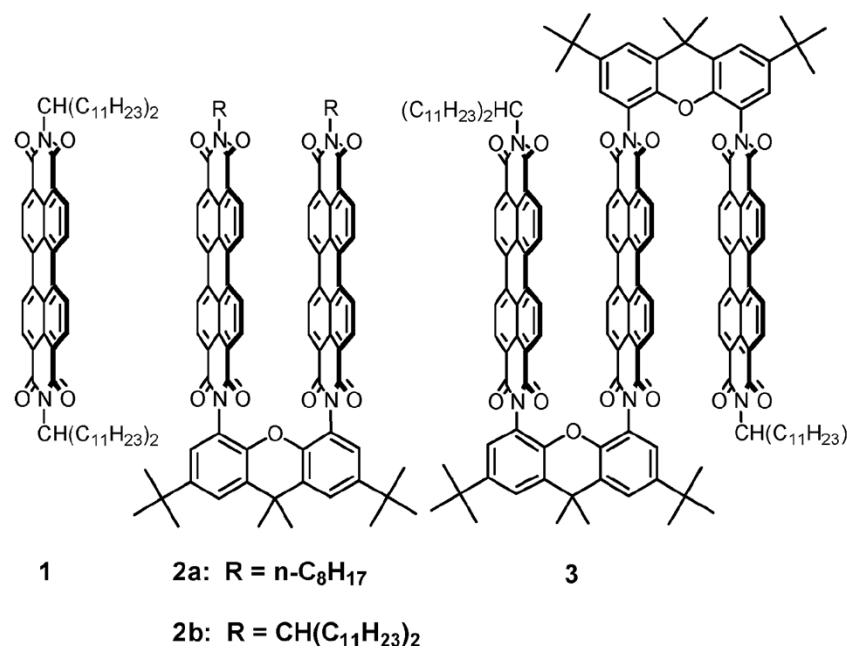


$h\nu$   
(excimer)

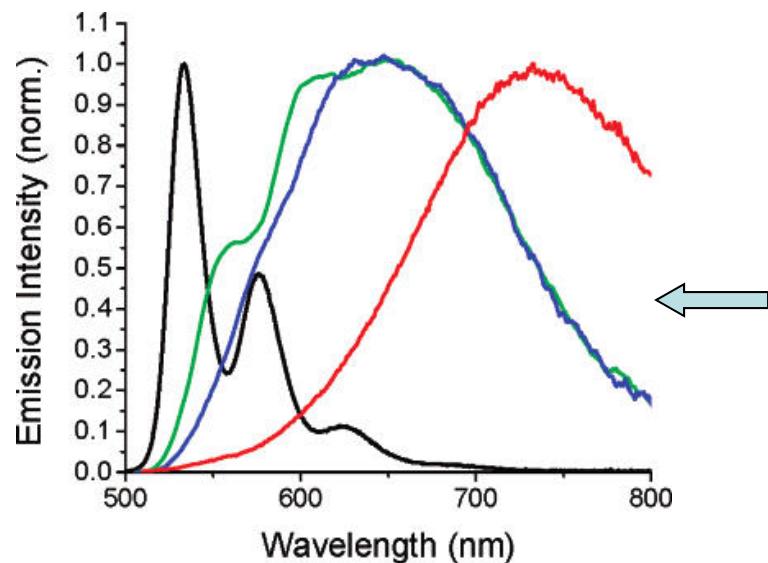
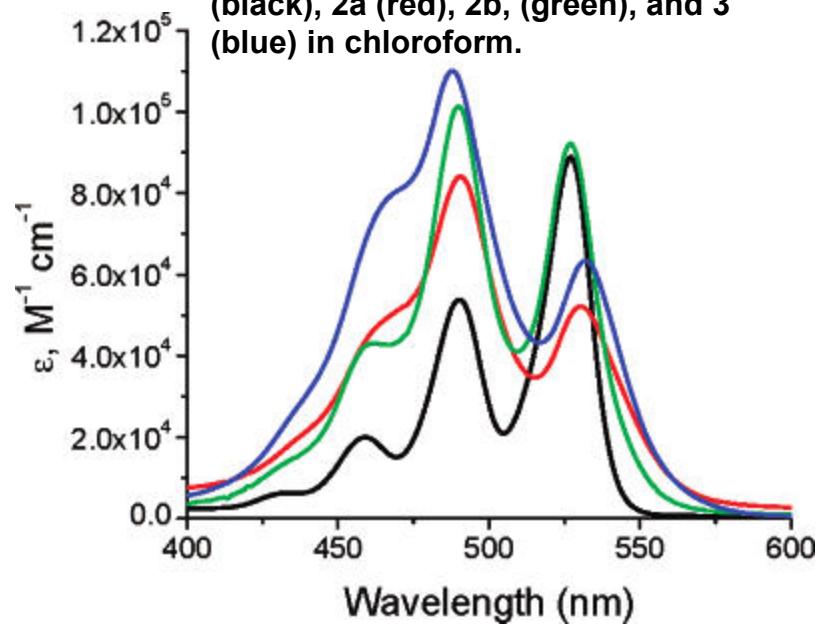


$$\begin{aligned}\Delta G &= \Delta H - T\Delta S \\ &= -10 - 0.3(-20) \\ &= -4 \text{ kcal/mole}\end{aligned}$$





**Ground-state absorption spectra of 1 (black), 2a (red), 2b, (green), and 3 (blue) in chloroform.**



Fluorescence spectra of 1 (black), 2a (red), 2b (green), and 3 (blue) in toluene (excited at 490 nm). The emission intensities are normalized at their maxima.

**Wasielewski et al.**

Excitons (excitation energy)  
can travel

Energy transfer

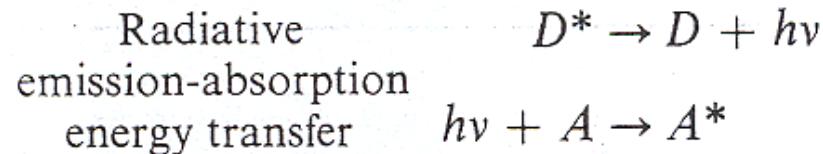
# Energy Transfer



A can be viewed as a quencher  
for the excited state of D

Energy transfer can occur via both  
radiative and nonradiative mechanisms

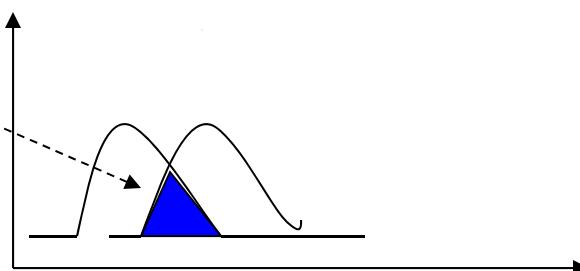
# Radiative Energy Transfer (“trivial”)



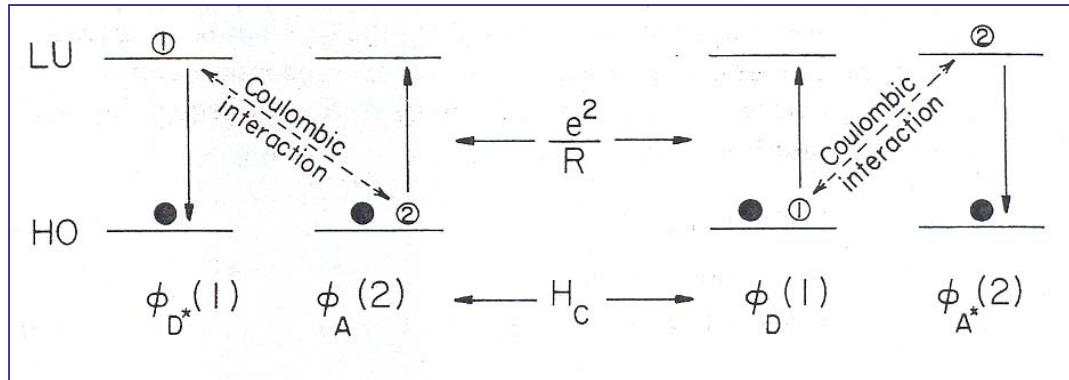
It is clear that trivial transfer is favored when each of these four factors is maximized, i.e.,  $\Phi_e^D \sim 1$ , high concentration of  $A$ , high extinction coefficient of  $A$ , and good overlap between the emission of  $D^*$  and absorption of  $A$ . The last factor may be quantified in terms of the spectral overlap integral,  $J$ , which is the *integrated* overlap of the experimental absorption and emission curves (Fig. 9.3).<sup>1a</sup>

Mathematically,  $J$  is given by

$$J \equiv \int_0^\infty I_D \varepsilon_A d\bar{\nu} \quad (9.3)$$

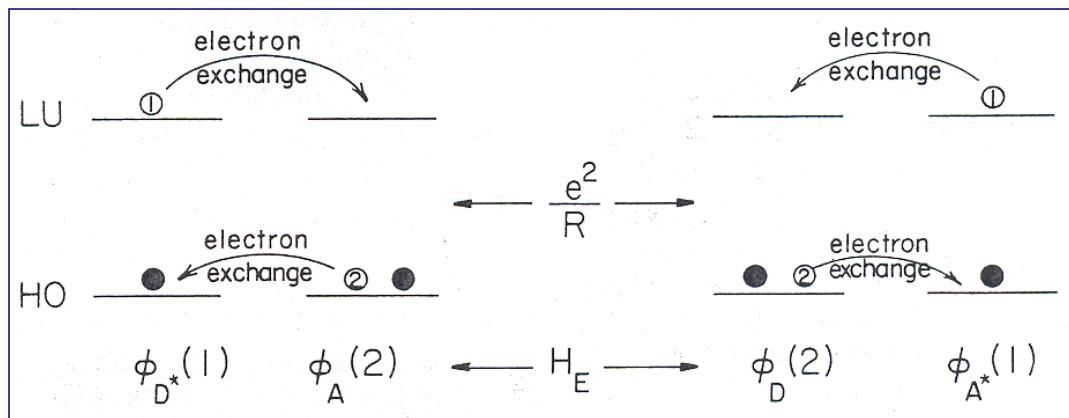


# Nonradiative energy transfer: Förster and Dexter mechanisms



**Coulombic mechanism (dipole-dipole) is called Förster energy transfer mechanism**

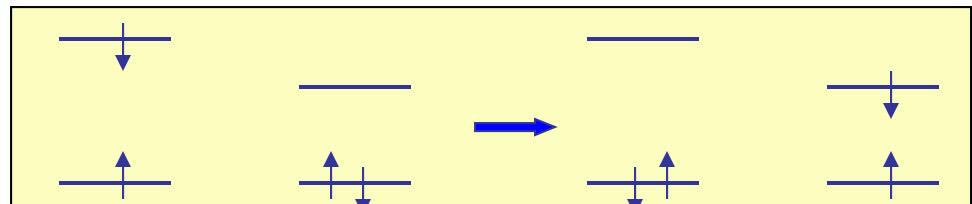
**No direct orbital overlap necessary**



**Exchange mechanism is called Dexter energy transfer mechanism**

**Direct orbital overlap necessary**

Energy gradient (driving force) is important for energy transfer observation



# Theodor Förster

- Förster grew interested in the energy transfer because of ***the efficient photosynthetic process***. He was aware from previous experiments, that leaves capture and use light energy much more effectively than would be expected, even if photons hit the reaction centers precisely. The efficient transfer of energy between the closely spaced chlorophyll molecules must be responsible, allowing the absorbed energy to diffuse into the relatively sparse reaction centers by hopping rapidly between molecules.



# FRET review

- FRET is a near-field interaction ( $r \ll \lambda$ ) and does not involve any photons because the energy is transferred non-radiatively. There is no emission during FRET.
- It involves ***interaction between the electric fields of the transition dipole of donor and acceptor.***
- The first step of a FRET molecular process is the absorption of a quantum of energy by the donor molecules. Once the excited molecule is in thermal equilibrium with the surrounding medium, it will return to the ground state by spontaneous radiative or nonradiative process.
- There is interaction between the electric fields of the transition dipole moments of the donor (emission) and acceptor (absorption) leading to energy transfer (dipole induces another dipole).
- Förster resonance energy transfer can be used as a ***spectroscopic ruler*** in the range of 10 – 100 Å - using the variations in transfer efficiency.

# Förster resonance energy transfer (FRET). Dominating mechanism.

The **FRET efficiency** is determined by three parameters:

- 1.The distance between the donor and the acceptor.
- 2.The spectral overlap of the donor emission spectrum and the acceptor absorption spectrum.
- 3.The relative orientation of the donor emission dipole moment and the acceptor absorption dipole moment.

**k (Coulombic ET)  $\sim 1/R^6$ , where R=donor-acceptor distance.**

The FRET efficiency  $E$ , which is defined as

$$E = 1 - \tau'_D / \tau_D$$

where  $\tau'_D$  and  $\tau_D$  are the donor fluorescence lifetimes in the presence and absence of an acceptor, respectively, or as

$$E = 1 - F'_D / F_D$$

where  $F'_D$  and  $F_D$  are the donor fluorescence intensities with and without an acceptor, respectively.  $E$  depends on the donor-to-acceptor separation distance  $r$  with an inverse 6th order law due to the dipole-dipole coupling mechanism:

$$E = \frac{1}{1 + (r/R_0)^6}$$

with  $R_0$  being the Förster distance of this pair of donor and acceptor at which the FRET efficiency is 50%. The Förster distance depends on the overlap integral of the donor emission spectrum with the acceptor absorption spectrum and their mutual molecular orientation as expressed by the following equation:

$$R_0^6 = 8.8 \times 10^{23} \kappa^2 n^{-4} Q_0 J$$

where  $\kappa^2$  is the dipole orientation factor,  $n$  is the refractive index of the medium,  $Q_0$  is the fluorescence quantum yield of the donor in the absence of the acceptor, and  $J$  is the spectral overlap integral.

### Orientation Factor Critical Angles

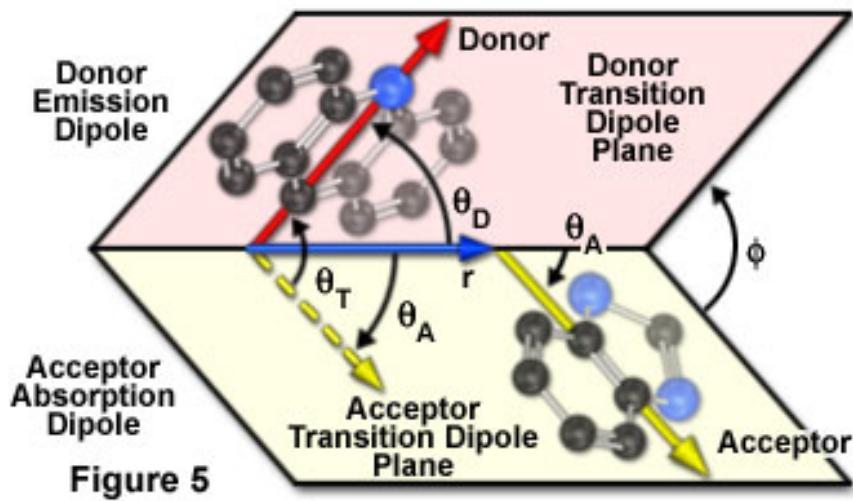


Figure 5

$$k^2 = (\cos\theta_T - 3\cos\theta_D\cos\theta_A)^2 = (\sin\theta_D\sin\theta_A \cos \varphi - 2\cos\theta_D\cos\theta_A)^2$$

# Several points to notice

- Fluorescence resonance energy transfer (FRET) is a nonradiative process whereby an excited state donor D (usually a fluorophore) transfers energy to a proximal ground state acceptor A through long-range dipole–dipole interactions
- The uncertainty in evaluating the orientation factor ( $k$ -squared) has been discussed extensively in the literature, and in spite of experimental evidence that the Förster theory is valid and applicable to distance measurement, this variable has continued to be somewhat controversial. It is important to recognize that Förster distances are usually given for an assumed value of  $k$ -squared, typically the dynamically averaged value of 2/3 (0.67). This assumed value results from randomization of donor and acceptor orientation by rotational diffusion prior to energy transfer. The orientation factor depends upon the relative orientations in space of the donor emission dipole and the acceptor absorption dipole, and can range from zero to 4. A value of 1 corresponds to parallel transition dipoles, while a value of 4 results from dipoles that are both parallel and collinear.
- **The phenomenon of resonance energy transfer by the Förster mechanism is complex in some aspects, but simple and dependable in its resulting effect. Förster distances are accurately predictable from spectral properties of the donor and acceptor**

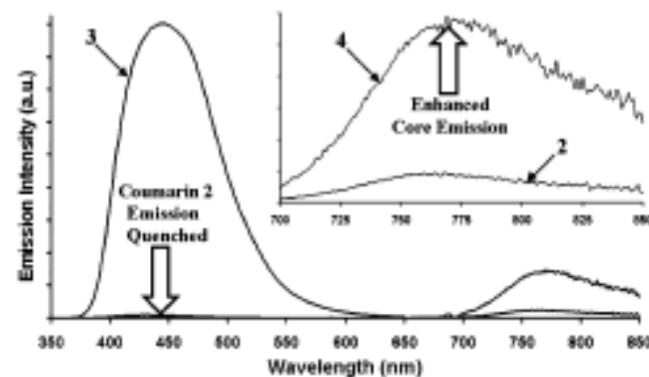
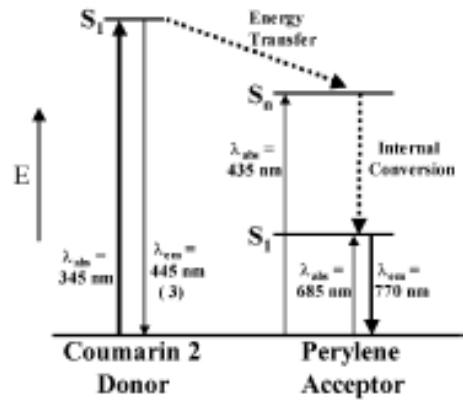
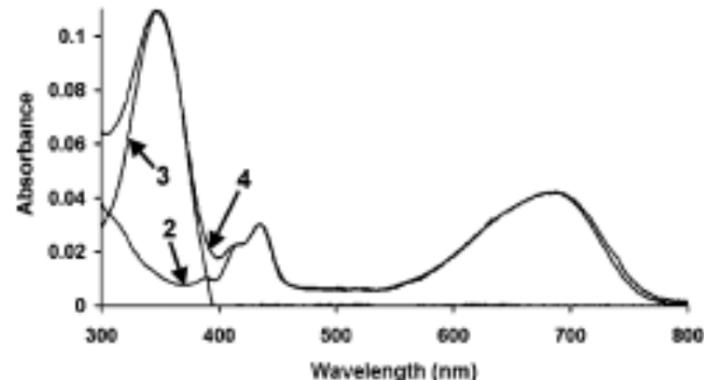
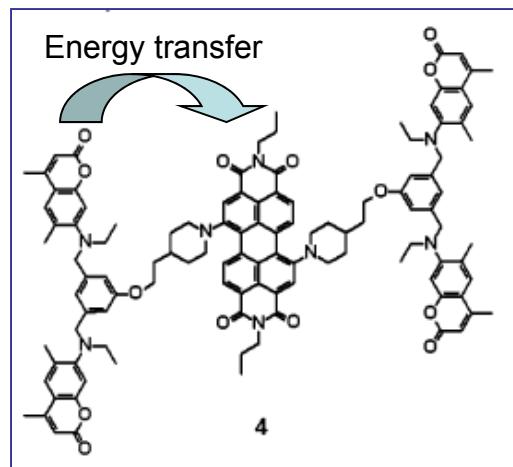
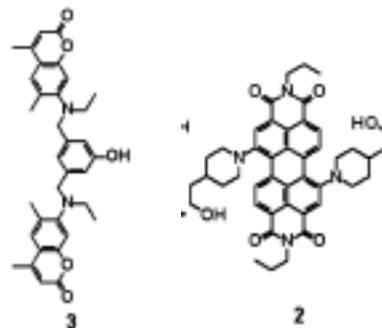
# FRET Example 1

A FRET-Based Ultraviolet to Near-Infrared Frequency Converter

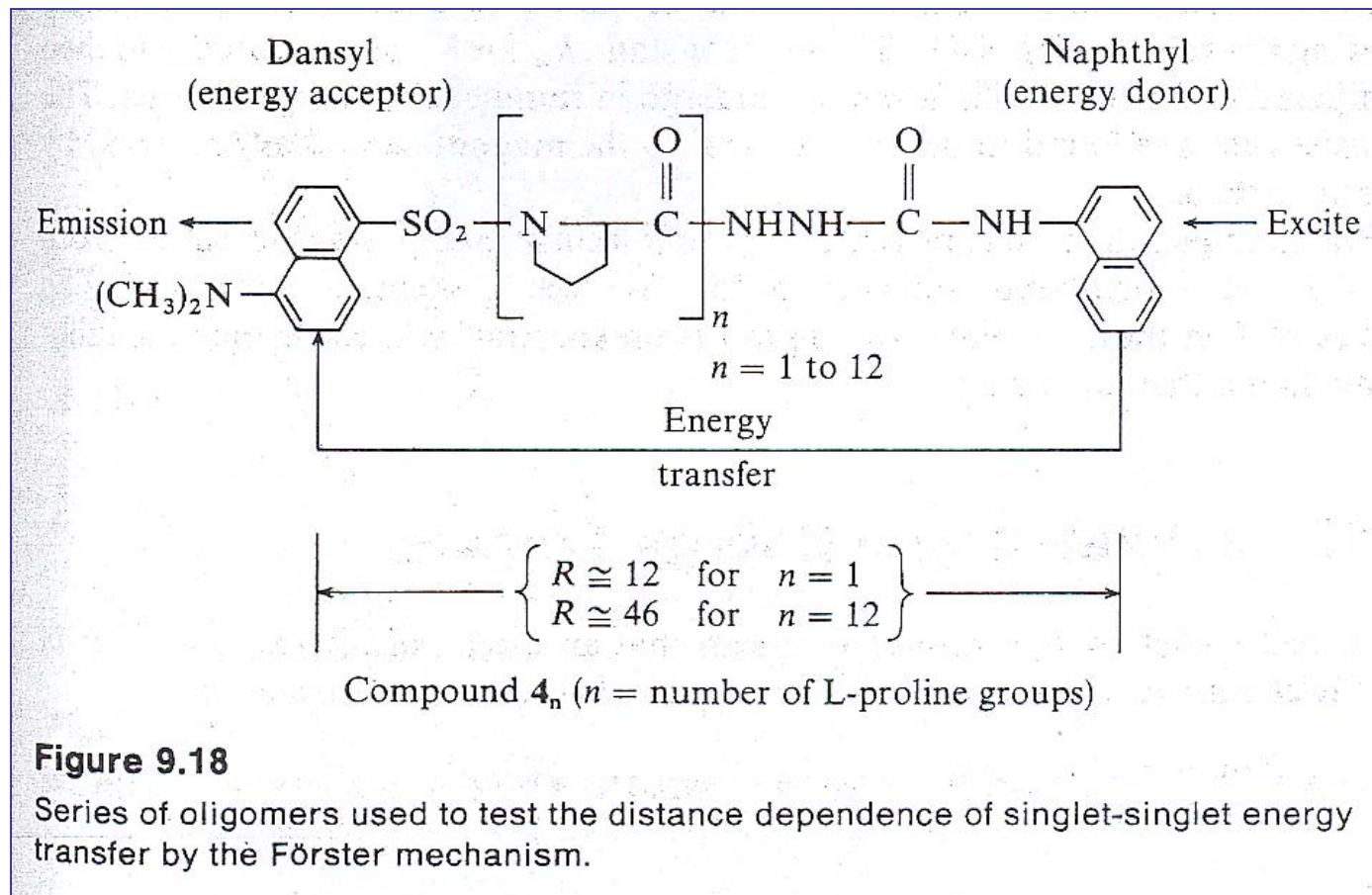
Jason M. Serin, Darryl W. Brousmiche, and Jean M. J. Fréchet\*

Department of Chemistry, University of California, Berkeley

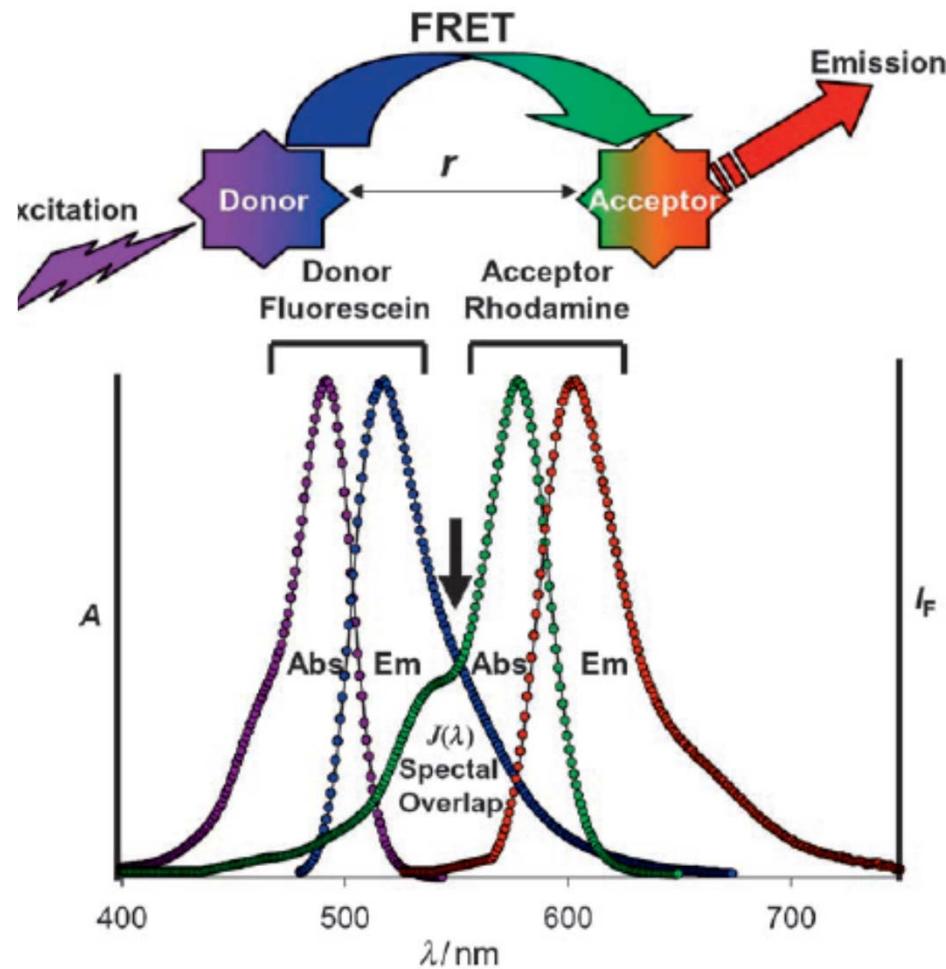
J. AM. CHEM. SOC. 2002, 124, 11848-11849



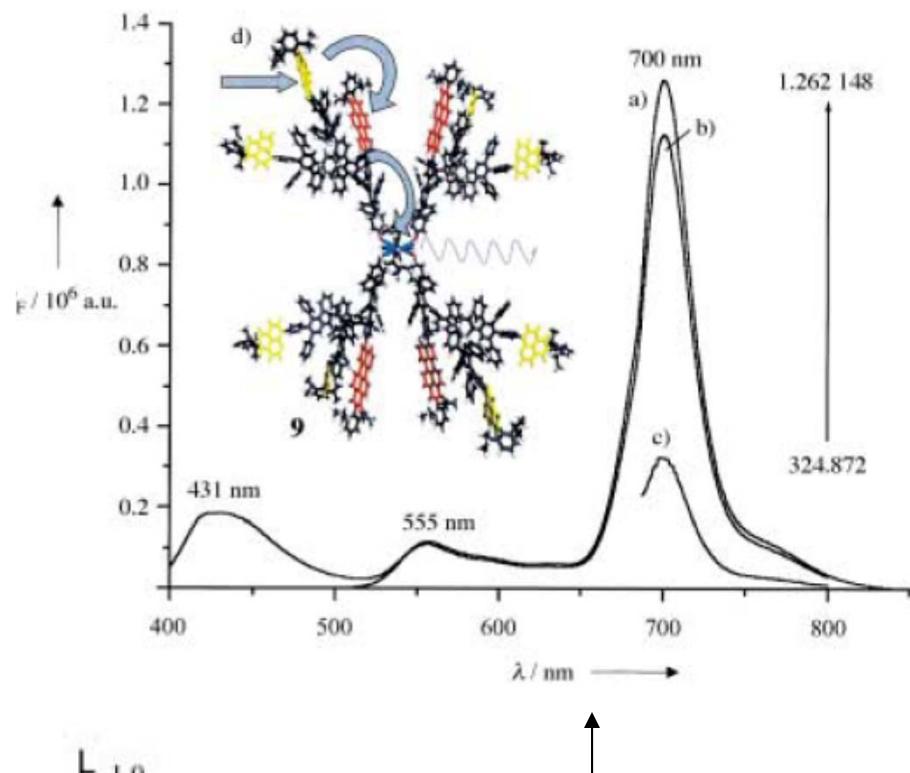
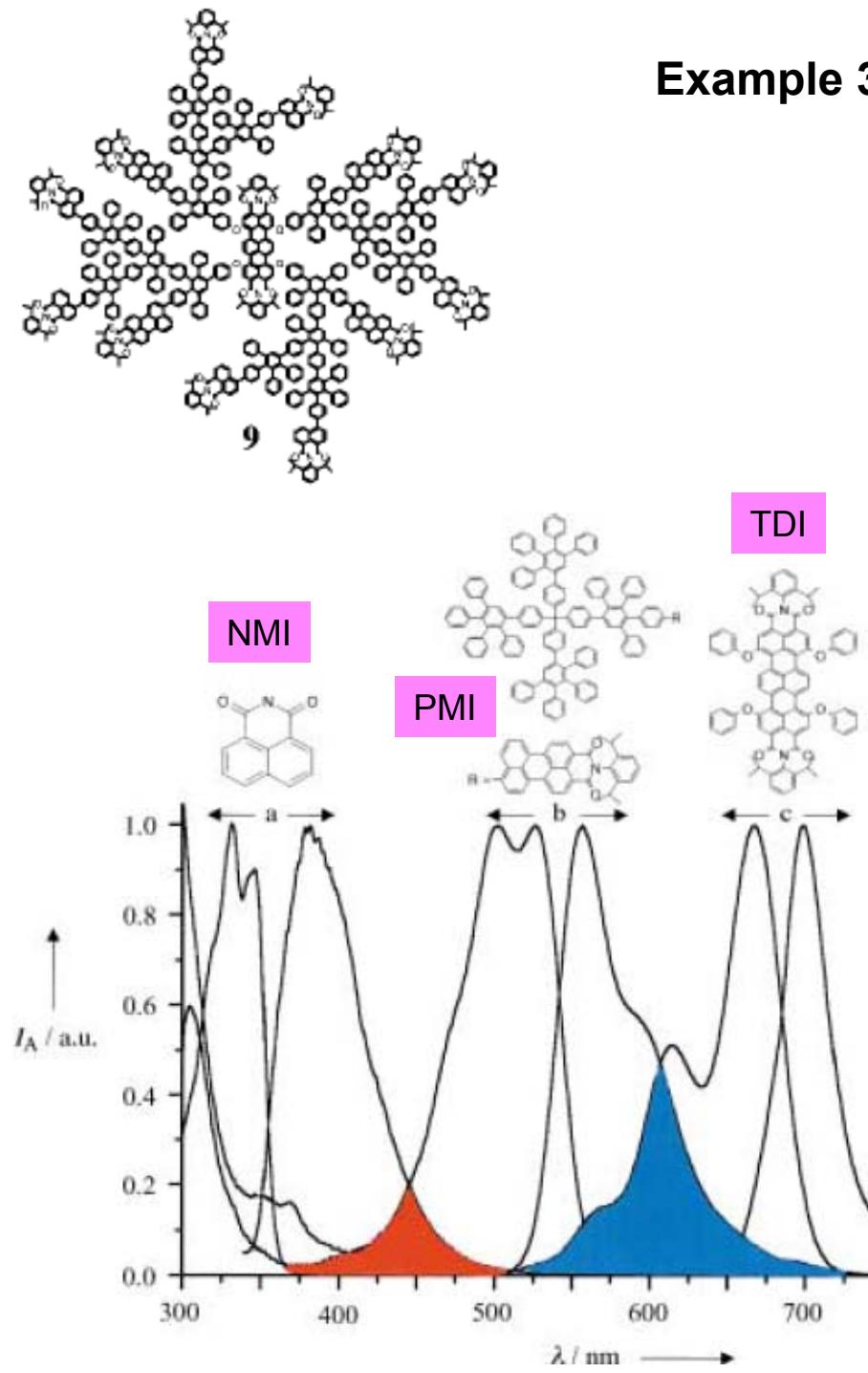
## FRET Example 2: spectroscopic ruler



Transfer efficiency depends on inverse sixth power of the distance between chromophores. Energy transfer efficiency can be used to estimate this distance.

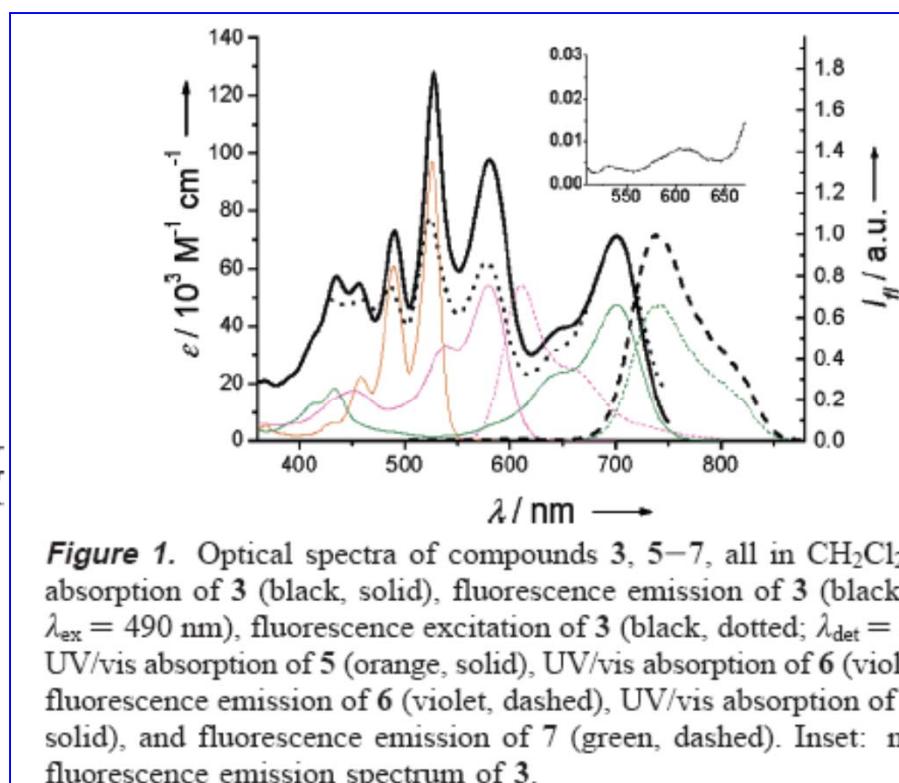
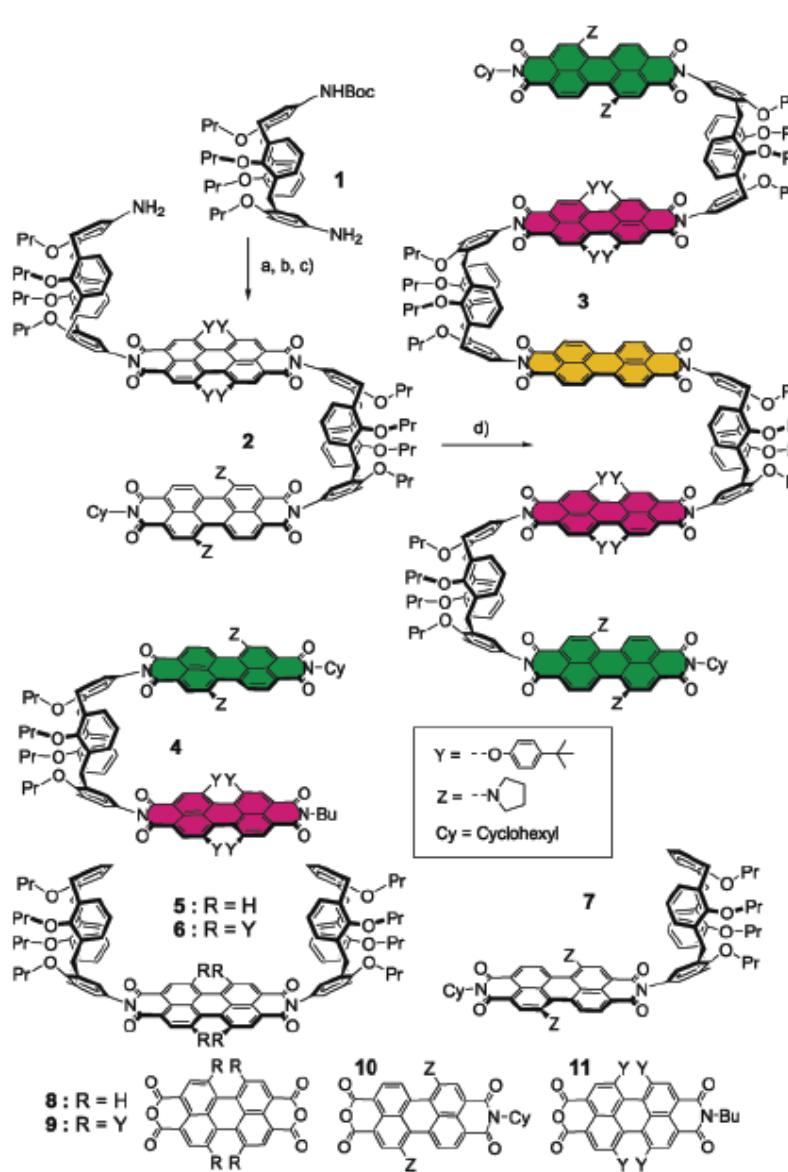


### Example 3



Emission spectra of 9 after excitation of a) the NMI chromophores at the periphery (exc370 nm), b) the PMI chromophores in the scaffold (exc480 nm), and c) the TDI chromophore in the center (exc665 nm). d) 3D structure of 9 obtained from molecular mechanics calculations and visualization of the vectorial energy transfer from the periphery towards the center.

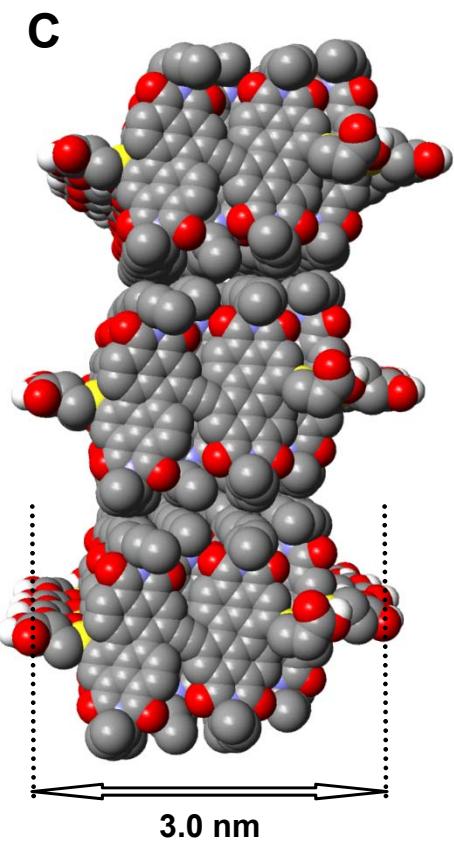
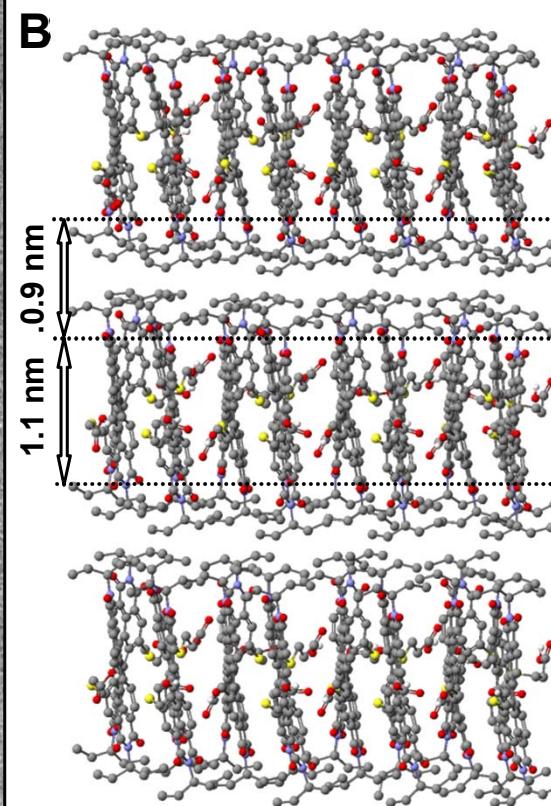
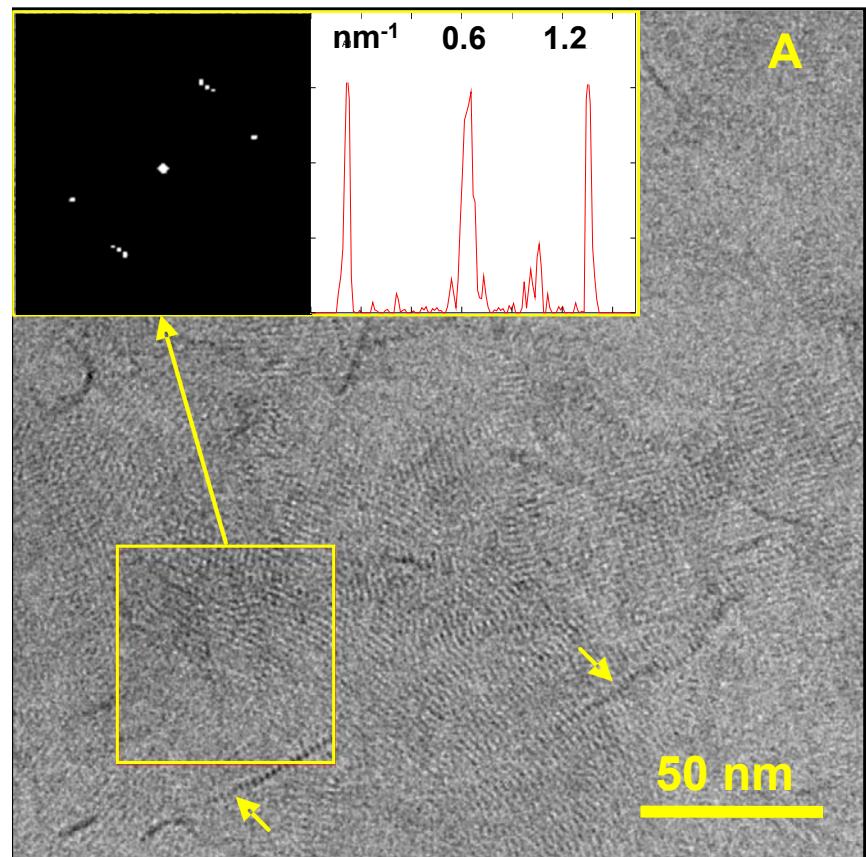
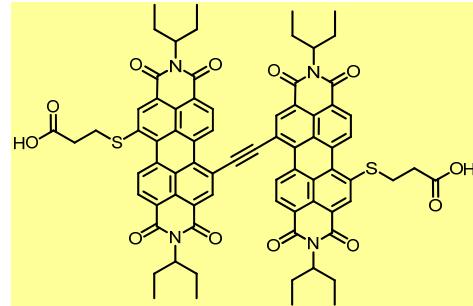
## Example 4



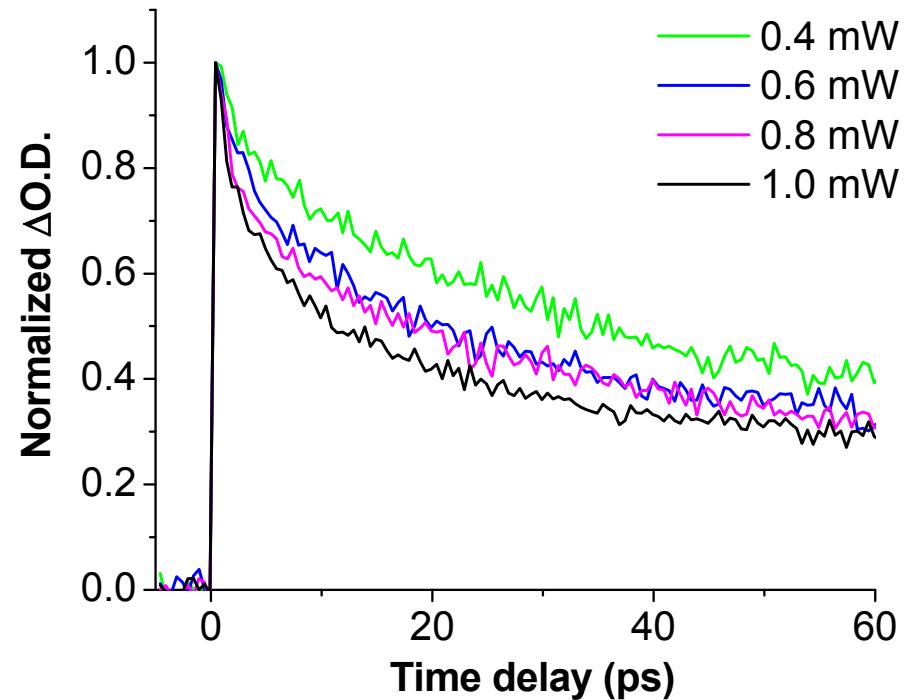
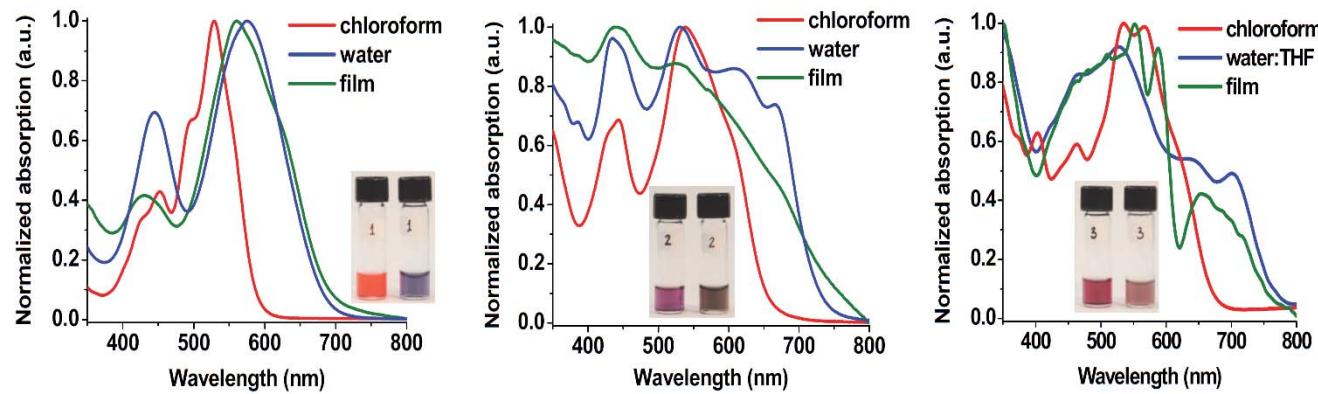
**Figure 1.** Optical spectra of compounds 3, 5–7, all in  $\text{CH}_2\text{Cl}_2$ . UV-vis absorption of 3 (black, solid), fluorescence emission of 3 (black, dashed;  $\lambda_{\text{ex}} = 490 \text{ nm}$ ), fluorescence excitation of 3 (black, dotted;  $\lambda_{\text{det}} = 850 \text{ nm}$ ), UV-vis absorption of 5 (orange, solid), UV-vis absorption of 6 (violet, solid), fluorescence emission of 6 (violet, dashed), UV-vis absorption of 7 (green, solid), and fluorescence emission of 7 (green, dashed). Inset: magnified fluorescence emission spectrum of 3.

# **How to probe exciton movement**

Let them kill each other....



# Exciton annihilation



The kinetics of crystalline assemblies of **2** and **3** exhibit fast decay component that is dependent on the laser power (Figures 8 and S10). In order to estimate exciton diffusion coefficient and diffusion length we used an analysis method, in which the data obtained at different laser powers were fitted using the bimolecular annihilation rate equation:<sup>15,48</sup>

$$\frac{d}{dt} n(t) = -\frac{n(t)}{\tau} - \gamma(t)n(t)^2 \quad (1)$$

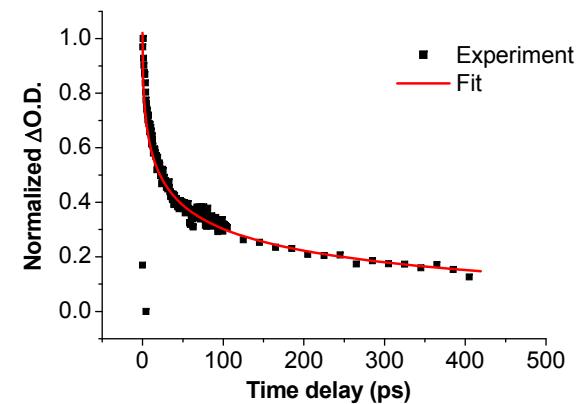
where  $n(t)$  is the exciton population at time  $t$ ,  $\tau$  is the intrinsic lifetime of an exciton (when no annihilation takes place) and  $\gamma(t)$  is the annihilation rate. Within this framework we utilized one-dimensional diffusion model for the annihilation rate:<sup>15,48</sup>

$$\gamma_{1D}(t) = \frac{1}{aN_0} \sqrt{\frac{4D}{\pi t}} \quad (2)$$

with  $a$  being the 1D lattice constant (distance between the adjacent molecules in the stack, 3.4 Å),  $D$  the exciton diffusion constant, and  $N_0$  the molecular density (number of molecules in the assembly per unit volume). This model fits best our data, which is in accordance with previous

	$D$ , cm <sup>2</sup> /s	$L_D^*$ , nm
<b>2</b>	$0.078 \pm 0.020$	<b><math>120 \pm 10</math></b>
<b>3</b>	$0.016 \pm 0.005$	<b><math>35 \pm 10</math></b>

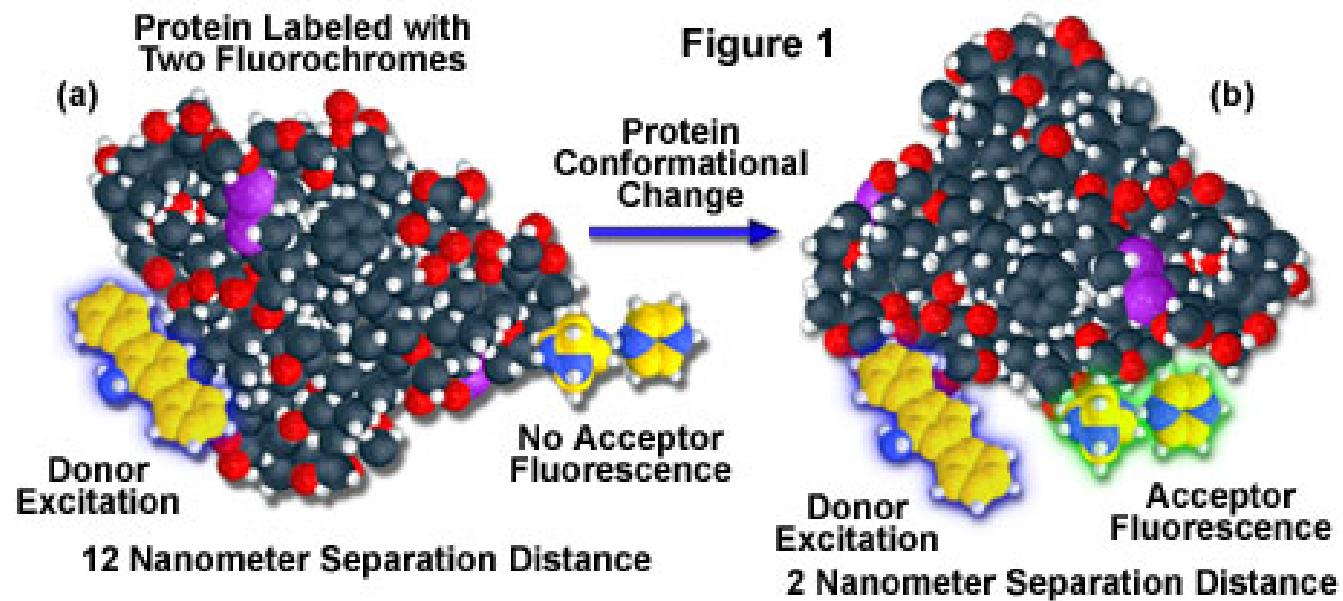
**Diffusion coefficient and exciton diffusion length for assemblies **2** and **3**.**



# FRET in biology

- FRET is a useful tool to quantify molecular dynamics such as protein-protein interactions, protein-DNA interactions, and protein conformational changes. For monitoring the complex formation between two molecules, one of them is labeled with a donor and the other with an acceptor, and these fluorophore-labeled molecules are mixed. When they are dissociated, the donor emission is detected upon the donor excitation. On the other hand, when the donor and acceptor are in close proximity (1-10 nm) due to the interaction of the two molecules, the acceptor emission is predominantly observed because of the intermolecular FRET from the donor to the acceptor.
- For monitoring protein conformational changes, the target protein is labeled with a donor and an acceptor at two loci. When a twist or bend of the protein brings the change in the distance or relative orientation of the donor and acceptor, FRET change is observed. If a molecular interaction or a protein conformational change is dependent on ligand binding, this FRET technique is applicable to fluorescent indicators for the ligand detection.

## Intramolecular Fluorescence Resonance Energy Transfer (FRET)



## Biomolecular Fluorescence Resonance Energy Transfer Applications

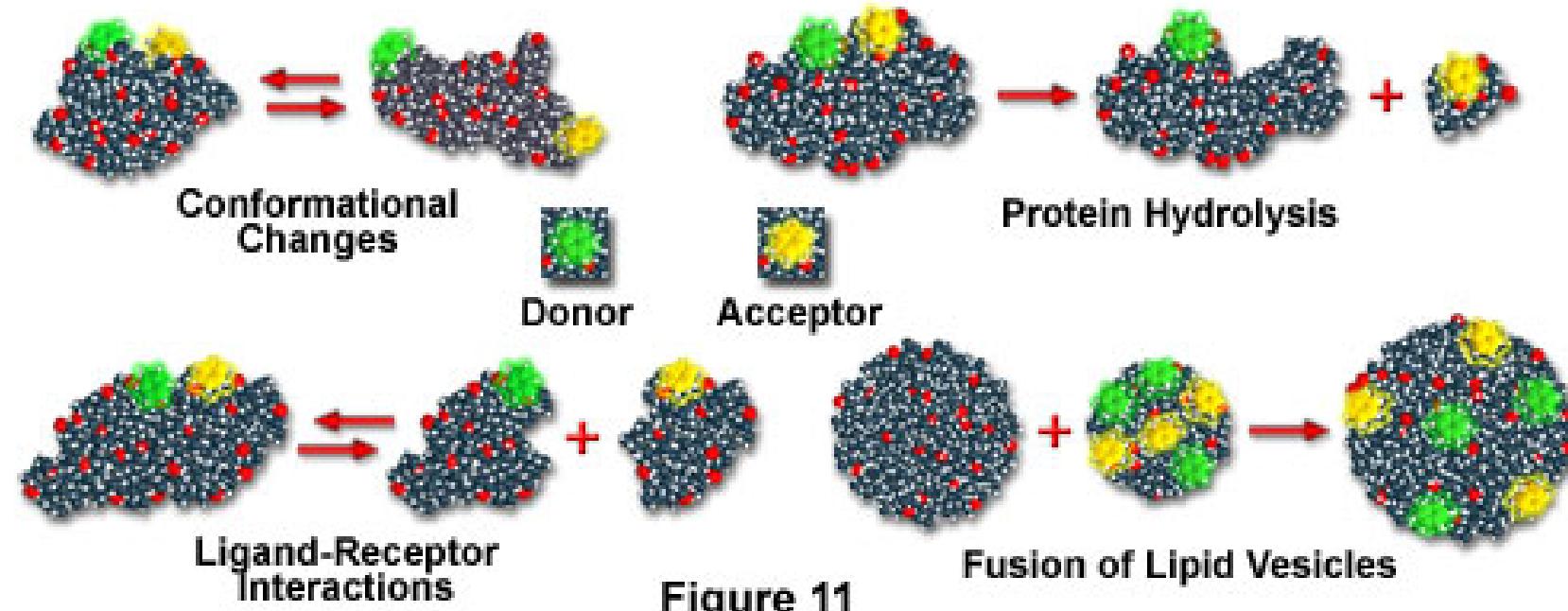
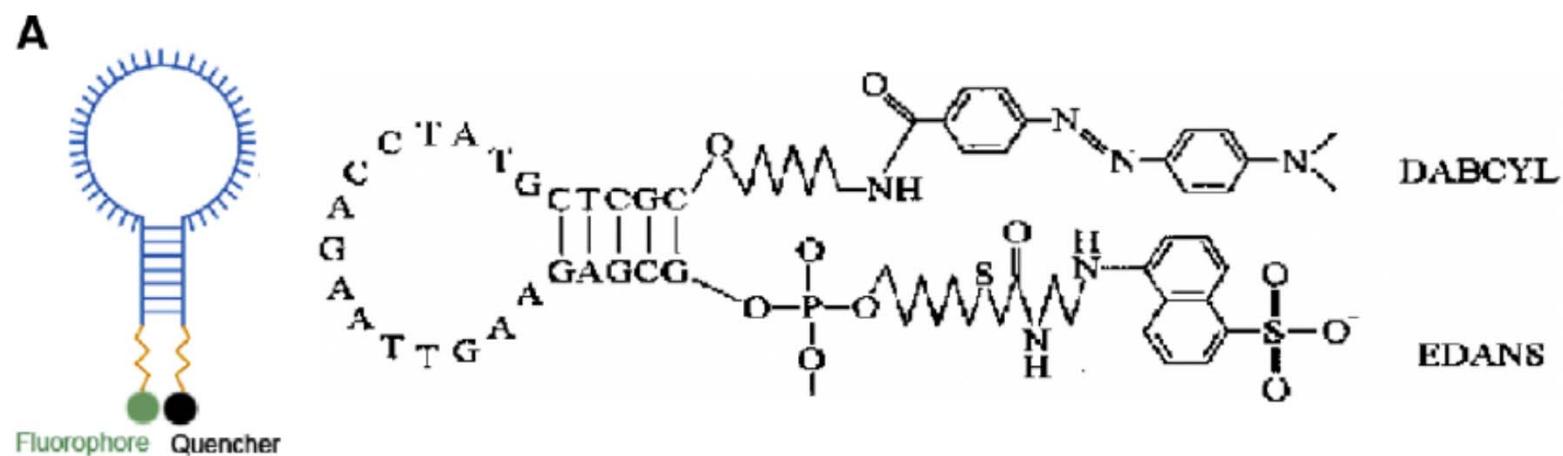
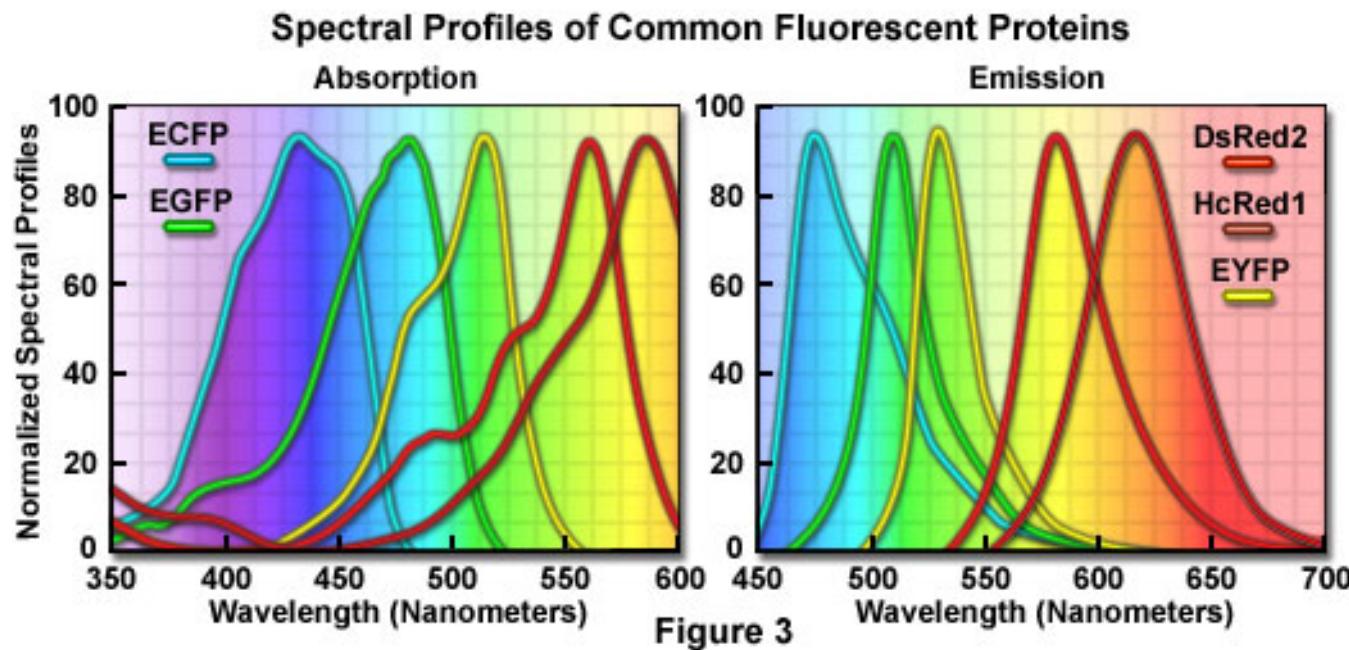


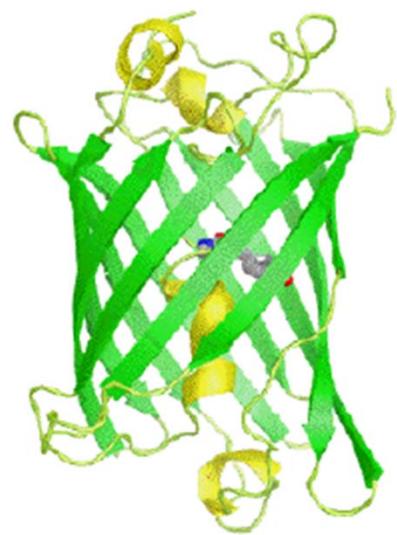
Figure 11



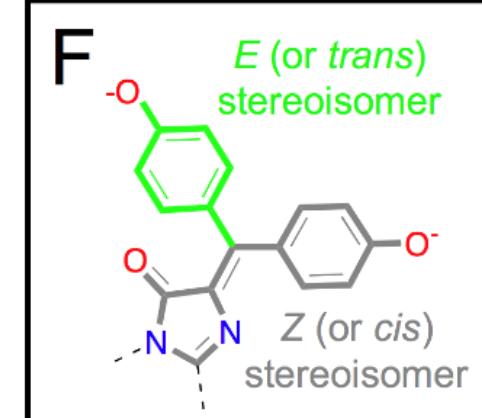
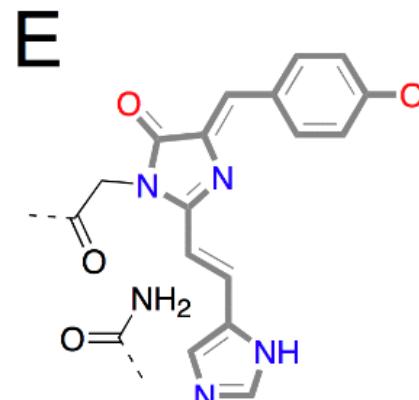
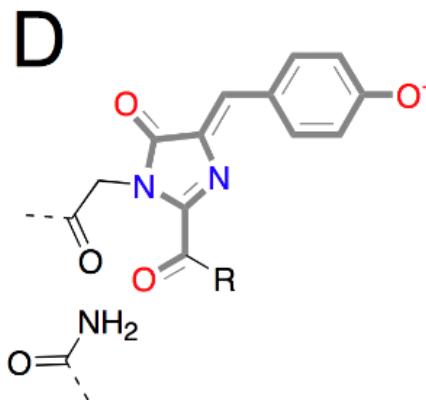
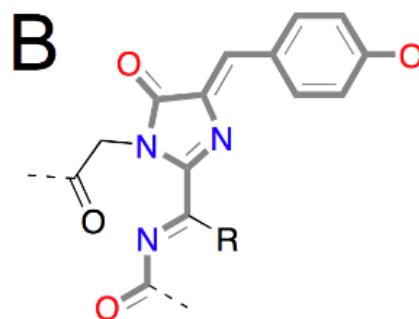
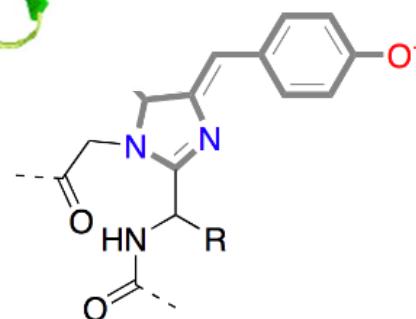
# Fluorescent proteins

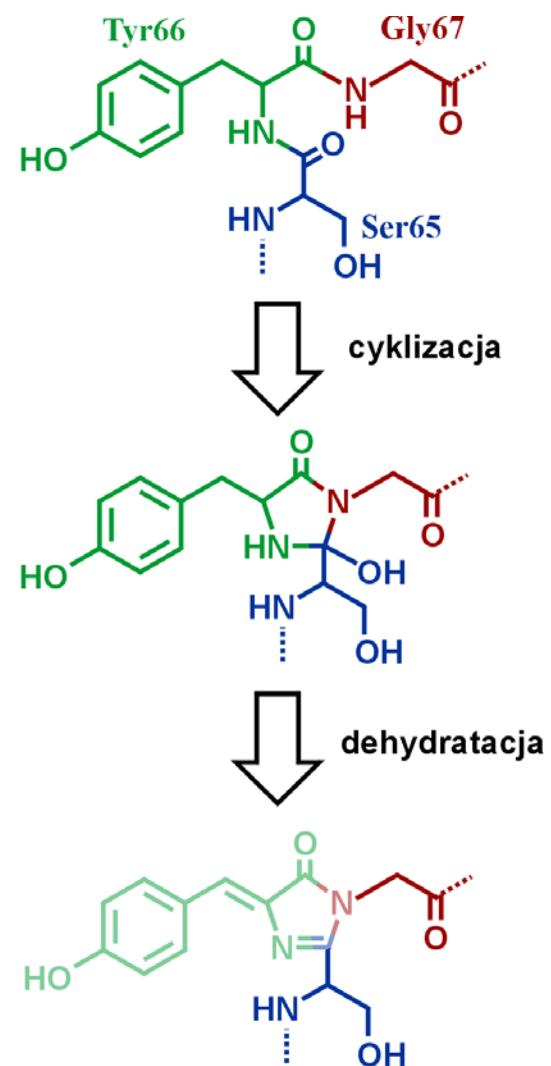
The discovery of green fluorescent protein in the early 1960s (Nobel prize in chemistry 2008) ultimately heralded a new era in cell biology by enabling investigators to apply molecular cloning methods, fusing the fluorophore moiety to a wide variety of protein and enzyme targets

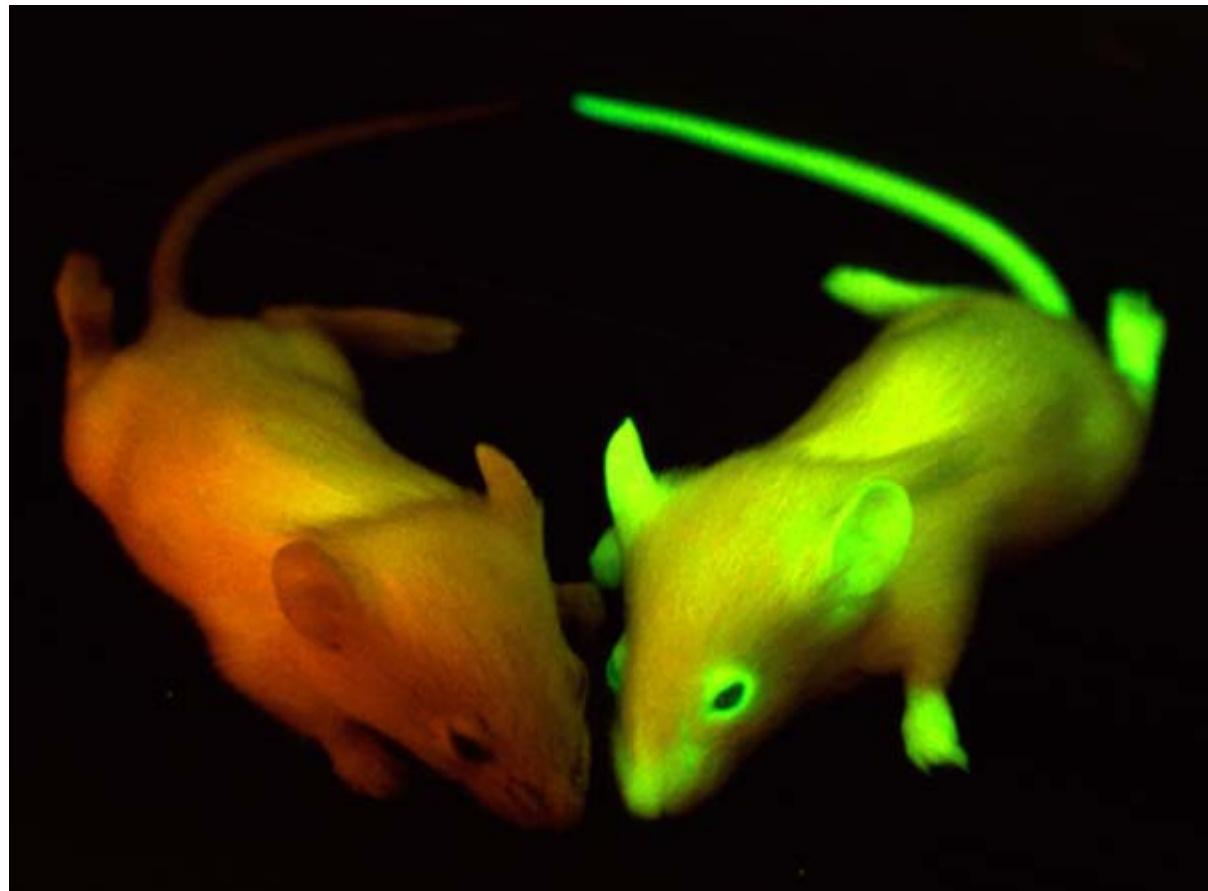


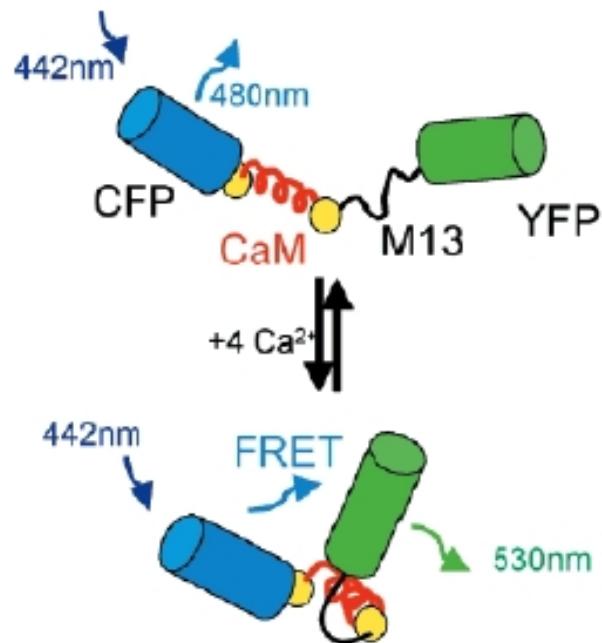


**Fluorescent proteins** are members of a structurally homologous class of proteins that share the unique property of being self-sufficient to form a visible wavelength chromophore from a sequence of 3 amino acids within their own polypeptide sequence. It is common research practice for biologists to introduce a gene (or a gene chimera) encoding an engineered fluorescent protein into living cells and subsequently visualize the location and dynamics of the gene product using fluorescence microscopy

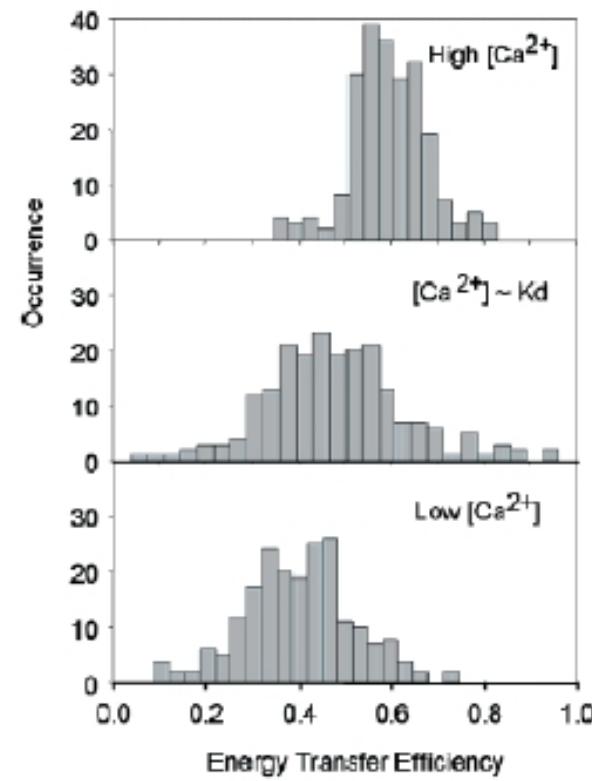








(a)



(b)

# Exchange Mechanism: Dexter

Also called overlap or collision mechanism. This is because in the exchange mechanism orbital **overlap** is needed which is achieved by **collisions** of molecules. This is a **short range** mechanism as opposed to **long range** Förster.

$$k_{ET}(\text{exchange}) = K J e^{(-2R/L)}$$

K=specific orbital interaction coefficient

J=overlap integral

R=distance between energy donor and acceptor

L=van der Waals radii

## ***Comparison of Dexter and Förster:***

- Different R dependence, only Förster is dependent on oscillator strength, both mechanisms depend on overlap integral.
- Most importantly: Dexter is strongly influenced by diffusion. E.g. solvent viscosity should significantly change Dexter energy transfer rates, while Förster rates should be only slightly changed.

# Triplet-Triplet energy transfer

- If you excite a system that gives triplets efficiently it will transfer energy to another triplet state if it can. In this way you can indirectly produce triplets:
- Excite benzophenone and it will transfer energy to triplet states not singlets!
- Perylene – direct excitation cannot produce triplets, but they can be sensitized using benzophenone
- Almost always – Dexter mechanism