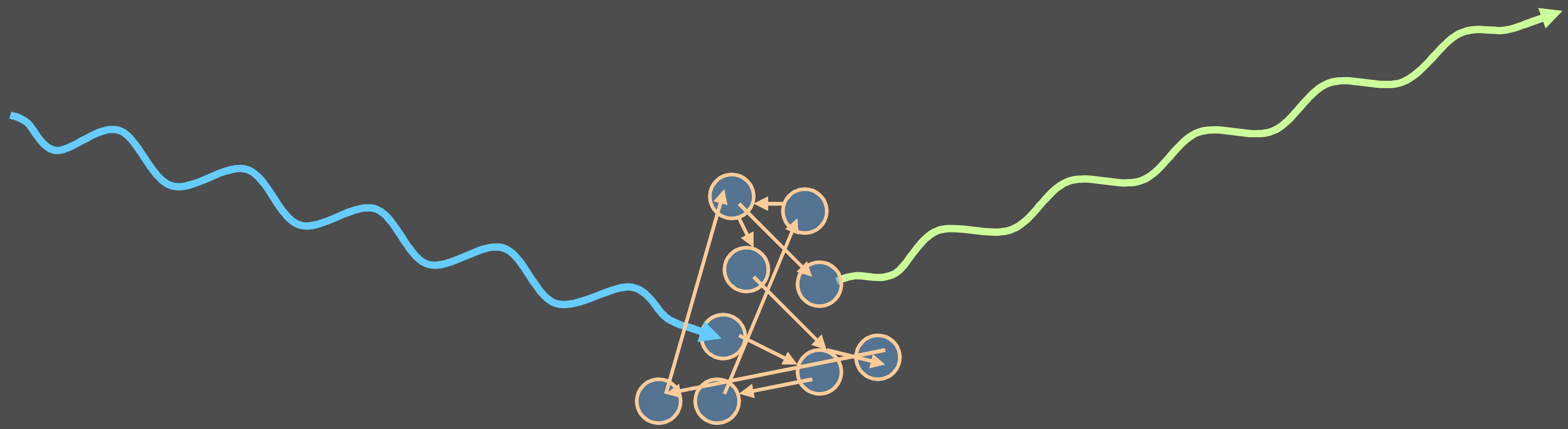
# Förster (Fluorescence) Resonance Energy Transfer (FRET)

- Great method for the detection of:
  - Protein-protein interactions
  - Enzymatic activity
  - Small molecules interacting inside a cell

Resonance Energy Transfer (non-radiative)

The Bad: Self-quenching



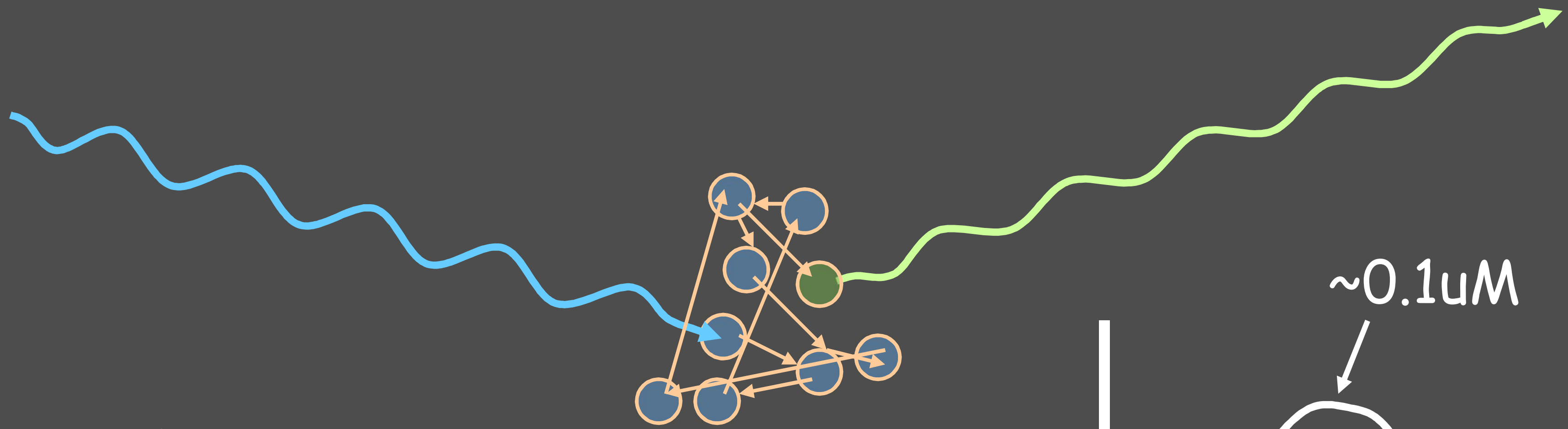
If dye at high concentration

“hot-potato” the energy until lost

Resonance Energy Transfer (non-radiative)

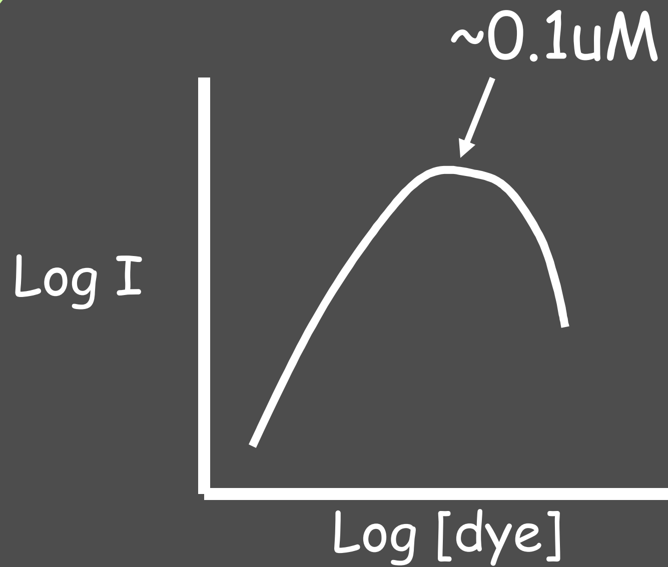
“Self-quenching” of dye

(“hot-potato” the energy until lost)



Depends on:

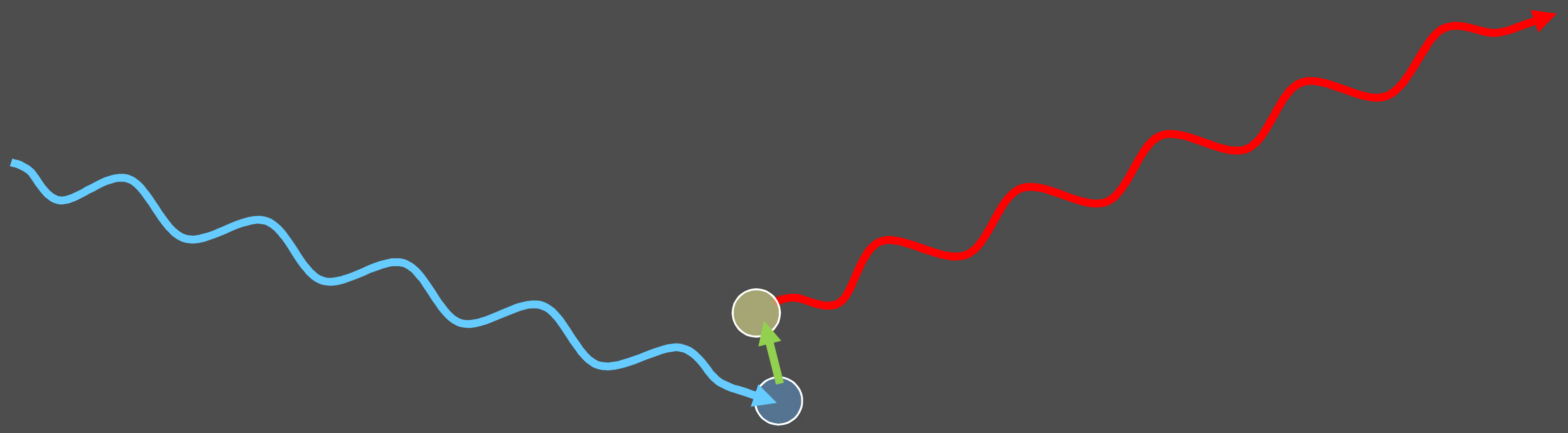
- Dye Concentration
- Geometry
- Environment



FRET:

Resonance Energy Transfer (non-radiative)

The Good:  FRET as a molecular yardstick



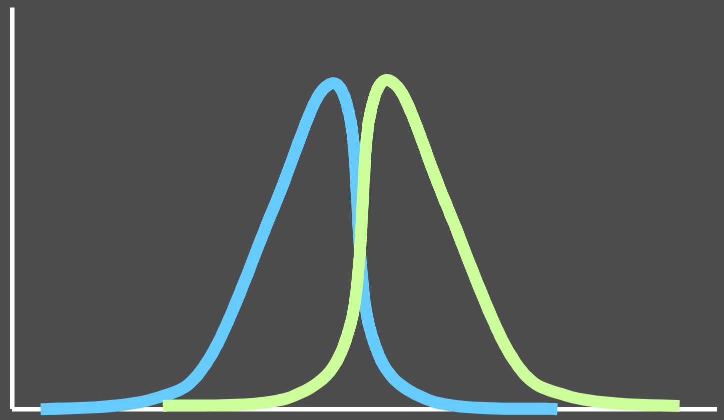
Transfer of energy from one dye to another

Depends on:

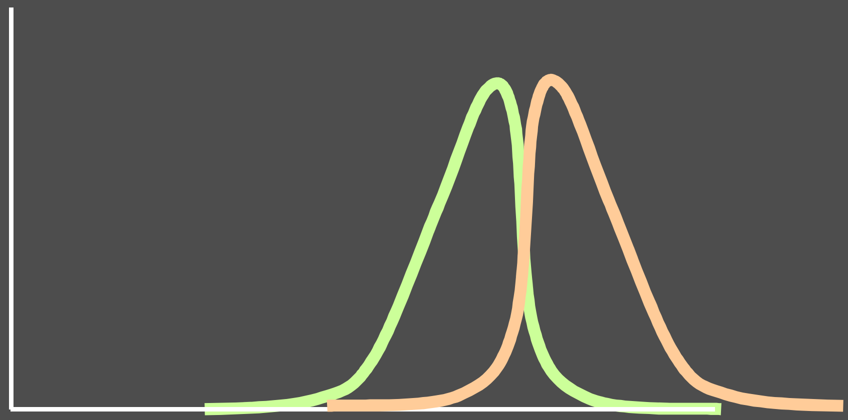
Spectral overlap

Distance

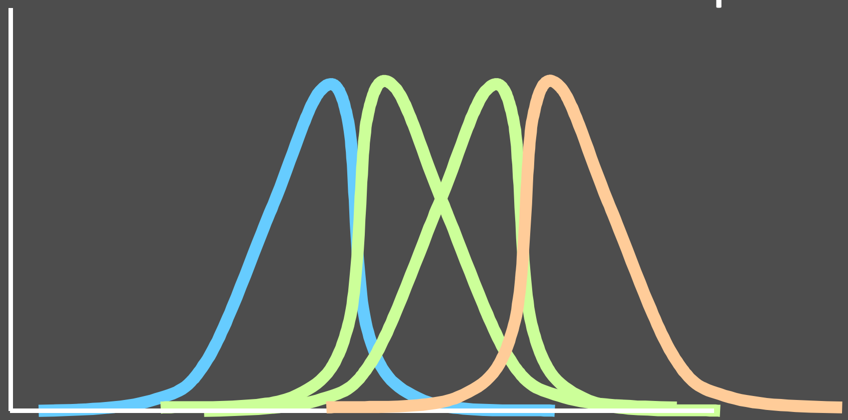
Alignment



donor



acceptor



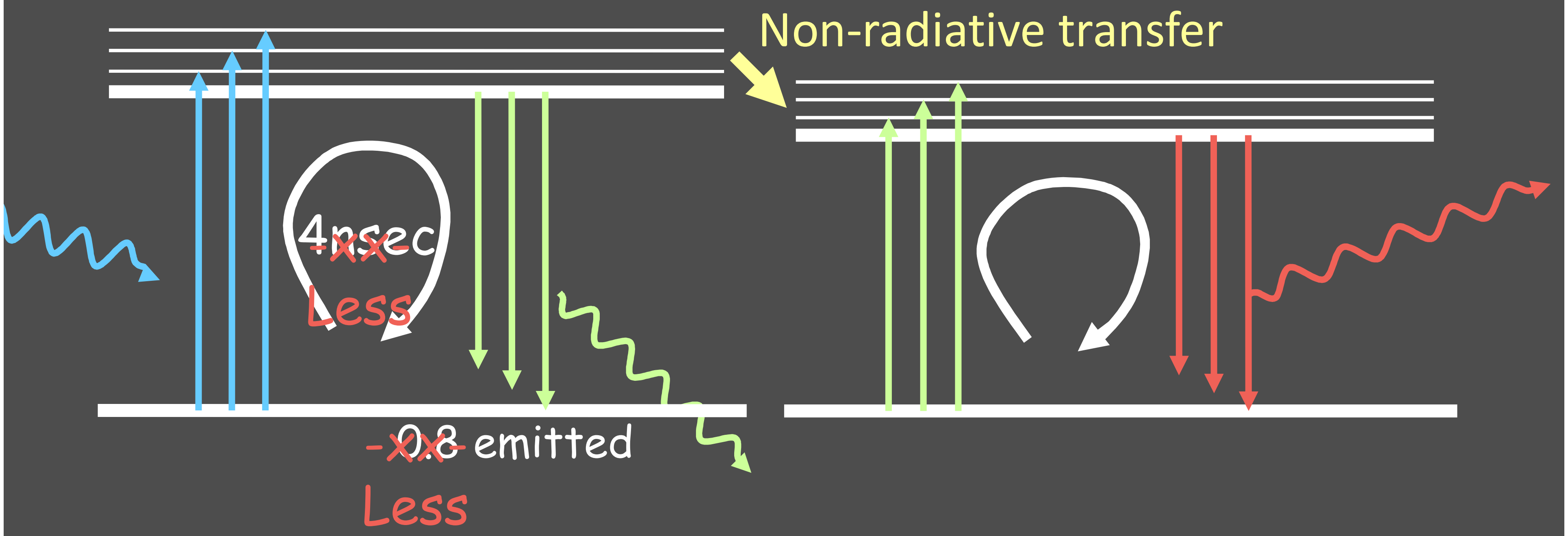
FRET:

Optimize spectral overlap

Optimize $\kappa^2$ -- alignment of dipoles

Minimize direct excitement of the acceptor
(extra challenge for filter design)

# FRET Diagram



The Förster Equations.

$$K_T = (1/\tau_D) \bullet [R_0/r]^6$$

$$E = \frac{1}{1 + (r/R_0)^6} = 1 - \frac{\tau'_D}{\tau_D}$$

$$R_0 = 2.11 \times 10^{-2} \bullet [\kappa^2 \bullet J(\lambda) \bullet \eta^{-4} \bullet Q_D]^{1/6}$$

$$J(\lambda) = \int f_D(\lambda)\, \varepsilon_A(\lambda)\, \lambda^4\, d\lambda$$

$$E = 1 - \frac{F'_D}{F_D}$$

r is the center-to-center distance (in cm) between the donor and acceptor
$\tau_D$ is the fluorescence lifetime of the donor in the absence of FRET
$\kappa^2$ is the dipole-dipole orientation factor,
$Q_D$ is the quantum yield of the donor in the absence of the acceptor
$\eta$ is the refractive index of the intervening medium,
$F_D$ ($\lambda$) is the fluorescence emission intensity at a given wavelength $\lambda$ (in cm)
$\varepsilon_A$ ($\lambda$) is the extinction coefficient of the acceptor (in $cm^{-1}$ $M^{-1}$).

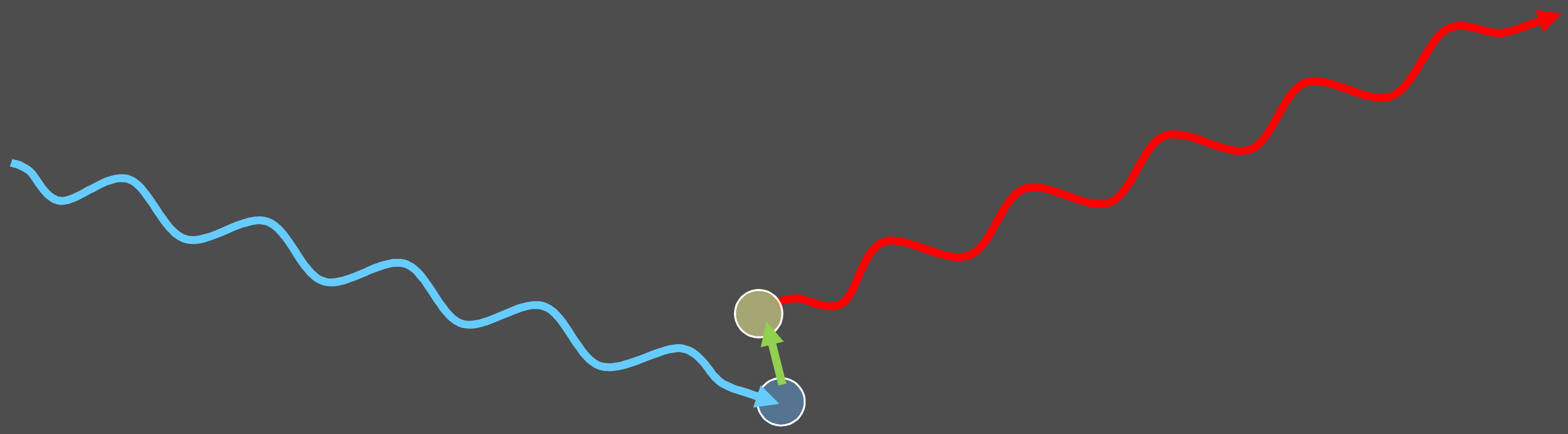
The orientation factor $\kappa^2$ can vary between 0 and 4, but
typically $\kappa^2$ = 2/3 for randomly oriented molecules (Stryer, 1978).

When r = $R_0$, the efficiency of FRET is 50%
(fluorescein-tetramethylrhodamine pair is 55 Å)

FRET:

Resonance Energy Transfer (non-radiative)

The Good:  FRET as a molecular yardstick



Transfer of energy from one dye to another

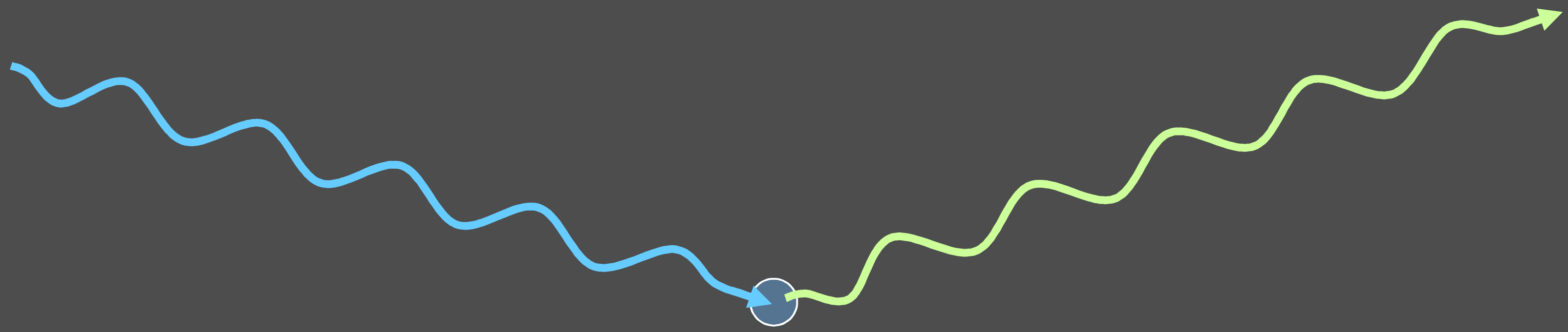
Depends on:

Spectral overlap

Distance

Alignment

# How dipole affects FRET as a molecular yardstick



Fluorescent Dye

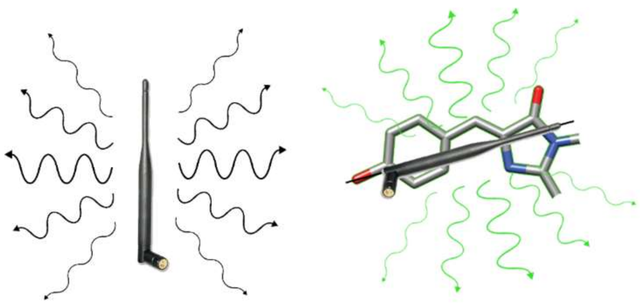
Dipole antenna

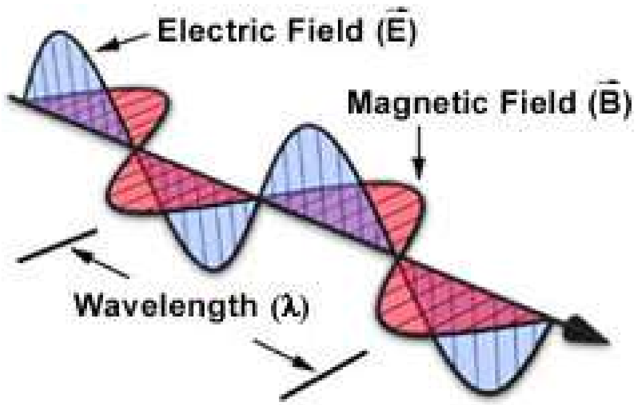
Delocalized electrons

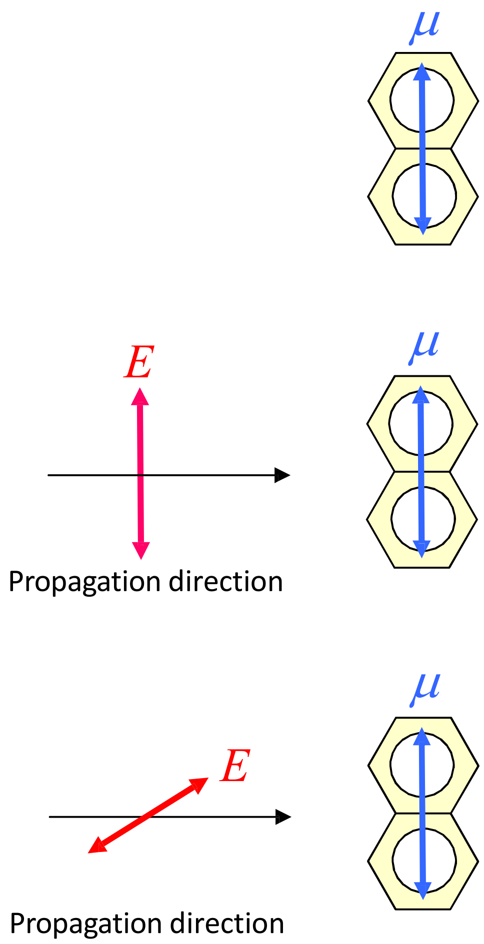
Longer dipole, longer λ

# Fluorescent dye as dipole antenna

- Absorption depends on orientation

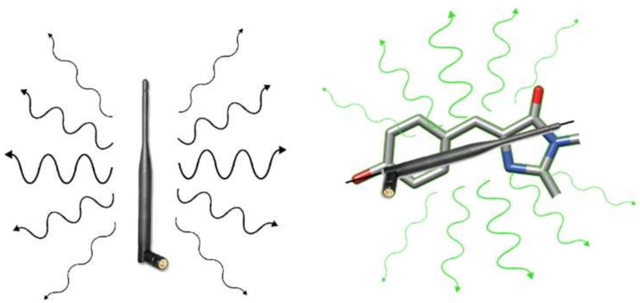


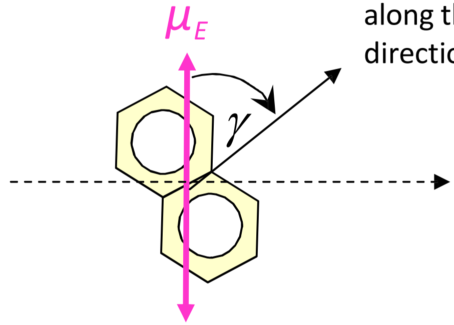


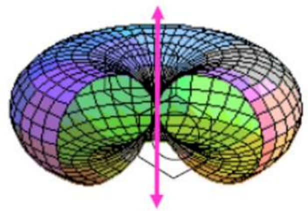
# Fluorescent dye as dipole antenna

- Orientation of fluorescence emission



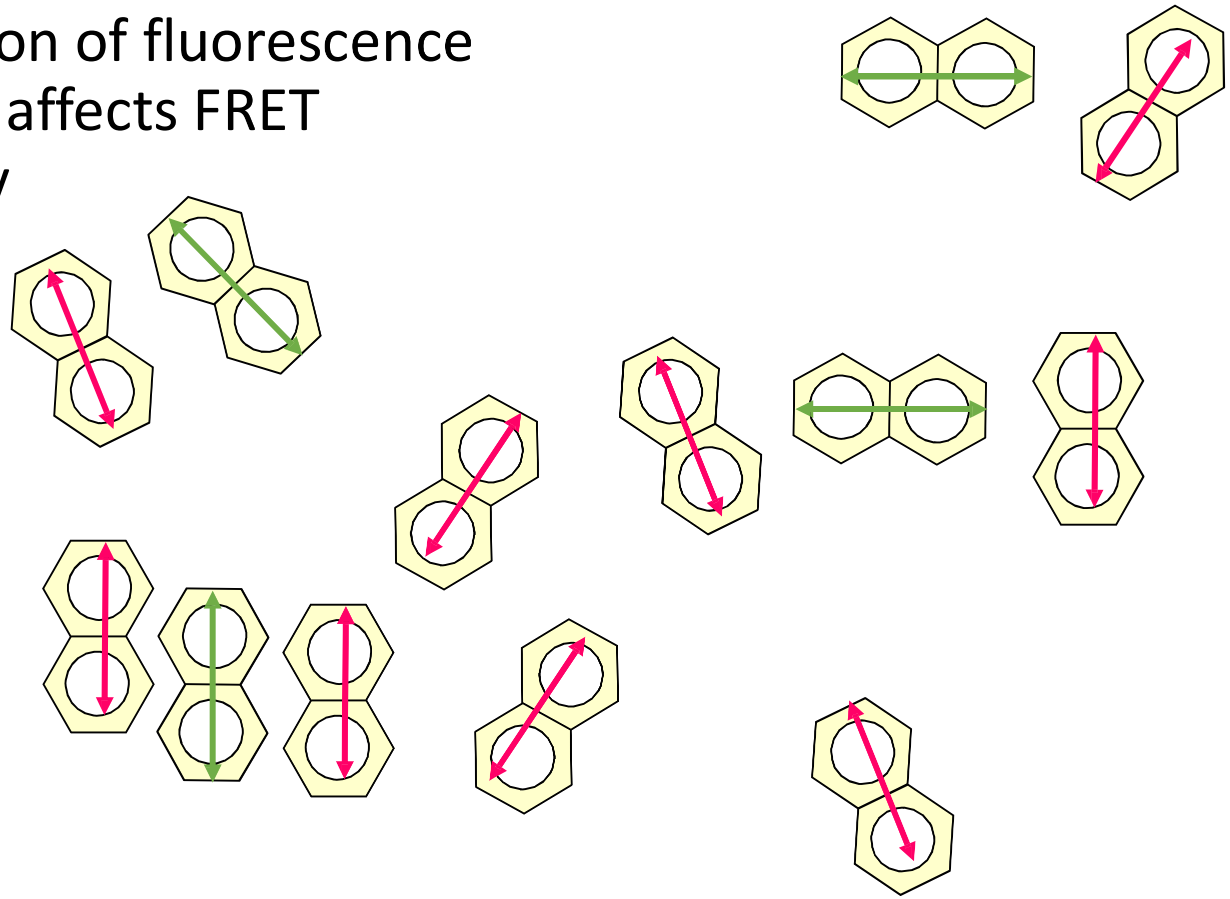



Dipole radiation pattern

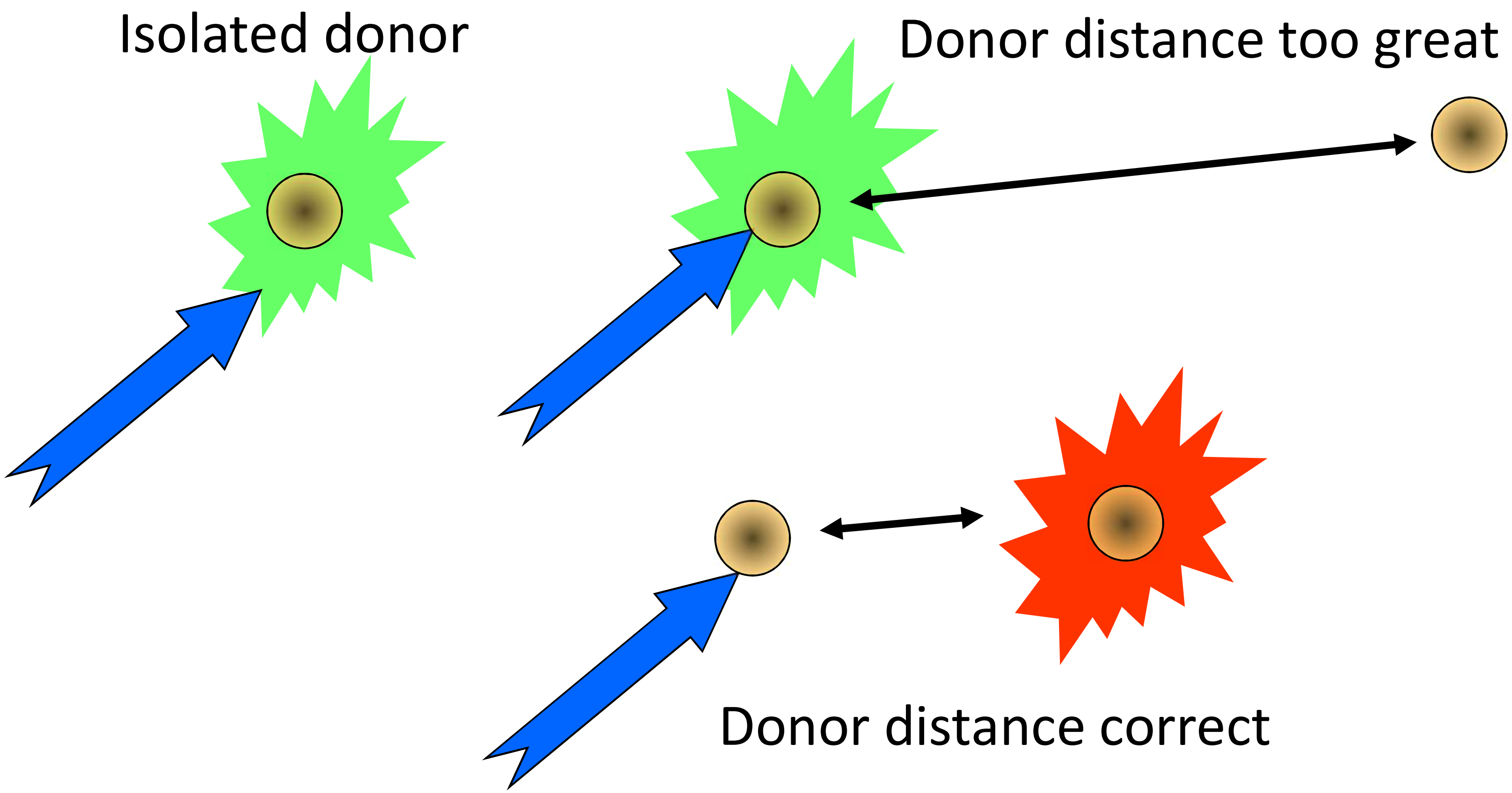


# Fluorescent dye as dipole antenna

- Orientation of fluorescence emission affects FRET efficiency



# More about FRET (Förster Resonance Energy Transfer)




**Effective between 10-100 Å only**

Emission and excitation spectrum must significantly overlap

Note: donor transfers non-radiatively to the acceptor

From J. Paul Robinson, Purdue University

# FRET efficiency and the Förster Equations

- Distance between donor and acceptor
- When $r = R_0$, the efficiency of FRET is 50%
- When $R < R_0$, $E_{FRET} > 0.50$
- When $R > R_0$, $E_{FRET} < 0.50$

$$K_T = (1/\tau_D) \bullet [R_0/r]^6$$

$$E = \frac{1}{1 + (r/R_0)^6} = 1 - \frac{\tau'_D}{\tau_D}$$

$$R_0 = 2.11 \times 10^{-2} \bullet [\kappa^2 \bullet J(\lambda) \bullet \eta^{-4} \bullet Q_D]^{1/6}$$

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