Define Quantum Yield

$$\phi_f = \frac{\#\text{ photons fluoresced}}{\#\text{ photons absorbed}} = \frac{\#\text{ decays via fluorescence}}{\text{total }\#\text{ of decays}}$$

$$= \frac{k_f[S_1]}{k_d[S_1]} = \frac{k_f}{k_d} = \frac{k_f}{k_f + k_t + k_p + k_Q[Q]}$$

$\therefore$ $$\tau = \text{fluoresc lifetime} = \frac{1}{k_d} = \frac{1}{k_f + k_t + k_p + k_Q[Q]}$$

$$\phi_f = \frac{\tau}{\tau_0}$$

12/5/01

Fluorescence Quenching

$$h\nu + M \longrightarrow M^* \quad \text{(excitation)}$$

$$M^* \xrightarrow{k_f} M + h\nu \quad \text{(fluorescence)}$$

OR

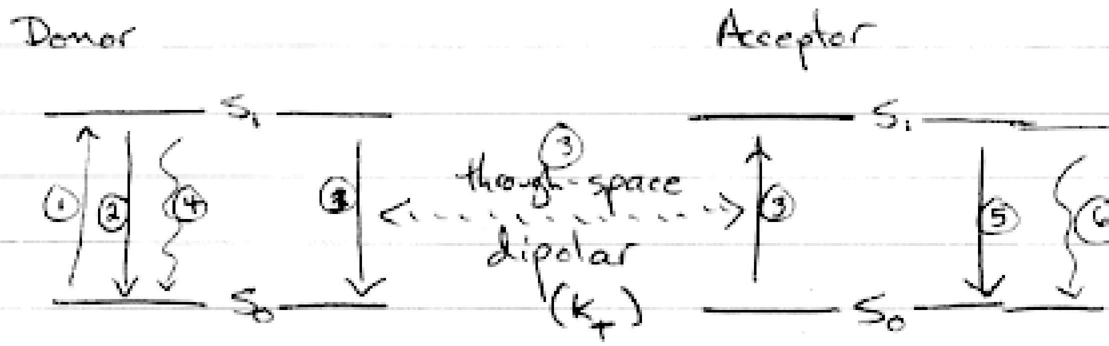
$$M^* + Q \xrightarrow{k_Q} M + Q^*$$

$$\phi_f^\circ \text{ (absence of quencher)} = \frac{k_f}{k_f + k_t + k_p}$$

$$\phi_f \text{ (w/ quencher)} = \frac{k_f}{k_f + k_t + k_p + k_Q Q}$$

$$\frac{\phi_f^\circ}{\phi_f} = \frac{k_f + k_t + k_p + k_Q Q}{k_f + k_t + k_p} = 1 + \frac{k_Q Q}{k_f + k_t + k_p}$$

Excitation Transfer



Equations

$D \xrightarrow{h\nu} D^*$ — ① Donor absorption

$D^* \xrightarrow{k_f} D + h\nu'$ — ② Donor fluorescence

OR

$D^* + A \xrightarrow{k_T} D + A^*$ — ③ Excitation Transfer

$D^* \rightarrow D$ — ④ All other deexcitation

$A^* \rightarrow A + h\nu''$ — ⑤ Fluorescence of Acceptor

$A^* \rightarrow A$ — ⑥ All other deexcitation

$$\text{Efficiency} = \frac{k_T}{k_T + k_d} = \frac{\text{deexcitation by xfer}}{\text{all donor deexcitation}}$$

$$\phi_D = \frac{k_f}{k_d} \quad \text{(Alone)}$$

$$\phi_{D+A} = \frac{k_f}{k_d + k_T} \quad \text{(Presence of Acceptor)}$$

$$E_{ff} = 1 - \frac{\phi_{D+A}}{\phi_D}$$

$$\text{Eff} = 1 - \frac{\tau_{D+A}}{\tau_D} = \frac{r_0^6}{r_0^6 + r^6}$$

↑ From Theory

$0 \le \text{Eff} \le 1$

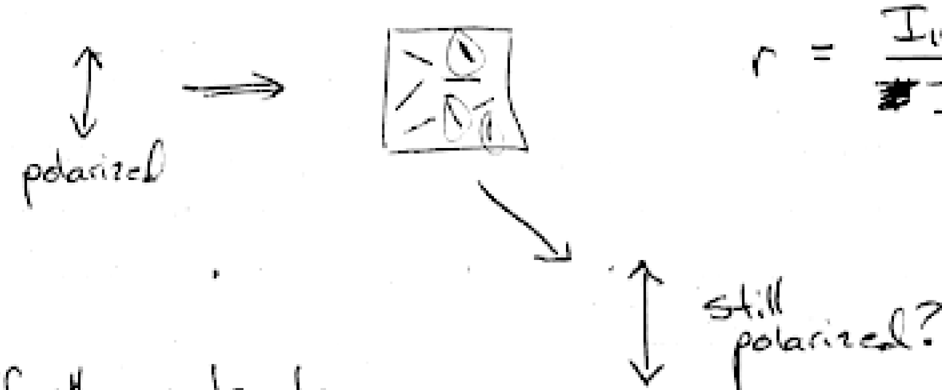
r_0 = Constant → characteristic of a particular donor-acceptor pair

r = distance between donor and acceptor

There is a theory for r_0, but often it's (r_0) determined empirically.

Molecular Rulers/Beacons

Fluorescence Polarization / Anisotropy



$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}}$$

Measure

Ⓐ Parallel ↕ $I_{\parallel}$

Ⓑ Perpendicular ↔ $I_{\perp}$

If the molecule tumbles during its lifetime, then polarization is scrambled

$I_{\parallel} = I_{\perp}$ $r = 0$

If molecule does not tumble within that time

$I_{\perp} = 0$ $r = 1$

Intermediate values tell you about intermediate rotation

Single Molecule Approaches (Fluorescence)

VERY useful and new.

Providing a lot of very useful information.

No longer restricted to "ensemble" measurements.