Lecture 15 2/20/09

A. Fluorescence Quenching

- The excited state singlet S_1 can decay by fluorescence (f), internal conversion (ic), and intersystem crossing (isc). The intensity S_1 versus time is given by a first order decay equation:

$$-\frac{d[S_1]}{dt} = \left(k_f + k_{ic} + k_{isc}\right)[S_1] = \frac{[S_1]}{\tau_0}$$

- τ_0 is called the fluorescence life time: $\dfrac{1}{\tau_0} = k_f + k_{ic} + k_{isc}$

- The excited singlet state S_1 can also be removed by collision with another molecule that we will simply call Q. The reaction is

$$S_1 + Q \xrightarrow{\ k_q\ } S_0 + Q + heat$$
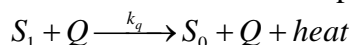
- This process is called quenching. If we incorporate quenching into the rate equation:

$$-\frac{d[S_1]}{dt} = \left(k_f + k_{ic} + k_{isc} + k_q[Q]\right)[S_1] = \frac{[S_1]}{\tau_f}$$

- Now the fluorescence life time τ has the form: $\dfrac{1}{\tau_f} = k_f + k_{ic} + k_{isc} + k_q[Q] = \dfrac{1}{\tau_0} + k_q[Q]$.

- The fluorescence lifetime has been shortened from τ_0 to τ_f by the addition of a quenching molecule Q.

- Assuming $k_f \gg k_{ic}, k_{isc}$ $\dfrac{1}{\tau_f} \approx k_f + k_q[Q]$. This equation implies that plotting the inverse of τ_f versus Q yields k_q and k_f.

- Fluorescence quenching experiments can give information on the accessibility of chromophores to the quencher Q. Therefore quenching is dependent on structure and is sensiitive to structural changes.

B. Quantum Yield & the Stern-Volmer Equation

- Quantum yield: An important parameter characterizing fluorescent transitions is quantum yield Q defined as $\phi_f = \dfrac{Number \ \ of \ \ Photons \ \ Emitted}{Number \ of \ \ Photons \ \ Absorbed} = \dfrac{N_{flr}}{N_{abs}}$

- ϕ_f varies from 1 (no radiationless pathway) to 0 (no pathway for emission).

- Quantum yield can be related to the kinetic parameters above as follows. S1 is produced by absorption and depleted by a combination of fluorescence, internal conversion, and inter-system crossing. Including the supply and removal of S1 in the rate equation we get

$$\left(\frac{d[S_1]}{dt}\right)_{Total} = k_{abs}[S_0] - k_f[S_1] - k_{ic}[S_1] - k_{isc}[S_1] - k_q[Q][S_1]$$

  where $k_{abs}[S_0]$ is the rate at which S_1 is produced by radiation absorption and $[S_1](k_{flr} + k_{ic} + k_{isc} + k_q[Q])$ is the rate at which S_1 disappears from fluorescence, ic, isc, and collisions with quenchers.

- If the system is continuously irradiated and the rate of S_1 formed by absorption is exactly equal to the rate of removal by any of the mechanisms we have discussed, then S_1 exists at a fixed, steady state level and the two rates are equal:

$$k_{abs}[S_0] = [S_1](k_f + k_{ic} + k_{isc} + k_q[Q])$$

- The quantum yield is

$$\phi_f = \frac{\text{fluorescence rate}}{\text{absorption rate}} = \frac{k_f[S_1]}{k_{abs}[S_0]} = \frac{k_f[S_1]}{(k_f + k_{ic} + k_{isc} + k_q[Q])[S_1]} = \frac{k_f}{(k_f + k_{ic} + k_{isc} + k_q[Q])}$$

- In the absence of quencher the quantum yield is

$$\phi_{f,0} = \frac{k_f}{(k_f + k_{ic} + k_{isc})}$$

- If we take the ratio of these two quantum yields we get

$$\frac{\phi_{f,0}}{\phi_f} = \frac{k_f + k_{ic} + k_{isc} + k_q[Q]}{k_f + k_{ic} + k_{isc}} = 1 + \frac{k_q[Q]}{k_f + k_{ic} + k_{isc}} \approx 1 + \frac{k_q[Q]}{k_f}$$

where the last step was obtained by assuming $k_f \gg k_{ic}, k_{isc}$.

- $\dfrac{\phi_{f,0}}{\phi_f} = 1 + \dfrac{k_q[Q]}{k_{flr}}$ is the Stern-Volmer equation. Quantum yield measurements

  obtain with and without Q, together with a measurement of the fluorescent life time $\dfrac{1}{\tau_f} \approx k_f + k_q[Q]$ is sufficient for measuring k_f and k_q.

C .Fluorescent Resonant Energy Transfers (FRET)

- In addition to fluorescence, quenching, ic, and isc, under certain conditions, S_1 can be removed by transferring absorbed photons to another nearby chromophore which subsequently fluoresces. This mechanism is called fluorescent resonance energy transfer or FRET.
- The mechanism for FRET is

$$D \xrightarrow{k_{abs}} D^*$$

$$D^* \xrightarrow{k_f} D$$

$$D^* \xrightarrow{k_d} D$$

$$D^* + A \xrightarrow{k_T} D + A^*$$

$$A^* \xrightarrow{k} A$$
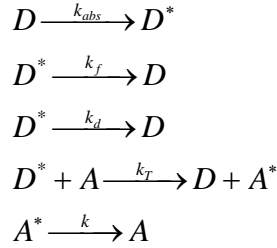
where k_d represents mechanisms for removing D* including ic, isc, etc.

- As before the quantum yield for D in the absence os A is

$$\phi_f = \frac{k_f}{k_f + k_d}$$

- In the presence of A the quantum yield of D is

$$\phi_{f/FRET} = \frac{k_f}{k_f + k_d + k_T}$$

- The efficiency of energy transfer is defined as

$$E_t = \frac{Number \quad of \quad Photons \quad Transferred \quad to \quad A}{Number \quad of \quad Photons \quad Absorbed \quad by \quad D} = \frac{\phi_f - \phi_{f/FRET}}{\phi_f} = 1 - \frac{\phi_{f/FRET}}{\phi_f}$$

- Because FRET involves the direct through-space transfer of photons between chromophores, the efficiency falls off rapidly with the distance r between the two chromophores A and D. In a theory due to T. Forster, the transfer efficiency is related to the inverse sixth power of r and a number of physical factors lumped into a constant R_0:

$$E_t = \frac{R_0^6}{R_0^6 + r^6}$$

- By measuring E_t, FRET, can be used to probe the distance r between the donor D and acceptor A, but this requires a knowledge of the constant R_0:
  - R_0 is the Forster distance, defined as the distance at which $E_t=0.5$. Typical values for R_0 are in the range 10-50Å.
  - The Forster distance is a rather complicated function of several quantities $R_0 = 9.79 \times 10^3 \left(J n^{-4} \kappa^2 \phi_f \right)^{1/6}$ where
    - n is the refractive index of the medium
    - κ^2 is a function of the mutual orientation of the transition dipole moments of the donor and acceptor chromophores. The parameter κ^2 can vary from 0 (dipoles perpendicular) to 4 (dipoles parallel).
    - As before ϕ_f is the quantum yield of the donor.
    - J is the spectral overlap integral which measures the degree of overlap between the fluorescence spectrum of the donor D and the absorption spectrum of the acceptor A.