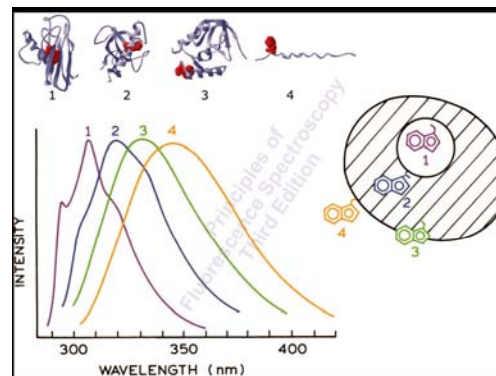


# Intramolecular interactions and Interaction with other molecules: cooperativity and binding

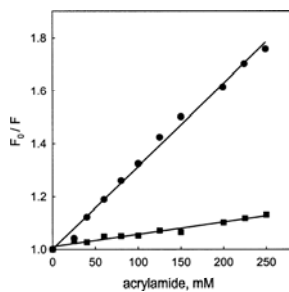
Overview of Fluorescence Spectroscopy. FRET

Principles of Fluorescence Spectroscopy 3<sup>rd</sup>. Ed. Lakowicz Ch 1  
Proteins. Creighton Ch. 4.4 , Ch. 8.2 to 8.2.2

## Effect of Trp environment on the emission spectra of proteins



## Collisional quenching measures solvent accessibility (for water-soluble quenchers)



slope =  $K_{sv}$

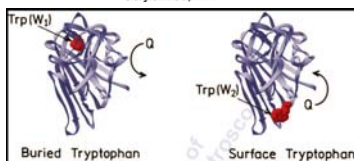
Stern-Volmer equation

$$F_0/F = 1 + K_{sv} [Q] = 1 + \tau_0 k_q [Q]$$

$$K_{sv} = \sum k_q$$

$\tau_0$  lifetime in the absence of Q  
If  $\tau_0$  is the same in both situations:

You can compare  $K_{sv}$ ,  
if not  
you must compare  $k_q$



## Fluorescence anisotropy measures mobility of the probe

$$r = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + 2I_{\perp})$$

$$r = 0.40$$

- in a totally rigid system, if the emission transition dipole is  $\parallel$  to the absorption emission dipole

$$r = -0.20$$

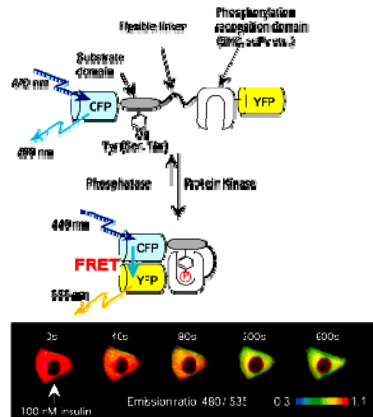
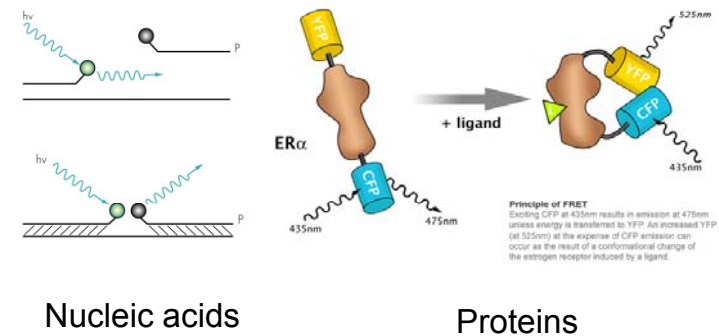
- in a totally rigid system, if the emission transition dipole is  $\perp$  to the absorption emission dipole

$$0.40 > r > -0.20$$

## Fluorescence anisotropy

- Provides information on
  - Size/shape of proteins
  - Rigidity of various molecular environment
  - Protein-small molecule association
  - Immunoassays
  - Fluidity in membranes
- If mobility increases:  $r \rightarrow 0$

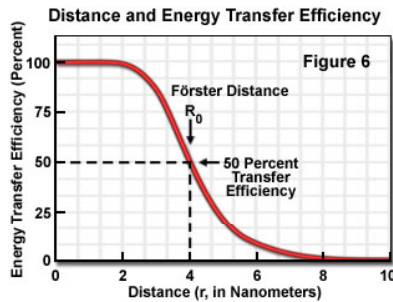
## FRET multiple applications



## Forster Resonance Energy Transfer

- FRET does not involve emission of light by the donor
  - Provides information about **proximity** between the donor and the acceptor dye
  - Useful range from  $\sim 10$  to  $100 \text{ \AA}$
  - Efficiency of FRET =  $1 - I_{DA}/I_D = 1 - \tau_{DA}/\tau_D$
  - $E = R_0^6 / (R_0^6 + r^6)$
- where  $r$  is the distance between D and A, and  $R_0$  is the characteristic transfer distance for the D:A pair

# FRET efficiency and distance

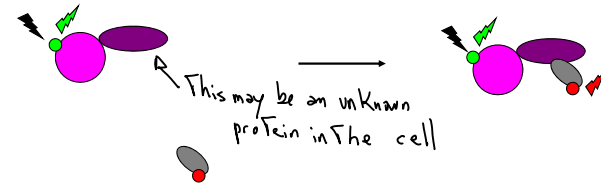
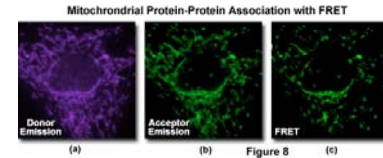


E  
 Less than  $0.5 R_0 = 100\%$   
 $R_0 = 50\%$   
 More than  $2 R_0 = 0\%$

Example  
 Trp and dansyl  $R_0 \approx 2 \text{ nm}$   $\rightarrow$  max distance  $2 \times 2 = 4 \text{ nm}$   
 to detect FRET

# FRET

- Proximity not always means "interaction"



# Intramolecular interactions

- Electrostatic
- Van der Waals
- Hydrogen bond

These interactions are weak in the presence of water

- Hydrophobic interactions are not very strong

How can they produce a stable folded conformation?

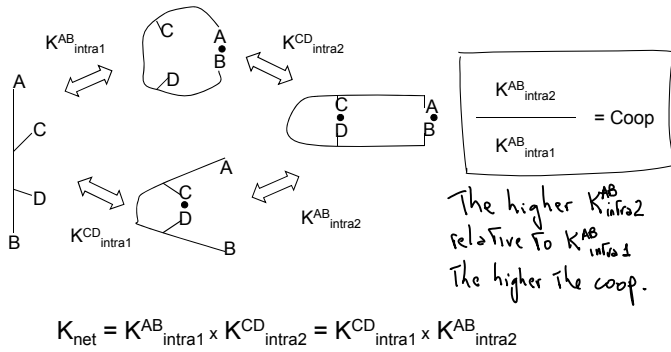
# Effective concentrations



For molecules to interact: they must lose entropy

$$\text{Effective concentration} = K_{\text{intra}} / K_{\text{inter}} = \frac{10^{-3}}{10^{-5}} = 100$$

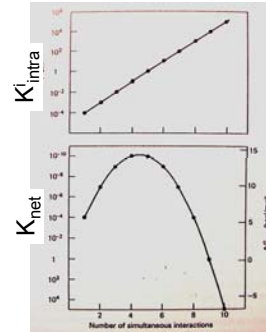
## Cooperativity of multiple interactions



## For multiple interacting groups in a polypeptide

- $K_{net} = K_{intra}^{AB} \times K_{intra}^{CD} \times K_{intra}^{EF} \times \dots$
- $K_{net}$  is independent of the reaction pathway

$K^{AB} = 10^{-4}$  1<sup>st</sup>  
 $K^{CD} = 10^{-3}$  2<sup>nd</sup>  
 $\vdots$  3<sup>rd</sup>  
 $\vdots$



$K_{net} = 10^{-4} \times 10^{-3} \times 10^{-2} \times 10^{-1} \times 10^0 \times 10^1 \times 10^2 \dots$