Intramolecular interactions and Interaction with other molecules: cooperativity and binding

Overview of Fluorescence Spectroscopy. FRET

Principles of Fluorescence Spectroscopy 3rd. 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**

slope = $K_{sv}$

Stern-Volmer equation

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

If $\tau_0$ is the same in both situations:

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

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Fluorescence anisotropy measures

**mobility** of the probe

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

- $r = 0.40$
  - 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

Nucleic acids

Proteins
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 ~10 to 100 Å
- Efficiency of FRET = $1 - \frac{I_{DA}}{I_D} = 1 - \frac{\tau_{DA}}{\tau_D}$
- $E = \frac{R_0^6}{(R_0^6 + r^6)}$
  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

Less than 0.5 $R_0 = 100\%$

$R_0 = 50\%$

More than 2 $R_0 = 0\%$

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

\[ A + B \leftrightarrow A\cdot B \quad K_{\text{inter}} \]

\[ A - B \leftrightarrow A\cdot B \quad K_{\text{intra}} \]

For molecules to interact: they must lose entropy

Effective concentration = \( \frac{K_{\text{intra}}}{K_{\text{inter}}} \)
Cooperativity of multiple interactions

For multiple interacting groups in a polypeptide

- \( K_{\text{net}} = K_{\text{intra}}^{AB} \times K_{\text{intra}}^{CD} \times K_{\text{intra}}^{EF} \times \cdots \)

- \( K_{\text{net}} \) is independent of the reaction pathway
Energetics and Dynamics of Binding

• Protein-ligand interaction: affinity

• Whether or not any particular affinity of a protein for a ligand is significant depends on the concentration of the ligand that the protein is likely to encounter

\[ P \cdot L \rightleftharpoons P + L \quad K_d = \frac{[P][L]}{[P \cdot L]} \]

Binding affinities

\[ K_d = 0.1 \, \mu M \]

[L] = Kd \rightarrow 50 \% protein molecules have bound ligand
[L] = 9 \times Kd \rightarrow 90 \%
[L] = 99 \times Kd \rightarrow 99 \%
A general consequence of ligand binding is that the protein is stabilized against unfolding

\[
N \cdot L \xrightarrow{\frac{L}{K_d}} N \xleftarrow{K_u} U
\]

\[
K_{app} = \frac{[N \cdot L] + [N]}{[U]} = K_u \left(1 + \frac{[L]}{K_d}\right)
\]

Binding of two different ligands

\[
P \cdot A \xleftrightarrow{K_d} P \xleftrightarrow{K_d} P \cdot B
\]

\[
[P] = \frac{[P \cdot A]K_d}{[A]} = \frac{[P \cdot B]K_d}{[B]}
\]

\[
\frac{[P \cdot A]}{[P \cdot B]} = \frac{K_d[A]}{K_d[B]}
\]