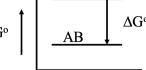


1 Molecular interactions

$$A + B \xrightleftharpoons[k_1]{k_2} AB, K = \frac{[AB]}{[A][B]} = \frac{k_2}{k_1}$$

$$\Delta G^\circ = -RT \ln K$$

The AB complex is stable relative to A + B, k_1 , the on-rate constant: $A + B \xrightarrow{k_1} AB$ (on-rate: k_1)



The rate of complex formation (or any bimolecular reaction) is limited by the diffusional limit of the rate of collisions

1.1 Translation diffusion

- Fick's first law: concentration gradient across the reference plane $J = -D \left(\frac{dn}{dx} \right)$ = moles (net) area \cdot time \cdot D: diffusion coefficient, cm^2/s
- (PS: Use J at boundary conditions to solve for 2nd law)
- Fick's second law: $\left(\frac{dn}{dt} \right)_t = -\frac{dJ}{dx} = D \left(\frac{\partial^2 n}{\partial x^2} \right)$

change in # particles per unit volume per unit time

- Constant gradient: same amount leaves as enters the box = concentration is constant
- Gradient higher on left: more enters the box than leaves = concentration changes

Example: one-dimensional diffusion in an infinite pipe (initial cond. $n_0 = \frac{N}{V}$)

$$\frac{dn(x,t)}{dt} = D \left(\frac{d^2 n(x,t)}{dx^2} \right)$$

Diffusion only in the x direction: $n(x,t)$ = number of particles per unit area between x and $x+dx$ at time t

$$\text{SOLUTION: } n(x,t) = \frac{n_0}{\sqrt{4\pi Dt}} e^{-x^2/4Dt}$$

Particles per unit area, where $n_0 = \frac{N}{V}$

$$n(x,t) = \frac{1}{\sqrt{4\pi Dt}} e^{-x^2/4Dt} = p(x,t)$$

Units: fraction of molecules per unit distance, cm⁻¹

$$\langle x^2 \rangle = \int_{-\infty}^{+\infty} x^2 p(x,t) dx = \int_{-\infty}^{+\infty} x^2 \frac{1}{\sqrt{4\pi Dt}} e^{-x^2/4Dt} dx = 2Dt$$

3D diffusion: l is proportional to \sqrt{t}

$$D_s = D_r = D_t, \text{ isotropic diffusion}$$

$$\langle x^2 \rangle = 2Dt$$

$$\langle y^2 \rangle = 2Dt$$

$$\langle z^2 \rangle = 2Dt$$

$$\langle x^2 \rangle + \langle y^2 \rangle + \langle z^2 \rangle = \langle l^2 \rangle \quad \sqrt{\langle l^2 \rangle} = \langle l \rangle = \sqrt{6Dt}$$

$$\langle l^2 \rangle = 6Dt \quad \langle l \rangle = \sqrt{6D}\sqrt{t}$$

$$D = \frac{\langle l^2 \rangle}{6t} \quad \text{Units of the diffusion coefficient}$$

$$\text{cm}^2/\text{s}$$

2 Diffusion

1. 1 dimension: protein along DNA or RNA

2. 2 dimensions: lipid or protein in a membrane bilayer

3. 3 dimensions: molecules in the cytoplasm

a small molecule in solution: $D = 10^{-5} \text{ cm}^2/\text{sec}$, 3 days to travel 4 cm

for a protein $10^{-6} - 10^{-7} \text{ cm}^2/\text{sec}$

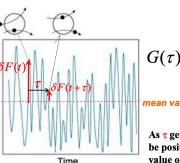
Measuring D of molecules in solution

1. Methods that depend on measuring local fluctuations in concentration which depends on the same driving forces that determine the diffusion coefficient

• E.g. Fluorescence correlation spectroscopy FCS (single molecule): Watch fluorescence from single molecules (by selecting low concentration) as they pass through a very small excitation volume (10 - 15 liter)

2. Fluorescence recovery after photobleaching (for membrane proteins)

3. Other single-molecule methods



$$\text{autocorrelation function: } G(\tau) = \frac{\langle \delta F(t) \cdot \delta F(t+\tau) \rangle}{\langle F(t)^2 \rangle}$$

- Rate of binding or association to form the PL complex: $r = k_1 [P] [L]$
- k_1 has units of s^{-1} ; Second order rate constant

Reverse reaction (dissociation):

- Rate of disappearance of the PL complex due to dissociation: $r = k_{-1} [PL]$

k_{-1} has units of s^{-1} ; First order rate constant

As τ gets larger, $\delta F(t+\tau)$ is equally likely to be positive or negative. Hence the average value of $\delta F(t) \cdot \delta F(t+\tau)$ will go to zero.

Take the average for all values of τ as a function of τ :

$G(\tau)$ decays rapidly as a function of time τ for a rapidly diffusing small molecule (short memory)

$G(\tau)$ decays slowly as a function of time τ for a slowly diffusing small molecule bound to a large protein (long memory)

$$k_{obs} = k_1 [L] \quad \text{measured}$$

$$\text{and } [PL] = [P][L] - [PL]k_{-1}$$

$$\frac{d[PL]}{dt} = [P][L]k_1 - [PL]k_{-1} = \frac{d[PL]}{dt}$$

$$[PL]_t = [P]_0 [L] - \frac{k_{-1}}{k_1 + k_{-1}} [P]_0 [L]$$

$$= \frac{k_1}{k_1 + k_{-1}} [P]_0 [L]$$

$$\text{where } k_{obs} = k_1 [L] + k_{-1}$$

$$= k_{obs} = k_1 [L] + k_{-1}$$

steepness is a measure of cooperativity, and is limited by N

2. Heterotropic cooperativity

ligand binding influences the subsequent binding of a ligand of a different type (e.g., substrate binding and allosteric effector)

1. Binding sites for different ligands

2. Occupancy of one site alters the binding of the second ligand

3. The coupling free energy must be the same for each ligand

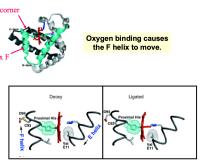
$$\Delta G_B^o(A+B) - \Delta G_B^o = \Delta G_A^o(A+B) - \Delta G_A^o \approx \Delta G_{\text{coupling}}^o$$

Same coupling free energy for A→B and B→A

$$\Delta G_A^o + \Delta G_{(A+B)}^o = \Delta G_B^o + \Delta G_{(A+B)}^o$$

$$\Delta G_{\text{coupling}}^o = RT \ln \frac{K_A}{K_1} = RT \ln \frac{K_B}{K_2}$$

$$RT \ln \alpha = \Delta G_{\text{coupling}}^o$$



Competing "classical" models of allostery

- Sequential model: focuses on tertiary changes at one active site induced by the binding of a ligand at another binding site (KNF model) – induced fit

PRINCIPLE OF RECIPROCITY

$$\Delta G_{(B+A)}^o = RT \ln K_A$$

$$= RT \ln (K_1) + RT \ln (\alpha)$$

$$= RT \ln (K_1 \alpha)$$

$$= RT \ln K_B$$

$$= RT \ln (K_2) + RT \ln (\alpha)$$

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