MB&B 302: Principles of Biophysics

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Module I: Physical Principles and Biological Macromolecules

Lecture 1: Review of Classical and Statistical Physics

Review of physical forces

1. Force is transmitted through direct contact or a field.

Force	Expression	Magnitude (pN)
Elastic	$-kx$	$1-100$
Viscous	$-\gamma v$	$1-1000$
Thermal (Collisional)	$m\frac{dv}{dt}$	$100-1000$
Gravitational	mg	$< 10^{-9}$
"Centrifugal"	$m\omega^2 r$	$< 10^{-3}$
Magnetic	$qv \times B$	$\ll 10^{-6}$
Electrostatic	zE	$1-1000$

Note: $\gamma = 6\pi\eta r$ is the drag coefficient.

2. **Reynold's number (R_e)** characterizes the relative importance of friction and inertia.

$$R_e = \frac{\text{Inertial "Force"}}{\text{Viscous Force}} = \frac{(\text{velocity})(\text{particle size})(\text{fluid density})}{\text{fluid viscosity}} = \frac{vL\rho}{\eta}$$

   a. Inertial term scales with mass; viscous term scales with drag coefficient and velocity.
   b. R_e tells you what opposes acceleration:
      - Small R_e (e.g., most biological bodies) means drag dominates and systems are overdamped.
      - Large R_e (e.g., ocean liner) means mass dominates.

Review of thermodynamics

1. **Ideal Gas Law**: $PV = nRT = k_BT$
2. Both PV and k_BT are expressions of energy (units in Joules).
3. The energy of each molecule is $\frac{3}{2}k_BT$.
   - Multiply by 3 to account for x, y, and z axes.
   - Divide by 2 because pressure measurements double-count from $\Delta p = 2mv_x$.
4. **Equipartition Theorem**: The mean energy associated with each degree of freedom is $\frac{1}{2}k_BT$ (for a monatomic gas).
   - 3 dfs associated with translation
   - 3 dfs associated with rotation
5. **Background thermal energy** is $k_BT = \frac{RT}{N_A} \approx 0.6$ kcal/mol
   - 1 $k_BT \Rightarrow$ transient conformational changes.
   - 1 ATP = 20 $k_BT \Rightarrow$ stable confirmational changes.
   - 1 Glucose = 36 ATP = 720 $k_BT \Rightarrow$ support multiple enzymatic processes.

Lecture 2: Diffusion, Random Walks, and Brownian Motion

Relevance of thermodynamics

1. Background thermal noise provides kinetic energy to particles around and overcome activation barriers.
   - The unit of thermal energy is k_BT
   - This is the minimum stable energy for resisting thermal motion (increases binding affinity by 3x).
2. Molecular collisions result in random Brownian motion.
3. Random diffusive walks yield net displacement without a net change in mean position.

RMS velocity

1. Definition (for a collection of N particles):

$$v_{rms} = \sqrt{\frac{1}{N}\sum_{i=1}^{N} v_i^2}$$

2. Derivation
   - Set average kinetic energy equal to average thermal energy and solve:

$$KE = \frac{1}{N}\sum_{i=1}^{N}\left(\frac{1}{2}mv_i^2\right) = \frac{1}{2}m\left(v_{rms}^2\right) = \frac{3}{2}k_BT$$

$$v_{rms} = \sqrt{\frac{3k_BT}{m}} = \sqrt{\frac{3RT}{N_Am}} = \sqrt{\frac{3RT}{M}}$$

3. Examples:
   - For a lysozyme (M.W. = 14 kg/mol), v_{rms} = 23 m/s = 50 mph.
   - For an H_2O molecule (M.W. = 0.018 kg/mol), v_{rms} = 640 m/s = 1400 mph.

Aqueous collisions

Compute the density of water from 55 M:

$$\frac{55N_A}{1000\,\text{cm}^3} = \frac{55(6.022\times10^{23})\,\text{molecules}}{10^{24}\,\text{nm}^3} = 33.1\frac{\text{molecules}}{\text{nm}^3}$$

1. The average volume occupied (V_{H_2O}) = 1/33.1 = 0.03 nm^3. 2. The mean distance between the centers of water molecules (r_{H_2O}) = $\sqrt[3]{0.03}$ = 0.32 nm.

Random walks in 1D: theory

1. We can model the random walk as a discrete Markov chain:
   - State space: $\{\ldots, -3\delta, -2\delta, -\delta, \quad 0, +\delta, +2\delta, +3\delta, \ldots\}$
   - Transition probabilities: $P_{left} = P_{right} = 0.5$
2. In other words, $X_n = X_{n-1} + \delta I_\delta$
   - X_t = displacement at time t
   - I_δ = right movement (1) or left movement (-1)
   - $P(I_\delta = 1) = P(I_\delta = -1) = 0.5$
   - $E[I_\delta] = 0$

Result 1: Mean displacement: $E[X_n] = 0$

$$\begin{aligned} E[X_n] &= E[X_{n-1}] + \delta E[I_\delta] \\ &= E[X_{n-1}] + 0 \\ &= E[X_0] \ \text{(by recursion)} \\ E[X_n] &= 0 \ \text{(starting position = 0)} \end{aligned}$$

Result 2: Mean square displacement (MSD): $E[X_n^2] \propto n$

$$\begin{aligned} E[X_n^2] &= E[(X_{n-1} + \delta I_\delta)^2] \\ &= E[X_{n-1}^2 + 2X_{n-1}\delta I_\delta + \delta^2] \\ &= E[X_{n-1}^2] + 2X_{n-1}\delta E[I_\delta] + E[\delta^2] \\ &= E[X_{n-1}^2] + \delta^2 \ \text{(middle term = 0)} \\ E[X_n^2] &= n\delta^2 \ \text{(by recursion)} \end{aligned}$$

Result 3: Proportionality to time

$$\begin{aligned} t &= n\tau \\ E[X_t^2] &= \left(\frac{t}{\tau}\right)\delta^2 \\ E[X_t^2] &\propto t \\ RMS(X_t) &\propto \sqrt{t} \end{aligned}$$

Result 4: Simplification by diffusion constant (D)

$$\begin{aligned} D &= \frac{\delta^2}{2\tau} \\ E[X_t^2] &= 2Dt \\ RMS(X_t) &= \sqrt{2Dt} \end{aligned}$$

Result 5: Diffusion is driven by increasing $Var[X_t]$

- The variance of particle displacements increases linearly with time.
- This means that any initial distribution of particle positions will spread out over time.
- Proof: variance is the centered 2nd moment, so $Var[X_t] = E[X_t^2] - E[X_t]^2 = MSD \propto t$.

Random walks in N dimensions

If the particle moves independently in n dimensions, we have:

$$\begin{aligned} d_n^2 &= X_1^2 + X_2^2 + \cdots + X_n^2 \\ E[d_n^2] &= E[X_1^2] + E[X_2^2] + \cdots + E[X_n^2] \\ &= \sum_{i=1}^{n} 2Dt = 2nDt \end{aligned}$$

- $RMS(d_1) = \sqrt{2Dt}$
- $RMS(d_2) = \sqrt{4Dt}$
- $RMS(d_3) = \sqrt{6Dt}$

  ...
- $RMS(d_n) = \sqrt{2nDt}$

First passage time: the time it takes to diffuse over a given distance (solve for t in the above equations).

Diffusion constant and particle radius

The **Einstein relation** (for spherical particle):

$$D = \frac{k_B T}{\gamma}$$

For translational diffusion:

- $\gamma = 6\pi\eta r$
- D is inversely proportional to size/radius

For rotational diffusion:

- $\gamma_\theta = 8\pi\eta r^3$
- D is inversely proportional to size/radius CUBED

Time dependence

1. It takes 100x longer to diffuse 10x as far (in any number of dimensions).
2. Large cells adapted in two ways:
   a. Active transport
   b. Confinement
      - Constrains diffusive motion to "corrals"
      - Ex: vesicles and organelles
      - Confinement radius $\approx RMS = \sqrt{MSD}_{max}$.
3. Comparing diffusion and active transport
   a. Diffusion:
      - $RMS \propto \sqrt{t}$
      - $MSD \propto t$
   b. Active transport:
      - Velocity is constant
      - $RMS \propto t$
      - $MSD \propto t^2$

Lecture 3: Chemical Equilibria, Binding, and Kinetics

Review of equilibrium concepts

- **Ligand binding** is bimolecular: $M + L \rightleftharpoons ML$
- **Conformation changes** are unimolecular: $M \overset{K}{\rightleftharpoons} M'$
- **Structural state**: time-averaged conformational states about a stable eneregy minimum.
- **Equilibrium constant** measures interaction strength and stability:

$$K_a = \frac{[ML]}{[M][L]} \quad \rightarrow \quad K_d = \frac{[M][L]}{[ML]}$$

- **Equilibrium** is when chemical potentials are equal. **Chemical potential**:

$$\mu_i = \frac{\partial G}{\partial N_i}$$

- At constant temperature and pressure, we can use **molar free energy** instead of chemical potential:

$$G_{eq} = G_{M',eq} - G_{M,eq} = 0$$

Boltzmann's law

- **Boltzmann's Law** defines the probability of occupying any state (i) with energy U as:

$$p_i = \frac{1}{Z} \exp\left[\frac{-U_i}{k_B T}\right]$$

- Z is a normalizing constant called the "**partition function**," i.e., the sum of all probability mass contributions.

$$Z = \sum_i \exp\left[\frac{-U_i}{k_B T}\right]$$

- The probability of populating the less favorable energy state $U_2 > U_1$ can be predicted from ΔU:

$$\frac{p_2}{p_1} = \frac{[M']}{[M]} = K_a = \exp\left[\frac{-\Delta U}{k_B T}\right] = \exp\left[\frac{-\Delta G^o}{k_B T}\right]$$

- Smaller ΔU leads to larger fractions occupying the higher state (between 0-50%).
- Rotational $\Delta U <$ Vibrational $\Delta U <$ Chemical ΔU

4. Adding energy (e.g., work) can shift the equilibrium distribution of states:

$$K'_{eq} = \exp\left[\frac{-\Delta G^o + F\Delta x}{k_B T}\right] = K_0 \exp\left[\frac{F\Delta x}{k_B T}\right]$$

Module II: Experimental Measurement of Molecular Interactions

Lecture 4: Equilibrium-Binding (Single Site)

Why study equilibrium binding?

1. Nearly all biological reactions are controlled by ML binding.
   - Exceptions: unimolecular isomerizations.
2. Equilibrium-binding experiments provide a predictive and mechanistic framework for kinetics:
   a. **Stoichiometry (n)**
      - ligands per macromolecule.
      - overlapping sites and competition.
   b. **Binding affinities (K)**
      - *If* and the *extent* to which reactions occur.
      - Derive thermodynamic constants (ΔG^o, ΔH^o, ΔS^o, ΔC_p^o).
   c. **Cooperative interactions and linkage**
      - Chemical linkage: does binding of one ligand affect binding of another?
      - Activator, regulators, and modulators.

Binding Terminology

- **Binding density** (v): how many L are bound per M.

$$v = \frac{[\text{Bound Ligand}]}{[\text{Total Macromolecule}]} = \frac{[ML]}{[M] + [ML]}$$

- **Fractional Saturation** (θ): what fraction of the total M is bound with L.

$$\theta = \frac{[\text{Bound Macromolecule}]}{[\text{Total Macromolecule}]} = \frac{[ML]}{[M] + [ML]}$$

Note: $v = \theta \cdot n$; stoichiometry (n) is how many L binds to each M.

Logistics of a binding experiment

1. Approach: measure $\langle v \rangle$ as a function of $[L]$ (sample MUST be at equilibrium)
2. Measurement methods:
   a. Direct: physical separation
      - Mass (cosedimentation, filter binding, pull-down)
      - Migration (gel shift, chromatography)
      - Problem: separation removes substrates from true equilibrium
   b. Indirect: side-effects
      - Spectroscopy (fluorescence, absorbance, circular dichriosm): ideal when possible
      - Enzymatic activity (product formation, inhibition)
      - Problem: signal must precisely correspond to concentration.
      - Advantage: More sensitive (needs less material, even < 1 nM).
3. Method selection is difficult and non-standardized; depends on convenience and substrate properties.

Equilibrium-binding assays

1. Physical separation
   a. Gel shift assay
      - Bound proteins (ML) travel slower than free proteins (M)
      - Measure $[ML]_{eq}/[M]_{eq}$ for each given $[L]_0$ (per lane)
   b. Filter binding assay
      - Separates proteins and DNA/RNA
      - Double filter:
        – Nitrocellulose(-) binds proteins (ML + M)
        – DEAE(+) binds DNA/RNA (L)
   c. Pull-down and cosedimentation assays
      - Pellet contains (Bead)-M and (Bead)-ML. Supernatant contains L.
      - Everything remains in equilibrium!
      - Measure $[L]_{eq}$ from the supernatant.
      - Compute $[ML]_{eq}$ by subtraction and K_d by plotting ν vs. [(Bead)-M].

2. Indirect methods
   a. Equilibrium dialysis
      - Add M || L. Measure $[L]_{eq}$ on the right.
      - Compute $[ML]_{eq}$ from $[L]_{eq} = ([L]_0 - [ML])/2$.
      - Compute $[M]_{eq}$ by subtraction.
      - Disadvantages: slow, requires size difference, must label ligand.
   b. Spectroscopy (chromo/fluorophores)
      - May be intrinsic (usu. Trp) or conjugated.
      - Fluorescence intensity: bound form usually fluoresces more.
      - Fluorescence anisotropy: measures rotational diffusion.
        – Bound ML = less rotational diffusion = greater anisotropy.
      - Measures $[ML]_{eq}$ as a ratio of either $[M]_{eq}$ or $[L]_{eq}$ (whichever one is fluorescing).

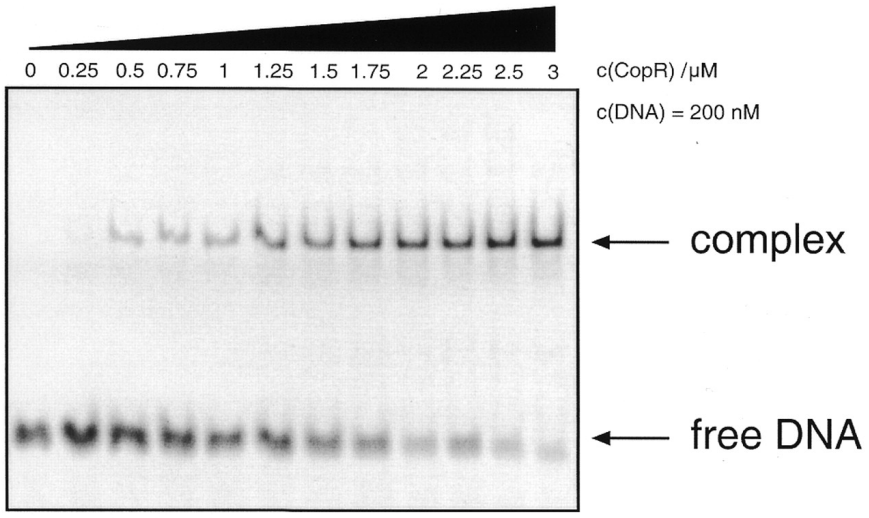
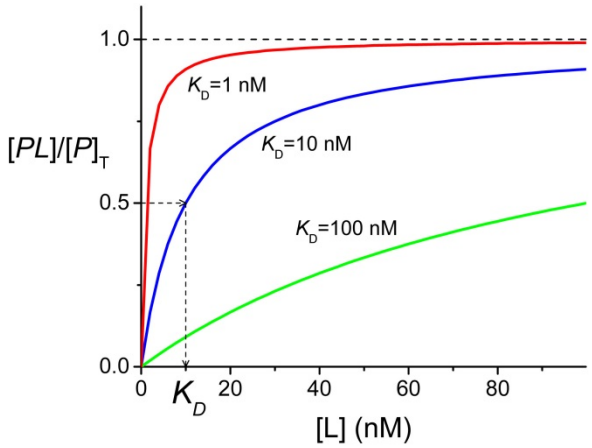


Figure 1: Gel Shift Assay

Analysis of binding data

1. Objective: measure binding density ν and its dependence on $[L]$. If the stoichiometry is 1:1, we can derive a rectangular hyperbola relationship for single-site binding:

$$\langle \nu \rangle = \frac{[ML]}{[M]+[ML]} = \frac{[L]}{\frac{[M][L]}{[ML]}+[L]} = \frac{[L]}{K_d+[L]}$$



2. Assumptions
   a. $[L]_0 \approx [L]_{free}$
      - We typically plot $[L]_0$ when $[L] \gg [M]$, and $[ML]$ accounts for little of $[L]_{tot}$.
   b. $[M] \ll K_d$
      - Otherwise, $[ML]$ and ν will just increase linearly with more $[L]$ until saturation at $[L]/[M] =$ stoichiometry(n).
      - This can be used to determine stoichiometry!

Predicting concentrations

1. Use $[ML] = K_a[M][L]$
   a. You need both concentrations and affinity.
   b. To find what fraction is bound, compute $\nu = [ML]/[M]_{tot} \approx K_a[L]$.
   c. Rules of Thumb:
      - 1% bound when $[L] = K_d/100$
      - 10% bound when $[L] = K_d/10$
      - 50% bound when $[L] = K_d$
      - 90% bound when $[L] = 10K_d$
      - 99% bound when $[L] = 100K_d$
2. Competition and Partitioning
   - Partitioning is determined by relative concentrations and affinities of L_A and L_B.

$$\frac{[ML_A]}{[ML_B]} = \frac{K_A[H_A]}{K_B[H_B]}$$

Binding affinity and interaction energies

1. Relating equilibrium constants to free energy changes:

$$\Delta G^o = -RT \ln K_a = RT \ln K_d = \Delta H^o - T\Delta S^o$$

2. ΔH^o is measured using an isothermal titration calorimeter (ITC):
   a. Add L to M and heat is absorbed or released.
   b. Measure the power required to maintain equal temperatures.
   c. Integrate to find total heat released per injection (spike).
      - *Note: peak minima will plateau at the heat of dilution.*
   d. Plot the CDF as a binding isotherm curve to find ΔG^o and ΔS^o.

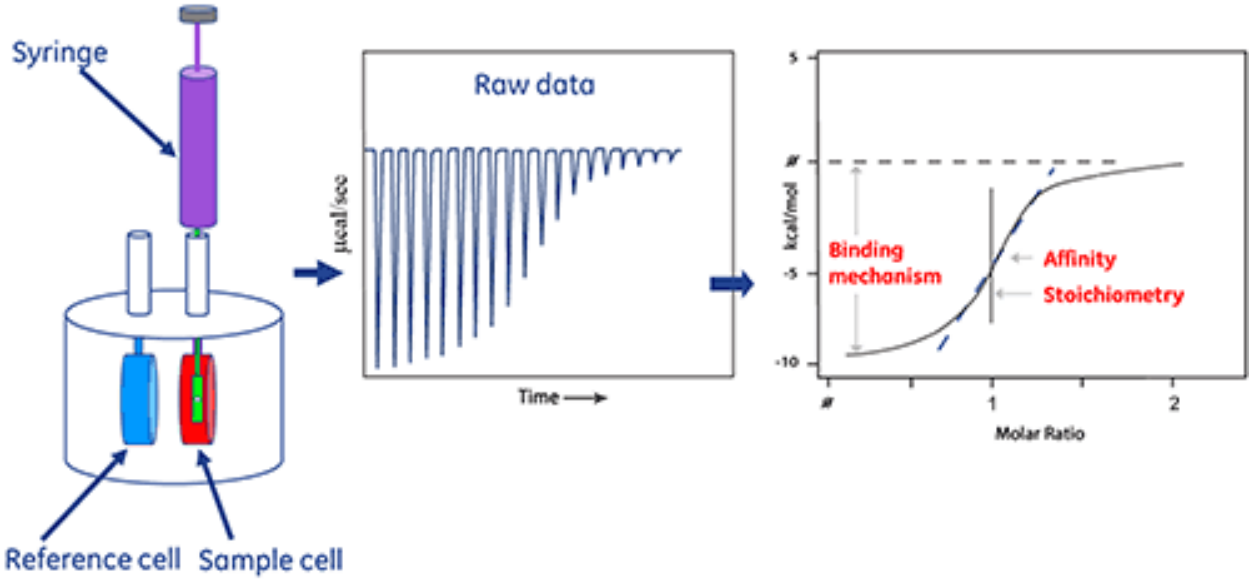


Figure 2: Isothermal Titration Calorimeter

Lecture 5: Multiple Site Binding and Cooperativity

Linkage Terminology

1. Linkage: ligand binding dependence.
   a. *Chemical linkage*: due to another ligand
      - *Homotropic*: same ligand X
      - *Heterotropic*: different ligand Y
        - *Identical linkage*: X and Y compete for the same site.
      - *Polysteric*: ligand binding tied to aggregation/dissociation/oligomerization.
   b. *Physical linkage*: due to temperature or pressure.
2. Binding Constants
   a. *Site* binding constant: one specific site.
   b. *Average* binding constant: averaged across multiple sites.

Binding constants

1. Overall/stoichiometric binding constant (β_i)
   - Describes simultaneous binding of iL.

$$M_0 + iL \overset{\beta_i}{\rightleftharpoons} ML_i \qquad \beta_i = \frac{ML_i}{[M_0][L]^i}$$

2. Step-wise macroscopic binding constant (K_i)
   - Describes sequential binding of L, using an average binding constant over multiple sites.

$$M_0 + L \overset{K_1}{\rightleftharpoons} ML \qquad K_1 = \frac{ML}{[M_0][L]}$$

$$ML + L \overset{K_2}{\rightleftharpoons} ML_2 \qquad K_2 = \frac{ML_2}{[ML][L]}$$

$$\beta_i = \prod_{i=1}^{n} K_n = K_1 K_2 \dots K_n$$

3. Step-wise microscopic binding constant (k_i)
   a. Same as K_i, but with binding constants specific to a single site (corrected statistical factors).
   b. Cooperativity
      - If $k_2 = k_1$, there is no cooperativity.
      - If $k_2 > k_1$, cooperativity is positive.
      - If $k_2 < k_1$, cooperativity is negative.
   c. Example
      - 2 ways to add the 1st ligand; K_1 is inflated by 2-fold.
      - 1 way to add the 2nd ligand despite 2 possible sites; K_2 is deflated by 2-fold.

$$\text{Statistical factor} = \frac{\text{Possible Product States}}{\text{Possible Reactant States}}$$

$$M_0 + 2L \overset{K_1}{\rightleftharpoons} ML + L \overset{K_2}{\rightleftharpoons} ML_2$$

Adair equation

1. Binding configurations
   - The number of possible configurations for i ligands bound and n total sites is: $\binom{n}{i}$.
   - As a result, the multiplicity for each reaction step can be found from row n of Pascal's Triangle. The statistical factor is therefore equal to the pairwise quotients.

2. The Adair equation models ligand binding as a sequential process with individual equilibrium constants.
   - Expression forms (take a subset of numerator terms for $[ML_i]/[M]_{tot}$)

$$\langle \nu \rangle = \frac{[L]_{\text{Bound}}}{[M]_{tot}}$$

$$\langle \nu \rangle = \frac{\sum_{i=0}^{n} i[ML_i]}{\sum_{i=0}^{n} [ML_i]} = \frac{[ML_1] + 2[ML_2] + 3[ML_3] + \dots}{[M_0] + [ML_1] + [ML_2] + [ML_3] + \dots}$$

$$\langle \nu \rangle = \frac{\sum_{i=0}^{n} i\beta_i[M_0][L]^i}{\sum_{i=0}^{n} \beta_i[M_0][L]^i} = \frac{\beta_1[L] + 2\beta_2[L]^2 + 3\beta_3[L]^3 + \dots}{1 + \beta_1[L] + \beta_2[L]^2 + \beta_3[L]^3 + \dots}$$

$$\langle \nu \rangle = \frac{\sum_{i=0}^{n} i \left(\prod_{j=1}^{i} K_j\right)[M_0][L]^i}{\sum_{i=0}^{n} \left(\prod_{j=1}^{i} K_j\right)[M_0][L]^i} = \frac{K_1[L] + 2K_1K_2[L]^2 + 3K_1K_2K_3[L]^3 + \dots}{1 + K_1[L] + K_1K_2[L]^2 + K_1K_2K_3[L]^3 + \dots}$$

Application to multiple site binding

1. General form (non-identical and interacting):
   - We can convert $K \to k$ for a given n.
   - For $n = 3$, $K_1 = 3k_1$, $K_2 = k_2$, and $K_3 = K_3/3$:

$$\langle \nu \rangle_{n=3} = \frac{3k_1[L] + 6k_1k_2[L]^2 + 3k_1k_2k_3[L]^3}{1 + 3k_1[L] + 3k_1k_2[L]^2 + k_1k_2k_3[L]^3}$$

2. Identical and non-interacting sites
   - Non-interaction: $k = k_1 = k_2 = \dots = k_n$
   - We can factor the binomial powers and cancel:

$$\langle \nu \rangle = \frac{nk[L](1 + k[L])^{n-1}}{(1 + k[L])^n} = n\left(\frac{k[L]}{1 + k[L]}\right) = n\left(\frac{[L]}{k_d + [L]}\right)$$

   - Observe that n binding sites simply scales $\langle \nu \rangle_{max}$ up by n, and otherwise preserves the binding function shape from the single-site case.
3. Non-identical and non-interacting sites
   - Take the weighted average across the individual sites.
   - For N sites types:

$$\langle \nu \rangle = \sum_{i=1}^{N} \left[n_i \left(\frac{k_i[L]}{1 + k_i[L]}\right)\right]$$

$$\langle \nu \rangle_{N=2} = n_1\left(\frac{k_1[L]}{1 + k_1[L]}\right) + n_2\left(\frac{k_2[L]}{1 + k_2[L]}\right)$$

Alternate cooperativity models

1. Cooperativity on a $\langle \nu \rangle$ vs. $[L]$ plot
   a. Linear scale: sigmoid behavior
   b. Log scale: steeper hyperbolic rise
2. Extrema
   a. No cooperativity (discussed above)
   b. Infinite cooperativity: - This model cannot account for negative cooperativity, predict site binding affinities, predict stoichiometries, or account for different ligands. - n_{Hill} is non-integer and the y-intercept is meaningless when the assumption fails.

$$M_0 + nL \overset{K_{app}}{\rightleftharpoons} ML_n \qquad K_{app} = \frac{[ML_0]}{[M_0][L]^n}$$

$$\langle \nu \rangle = n \left(\frac{[ML_n]}{[M_0] + [ML_n]} \right) = n \left(\frac{K_{app}[L]^{n_{Hill}}}{[M_0] + K_{app}[L]^{n_{Hill}}} \right)$$

Cooperativity and binding affinity

1. Simple System: 2 ligands (A and B) and 2 binding sites

$$M \overset{K_A}{\rightleftharpoons} MA \overset{K_{AB}}{\rightleftharpoons} MAB \qquad K_{AB} = cK_B$$

$$M \overset{K_B}{\rightleftharpoons} MB \overset{K_{BA}}{\rightleftharpoons} MAB \qquad K_{BA} = cK_A$$

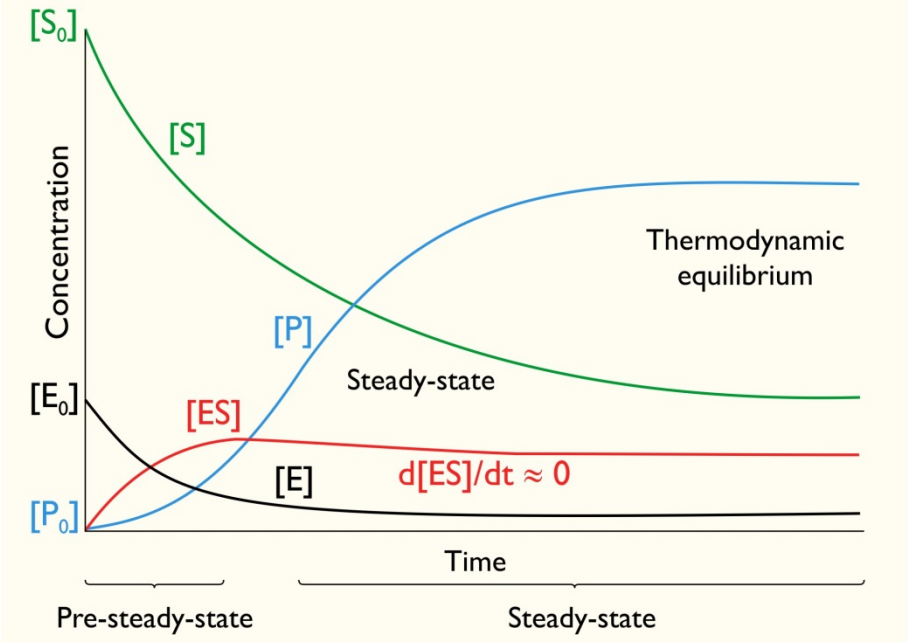
2. Cooperativity scales subsequent binding affinity by the coupling factor (c)
   - $c = 1$: no cooperativity
   - $c > 1$: positive cooperativity
   - $c < 1$: negative cooperativity
3. Cooperativity is proportional to conformational change energy

Lecture 6: Transient Kinetics I (Chemical Relaxations)

Steady-state enzyme kinetics

1. Steady-state approximation: $\frac{d[ES]}{dt} = 0$.
2. Two step mechanism:

$$E + S \underset{k_-}{\overset{k_+}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$



Transient (pre-steady-state) kinetics

1. Goals
   a. Identify the reaction mechanism in terms of elementary reactions.
   b. Compute the formation, loss, lifetime, and distribution of intermediates.
2. Signal: indicates biochemical state.
   a. Chemical: ATP hydrolysis, acetylation, base incorporation, peptide bond formation, etc.
   b. Optical: Absorbance, fluorescence, anisotropy, scattering, chemical shift, etc.
3. Rapid disruption
   a. Temperature jump
      - Uses laser
      - Requires $\Delta H_r^o \neq 0$ for an effect
      - Dead time (between stimulus and observation) $\approx 1\mu$s
   b. Pressure jump
      - Slow compression and quick release
      - Requires $\Delta V_r^o \neq 0$ for an effect
      - Dead time $\approx 50\mu$s
   c. Rapid mixing
      - Dead time $\approx 500 - 2000\mu$s.

4. Rapid mixing methods
    a. Continuous flow
        - Reaction solution is mixed and travels down a flow tube at constant speed.
        - Age is determined by distance of measurement. Can make continuous measurements.
        - Requires a lot of material.
    b. Stopped flow
        - Same as continuous flow, but the flow tube terminates in a "stop syringe," which fills and moves back to terminates the flow.
        - Requires little material.
    c. Quench flow
        - Same as continuous flow, but add a quench solution (e.g., acid) some distance/time away.
        - Allows direct detection of intermediates, but does not allow continuous measurement.
5. Chemical relaxations
    a. Relaxation is exponential (time $= e^{-kt}$) or sum of exponential.
    b. k reflects the reaction rate or "probability."

First-order reactions

$$M \xrightarrow{k} M^*$$

1. Differential/integrated rate law:

$$\begin{aligned} \frac{d[M]}{dt} &= -k[M] \\ \frac{1}{[M]} d[M] &= -k dt \\ \ln[M] &= -kt + C \\ [M] &= Ce^{-kt} \\ [M] &= [M_0]e^{-kt} \end{aligned}$$

2. Half-time $(t_{1/2}) = \frac{\ln 2}{k}$
3. Reversibility: $M^* \rightarrow M$ decreases k_{obs}.

$$M \underset{k_-}{\overset{k_+}{\rightleftharpoons}} M^* \qquad k_{obs} = k_+ + k_-$$

4. Measuring k
    a. Ligand dissociation with competition:

$$ML \underset{k_+}{\overset{k_-}{\rightleftharpoons}} M + L \rightarrow MX + L$$

    b. If $[X] \gg [L]$, dissociation is effectively irreversible.
        - k_{obs} is now just k_-, and k_+ can be computed by substraction.

Second-order reactions (with distinct reactants)

$$M + L \underset{k_-}{\overset{k_+}{\rightleftharpoons}} ML$$

1. Differential rate law:

$$\frac{d[ML]}{dt} = k_+[M][L] - k_-[ML]$$
$$k_{obs} = k_+[L] + k_-$$

2. Measuring k
   a. The integrated solution is complex and does not follow simple exponentials.
   b. BUT, k_{obs} depends linearly on $[L]$ for 2nd-order reactions
      - Measure and plot k_{obs} across different $[L]$. The slope will be k_+ and the intercept will be k_-
      - This must be done under pseudo-1st-order conditions ($[L] \gg [M]$ so that $[L]_{eq} \approx [L]_p$).
      - If the reaction is 1st-order, the slope will be 0.

Diffusion-limited reactions

1. Characteristics of diffusion-controlled reactions
   a. Magnitude $\approx 10^9\, M^{-1}s^{-1}$
   b. Weak temperature dependence ($k_{collision} \propto T$)
   c. Moderate solvent viscosity dependence ($k_{collision} \propto \eta^{-1}$)
2. Smoluchowski limit: describes the maximum bimolecular k for $A + B \longrightarrow AB$
   a. Assumptions: A and B are freely diffusing, uncharged, and will always react upon contact.
   b. Units: $\quad k_{collision} : M^{-1}s^{-1}, \quad D : cm^2s^{-1}, \quad r : cm$

$$\begin{aligned} k_{collision} &= 4\pi(D_A + D_B)(r_A + r_B)\frac{N_A}{1000} \\ &= 4\pi\left(\frac{k_BT}{6\pi\eta r}\right)\left(\frac{1}{r_A} + \frac{1}{r_B}\right)(r_A + r_B)\frac{N_A}{1000} \end{aligned}$$

   c. Rate depends on ratio of radii, not absolute radii.
      - Molecules of different size collide faster
      - If molecules are the same size ($r_A = r_B$):

$$\begin{aligned} k_{collision} &= \left(\frac{8k_BT}{3\eta}\right)\left(\frac{N_A}{1000}\right) \\ &= 6.6 \times 10^9\, M^{-1}s^{-1} \quad \text{(for } H_2O \text{ at } 25^o \text{ C)} \end{aligned}$$

3. Faster than the diffusion limit
   a. Biomolecular reactions can exceed the diffusion limit (e.g. $> 10^{10}\, M^{-1}s^{-1}$).
   b. Facilitated target location
      - Electrostatic steering
      - Dimensionality reduction (less sensitive to target size).

For a target (radius r) in a cell (radius R), mean time to encounter (τ):

$$\text{In 3D: } \tau_3 = \left(\frac{R^2}{3D_3}\right)\left(\frac{R}{r}\right)$$
$$\text{In 2D: } \tau_2 = \left(\frac{R^2}{3D_2}\right)\ln\left(\frac{R}{r}\right)$$
$$\text{In 1D: } \tau_1 = \left(\frac{R^2}{3D_1}\right)$$

3D is strongly dependent on r, 2D is weakly dependent, and 1D is independent.

4. Slower than the diffusion limit
   a. Molecules only react at binding sites; reduces reaction rates to $10^5 < k_{collision} < 10^7 M^{-1}s^{-1}$.
   b. Diffusion entrapment: microcollisions with reorientations speed up reactions.
   c. Thermal fluctuations make sites temporarily inaccessible due to "breathing" or "gating."
   d. Induced fit (multi-step binding)

Induced fit and two-step binding

1. Most bimolecular reactions involve multiple steps.
   - Involves multi-exponential behavior.
   - k_{obs} vs. $[L]$ is a hyperbola.
2. Isomerization to a stable complex:
   a. Steps: single-site binding and 1st-order isomerization

$$M + L \overset{K_d}{\rightleftharpoons} ML^* \underset{k_-}{\overset{k_+}{\rightleftharpoons}} ML$$

   b. k_+ is scaled down because ML* must form before ML. This scale factor is equal to the fraction of "isomerizable" macromolecule $\nu_{(ML)}$.

$$\begin{aligned} k_{obs} &= \frac{[(ML)]}{[M]_{tot}}k_+ + k_- \\ &= \frac{[L]}{K_d + [L]}k_+ + k_- \end{aligned}$$

   c. Implications

   (1) The asymptote is $(k_- + k_+)$. As $[L]$ increases, (ML) dominates and isomerization is rate-limiting.

$$\lim_{[L]\to\infty} k_{obs} = \max k_{obs} = k_+ + k_-$$

   (2) The y-intercept is (k_-). As $[L]$ decreases, M_{free} dominates and binding is rate-limiting.

$$\lim_{[L]\to 0} k_{obs} = \min k_{obs} = k_-$$

   (3) Scale the plot down to find K_d.

$$K_d = [L] \text{ when } k_{obs} = \frac{1}{2}k_+ + k_-$$

Module III: Mechanical Properties of Biological Polymers and Cells

Module IV: Introduction to Macromolecular Structure

Module V: Determining Macromolecular Structures

De La Cruz Quote Board

- I once wrote in a paper about the need to assess some kind of effect. I heard back from a reviewer who told me " 'assess' is not spelled 'asses' ".
- I spend a lot of my life thinking about drugs. Not drugs. Pharmaceutical agents.
- Gibb's equation is an equation for marriage. You need to have a great bond (ΔH^o), but they can't always be controlling you (ΔS^o).
- This understanding saved my life. Well, not really. But now I've got your attention.
- I'm gonna have to teach this next year. I don't know how I'm going to do that. I don't believe it.
- If you can name a macromolecule that binds only one ligand, I will give you a dollar right now. . . . Nobody? You're not even thinking about it are you?
- This protein is a beast. It just comes up from behind and GSHHHH. Rips it open. Like a crab.
- If someone tries to draw kinetics claims from thermodynamics, check your wallet. You can't trust 'em.
- [Stopped flow apparatuses] are really simple. I built one back in the day. Didn't work very well.