MB&B 302: Principles of Biophysics

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Contents

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.

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

Note: L refers to the characteristic length (usually diameter).

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 (df) is $\frac{1}{2}k_BT$ (for a monatomic gas).
     - 3 dfs associated with translation
     - 3 dfs associated with rotation (usually ignored)
5. **Background thermal energy** is $1k_BT \approx 0.6$ 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

Thermodynamics background

1. Background thermal noise provides kinetic energy to particles; may help overcome activation barriers.
   - The unit of thermal energy is k_BT
   - This is the minimum stable energy for resisting thermal motion (increases binding affinity).
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
   - The square root of the mean of the squared velocities 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.

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 energy 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
- 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: Single-Site Binding

Background and terminology

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

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

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

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

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

- Nearly all biological reactions are controlled by ML binding.
  - Exceptions: unimolecular isomerizations.
- 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.

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).

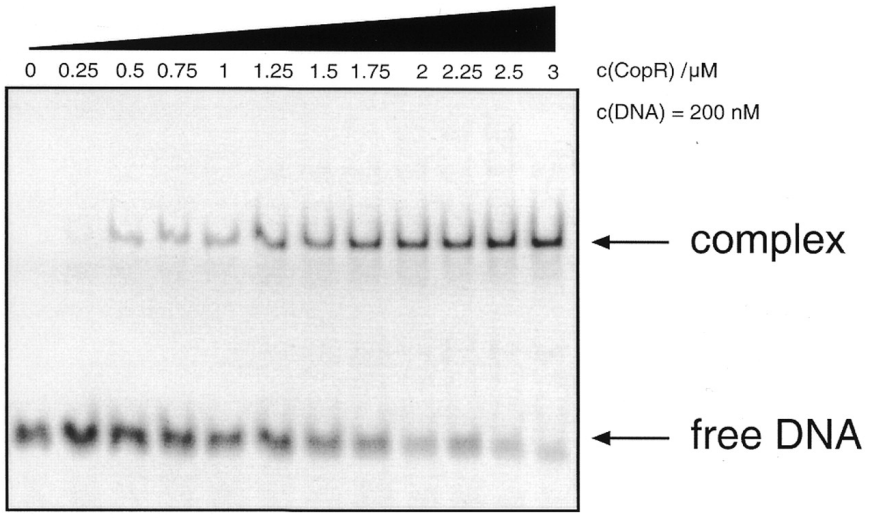
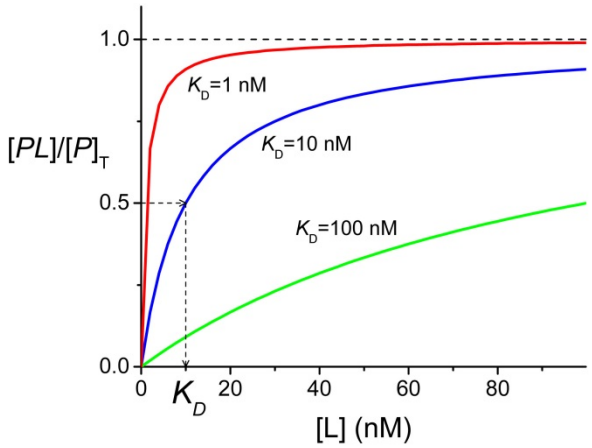


Figure 1: Gel Shift Assay

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.

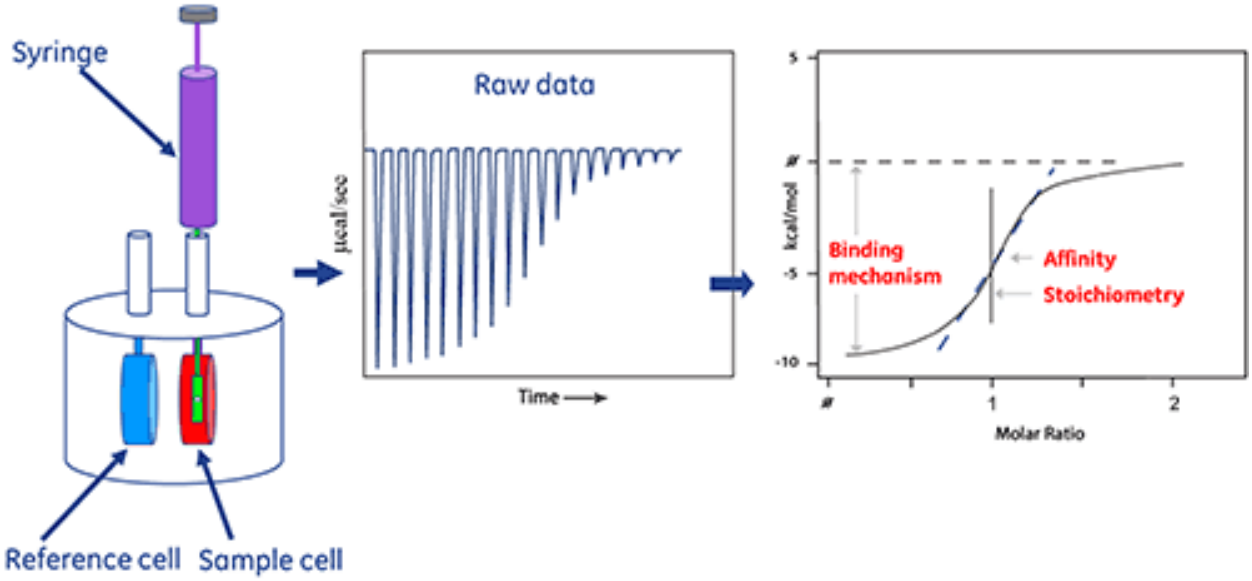


Figure 2: Isothermal Titration Calorimeter

Lecture 5: Multiple-Site Binding

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. Infinite cooperativity and the Hill coefficient:
   - 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.

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

$$\langle\nu\rangle = n\left(\frac{[ML_n]}{[M_0]+[ML_n]}\right) = n\left(\frac{K_{app}[L]^{n_{Hill}}}{1+K_{app}[L]^{n_{Hill}}}\right)$$

3. Simple system: 2 ligands (A and B) and 2 binding sites

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

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

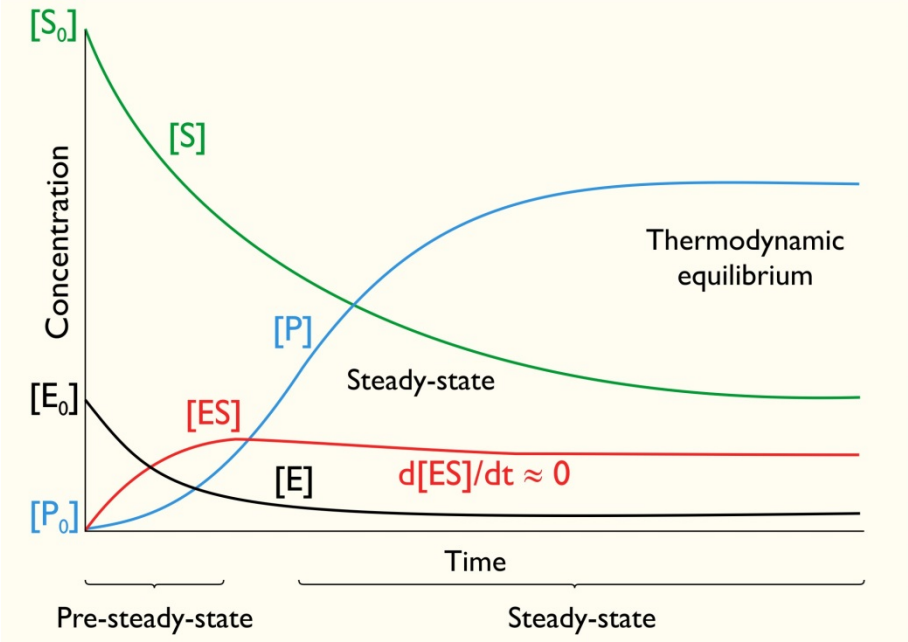
   - Cooperativity scales subsequent binding affinity by the coupling factor (c)
     - $c = 1$: no cooperativity
     - $c > 1$: positive cooperativity
     - $c < 1$: negative cooperativity
   - Cooperativity is proportional to conformational change energy

Lecture 6: Transient Kinetics

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:

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

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: $k_{collision} : M^{-1}s^{-1}, \quad D : cm^2s^{-1}, \quad r : cm$

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

   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. Thermal fluctuations make sites temporarily inaccessible due to "breathing" or "gating."
   c. 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

Lecture 7: Biopolymer Mechanics

Key material properties

1. The elastic (Young's) modulus (E) defines stiffness.

$$E = \frac{stress}{strain} = \frac{F/A}{\Delta L/L}$$

   - Cells: 100-1000 Pa (like pudding)
   - Cytoskeleton: 2 GPa (like plexiglass)
   - Teeth: 75 GPa (like quartz)
2. Second geometric moment (I): describes the cross-sectional area and its distribution. Units of m^4.
3. Flexural rigidity ($\kappa = EI$): the force couple required to bend a rod (analogous to spring constant).

Rods under load

1. Types of Motion
   - Bending, twisting, and stretching are all coupled, i.e., one will lead to another.
2. Buckling (from inward forces at the ends)
   - Critical (Euler) force: required to start buckling a rod. Additional bending requires little force.

$$F_c = \frac{\pi\kappa}{L^2}$$

3. Thermal "wiggling" and persistence length
   - Persistence length (L_p): when thermal forces become significant.
   - Angular correlation (C_s): cosine of angle between tangent lines at the two endpoints.

$$C(s) = cos(\angle A - \angle B) = \exp\left(-\frac{s}{2L_p}\right)$$

   - Longer L_p is stiffer; greater endpoint angle correlation.
4. Twisting
   - Torsional constant (α): torque required to twist 1 rod by 1 radian (analogous to E); units of $N \cdot m$.
   - Torsional ridigity ($C = \alpha h$): measures twisting elasticity (analogous to κ); units of $N \cdot m^2$.
   - Angular disorder ($\Delta\xi$):

$$\Delta\xi_{avg} = \sqrt{\frac{k_B T}{\alpha}}$$

5. Stretching
   - The energy stored in elastic stretching is:

$$\begin{aligned} U_{stretch} &= \frac{1}{2} F \Delta x \\ &= \frac{1}{2}(stress)(area)(\Delta x) \\ &= \frac{1}{2}\left(E * \frac{\Delta L}{L}\right)(\pi r^2)(\Delta L) \\ &= \frac{\pi r^2 E}{2L}(\Delta L)^2 \end{aligned}$$

   - This can be set equal to $\frac{1}{2}k_B T$ to solve for ΔL.

6. Deformation
   - The energy stored from elastic deformation is:

$$E_{free,elastic} = \frac{1}{2} k_B T (L_p(\kappa_1^2 + \kappa_2^2) + L_T \kappa_3^2)$$

$$\text{where: } \begin{cases} L_p = \text{Bending persistence length} \\ L_T = \text{Torsional persistence length} \\ \kappa_1, \kappa_2 = \text{Rotation strain vectors perpendicular to the main polymer axis} \\ \kappa_3 = \text{Rotation strain vector parallel to the main polymer axis} \end{cases}$$

7. Application examples
   - Ex 1: Actin bends from payload; monomer is added; actin bends back and increments payload.
   - Ex 2: Payload fluctuates forward; monomer is added; payload can no longer move back.

Non-elastic behavior

1. Viscoelasticity
   a. Definition and properties
      - Materials with both solid (elastic) and liquid (viscous) properties.
      - Deformation stores energy AND generates flow.
      - Results from self-interactions (entanglement, cross-linking) and interactions with solvent.
   b. Measuring viscoeasticity/viscosity
      - Rheometers (plate & cone, bob & cup).
      - Observe particle fluctations and solve for η from:

$$\Delta \langle x^2 \rangle_t = 4D\Delta t$$
$$D = \frac{k_B T}{6\pi\eta r_{bead}}$$

2. Freely-jointed and worm-like chains
   a. Worm-like chain: flexible polymers where $L \gg L_p$.
   b. Freely-jointed chain: straight segments separated by flexible joints; approximates worm-like chains.
   c. Entropic springs: polyers resist extension because $\Delta S_{extension} < 0$.
   d. Order of stretching response: (1) fiber alignment, (2) elastic stretching, (3) monomer unfolding.

Applications in cellular mechanics

1. Cells generate contractile forces.
   - Contraction is driven by myosin motors (inhibited by blebbistatin).
   - The extracellular matrix's elasticity/stiffness affects net force.
2. Applications
   - Adhesion, motility, migration/metastasis, and substrate-based stem cell differentiation.

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 in 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.
- I'm sorry for cancelling class last week... and also you're welcome.