Important Topics for the 2nd exam – Coverage: Lectures 11-21, Problem sets 5-7

Previous Material:
Amino Acids
- Names & Structures.
- Properties of sidechains.
  1. hydrogen bonding groups (i.e Thr).
  2. ionizable groups & their pKₐ's (Lys).
  3. non-polar groups (i.e. Phe).

Thermodynamics:
- ΔG° = -RTlnK_{EQ} = ΔH° - TΔS°.
- What does the sign of ΔG° tell you about the equilibrium position of a reaction?

Thermodynamics: Molecular aspects:
- Hydrogen bonds (ΔH°)
- van der Waals (ΔH°)
- Electrostatic interaction (ΔH°)
- Hydrophobic effect (ΔS°)
- Conformation entropy (ΔS°)

New material:
Ligand Binding
1. Association constant (K_{EQ} = K_A).
2. Dissociation constant, K_{D}.
3. Experimental measurement of K_{D} (equilibrium dialysis experiments, Absorbance measurements).
4. Thermodynamics aspects of ligand binding (all energy terms can be significant).
5. Definition of Y (fraction of sites occupied).

Oxygen Binding
1. Physiological role of myoglobin and hemoglobin.
2. General structure of myoglobin and hemoglobin.
3. General structure of heme.

Cooperativity & Allosteric effects:
1. Know how to distinguish cooperative from non-cooperative binding (Hill plot).
2. Homotropic allosteric effectors. Specific mechanism of cooperativity in oxygen binding to hemoglobin.
3. Heterotropic allosteric effectors.
   - Importance role of both cooperativity and allosteric mechanism in biochemistry.
   - Specific mechanism of bis-phosphoglycerate in regulating Hb oxygen binding & altitude adaptation.

Enzyme Kinetics
1. Transition state theory.
   \[ k_{CAT} \propto [X]^n \]
2. The transition state is stabilized by entropic (all enzymes) and enthalpic (some enzymes) effects.
3. Steady-state enzyme kinetics - assumptions of the model (d[ES]/dt = 0).

 tegen
- V_{MAX} = k_{cat} E_i : catalytic rate observed when all enzyme is in the [ES] form.
- K_{M} = (k_{-1} + k_{cat})/k_{1} : Substrate concentration that gives a velocity = V_{MAX}/2.0, enzyme is \( \frac{1}{2} \) saturated.

Enzyme Inhibitors
1. Molecular nature of competitive inhibitors. Why V_{max} is not affected.
2. Molecular nature of mixed competitive inhibitor. Why V_{max} is affected.
3. K_{i} - Dissociation constant (K_P) for inhibitor from [EI] complex (both types of inhibitor).
4. K_{i}' - Dissociation constant (K_D) for inhibitor from [ESI] complex (mixed).
5. Suicide inhibitors – covalent modification → inactivated enzyme

Serine Proteases
1. Nucleophilic attack (Ser195, H₂O)
2. Base catalyzed activation of nucleophile (His57, Asp102).
3. Acyl-enzyme intermediate.
4. Molecular nature of specificity for different proteases (e.g. trypsin versus elastase)
5. Overall reaction scheme (ES → E-acyl intermediate → Product).

HIV Life Cycle
1. vRNA →DNA→mRNA & vRNA → virus
2. Common drug targets:
   i) HIV reverse transcriptase
   ii) Integrase
   iii) HIV Protease
   - Unique enzyme activities not found in humans, reducing side-effects of drugs.
   - Error prone reverse transcriptase generates drug resistant viruses.

HIV (Aspartate) Protease
1. Nucleophilic attack by HO⁻ (hydroxide).
2. Base activation of nucleophile by Asp25'.
3. General structure of inhibitors (e.g. specificity).
4. Concept of genetic variation in virus population, reducing effectiveness of drugs.
5. General concept of drug design in response to genetic variation.

Protein Purification
1. Overall steps (salt ppte, chromatography).
2. Column chromatography & fractionation.
3. Concept & application of specific activity.
4. Principles of separation by:
   - Gel filtration (aka size exclusion)
   - Ion exchange (cation and anion)
   - Affinity Chromatography

You should come prepared to design a simple purification scheme.
Tight Binding: \( k_{\text{off}} \) (off-rate) will be small. \( K_D, K_M, K_I, K_I' \) will be small. 
\( K_D, K_M, K_I, K_I' \) all represent \( 1/2 \) saturation points: when \([L]=K_D\), \( Y=1/2 \), when \([S]=K_M, [ES]=[E_{\text{TOT}}]/2\).

### Non-Cooperative Ligand Binding

\[
[M] + [L] \leftrightarrow [ML]
\]

\[
K_D = \frac{k_{\text{OFF}}}{k_{\text{ON}}}
\]

**Binding curve:** \( Y = \frac{[L]}{K_D + [L]} \)

When \([L]=K_D\) then \( Y=1/2 \)

**Hill Plot:** \( \log(Y/(1-Y)) = \log K_{D, \text{AVE}} + n_h \log([L]) \)

x-intercept \( \rightarrow \log K_D \)
Slope @ \( \log(Y/(1-Y)) = 0 \) \( \rightarrow n_h \)
\( n_h = n, \infty \) pos. coop.
\( n_h > 1, \) pos. coop.
\( n_h = 1, \) non-coop.
\( n_h < 1, \) neg. coop.

### Cooperative Binding (2 sites), \( K_{D1} \neq K_{D2} \)

\[
[M] + [L] \leftrightarrow [ML] \leftrightarrow [ML_2]
\]

\[
K_{D1} = \frac{k_{1-\text{off}}}{k_{1-\text{on}}} \quad K_{D2} = \frac{k_{2-\text{off}}}{k_{2-\text{on}}}
\]

\[
K_D^{\text{obs}} = \sqrt{K_{D1} K_{D2}}
\]

\( K_D \) is \textit{always} the amount of ligand to \( 1/2 \) saturate.

### Enzyme Kinetics

\[
-K \rightarrow [U]
\]

\[
K_{EQ} = \frac{[U]/[N]}{K_{EQ} = [ML]/[M][L] \text{ (also called } K_a)}
\]

\[
K_D = 1/K_{EQ} \text{ for ligand binding to } [M]
\]

\[
K_{I} = k_{\text{off}}/k_{\text{on}} = k_i/k_1
\]

**Enzyme Kinetics**

\[
 [E] + [S] \leftrightarrow [ES] \rightarrow [EP]
\]

\[
K_M = \frac{k_{\text{OFF}}}{k_{\text{ON}}} + \frac{k_{\text{cat}}}{k_{\text{ON}}}
\]

**Velocity curve:**

\[
v = \frac{V_{\text{MAX}} [S]}{K_M + [S]} = \frac{E_{\text{TOT}} k_{\text{CAT}} [S]}{K_M + [S]}
\]

**Hill Plot:**

\[
\log(Y/(1-Y)) = \frac{-RT \ln K_{EQ}}{\Delta H^o - T \Delta S^o}
\]

**Enzyme Inhibition:**

\[
v = \frac{V_{\text{MAX}} [S]}{\alpha' K_M + [S]}
\]

\[
Y_{\text{int}} = \frac{1}{V_{\text{MAX}}}
\]

\[
\text{Slope} = \frac{K_M}{V_{\text{MAX}}}
\]

\[
\alpha' = \text{ratio of } y\text{-intercepts}
\]

\[
\alpha = \text{ratio of slopes}
\]
Summary of Enzyme Kinetics:

Raw Data:
- Measure \( \frac{dp}{dt} \) for different substrate concentrations.
- You must measure the \textit{initial rate} to ensure you are under steady-state conditions.

Equations and plots:

\[ v = \frac{[S]}{K_M + [S]} = \frac{k_{\text{cat}} E_{\text{TOT}} [S]}{K_M + [S]} \]

\[ \frac{1}{v} = \frac{K_M}{V_{\text{MAX}} [S]} + \frac{1}{V_{\text{MAX}}} \]

\[ \frac{1}{v} = \frac{K_M}{V_{\text{MAX}} [S]} + \frac{1}{V_{\text{MAX}}} \frac{1}{\alpha} \]

\[ \alpha = \text{ratio of the slopes.} \quad \alpha' = \text{ratio of Y-intercepts.} \]

\[ K_I = K_D \text{ for the inhibitor dissociation from the (EI) complex (both types of inhibitors).} \]
\[ K_I' = K_D \text{ for the inhibitor dissociation from the (EIS) complex (mixed only).} \]

\textit{Lower the } K_i \text{ the better the inhibitor binds.}