**Important Topics for the 2nd exam** – Coverage: Lectures 11-21. Problem sets 4(Q4&5) - 7

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

**Thermodynamics**:
- $\Delta G^0 = -RT\ln K_{eq} = \Delta H^0 - T\Delta S^0$.
- What does the sign of $\Delta G^0$ tell you about the equilibrium position of a reaction?

**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 bi-phosphoglycerate in regulating Hb oxygen binding & altitude adaption.

**Enzyme Kinetics**
1. Transition state theory.
   $$k_{cat} \propto [ES] \propto [X^2]$$
2. The transition state is stabilized by entropic (all enzymes) and enthalpic (some) effects.
3. Steady-state enzyme kinetics - assumptions of the model (d[ES]/dt = 0).
4. $V_{MAX} = k_{cat} E_s$: catalytic rate observed when all enzyme is in the [ES] form (saturated).

**Second Exam Review Topics**

**Enzyme Inhibitors**
1. Molecular nature of competitive inhibitors. Why Vmax is not affected.
2. Molecular nature of mixed competitive inhibitor. Why Vmax is affected.
3. $K_I$: Dissociation constant (Ki) for inhibitor from [EI] complex (both types of inhibitor).
4. $K_I'$: Dissociation constant (Kf) 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 (trypsin versus chymo versus elastase).
5. Overall reaction scheme (ES → E-acyl intermediate → Product).

**HIV Life Cycle**
1. vRNA → DNA → mRNA & vRNA → virus.
2. 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 ppt, 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.
**Biochemistry I**  
**Second Exam Review Topics**

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**Cooperative Binding (n_h)**

Ligand Binding ($K_D$) → Enzyme Kinetics ($K_M$, $k_{cat}$) → Enzyme Inhibition ($K_I$, $K_I'$)

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<table>
<thead>
<tr>
<th>Protein Folding</th>
<th>$[N] \rightarrow [U]$</th>
<th>$K_{EQ} = [U]/[N]$</th>
<th>$\Delta G^o = -RT \ln K_{EQ}$ = $\Delta H^o - T\Delta S^o$</th>
</tr>
</thead>
</table>

**Ligand Binding**

$[M] + [L] \leftrightarrow [ML]$  
$K_D = \frac{k_{ON}}{k_{OFF}}$

Enzyme-Substrate

$[E] + [S] \rightarrow [ES]$  
$K_M = (k_{OFF} + k_{CAT})/k_{ON} \approx K_D$

Competitive Inhibitor

$[E] + [I] \rightarrow [EI]$  
$K_I = K_D$ for inhibitor binding to $[E]$

Mixed Inhibitor

$[E] + [I] \rightarrow [EI]$  
$K_I = K_D$ for inhibitor binding to $[E]$

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**Enzyme Kinetics**

$[E] + [S] \rightarrow [ES] \rightarrow [EP]$  
$V = \frac{V_{MAX}[S]}{K_M + [S]}$

**Non-Cooperative Ligand Binding**

$[M] + [L] \leftrightarrow [ML]$  
$K_D = \frac{k_{OFF}}{k_{ON}}$

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

When $[L] = K_D$ then $Y = \frac{1}{2}$

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

x-intercept → $log K_D$

Slope @ $log(Y/(1-Y)) = 0$

$n_h$

$n_h = n, \infty$ pos. coop.  
$n_h > 1$, pos. coop.  
$n_h = 1$, non-coop.  
$n_h < 1$, neg. coop.

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

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

$K_{D1} = \frac{k_{on1}}{k_{off1}}$, $K_{D2} = \frac{k_{on2}}{k_{off2}}$

$K_{D,AVG}^2 = \sqrt{K_{D1}K_{D2}}$

$K_D$ is always the amount of ligand to $\frac{1}{2}$ saturate.

**Enzyme Inhibition:**

$V = \frac{V_{MAX}[S]}{K_M + [S]}$

$K_I = \frac{[I]}{(\alpha - 1)}$

$\alpha = \text{ratio of slopes}$

$K'_I = \frac{[I]}{(\alpha' - 1)}$

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

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Summary of Enzyme Kinetics:

\[ E + S \xrightarrow{k_{on}} (ES) \xrightarrow{k_{off}} (EP) \rightarrow E + P \quad v = k_{cat} [ES] \quad \frac{d[ES]}{dt} = 0 \quad [P] \]

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

**Equations and plots:**

**Velocity Curve**

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

**Double Recp. Plot**

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

**Summary of Parameters:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Related Formula</th>
<th>Importance</th>
<th>From Velocity Curve: ( v = \frac{V_{\text{MAX}} [S]}{(K_M + [S])} )</th>
<th>From Double Reciprocal Plot</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_{\text{MAX}} )</td>
<td>( k_{cat} [E_{\text{TOT}}] )</td>
<td>Maximum rate of product produced at a given enzyme concentration.</td>
<td>Reaction velocity at high substrate</td>
<td>( V_{\text{MAX}} = \frac{1}{Y\text{-int}} )</td>
</tr>
<tr>
<td>( k_{cat} )</td>
<td>( V_{\text{MAX}} / [E_{\text{TOT}}] )</td>
<td>Turnover number: Number of product molecules produced/sec by a single enzyme molecule. <strong>Higher ( k_{cat} ), more efficient the chemistry ([ES]→[EP])</strong></td>
<td>see ( V_{\text{MAX}} )</td>
<td>see ( V_{\text{MAX}} )</td>
</tr>
<tr>
<td>( K_M )</td>
<td>( \frac{(k_{OFF} + k_{cat})}{k_{ON}} = K_D )</td>
<td>Related to dissociation constant for substrate binding. <strong>Lower ( K_M ), better binding of [S]</strong></td>
<td>Substrate concentration to give a velocity that is ( \frac{1}{2} V_{\text{MAX}} )</td>
<td>( K_M = \text{slope} \cdot V_{\text{MAX}} = \text{slope} / Y\text{-int} )</td>
</tr>
</tbody>
</table>

**Inhibitor Analysis:** Comp: binds at active site, Mixed: Allosteric site, elsewhere.

**Raw data:**
1. Obtain \( v \) versus \([S]\) in the absence of inhibitor.
2. Obtain \( v \) versus \([S]\) in the presence of a fixed and known concentration of inhibitor, using the same \([S]\) as in step 1.
3. Plot both data sets on a double reciprocal plot.

**Equations and Double Reciprocal Plots:**

<table>
<thead>
<tr>
<th>No Inhibitor Present</th>
<th>Competitive Inhibitor</th>
<th>Mixed Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \frac{1}{v} = \frac{K_M}{V_{\text{MAX}}} \frac{1}{[S]} + \frac{1}{V_{\text{MAX}}} )</td>
<td>( \frac{1}{v} = \frac{\alpha K_M}{V_{\text{MAX}}} \frac{1}{[S]} + \frac{1}{V_{\text{MAX}}} )</td>
<td>( \frac{1}{v} = \frac{\alpha K_M}{V_{\text{MAX}}} \frac{1}{[S]} + \frac{\alpha'}{V_{\text{MAX}}} )</td>
</tr>
</tbody>
</table>

\( \alpha = \text{ratio of the slopes.} \quad \alpha' = \text{ratio of Y-intercepts.} \quad K_I = \frac{[I]}{(\alpha - 1)} \quad K_I' = \frac{[I]}{(\alpha' - 1)} \)

**Summary of Parameters:**

\( K_I = K_D \) for the inhibitor dissociation from the (EI) complex (both types of inhibitors).
\( K_I' = K_0 \) for the inhibitor dissociation from the (EIS) complex (mixed only).

**Lower the \( K_I \) the better the inhibitor binds, it has a smaller off-rate.**