Important Topics for the 2nd exam – Coverage: Lectures 11-21

Previous Material:

Amino Acids

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

Thermodynamics:

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

Thermodynamics: Molecular aspects:

- Hydrogen bonds (ΔH^o)
- van der Waals (ΔH^o)
- Electrostatic interaction (ΔH^o)
- Hydrophobic effect (ΔS^o)
- Conformation entropy (ΔS^o)

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.

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 adapation.

Enzyme Kinetics

1. Transition state theory. $k_{CAT} \propto [X^{\ddagger}]$
2. The transition state is stabilized by entropic (all enzymes) and enthalpic (some enzymes) effects.
3. Steady-state enzyme kinetics - assumptions (d[ES]/dt = 0).
4. $V_{MAX} = k_{cat} E_t$: catalytic rate observed when all enzyme is in the [ES] form.
5. k_{cat} – number of products produced/one E
6. $K_M = (k_{-1}+k_{cat})/k_1$: Substrate concentration that gives a velocity = $V_{MAX}/2.0$, enzyme is ½ 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_D) 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

Computational Data Analysis

1. Fitting data to models using solver.

Serine Proteases

1. Nucleophilic attack (Ser195, H_2O)
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.
   - 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.
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.

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

Protein Folding	[N] → [U]	K_{EQ}=[U]/[N]	**$\Delta G^o = -RT\,ln\,K_{EQ}$ $= \Delta H^o - T\Delta S^o$** **Molecular complementarily involving:** • hydrophobic effects • van der Waals • hydrogen bonds • electrostatics
Ligand Binding	[M] + [L] ⇌ [ML] (k_{on} (k_1), k_{OFF} (k_{-1}))	K_{EQ} = [ML]/[M][L] (also called K_A) $K_D = 1/K_{EQ}$ for ligand binding to [M] $K_D = k_{off}/k_{on} = k_{-1}/k_1$	
Enzyme-Substrate	[E] + [S] → [ES]	$K_M = (k_{OFF} + k_{CAT})/k_{ON} \approx K_D$	
Competitive Inhibitor	[E] + [I] → [EI]	$K_I = K_D$ for inhibitor binding to [E]	
Mixed Inhibitor	[E] + [I] → [EI] [ES]+[I] → [ESI]	$K_I = K_D$ for inhibitor binding to [E] $K_I' = K_D$ for inhibitor binding to [ES]	

Tight Binding: k_{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 ½ saturation points: when [L]=K_D, Y=1/2, when [S]=K_M, [ES]=[E_{TOT}]/2.

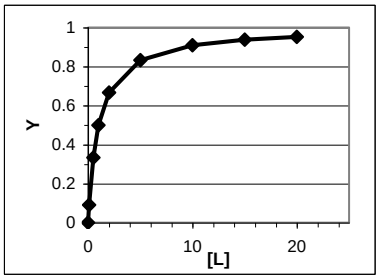
Non-Cooperative Ligand Binding

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

Enzyme Kinetics

$$[E]+[S] \underset{k_{OFF}}{\overset{k_{ON}}{\leftrightarrow}} [ES] \overset{k_{cat}}{\rightarrow} [EP] \qquad K_M = \frac{k_{OFF}}{k_{ON}} + \frac{k_{cat}}{k_{ON}}$$

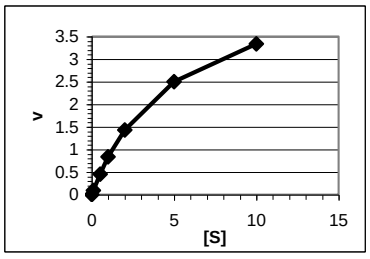
Binding curve: $Y = \frac{[L]}{K_D + [L]}$



When **[L]=K_D** then **Y=1/2**

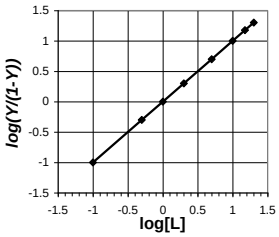
Velocity curve:

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



When **[S]=K_M** then **v=V_{max}/2, [ES]=[E_T]/2**

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

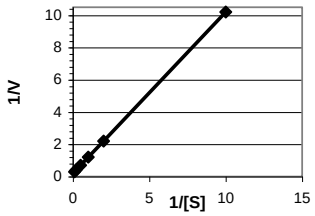


x-intercept → *log* K_D

Slope @ *log(Y/(1-Y))* = 0 → n_h

n_h = n, ∞ pos. coop.
n_h >1, pos. coop.
n_h =1, non-coop.
n_h <1, neg. coop.

Double Reciprocal Plot: $\frac{1}{v} = \left[\frac{K_M}{V_{MAX}}\right]\frac{1}{[S]} + \frac{1}{V_{MAX}}$



Slope = K_M/V_{MAX}

$Y_{INT} = 1/V_{MAX}$

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

$$[M]+[L] \underset{k_{1-OFF}}{\overset{k_{1-ON}}{\leftrightarrow}} [ML] \underset{k_{2-OFF}}{\overset{k_{2-ON}}{\leftrightarrow}} [ML_2]$$

$$K_{D1} = \frac{k_{1-off}}{k_{1-on}}, K_{D2} = \frac{k_{2-off}}{k_{2-on}}$$

$$K_D^{OBS} = \sqrt{K_{D1}K_{D2}}$$

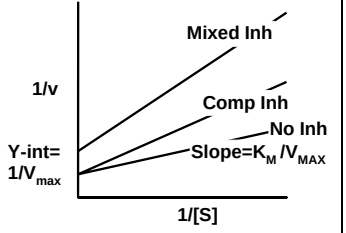
K_D is ***always*** the amount of ligand to ½ saturate.

Enzyme Inhibition:

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

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

α = ratio of slopes



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

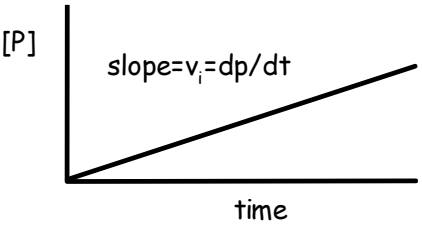
α' = ratio of y-intercepts

Summary of Enzyme Kinetics:

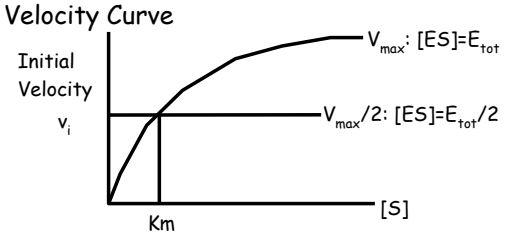
$$E + S \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} (ES) \xrightarrow{k_{CAT}} (EP) \longrightarrow E + P \qquad v = k_{CAT}[ES] \qquad d[ES]/dt = 0$$

Raw Data:

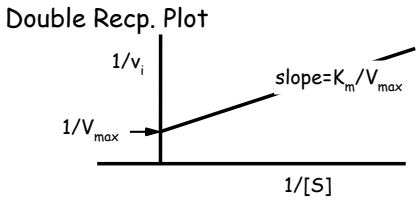
- Measure dp/dt for different substrate concentrations.
- You must measure the ***initial rate*** to ensure you are under steady-state conditions with the starting amount of substrate and no product inhibition.



Equations and plots:



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

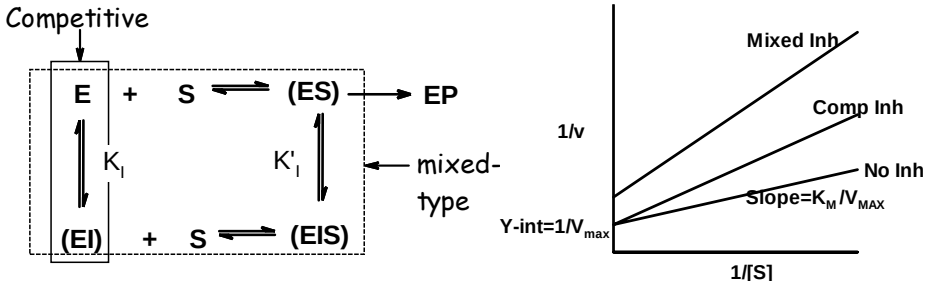


$$\frac{1}{v} = \frac{K_M}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}}$$

Summary of Parameters:

Parameter	Related Formula	Importance	From Velocity Curve: $v=V_{MAX}[S]/(K_M+[S])$	From Double Reciprocal Plot
V_{MAX}	= $k_{cat}[E_{total}]$	Maximum rate of product produced at a given enzyme concentration.	Reaction velocity at high substrate	V_{MAX}=1/Y-int
k_{cat}	=$V_{max}/[E_{Total}]$	Turnover number: Number of product molecules produced/sec by a single enzyme molecule. ***Higher k_{CAT}, more efficient the chemistry*** **([ES]→[EP])**	see V_{MAX}	see V_{MAX}
K_M	= $(k_{OFF}+k_{cat})/k_{ON}$ ≈K_D	Related to dissociation constant for substrate binding. ***Lower K_M, better binding of*** **[S]**	Substrate concentration to give a velocity that is ½ V_{MAX}	K_M = slope*V_{MAX} = slope/Y-int

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.

Analysis:

Computational – Best fit with Solver, minimizing difference between observed & predicted.

Graphical - Plot both data sets on a double reciprocal plot:

No Inhibitor Present

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

Competitive Inhibitor

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

Mixed Inhibitor

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

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

Summary of Parameters:

$K_I = K_D$ for the inhibitor dissociation from the (EI) complex (both types of inhibitors).

$K_I' = K_D$ for the inhibitor dissociation from the (EIS) complex (mixed only).

Lower the K_I the better the inhibitor binds.