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Kirk's Amazing Notes for Problem Set 2

Check which version (bottom-right corner) you have to make sure you have the most updated notes!

Problem 1

A protein contains a 15-residue long α-helix with the following sequence: **QEANIKQRLSTEYKW**

α-helix characteristics:
residues per turn: _____
pitch (distance between turns): _____ nm

β-sheet characteristics:
residues per repeat: _____
repeat length: _____ nm (antiparallel)

Problem 1A:
How many full turns are in this α-helix?

Number Bank for 1:
0.54
2
3.6
0.695

Given:
residues per turn: _____
number of residues in this protein: _____ residues

_____ residues / _____ residues per turn = _____ turns → _____ full turns (round down)

Problem 1B:
What is the length of the helix (in Å) in the direction of the helix axis?

Given:
residues per turn: _____
number of residues in this protein: _____ residues
pitch (distance between turns): _____ nm x (10Å / 1nm) = _____ Å

_____ residues / _____ residues per turn x _____ Å in an α-helix pitch = _____ Å

Problem 1C:
How many hydrogen bonds between the backbone atoms are in this helix?

Symbols:
○ = 1 amino acid residue

C=O ← H-N
hydrogen bond from the amide of one amino acid to the carbonyl of another amino acid

| = indicates the start of a new turn in an α-helix

Directions:
Draw an arrow from the fifth amino acid to the first amino acid. This represents one hydrogen bond. Then draw an arrow from the sixth amino acid to the second amino acid. Repeat until the last (fifteenth) amino acid. Note the dashes are supposed to be at every 3.6 amino acid residues (turn = 3.6 amino acids) and not every 4 amino acid residues (as shown in the drawing). Thus, the dashes are placed at every 4 for drawing purposes.

Count the number of arrows: _____ How many total hydrogen bonds? _____ hydrogen bonds

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Problem 2

Which of the following polypeptide is most likely to form an α-helix? How about the other two? Explain your reasoning.

(a) CRAGNRKIVLETY
(b) SEDNFGAPKSILW
(c) QKASVEMAVRNSG

Analyzing each of the three polypeptides by primary structure:

Word Bank for 2 (circle):
(1) aliphatic | acidic | basic | aromatic
(2) stabilizes | destabilizes

(a) C R A G N **R** **K** I V L E T Y

Arginine and Lysine are adjacent (1)_____ amino acids residues, which (2)_____ an α-helix?

(b) S E D N F **G** A **P** K S I L W

Looking at table 6.1 to the right, what are the helix behaviors of Glycine and Proline?

H = helix former
I = indifferent
B = helix breaking
C = random coil

Glycine: _____

Proline: _____

What can you conclude about the Gly and Pro on the stabilization of an α-helix?

_____

_____

TABLE 6.1 Helix-Forming and Helix-Breaking Behavior of the Amino Acids

Amino Acid		Helix Behavior*	
A	Ala	H	(I)
C	Cys	Variable	
D	Asp	Variable	
E	Glu	H	
F	Phe	H	
G	Gly	I	(B)
H	His	H	(I)
I	Ile	H	(C)
K	Lys	Variable	
L	Leu	H	
M	Met	H	
N	Asn	C	(I)
P	Pro	B	
Q	Gln	H	(I)
R	Arg	H	(I)
S	Ser	C	(B)
T	Thr	Variable	
V	Val	Variable	
W	Trp	H	(C)
Y	Tyr	H	(C)

*H = helix former; I = indifferent; B = helix breaker; C = random coil; ( ) = secondary tendency.

Word Bank for 2 (circle):
(3) charged | uncharged
(4) stabilizes | destabilizes

(c) Q **K** A S V **E** M A V **R** N S G

Lysine, Glutamic Acid, and Arginine are (3)_____ amino acids residues at physiological conditions and aligned on one face of the helix, which (4)_____ an α-helix?

Which of the three polypeptides are most likely to form an α-helix? _____ Explain why.

Problem 3

A 14-residue peptide, **IFILYKDGEALRSL**, in the context of a protein forms a β-hairpin, consisting of two antiparallel β-strands connected by a β-turn. You have found that the amide nitrogen of phenylalanine forms a hydrogen bond with the carbonyl oxygen of serine. Based on your finding, characterize the structure of the β-hairpin:

Fun facts about β-hairpins, β-strands, and β-turns:

A β-hairpin is made of only two β-strands. β-strands refer to a single continuous polypeptide chain of typically 3-10 amino acids adopting a fully extended conformation and involved in backbone hydrogen bonding to at least one other strand. β-strands form an extensive hydrogen bond network with other strands in which the N-H groups in the backbone establish hydrogen bonds with the C=O groups of the other backbone. In other words, the extended conformation is only stable as part of a β-sheet where contributions from hydrogen bonds and van der Waals interactions between aligned strands exert a stabilizing influence. A β-turn is used to change peptide direction in a β-hairpin.

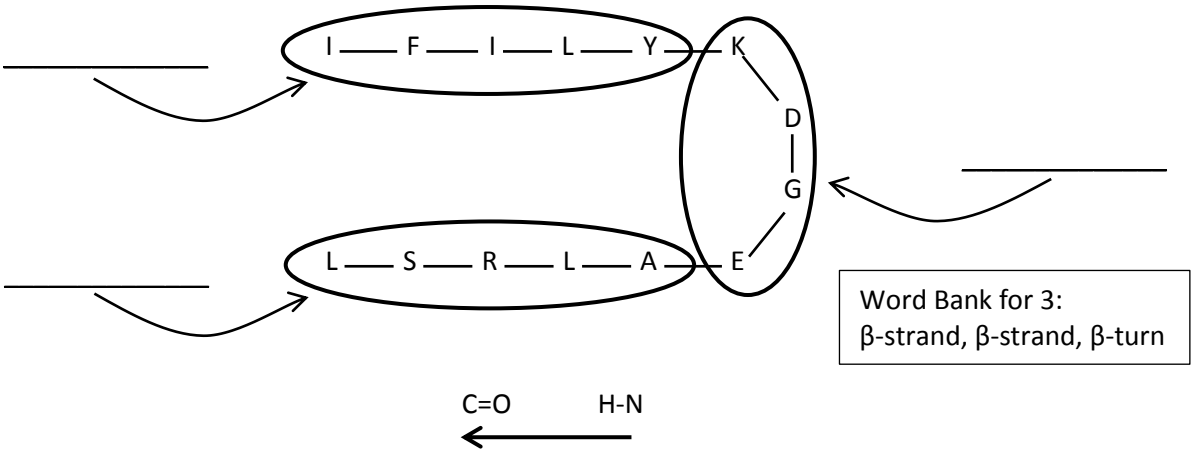
β-sheets refer to an assembly of at least two β-strands that are laterally connected by backbone hydrogen bonding to each other. This forms a generally twisted, pleated sheet.

Problem 3A:

In the peptide sequence below clearly indicate, which residues belong to the two β-strands and which to the β-turn. **IFILYKDGEALRSL**

Problem 3B:

Draw *schematically* the hydrogen bonding pattern for this β-hairpin. Do not draw the side chains – just indicate what residues are connected by H-bonds. How many hydrogen bonds between the backbone atoms are present in the structure? Explain your reasoning.



Directions:

First, label which residues belong to the two β-strands and which belongs to the β-turn. Second, draw an arrow from the amide nitrogen of F (phenylalanine) to the carbonyl oxygen of S (serine) to indicate one hydrogen bond. Then, draw an arrow from the amide nitrogen of S (serine) to the carbonyl oxygen of F (phenylalanine) to indicate the second hydrogen bond. Since the amide and carbonyl groups of β-strands face the interior on alternating residues, there are no hydrogen bonds for every residue. Draw additional arrows as necessary.

Count the number of arrows: _____ How many total hydrogen bonds? _____ hydrogen bonds

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$ E = enzyme, S = substrate, ES = enzyme-substrate complex, P = product

Assuming that [S] >> $[E]_o$, E binds to S to form ES complex with a 2nd order rate constant of k_1. ES can either (1) dissociate back into E and S with a 1st order rate constant of k_{-1} or it can (2) form E and P with a 1st order rate constant of k_2. In rapid equilibrium, we assume $k_{-1} >> k_2$ and that the ES complex falls apart much more quickly than S is converted to P. The rate limiting step is k_2 (also called k_{cat}). In steady-state equilibrium, we do NOT assume $k_{-1} >> k_2$ and that the ES complex converts S to P either more or less quickly than the ES complex falling apart back into E + S.

Definitions:

K_m: Michaelis constant with units in Molarity. It is the substrate concentration at which the initial velocity is half of its V_{max}. The lower the K_m, the higher affinity of the enzyme for the substrate and greater rate of reaction. It is important to note that K_m in the general equation does not equal K_S.

V_{max}: represents the maximum rate achieved by the system, at maximum (saturating) substrate concentrations

k_{cat}: catalytic rate constant (also called the turnover number) with units in s^{-1}; measure of how many bound substrate molecules turnover or form products in 1 second. In most cases, $k_2 = k_{cat}$ so that $v_0 = k_{cat}[ES]$. ES converts to form E and P with a 1st order rate constant of k_2.

k_{cat}/k_m: measures enzyme efficiency. If [S] << K_m then $v_o = k_{cat}/k_m\ [E]_t[S]$ and k_{cat}/k_m is a 2nd order rate constant with units of $M^{-1}s^{-1}$. A perfect enzyme has an efficiency of 10^8-10^9 and is only limited by diffusion.

Rapid Equilibrium (Michaelis-Menten):

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$ Rapid equilibrium means $k_2 = k_{cat}$ = rate limiting; k_1 & $k_{-1} >> k_2$

$[E][S]k_1 = [ES](k_{-1})$

$$\frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_1} = K_S$$ k_S = substrate dissociation constant

$$v_o = \frac{k_2[E]_{total}[S]}{\frac{k_{-1}}{k_1}+[S]}$$ (V_{max} = $k_2[E]_{total}$; K_S = $\frac{k_{-1}}{k_1}$)

If $k_{-1} >> k_2$, $k_S = K_m$ (follows rapid equilibrium assumption)

Steady-State Equilibrium (Briggs & Haldane):

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$ Steady-state equilibrium means ES formation = rate of its breakdown; k_2 has to be relatively large enough to consider steady-state eq.

$[E][S]k_1 = [ES](k_{-1}+k_2)$

$$\frac{[E][S]}{[ES]} = \frac{k_{-1}+k_2}{k_1} = K_m$$ K_m = Michaelis constant

$$v_o = \frac{k_2[E]_{total}[S]}{\frac{k_{-1}+k_2}{k_1}+[S]}$$

V_{max}

K_m

If $k_{-1} >> k_2$, $K_m = K_S$ (follows rapid equilibrium assumption)

Problem 4

An enzyme is found that catalyze the following chemical reaction and experiments were conducted to characterize this enzyme: A ↔ B

Problem 4A:

In the first experiment, with $[E_t]$ at 4 nM, the V_{max} was found to be 1.6 µM•s^{-1}. Based on this experiment, what is the k_{cat} for this enzyme.

Given:

$[E]_{total}$: 4 nM x ($1M/10^9nM$) = _____ M

V_{max}: 1.6 µM·s^{-1} x ($1M/10^9nM$) = _____ M·s^{-1}

k_{cat} = ___?___ s^{-1}

$V_{max} = k_{cat}[E]_{total}$ → $k_{cat} = V_{max}/[E]_{total}$

k_{cat} = _____ M·s^{-1} / _____ M = _____ s^{-1}

Problem 4B:

In second experiment, with $[E_t]$ at 1 nM and [A] at 30 µM, the researchers found that v = 300 nM•s^{-1}. What is the measured K_m of this enzyme for the substrate A?

Since the V_{max} is dependent on the total enzyme concentration and $[E]_{total}$ changes, then V_{max} changes as well.

Given:

v = 300 nM·s^{-1} x ($1M/10^9nM$) = _____ M·s^{-1}

V_{max} = _____ M·s^{-1}

$[E]_{total}$: 1 nM x ($1M/10^9nM$) = _____ M

[A] (substrate) = 30 µM x ($1M/10^6µM$) = _____ M

k_{cat} = _____ s^{-1} (from problem 4A)

K_m = ___?___ M

Calculating for the new V_{max}:

$V_{max} = k_{cat}[E]_{total}$

V_{max} = _____ s^{-1} x _____ M = _____ M·s^{-1}

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Calculating for the new K_m using the Michaelis-Menten equation:

K_m: Michaelis constant with units in Molarity. It is the substrate concentration at which the initial velocity is half of its V_{max}. The lower the K_m, the higher affinity of the enzyme for the substrate and greater rate of reaction.

Basis for enzyme assay:

$$v_o = \frac{k_2[E]_{total}[S]}{K_m + [S]}$$ (V_{max})

$V_{max} = k_2[E]_{total}$ and K_m depends on k_1, k_{-1}, and k_2

Rearranging the Michaelis-Menten equation to solve for K_m:

$$K_m = [S]\left(\frac{V_{max}}{v_0} - 1\right) = \_\_\_\_\_\ M\left(\frac{\_\_\_\_\_\ M\cdot s^{-1}}{\_\_\_\_\_\ M\cdot s^{-1}} - 1\right) = \_\_\_\_\_\ M$$

Problem 4C:
Calculate the catalytic efficiency value of this enzyme. Would you rate it a perfect enzyme? Explain.

k_{cat}/k_m: measures enzyme efficiency. If [S] << K_m then $v_o = k_{cat}/k_m\,[E]_t[S]$ and k_{cat}/k_m is a 2nd order rate constant with units of $M^{-1}s^{-1}$. A perfect enzyme has an efficiency of 10^8-10^9 and is only limited by diffusion.

Enzyme efficiency:

$$\frac{k_{cat}}{K_m} = \frac{\_\_\_\_\_\ sec^{-1}}{\_\_\_\_\_\ M} = \_\_\_\_\_\ M^{-1}\ sec^{-1}$$

A perfect enzyme has an efficiency ratio of 10^8-10^9 $M^{-1}\cdot sec^{-1}$ and can only be limited by rate of diffusion. Can this enzyme rated as a perfect enzyme?

Yes or no? _____ because…_____

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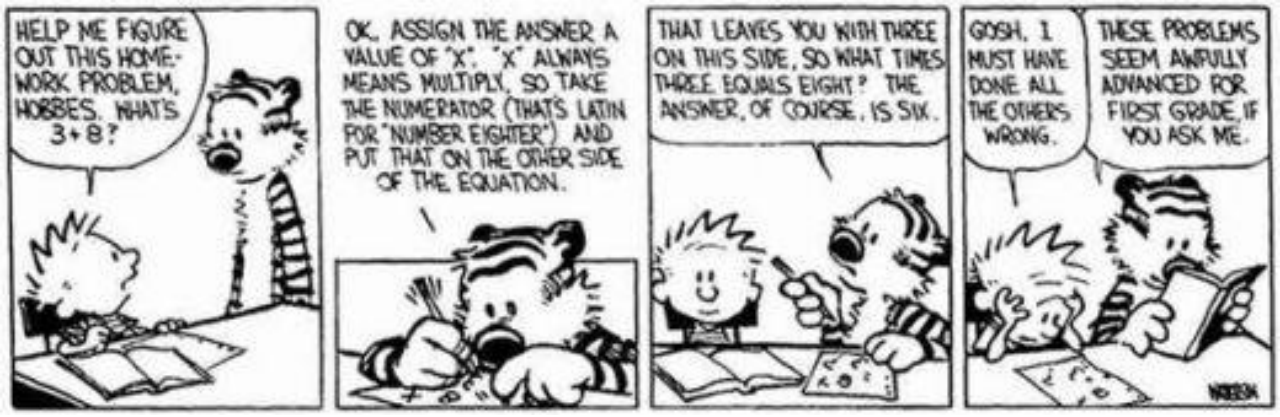

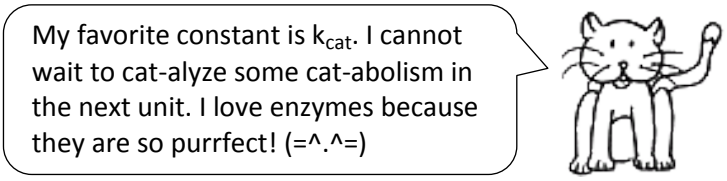

Show work and solution on your own paper!

Problem 5

The plot of 1/v versus 1/[S] is called a Lineweaver-Burk plot. Another way of expressing the kinetic data is to plot v versus v/[S], which is known as an Eadie-Hofstee plot.

Problem 5A:

Rearrange the Michaelis-Menten equation to give v as a function of v/[S].

$$v_o = \frac{k_2[E]_{total}[S]}{K_M + [S]} \quad \rightarrow \quad v_o = \frac{v_{max}[S]}{K_M + [S]}$$

Step 1: Invert and multiply both sides by V_{max}

Step 2: Simplify the right side by canceling the V_{max} on the top and bottom

Step 3: Move the v from the left side to the right side

Step 4: Split the fraction on the right side

Step 5: Simplify the second term on the right side by canceling the [S] on the top and bottom

Step 6: Solve for the second term on the right side (v) to get it into a slope-intercept form (y = mx + b)

What is the equation in terms of v as a function of v/[S]? _____ = _____ _____ + _____

y = m x + b

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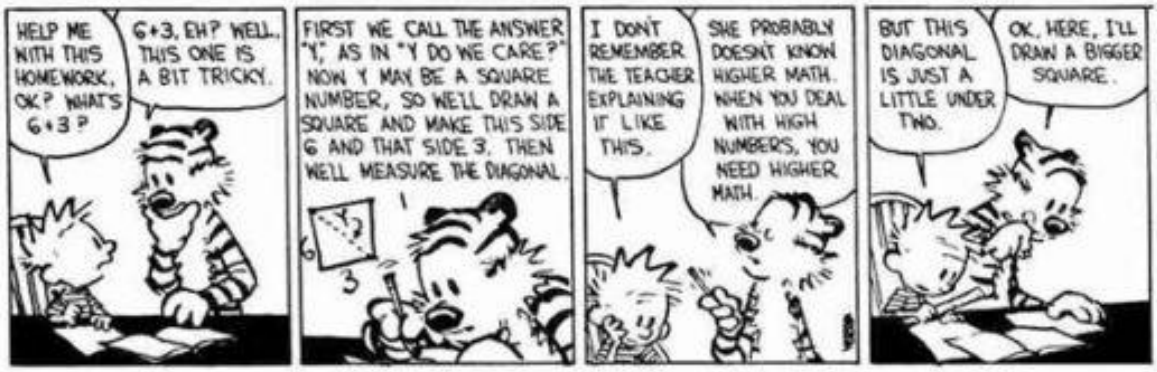
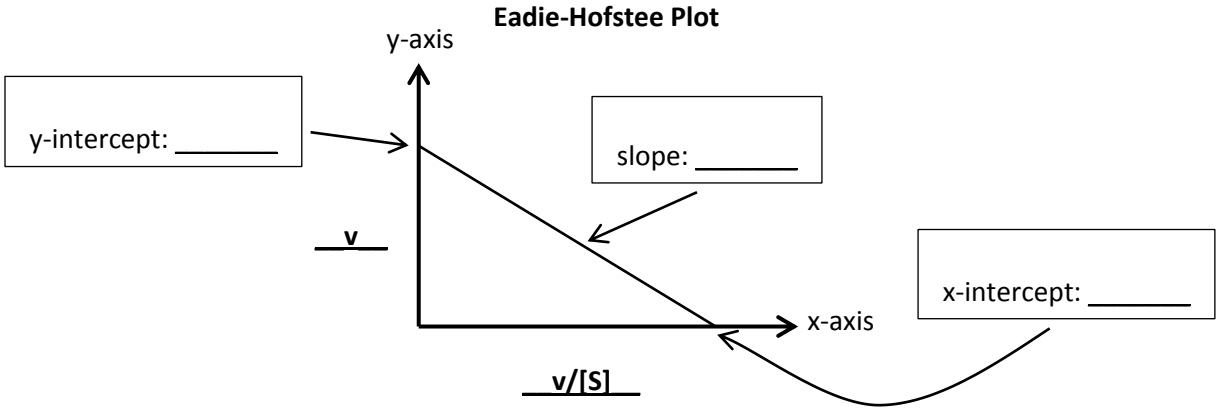


Problem 5B:
Draw a theoretical Eadie-Hofstee plot (v versus v/[S]). What is the significance of the slope, the x-intercept, and the y-intercept in a plot of v versus of v/[S]?



Slope (m) = _____ x-intercept (-b/m) = _____ y-intercept (b) = _____

Enzyme Kinetics – Reversible Inhibition
Inhibitor: Any ligand, natural or synthetic, that decreases the velocity of an enzyme-catalyzed reaction
Effect: A change in K_m and/or V_{max} (apparent K_m (written as $K_{(m)}$ or $K_{m(app)}$)means presence of an inhibitor)

Types of Inhibitors:
Competitive – K_m ↑, V_{max} ↔
A direct competition exists between substrate and inhibitor for binding to the free enzyme. Thus, a higher level of substrate is required to obtain ½V_{max}. In most cases, the inhibitor binds in the active site.

Uncompetitive – K_m and V_{max} ↓ by same proportion
Inhibitor binds to the ES complex preventing the catalytic breakdown of substrate to the product. This results in a lower V_{max}. Since the substrate cannot compete with the inhibitor for binding (i.e., it is uncompetitive), the K_m, being [S] at ½V_{max}, is lowered proportionally.

Noncompetitive – K_m ↔ or ↑ or ↓ V_{max} ↓ proportionally to inhibitor concentration
Inhibitor binds to both free enzymes and ES complex outside the active site, modifying the structure of the enzyme so that substrate affinity is unchanged, reduced, or increased and product formation is prevented.

Two types of noncompetitive inhibition occur:

Pure (aka classic) – K_m ↔. This holds when $\alpha = 1$.

Mixed - $K_{(m)}$ ↑. This holds when $\alpha > 1$. Higher affinity for free enzyme.

$K_{(m)}$ ↓. This holds when $\alpha < 1$. Higher affinity for ES complex.

K_I: dissociation constant for enzyme inhibitor complex. Effects best observed in Lineweaver-Burk plots.

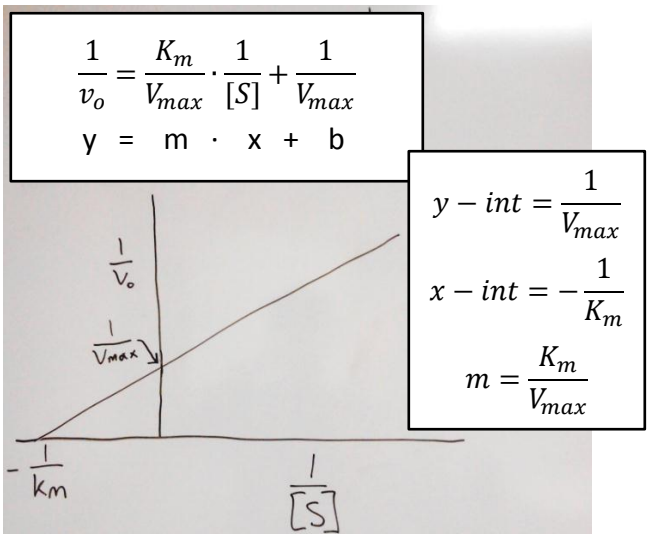
How can V_{max} and K_m be determined from experimental data?

- nonlinear hyperbolic fit – plots v_o vs. [S]
  - $v_o = k_1$[reactant]

- double reciprocal plot (Lineweaver-Burk plot)
  - rearrange Michaelis-Menten equation
  - $\frac{1}{v_o} = \frac{K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$

- Scatchard plot – plots $[S]_{bound} / [S]_{free}$ vs. $[S]_{bound}$
  - used to determine no. of binding sites and K_S of a substrate or any other ligand
  - $[S]_{bound} = \frac{[S]_{free}}{K_S}[E]_{free}$

Lineweaver-Burk Plot:



Problem 6

The following data were collected for the sphingosine kinase reaction in the presence or absence of threo-sphingosine, a stereoisomer of sphingosine that inhibits the enzyme.

[Sphingosine] (μM)	1/[Sphingosine] (μM^{-1})	v (mg/min) (no inhibitor)	1/v (min/mg)	v (mg/min) (with threo-sphingosine)	1/v (min/mg) (with threo-sphingosine)
2.5		32.3		8.5	
3.5		40		11.5	
5		50.8		14.6	
10		72		25.4	
20		87.7		43.9	
50		115.4		70.8	

Find the reciprocal for [Sphingosine], v without inhibitor, and v with inhibitor.

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Problem 6A:
Construct a Lineweaver-Burk plot. What are the apparent K_m and V_{max} values in the presence and absence of the inhibitor?

Graph the values according to the Lineweaver-Burk plot: $\frac{1}{v_o} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$

$y = \frac{1}{v_o} \quad m = \frac{k_m}{V_{max}} \quad x = \frac{1}{[S]} \quad b = \frac{1}{V_{max}}$

The x-axis has a scale ranging from -0.2 to 0.5 with the units as 1/[S], μM^{-1}.
The y-axis has a scale ranging from -0.02 to 0.12 with the units as 1/v, min/mg.

Add a linear trendline and the linear equation (y = mx + b) for the sets of data.

Linear trendline (without inhibitor): _____

Linear trendline (with inhibitor): _____

Determine the values of V_{max} and K_m for enzyme w/o inhibitor and $V_{(max)}$ and $K_{(m)}$ in presence of inhibitor:
Units for V_{max} and $V_{(max)}$ are in mg/min.
Units for K_m and $K_{(m)}$ are in µM.

Check your work by comparing graph and linear equation:
V_{max} (graph) ≈ V_{max} (linear equation) & $V_{(max)}$ (graph) ≈ $V_{(max)}$ (linear equation)
K_m (graph) ≈ K_m (linear equation) & $K_{(m)}$ (graph) ≈ $K_{(m)}$ (linear equation)

From the graph:

Without Inhibitor threo-sphingosine

$y - intercept = \frac{1}{V_{max}}$

Reciprocal of the y-int gives the V_{max}.

V_{max} = _____ mg/min

$x - intercept = -\frac{1}{K_m}$

Negative reciprocal of the x-int gives the K_m.

K_m = _____ µM

With Inhibitor threo-sphingosine

$y - intercept = \frac{1}{V_{(max)}}$

Reciprocal of the y-int gives the $V_{(max)}$.

$V_{(max)}$ = _____ mg/min

$x - intercept = -\frac{1}{K_{(m)}}$

Negative reciprocal of the x-int gives the $K_{(m)}$.

$K_{(m)}$ = _____ µM

From the linear equation (y = mx + b):

Without Inhibitor threo-sphingosine

$b = y - intercept = \frac{1}{V_{max}}$

Reciprocal of the y-int gives the V_{max}.

V_{max} = _____ mg/min

To find the K_m, find the x-int by plugging in 0 for y and solving for x.

$\frac{-b}{m} = x - intercept = -\frac{1}{K_m}$

Negative reciprocal of the x-int gives the K_m.

K_m = _____ µM

With Inhibitor threo-sphingosine

$b = y - intercept = \frac{1}{V_{(max)}}$

Reciprocal of the y-int gives the $V_{(max)}$.

$V_{(max)}$ = _____ mg/min

To find the $K_{(m)}$, find the x-int by plugging in 0 for y and solving for x.

$\frac{-b}{m} = x - intercept = -\frac{1}{K_{(m)}}$

Negative reciprocal of the x-int gives the $K_{(m)}$.

$K_{(m)}$ = _____ µM

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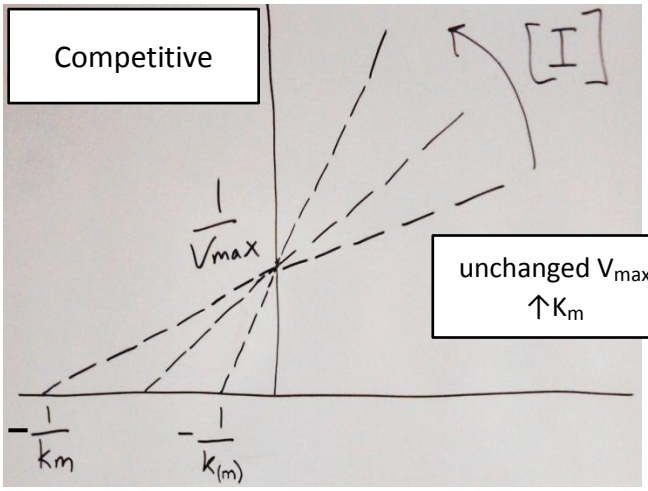
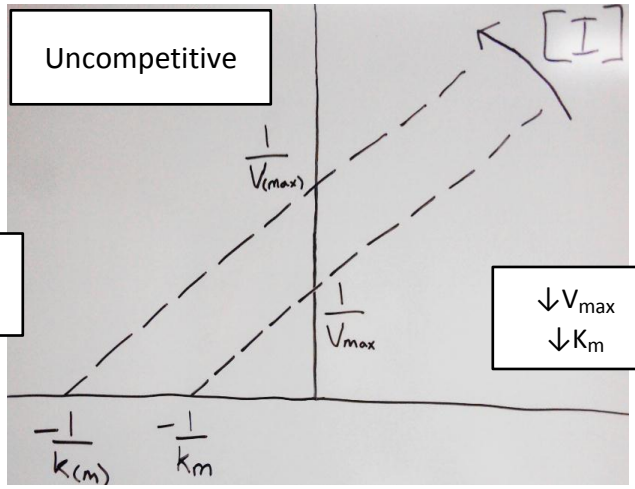
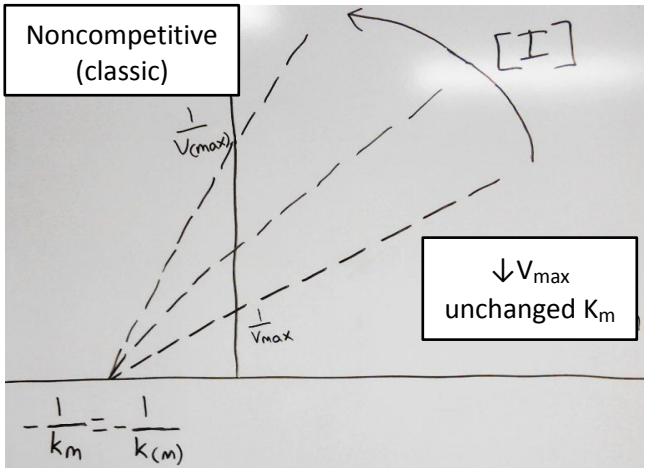






Which type of inhibitor (competitive, uncompetitive, or noncompetitive) is the threo-sphingosine?

Can be determined from the trendlines on the graph and from the calculated V_{max}, K_m, $V_{(max)}$, and $K_{(m)}$.

Does V_{max} increase, decrease, or unchanged?

V_{max} → ____ $V_{(max)}$

Does K_m increase, decrease, or unchanged?

K_m → ____ $K_{(m)}$

Graph not shown for noncompetitive (mixed)

↓V_{max}

↑K_m if $\alpha > 1$ ↓K_m if $\alpha < 1$

Which type of inhibitor is threo-sphingosine? _____ inhibitor

Problem 6B:

What kind of an inhibitor is threo-sphingosin? Provide two reasons.

Threo-sphingosine is a _____ inhibitor because…

Reason 1:

Compare the V_{max} in the absence of the inhibitor and in the presence of the inhibitor. Does it increase, decrease, or remain unchanged? _____

Compare the K_m in the absence of the inhibitor and in the presence of the inhibitor. Does it increase, decrease, or remain unchanged? _____

Reason 2:
Explain what happens in (1)_____ inhibition.
Are there structural similarities between the substrate and the inhibitor? (2)_____ The inhibitor threo-spingosine (3)_____ competes against the substrate in binding to the enzyme. The inhibitor binds to the (4)_____. It binds to an (5)_____ on the enzyme.

Word Bank for 6B (circle):
(1) competitive | uncompetitive | noncompetitive
(2) Yes | No
(3) directly | indirectly
(4) free enzyme | ES complex | both free enzyme and ES complex
(5) active site | allosteric (effector) site

Problem 7

A reversible inhibitor (inhibitor A) of acid phosphates was investigated. The rate was measured at two substrate concentrations, a low concentration and a high concentration to give the following data. Indicate the type of inhibitor and explain your reasoning.

Low [S]
Rate with no inhibitor present = 0.0364 µmoles•min^{-1}
Rate with inhibitor A present = 0.0308 µmolesmin^{-1}

High [S]
Rate with no inhibitor present = 0.2000 µmoles•min^{-1}
Rate with inhibitor A present = 0.1000 µmolesmin^{-1}

Comparing the decrease in rates in low [S] and high [S]:
In low [S], the rate decreases by (0.0364 – 0.0308) = _____ µmoles·min^{-1}
In high [S], the rate decreases by (0.2000 – 0.1000) = _____ µmoles·min^{-1}

Which rate decreases more? _____ [S]

Comparing the ratio of V_I (velocity with inhibitor) to V_0 (velocity without inhibitor):
In low [S], the V_I/V_0 ratio is (0.0308/0.0364) = _____
In high [S], the V_I/V_0 ratio is (0.1000/0.2000) = _____

Does the V_I/V_0 ratio increase, decrease, or remain the same from low [S] to high [S]? _____

In **competitive** inhibition, there is a direct competition between substrate and inhibitor for binding to the free enzyme. Increasing the [S] concentration overcomes the inhibition because there are more substrates in overwhelming inhibition to move the reaction in the forward direction. This means the rate decrease in high [S] is less than the rate decrease in low [S] and the V_I/V_0 ratio increases.

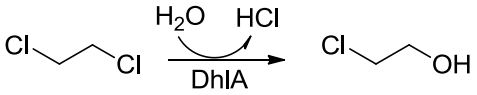
In **uncompetitive** inhibition, the inhibitor binds to the ES complex and prevents the catalytic breakdown of substrate to the product. Increasing the [S] concentration converts more free enzymes to the ES complex. This means the rate decrease in high [S] is more than the rate decrease in low [S] and the V_I/V_0 ratio decreases.

In **noncompetitive** inhibition, the inhibitor binds to both free enzymes and ES complex outside the active site. Increasing the [S] concentration has no effect (depending on the α-value) on the inhibition. This means the rate decrease in high [S] is the same as the rate decrease in high [S] and the V_I/V_0 ratio remains the same.

Which type of inhibitor is inhibitor A? _____ inhibitor Give a few reasons why.

Problem 8

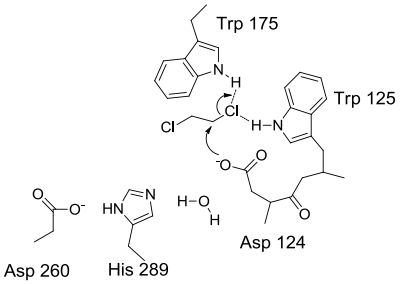
The bacterial degradation of various haloalkanes is initiated by the activity of a haloalkane dehalogenase (DhlA). This enzyme cleaves a carbon-halogen bond with water as the sole co-substrate.

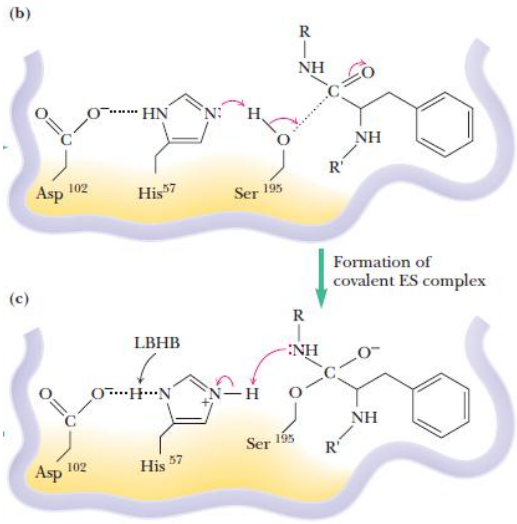


It is known that catalysis of this enzyme reaction is mediated by Asp-His-Asp catalytic triad. Unlike in the related serine hydrolases, aspartate carboxylate (Asp 124) is the nucleophile that attacks the substrate.

Problem 8A:

Based on the drawing below, complete the reaction mechanism of DhlA for this reaction by locating curved arrows to show the correct movement of electrons.





Serine proteases: contain an Asp-His-Ser catalytic triad at the active site. Asp-102, His-57, and Ser-195 engage directly with the peptide in a mixture of covalent and general acid-base catalysis. Asp-102 functions to immobilize and orient His-57 via charge interaction. His-57 acts as a general acid and base. Ser-195 forms a covalent bond with peptide to be cleaved.

In this enzymatic reaction, the catalytic triad is Asp-His-Asp. Asp replaces Ser as the nucleophile that attacks the substrate.

Using the reaction mechanism for chymotrypsin (Figure 14.21) to the left as an analogy, sketch the next step for DhlA. Follow the steps: 1. Binding of substrate, 2. Formation of covalent ES complex (note the low-barrier hydrogen bond here), 3. Proton acceptance by His-289, 4. nucleophilic attack by water to cleave C–O bond, 6. Release of product

Kirk's Amazing Notes for Problem Set 2

Problem 8B:
What are two roles of Asp 260 residue in this enzymatic reaction?

Serine proteases: contain an Asp-His-Ser catalytic triad at the active site. Asp-102, His-57, and Ser-195 engage directly with the peptide in a mixture of covalent and general acid-base catalysis. Asp-102 functions to immobilize and orient His-57 via charge interaction. His-57 acts as a general acid and base. Ser-195 forms a covalent bond with peptide to be cleaved.

In this enzymatic reaction, Asp-260 acts to (1)_____ and (2)_____ His-289 via charge interaction. Asp-260 also (3)_____ the (4)_____ charge that develops on His-289 during hydrolysis.

Word Bank for 8B (circle):
(1) mobilize | immobilize
(2) orient | disorient
(3) stabilizes | destabilizes
(4) positive | negative

Problem 8C:
What is a role of His 289 residue in this enzymatic reaction?

Serine proteases: contain an Asp-His-Ser catalytic triad at the active site. Asp-102, His-57, and Ser-195 engage directly with the peptide in a mixture of covalent and general acid-base catalysis. Asp-102 functions to immobilize and orient His-57 via charge interaction. His-57 acts as a general acid and base. Ser-195 forms a covalent bond with peptide to be cleaved.

In this enzymatic reaction, His-289 acts as a general (3)_____ by (4)_____ a proton to/from the water molecule. The water molecule then functions as a (5)_____ and attacks the (6)_____ carbonyl group of the chloroethyl-enzyme ester.

Word Bank for 8C (circle):
(3) acid | base
(4) accepting | donating
(5) nucleophile | electrophile
(6) nucleophilic | electrophilic

Problem 8D:
If you mutate the His 289 to Phe, what is the expected product of this enzymatic reaction? Explain your reasoning

Compare the structures of Histidine and Phenylalanine. His can act as a general acid and base. Phe has a cyclic stable ring for its side chain that does not like to react.

What property does His have that Phe does not have? _____

What is the expected product? _____

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Problem Set #2: Due Friday 10/19 at 5:00PM in FO 3.602
Exam #2 Review: TBA
Exam #2: Monday 10/22 at 10:00AM (Lee) in normal classroom
Tuesday 10/23 at 1:00PM (Marsh) in normal classroom