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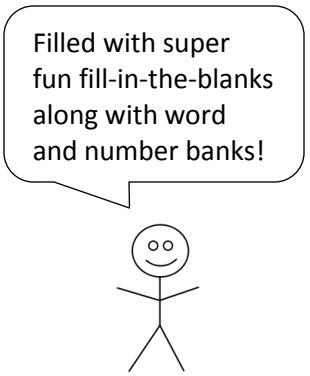
Kirk's Amazing Notes for Problem Set 2

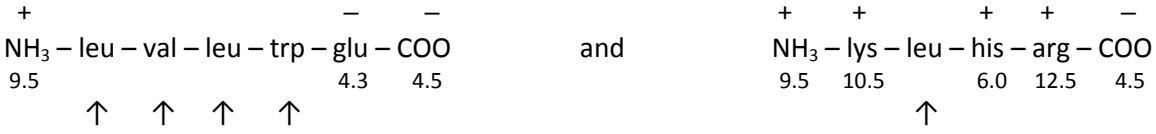


Problem 1

nonapeptide: leu – val – leu – trp – glu – lys – leu – his – arg
pKas: N-termini - 9.5, C-termini - 4.5, glu - 4.3, lys - 10.5, his - 6.0, arg - 12.5

V8 protease cleaves peptides after acidic residues (asp, glu)

At pH = 5.5: (pH > pKa ---> deprotonate)



4 hydrophobic, less polar

1 hydrophobic, more polar

↑ denotes hydrophobic residues

Reverse-phase chromatography: uses a non-polar stationary phase, meaning the non-polar compounds bind with higher affinity to the immobile non-polar stationary resin. This also means the <u>more polar compounds elute first</u> from the column. Which of the two post-cleaved peptides is more polar and emerges from the column first? _____

Leucine zipper: a structural motif occurring in many transcription factors. It has heptad repeats of leucine residues at positions 1 and 4. These hydrophobic leucine regions provide an area of dimerization via adhesion between two alpha helices and allow the motifs to "zip" together. Does the original nonapeptide contain leucine residues at residue positions 1 and 4? _____ (yes = high chance, no = low chance)

Problem 2

leu – val – leu – trp – glu – lys – leu – his – arg
pKas: N-termini - 9.5, C-termini - 4.5, glu - 4.3, lys - 10.5, his - 6.0, arg - 12.5

Trypsin cleaves peptides after arg, lys, and AEcys.

At pH = very low: (pH < pKa ---> protonate)

CM-chromatography (also called cation exchange chromatography): separates ions and polar molecules based on their charge. It uses a negatively charged stationary resin, meaning the more positively charged compounds bind with higher affinity to the resin. This also means the more <u>negatively charged compounds elute first</u> from the column.

Finding the pH range:
chromatography resin pKa: 4.5 → This means that at a pKa of 5.5, 90% of the CM groups on the resin will deprotonate and carry a negative charge. The pH range will include a pH of 5.5. The <u>low mark for the pH range will be 4.5</u> because any pH less than 4.5 will protonate the CM groups to give them a neutral charge. Since the CM groups are mostly negatively charged then the two post-cleaved peptides must carry a net

positive charge in order to bind to the CM resin. Finding the lower pI between the two peptides serves as the high mark for the pH range.

Finding the pI of the first peptide:

$\overset{+}{NH_3}$ – leu – val – leu – trp – glu – $\overset{+}{lys}$ – COOH
9.5 4.3 10.5 4.5

Number Bank:
+2
+0
+1

pH < 4.3: net charge of _____
4.3 < pH < 4.5: glu deprotonates, net charge of _____
4.5 < pH < 9.5: COOH deprotonates, net charge of _____
pI = $(pKa_{COOH} + pKa_{NH3})/2$ = _____ pI of first peptide

Finding the pI of the second peptide:

$\overset{+}{NH_3}$ – leu – $\overset{+}{his}$ – $\overset{+}{arg}$ – COOH
9.5 6.0 12.5 4.5

Number Bank:
+1
+0
+2
+3

pH < 4.5: net charge of _____
4.5 < pH < 6.0: COOH deprotonates, net charge of _____
6.0 < pH < 9.5: his deprotonates, net charge of _____
9.5 < pH < 12.5: NH_3 deprotonates, net charge of _____
pI = $(pKa_{NH3} + pKa_{arg})/2$ = _____ pI of second peptide

Which pI is lower? _____ This serves as the high end of the pH range. Any pH higher than both pI will give the peptides a net negative charge. Any pH between the two pI will give the peptide with the lower pI a net negative charge. Thus, the high end of pH range has to be lower than the pI of both peptides so that the peptides bind to the negatively charged CM resin. 4.5 < pH range < lower pI
Thus, the pH range is between _____ and _____.

Which of the two post-cleaved peptides is more negatively charged (or less positively charged) and emerges from the column first? _____

Problem 3

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

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

Given:
lipid-membrane thickness: _____ nm
mass of protein: 90 kilodaltons or 90,000 daltons
average mass of residue: _____ daltons

Number Bank:
0.54
2
3.6
120
0.695
3.0

Finding the number of residues in 6 membrane-spanning α-helices:
First, find the amount of total residues:
6 α-helices x 3 nm thickness = _____ nm thickness of α-helices
_____ nm α-helices / 0.54 nm in a pitch = _____ no. of pitches
_____ no. of pitches x 3.6 residues per turn = _____ amount of residues

Second, find the mass of total residues:
_____ amount of residues x 120 daltons per residue = _____ daltons of residues

Third, find the percent of mass:
_____ daltons of residues / 90,000 daltons of protein x 100 = _____ %

Finding the number of residues in 12 membrane-spanning β strands:
First, find the amount of total residues:
12 β-strands x 3 nm thickness = _____ nm thickness of β-strands
_____ nm β-strands / 0.695 nm in a repeat = _____ no. of repeats
_____ no. of repeats x 2.2 residues per repeat = _____ amount of residues

Second, find the mass of total residues:
_____ amount of residues x 120 daltons per residue = _____ daltons of residues

Third, find the percent of mass:
_____ daltons of residues / 90,000 daltons of protein x 100 = _____ %

Problem 4

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{E} + \mathrm{P}$$

E = enzyme, S = substrate, ES = enzyme-substrate complex, P = product

Examination of the reaction:
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) convert to 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}. It is important to note that K_m in the general equation does not equal K_S.
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 $v_0 = k_{cat}[ES]$.
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 $M^{-1}s^{-1}$.

Rapid Equilibrium (Michaelis-Menten):

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{E} + \mathrm{P}$$

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

Show work and solution on your own paper!

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

V_{max} points to $k_2[E]_{total}$; K_S points to $\frac{k_{-1}}{k_1}$

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} points to $k_2[E]_{total}$; K_m points to $\frac{k_{-1}+k_2}{k_1}$

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

Basis for enzyme assay:

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

V_{max} points to $k_2[E]_{total}$

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

Given:

$K_1 = 2.0 \times 10^9\ M^{-1}\ s^{-1}$

$k_{-1} = 5.0 \times 10^4\ s^{-1}$

$k_2 = 4.0 \times 10^2\ s^{-1}$

Finding K_S, the substrate dissociative constant:

$K_S = \frac{k_{-1}}{k_1} =$ _____ M

Finding K_m, the Michaelis constant:

$K_m = \frac{k_{-1}+k_2}{k_1} =$ _____ M

If $K_S \approx K_m$ then It means $k_{-1} >> k_2$ and the enzyme follows rapid-state equilibrium.

If $K_S \neq K_m$ then it means k_2 is large enough for the enzyme to be follow steady-state.

Show work and solution on your own paper!

Problem 5

As the concentration of substrate approaches infinity (S >> K_m), initial velocity reaches maximal velocity and the reaction is dependent only on E ($v_o = V_{max} = k_2[E]_o$). If the concentration of substrate is decreased drastically (S << K_m), then the reaction is bimolecular and dependent on both S and E ($v_o = V_{max}[S]/K_m = k_2[E]_oS/ K_m$).

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

Find factor at which concentration of substrate increases in order for $v_o = 0.25V_{max} \rightarrow v_o = 0.75V_{max}$

For 75% of V_{max}:

$$\frac{v_o}{v_{max}} = 0.75 \quad \rightarrow \quad 0.75 = \frac{[S]_{0.75}}{K_m+[S]_{0.75}} \quad \rightarrow$$ Solve for $[S]_{0.75}$ = _____ K_m

For 25% of V_{max}:

$$\frac{v_o}{v_{max}} = 0.25 \quad \rightarrow \quad 0.25 = \frac{[S]_{0.25}}{K_m+[S]_{0.25}} \quad \rightarrow$$ Solve for $[S]_{0.25}$ = _____ K_m

Divide to find the factor:

$\frac{[S]_{0.75} = \_\_\_\_\ K_m}{[S]_{0.25} = \_\_\_\_\ K_m}$ = _____ x more [S]

Problem 6

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 – Km ↑, Vmax ↔

A direct competition exists between substrate and inhibitor for binding to the free enzyme. Thus, a higher level of substrate is required to obtain 1/2 V_{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 $V_{max}/2$, 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) – Km ↔. This holds when α = 1.

Mixed - K(m) ↑. This holds when α > 1.

K(m) ↓. This holds when α < 1.

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

Show work and solution on your own paper!

Kirk's Amazing Notes for Problem Set 2

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]

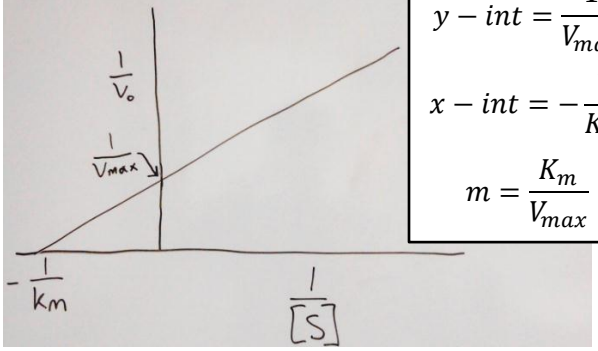
- double reciprocal plot (Lineweaver-Burk plot)
  - rearrange Michaelis-Menten equation
  - $\frac{1}{v_o} = \frac{K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$

- 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
  - $[S]_{bound} = \frac{[S]_{free}}{K_S}[E]_{free}$

<u>Lineweaver-Burk Plot</u>:

$$\frac{1}{v_o} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

y = m · x + b



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

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

$$m = \frac{K_m}{V_{max}}$$

[Arachidonate] mM	1/[Arachidonate] mM^{-1}	v_o mM/min w/o Ibuprofen	$1/v_o$ min/mM w/o Ibuprofen	v_o mM/min w/ Ibuprofen	$1/v_o$ min/mM w/ Ibuprofen
0.5		23.5		16.67	
1		32.2		25.25	
1.5		36.9		30.49	
2.5		41.8		37.04	
3.5		44		38.91	

Find the reciprocal for [Arachidonate], v_o without Ibuprofen, and v_o with Ibuprofen.

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

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

The x-axis has a scale ranging from -2.00 to 3.00 with the units as 1/[Arachidonate], mM^{-1}.

Show work and solution on your own paper!

Kirk's Amazing Notes for Problem Set 2

The y-axis has a scale ranging from -0.01 to 0.07 with the units as $1/v_o$ min/mM.

Add a linear trendline and the linear equation (y = mx + b) for both sets of data.
Linear trendline (w/ Ibuprofen): _____ Linear trendline (w/o Ibuprofen): _____

Given:
COX-2: _____
Arachidonate: _____
Prostaglandin G_2 (PPG_2): _____
Ibuprofen: _____; M_r 206 mg/mmol, presence of 10 mg/ml

Word Bank:
product
substrate
Michaelis enzyme
inhibitor

Determine the values of V_{max} and K_m for COX-2 w/o Ibuprofen and $V_{(max)}$ and $K_{(m)}$ in presence of Ibuprofen:

From the graph:

For COX-2 without Ibuprofen

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

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

V_{max} = _____ mM/min

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

Negative reciprocal of the x-int gives the K_m.

K_m = _____ mM

For COX-2 with Ibuprofen

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

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

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

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

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

$K_{(m)}$ = _____ mM

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

For COX-2 without Ibuprofen

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

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

V_{max} = _____ mM/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 = _____ mM

For COX-2 with Ibuprofen

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

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

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

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

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

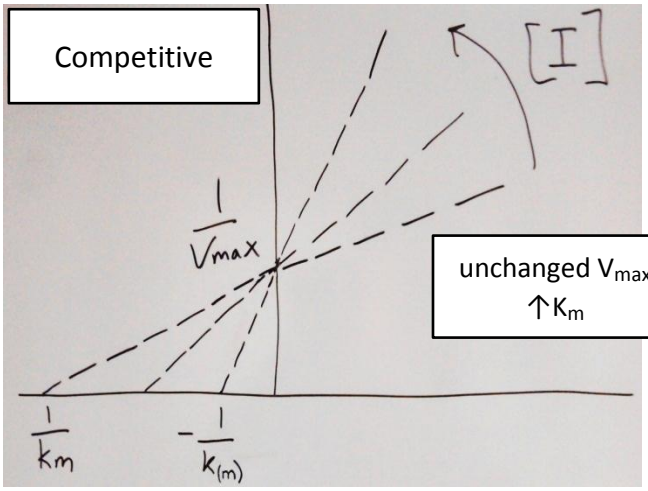
Negative reciprocal of the x-int gives the $K_{(m)}$.

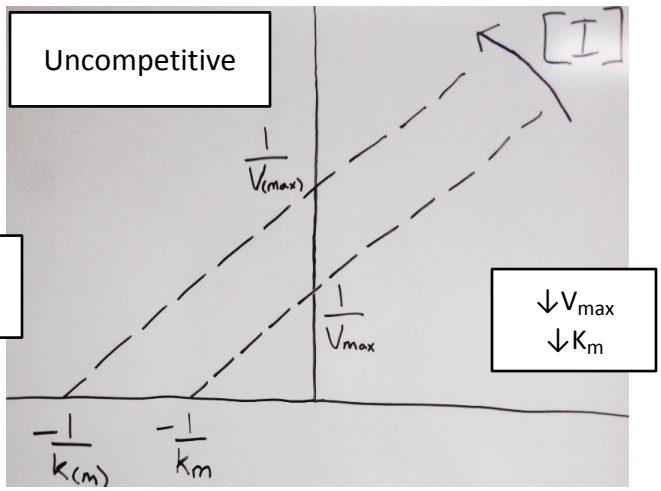
$K_{(m)}$ = _____ mM

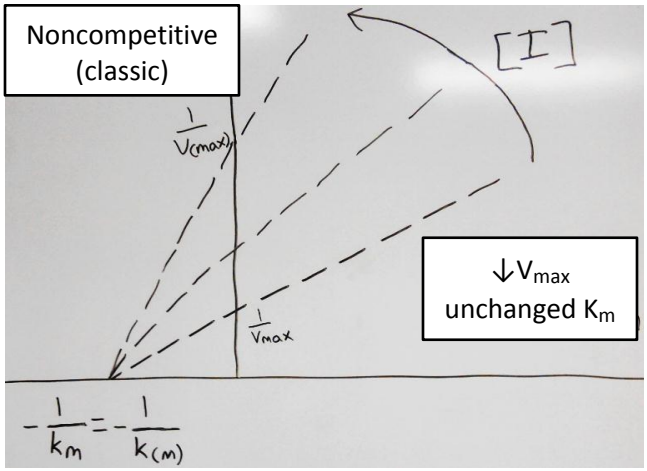
Units for Vmax and $V_{(max)}$ are in mM/min.
Units for Km and $K_{(m)}$ are in mM.

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)

Show work and solution on your own paper!







Which type of inhibitor (competitive, uncompetitive, or noncompetitive) is Ibuprofen?

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 _____ $K_{(m)}$

<u>Finding the K_I for Ibuprofen with COX-2</u>:

Depending on which type of inhibitor Ibuprofen is, use the table below to solve for K_I.

$K_{(m)}$: determined from the graph and linear equation for COX-2 with Ibuprofen; _____ mM

K_m: determined from the graph and linear equations for COX-2 w/o Ibuprofen; _____ mM

[I]: concentration of inhibitor Ibuprofen = $\frac{10\frac{mg}{ml}}{206\frac{mg}{mmol}} = 48.5mM$

K_I: dissociation constant for enzyme inhibitor complex with units in mM. **<u>Solve for this</u>**.

TABLE 13.6 The Effect of Various Types of Inhibitors on Apparent K_m and Apparent V_{max}

Inhibition Type	Apparent K_m	Apparent V_{max}
None	K_m	V_{max}
Competitive	$K_m(1 + [I]/K_I)$	V_{max}
Noncompetitive	K_m	$V_{max}/(1 + [I]/K_I)$
Mixed	$K_m(1 + [I]/K_I)/(1 + [I]/\alpha K_I)$	$V_{max}/(1 + [I]/\alpha K_I)$
Uncompetitive	$K_m/(1 + [I]/K_I)$	$V_{max}/(1 + [I]/K_I)$

Show work and solution on your own paper!

Problem 7

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 $v_0 = k_{cat}[ES]$.

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

Given:
COX-2: homodimer enzyme with 2 active sites; M_r 140,000 g/mol
k_{cat}: 5.0 x 10^7 min^{-1}
V_{max}: determined the graph and linear equations for COX-2 w/o Ibuprofen in problem 6A
_____ mM min^{-1}

$V_{max} = 2k_{cat}[E]_{total}$
(k_{cat} multiplied by 2 because there are 2 active sites per COX-2 molecule)

Finding the amount of COX-2 (g/mL) present in the assays:
First, solve for the total concentration of COX-2:
_____ mM min^{-1} = 2 x _____ min^{-1} $[COX\text{-}2]_{total}$
$[COX\text{-}2]_{total}$ = _____ mM / 1000 (convert to M)
$[COX\text{-}2]_{total}$ = _____ M

Second, solve for the grams of COX-2 per ml present in assays:
_____ $[COX\text{-}2]_{total}$ mol/L x _____ M_r g/mol = _____ COX-2 g/L
_____ COX-2 g/L x 1/1000 (convert to g/ml)
_____ COX-2 g/mL

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 $M^{-1}s^{-1}$.

Given:
k_{cat}: 5.0 x 10^7 min^{-1} x 1/60 (convert to sec^{-1}) = _____ sec^{-1}
K_m: determined from the graph and linear equations for COX-2 w/o Ibuprofen in problem 6A
_____ mM x 1/1000 (convert to M) = _____ M

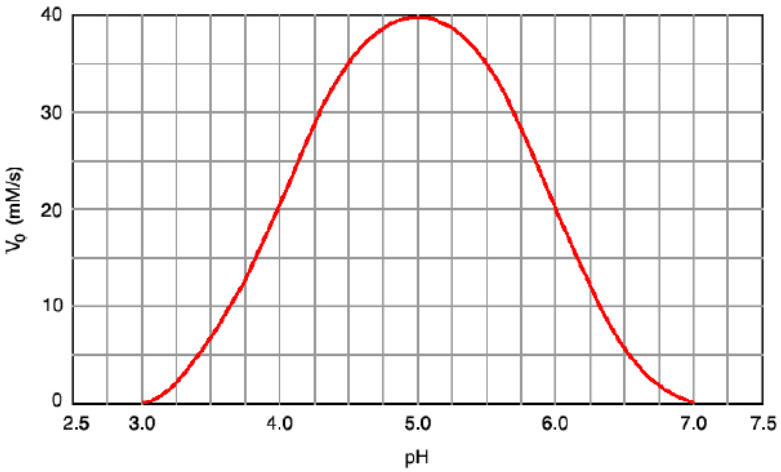
Enzyme efficiency:

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

A perfect enzyme has an efficiency ratio of 1 x 10^9 M^{-1} sec^{-1} and can only be limited by diffusion.
Can COX-2 be rated as a perfect enzyme?
Yes or no? _____ because…

Show work and solution on your own paper!

Problem 8



Amino acids containing side chains with pKa values:

asp - 3.9, glu - 4.3, his - 6.0, cys - 8.3, tyr - 10.1, lys - 10.5, arg - 12.5, ser - 13, thr - 13

Assuming the concentration of the substrate is very high ($S >> K_m$), initial velocity reaches maximal velocity and the reaction is dependent only on E ($v_o = V_{max} = k_2[E]_o$). The optimal pH range for this enzyme is the optimal pH ± 0.5. The optimal pH is the pH at which the reaction proceeds at maximal velocity ($v_o = v_{max}$). Thus, the optimal pH is _____ and the optimal pH range for this enzyme is between _____ and _____.

The two residues most likely involved in the enzyme reaction are the two residues containing side chains with pKa values closest to the pH range. Therefore, the two amino acids are _____ with a side chain pKa of _____ and _____ with a side chain pKa of _____. This is because these two residues would be ideal to function within the pH range and they would both be charged at an optimal pH range of ____-____. Amino acids with a side chain pKa farther from the optimal pH range would decrease the initial velocity.

Oligosaccharide	Rate Constant, k_{cat}
$(A\text{-}B)_6$	0.5
$(A\text{-}B)_4$	0.5
$(A\text{-}B)_2$	10^{-5}
A_8	0.3
A_7	0.3
A_6	0.3
A_5	0.025
A_4	10^{-7}
A_3	10^{-8}

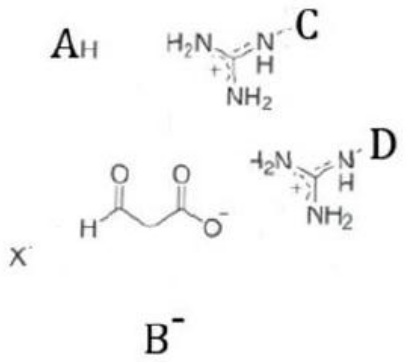
The enzyme Q hydrolyzes polysaccharides $(A\text{-}B)_x$ or $(A\text{-}A)_x$. To the left is a table of rate constants of the enzyme Q for several oligosaccharides.

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 $v_0 = k_{cat}[ES]$.

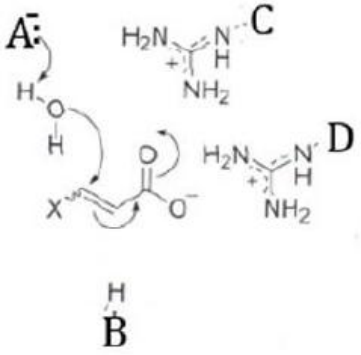
The minimal length for the substrate is the <u>least</u> residues forming the substrate in order to have a rate constant (k_{cat}) that is considerably high enough to create product formation (steady-state equilibrium). For example, the minimal length of the substrate cannot be 3 or 4 because A_3 and A_4 display low turnover rates and hardly any products will form. The minimal length could be 12 because $(A\text{-}B)_6$ has the necessary rate constant for product formation but it is <u>not</u> the absolute minimal length. Therefore, the minimal length of the substrate is _____ residues. Product formation can also inhibit forward reactions and this is evident in the low turnover rates for the inhibitory effects shown by the shorter substrates.

Problem 9

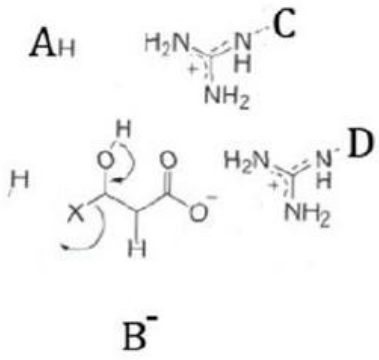
(a)



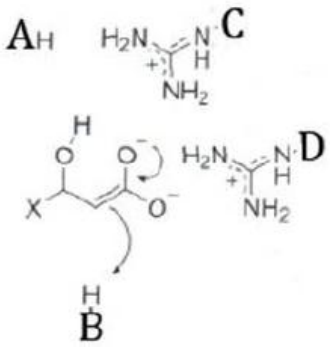
(b)



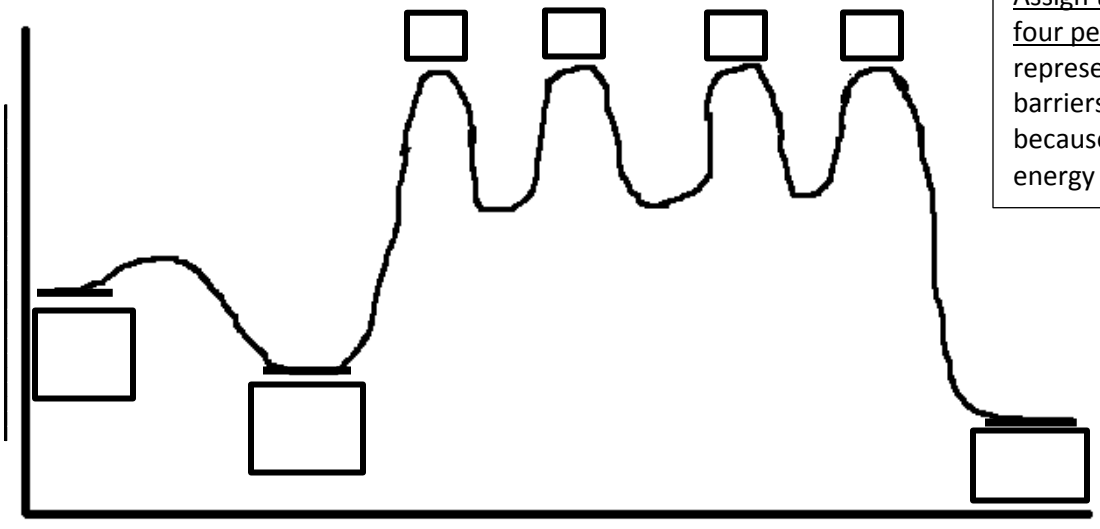
(c)



(d)



Free Energy Diagram



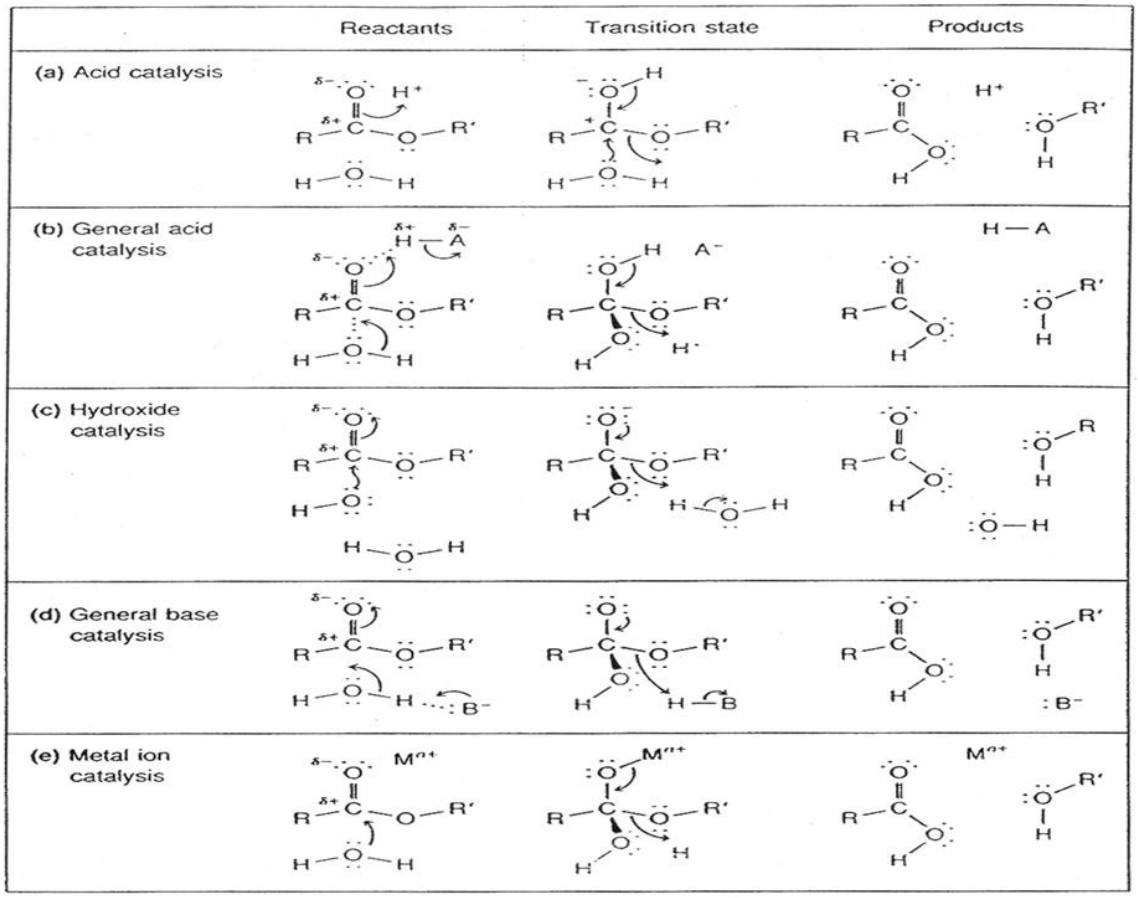
Assign the four stages to the four peaks. The peaks represent activation energy barriers, at the same height because their relative energy values are not given.

Word Bank:
E + P
Free Energy →
ES complex
E + S
Stage of Reaction
A, B, C, D

Show work and solution on your own paper!

Kirk's Amazing Notes for Problem Set 2

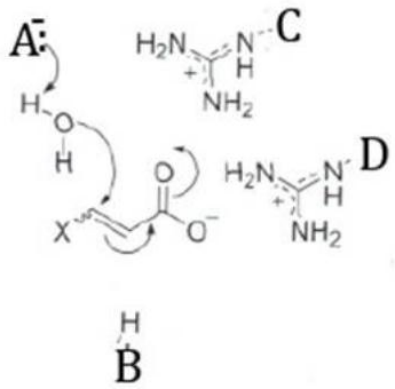
Catalytic Mechanisms



4

In the catalytic mechanism shown in stage (b) of the reaction, H-O-H acts as a(n) _____ because it attacks an electron-deficient carbon center. The process forms a(n) _____ in the transition state and this molecule is what catalyzes the reaction. H-O-H also acts as a(n) _____ because it _____ an H^+ to molecule A. As a result, molecule A acts as a(n) _____ because it _____ an H^+ from water. Thus, the catalytic mechanism shown here must be _____ (not in word bank, use table above).

(b)



Word Bank:
donates
acid
nucleophile
base
accepts
hydroxide

Show work and solution on your own paper!

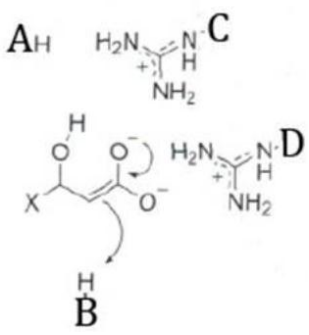
Number	Classification	Biochemical Properties
1	Oxidoreductases	Act on many chemical groupings to add or remove hydrogen atoms. $A^- + B \leftrightarrow A + B^-$
2	Transferases	Transfer functional groups between donor and acceptor molecules. Kinases are specialized transferases that regulate metabolism by transferring phosphate from ATP to other molecules. $A\text{-}B + C \leftrightarrow A + B\text{-}C$
3	Hydrolases	Add water across a bond, hydrolyzing it. $A\text{-}B + H_2O \leftrightarrow A\text{-}H + B\text{-}OH$
4	Lyases	Add water, ammonia or carbon dioxide across double bonds, or remove these elements to produce double bonds. $\begin{matrix} X & Y \\ \vert & \vert \\ A\text{-}B \end{matrix} \leftrightarrow A{=}B + X\text{-}Y$
5	Isomerases	Carry out many kinds of isomerization: L to D isomerizations, mutase reactions (shifts of chemical groups) and others. $\begin{matrix} X & Y \\ \vert & \vert \\ A\text{-}B \end{matrix} \leftrightarrow \begin{matrix} Y & X \\ \vert & \vert \\ A\text{-}B \end{matrix}$
6	Ligases	Catalyze reactions in which two chemical groups are joined (or ligated) with the use of energy from ATP. $A + B \leftrightarrow AB$

Using the enzyme-classification table above and the wisdom gained in problem 9B, under what EC type would this enzyme be classified?

The enzyme catalyzes a _____ reaction so it must be a(n) _____ type of enzyme!

Three amino acids that could function as B residue in stage (d). Specific role in the catalysis?

(d)



The B residue serves to protonate the double bond of the molecule. Which amino acids can donate an H^+ and act similarly to the B residue?

The general acidic amino acids can readily donate a proton due to their acidic character (low pKa). His has a low pKa for a basic amino acid and can deprotonate to donate a proton. Cys and Tyr can also donate a proton but are less likely due to their very basic character (higher pKa) and thus, higher affinity to stay protonated.

Problem 10

Serine proteases: contain an AspHisSer 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 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.

Use the detailed mechanism for the chymotrypsin reaction (Figure 14.21) as an analogy to sketch the steps for this serine protease and PMSF. Follow the steps and know that the reaction stops at (e) because there is no second release: 1. Binding of substrate, 2. Formation of covalent ES complex (note the low-barrier hydrogen bond here), 3. Proton donation by His-57. 4. S–F bond cleavage, and 5. Release of product.