

You will have 2 hours to complete this exam. There are **18** pages containing 8 questions worth 200 points and bonus questions worth 20 points. A 3 page supplement contains reference material and figures that might not reproduce well. Please answer the questions in the spaces provided. There are 2 blank pages at the back in case you need additional space. Be sure to indicate that you have completed a problem on these pages to insure that you receive credit for the work. Please show all of your work, including units, in order to receive full credit. You may use a calculator but you may NOT share a calculator with your neighbor.

READ THE QUESTIONS CAREFULLY! In several cases, you have the choice of which question you want to answer.

Pace yourself. Many of the questions have multiple parts, so do what you can on one question and then move to the next. The exam will most likely take you the full 2 hours to complete.

Please try to be neat. If we cannot read your writing, we cannot award you credit for your answer.

	Question 1	_____	/15
	Question 2	_____	/24
	Question 3	_____	/35
	Question 4	_____	/35
	Question 5	_____	/25
A / B	Question 6	_____	/24
A / B	Question 7	_____	/32
	Question 8	_____	/10
	Bonus	_____	/20
	TOTAL:	_____	/200

1) Define **any 5** the following terms: **(3pts. each/15 pts. total)**

a) transition state

b) cooperative binding

c) rate-determining step

d) intrinsic binding energy

e) effective concentration

f) zymogen

g) steady-state approximation

h) Michaelis Constant

2) Each of the statements below is untrue. For 6 of the statements below, fix them to make the statements true or explain why the statement is incorrect (**4 pts. Each/24 pts total**)

- a) The directionality of biomolecules is of great importance. By convention, unless labeled otherwise, proteins are represented from C → N and nucleic acids 5' → 3' and sugars from non-reducing → reducing ends.
- b) The pH titration curve of Asn exhibits 3 buffering regions centered around the pK_a of the titratable groups at 2.1, 3.9 and 9.8 and a pI of 6.9.

- c) Ramachandran plots are graphical representations of protein structures that plot ψ (C_O-C_α angle) versus ϕ ($N-C_\alpha$ angle) and allow the determination of tertiary structure types since these structures cluster in specific regions of the plot.
- d) Alpha-amylase and glycogen are both homoglycans of D-glucose, but differ in the stereochemistry of the anomeric carbons as well as the extent of their branching.
- e) The facile interconversion of furanose and pyranose forms of hexoses such as glucose and fructose relies on the kinetic instability of the glycosidic bonds found in the cyclic monosaccharides.
- f) The incorporation of unsaturated fatty acids into triacylglycerides results in lipid bilayers with higher melting temperature due to the poor ability of these unsaturated fatty acids to pack efficiently with the more common saturated fatty acid chains.

- b) Identify the catalytic function for each member of the triad you drew in part b. **(6 pts)**
- c) What are low-barrier hydrogen bonds and how do they relate to the mechanism of serine proteases? **(7 pts)**
- d) Describe the oxyanion hole and explain from a mechanistic standpoint its role in the peptide hydrolysis reaction. **(7 pts)**

- e) Mechanistically speaking, how does the aspartic protease of HIV-1 differ from serine proteases? **(7 pts)**

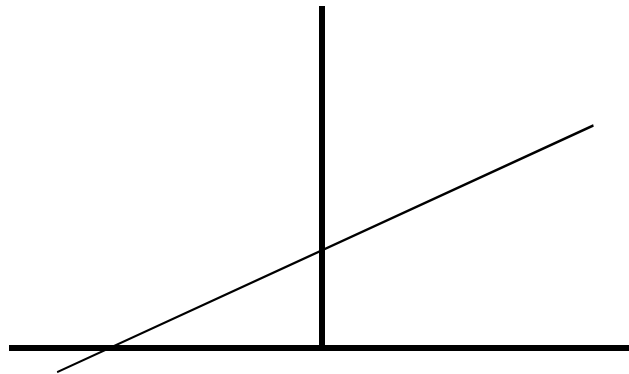
4) Enzyme kinetics and inhibition question **(35 pts.)**

- a) What is the Michaelis-Menten equation? Define all of the terms. **(7 pts)**

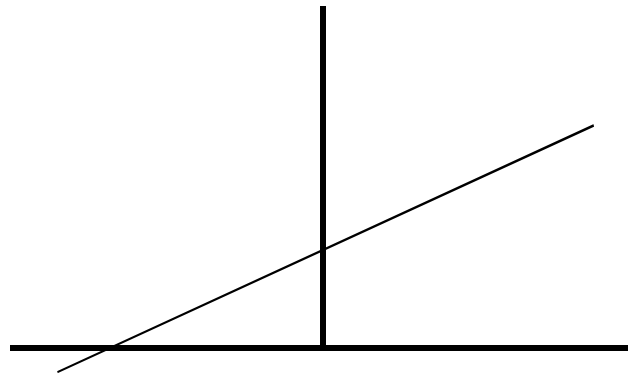
- b) Identify 2 approximations incorporated into the Michaelis-Menten equation and explain how you would insure experimentally that these approximations are satisfied in a hypothetical kinetic study you are designing. **(6 pts)**

- c) The rate data for a hypothetical enzyme kinetics study derive from progress curves. Draw a typical progress curve (be sure to label the axes appropriately) and identify which data you will use for your Michaelis-Menten analysis. (4 pts)

- d) A Lineweaver-Burke plot is shown below. Label the axes of this plot (including units) and identify how V_{\max} and K_M are obtained from it. (6 pts)

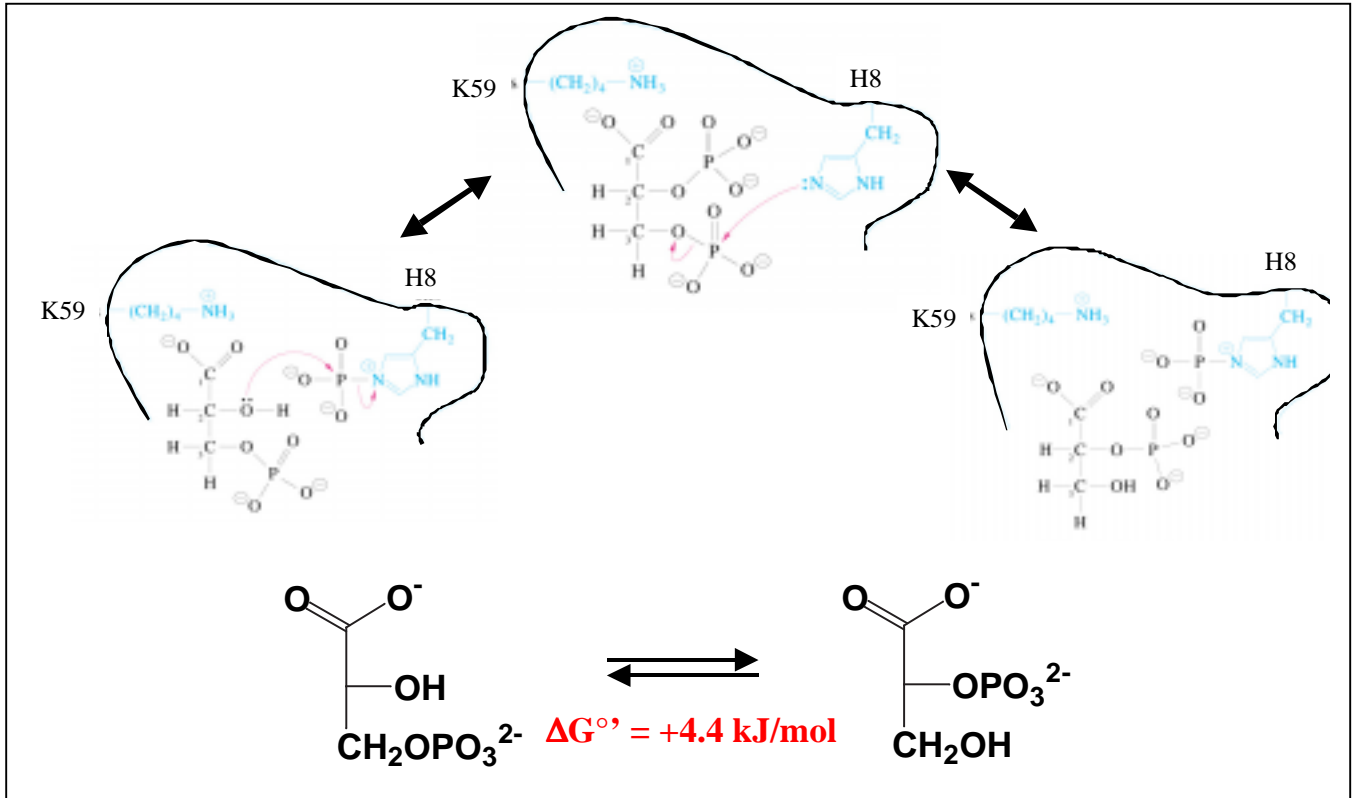


- e) Add to the **Lineweaver-Burk plot** lines that would be indicative of a competitive inhibitor at 5, 15 and 30 μM if the $K_i = 15 \mu\text{M}$. Indicate the value of α for each line and draw the lines to scale to the best of your ability. (8 pts)



- f) Transition state analogs are often considered among the best enzyme inhibitors. What category of inhibitors would a transition state analog fall under and why are they such good inhibitors of their target enzymes? (4 pts)

5) Phosphoglycerate mutase is an enzyme that catalyzes the 8 step of glycolysis. The yeast enzyme converts 3-phosphoglycerate to 2-phosphoglycerate as shown in the reaction mechanism below. Use this figure and your knowledge of mechanistic enzymology to discuss this reaction and make hypotheses regarding specific proposed mutations. The figure is reproduced in the supplement if it is hard to read it here. **(25 pts)**



a) What is the role of Lys59 in the mechanism? **(2 pts)**

b) You perform a multiple sequence alignment and you find that another histidine is 100% conserved. Superimposing this alignment on the crystal structure, you find that it is located in the active site about 5 Å from H8. Add H181 to the diagram above and show how it might participate in this reaction. **(5 pts)**

c) Identify the modes of catalysis apparent in this enzymatic transformation. (4 pts)

d) You isolate a sample of phosphoglycerate mutase and store it in your lab's freezer. When you take it out of the freezer, you find that it is inactive. However, if you add a small amount of 2,3-bisphosphoglycerate, the enzyme can then convert 3-phosphoglycerate to 2-phosphoglycerate at its normal rate. Explain these results based on the diagram above and your understanding of enzyme mechanisms and thermodynamics. (6 pts)

e) Predict whether each of the following mutations will affect K_M , k_{cat} or both. (8 pts)

Mutation	K_M	k_{cat}	why?
example: H8A	no change	inactive enzyme	no phosphate donor for 1st step
H8S			
K59R			
K59A			
H181E			

6) Answer **EITHER A OR B** on the next page. Please indicate on the front cover of the exam which question you have answered. (24 pts.)

A. Buffers and pH

- a. Tris is a common biological buffer with a pK_a of 8.1 (MW = 121.1). If 6.06 g of Tris were dissolved in 1 L of water, what would the pH of the solution be?
- b. How much 1 N HCl would you have to add to adjust the pH to 7.5?
- c. In enzyme active sites, the pK_a of amino acid side chains are often altered from their “normal” values as would be measured free in solution. Explain how enzymes shift the pK_a 's of these functional groups and give a discrete example from an enzyme we have studied.

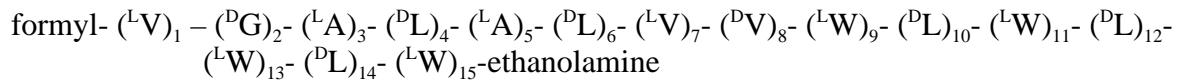
B. Thermodynamics and coupled reactions

- a. What are the differences between ΔG , ΔG° and $\Delta G^{\circ'}$?
- b. In question 5 you saw the enzyme phosphoglycerate mutase that converts 3-phosphoglycerate into 2-phosphoglycerate. $\Delta G^{\circ'} = +4.4 \text{ kJ mol}^{-1}$ for this reaction. Calculate the equilibrium constant for this reaction at 37 °C.
- c. Calculate ΔG for this transformation under physiological conditions (T = 37 °C, pH 7).
- d. Is ΔG for this reaction pH dependent? Why or why not?

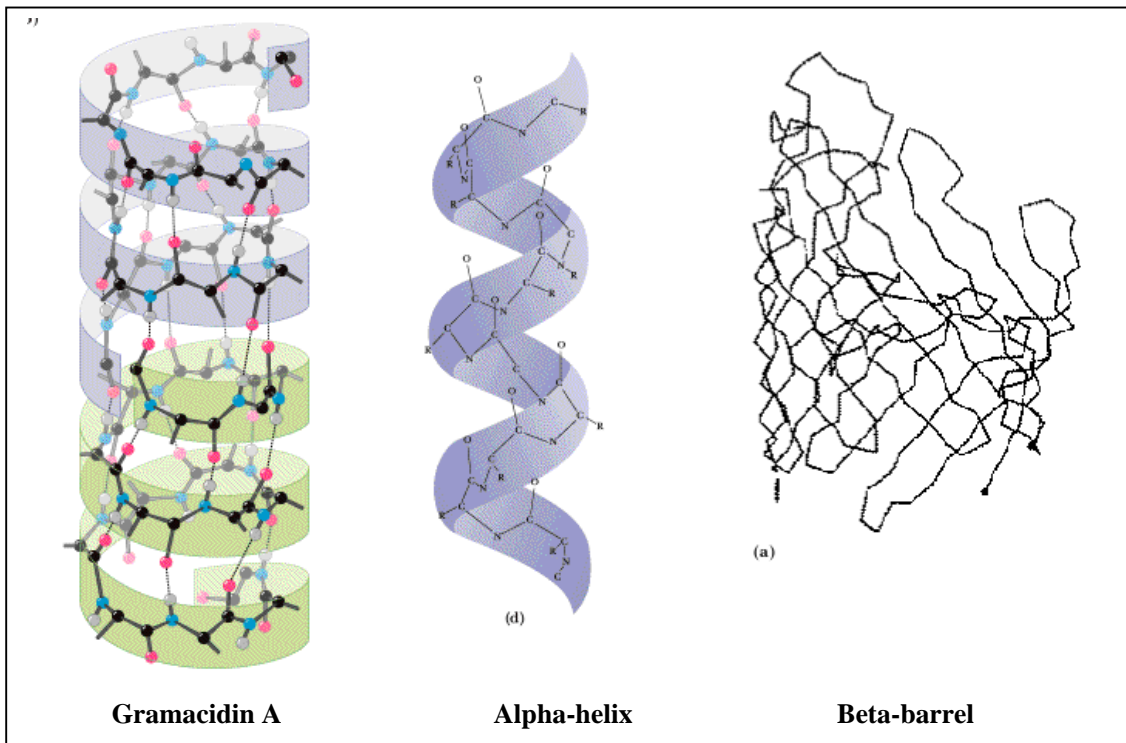
7) Comparison and contrast questions. **ANSWER EITHER A OR B.** Please indicate on the front cover of the exam which question you have answered. (32 pts.)

- A.** The hammerhead ribozyme with RNase A both catalyze the cleavage of RNA phosphodiester bonds. The structure figures can be found in the exam supplement and will be projected on the front screen during the exam. Compare and contrast the structures of these two molecules and the way they catalyze their respective cleavage reactions.
- B.** Gramicidin is a short (15 residues) peptide of alternating L- and D-amino acids modified at the N- and C-termini as indicated. It is an example of a channel forming ionophore whose structure we did not discuss explicitly in class. Using the figure below to compare and contrast the structures of the gramicidin helix, a membrane spanning α -helix, and β -barrel such as that from a porin.

Gramacidin A:



The superscript indicates the stereochemistry of the amino acid



8) This exam period is too short to cover every topic we learned this semester. So, write a short answer question on a topic that you prepared for but **DOES NOT APPEAR ANYWHERE ELSE ON THIS EXAM** and then **ANSWER IT**. If you repeat a question from a previous exam in this course, you will receive no credit due to lack of creativity. Be original. Use only the space provided on this page. **(10 pts)**

BONUS QUESTIONS (Answer one or both as time allows.):

A) Biochemical Structures (0.5 pt each, 10 pts total)

Ala	Cys	Glu
Asp	Trp	Tyr
Leu	Ile	Val
Met	Arg	Thr
ATP	Guanosine	Uracil
Cytidine	Fructofuranose (Haworth)	Phosphatidylserine (1 pts)
Aracidonic acid (24:4 $\Delta^{5,8,11,14}$)		

Physical constant and data that might be required for certain problems on this exam.

Amino Acid Hydrophathies

Amino Acid Hydrophathy	KJ/mol
Alanine	1.0
Arginine	-7.5
Asparagine	-2.7
Aspartic Acid	-3.0
Cysteine	0.17
Glutamic Acid	-2.6
Glutamine	-2.9
Glycine	0.67
Histidine	-1.7
Isoleucine	3.1
Leucine	2.2
Lysine	-4.6
Methionine	1.1
Phenylalanine	2.5
Proline	-0.29
Serine	-1.1
Threonine	-0.75
Tryptophan	1.5
Tyrosine	0.08
Valine	2.3

Concentrations of metabolites

Metabolite	Conc. μM
1,3-bisphosphoglycerate	1.0
2-phosphoglycerate	30
3-phosphoglycerate	120
ADP	140
ATP	1850
Dihydroxyacetone phosphate	140
Fructose-1,6-P	31
Fructose-6-P	14
Glucose	5000
Glucose-6-P	83
Glyceraldehyde-3-P	19
Glycerol-3-phosphate	25
Lactate	2900
NAD ⁺	540
NADH	50
Phosphoenolpyruvate	23
P _i	1000
Pyruvate	51

Standard Free Energies of hydrolysis for common metabolites

Metabolite	$\Delta G^{\circ'}_{\text{hyd}}$ kJ mol ⁻¹
Phosphoenolpyruvate	-62
1,3-bisphosphoglycerate	-49
Phosphocreatine	-43
Pyrophosphate	-33
Phosphoarginine	-32
Phosphohistidine	-32
ATP \rightarrow AMP + PP _i	-32
Acetyl-CoA	-32
ATP \rightarrow ADP + P _i	-30
Glucose-1-Phosphate	-21
Glucose-6-Phosphate	-14
AMP \rightarrow Adenosine + P _i	-14
Glycerol-3-phosphate	-9

Constants:

$R = 8.315 \text{ J mol}^{-1} \text{ K}^{-1}$

$F = 96.48 \text{ kJ V}^{-1} \text{ mol}^{-1}$

$k = 1.381 \times 10^{-23} \text{ J K}^{-1}$

Conversion Factors:

1 cal = 4.184 J

T in K = °C + 273

Relevant Equations:

$\text{pH} = \text{pK}_a + \log\left[\frac{[A^-]}{[HA]}\right]$

$\Delta G^{\circ'} = -RT \ln(K_{\text{eq}})$

$\Delta G = \Delta G^{\circ'} + RT \ln(Q)$

$\Delta G^{\circ'} = \Delta G^{\circ} + RT \ln[H^+]$

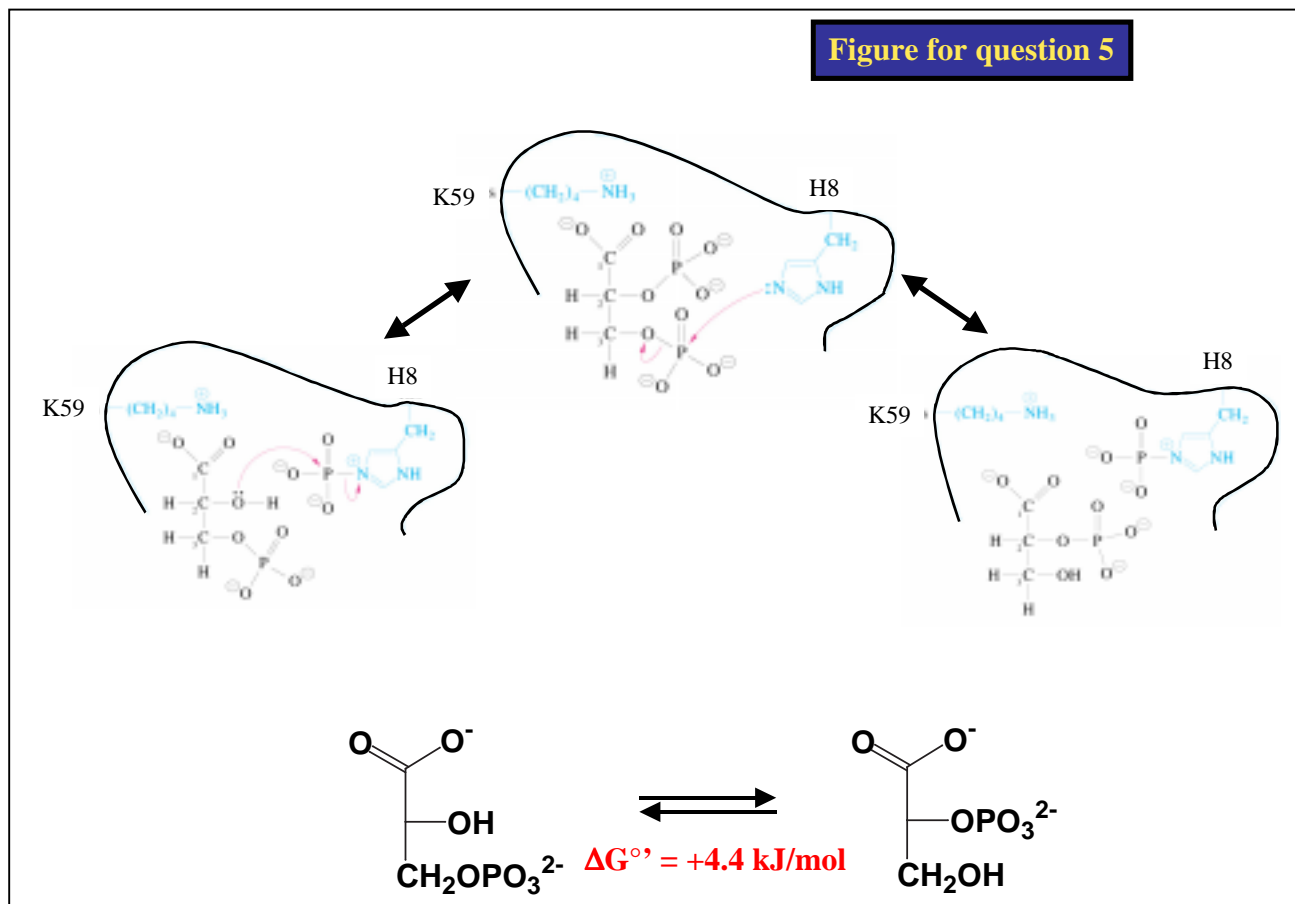
Enzyme Inhibition Equations:

Competitive: $\frac{1}{v_0} = \frac{\alpha K_M}{V_{\text{max}}} \frac{1}{[S]} + \frac{1}{V_{\text{max}}}$

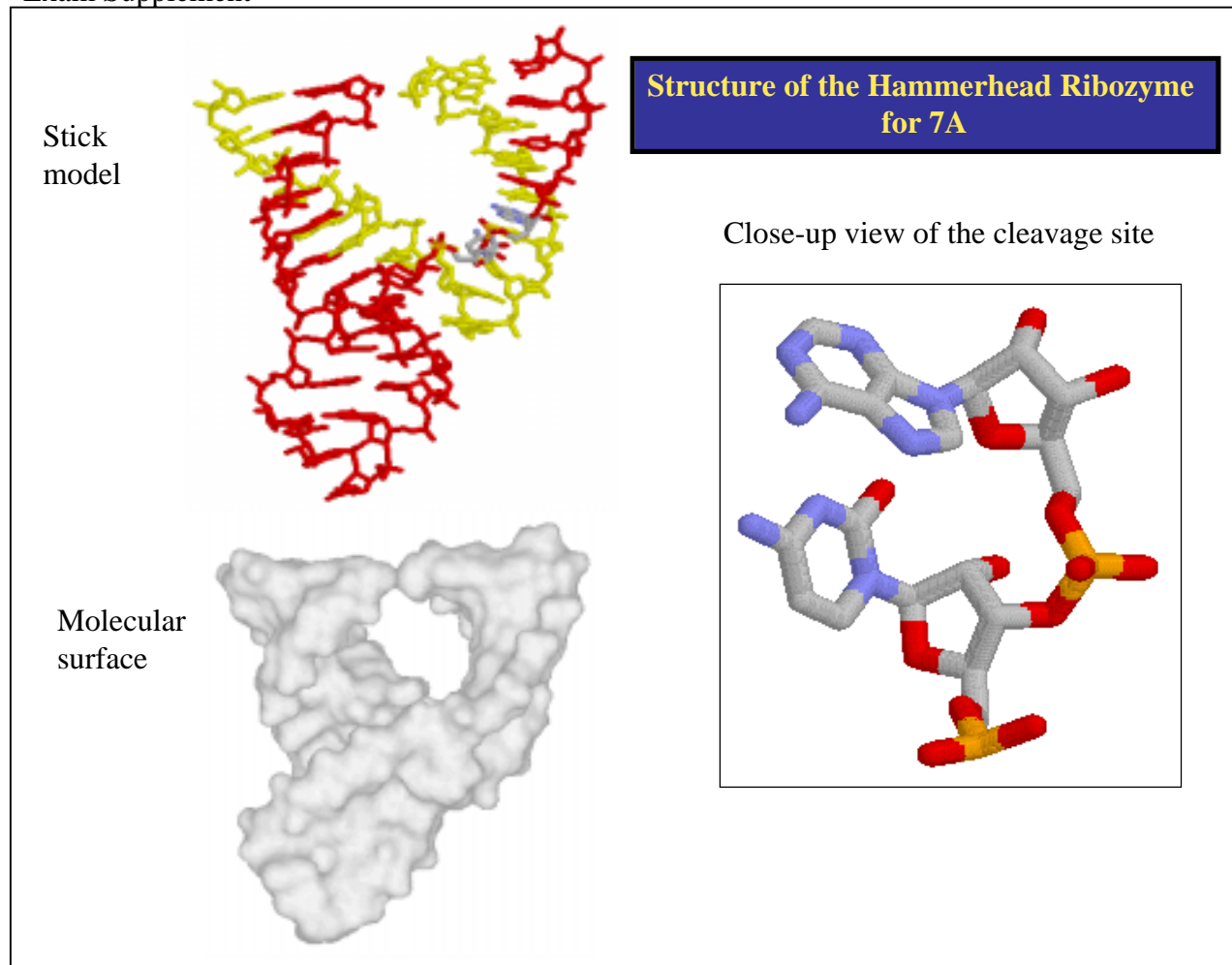
Uncompetitive: $\frac{1}{v_0} = \frac{K_M}{V_{\text{max}}} \frac{1}{[S]} + \frac{\alpha'}{V_{\text{max}}}$

Mixed: $\frac{1}{v_0} = \frac{\alpha K_M}{V_{\text{max}}} \frac{1}{[S]} + \frac{\alpha'}{V_{\text{max}}}$

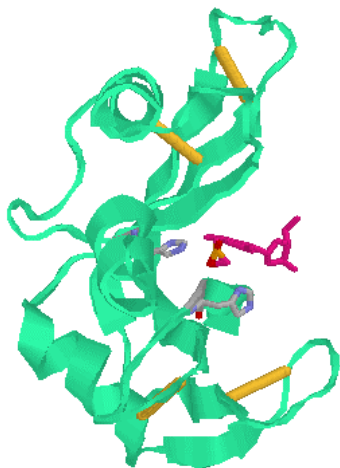
Exam Supplement



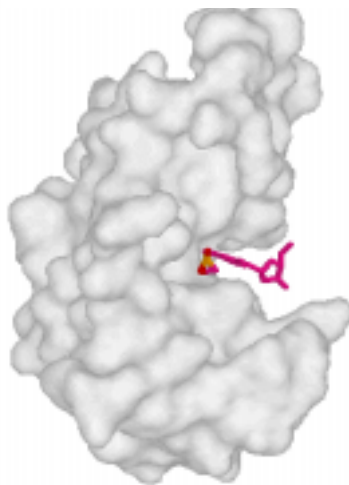
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Cartoon
model

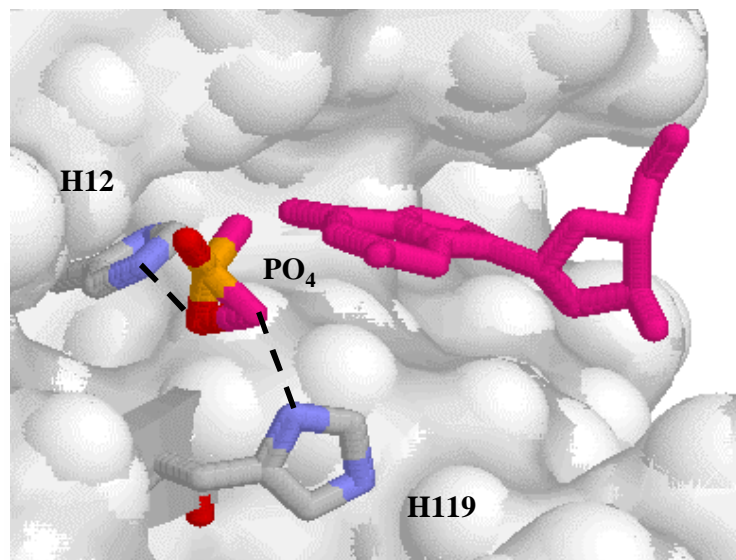


Molecular
surface



Structure of RNase A for 7A

Close-up view of the cleavage site



The nucleoside in the active site does not represent the appropriate binding mode for an RNA substrate. H12 and H119 are catalytically important residues.

Exam Supplement

