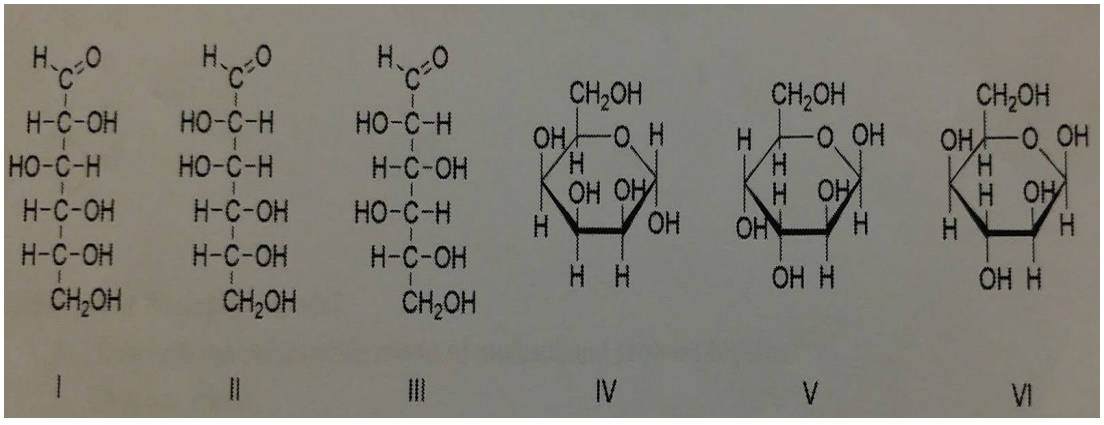
Midterm 1

Multiple Choice Questions. Once correct answer per question. (1 Mark each unless otherwise stated):

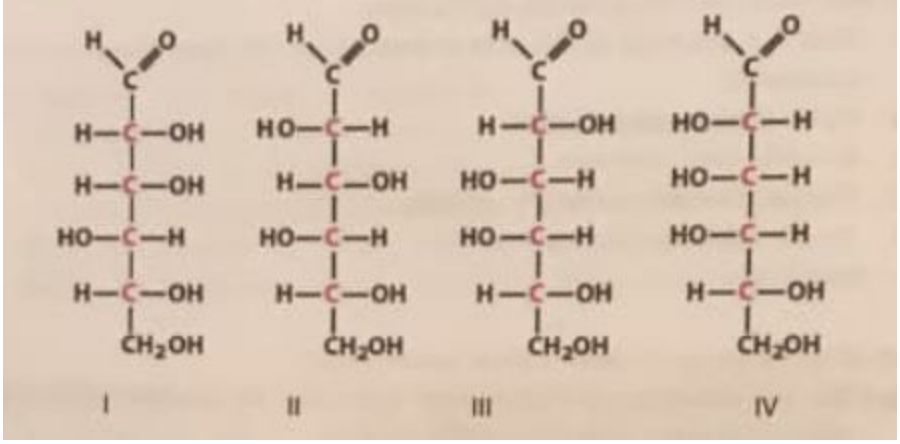
1. Which amino acid has three pK_a's?
    a. Leucine
    b. Proline
    c. Cysteine
    d. Threonine
    e. Glycine
2. What is suggested in the "RNA world" hypothesis?
    a. Arginine, asparagine, and alanine were the first three amino acids
    b. Life was thought to evolve 6 billion years ago
    c. DNA evolved later due to the instability of RNA
    d. RNA was introduced to the early Earth from a meteorite
    e. Chemical building blocks can be derived only from complex organic compounds
3. At pH 2.2, the concentration of lactic acid is 0.60 M and the concentration of lactate is 0.01 M. What is the pK_a of lactic acid?
    a. 0.4
    b. 2.5
    c. 3.1
    d. 4.0
    e. 4.8
4. What is true for a spontaneous process?
    a. $\Delta G > 0$
    b. If $\Delta S < 0$, ΔH must be < 0
    c. It is called an endergonic process
    d. It is always linked to an endergonic process
    e. The reaction happens faster than an exergonic process
5. Both D-ribose and D-glucose
    a. Can form intramolecular hemiacetal bonds
    b. Are hexoses
    c. Lack functional groups that can form glycosidic linkages
    d. Exist mainly in their pyranose form in biopolymers
    e. Are crucial building blocks for nucleic acids

6. For which of the following pairs can the amino acid side chains interact via electrostatic interactions at neutral pH?
    a. Leu - Met
    b. Cys - Val
    c. Asp - Arg
    d. Tyr - Gly
    e. Glu - Ala
7. What statement concerning biopolymers is true?
    a. Their formation was shown to be thermodynamically spontaneous in the Miller-Urey experiment
    b. RNA is more stable than DNA
    c. $\Delta S > 0$ for their formation
    d. They are thermodynamically unstable
    e. Their breakdown releases water
8. Which of the following statements about water is false?
    a. The high dielectric constant of water means that electrostatic interactions are stronger in water than in non-polar solvents
    b. The hydrophobic effect is driven by the entropy of the aqueous solvent
    c. Water molecules form more hydrogen bonds in ice than in liquid form
    d. Water is both an efficient hydrogen bond donor and hydrogen bond acceptor
    e. The hydrogen bonding network among water molecules enables very fast movement of H^+ and OH^-
9. Identify the pair that shows the same monosaccharide in the linear (Fischer) and the cyclic form, respectively.



    a. I and IV
    b. II and V
    c. III and IV
    d. III and VI
    e. II and VI

10. If a polypeptide has 400 amino acid residues, what is the approximate molecular weight?
    a. 11,000
    b. 22,000
    c. 44,000
    d. 88,000
    e. 176,000
11. What is true for the illustrated monosaccharides?



    a. (I) is an aldopentose
    b. (II) and (III) are enantiomers
    c. (I) and (IV) are epimers
    d. (III) and (IV) are epimers
    e. They are all L-sugars
12. At pH 1, what is the net charge of WINTER? (2 marks)
    a. -2
    b. -1
    c. 0
    d. +1
    e. +2
13. What is true for peptide bonds?
    a. They hold the polypeptide chains of multisubunit proteins together
    b. They are planar
    c. They are formed by a hydrolysis reaction
    d. Different types are found in different proteins
    e. Both (b) and (c) are correct

14. What is true for nucleosides?
    a. They contain phosphates
    b. They contain nitrogenous bases and monosaccharides
    c. They can be linked together to form nucleic acids
    d. They contain hexose sugars
    e. All of the above
15. Which of the following pairs of amino acid residues are most likely to be found at the surface of a water-soluble protein?
    a. Thr and Asn
    b. Thr and Ile
    c. Val and Ile
    d. Gln and Val
    e. Val and Thr
16. At pH 10.5, what is the net charge of CINEMA? (2 marks)
    a. -4
    b. -3
    c. -1
    d. 0
    e. +3
17. Which bases pair together in DNA?
    a. Guanine and cysteine
    b. Adenine and uracil
    c. Guanine and adenine
    d. Cysteine and thymine
    e. Thymine and adenine

True/False Questions (2 Marks Each):

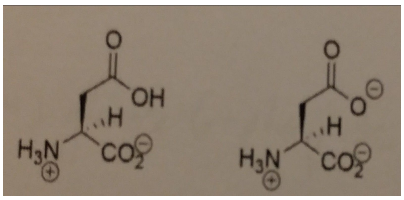
18. The isoelectric points for the free amino acids S and Ile will be approximately the same. T/F? Explain.
    True. Neither of the side chains are ionizable, making the isoelectric point depend on the backbone amine and carboxylate pK_a values, which are almost the same for both.

19. The bases A, G, and T were found in a nucleic acid. The nucleic acid was most likely RNA. T/F? Explain.
    False. The presence of T suggest that it is DNA

20. Considering only sidechain interactions between the pairs listed: Ser and Gly will interact stronger with each other than Leu and Phe. T/F? Explain.

False. Gly doesn't have a side chain. Leu and Phe will make favourable van der Waal's and hydrophobic interactions.

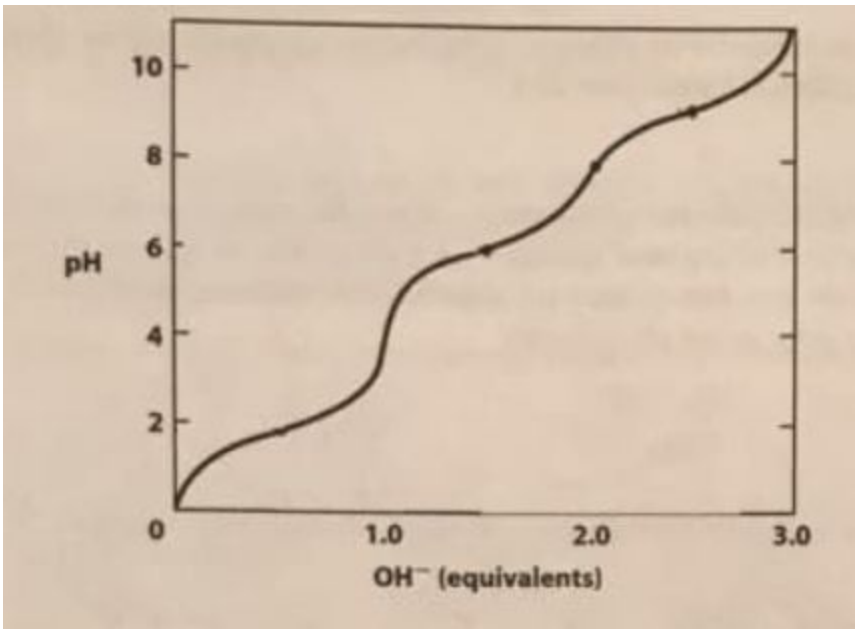
21. A mixture of the two compounds shown below would be ideal for making a buffer at pH 6. T/F? Explain.



False. The pK_a for the sidechain is around 4 (3.65 according to the textbook), meaning that there would be no buffer capacity left at pH 6

Short Answer Questions:

22. A titration curve for an amino acid is illustrated below. Which amino acid is it most likely to be (name, 3 letter code, & one letter code)? What is the side chain pK_a value for this amino acid? (4 marks)



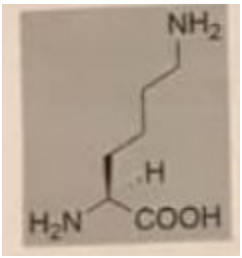
Histidine, His, H. Side chain pK_a = 6

23. The hexokinase enzyme catalyses the phosphorylation of glucose to glucose-6-phosphate. For the reaction between glucose and ATP, forming glucose-6-phosphate and ADP, calculate ΔG° and the equilibrium constant K_{eq} at 25°C based on the given values for the following reactions (4 marks):

1) Glucose-6-phosphate + H_2O -> glucose + P_i ΔG° = -16.7 kJ/mol
2) ATP + H_2O -> ADP + P_i ΔG° = -30.5 kJ/mol

$$\Delta G° = \Delta G°_{2} - \Delta G°_{1} = -30.5 - (-16.7) = -13.8 kJ/mol$$
$$\Delta G° = -RT\ln K,\ K = e^{(-\Delta G/RT)} = e^{(-(-13.8)/(8.316*10^{-3})(298))} = 262$$

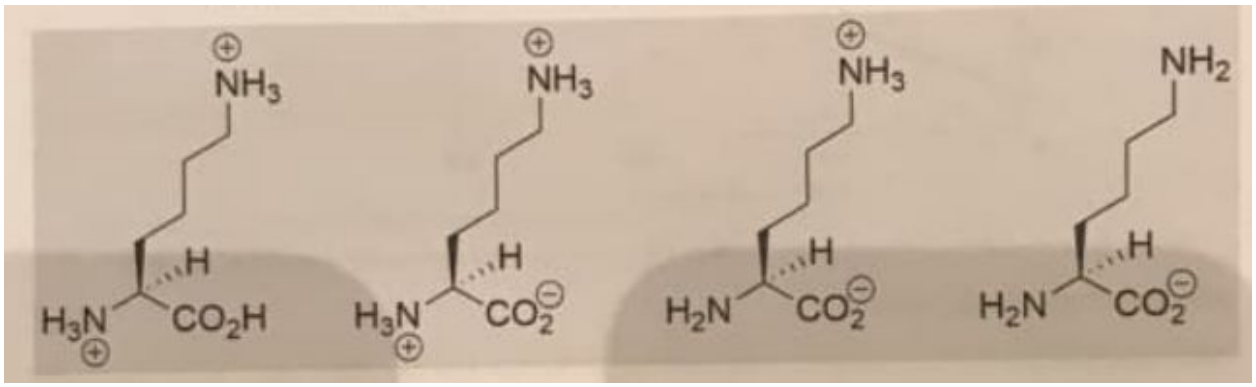
24. The structure of an amino acid in its neutral state is given below.



a. Give the name, three letter code and one letter code for the amino acid. (2 marks)
Lysine, Lys, K

b. List the pK_a values for the different ionisable groups in the amino acid. (2 marks)
Amine: 9.4, COOH: 2.2, side chain: 10.5

c. Draw the different charged states of the amino acid that would be present during a complete titration with strong base, starting at pH 1 and continuing until the titration is complete. Order the ionization states in the order they would appear in the titration, starting at the lowest pH. (7 marks)



25. For the pentapeptide Glu-Met-Arg-Thr-Gly,

a. Name the carboxyl terminal residue. (1 mark)
Glycine (Gly, G)

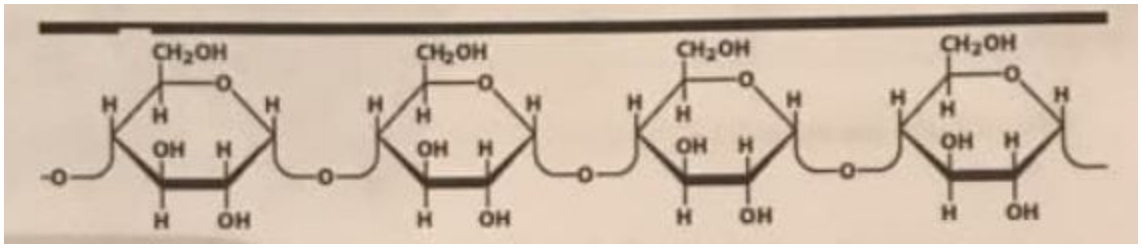
b. Name the amino terminal residue. (1 mark)
Glutamate (Glu, E)

c. Which groups will be charged at pH 1 (including the termini if relevant)? (2 marks)
Backbone amine (+1), Arg sidechain (+1)

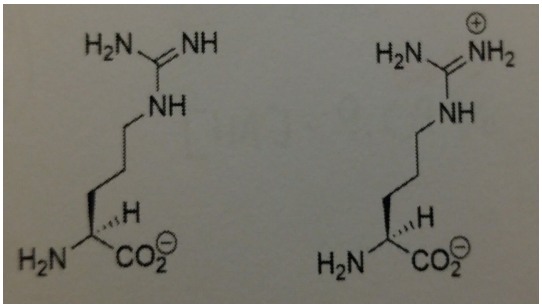
26. Part of a polysaccharide is shown below.

a. What kind of bond is found between the monomers and what would you name it?
Bond: O-glycosidic bond (Glycosidic bond is acceptable)
Name: α1->4

b. Given the nature of the bond named in (a), is the polysaccharide likely to be digestible by humans? Why/Why not? (2 marks)
Yes, it is likely to be digestible by humans. The bonds are the same type as in glycogen and starch, which can be digested.

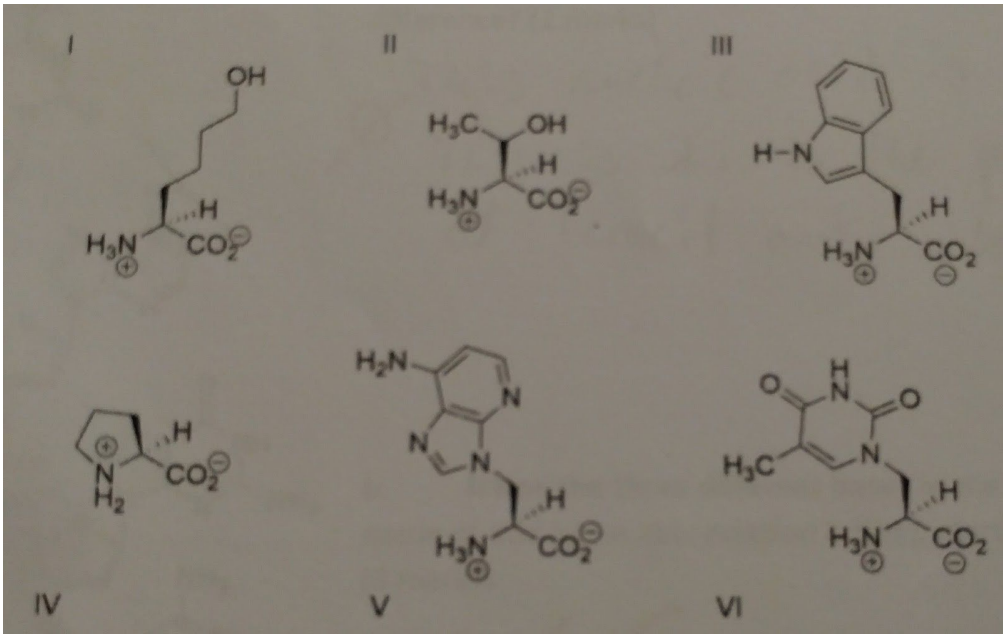


27. During a titration with strong base, at which pH would the concentration of the two compounds shown below be equal? (1 mark)



At 12.5 (pK_a of Arg sidechain)

28. Among the six structures shown below, three are proteogenic amino acids. Fill in the table with their names, three letter codes, and one letter codes. (6 marks)



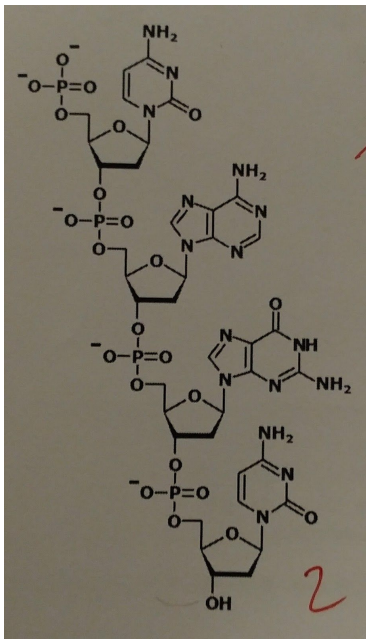
Compound number	II	III	IV
Name	Threonine	Tryptophan	Proline
3-letter code	Thr	Trp	Pro
1-letter code	T	W	P

29. The charged form of the imidazole ring on the side chain of histidine is believed to participate in a reaction catalyzed by an enzyme. At pH 7, what (approximate) proportion of the imidazole ring will be charged? (2 marks)

$pH = pK_a + log([A^-]/[HA])$, $[A^-]/[HA] = 10^{pH-pK_a} = 10^{7-6} = 10$, $[A^-] = 10[HA]$

$[HA]/([A^-] + [HA]) = [HA]/11[HA] = 1/11 = 9.1\%$

30. The structure below shows parts of a nucleic acid.



a. Is the nucleic acid DNA or RNA? How can one tell the difference? (2 marks)

It is DNA. It lacks the 2' OH group.

b. Name the three different bases in the structure (full name or one letter abbreviation is fine), starting from the top. (3 marks)

1. Cytosine (C) 2. Adenine (A) 3. Guanine (G)

c. Where in the figure in the 3' end? And the 5' end?

The 3' end is the lower end. The 5' end is the upper end.

Midterm 2

If you need the gas constant, the following value can be used: $R = 8.316\ J/(mol \cdot K)$

Multiple Choice Questions. One correct answer per question. (1 mark each)

1. What is true for DNA?
    a. DNA in the B-DNA form is rarely observed in cells
    b. The two chains in the DNA double helix are identical, wherefore each can serve as a template in replication.
    c. Due to the double helical structure, DNA is generally a very rigid molecule
    d. Hairpin structures can form upon intrastrand base pairing
    e. All of the above are true
2. Fatty acids
    a. Are found in all lipid molecules
    b. Are classified as omega-3 fatty acids if they have three double bonds
    c. Generally only have trans double bonds
    d. Have higher melting points the more carbon atoms they have
    e. Most often have more than 28 carbon atoms
3. Which three amino acids all have hydrogen bond donors in their side chains at pH 7.4?
    a. TND
    b. RGS
    c. WTF
    d. YES
    e. HKQ
4. RNA
    a. Can have enzymatic activity
    b. Can form complex structures even when single stranded
    c. Of the messenger class (mRNA) can be seen as an intermediate between DNA and protein
    d. Undergoes hydrolysis at high pH
    e. All of the above are true

5. Which of the following statements about lipids is true?
    a. **Storage lipids are only used for energy storage**
    b. Diversity in structural lipids are caused only by the variation in head group
    c. One of the most important types of structural lipids are made from glycerol, fatty acids and phosphate
    d. A triacylglycerol made from three trans monounsaturated fatty acids with 18 carbon atoms will have the same melting point as the triacylglycerol made from the same fatty acids in the cis configuration
    e. All lipids in membranes have only one polar end (the head) and one apolar end (the tail)
6. Which of the following statements about fibrous proteins is true?
    a. Fibrous proteins are always helical
    b. Fibrous proteins tend to be water soluble due to the high occurrence of charged residues
    c. **Fibrous proteins sometimes form left-handed helices**
    d. Fibrous proteins are only synthesized by mammals
    e. Serine is crucial for the correct structure of collagen to allow for tight junctions in the coiled coil
7. Which of the following statements about protein folding and denaturation is false?
    a. **Denaturation causes irreversible loss of function for any protein with defined structure**
    b. The native state is constantly in equilibrium with partially folded states
    c. Protein aggregates can be broken up into the individual proteins by chaperones
    d. Organic solvents can denature proteins by disturbing the packing of hydrophobic core
    e. The formation of hydrogen bonds is important for secondary structure formation

"Fill in the Blank" Questions (1 mark each)

8. How many full turns does an α-helix of 23 residues make? _____**6**_____
9. How many residues does it take to form an α-helix which is 18 Å long? __**12 residues**__
10. How long is a β-strand made from 7 residues in a antiparallel β-sheet? ____**24.5 Å**____
11. The helix dipole moment causes one end of an α-helix to be slightly positive and the other end to be slightly negative. Which end is positive? ____**N/Amino terminal**_____
12. Name the given fatty acid using standard nomenclature (Δ). _____**C18:3(Δ^{6,9,12})**_____

13. Is the fatty acid shown in question 12 an ω-3 fatty acid? _____**No**_____

14. For a newly identified protein, each chain was found to have 479 residues. The molecular weight of the protein was around 53000. What is the highest level of protein structure for this protein?_____**Tertiary**_____

True/False Questions. Briefly explain your choice. (2 marks each)

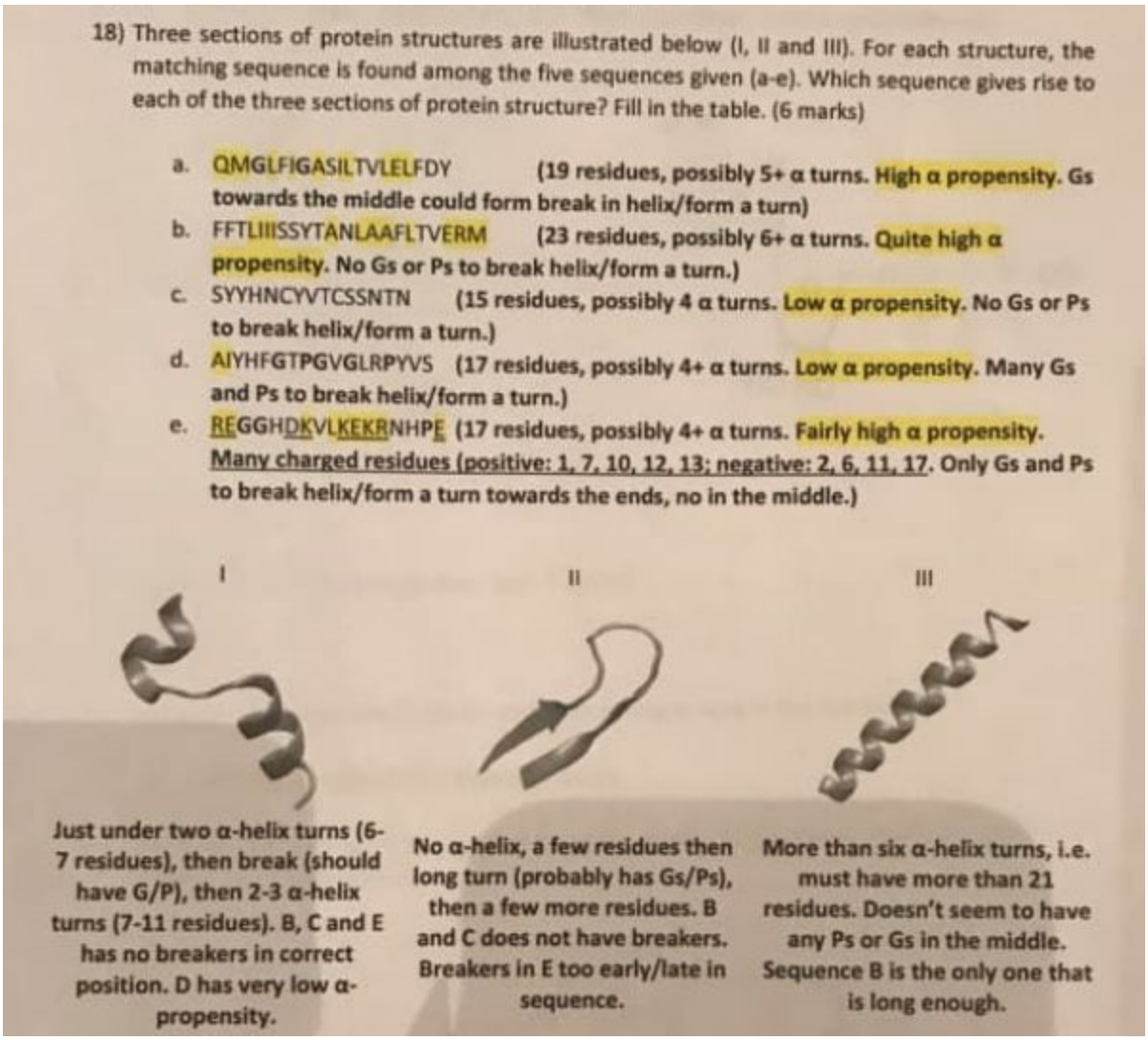
15. Having a K in position 4 in an α-helix and an R in position 7 can stabilise the helix. T/F? Explain.
    False. The two residues will sit in close proximity of each other in the helix due to being 3 residues apart. As they are both positively charged, they would repel one another and destabilize the helix

16. X binds to Y with an association constant of 0.7 μM^{-1}. We would hence characterize X as a strong binder. T/F? Explain.
    False. A strong binder is classified as having a dissociation constant (K_D) < 10 nM. $K_D = 1/K_A = 1/0.7\mu M^{-1} = 1.43*10^6 M$, i.e. X is a very weak binder.

17. For a β-sheet to have a hydrophilic side and a hydrophobic side, every second amino acid sidechain has to be hydrophilic and the other ones hydrophobic. T/F? Explain.
    True. For β-sheets, the amino acid side chains sit on alternating sides of the sheet. Therefore, if every second sidechain is hydrophilic and the others hydrophobic, a hydrophilic and hydrophobic side will be formed

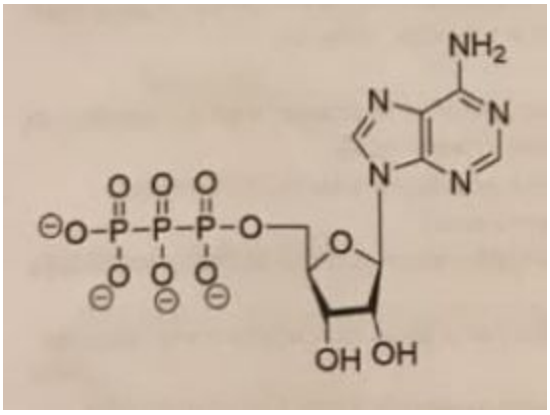
Short Answer Questions

18. Three sections of protein structures are illustrated below (I, II, and III). For each structure, the matching sequence is found among the five sequences given (a-e). Which sequence gives rise to each of the three sections of protein structure? Fill in the label. (6 marks)

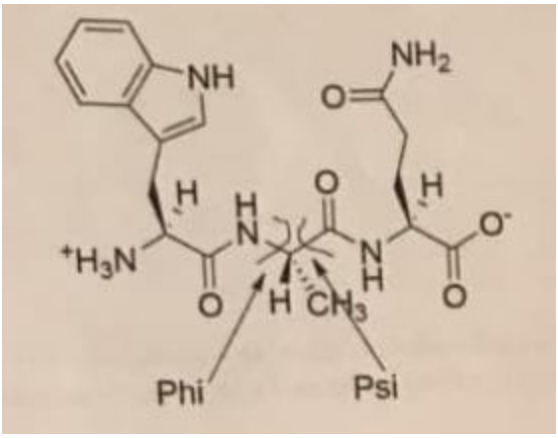


Protein	Sequence
I	**A**
II	**D**
III	**B**

19. Energy required for biochemical reactions is often acquired from hydrolysis of a particular nucleotide. Draw this nucleotide as it appears before hydrolysis. (2 marks)



20. Draw the structure of the tripeptide WAQ at neutral pH, using correct stereochemistry. For the central residue, show on your drawing where the phi and psi angles are. (6 marks)



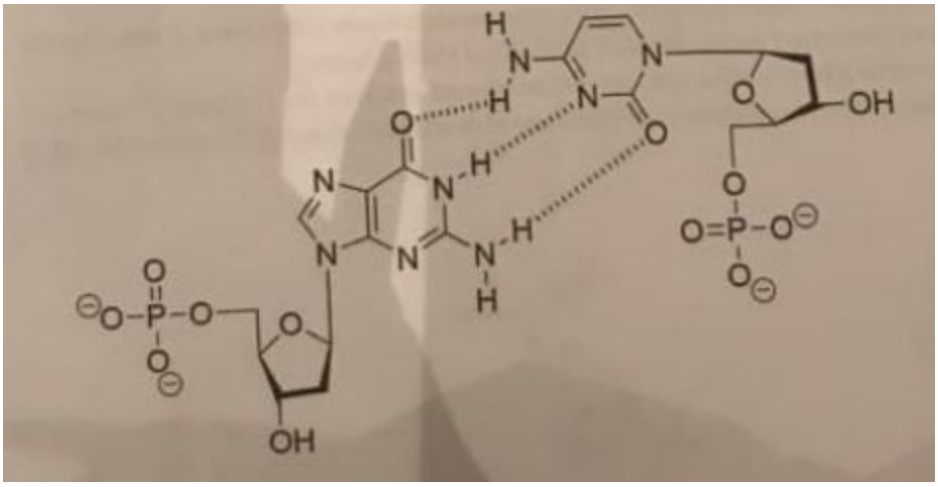
21. (9 marks) DNA has two base pairs - which?

A-T & G-C

How many hydrogen bonds are formed upon pairing in each of the two pairs?

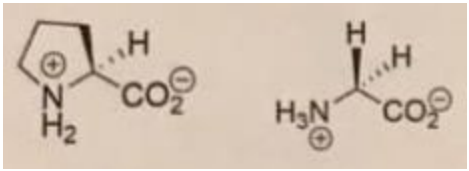
A-T: 2 hydrogen bonds; G-C: 3 hydrogen bonds

Draw the base pairing for the pair that forms the most hydrogen bonds. Use nucleotide structures for your illustration



22. Two amino acid residues are rarely found in α helices. One of them is known as a "helix-breaker", the other one is highly flexible. Give the one letter code for these two residues and draw their structures with correct stereochemistry and protonation states at neutral pH. (3 marks)

P and G



23. Enkephalins are pentapeptides which interact with opioid receptors. An enkephalin was digested with chymotrypsin, which gave three fragments for which the amino acid composition was as follows
    i. L
    ii. Y
    iii. F, G, G

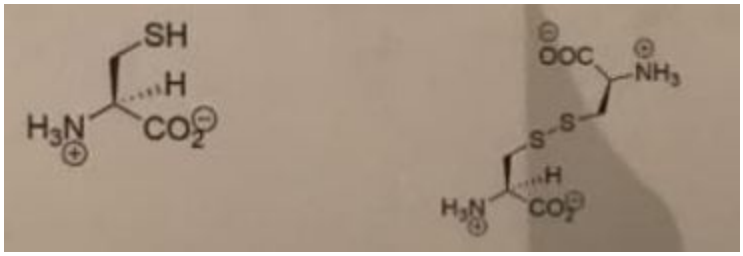
    The order of the fragments, as well as the order of the individual amino acids, is unknown. However, the sidechain of the N-terminal residue is known to be relatively large. Determine the sequence of the given enkephalin, and note down the sequence with the full names for the amino acids. Determine the charge of this enkephalin at pH 1. (6 marks)

    Tyrosine-glycine-glycine-phenylalanine-leucine

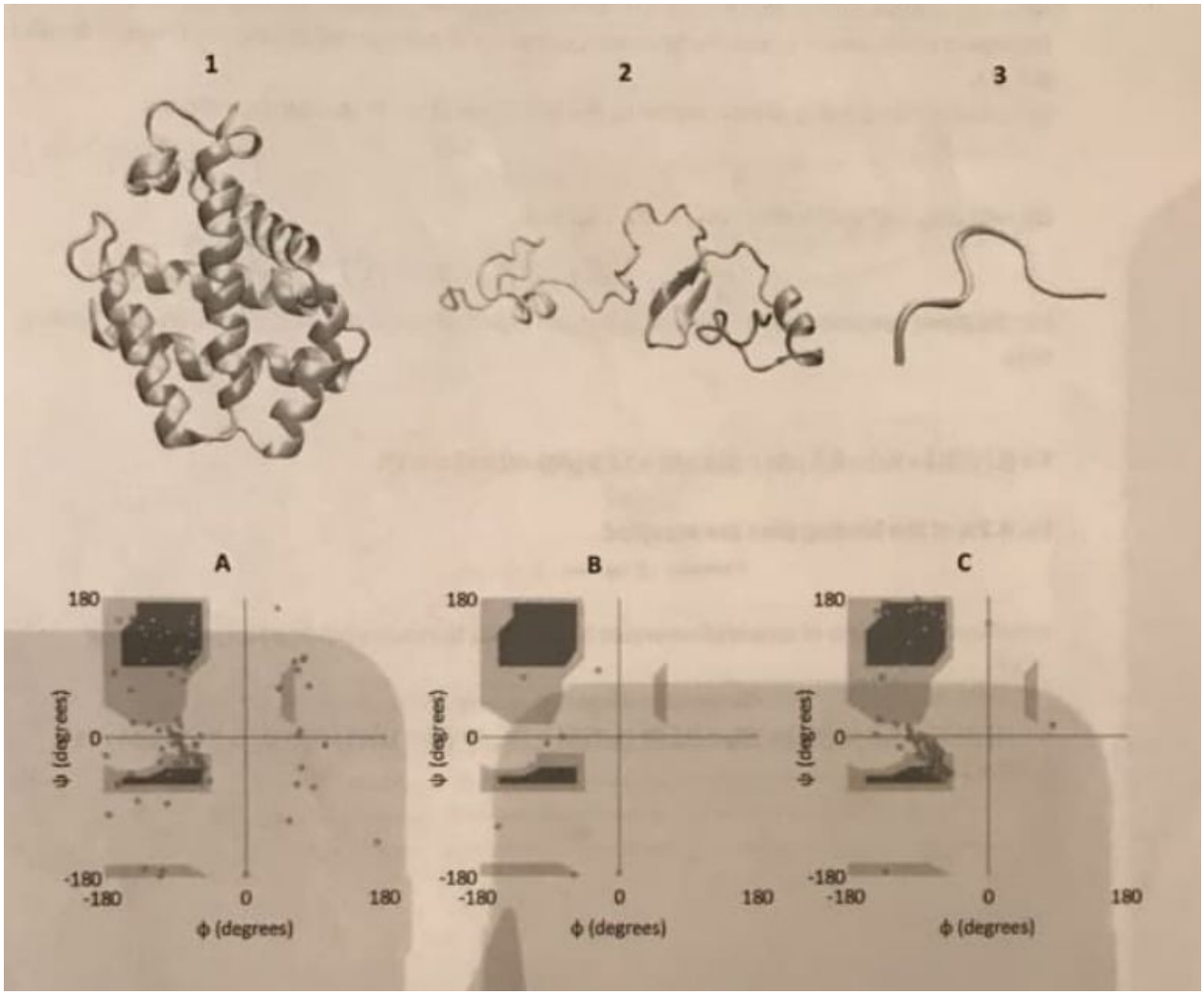
    Charge at pH 1: +1

24. One amino acid sidechain is capable of forming covalent bonds which are important for the stability of the tertiary and quaternary structure of some proteins. Give the name and one letter code for this amino acid and draw the structure with correct stereochemistry at neutral pH. Then, draw two such residues, showing the covalent bond. (3 marks)

    Cysteine, C.



25. Match the structures given below with their Ramachandran plots by filling in the table. (6 marks)



Protein	Ramachandran Plot
1	**C**
2	**A**
3	**B**

26. (6 marks) The NMDA receptor in the brain can be blocked by e.g. memantine and amantadine with dissociation constants of 17.9 µM and 694 µM, respectively. Memantine is used in the treatment of Alzheimer's, and the brain concentration of memantine during treatment is around 0.8 µM. Calculate ΔG for binding of memantine to the NMDA receptor at standard concentrations.

$\Delta G° RT ln K_D = 8.314(298 ln(17.9 * 10^{-6})) = -27.1 kJ/mol$

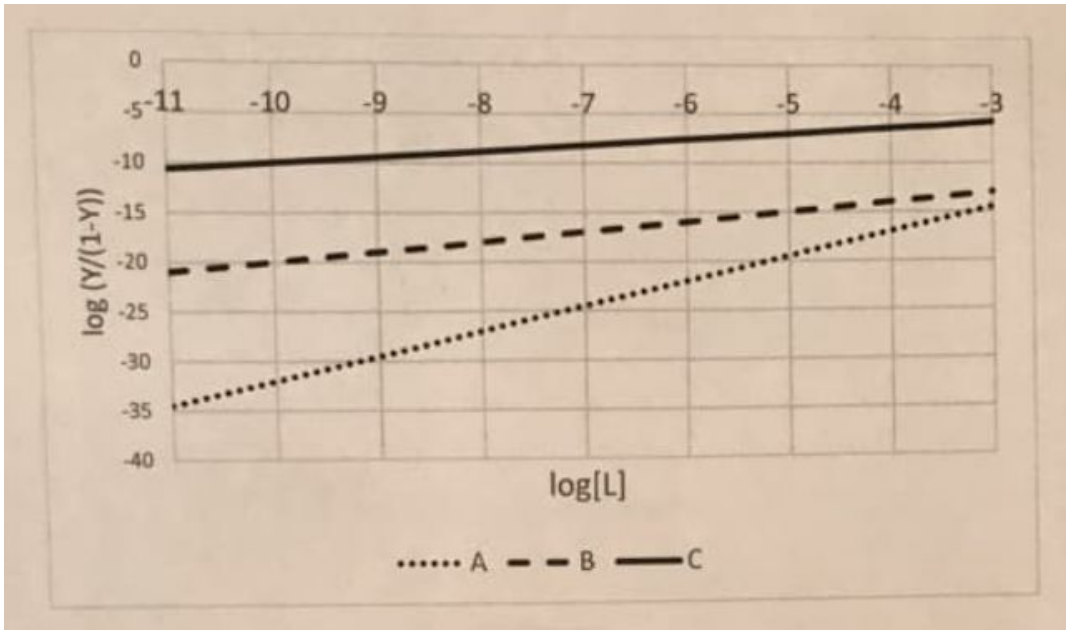
For the given concentration memantine in the brain, calculate the fraction of occupied binding sites.

$Y = [L]/([L] + K_D) = 0.8/(0.8 + 17.9) = 0.042 = 4.2\%$

Which concentration of amantadine would be required to reach a binding site saturation of 75%?

$Y = [L]/([L] + K_D),\ [L] = Y K_D/(1 - Y) = \frac{0.75(694 \mu M)}{1-0.75} = 2.08 mM$

27. Using the plot illustrated below, determine whether the three proteins (A, B, and C) show cooperativity when binding with ligand L. (3 marks)



Protein	Positive cooperativity	Negative cooperativity	No cooperativity
A	**A**		
B			**B**
C		**C**	

Midterm 3

If you need to use the gas constant, the following value can be used: $R = 8.314\ J/(mol \cdot K)$

Multiple Choice Questions. One correct answer per question. (1 mark each)

1. Which of the following statements are true for enzyme catalysis?
    a. Enzymes increase reaction rates by binding the product very tightly, favouring the product state over the reactants.
    b. Enzymes increase reaction rates by lowering the activation barrier through stabilizing the transition state
    c. Negatively charged metal ions can be crucial for metal ion catalysis
    d. Enzymes can change an overall reaction from being nonspontaneous to being spontaneous
    e. Enzymes are catalysts and hence never from covalent bonds to the reactants
2. The entropy of a reaction refers to
    a. The energy of the transition state
    b. The effect of temperature on the rate of the reaction
    c. The tendency of the system to move towards maximal randomness
    d. The difference in energy between reactants and products
    e. The heat given off by the reaction
3. Hemoglobin transports both O_2, H^+, and CO_2. Which of the following statements regarding hemoglobin transport function is false?
    a. Upon reaction between CO_2 and water, H^+ is released. The increase in $[H^+]$ contributes to a reduction in O_2 affinity
    b. O_2 binding causes a flattening of the heme group which is converted to larger conformational changes at the subunit interfaces
    c. H^+ binding to ionisable sidechains can stabilize hemoglobin in the T-state
    d. CO_2 binds covalently to the N-terminal and stabilizes the T-state by causing a change in electrostatic interactions
    e. O_2 binds cooperatively; i.e. binding of O_2 to one subunit increases K_D for O_2 binding to the other subunits
4. Which statement concerning irreversible inhibition is false?
    a. Irreversible inhibition is a mix between competitive and uncompetitive inhibition
    b. Transition state analogs sometimes bind tightly enough to be considered irreversible inhibitors
    c. Suicide inhibitors react with the enzyme and form a covalent bond to the enzyme
    d. Penicillin is an example of an irreversible inhibitor
    e. Irreversible inhibition can destroy catalytically important functional groups

5. Myoglobin is great for storing oxygen, and the p_{50} is 0.26 kPa. The minimal partial pressure required for maintaining conciousness is around 2.65 kPa. What is the fractional occupancy of O_2 sites in myoglobin under these conditions?
    a. 0.23
    b. 0.41
    c. 0.58
    d. 0.74
    e. 0.91
6. What is $[S]/K_m$ when the initial velocity of an enzyme-catalyzed reaction is 80% of V_{max}?
    a. 0.25
    b. 0.8
    c. 2.5
    d. 4.0
    e. 5.2
7. 2,3-bisphosphoglycerate (BPG) is an allosteric modulator for hemoglobin. Changes in the BPG concentration contributes to altitude adaption (where pO_2 is reduced). What is true for this modulation?
    a. BPG stabilizes the R state, increasing the O_2 affinity, hence enabling better transport of O_2 to tissue at high altitude
    b. BPG binds to the heme group and reduced O_2 binding by competing for the binding site
    c. BPG stabilizes the low-affinity T state and enables a more efficient O_2 transport to tissue
    d. The Bohr Effect explains why O_2 release in tissue is more efficient at higher [BPG]
    e. At higher [BPG], more O_2 binds to heme in the lungs and more O_2 is released in tissue, which increases the amount of O_2 delivered to tissue

True/False Questions. Give a brief explanation. (2 marks each)

8. For a standard enzyme reaction scheme, K_m equals the dissociation constant for the ES complex when k_{-1} is approximately equal to k_2. True/False? Briefly explain why/why not.
    False. $K_m = (k_{-1}+k_2)/k_1$, and $K_D = k_{-1}/k_1$. Hence, $K_m = K_D$ when $k_2 <<< k_{-1}$
    i.e. the Michaelis constant is the dissociation constant when k_2 is rate-limiting, not when $k_{-1}=k_2$

9. In the sequential model for cooperativity, all subunits undergo a conformational change simultaneously. True/False? Briefly explain why/why not.
    False. In the sequential model, conformational change in one subunit increases the likelihood of conformational change in the other subunits. All subunits undergo a simultaneous conformational change in the concerted model.

10. The Fab fragments of immunoglobulins recognize antigens, and the binding affinity is strengthened through induced fit. True/False? Briefly explain why/why not
    True. Fab fragments recognize antigens and the binding site of the Fab fragment changes upon binding to optimize the binding affinity (= induced fit).

11. In uncompetitive inhibition, K_m increases as an inhibitor is added. True/False? Briefly explain why/why not
    False. In uncompetitive inhibition, I binds to ES, effectively reducing [E]. This reduces V_{max} and hence reduces the [S] required for $0.5V_{max}$, i.e. K_m

12. The HIV protease is used by the virus to form the carbohydrate molecules to be displayed at the surface of the virus. True/False? Briefly explain why/why not.
    False. The HIV protease is a protease which cleaves peptide bonds. Hence, the substrates are peptides/proteins, not carbohydrate molecules.

13. A stimulatory allosteric enzyme modulator can cause the sigmoidal dependence of V_0 on [S] to become almost hyperbolic. True/False? Briefly explain why/why not
    True. A stimulatory allosteric modulator can shift the equilibrium for the enzyme to be almost fully in the R state (e.g. ATP for ATCase), causing the dependence of V_0 on [S] to be hyperbolic.

14. The majority of enzyme-catalyzed reactions involves two substrates. For an enzyme to catalyze a reaction involving two molecules, the two molecules must be bound to the enzyme at the same time. True/False? Briefly explain why/why not.
    False. The ping-pong mechanism is an example of a two substrate reaction for which only one substrate binds at the time. $E+S_1 \rightarrow E^*+P_1$; S2 binds => $E^*+S_2 \rightarrow E+P_2$. *enzyme modification

15. Enzymes can be covalently modified by being linked to other proteins for regulatory purposes. True/False? Briefly explain why/why not.
    True. Possible covalent modifications for enzyme regulation include the attachment of small proteins. (e.g. ubiquitin, sumo).

Short Answer Questions

16. For enzyme regulation, what is the most common type of reversible covalent modification? (1 mark)
    Phosphorylation

17. Molecular recognition is dependent on the so-called weak interactions. For each of the mentioned types of interactions in the table below, indicate all the characteristics (a-h) that apply to that interaction (you can list a given characteristic for several interaction types). (3 marks)
    a. Require nonpolar species
    b. Involve charged species only
    c. Require polar or charged species
    d. Involve either O and H or N and H atoms
    e. Involve nonspecific atoms
    f. Are also called electrostatic interactions
    g. Only exist in water
    h. Are weakened in water

Interaction	Characteristics
Salt bridges	**b,f,h**
Hydrophobic interactions	**a,e,g**
Van der Waal's interactions	**e**
Hydrogen bonds	**c,d,h**

18. Sometimes it is said for protein-ligand interactions that some types of interactions are mostly important for specificity, while other types of interactions are those which provide the "glue", i.e. increase the binding affinity. (4 marks)
    Based on the table in question 17, which types of interactions would be most important for specificity?
    Salt bridges, hydrogen bonds

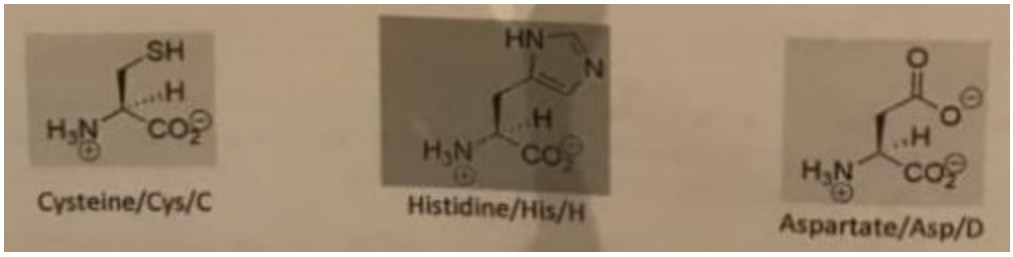
    And which would be most important for ensuring a strong binding?
    Hydrophobic interactions, van der Waal's interactions

19. Acetylcholinesterase, which cleaves acetylcholine, has a turnover number of $1.4 \times 10^4\ s^{-1}$. (3 marks)
What does this number tell us?
The number of acetylcholine molecules converted per second

Suppose the concentration of acetylcholinesterase is 0.3 nM. What would V_{max} be in the given situation?
$k_{cat} = V_{max}/[E_{tot}]$, $V_{max} = k_{cat}[E_{tot}] = (1.4 \times 10^4\ s^{-1})(0.3 \times 10^{-9} M) = 4.2 \times 10^{-6}$ M/s

20. For two substrates of the enzyme fumarase, the turnover numbers and the Michaelis constants differ as listed: fumarate (turnover number: $8 \times 10^2\ s^{-1}$; Michaelis constant: 5×10^{-6} M) and malate (turnover number: $9 \times 10^2\ s^{-1}$; Michaelis constant: 2.5×10^{-5} M). For which substrate does the enzyme display the highest catalytic efficiency? (2 marks)
$CE = k_{cat}/K_m$
Fumarate: $CE = (8.2 \times 10^2\ s^{-1})/(5 \times 10^{-6}) = 1.64 \times 10^8\ s^{-1}\ M^{-1}$
Malate: $CE = (9 \times 10^2\ s^{-1})/(2.5 \times 10^{-6}) = 3.6 \times 10^7\ s^{-1}\ M^{-1}$
The enzyme with the highest catalytic activity with fumarate

21. (10 marks) Cysteine proteases catalyze the cleavage of peptide bonds. Three residues form the catalytic triad in the active site of the enzyme. Draw these three residues (using correct stereochemistry and the standard protonation state at pH 7).

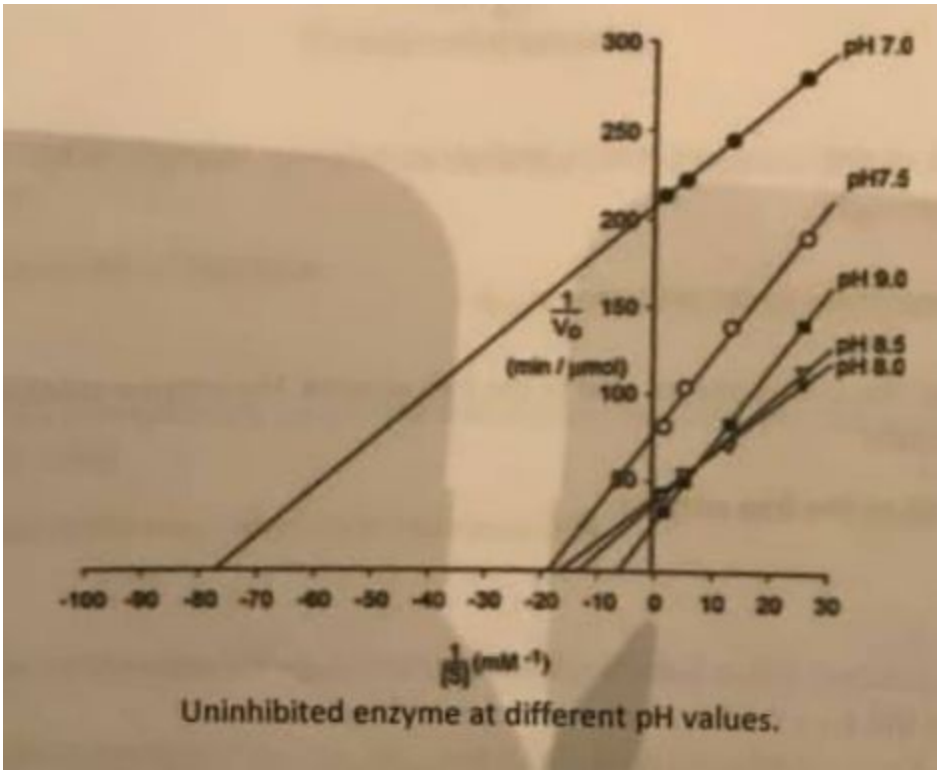


Briefly state the main role of each of these residues for the catalysis reaction.
Asp: forms hydrogen bond to His sidechain, hold His in place and increase His pK_a
His: general acid/base catalyst. Take the H from Cys to make it a stronger nucleophile
Cys: nucleophile, attack the electrophilic peptide carbon, form covalent bond

List the two most important mechanisms of catalysis expected for a cysteine protease.
Covalent catalysis, several steps of acid/base catalysis. Also some transition state stabilization.

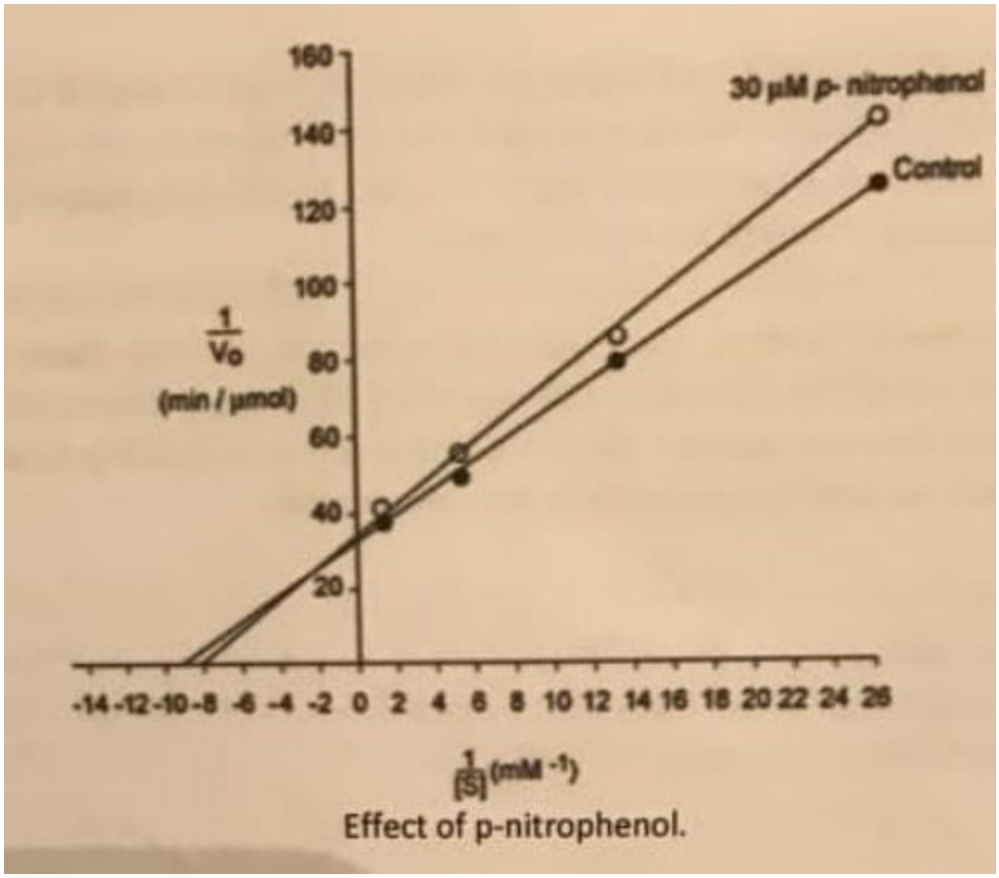
22. (2 marks) For a given protein, tests suggest a molecular weight of around 67,000. However, after treatment with a chemical that cleaves disulfide bonds, the molecular weight is a bit less than 34,000. The protein is known to bind to ligand L. Based on the information given above, could L possibly bind cooperatively to the native protein?
Yes. for cooperative binding, more than one subunit is required. Given that the molecular weight is approximately halved upon cleavage of disulfide bonds, it seems likely that the protein has two nearly identical subunits which are held together by disulfide bonds. Hence, it seems possible that L can bind cooperatively to the naitive protein.

23. The following questions (a-k) concern the hydrolysis of p-nitrophenyl phosphate, catalyzed by the enzyme alkaline phosphatase. (Data from Dean, *Biochem. Mol. Biol. Edu.* (2002)). Use the plots provided to answer the questions. (14 marks)



Uninhibited enzyme at different pH values.

a. At which pH value is the highest possible reaction rate observed?
pH 9
b. What is the approximate V_{max} at this pH value?
y-intercept = $1/V_{max}$, V_{max} = 1/y-intercept = 1/(25 min/μmol) = 0.04 μmol/min
c. What is the approximate K_m value?
m = $(y_2-y_1)/(x_2-x_1)$ = 25 mM min/6 μmol = K_m/V_{max}, $K_m = 25V_{max}/6$ = 25(0.04)/6
K_m = 0.167 mM

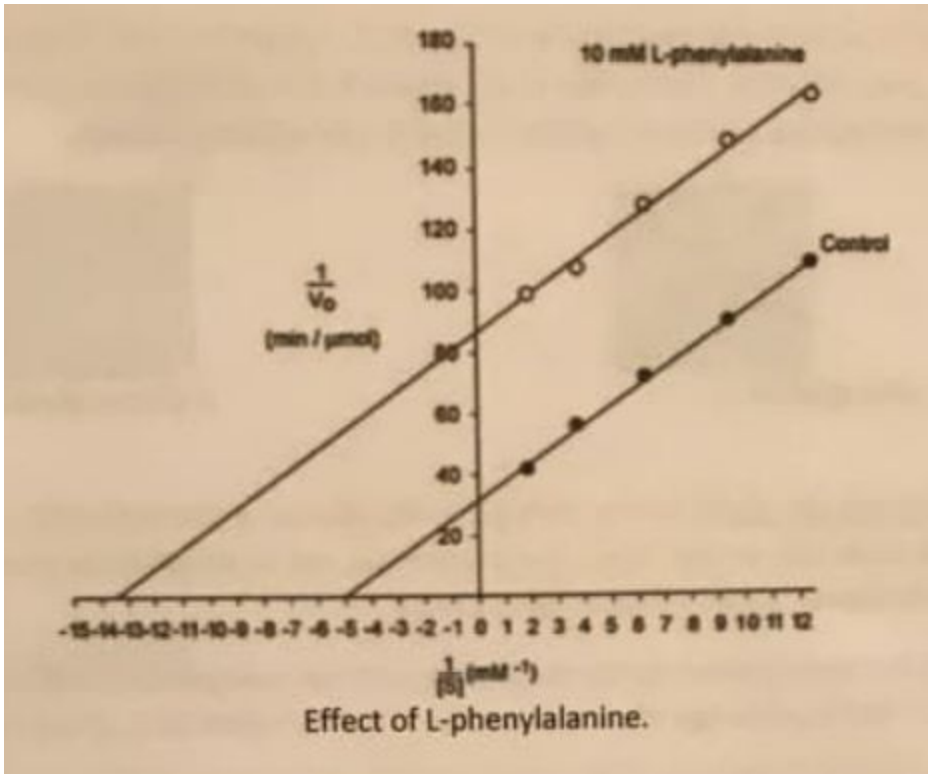


Effect of p-nitrophenol.

d. One of the products, p-nitrophenol, inhibits the reaction. What type of inhibition is occurring?
Competitive inhibition (same V_{max}). (Mixed accepted for 0.5 mark, if following questions answered under the assumption it is mixed, full marks will be awarded)

e. Does the p-nitrophenol bind to the free enzyme, the enzyme-substrate complex only, or to both?
Binds to the free enzyme (Mixed binds to both)

f. Can this type of inhibition be overcome? If yes, then how?
Yes. A competitive inhibitor can be outcompeted by adding more substrate. (No, a mixed inhibitor can not be outcompeted)



Effect of L-phenylalanine.

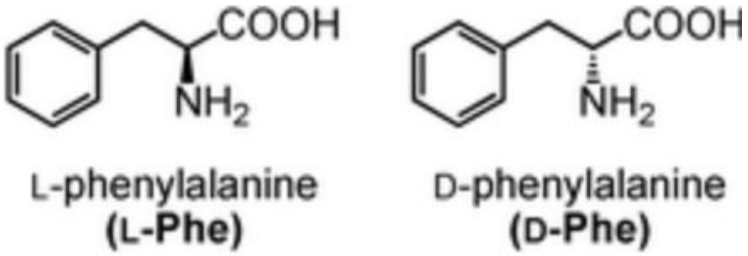
g. L-phenylalanine also inhibits the reaction. What type of inhibition does L-phenylalanine cause?
Uncompetitive inhibition

h. Does L-phenylalanine bind to the free enzyme only, the enzyme-substrate complex only, or to both?
Binds to the enzyme-substrate complex only.

i. How are the apparent values for V_{max} and K_m affected by the addition of L-phenylalanine?
In uncompetitive inhibition, V_{max} and K_m are both reduced (by 1/α')

j. Given that 10 mM L-phenylalanine was used for the experiments, calculate the approximate inhibition constant (K_I or K_I').
$\alpha = V_{max}/(\text{apparent } V_{max}) = [I]/(1+K_I')$,
$K_I' = \{(\text{apparent } V_{max})[I]/V_{max}\} - 1 = (32*10 \text{ mM}/85) - 1 = 6.0 \text{ mM}$

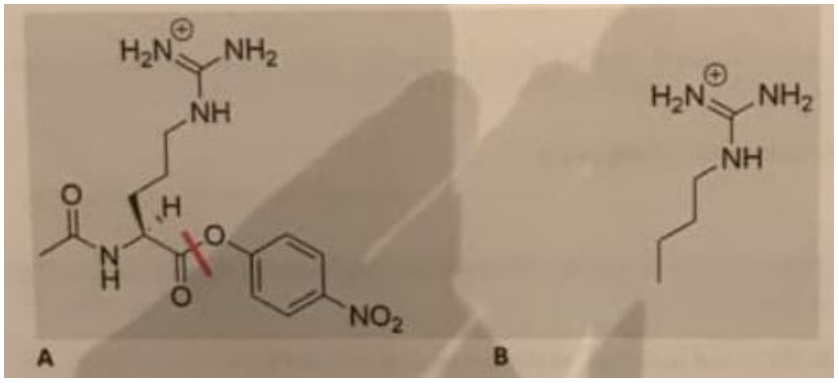
k. The D-isomer of phenylalanine is, however, not an inhibitor. Draw the D- and L-isomers of phenylalanine (remember to state which is D and which is L). Briefly explain how L-phenylalanine can be on inhibitor when D-phenylalanine is not.



L-phenylalanine (L-Phe) D-phenylalanine (D-Phe)

Enzymes are chiral, making them interact differently with two enantiomers of the same compound. Thus, one enantiomer can be an inhibitor and the other enantiomer have no effect.

24. Compound **A** is a good substrate for the trypsin protease. Compound **B** is an effective inhibitor of trypsin. ΔG°' for the cleavage of compound A is -20.92 kJ/mol at 25°C. (8 marks)



a. Which amino acid forms part of compound **A**? Illustrate clearly on the drawing above where trypsin would cleave. From which other amino acid can one design a similar substrate for trypsin?
Arginine. A similar substrate could be designed from Lysine

b. Explain briefly why compound **B** is an inhibitor for trypsin, and whether the inhibition will be competitive or uncompetitive.
B does not have a bond to cleave, but would bind well in the pocket that recognizes Lys/Arg, blocking substrate access. It would be a competitive inhibitor

c. Calculate the equilibrium constant, K_{eq}' for the cleavage of **A** at 25°C. Once at equilibrium, will there be more reactant or more product?
$\Delta G°' = -RT\ln K_{eq}'$, $K_{eq}' = e^{(-\Delta G°'/RT)} = e^{(-20920/8.314*298)} = 4646$. There will be mostly product