

I. Amino Acids, Peptides and Proteins [21 points]

- (1) An oligopeptide has been shown to have the following sequence:

TTYADFIASGRTGRRNAIHD

- (a) Estimate its net charge at pH 7, pH 8, pH 9, and pH 10. Show your analysis. [4 points]

| | NH ₂ | Y | D | R | R | R | H | D | COOH | net | |
|------|-----------------|------|----|----|----|----|---|----|------|------|--|
| pH 7 | +1 | 0 | -1 | +1 | +1 | +1 | 0 | -1 | -1 | +1 | Answer based on rounded pKa of α-amino = 9, Tyr = 10. Using pKa = 10 for α-amino gives pI = 10.0 which is also acceptable. |
| 8 | 1 | 0 | -1 | 1 | 1 | 1 | 0 | -1 | -1 | +1 | |
| 9 | 0.5 | 0 | -1 | 1 | 1 | 1 | 0 | -1 | -1 | +0.5 | |
| 10 | 0 | -0.5 | -1 | 1 | 1 | 1 | 0 | -1 | -1 | -0.5 | |

- (b) Estimate its isoelectric point [1 point]

Midpoint between 9 and 10: 9.5

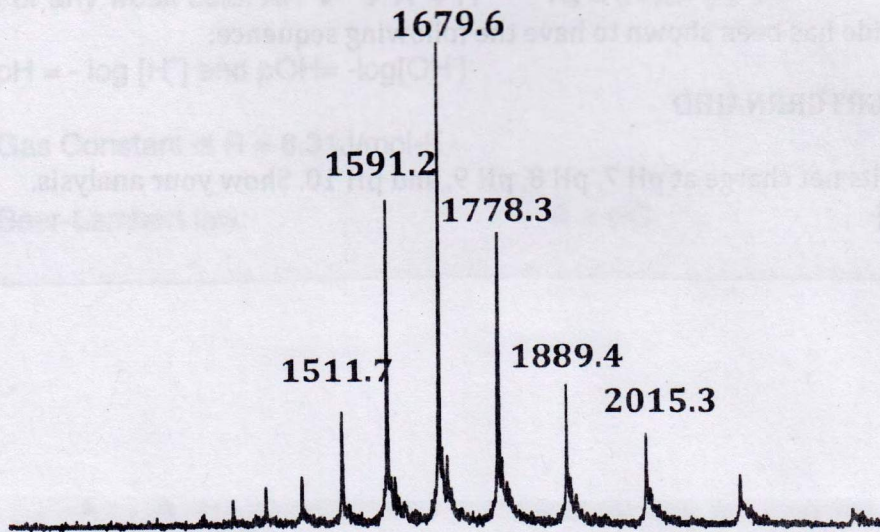
- (2) The crystal structure of a protein has been determined to atomic resolution. Analysis of the structure reveals several ionizable sidechains in various positions in the protein. Indicate whether the pKa of the following side chains will increase, decrease or remain unchanged relative to their value when totally exposed to water. [2 points, 0.5 points each]

- (a) a Glu side chain in a protein pocket containing a Lys nearby *decrease*
 (b) an Arg side chain found in a hydrophobic pocket *decrease*
 (c) an Asp side chain engaged in a O-H---N hydrogen bond *increase*
 (d) a His residue on the surface of the protein *remain unchanged*

- (3) Hydrolysis of a peptide bond is known to be exergonic, but its average half life-time under most intracellular conditions is ~ 7 years. What factor is responsible for this long half life-time (Circle the correct answer). [1 point]

- a) a negative ΔG for the hydrolysis reaction
 b) a large activation energy for the hydrolysis reaction
 c) a large positive ΔS for the hydrolysis reaction
 d) a large negative ΔH of the hydrolysis reaction
 e) the double bond character of the peptide bond

- (4) Determine the molecular weight of a protein for which the following mass spectrum has been obtained [4 points]:



- a) 25,214
b) 23,214
c) 24,612
d) 35,235
e) 30,215
- (5) Consider the following polypeptides:
1) ATKNRASCLVPKLGALMFWRHKQLVSDPILQKRQHILVCRNAAG
2) GPYFGDEPLDVHDEPEEG
3) PHLLSAWKGMGVGKSQSFAALIVILA

Of the three which one would migrate most slowly during chromatography through [1 point each]:

- (a) a carboxymethyl cellulose resin *N°1*
(b) a diethylaminoethyl agarose column *N°2*
(c) a size exclusion (gel filtration) resin for peptides of this size? *N°2*
- (6) A biochemist is attempting to separate a DNA-binding protein (protein X) from other proteins in a solution. Only three other proteins (A, B, and C) are present. The proteins have the following properties:

| | pI (isoelectric point) | Size M_r | Bind to DNA? |
|-----------|------------------------------|---------------|-----------------|
| protein A | 7.4 | 82,000 | yes |
| protein B | 3.8 | 21,500 | yes |
| protein C | 7.9 | 23,000 | no |
| protein X | 7.8 | 22,000 | yes |

What type of protein separation techniques might she use to separate:

- (a) protein X from protein A? [1 points] *size exclusion chromatography*
 (b) protein X from protein B? [1 points] *ion exchange chromatography or isoelectric focussing*
 (c) protein X from protein C? [1 points] *affinity chromatography with immobilized DNA*

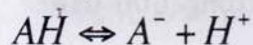
(7) Choose the best answer to fill in each blank:

- A. Alanine
 B. Proline
 C. Glycine

- a) this amino acid has the highest α -helix propensity: A [0.5 point]
 b) these two amino acids have the lowest α -helix propensity: B and C [0.5 point each]
 c) these two amino acids often occur in β turns: B and C [0.5 point each]
 d) The peptide bond nearly always has the trans configuration since it is more favorable than cis, which is sometimes found to occur with this residue B [0.5 point]

II. Acid-Base Equilibria [13 points]

(8) An acid has a dissociation constant $K_a = 10^{-4}$ and dissociates in water according to:



Provide a numerical answer (you do not need a calculator):

a) what would be the pH of a 1 M solution of this acid? [2 points]

$$K_a = \frac{[A^-][H^+]}{[AH]} = \frac{x \cdot x}{1M - x} = \frac{x^2}{1} = 10^{-4}; x = 10^{-2}$$

$$[H^+] = 10^{-2}$$

$$\boxed{pH = 2}$$

- b) if you dilute this solution 100 times, would its fractional dissociation, $[A^-]/([A^-] + [AH])$, increase, decrease or remain the same? [2 points]

increase

- c) What would be the pH of this dilute solution? [2 points]

$$K_a = \frac{[H^+][A^-]}{[AH]} = \frac{x \cdot x}{0.01 - x} \approx \frac{x^2}{0.01}; \quad x^2 = 0.01 \times 10^{-4} = 10^{-6}$$

$$x = [H^+] = 10^{-3}$$

$$pH = 3$$

- (9) A biochemist prepares a 10^{-8} M aqueous solution of the strong acid HCl.
a) Would the pH of the solution be acid, neutral or alkaline? Explain. [3 points]

It will be acid, because to the $[H^+]$ of pure water which is 10^{-7} M, he adds 10^{-8} M more or 0.1×10^{-7} M so the final $[H^+] = 1.1 \times 10^{-7}$ M and thus the pH < 7

- (10) Indicate whether the following statements are true (T) or false (F) [0.5 points each]:
- T The ionic product of water K_w is the 10^{-14} M
 - F A buffer results from making a very dilute solution of a strong acid
 - F The pOH of a solution is the negative of its pH
 - T The lower the pKa of an acid the stronger the acid
 - F The dissociated form A^- of an acid AH is called its conjugate acid?
 - T The dissociated form A^- of an acid AH is called its conjugate base?
 - F A buffer solution made with a monoprotic acid AH has two buffering regions?
 - F The lower the pKa of a base the stronger the base

III. Protein Structure and Folding [20 points]

- (11) Provide the following data about alpha helices [0.5 points each]:

- a) periodicity of the helix in Angstroms: 5.4 \AA
 b) handedness (right or left): *right-handed*
 c) number of residues per turn: $3.6 \text{ residues/turn}$
 d) rise in Angstroms per 100° rotation: 1.5 \AA
 e) diameter of the helix: 5 \AA
 f) value and sign of ϕ angle: -57°
 g) value and sign of the ψ angle: -47°
 h) maximum number of H-bonds a 10-residue alpha helix can make is: 7

(12) Indicate True (T) or False (F) [0.5 points each]:

Chris Anfinsen used the enzyme pancreatic ribonuclease to show that:

- F it cleaves RNA in the presence of urea
F it can reform all its native disulfide bonds if allowed to refold fast
T it can reform all its native disulfide bonds if allowed to refold slowly
T the native structures of proteins are thermodynamically determined
F the native structures of proteins are kinetically determined
F only proteins with disulfide bonds can attain the native state

(13) The maximum number of random disulfide bonds that a protein containing 10 cysteines can make is [2 points]:

$$9 \times 7 \times 5 \times 3 \times 1 = 945$$

(14) If the configurational unfolding free energy of a polypeptide is -1.2 Kcal/mol residue, use the following table showing the free-energy change for transferring amino acids from the hydrophobic interior of proteins to the solvent to determine if the following polypeptide will fold or remain unfolded (Show your reasoning) [3 points]:

RRKKAGIVHLVHRIDHAELA

| Residue | $\Delta G_{\text{transfer}}$ |
|---------|------------------------------|
| Ala | +0.730 Kcal/mol |
| Val | +1.69 Kcal/mol |
| Leu | +2.4 Kcal/mol |
| Ileu | +2.97 Kcal/mol |
| Ser | +0.040 Kcal/mol |

There are 20 aa in the peptide: Therefore ΔG_{conf} for unfolding is
 $20 \times -1.2 \text{ Kcal/mol} = -24 \text{ Kcal/mol}$.

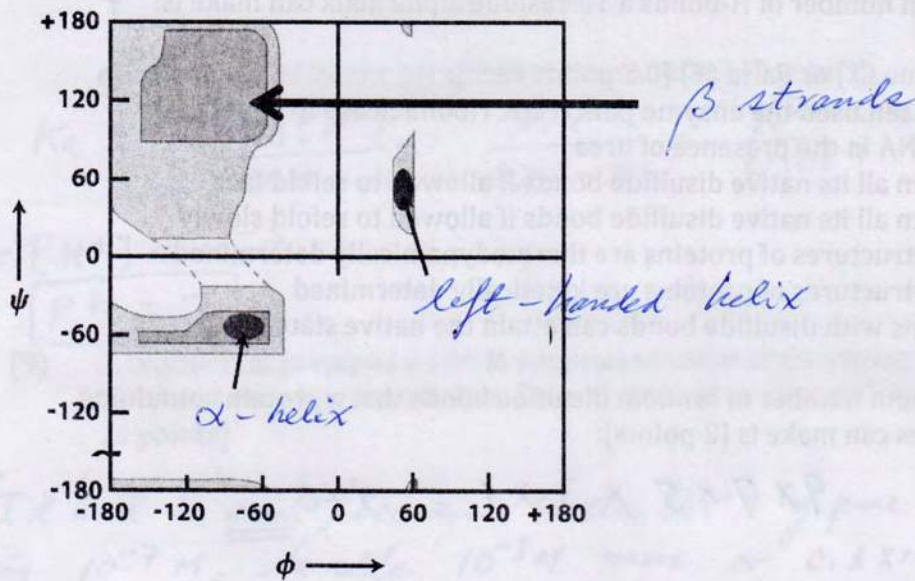
$\Delta G_{\text{transfer}}$ for 3 Ala, 2 Val, 2 Leu and 2 Ileu is:

$$\Delta G_{\text{transfer}} = 3 \times 0.73 \text{ Kcal/mol} + 2 \times 1.69 \text{ Kcal/mol} + 2 \times 2.4 \text{ Kcal/mol} + 2 \times 2.97 \text{ Kcal/mol} = +16.3 \text{ Kcal/mol}$$

$$\Delta G_{\text{net}} = \Delta G_{\text{conf}} + \Delta G_{\text{transfer}} = -24 \text{ Kcal/mol} + 16.3 \text{ Kcal/mol} = -7.7 \text{ Kcal/mol}$$

So the peptide will NOT FOLD!

- (15) In the following Ramachandran diagram write down to what structures correspond the areas indicated by the arrows. [2 points]



- (16) Complete the following descriptions [1 point each]:

a) In the Koshland model, the cooperativity arises because....

Binding of the ligand to a subunit increases the affinity of the neighboring subunits.

b) In the Monod model, the cooperativity arises because....

Preferential binding of the ligand to the high affinity form, tilts the equilibrium towards this form, making it easier for the next ligand to bind.

- (17) Indicate whether the following statements are true (T) or false (F) [0.5 points each]:

- T Unlike myoglobin, hemoglobin displays binding cooperativity to oxygen
- F ATP is an allosteric effector of hemoglobin
- T BPG is an allosteric effector of hemoglobin
- T Bohr's effect is the decrease of oxygen affinity of hemoglobin with lower pH
- T BPG increases at high altitude to decrease the affinity of hemoglobin for oxygen
- F The Monod or concerted model can only describe negative binding cooperativity
- F The Koshland or induced-fit model can only describe positive cooperativity
- T At a ligand concentration equal to the twice the value of the dissociation constant of a protein, the fractional saturation of the protein is 66.66%

IV. Enzyme Kinetics and Mechanism [22 points]

- (18) Indicate which of the following sentences are true [0.5 points each]:
- F the Haldane-Pauling hypothesis states that the higher the energy of the transition state the higher the specificity of the enzyme for its substrate.
 - F enzymes catalyze chemical reactions by making the strongest bonding interactions with the substrate.
 - F enzymes have optimal efficiency at pH = 7.
 - T some enzymes speed reactions up by changing the reaction pathway.
 - T the affinity of the enzyme for the substrate is often used to pay for the cost of desolvating it.
 - T part of the energy of binding of the enzyme is used to introduce strain into the substrate.
 - T Emil Fischer proposed that the enzymes are specific because they fit to the substrate like a lock to its key.
 - F catalytic antibodies were proposed by William Jencks to explain the phenomenon of autoimmune diseases.

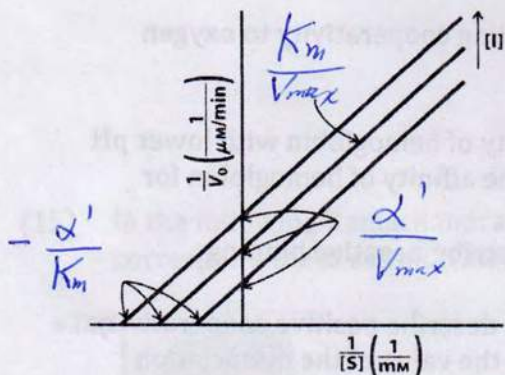
- (19) An enzyme is found to bind to the substrate with an energy of -10 kJ/mol and to the transition state with an energy of -34 kJ/mol.
- a) will the enzyme accelerate or slow down the reaction? Explain. [1 point]

Accelerate it

- c) by approximately what factor would the enzyme change the rate of the reaction? You do not need a calculator [1 point].

10⁴ times

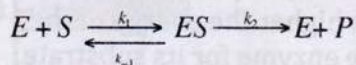
- (20) a) To what kind of inhibitor this kinetic plot corresponds to [1 point]:



Uncompetitive inhibition

b) Write down next to the arrows how are the slope and intercepts affected by the concentration of the inhibitor and its dissociation constant. [2 points].

(21) Consider the simple Michaelis-Menten mechanism for an enzyme-catalyzed reaction



the following data were obtained:

k_1, k_{-1} very fast

$k_2 = 100 \text{ s}^{-1}, K_M = 1.0 \times 10^{-4} \text{ M}$ at 280 K

$k_2 = 200 \text{ s}^{-1}, K_M = 1.5 \times 10^{-4} \text{ M}$ at 300 K

a) For $[S] = 0.10 \text{ M}$ and $[E_T] = 1.0 \times 10^{-5} \text{ M}$, calculate the rate of formation of product and 280 K. [2 points]

$$v = \frac{V_{max} [S]}{K_M + [S]} = \frac{1.0 \times 10^{-5} \text{ M} \times 100 \text{ s}^{-1} \times 0.1 \text{ M}}{10^{-4} \text{ M} + 10^{-1} \text{ M}} = \frac{10^{-6}}{10^{-1}} = 10^{-5} \text{ M s}^{-1}$$

b) Using the fact that $\ln 2 = 0.69$, calculate the activation energy for k_2 .

Assume that $k_2 = \text{constant} \times e^{-\Delta G^\ddagger / RT}$. You just need to show what you will do to solve this problem. You don't have to get a numerical answer. [3 points]

$$\text{From equation provided: } \ln k_2(T_2) = -\frac{\Delta G^\ddagger}{RT_2}$$

$$\ln k_2(T_1) = -\frac{\Delta G^\ddagger}{RT_1}$$

$$\ln \left(\frac{k_2}{k_1} \right) = \frac{\Delta G^\ddagger}{R} \left(\frac{1}{T_1} - \frac{1}{T_2} \right)$$

$$\Delta G^\ddagger = \frac{R \ln 2}{\left(\frac{1}{T_1} - \frac{1}{T_2} \right)}$$

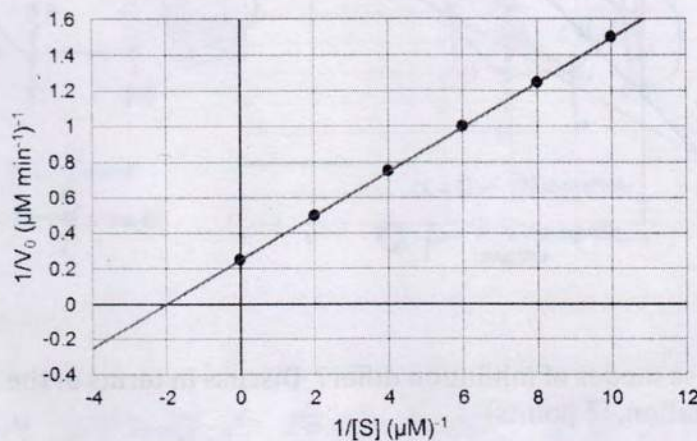
- c) Provide an approximate value of the equilibrium constant at 280K for the formation of the Enzyme-Substrate complex ES from E and S? Hint: start with the expression for K_M . [2 points]

$$K_{Mn} = \frac{k_{+2} + k_{-1}}{k_1}, \text{ since } k_{-1} \gg k_2, \text{ then}$$

$$K_{Mn} \approx \frac{k_{-1}}{k_1} \equiv K_d \text{ for the ES complex. So,}$$

$$K_d = 1 \times 10^{-4} \text{ M}$$

- (22) You measure the kinetics of $1 \mu\text{M}$ enzyme E as a function of substrate concentration and plot the results as a Lineweaver-Burk plot.



The trend line you obtain has the equation $y = 0.125x + 0.25$
From this plot, determine V_{\max} and K_m . [2 points]

$$V_{\max} = \frac{1}{0.25} \frac{\mu\text{M}}{\text{min}} = \underline{4 \frac{\mu\text{M}}{\text{min}}}$$

$$\frac{K_m}{V_{\max}} = 0.125 \text{ min} ; K_m = 0.125 \text{ min} \times \frac{4 \mu\text{M}}{\text{min}}$$

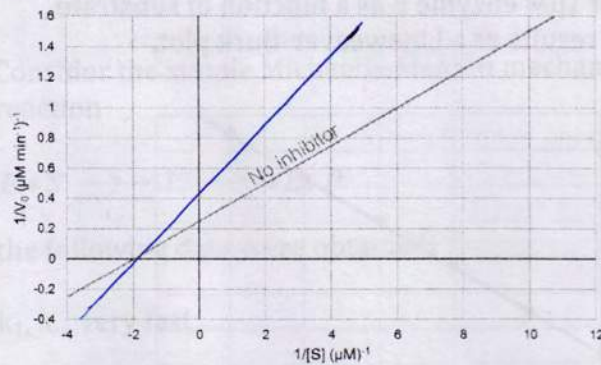
$$K_m = \underline{0.5 \mu\text{M}}$$

You are interested in determining how enzyme E is inhibited. On the following Lineweaver-Burk plots, draw the trend lines you expect under competitive or non-competitive inhibition. [2 points]

Competitive:



Non-competitive



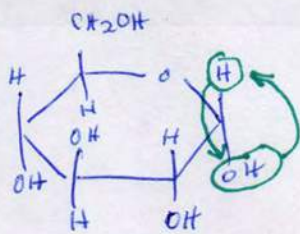
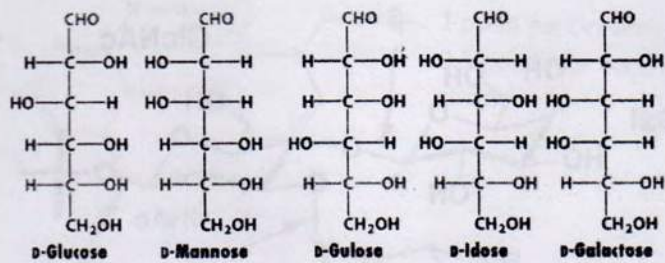
How do these modes of inhibition differ? Discuss in terms of the Michaelis-Menten equation. [2 points]

For competitive inhibition: $v = \frac{V_{max}}{\alpha K_m + [S]}$; $\alpha = \left(1 + \frac{I}{K_I}\right)$

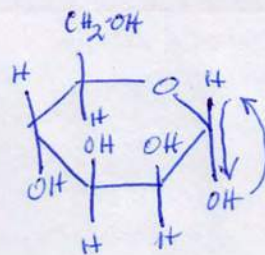
For non-competitive inhibition: $v = \frac{V_{max}}{\alpha(K_m + [S])}$; $\alpha = \left(1 + \frac{I}{K_I}\right)$

V. Carbohydrates [10 points]

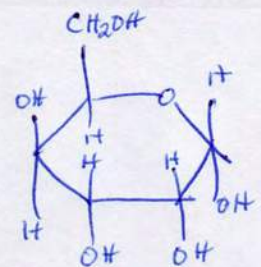
- (23) a) Write the Haworth cyclic form for D-Glucose, D-Manose, D-Gulose [1 point each]:



either one acceptable } α -D-glucose
 β -D-glucose



α -D-mannose
 β -D-mannose



α -D-galactose

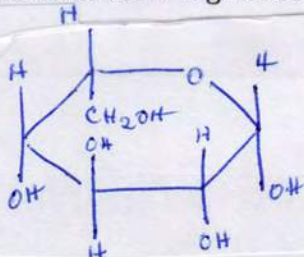
They were not asked to indicate α or β , so either of the two is OK.

b) Indicate the two epimers of glucose. [0.5 points each]

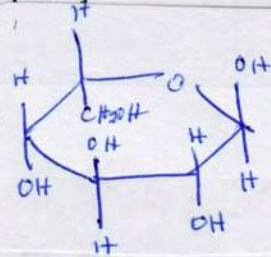
mannose and galactose

d) Write the formula of L-glucose in Haworth cyclic form. [2 point]

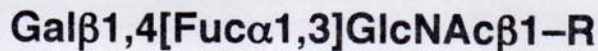
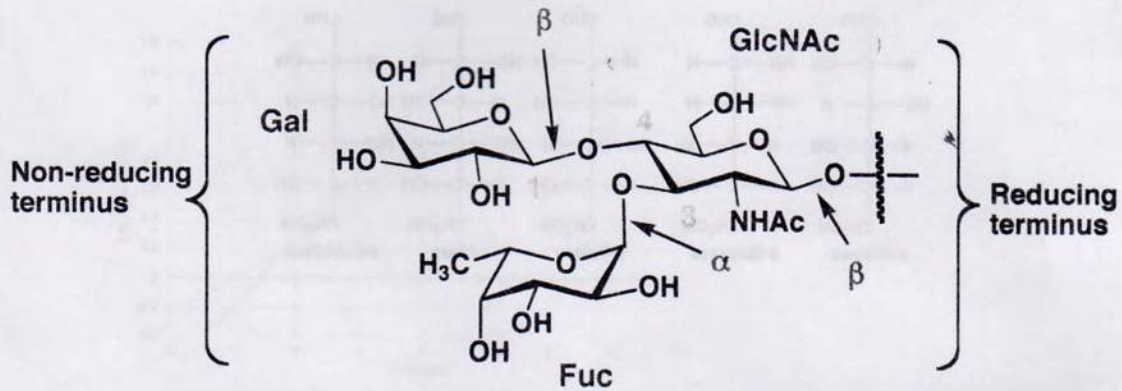
α -L-glucose



or β -L-glucose



(24) The following is the central tri-carbohydrate core of all blood groups:



Indicate what are the substituents that are added to the following blood types [0.5 point each]:

- a) A *Galactosamine*
 b) B *Galactose*
 c) AB *Galactosamine and galactose*
 d) O *no substituent*

- (25) a) Two proteins play a central role in the escape step of the virus from an infected cell. Name them. [0.5 points each].

Hemagglutinin and Neuraminidase

- b) Succinctly indicate what are their functions [1 point].

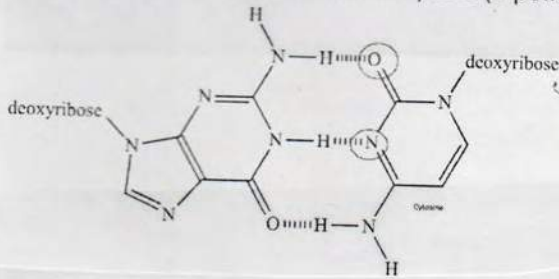
Hemagglutinin binds sialic acid on cell surface to invade the cell.

Neuraminidase, cleaves sialic acid to allow the newly formed viruses to escape the infected cell.

VI. Nucleic Acids [6 points]

- (26) Which Nucleotide has the most H-bond acceptors in a base pair? (1 point) Draw the structure and circle the H-bond acceptors. [2 points]

Cytosine has the most H-bond acceptors (1 point)

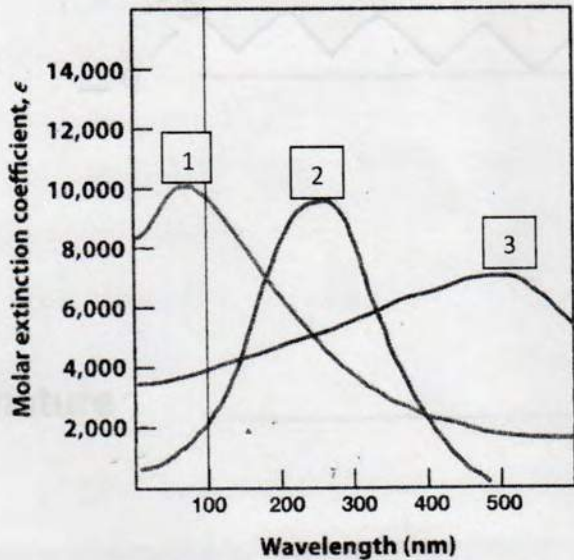


1 point for Drawing Cytosine Structure
0.5 points for each H-bond acceptor circled

- [27] Based on evolution, why is it better that DNA and not RNA the ultimate depository of genetic info? [2 points]

Because DNA is much more stable than RNA. RNA can be cleaved in basic medium.

- [28] MCB102)-MP is a new nucleic acid that has similar UV absorption properties as other known nucleic acids. Given the following UV Absorption Spectrum of a mixed solution with (MCB102)-MP in it, find the Absorbance at 100nm of 1mM of (MCB102)-MP in a 1mm path length cuvette. [2 points]



Substance 1: Peak at 100nm

$$\epsilon_{100} = 10,000 \text{ M}^{-1}\text{cm}^{-1}$$

Substance 2: Peak at 280nm

$$\epsilon_{200} = 2,000 \text{ M}^{-1}\text{cm}^{-1}$$

Substance 3: Peak at 500nm

$$\epsilon_{300} = 4,000 \text{ M}^{-1}\text{cm}^{-1}$$

The nucleic acid corresponds to spectrum 2. (1 point)

$$Abs = \epsilon c l = 2000 \text{ M}^{-1}\text{cm}^{-1} \times 10^{-3} \text{ M} \times 0.1 \text{ cm}$$

$$Abs = 0.2 \quad (2 \text{ points})$$

VII. LIPIDS [8 points]

(29) The energy is stored in lipids in the form of triglycerides [1 point]

[30] Cellular membranes are made of three major classes of lipids:

a) Phospholipids [1 point],

b) Glycolipids [1 point],

c) Sterols [1 point].

[31] The major component of the inner membrane of the mitochondria is: [2 points]

Glycerol phospholipids

[32] Write down the schematic formula ($\text{---}\backslash\backslash\backslash\backslash\backslash\text{COO}$.) for palmitic acid. [2 points]

