KEY
Exam I

1. Name the following molecules (abbreviations are OK for those that are known by their abbreviations) and state what they are used for in the biochemistry laboratory.

A)

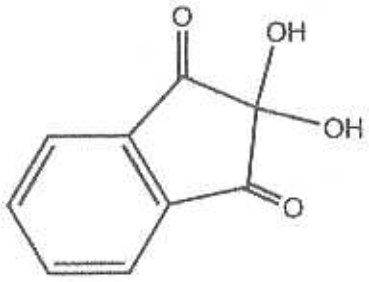
DCC

Used to activate carboxylate group (make O a good leaving group) for peptide synthesis

B) CNBr Cyanogen bromide

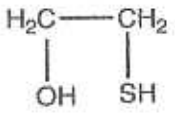
Used to break larger peptide into small pieces, breaks chain on the C side of methionine (forms homoserine lactone) from Met.

C)



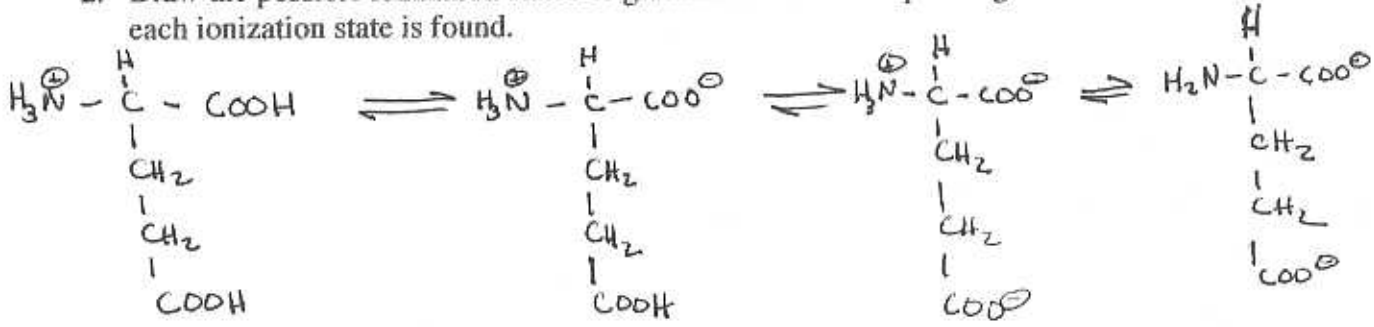
Ninhydrin
Used in amino acid detection

D)



R -mercapto ethanol
BME
Used to reduce disulfide bonds

2. Draw the possible ionization states of glutamate. Show the pH range in which each ionization state is found.



less than pH 2

between 2 and ~4

between ~4 and ~9

above pH 9

3. Information necessary for this problem
pKa MOPS = 7.2

A) You have 500 ml of a 0.050 M solution of MOPS base. What is the pH?

$pK_a + pK_b = 14 \qquad pK_b = 6.8$

$MOPS + H_2O \rightarrow \underset{\text{MOPS acid}}{MOPS\text{-}H^+} + OH^-$

$MOPS + H_2O \rightleftharpoons MOPS\text{-}H^+ + OH^-$

$0.050 - x \qquad x \qquad x$

$10^{-6.8} = \frac{x^2}{0.050 - x} \qquad x = [OH^-] = 8.90 \times 10^{-5}$

$pOH = -\log[OH^-] = -\log[8.90 \times 10^{-5}] = 4.05$

$pH = 14 - pOH = \underline{9.95}$

B) You add 2.0 ml of 6.0 M HCl to the solution in A). What is the pH?

moles MOPS base $= \left(0.050 \frac{mol}{L}\right)(0.5\ L) = 0.025$ mol

moles HCl $= (0.002\ L)\left(6 \frac{mol}{L}\right) = 0.012$ mol

moles MOPS base after HCl is added $= 0.025 - 0.012 = 0.013$ mol

moles MOPS acid after HCl is added $= 0.012$ mol

$$pH = pK_a + \log\frac{[A^-]}{[HA]} = 7.2 + \log\frac{0.013\ mol/Volume}{0.012\ mol/Volume}$$

$$= 7.2 + 0.03 = 7.23$$

(or to the sig. fig. of pH originally given $\underline{7.2}$.

C) You add 1.0 ml of 6.0 M NaOH to the solution created in B). What is the new pH?

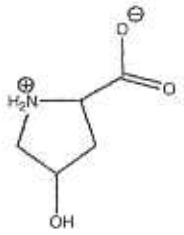
Initial { moles HA = 0.012 mol; moles A^- = 0.013 mol } + moles NaOH 0.006 mol $\rightarrow$ 0.006 mol HA, 0.019 mol A^-

$$pH = pK_a + \log\frac{0.019/V}{0.006/V} = 7.2 + 0.5 = \underline{7.7}$$

Still a buffer!

4. A) Name the following modified amino acid.

4-hydroxyproline



B) Where (in what protein) is this amino acid found? What structural purpose does it serve in this protein?

It is found in collagen. The addition of the OH group causes the 5-membered ring to pucker differently. The alteration of conformation helps stabilize the triple-helix (superhelix). Additionally, the OH group can participate in H-bonding.

C) How is this amino acid made? What vitamin is necessary for modification process? Why is this vitamin needed?

This modification is catalyzes by the enzyme prolyl hydroxylase. This enzyme has an iron cofactor that must be kept in the reduced state for the enzyme to be active. Vitamin C (a reductant) keeps the enzyme in the reduced form.

5. (20 pts)

A	B	C	D	E
Gly-Pro-Arg	Asp-Trp-Tyr	Leu-Val-Phe	Tyr-Lys-Met	Asp-His-Glu

Which one of the above tripeptides:

E is most negatively charged at pH 7?

D will yield DNP-tyrosine when reacted with 1-fluoro-2,4-dinitrobenzene and hydrolyzed in acid?

C contains the largest number of nonpolar R groups?

D contains sulfur?

B will have the greatest light absorbance at 280 nm?

6. (15pts) Oligomeric protein A dissociates to its component subunits when it is exposed to low concentrations of detergent such as SDS, but does not dissociate when the pH is changed significantly. Oligomeric protein B dissociates when the pH is changed, but not in the presence of low concentrations of SDS.

A) What experimental technique would you use to observe the results described above?

Experimental techniques: Size exclusion chromatography; analytical ultracentrifugation

Note: gel electrophoresis (PAGE) would not be an option as it is a high concentration of SDS and would totally denature a protein.

B) What do these results suggest about the interactions that stabilize the quaternary structure of the each protein?

Oligomeric protein A is held together by hydrophobic interactions, which are disrupted by low concentrations of SDS.

Oligomeric protein B is held together by hydrogen bonds or salt bridges. A change in pH will change the protonation states of the residues involved in the salt bridge or the hydrogen bond.

7. (12pts) You decide to do some mutagenesis experiments on a protein with a known structure. You make the following amino changes. Predict the effects you will see on the secondary structure. Briefly explain why you will see these changes.

A) Changing a Glu to a Lys. It is known that the Glu is located at the amino end of an α-helix.

A Glu at the amino end of an α-helix (+ part of the helix dipole) helps to stabilize the helix. Placing a + charged Lys will lower helix stability.

B) An Gly is changed to a Pro. This Gly is found in the middle of a β-sheet.

The change of a small amino acid, with many accessible ψ and ϕ angles, to one which has very constrained ψ & ϕ angles will disrupt the β-sheet.

C) A Lys is changed to a Glu. This Lys is in an α-helix which has the sequence: SRLAKVLSLIANT

Arg + Lys +

The Arg + Lys are separated by 3 residues so they will be on the same side of the α-helix. Two + charges are destabilizing. Changing the Lys to − (Glu) will help stabilize the helix.

8. List three differences between prokaryotes and eukaryotes.

Prokaryotes	Eukaryotes
No membrane bound nucleus	Membrane bound nucleus
No membrane bound organelles	Membrane bound organelles
Small, circular chromosome	large linear chromosome
no introns in DNA	introns in DNA
post-translation modification of protein not complex	post-translation modification can be complex (add lipid tails, sugars etc.)