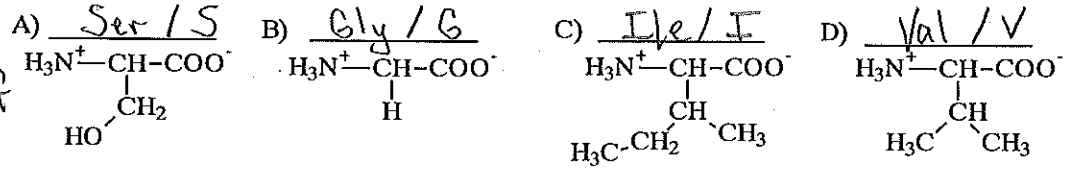
Fall '14 BCH370 Name KEY
Hackert Exam I UTeID _____

Given: $c = 3.0 \times 10^{10}$ cm/sec; $k = 1.38 \times 10^{-23}$ J/K; $h = 6.63 \times 10^{-34}$ J-sec; $No = 6.02 \times 10^{23}$ /m
$|E(1\%) \times MW| = |10 \times \varepsilon|$; Eff. = 1 / (1 + (R/Ro)^6); E = hv

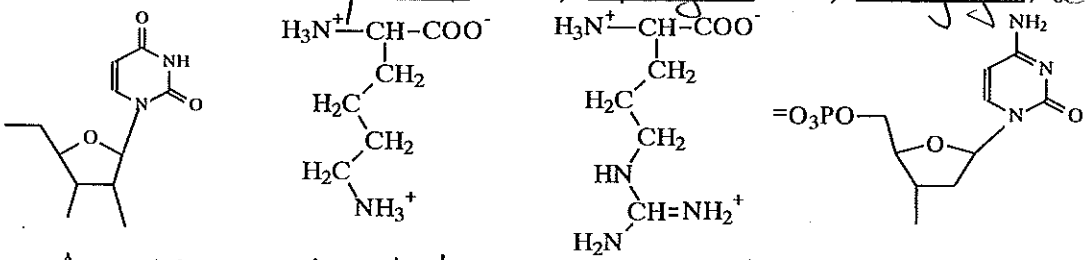
1. Identify each **amino acid** by its **three and one letter codes** (e.g. **Ala / A**, etc.) and each **N base, nucleoside** or **nucleotide** by its **full name** in the blanks above or beside the structures (**22 pts**).

1/2 pt each half

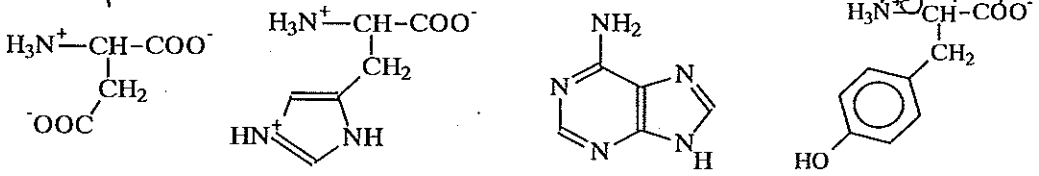
A) Ser / S B) Gly / G C) Ile / I D) Val / V



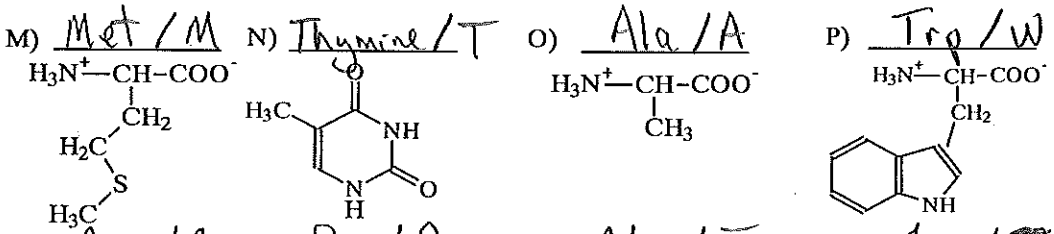
E) Uridine F) Lys / K G) Arg / R H) deoxycytidine MP / dCMP



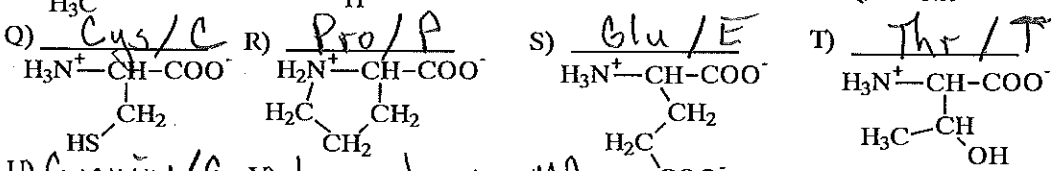
I) Asp / D J) His / H K) Adenine / A L) Tyr / Y



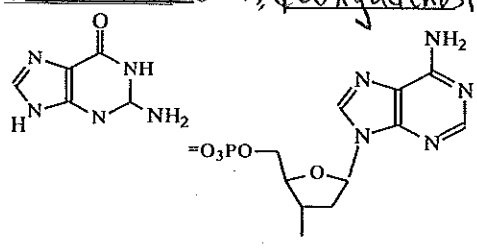
M) Met / M N) Thymine / T O) Ala / A P) Trp / W



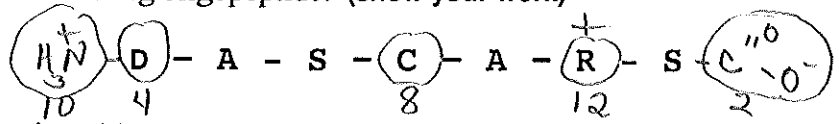
Q) Cys / C R) Pro / P S) Glu / E T) Thr / T



U) Guanine / G V) deoxyadenosine MP /dAMP



2. Consider the following oligopeptide: **(show your work)**



(1/1)

a) How many titratable protons are there in this oligopeptide under normal conditions? 5
(2) (Circle the groups involved.)

b) What is the net charge on this oligopeptide at very low pH (pH ~ 1.0)? +2 (2)
(2)

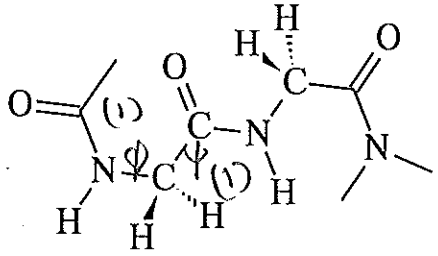
c) What is the approximate pI for this oligopeptide? 6 (3)
(3)

+2 (2) → +1 (4) → 0 (8) → -1 (10) → -2 (12) → -3

d) Describe how you could take advantage the charge properties resulting from the amino acid content of this oligopeptide to purify it by ion exchange chromatography. Describe a chromatography experiment to enable this oligo to bind and then be eluted from your column by describing the materials and conditions (pH, etc) needed.

(4) You want peptide to bind to column. Since pI ~ 6, run anion exchange col. like DEAE matrix at pH = 8 so peptide binds, then elute with high salt.
At pH ~ 4, could use cation exch. col. like CMC.

3. Label the phi (φ) and psi (ψ) angles on the ***first glycyl residue*** shown below. In the blanks provided, indicate the values of φ = 0° and ψ = 180° for this residue:
(4) (1) (1)



4. A classmate asks for your help understanding the relationships and differences between using Maxam-Gilbert (MG), dideoxy (DD) nucleotides and pyrosequencing of DNA.
a) For each of the following terms, place a "DD" for dideoxy, "MG" for Maxam-Gilbert and/or "PY" depending on which sequencing method the terms apply to. Note: more than one method may apply a term.
(6)

i) use of DNA polymerase	DD, PY	ii) "ladder" sequencing	MG, DD
iii) 5'-primer	DD, PY	iv) cleavage reactions	MG
v) luciferase	PY	vi) emulsion PCR	PY

1 pt each if correct
½ pt if partly correct
2

5. DNA structure: When someone discusses the "melting temperature" of DNA, a) what is being measured, and b) what factors would contribute to a higher Tm for a DNA molecule? c) Describe one feature of A-DNA that distinguishes it from B-DNA?

(6) a) Unstacking of base pairs / unfolding / Abs. inc. as base unstack, T_m is inflection pt. when ½ DNA has unfolded.

b) More G≡C base pairs

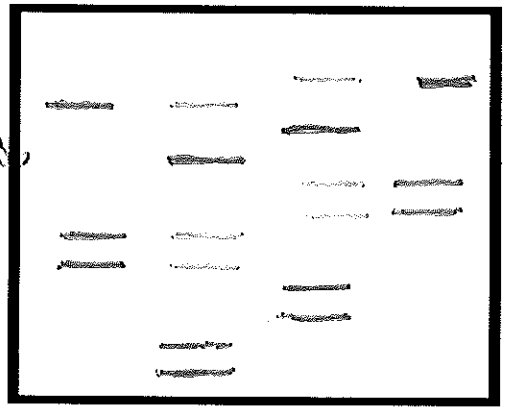
c) A-DNA: C3'endo sugar pucker / ~23Å dia / 28Å pitch / major minor gr

B-DNA: C2' " " " / ~20Å dia / 34Å pitch / large major groove

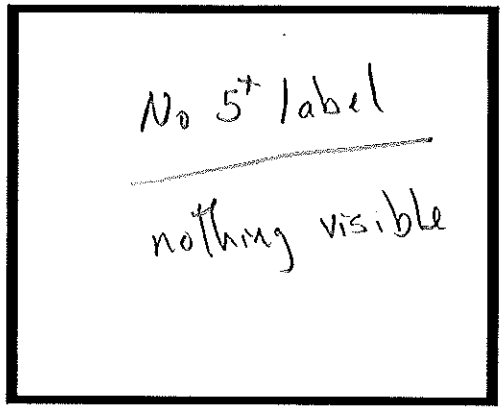
6. Consider the following nucleic acid sample: 5'-AATTGGCCATGC-3' with a 5' radiolabel. On gel "a" below, draw the expected gel pattern that would be obtained if the student carried out Maxam-Gilbert sequencing on this sample. On gel "b" below, draw the expected gel pattern that would occur if the student forgot to add the 5' radiolabel to the oligonucleotide

(4 + 3)

a) G rxn (1) G+A rxn (1) T+C rnx (1) C rxn (1)



b) G rxn G+A rxn T+C rnx C rxn



7. **Complete the table below** by calculating the specific activities for the following steps used to purify an enzyme, and **then answer** the questions below: **(Show your work)**

Step	[Protein] (mg/mL)	Volume (mL)	Total Activity (units)	Specific Activity
1. Crude extract	5.2	285	56,550	38
2. Salt ppt	10.4	38	35,750	90
3. Ion exhange Chrom	7.5	26	31,250	160
4. Affinity Chrom.	2.6	4.2	23,400	2143
5. Gel filtration	1.9	4.8	21,600	2368

(3) Fill in the table with the specific activity values.

(2) a) What is the overall percentage "yield" for this purification scheme? 38%

(2) b) What is the number fold improvement for this purification scheme? 62 X

8. **Recognition of Terms:** Match each of the first six terms with the phrase that best describes it.

(5) 1 pt each

f PAM100	a) arrangement of subunits
b ClustalW	b) multiple sequence alignments
d primary structure	c) independent folding unit with a subunit
c domain	d) covalent structure
e BLAST	e) sequence comparison algorithm / search sequence databases
	f) substitution matrix

9. Consider a "gel filtration" column that is 120 cm in length and 2.0 cm in diameter. It is packed with spherical beads that are 0.14 mm in diameter with a V_o that is 35% of V_{tot}. The column is calibrated with trypsin inhibitor (~21.5 kD) and β-galactosidase (~116 kD) which gave Ve /Vo values of 2.39 and 1.42, respectively. Calculate the expected partition coefficients and elution volumes for the **dimeric and hexameric** forms of a protein whose **subunit size** is 14,500 Da. (Show work for credit and Draw a Box around your answer).

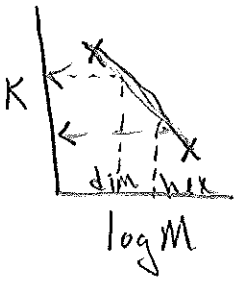
a) Estimate the **partition coefficients** for the dimeric and hexameric forms of the protein.

(6) $V_T = h \cdot \pi \cdot r^2 = 377$

$V_o = .35 \cdot V_T = 132$

$K = \frac{V_e - V_o}{V_T - V_o (245)}$

Protein	MW	logM	V_e	K
Trypsin	21,500	4.33	315	.75
dimer	29,000	4.46		.66
hexamer	87,000	4.94		.31
β-gal	116,000	5.06	187	.22



b) Estimate the elution volumes for both the dimeric and hexameric forms of the protein.

(2) $V_e = V_o + K(V_T - V_o)$

$V_{e,dimer} = 132 + .66(245) = 294\ mL$

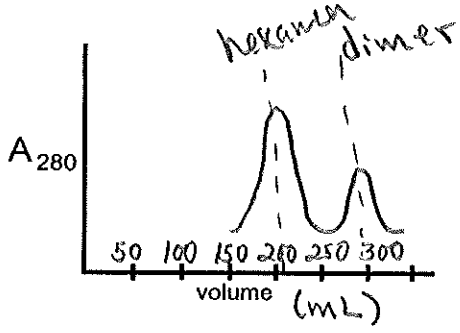
$V_{e,hex.} = 132 + .31(245) = 208\ mL$

c) Illustrate on the graph what A280 profile peaks would be expected if your sample contained equal numbers of dimers and hexamers.

(2)

Equal numbers of dimers & hexamers

⇒ 3x as much protein by weight in hexamer band



1 pt. order
1 pt. rel. heights

10. Given the sizes and pI's of the following proteins, predict the order in which these proteins would be eluted off a DEAE (diethylaminoethyl) column run at pH 7.0 and eluted with a salt gradient from 0.10 M NaCl to 2.0 M NaCl.

	A) Hemoglobin	B) Serum albumin	C) Chymotrypsin
Size (kD):	64.5	68.5	23
pI:	6.8 ~0	4.9 (−)	9.5 (+)

(2) Order off column: (1st off) C A B (last off)

Since DEAE col. is (+) charged, only albumin binds tightly

11. Expression:

a) What are two advantages and two disadvantages of using *E. coli* to express eukaryotic proteins?

(2) Advantages i) Cheap

ii) Fast / easy to manipulate

(2) Disadvantages i) Codon usage / misfolding / Toxicity

ii) Sugar linkages / post-trans. mod.

b) Your protein contains an N-terminal proline that is crucial for activity, but you want to use an affinity tag to improve the purification of your expressed protein. Consider the following vector, what restriction sites should you use to add a C-terminal His tag?

(4) EcoRI or NdeI to insert your protein's DNA before His_6 tag so tag is on C-terminus.

12. A protein has a molar extinction coefficient of 98,600 $M^{-1}cm^{-1}$ at 280 nm. A sample in a standard 0.50 cm cuvette was found to have a T of 62% at a wavelength of 280 nm. The molar extinction coefficients of tryptophan ($\varepsilon = 5.6 \times 10^3\ M^{-1}cm^{-1}$) and tyrosine ($\varepsilon = 1.4 \times 10^3\ M^{-1}cm^{-1}$) at 280 nm are as given, and the molecular weight of the protein is 92,350.

a) What is the **absorbance** for this sample protein solution?

(3) $A = -\log T = -\log(.62) = 0.21$

b) Calculate the **E(1%)** extinction coefficient for this protein at 280 nm.

(2) $|E^{1\%}| \cdot MW = |\varepsilon| \cdot 10 \Rightarrow |E^{1\%}| = \frac{98,600\,(10)}{92,350} = 10.7\ (g/dL)^{-1}cm^{-1}$

c) Calculate the concentration of this protein solution in **mg/mL**.

(3) $A = E^{1\%} \cdot c \cdot l \Rightarrow c = \frac{(0.21)}{10.7\ (g/dL)^{-1}cm^{-1}\,(.5cm)} = 0.039\ g/dL \Rightarrow 0.39\ mg/mL$

13. You are running a Next Gen sequencing experiment. When you image your plate, you see a bunch of colorful spots.

a) What does each spot represent? Amplification of a single DNA fragment
(2)

b) What does the color represent? Identifies last nucleotide incorporated during replication event
(2)

(Please sign your name on the back of your exam in a manner that you can recognize it when it is returned.)