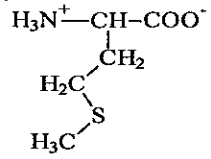
Fall '12 **CH370** Name KEY
Hackert **Exam I** UTeID _____

1. Identify the numbered compounds from the list of 1-26 below, identifying 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 provided (20 pts).

(21)

1 = Met / M	2 = Adenine	3 = Pro / P	5 = Lys / K
6 = deoxythymidine	7 = Gln / Q	9 = Phe / F	10 = Trp / W
11 = Guanine	12 = Tyr / Y	13 = Glu / E	14 = Cys / C
15 = Uracil	16 = Ser / S	17 = Glu / G	19 = Asn / N
21 = Thymine	23 = Ile / I	25 = Cytidine	26 = Leu / L

1)


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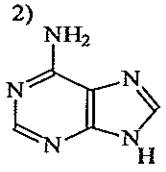

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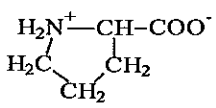

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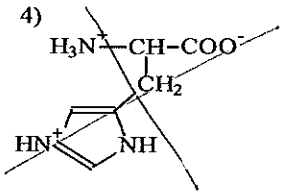

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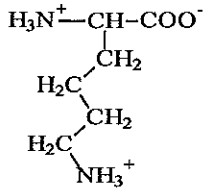

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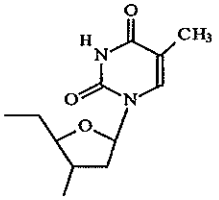

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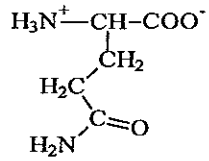

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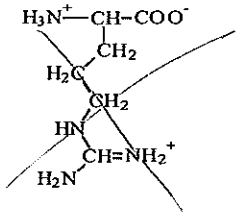

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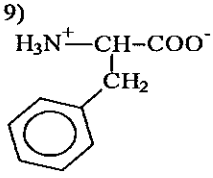

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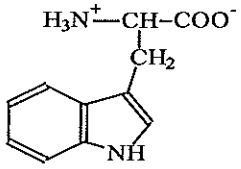

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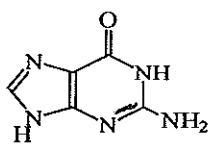

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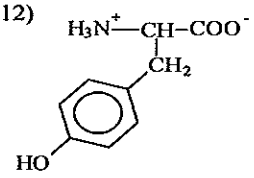

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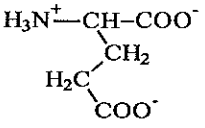

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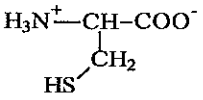

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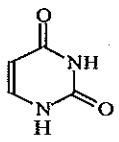

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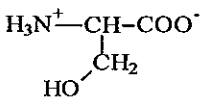

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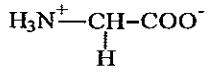

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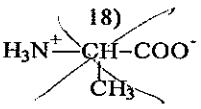

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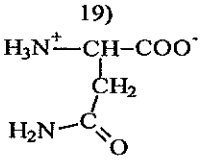

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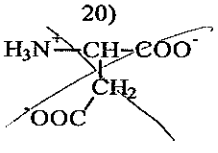

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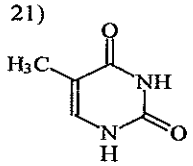

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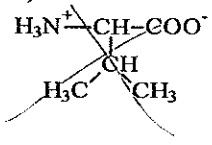

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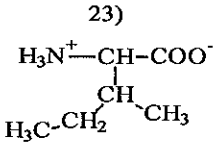

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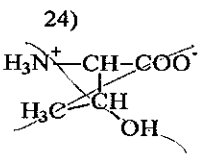

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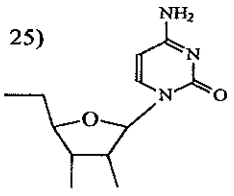

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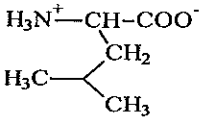


2. a) Estimate the charge of the oligopeptide at pH 1 and pH 14.

$^{+}H_3N$-**RAMACHANDRAN**-COOH 2pts pKa indicated

~pKa 3 10 12 8 6 4 12 2

+ + 0 + 0 + 0

(4) **pH 1:** +4 (1) **pH 14:** -3 (1)

b) Estimate the isoelectric point of the oligopeptide above 9 (2).

(4) +4 $\xrightarrow{2}$ +3 $\xrightarrow{4}$ +2 $\xrightarrow{6}$ +1 $\xrightarrow{8}$ 0 $\xrightarrow{10}$ -1 $\xrightarrow{12}$ -2 $\xrightarrow{12}$ 3 2pts work

Av. ~ 9

c) If you want the oligopeptide to bind to a carboxymethyl cellulose ion exchange column, what pH buffer should you use?

CMC is ⊖, so peptide ⊕ → pH < pI

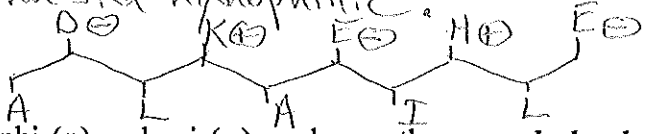
(3) Buffer pH ~ 7 (< 9) (2) (1)

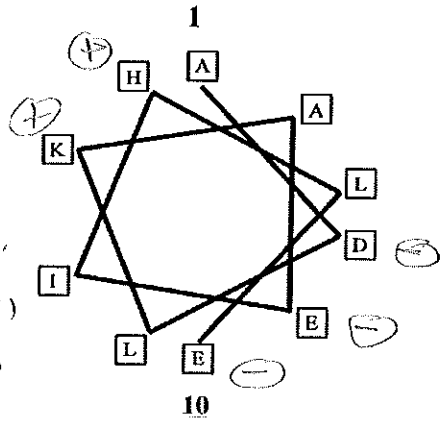
3. Consider the decapeptide sequence ADLKAEIHLE, also presented on the helical wheel shown below, what should you conclude about the most likely secondary structure for this decapeptide – helix or sheet? Why?

Sheet, as helical wheel shows these residues in a helix pack ⊖ side chains next to one another, similarly ⊕ side chains. In a sheet – one side hydrophobic, other side hydrophilic.

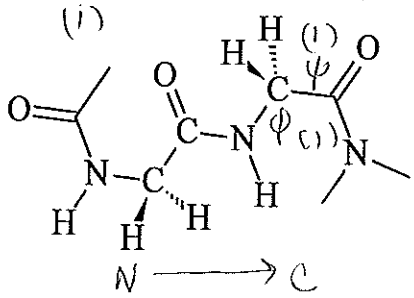
1-2 for helix with good why → 3 sheet 4-5 good why.

(5)



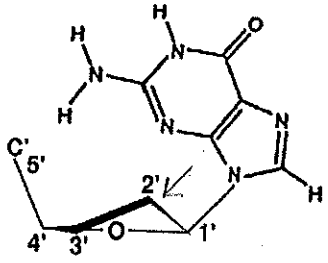


4. Label the phi (φ) and psi (ψ) angles on the ***second glycyl residue*** below. In the blanks provided, indicate the values of φ = 180° (1) and ψ = 0° (1) for this residue:



(4)

5. Identify the sugar pucker / base conformation shown below:



(1) (1) (2)

(4) C2' endo / Syn

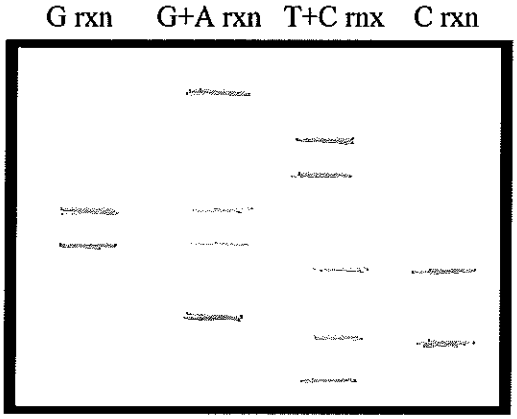
6. For each of the following terms, place a "*DD*" for dideoxy **and/or** a "*MG*" for Maxam-Gilbert **and/or** "*PS*" for pyrosequencing **and/or** "*IL*" for Illumina sequencing depending on which sequencing method the terms apply to.

(6) 1 each or ½ +/- i) use of DNA polymerase DD / PS / IL ii) "ladder" sequencing DD / MG
iii) bridge amplication IL iv) luciferase PS
v) emulsion PCR PS vi) reversible terminators IL

7. Consider the following nucleic acid sample: 5'-ATTGGCACT-3' with an ***unusual 3' radiolabel.*** On the "gel" below, draw the expected gel pattern that *would occur if* the student carried out Maxam-Gilbert sequencing on this sample with the ***unusual 3' radiolabel.***

(5)

3' label / chemical cleavage → 3' pieces indicated on gel



MG - correct 5
MG - reversed 3
DD - correct 2
3' label on primer

8. When someone discusses the "melting temperature" of DNA, a) what property of DNA is changing that is really being referred to, b) what physical property of DNA is actually being measured, and c) what factors contribute to a higher Tm for a DNA molecule?

(6) 2 each
a) Denaturation of double helix or base stacking
b) Absorbance (A_{260}) changes when stacked bases unstack
c) High G≡C content increases T_m

9. Expression:

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

(4) Advantages 1 each
i) Easy / Fast
ii) Cheap / Not contaminated easily

(4) Disadvantages
i) Codon usage / Post trans. mod.
ii) Toxicity / Folding (-S-S-)

10. **Recognition of Terms**: Match each of the ~~six~~ five terms with the phrase that best describes it.

(5) f PAM
b ClustalW
d primary structure
c domain
e BLAST

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

11. You have visited your doctor about a "lump" on your back. She runs a genomics marker test using a DNA microarray to compare "normal" cells vs. "lump" cells. After 24 hours exposure, mRNA is harvested, cDNA prepared using red-dye markers for the "normal" cell sample and green-dye markers for "your lump" cells. Any gene product that shows **no difference** in expression between the two cell lines would be indicated by a Yellow spot.

(3) A) red (B) yellow C) green spot D) white E) black

12. For the 5-step enzyme purification shown, **answer** the questions below:

Step	Protein (mg/mL)	Volume (mL)	Total Activity (units)	Specific Activity
1. Crude extract	9.2	268	87,737	36
2. Salt ppt	23.4	42.8	55,650	56
3. Ion exhange Chrom	22.7	14.3	35,552	109
4. Affinity Chrom.	8.3	8.4	24,890	357
5. Gel filtration	3.4	4.3	23,285	1593

(1) a) Which step (#) exploited the differences in charge? 3

(1) b) Which step (#) exploited the differences in size? 5

(2) c) Complete the purification table by filling in the remaining blanks. ✓

(2) d) What is the overall percentage "yield" for this purification scheme? $\frac{23285}{87737} = 27\%$

(2) e) What is the fold (number) purification for this purification scheme? $\frac{1593}{36} = 44$

(1) f) Which step (#) of the purification produced the largest % increase in specific activity? 5

13. 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 (+)

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

(2) (2)

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14. Consider a "gel filtration" column that is 120 cm in length and 2.60 cm in diameter with a total volume of 504 cm^3. It is packed with spherical beads that are 0.13 mm in diameter with a V_o that is 34.0% of V_{tot}. The column is calibrated with two standards, "Std A" (~18,500 Da) and "Std B" (~163,800 Da) which gave Ve /Vo values of 2.54 and 1.39, respectively. An unknown protein is run on the same column and gave a Ve /Vo value of 2.16. (Show work / draw a Box around answers).

$V_T = 637\ cm^3$ $V_o = 217\ cm^3$ $V_T - V_o = 420\ cm^3$

a) Calculate the elution volumes for the two standards and the unknown.

1 each (3)

$V_{e,A} = 217\ cm^3 \times 2.54 = 551\ cm^3$

$V_{e,B} = 217 \times 1.39 = 302\ cm^3$

$V_{e,unk} = 217 \times 2.16 = 469\ cm^3$

b) Calculate the **partition coefficients** for the two standards and the unknown.

(3) 1 each

$K = \frac{V_e - V_o}{V_T - V_o} = \frac{V_e - V_o}{420}$

$K_A = .80$ 18.5 kDa

$K_B = .20$ 164 kDa

$K_{unk} = .60$ (unk)

c) Estimate the molecular weight of the unknown protein.

(4)

	K	log M (1) for using log M
A	.80	4.27
B	.20	5.21
unk	.60	4.58

.94 → .31

→ M ~ 38,300 Da

> 100 kDa (-2 pts)

(-1 math)

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