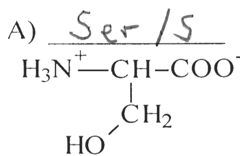
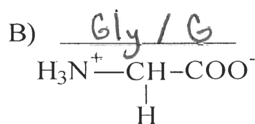
Spring '05 **CH370 / CH387D** Name KEY
Hackert **Exam I**

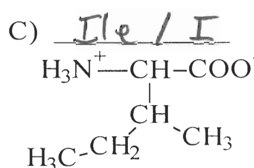
1. Identify each **amino acid** by its **three and one letter codes** (e.g. **Ala / A**, etc.) and each **N base, nucleoside** or **nucleotide** by its **full name** in the blanks **ABOVE** the structures (26 pts).

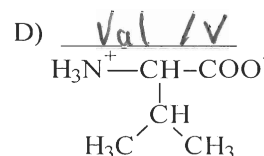
(26)

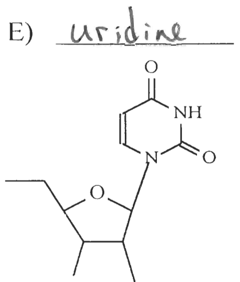
½ / ½ or 1pt each

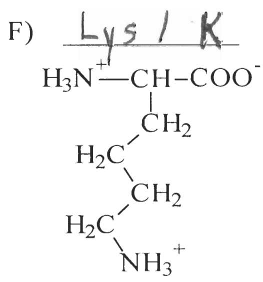
A) Ser / S


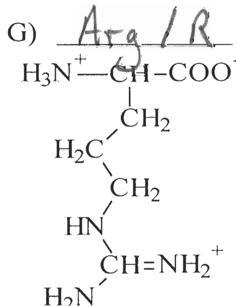
B) Gly / G


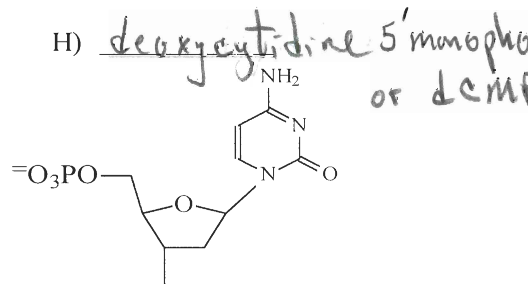
C) Ile / I


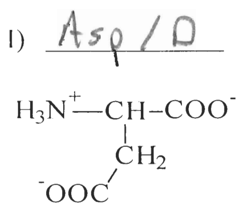
D) Val / V


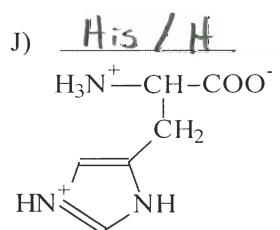
E) uridine


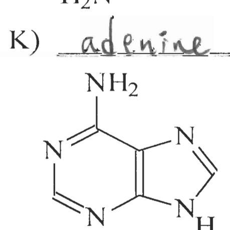
F) Lys / K


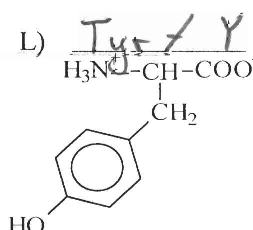
G) Arg / R


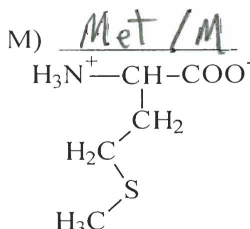
H) deoxycytidine 5'monopho[sphate] or dCMP


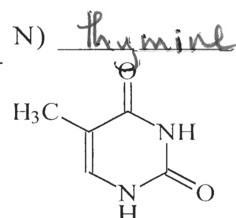
I) Asp / D


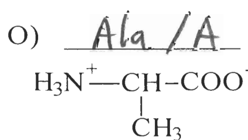
J) His / H


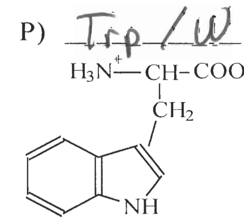
K) adenine


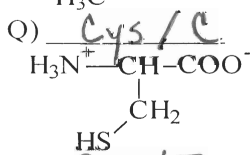
L) Tyr / Y


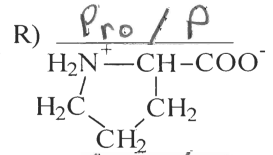
M) Met / M


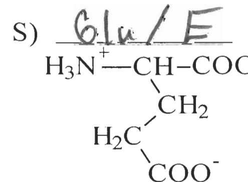
N) thymine


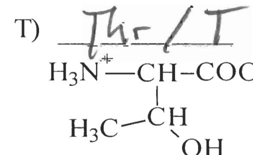
O) Ala / A


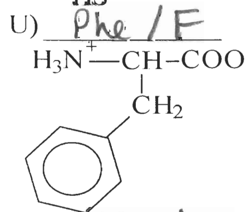
P) Trp / W


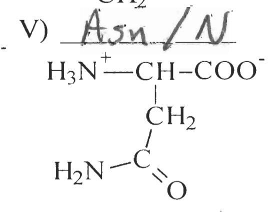
Q) Cys / C


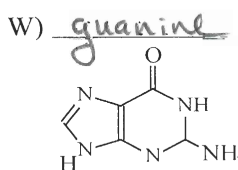
R) Pro / P


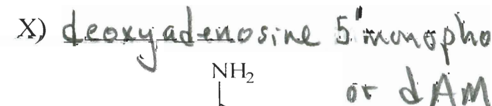
S) Glu / E


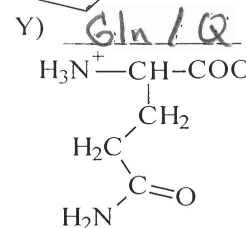
T) Thr / T


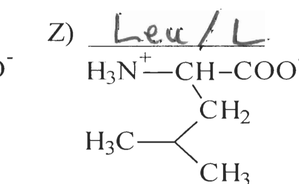
U) Phe / F


V) Asn / N


W) guanine


X) deoxyadenosine 5'monopho[sphate] or dAM[P]


Y) Gln / Q


Z) Leu / L


2. a) Which **one** amino acid is primarily responsible for the adsorption by proteins at 280 nm?
(2) Trp / W

b) Identify the 3-letter code of **one** amino acid for the approx. pKa's listed for their side chains.
1 each (5) 4: Glu Asp 6: His 8: Cys 10: Lys Tyr 12: Arg

3. You are trying to purify a mixture of the following two polypeptides:

RAMACHANDRAN and **TEAMLEADER**

a) Estimate the charge of each peptide at pH 14.

2 each (4) **RAMACHANDRAN** -3
TEAMLEADER -5

b) Estimate (± 1 pH) the isoelectric point of each peptide.

2 each (4) **RAMACHANDRAN** ~9
TEAMLEADER ~4

Describe how you could take advantage the charge properties resulting from the amino acid content of these peptides to separate them. Recommend a chromatography method and conditions (pH, etc.) for their separation.

(4) Since the two isoelectric points are very different, separate by charge using an ion-exchange column. At pH ~6.5 (any pH between 4 and 9) the two peptides have opposite charges. e.g. On a DEAE anion exchange column, "TEAMLEADER" is neg. charged and would bind but "RAMACHANDRAN" would pass through

4. Define the difference between a folding "**domain**" and a "**motif**". Name one example of each.
(6)

2x [2 - def. 1 - example]

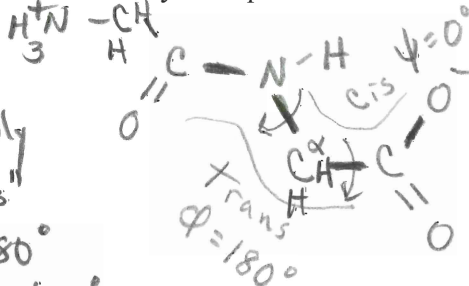
Domain - an independently folded unit or portion of a larger subunit - often possessing a defined func (nucleotide binding domain)

Motif - a conserved, short region (sequence) or portions of seq. that are conserved - part of a domain. Leu zip

5. Draw the structure of the dipeptide glycylglycine in a conformation that has a value of phi $\varphi = 180^o$ and psi $\psi = 0^o$ with the peptide bond in a trans ($\omega = 180^o$) configuration. In what type of protein structure would you expect to find this conformation to be present?

(6)

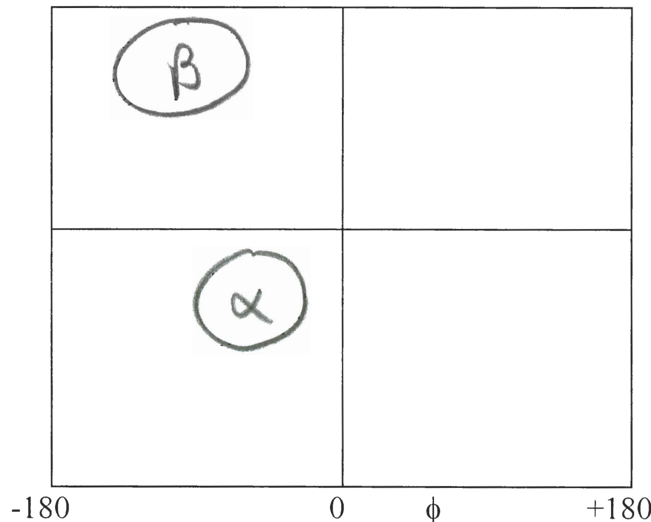
1 pt GlyGly
2 pts "trans" $\varphi = 180^\circ$
2 pts "cis" $\psi = 0^\circ$



None, This is not a favorable conformation. In an extended protein, the N-H H-N will conflict

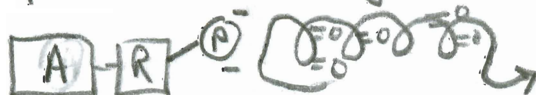
6. A common method of evaluating the stereochemistry of a protein structural model is to compute a ___Ramachandran___ plot? Draw a circle on the plot below the approximate regions for β-sheet and right handed α-helix.

(6)



7. You are modeling the X-ray structure of an AMP-dependent enzyme and are stuck having to work with a low-resolution (poor) electron density map. You can make out some features that appear to be helices and sheets, but the resolution is too poor to tell the direction of the polypeptide chain. However, the electron density for the phosphate of the AMP moiety shows up strong in the map, and appears to be positioned at one end of a helix. How does this information help you assign the direction of the polypeptide chain for that helix? Why does the phosphate prefer that binding location?

(5) The phosphate of AMP is neg. and will prefer the N-terminal (not N-Terminus) end of helix due to partial + charge arising from the aligned dipoles



8. Some oligomeric proteins are known to "fall apart" when stored in the cold and are more stable in solution at room temperature. Speculate on what types of non-covalent forces might dominate oligomer formation for such systems and explain this in terms of Gibb's free energy.

(5) $\Delta G = \Delta H - T\Delta S$

ΔG more neg. for more favorable process

⇒ to be more favorable at higher Temp ⇒ ΔS (+)

this is due to solvent entropy → dimerization

9. You have the following amino acid sequence from a protein that you believe is part of a "smart hormone" that recognizes DNA. P-Y-V-N-V-K-L-P-G-R-S-D-E-Q-L-K-N-L-V-S-E-V-T-D-A-V-E. You want to search for homologues to find out more about the properties of this protein's relatives. You use your web browser to go to the N C B I site that is a well known bioinformatics site providing access to many sequence databases. You search for homologues using the BLAST routine and obtain the following results.

1 pt each

(2)

```
                                                          E - score
1. gi[1042879] HUMAN PROTEIN                            482   2e-85
2. gi[2346879] HYPOTHETICAL PROTEIN Mus                 123   4e-18
3. gi[5310428] UNKNOWN PROTEIN FROM 2D-PAGE              79   7e-13
4. gi[2385419] Y531  protein [Methanobacterium]          55    1.45
```

a) What can you conclude from the search results? Are there any significant homologues?

(3) Yes, there are 3 significant hits - those with "Expect" values < 1.

b) Aligning up the amino acid sequences gives the following results:

```
                  1     5         10        15        20        25
                                   *         *         *
1. gi[1042879] P-Y-V-K-V-Q-L-P-G-P-[S]-D-E-Q-L-[K]-N-L-V-[R]-E-V-T-D-A-V-E
2. gi[2346879] P-F-L-S-L-R-L-P-G-P-[S]-N-E-Q-L-[K]-N-L-V-[R]-E-L-S-E-A-V-E
3. gi[5310428] P-F-I-N-V-K-L-P-G-P-[S]-S-E-Q-L-[K]-D-I-V-[R]-E-I-T-Q-A-V-E
```

ignore ~~4. gi[2385419] P-I-V-N-V-I-T-G-G-D-V-A-H-E-S-P-L-V-S-M-V-I-K-A-D-V-A~~

The protein is believed to bind non-specifically to the phosphate backbone of nucleic acids, place an (*) **above those residues most likely to be involved in this binding**. Why?

(4) Since the phosphate backbone is ⊖ charged, non-specific binding would most likely involve conserved ⊕ charges like K16 or R20 and maybe Ser 11.

+4 K,R

10. Name four general properties of proteins whose differences can be exploited to separate and purify proteins. Also, name one technique based on each of these properties.

(8)

1 pt each

A) solubility - salt cuts (AS)

B) size - size exclusion / "gel filtration" chrom

C) charge - ion exchange chrom

D) affinity - IMAC / PLP-affinity / etc

-1 for using affinity and specificity

11. A classmate asks for you help. They are having trouble understanding the use of dideoxy nucleotides and how that relates to the Maxim-Gilbert method of DNA sequencing. Explain their misunderstanding by contrasting the difference between the Maxim-Gilbert and Sanger methods of DNA sequencing.

(5)

a) Maxam - Gilbert method relies on chemical treatment to create seq. specific fragments that can then be seq. using "ladder - seq." tech.

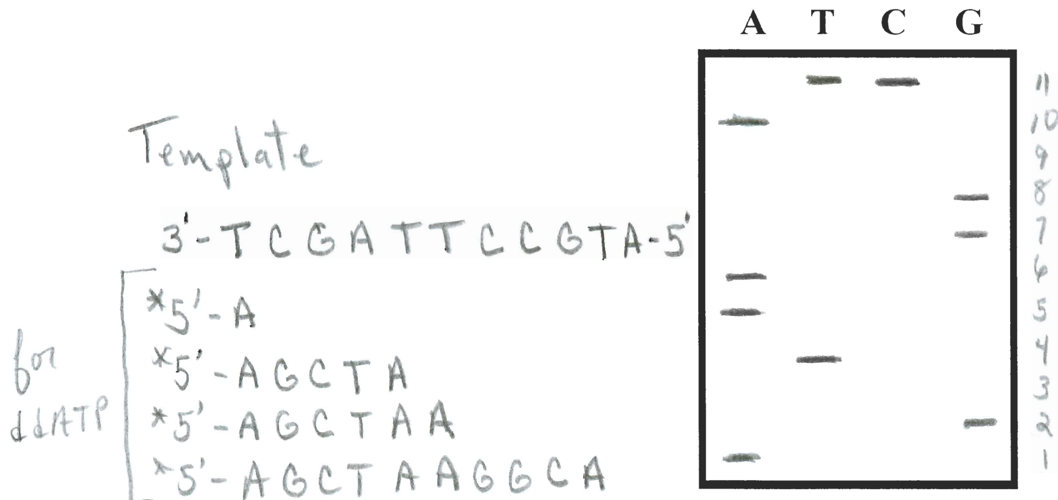
2

b) Sanger method uses DNA as template and dideoxy nucl. to terminate growing chains.
"Chain termination method" - also uses "ladder sequencing" but now labelled fragments are of the newly synth. complement to the template.

3

Consider the following nucleic acid sample: 5'-ATGCCTTAGCT-3' used as the template in a dideoxy sequencing experiment by an undergraduate assistant. On the "gel" below, draw the expected gel pattern that *would occur if* the student used 5'-primer with a fluorescent label, but forgot to add the dideoxy CTP to that reaction mixture.

(5) [DNA polymerase I + 4 dNTPs + ddATP ddTTP ddCTP ddGTP]



-3 for not using template

-1 for more than one band in "C" lane