HW3: Chapter 3: 2, 7, 8, 9, 11, 15, 18, 21, 22

(2) What do we have?

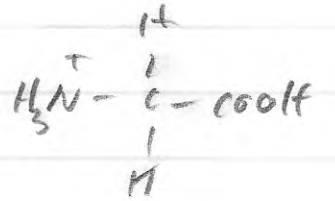
0.1L of 0.1M glycine @ pH 7.2

What are we doing?

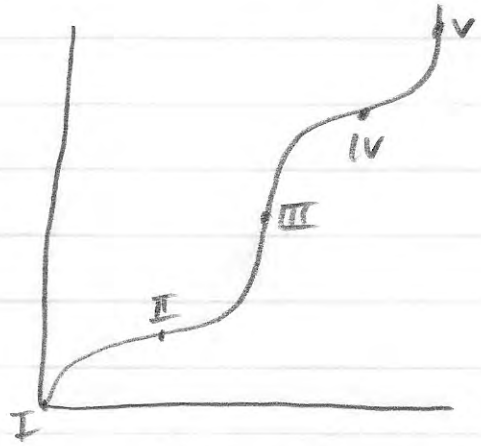
Adding 2M NaOH

What will happen?

@ pH 2 glycine looks like:



- Evaluate the titration curve:



@ I: 100% Species I

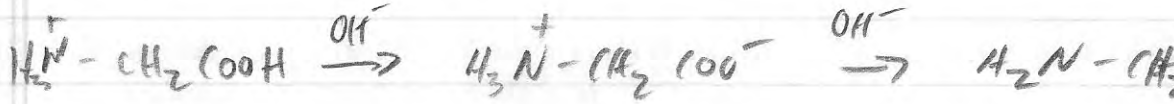
@ II: 50% Species I 50% Species 2

@ III: 100% Species 2

@ IV: 50% Species 2 50% Species 3

@ V: 100% Species 3

Titration will result in:

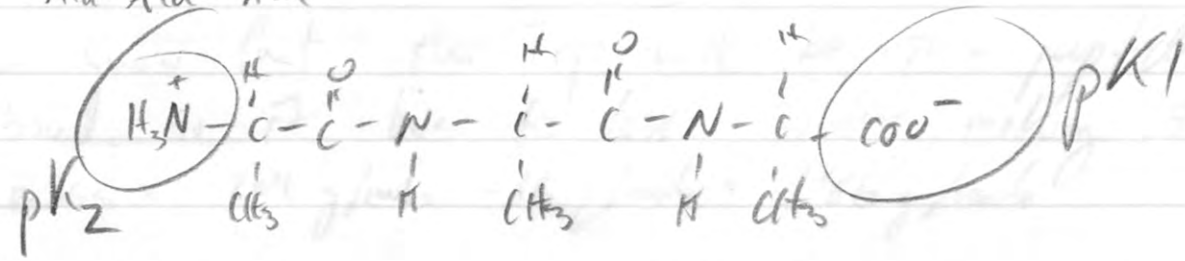


Species 1 Species 2 Species 3

Using the previous page as a guide:

a) I
b) II
c) IV
d) II
e) IV
f) II and IV
g) II
h) III
i) V
j) III
k) V
l) II
m) III
n) V
o) I, III & V

(P) a) Ala-Ala-Ala



b) The electron withdrawing effect of the α-amino group is decreased once it becomes involved in an amide bond.

c) The pKa of the α-amino groups decreases because the carboxylate groups moves further away from it with the addition of each successive Alanine residue. The negative charge of the carboxylate stabilizes the positive charge of the amino groups.

(8) Average molecular mass of an amino acid = 110 g/mole

682 amino acids × $\frac{110\ g}{mole}$ = $\frac{75020\ g}{mole}$ or 75020 Daltons

(9) BSA contains 0.58% Trp (204 g/mole)

a) Calculate the minimum mw of BSA assuming there is only a single Trp per molecule of BSA

We know that the Trp will be in a peptide bond, so it has to lose water, making its mass = 204 g/mole − 18 g/mole = 186 g/mole

$\frac{\text{Amount of Trp}}{\text{Amount of Protein}} \Rightarrow \frac{0.58\ g}{100\ g} = \frac{186\ g/mole}{x\ g/mole} \Leftarrow \frac{\text{mass of Trp}}{\text{mass of protein}}$

x = 32069 g

b) The mass value of 70,000, is roughly double the mass we calculated in part a), so there must be 2 Trp residues in BSA

(11) a) $^{9.67}NH_3^+$-Glu-His-Trp-Ser-Gly-Leu-Arg-Pro-Gly-COOH (2.34)

COOH 4.25; HisH$^+$ 6.00; NH_2^+ 12

pH3, the side chains will all be protonated so the charge is: +2

pH 8: 0

pH 11: -1

b) pI = Must be between the Glu amino terminus and the His side chain pKa's.

$pI = (9.67 + 6.0)/2 = 7.8$

(12) a)

	Sp. Activity (units/mg)	
Step 1	200	
2	600	
3	250	
4	4000	← Largest jump (from step 3 to step 4)
5	15000	
6	15000	

b) Step 4 has the largest fold increase

c) Step 3 is least effective

d) Since the specific activity didn't increase between steps 5 and 6, the protein is likely pure. Running an SDS-PAGE gel would confirm that.

(12) a) Hydrolysis gives Gly / Leu / Phe / Tyr
2 : 1 : 1 : 1

b) 2,4, DNP tyrosine = Tyrosine is on the amino terminus

c) Free Tyr and Free Leucine
Chymotrypsin cleaves on the carboxyl side of Phe, Tyr and Trp. Therefore, the 1st residue is Tyr and the last is Leu. Phe must be the penultimate residue.

Sequence: Tyr - Gly - Gly - Phe - Leu

(71) a) Amino acids that are conserved only show a single large letter at that arrangement.

Positions: 1, 7, 9

b) Positive residues are found in positions 4, 9
Lysine is preferred @ position 4
Arginine is always found @ position 9

c) Negatively charged amino acids are found @:

Position 5 (Glu preferred)
Position 10 (Glu preferred)

d) Position 2 can be any amino acid (Hence the "X" designation) but Serine is preferred

(22) a) Anion exchange chromatography
- count the negative charges on each peptide, the one with the most charges will move slowest

Peptide 1: 1 (-) charge
Peptide 2: 5 (-) charges
Peptide 3: 1 (-) charge

← Peptide 2 would move the slowest

b) Cation exchange chromatography. Count the (+) charges on each

Peptide 1: 8 (+) charges
Peptide 2: 0 (+) charges
Peptide 3: 2 (+) charges

← Peptide 1 would move the slowest

c) The smallest peptide would move the slowest so Peptide 2

d) Peptide 3 has that motif:
GMEGVGKS

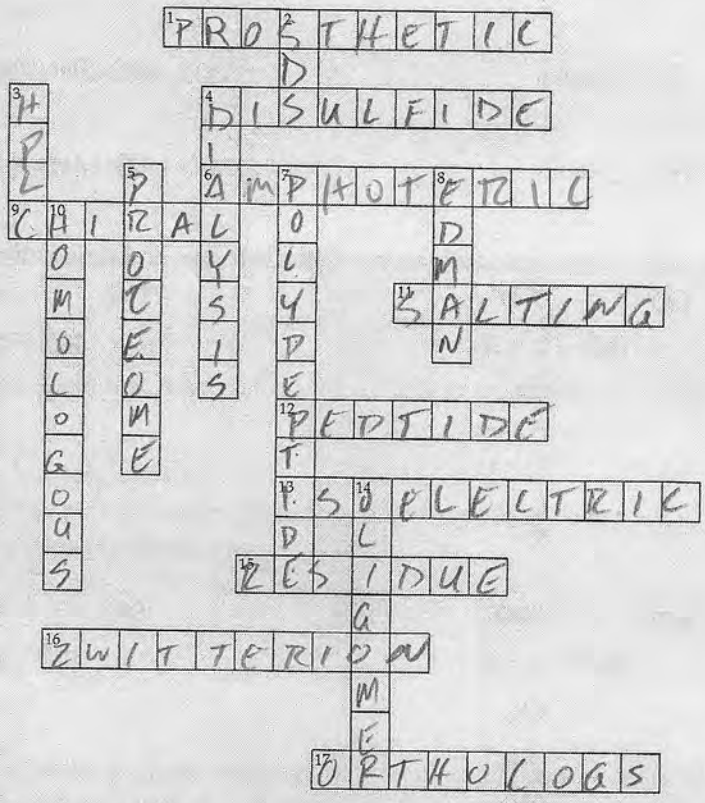
Crossword 3

SELF-TEST

Do You Know the Terms?

ACROSS

1. The zinc ion in a zinc finger protein is an example of a _____ group.
4. A covalent bond between two nonadjacent cysteines in a polypeptide chain is a _____ bond.
6. An example of a(n) _____ amino acid is histidine, which can either accept protons or donate them at a pH that is close to physiological pH values.
9. All stereoisomers must have at least one _____ center.
11. _____ out is a technique that selectively precipitates some proteins, while others remain in solution. Ammonium sulfate ($(NH_4)_2SO_4$) is often used for this purpose.
12. The bond type that forms the primary structure.
13. The pH at which the numbers of positive and negative charges on an amino acid are equal is referred to as the _____ point or pI.
15. A single unit within a polymer: for example, lysine in a protein molecule.
16. At pH 7, any amino acid with an uncharged R group is a _____.
17. Proteins from different species that have similar amino acid sequences and functions are referred to as _____.



DOWN

2. A reagent used in electrophoresis to separate polypeptides on the basis of mass. (abbr.)
3. Many types of separation can be done using this chromatographic technique; its advantage lies in the reduction of transit time on the column, limiting diffusional spreading of protein bands and improving resolution. (abbr.)
4. After "salting out" proteins, removal of excess ammonium sulfate can be accomplished by _____ of the protein-salt solution overnight against large volumes of buffer.
5. The whole assortment of proteins in an organism; analogous to the genome.
7. A linear chain of amino acid residues that usually has a molecular weight less than 10,000 daltons.
8. The _____ degradation procedure provides information about a protein's primary structure.
10. Insulin obtained from sheep can be used to treat human diabetics because sheep and human insulin are _____ proteins.
14. Hemoglobin, which contains two sets of identical subunits, is often referred to as a(n) _____.