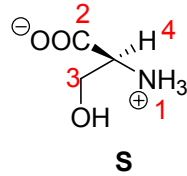
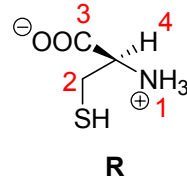
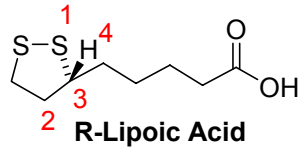
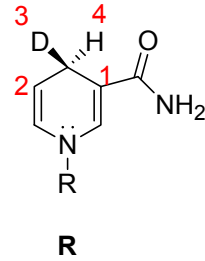
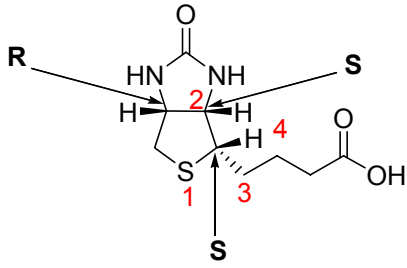


Problem Set 2
Answer Key

2.



3.

1. A-F-C-M-Y-C-L-W-C-N

2. V-C-W-V-I-F-G-C-K

Chymotrypsin Digest

Fragmentation: A,F

Only fragments with C2 will have Disulfide bonds

- N, C2, M, Y
- C, G, K
- C2, L, W2, V
- I, F, V

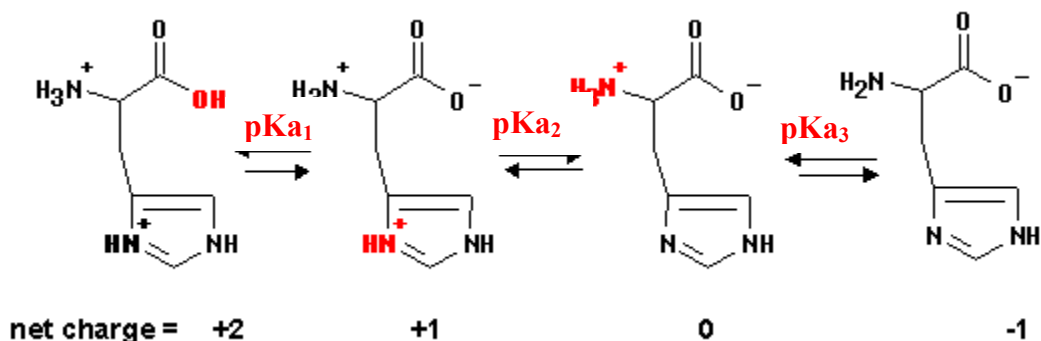
Disulfide 1

1. A-F-C-M-Y-C-L-W-C-N

2. V-C-W-V-I-F-G-C-K

Disulfide 2

4. First calculate the pI of Histidine:



Adapted from: <http://wwwchem.csustan.edu/chem4400/aapl.htm>

At the pI the net charge of the molecule is zero. To find the pI, average the two pKa values on either side of the neutral form of histidine.

For histidine $pK_1=1.82$, $pK_2=6.0$ and $pK_3=9.17$

$$(pK_2 + pK_3)/2 = pI$$

$$(6 + 9.17)/2 = 7.59$$

First find out the number of moles of Histidine in the solution:

$$0.050 \text{ L (0.010 mol/L)} = 0.0005 \text{ mol Histidine}$$

At the pI the carboxylic acid will be fully deprotonated, you can find the percentage of Histidine and the amine that are deprotonated.

For Histidine, $pH = pK_a + \log [A^-]/[HA]$

$$7.59 = 6.0 + \log [A^-]/[HA]$$

$$1.59 = \log [A^-]/[HA]$$

$$[A^-]/[HA] = 38.9$$

$$A^- + HA = 0.01 \quad \text{rearranging to} \quad HA = 0.01 - A^-$$

$$A^- = 38.9 (0.01 - A^-)$$

$$A^- = 0.00975M \text{ or } 0.00975 \text{ mol/L (0.05 L)} = 0.000487 \text{ mol}$$

It will take 0.0004887 mol of NaOH to titrate the His R-group to pH 7.59



5. First calculate the pI of each amino acid:

Amino Acid	pI	Order Elution
D	2.77	5 th
H	7.59	2 nd
M	5.74	3 rd
C	5.07	4 th
K	9.74	1 st

Elution is based on the pI of each amino acid. (e.g. Lysine is the most positively charged amino acid and will not interact with the column, eluting 1st)

6. At the pI the net charge of the molecule is zero.

$$\text{For Arginine: } pI = 9.04 + 12.48/2 = 10.76$$

$$\text{Histidine: } pI = 6.0 + 9.17/2 = 7.59$$

$$\text{Glutamate: } pI = 2.19 + 4.25/2 = 3.22$$

7. The concentration of a given amino acid residue after acid hydrolysis divided by the number of residues in the protein will give you the concentration of the protein in the original sample. Remember that Glutamine and Asparagine will be hydrolyzed to give Glutamate and Aspartate, respectively, during acid hydrolysis.

$$20F/(200 \mu\text{M}) = 10 \mu\text{M}$$

$$24R/(240 \mu\text{M}) = 10 \mu\text{M}$$

$$29D + 19N/(480\mu\text{M}) = 10 \mu\text{M}$$

$$21E + 16Q/(370\mu\text{M}) = 10 \mu\text{M}$$

$$29A/(290\mu\text{M}) = 10 \mu\text{M}$$

Using Beers Law: $A = \epsilon \times l \times c$

$$1.5 = \epsilon \times 1\text{cm} \times 10 \mu\text{M}$$

$$\epsilon = 150,000 \text{ M}^{-1} \text{ cm}^{-1}$$

8. First find the amount of protonated and deprotonated forms of tyrosine.

$$\text{pH} = \text{pK}_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

$$9.5 = 10.07 + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

$$-0.57 = \log \frac{[\text{A}^-]}{[\text{HA}]}$$

$$0.269 = \frac{[\text{A}^-]}{[\text{HA}]} \text{ or } [\text{HA}] (0.269) = [\text{A}^-]$$

$$[\text{A}^-] = 0.001\text{M} - [\text{HA}]$$

$$[\text{HA}] (0.269) = 0.001\text{M} - [\text{HA}]$$

$$1.269 [\text{HA}] + 0.001$$

$$[\text{HA}] = 0.000788\text{M}$$

$$[\text{A}^-] = 0.000212\text{M}$$

Note: Absorbance is additive, therefore the total absorbance will equal the sum of the absorbances of both species or:

$$A = (\epsilon_{\text{protonated}}) (C_{\text{protonated}}) (l) + (\epsilon_{\text{deprotonated}}) (C_{\text{deprotonated}}) (l)$$

$$= (1400 \text{ M}^{-1} \text{ cm}^{-1}) (0.000788\text{M}) \times 1\text{cm} + (2400\text{cm}^{-1}) (0.000212\text{M}) \times 1\text{cm}$$

$$= 1.103 + 0.5088$$

$$= 1.61$$