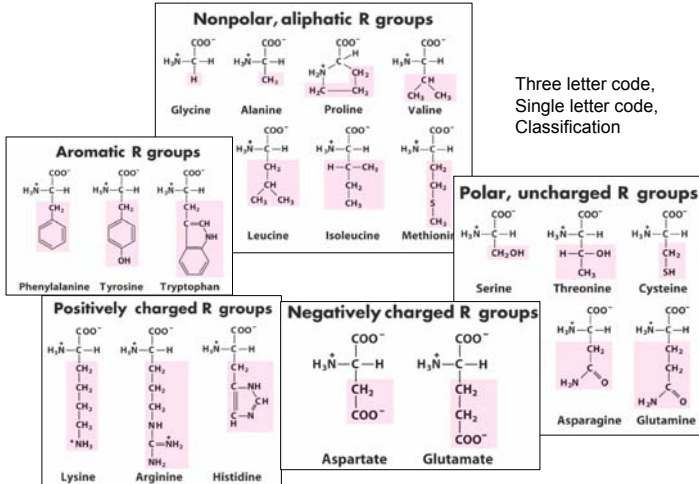
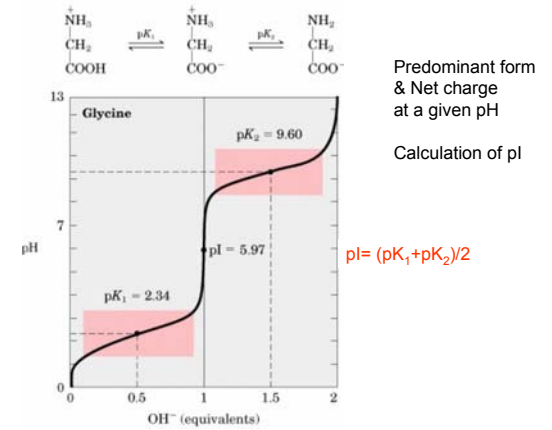


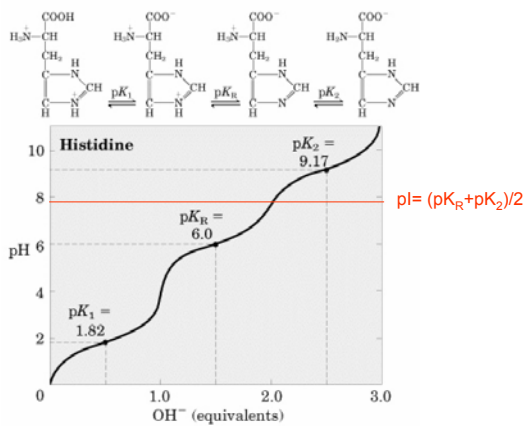
## Amino acid Classification by R group



## Amino acids as Acids and Bases



## Amino acids as Acids and Bases



## Amino acids as Acids and Bases

• Remember  $pH = pK_a + \log \left( \frac{[A^-]}{[HA]} \right)$

• For example, at  $pH = 2$ ,

$\text{COOH}: 2 = 1.82 + \log \left( \frac{[\text{COO}^-]}{[\text{COOH}]} \right)$

$\text{NH}_3^+: 2 = 9.17 + \log \left( \frac{[\text{NH}_2]}{[\text{NH}_3^+]} \right)$

$\text{NH} \text{ (R group)}: 2 = 6 + \log \left( \frac{[\text{N}]}{[\text{NH}]} \right)$

## Amino acids as Acids and Bases

When  $pK_a > pH$ , protonated  
(low  $pH \rightarrow$  more  $H^+ \rightarrow$  more likely to be protonated)

When  $pK_a < pH$ , deprotonated

## Peptides and Proteins

- Peptide bonds
- Calculation of net charge and pI
- Four levels of protein structure
- Polypeptide sequencing
- Protein purification

## Peptides and Proteins - calculation of net charge and pI

To calculate net charge,

When  $\text{pH} > \text{pK}_a$ , ionizing group lose their protons.

Look at amino term ( $\sim 9$ ), carboxyl term ( $\sim 2$ ), and R groups ( $\text{pK}_R$ )

& add up all the charges from each AA

To calculate pI,

Find the two ionizable groups with  $\text{pK}_a$  values that straddle the point at which net peptide charge = 0

& take an average

## Peptides and Proteins - calculation of net charge and pI

### Example Q

• Calculate the net charge of the following peptide at pH 3, 8 and 11.

• Estimate the pI for this peptide.

Glu-His-Trp-Ser-Gly-Leu-Arg-Pro-Gly

## Peptides and Proteins : polypeptide sequencing

• Sanger: by using FDNB (dinitrophenyl derivative), identify N-term AA

(pay attention to  $\delta$  amino group of Lysine)

• Edman: by using PTC (phenylisothiocyanate), identify AA sequentially at N term

• Trypsin: Lys, Arg (C)

• Chymotrypsin: Phe, Trp, Tyr (C)

• Cyanogen Bromide: Met (C)

## Peptides and Proteins : polypeptide sequencing

### Example Q

• Acid hydrolysis and AA analysis of a peptide yielded:  
Arg, Glu, 2 Val, Gly, Lys, Tyr, Thr & Phe.

• Cleavage of peptide J with trypsin gave three peptides: T1, T2, T3

T1 was a tripeptide; T2 a dipeptide; T3 a tetrapeptide

T1 had AA composition of Arg, Tyr, Glu

N terminal of T2 was Val

Edman degradation of T3 gave Phe. Its C term was Thr.

• Cleavage of peptide J with chymotrypsin gave three peptides: C1, C2, C3

C1 was a tripeptide, C2 a dipeptide, C3 a tetrapeptide

N term of C1 was Gly, while C term was Thr

AA composition of C2 was Tyr, Glu

N term of C3 was Arg

## Peptides and Proteins : polypeptide sequencing

### Example Q

What is the AA sequence of peptide J?

What is the net charge of peptide J at pH7?

What is pI?

## Protein Purification

• Ion exchange chromatography

cation exchange column:

(-) charged beads  $\rightarrow$  positively charged protein eluted later

anion exchange column:

• Size exclusion chromatography

porous beads: larger protein elute first

• Affinity chromatography

beads with ligand specific for protein of interest

• SDS-PAGE (polyacrylamide gel electrophoresis)

separate by charge to mass ratio

• Isoelectric focusing electrophoresis

pH gradient and pI of the protein

• 2D electrophoresis

first by isoelectric focusing, second by SDS PAGE

## Protein Purification

### Example Q

#### Separation of AAs by ion exchange chromatography

AAs placed on a cation exchange resin flow down the column at different rates due to 1) ionic attraction and 2) hydrophobic interaction.

For each pair listed below, determine which will be eluted first using pH 7 buffer.

Asp, Lys  
Arg, Met  
Gly, Leu  
Ser, Ala

## Protein purification

1. Net charge of AA at a given pH  
(negatively charged AA eluted first)
2. If both AAs are neutral, look at polarity  
(hydrophobic AA eluted later)

## Answers

1. Net charge: +2 (@pH=3), 0 (@pH=8), -1 (@pH=11); pI=7.8
2. Glu-Tyr-Arg-Val-Lys-Phe-Gly-Val-Thr (Always double check your answer)
3. Asp, Met, Gly, Ser