

Amino Acids 1: Intro and Acid-Base Properties

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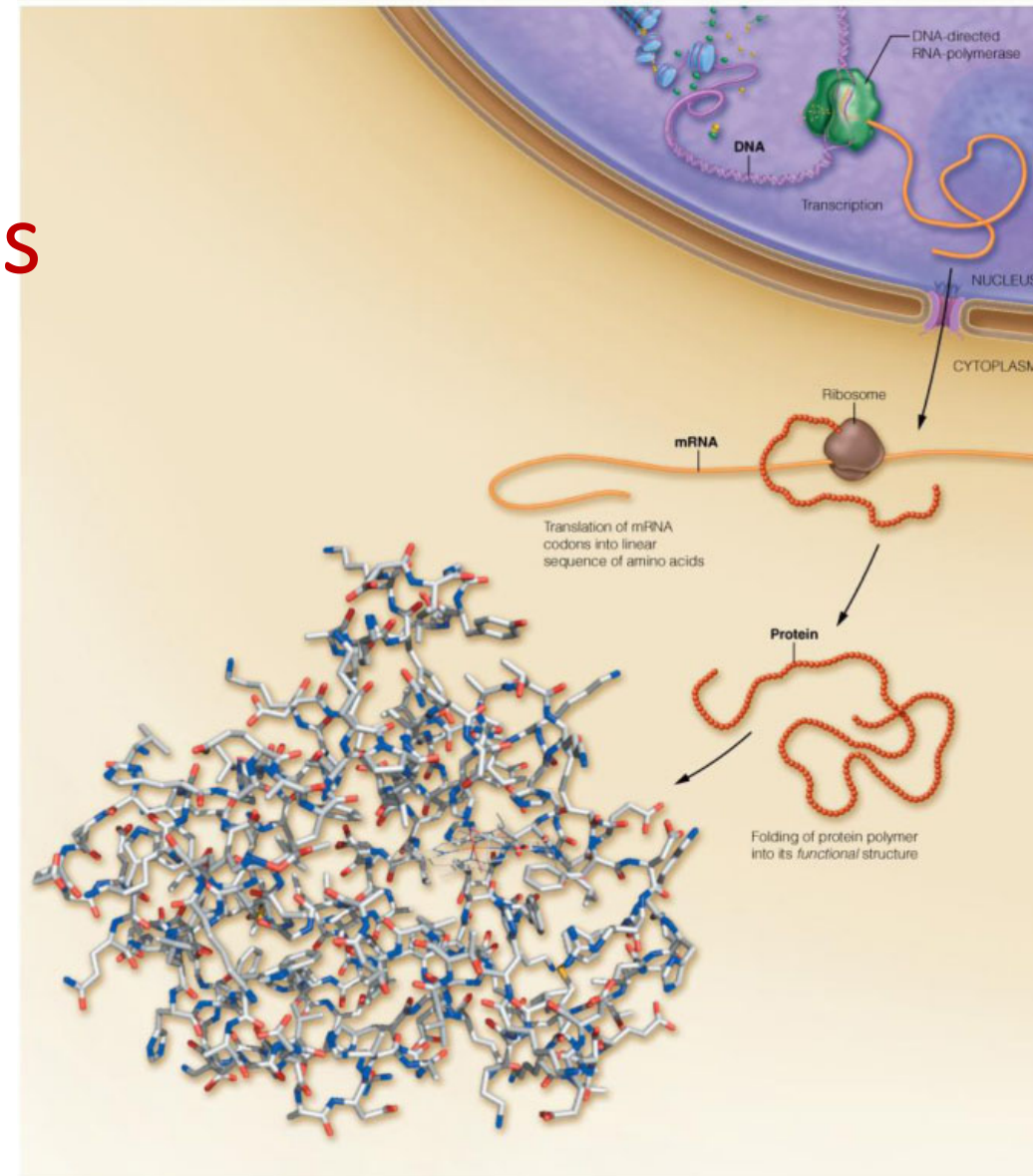
Objectives

- What are amino acids?
 - How are amino acids linked to form polymeric chains (peptides or proteins)?
- What are the acid-base properties of specific amino acids as well as the peptides and proteins they form?
 - Theory for Prac 1 starting Week 2.

AAM: Chapters 4 and 2

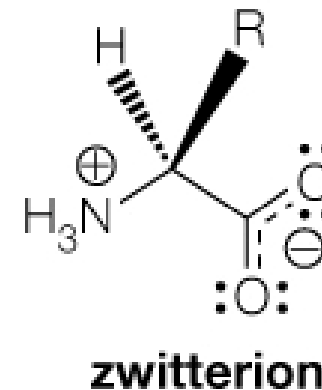
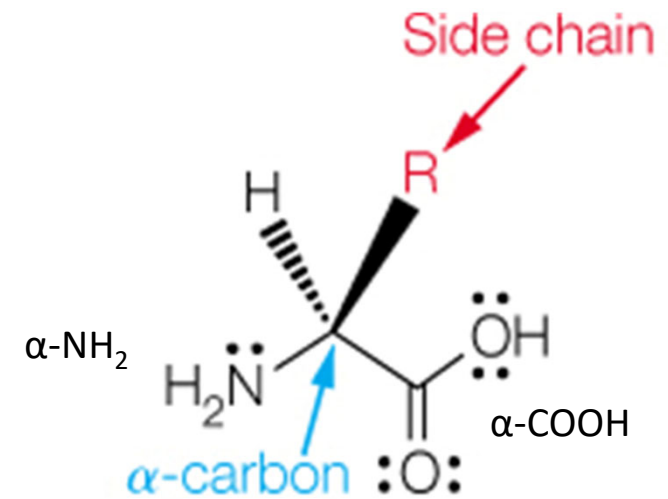
Amino acids are the building blocks of proteins

- DNA is transcribed to form messenger RNA (mRNA) in the nucleus
- mRNA is exported to the cytoplasm, where it is bound to the ribosome
- mRNA is translated into a linear sequence of amino acids that folds into a 3D structure



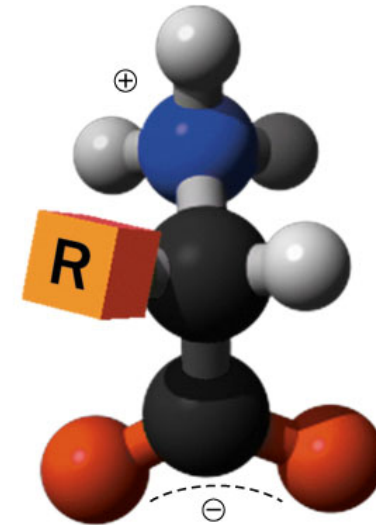
What are amino acids?

- The **α -Carbon** is central to an amino acid: amino group, carboxylic acid, and amino acid-specific side chain (R group) are attached to it
 - when R is not a hydrogen atom, the α -carbon is an **asymmetric** (chiral) centre
- At neutral pH (~ 7), the amino group nitrogen is protonated and the carboxylic acid is deprotonated to yield a “zwitterion” which has 0 net charge!

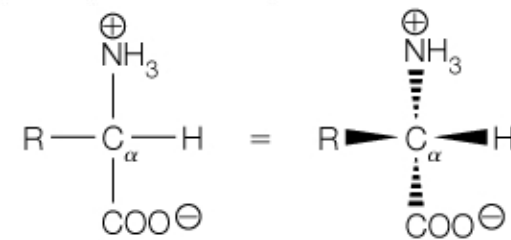


α -Amino Acid Stereochemistry - 1

- The ball and stick model shows the 3D arrangement of the atoms in an α -amino acid
- When four different groups are attached to a carbon atom, that atom is said to be chiral, or a stereocenter, or an asymmetric carbon
- The Fischer projection in the bottom figure (right) is a 2D stereochemistry representation



(a) This ball-and-stick model shows the three-dimensional arrangement of the atoms, with C atoms in black, H atoms in gray, the N atom in blue, and O atoms in red. The α -carbon is asymmetric, with tetrahedral bonding geometry. The variable R group is shown as an orange cube.

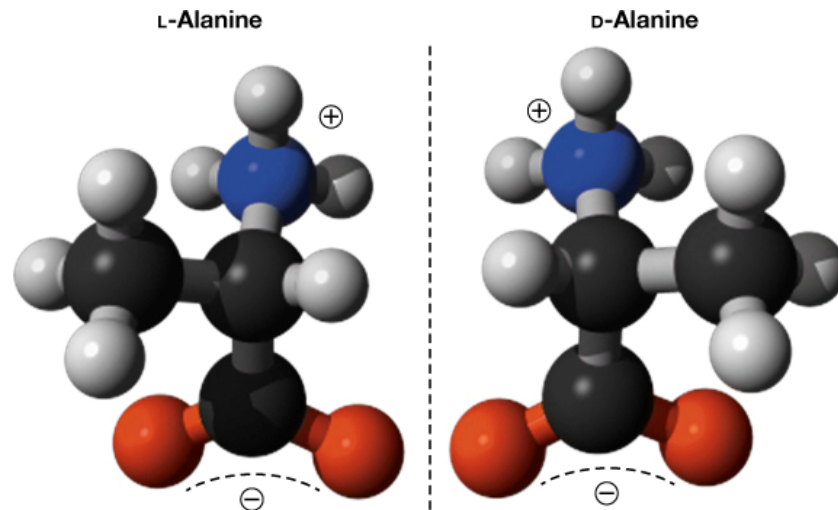


(b) In a Fischer projection (left), the horizontal bonds project toward the viewer, and the vertical bonds project away from the viewer. This orientation of bonds in the Fischer projection is represented on the right by solid and dashed wedges, respectively.

FIGURE 5.4 Three-dimensional representations of α -amino acids.

α -Amino Acid Stereochemistry - 2

- All α -amino acids (20), except glycine, contain an asymmetric α -carbon
- When $R = -CH_3$ (methyl group), we have the amino acid alanine
- L-alanine is the mirror image of D-alanine; they are enantiomers



(a) L-Alanine and its enantiomer D-alanine are shown as ball-and-stick models. The alanine side chain is $-CH_3$. The two models are nonsuperimposable mirror images. The mirror plane is represented by the vertical dashed line.



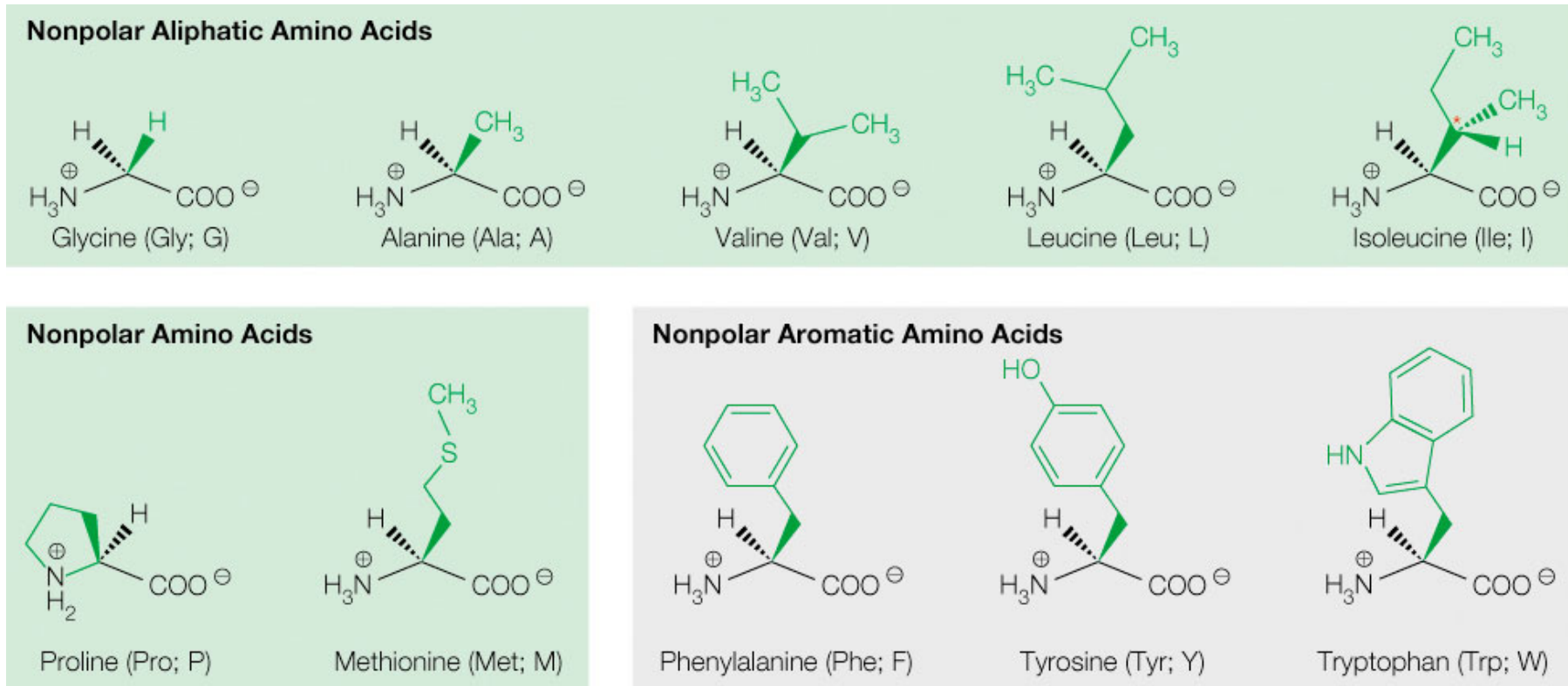
(b) The same two enantiomers in Fischer projections.

FIGURE 5.5 Stereoisomers of α -amino acids.



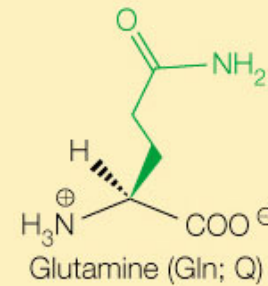
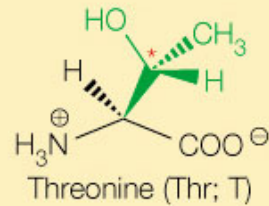
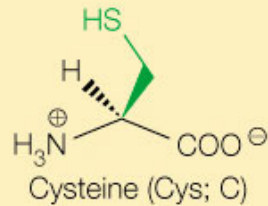
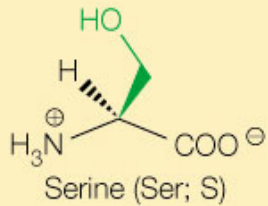
Classification of the 20 Naturally Occurring Amino Acids - 1

FIGURE 5.3 The 20 common amino acids found in proteins.

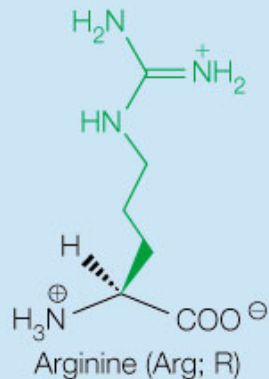
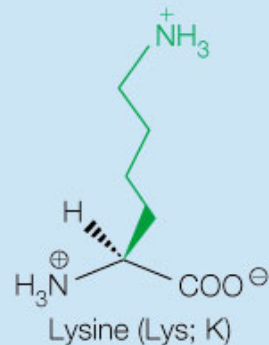
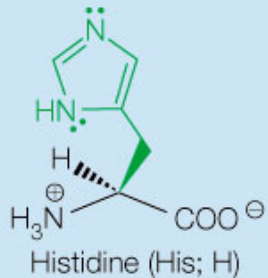


Classification of the 20 Naturally Occurring Amino Acids - 2

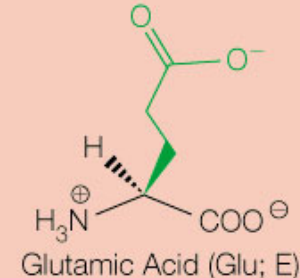
Polar Amino Acids



Positively Charged Polar Amino Acids



Negatively Charged Polar Amino Acids



General Properties of Amino Acids

TABLE 5.1 Properties of the common amino acids found in proteins

Name	Abbreviations: 1- and 3-letter codes	pK _a of α-COOH Group ^a	pK _a of α-NH ₃ ⁺ Group ^a	pK _a of Ionizing Side Chain ^a	Residue ^b Mass (daltons)	Occurrence ^c in Proteins (mol %)
Alanine	A, Ala	2.3	9.7	—	71.08	8.7
Arginine	R, Arg	2.2	9.0	12.5	156.20	5.0
Asparagine	N, Asn	2.0	8.8	—	114.11	4.2
Aspartic acid	D, Asp	2.1	9.8	3.9	115.09	5.9
Cysteine	C, Cys	1.8	10.8	8.3	103.14	1.3
Glutamine	Q, Gln	2.2	9.1	—	128.14	3.7
Glutamic acid	E, Glu	2.2	9.7	4.2	129.12	6.6
Glycine	G, Gly	2.3	9.6	—	57.06	7.9
Histidine	H, His	1.8	9.2	6.0	137.15	2.4
Isoleucine	I, Ile	2.4	9.7	—	113.17	5.5
Leucine	L, Leu	2.4	9.6	—	113.17	8.9
Lysine	K, Lys	2.2	9.0	10.0	128.18	5.5
Methionine	M, Met	2.3	9.2	—	131.21	2.0
Phenylalanine	F, Phe	1.8	9.1	—	147.18	4.0
Proline	P, Pro	2.0	10.6	—	97.12	4.7
Serine	S, Ser	2.2	9.2	—	87.08	5.8
Threonine	T, Thr	2.6	10.4	—	101.11	5.6
Tryptophan	W, Trp	2.4	9.4	—	186.21	1.5
Tyrosine	Y, Tyr	2.2	9.1	10.1	163.18	3.5
Valine	V, Val	2.3	9.6	—	99.14	7.2

^aApproximate values found for side chains on the free amino acids. W. P. Jencks and J. Regenstein (1976) Ionization constants of acids and bases in *Handbook of Biochemistry and Molecular Biology*, 3rd ed., G. Fasman (ed.), CRC Press, Boca Raton, FL.

^bTo obtain the mass of the amino acid itself, add the mass of a molecule of water, 18.02 daltons. The values given are for neutral side chains; slightly different masses are observed at pH values where protons have been gained or lost from the side chains.

^cAverage for a large number of proteins. Individual proteins can show large deviations from these values. Data from J. M. Otaki, M. Tsutsumi, T. Gotoh, and H. Yamamoto, Secondary structure characterization based on amino acid composition and availability in proteins (2010) *Journal of Chemical Information and Modeling* 50:690–700 © 2010 American Chemical Society.



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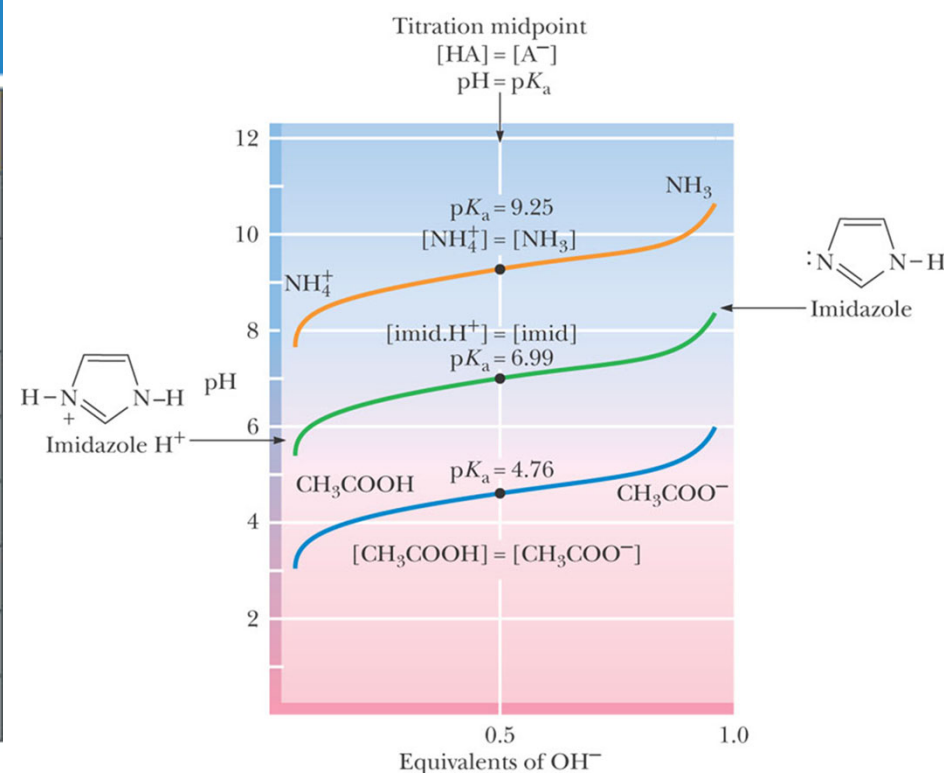
Ionizable groups of Amino Acids

pK_a values of ionizable amino acid groups: Prac 1 theory!

TABLE 5.2 Typical ranges observed for pK_a values of ionizable groups in proteins

Group Type	Typical pK_a Range ^a
α -Carboxyl	3.5–4.0
Side-chain carboxyl (aspartic and glutamic acids)	4.0–4.8
Imidazole (histidine)	6.5–7.4
Cysteine (–SH)	8.5–9.0
Phenolic (tyrosine)	9.5–10.5
α -Amino	8.0–9.0
Side-chain amino (lysine)	9.8–10.4
Guanidinyll (arginine)	~12

^aValues outside these ranges are observed. For example, side-chain carboxyls have been reported with pK_a values as high as 7.3.



Amino acids - summary

- The 20 standard amino acids share a common structure but differ in their side chains.
- Some amino acid side chains contain ionizable groups whose pK values may vary.

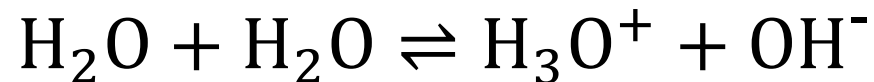
Acid-Base Equilibria

Acid and Bases: Proton Donors and Acceptors

- The behavior of molecules in biochemical processes occurring in aqueous environment depends on their state of ionization, particularly protonation
- **Acids** are **proton (H^+) donors** and **bases** are **proton acceptors** (“Brønsted-Lowry definition”)
- A strong acid dissociates (almost) completely into a proton and a conjugate base, while a weak acid dissociates only partially
 - the dissociated proton is transferred to water (H_2O) to yield a hydronium ion (H_3O^+)

Acid and Bases: Proton Donors and Acceptors

- A strong base ionizes entirely, generating an OH^- (hydroxide ion) and a conjugate acid, while a weak base ionizes only partially
- **Water** itself can act as both a weak acid or weak base
- It is **amphiprotic** and **autoionizes**:



Water as a Proton Donor and Acceptor

- At equilibrium:

$$K_w = \frac{(a_{\text{H}^+})(a_{\text{OH}^-})}{(a_{\text{H}_2\text{O}})} = 10^{-14}$$

$$K_w \cong \frac{[\text{H}^+][\text{OH}^-]}{1} = 10^{-14}$$

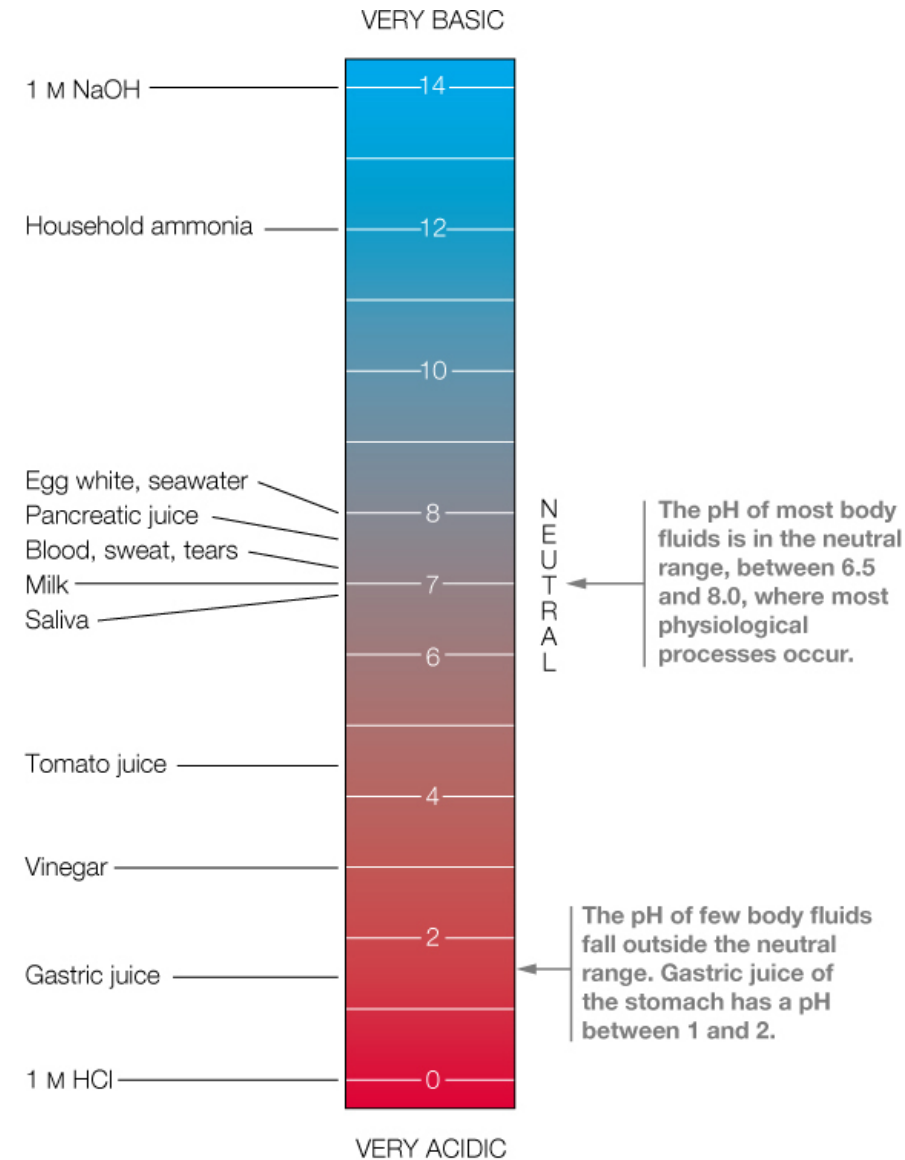
- K_w is the ion product (or ion-product constant) for water

The pH Scale and the Physiological pH Range

- The hydrogen ion (H^+ , or hydronium ion, H_3O^+) concentration can be expressed in terms of pH:

$$\text{pH} = -\log(a_{\text{H}^+}) \cong -\log[\text{H}^+]$$

- The higher** $[\text{H}^+]$, the lower the pH (and vice versa)
 - Acidic solutions have a low pH (<7), while basic solutions have a high pH (>7)
- Most biological reactions take place between **pH 6.5 and 8.0**, which is the physiological pH range



Molecular charge depends on pH

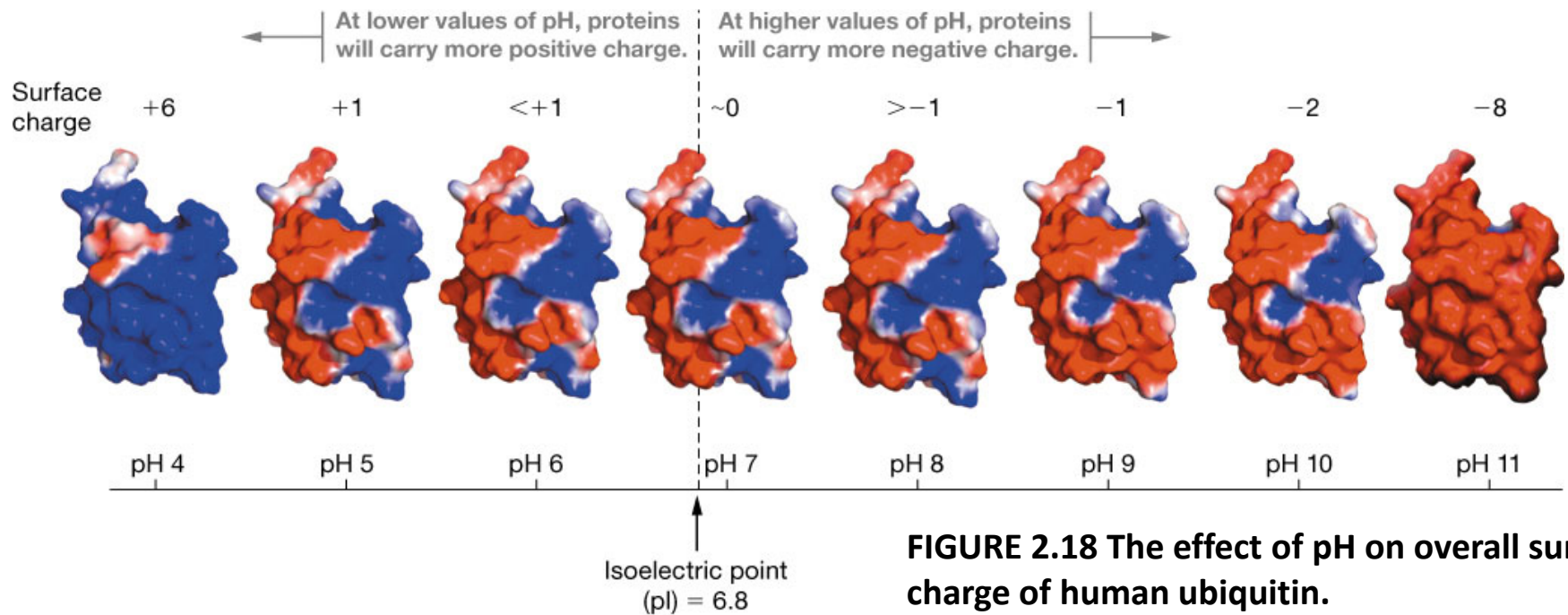


FIGURE 2.18 The effect of pH on overall surface charge of human ubiquitin.

The relationship between solution pH and molecular charge explains many features of biomolecular interactions

Weak Acid and Base Equilibria: K_a and pK_a

- Consider the dissociation of a weak acid:



- The equilibrium for the dissociation of a weak acid (K_a or the acid dissociation constant) is defined as:

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

- The strength of acids is expressed as $pK_a = -\log K_a$

K_a and pK_a for some common weak acids

TABLE 2.6 Some weak acids and their conjugate bases

Acid (Proton Donor)		Conjugate Base (Proton Acceptor)		pK_a	K_a (M)
HCOOH Formic acid	\rightleftharpoons	HCOO ⁻ Formate ion	+H ⁺	3.75	1.78×10^{-4}
CH ₃ COOH Acetic acid	\rightleftharpoons	CH ₃ COO ⁻ Acetate ion	+H ⁺	4.76	1.74×10^{-5}
$\begin{array}{c} \text{OH} \\ \\ \text{CH}_3\text{CH}-\text{COOH} \end{array}$ Lactic acid	\rightleftharpoons	$\begin{array}{c} \text{OH} \\ \\ \text{CH}_3\text{CH}-\text{COO}^- \end{array}$ Lactate ion	+H ⁺	3.86	1.38×10^{-4}
H ₃ PO ₄ Phosphoric acid	\rightleftharpoons	H ₂ PO ₄ ⁻ Dihydrogen phosphate ion	+H ⁺	2.14	7.24×10^{-3}
H ₂ PO ₄ ⁻ Dihydrogen phosphate ion	\rightleftharpoons	HPO ₄ ²⁻ Monohydrogen phosphate ion	+H ⁺	6.86	1.38×10^{-7}
HPO ₄ ²⁻ Monohydrogen phosphate ion	\rightleftharpoons	PO ₄ ³⁻ Phosphate ion	+H ⁺	12.4	3.98×10^{-13}
H ₂ CO ₃ Carbonic acid	\rightleftharpoons	HCO ₃ ⁻ Bicarbonate ion	+H ⁺	6.3*	$5.1 \times 10^{-7*}$
HCO ₃ ⁻ Bicarbonate ion	\rightleftharpoons	CO ₃ ²⁻ Carbonate ion	+H ⁺	10.25	5.62×10^{-11}
C ₆ H ₅ OH Phenol	\rightleftharpoons	C ₆ H ₅ O ⁻ Phenolate ion	+H ⁺	9.89	1.29×10^{-10}
NH ₄ ⁺ Ammonium ion	\rightleftharpoons	NH ₃ Ammonia	+H ⁺	9.25	5.62×10^{-10}

*Apparent pK_a and K_a values (see text for explanation).



Acid–Base Titrations

$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}$$

$$\text{pH} = -\log[\text{H}^+] \quad \text{and} \quad \text{p}K_a = -\log K_a$$

$$-\log [\text{H}^+] = -\log K_a + \log \frac{[\text{A}^-]}{[\text{HA}]} \quad \text{or} \quad \text{pH} = \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

- The equation to the right is known as the **Henderson–Hasselbalch equation**

Acid–Base Titrations can help determine pK_a

- The Henderson–Hasselbalch equation relates pH, pK_a and $[A^-]/[HA]$
- When:
 - $pH < pK_a$, $[HA] > [A^-]$
 - $pH = pK_a$, $[HA] = [A^-]$
 - $pH > pK_a$, $[HA] < [A^-]$
- A titration of a weak acid can determine when $pH = pK_a$, $[HA] = [A^-]$

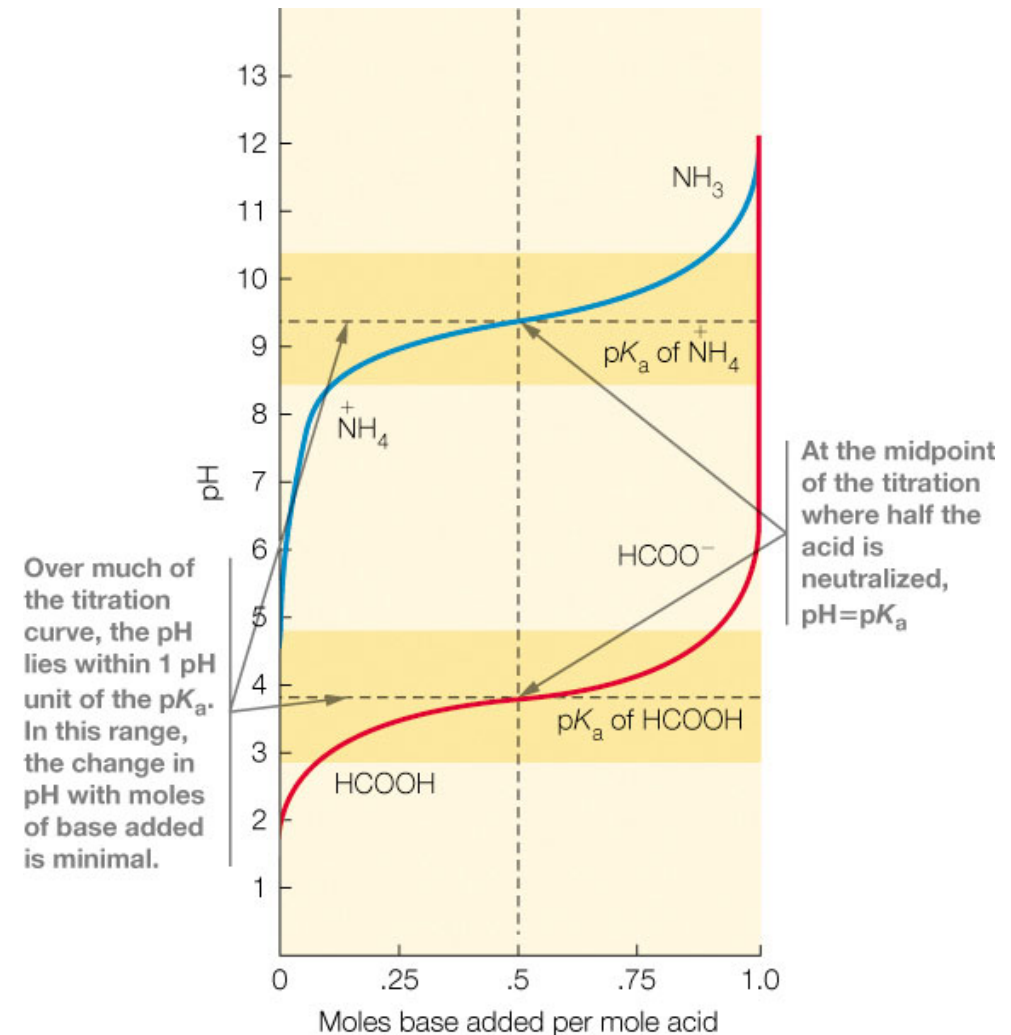
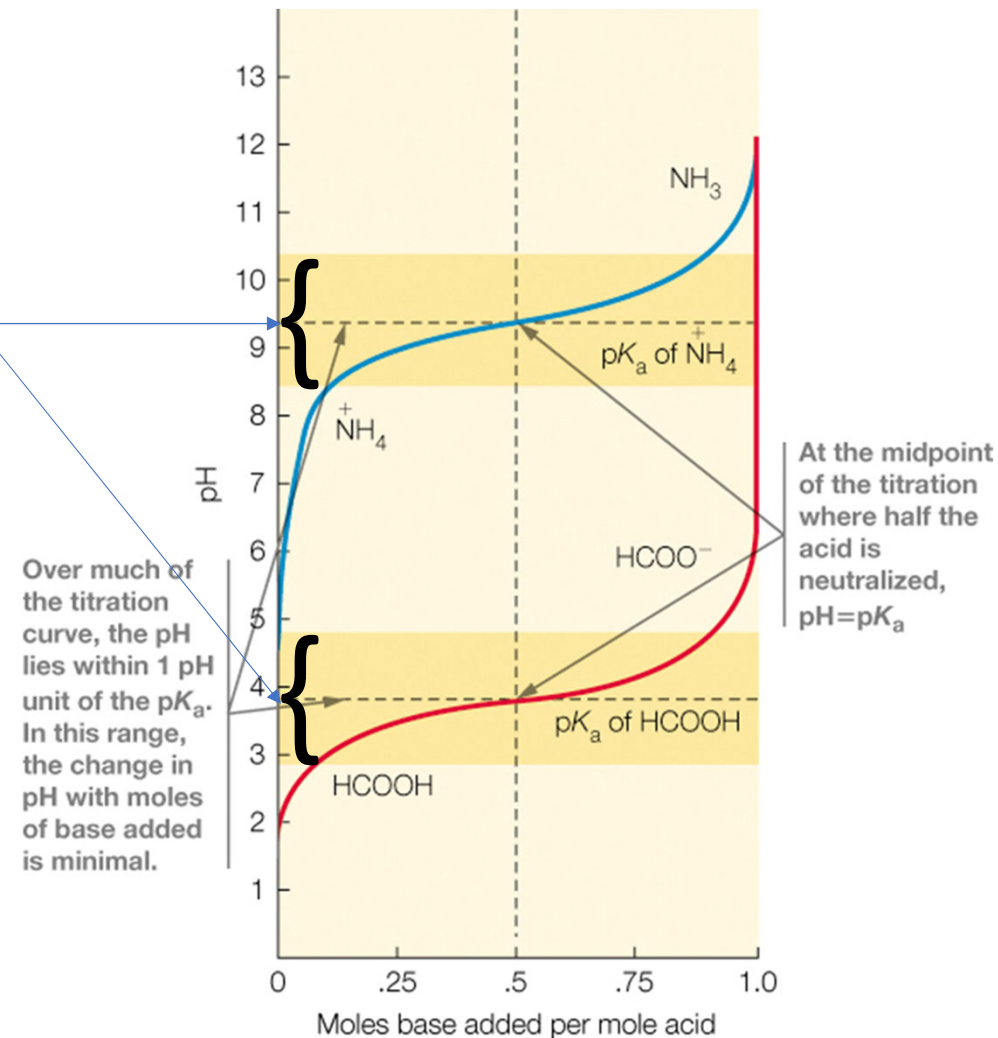


FIGURE 2.19 Titration curves of weak acids.

Buffer Solutions

- Buffers will **resist pH change following the addition of acid or base** within about ± 1 pH unit of the pK_a of a solution
- at pH values ± 3 pH units from pK_a , the group is essentially fully deprotonated or fully protonated
- at $pH = pK_a$, the group is 50% protonated, thus it is 50% ionized
- The Henderson–Hasselbalch equation can be used to calculate the average charge on an ionizable group at any pH

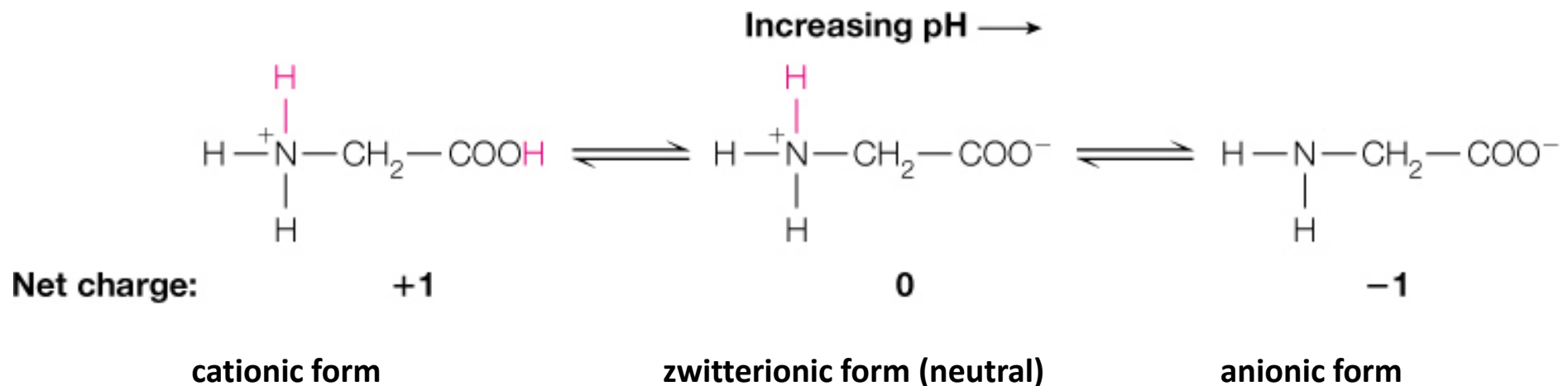


The pH of a buffer — An example calculation

- Consider an acetic acid buffer ($pK_a = 4.76$) containing 1 M HOAc and 1 M Na^+OAc^-
- $\text{pH} = pK_a + \log[1 \text{ M}/1 \text{ M}] = 4.76$
- Now add 0.1 mol of HCl to 1.0 liter of this buffer; H^+ reacts quantitatively with OAc^- to drive the pH down:
 - $[\text{HOAc}] = 1 \text{ M} + 0.1 \text{ M} = 1.1 \text{ M}$
 - $[\text{OAc}^-] = 1 \text{ M} - 0.1 \text{ M} = 0.9 \text{ M}$
- $\text{pH} = 4.76 + \log(0.9/1.1) = 4.76 - 0.087 = 4.673$

Amino Acids: Multiple Ionizing Groups

- An **ampholyte** is a molecule that contains groups with both acidic and basic pK_a values
 - many biologically relevant molecules are ampholytes
 - Amino acids are ampholytes: e.g. glycine (R group = H)



The neutral form is an identifiable characteristic

- The **isoelectric point** (pI) is the pH at which the average charge on the molecule is zero
- For a simple molecule, it is the average of the two pK_a values surrounding the isoelectric species
- For glycine:
 - $pI = (2.3 + 9.6)/2 = 5.95$
 - pK_a values can be determined by titration (Prac 1).

$$pI = \frac{pK_{\text{COOH}} + pK_{\text{NH}_3^+}}{2}$$

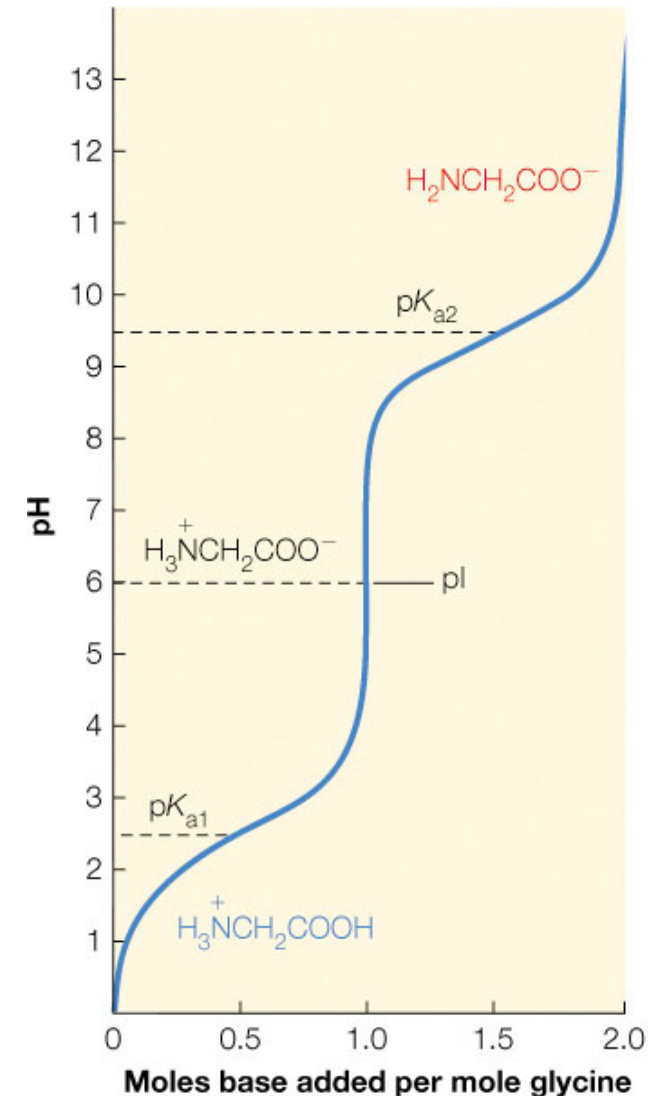


FIGURE 2.20 Titration of the ampholyte glycine.



Which Group is Ionized?

Relative concentrations of glycine species are a function of pH

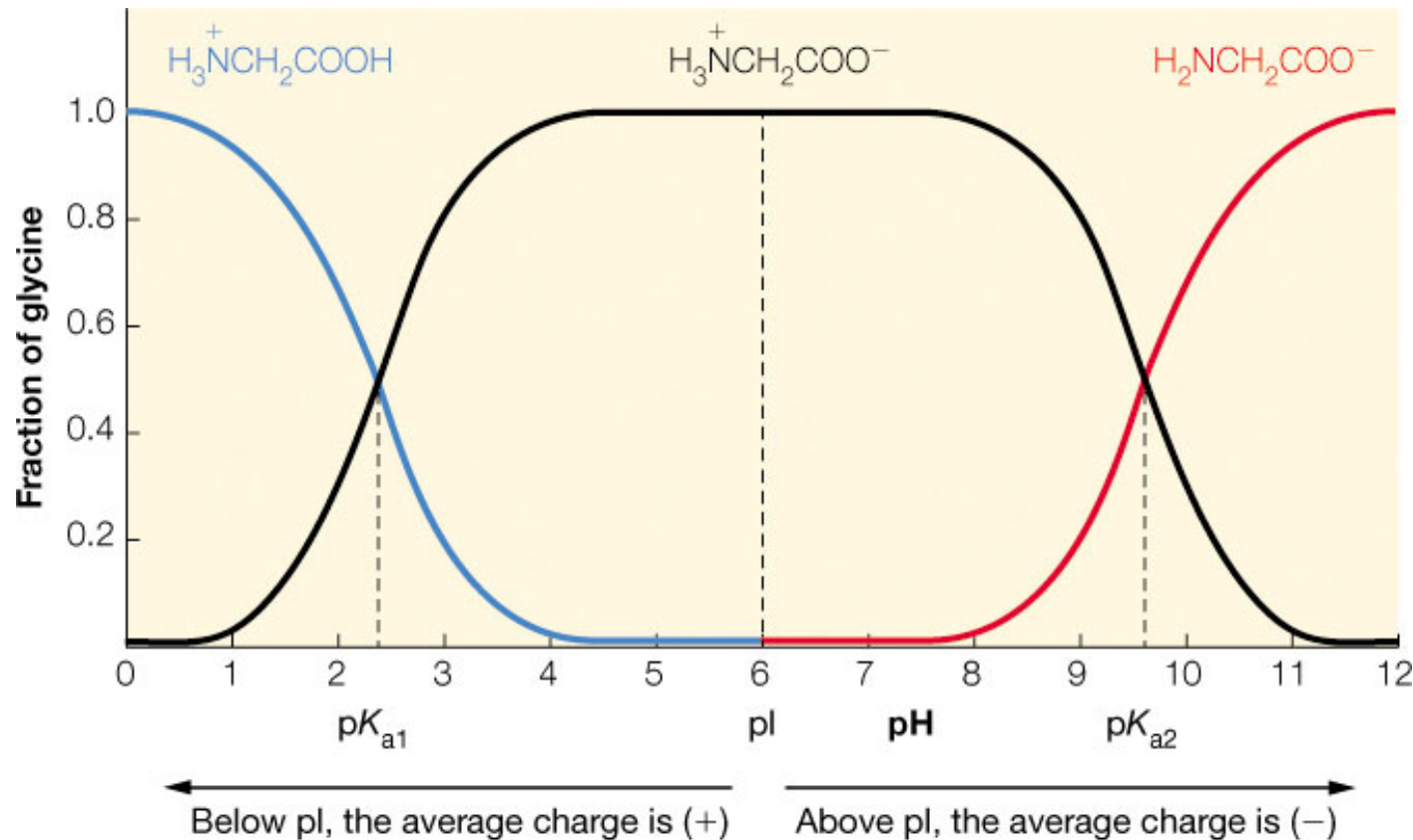


FIGURE 2.21 The relative concentrations of the three forms of glycine as a function of pH.

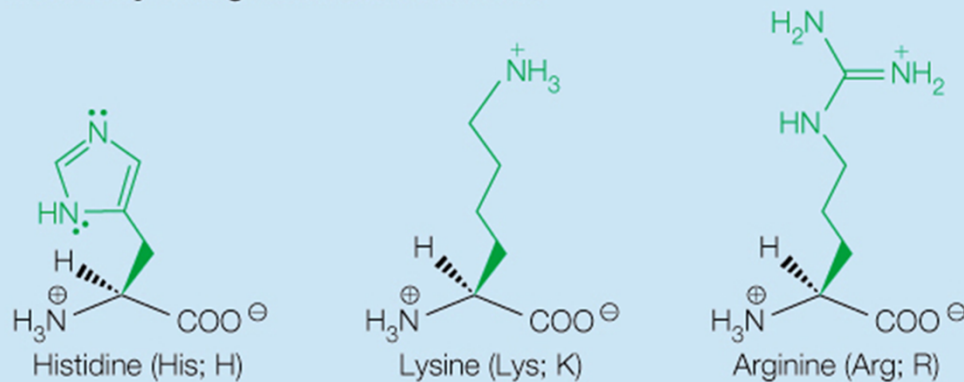


Five Amino Acids have R groups with Acid/Base properties

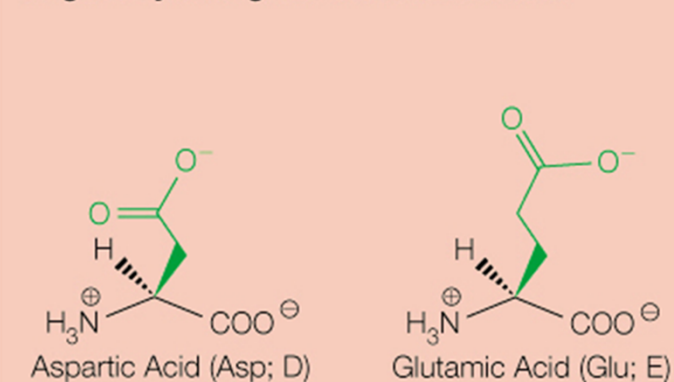
TABLE 5.1 Properties of the common amino acids found in proteins

Name	Abbreviations: 1- and 3-letter codes	pK _a of α-COOH Group ^a	pK _a of α-NH ₃ ⁺ Group ^a	pK _a of Ionizing Side Chain ^a
Alanine	A, Ala	2.3	9.7	—
Arginine	R, Arg	2.2	9.0	12.5
Asparagine	N, Asn	2.0	8.8	—
Aspartic acid	D, Asp	2.1	9.8	3.9
Cysteine	C, Cys	1.8	10.8	8.3
Glutamine	Q, Gln	2.2	9.1	—
Glutamic acid	E, Glu	2.2	9.7	4.2
Glycine	G, Gly	2.3	9.6	—
Histidine	H, His	1.8	9.2	6.0
Isoleucine	I, Ile	2.4	9.7	—
Leucine	L, Leu	2.4	9.6	—
Lysine	K, Lys	2.2	9.0	10.0

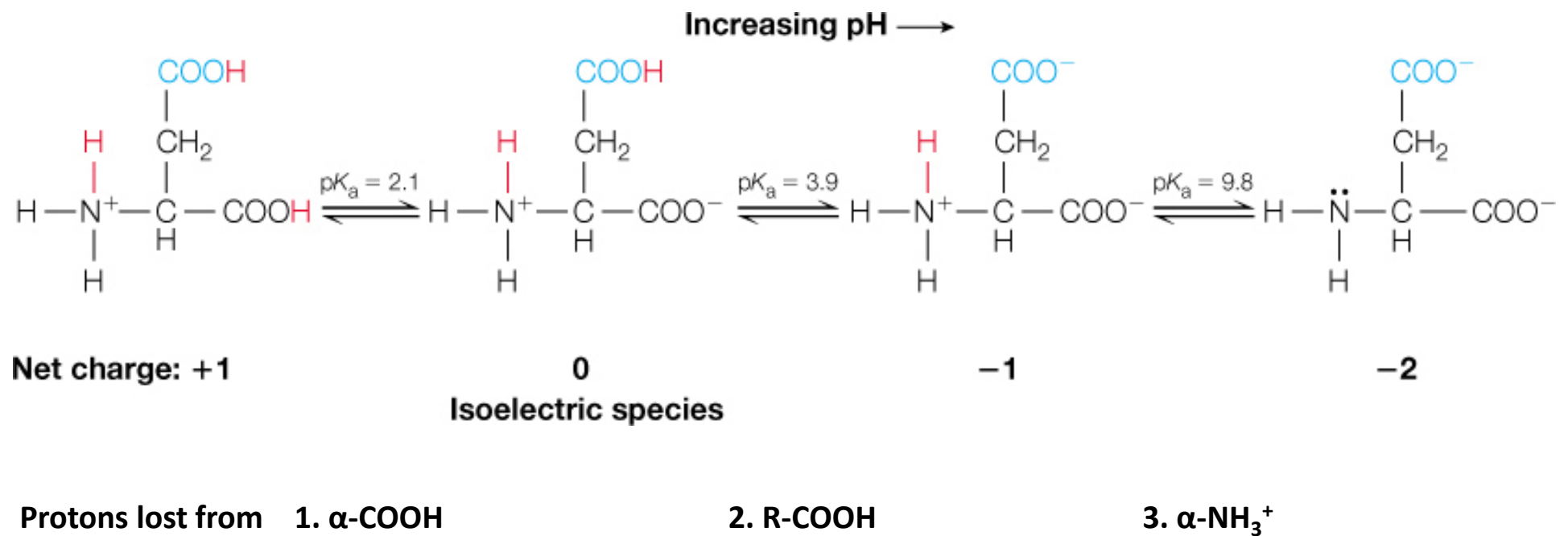
Positively Charged Polar Amino Acids



Negatively Charged Polar Amino Acids



Aspartic Acid has three Ionizing Groups



Aspartic Acid Titration Curve

pl = 3.0 (acidic!)

- The charge on aspartic acid (or aspartate) varies from +1 to -2, depending on pH
- Blue dots correspond to the pK_a values for the red hydrogen atoms
- The green dot corresponds to the pI (net charge on the molecule is zero)

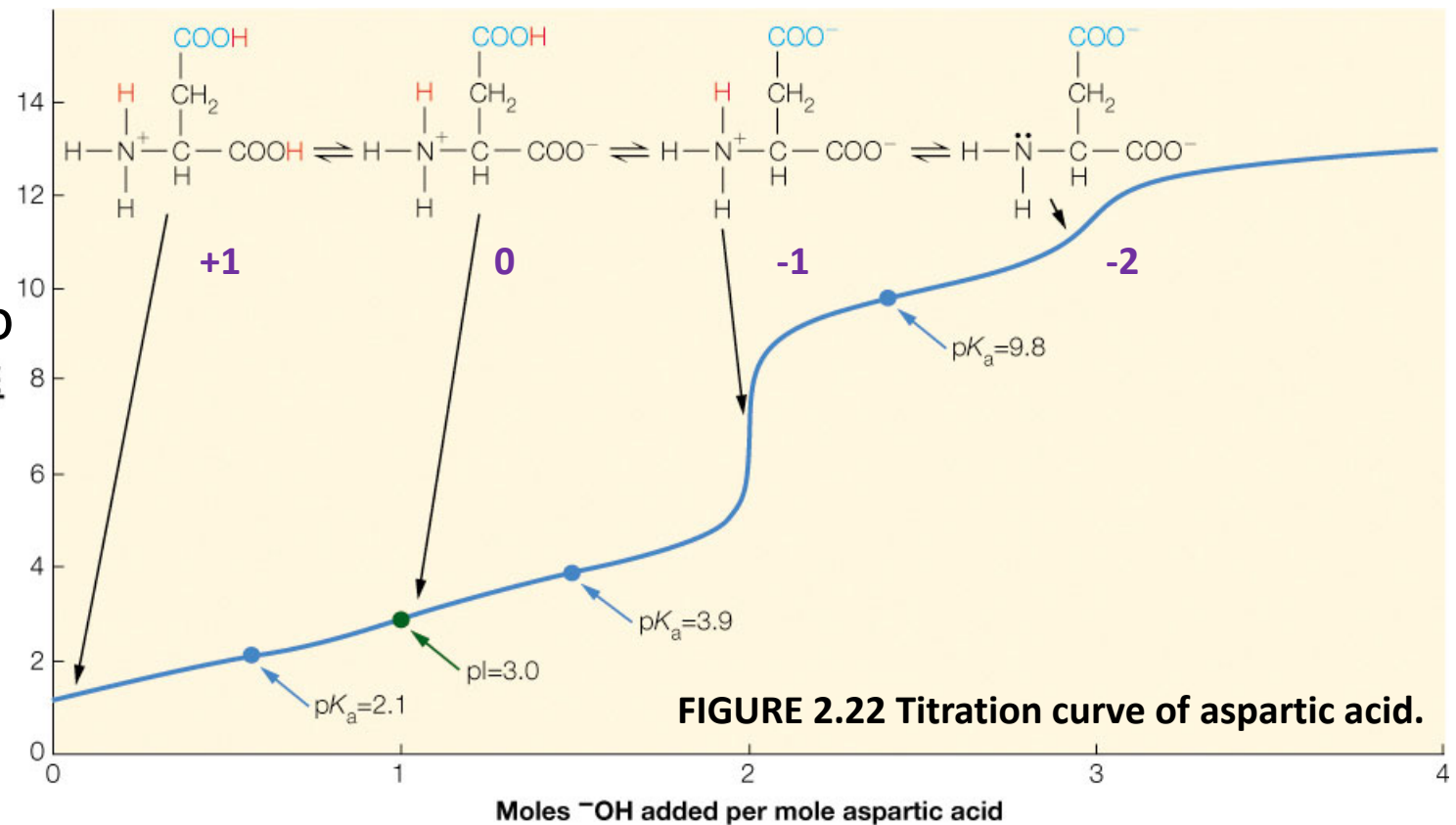
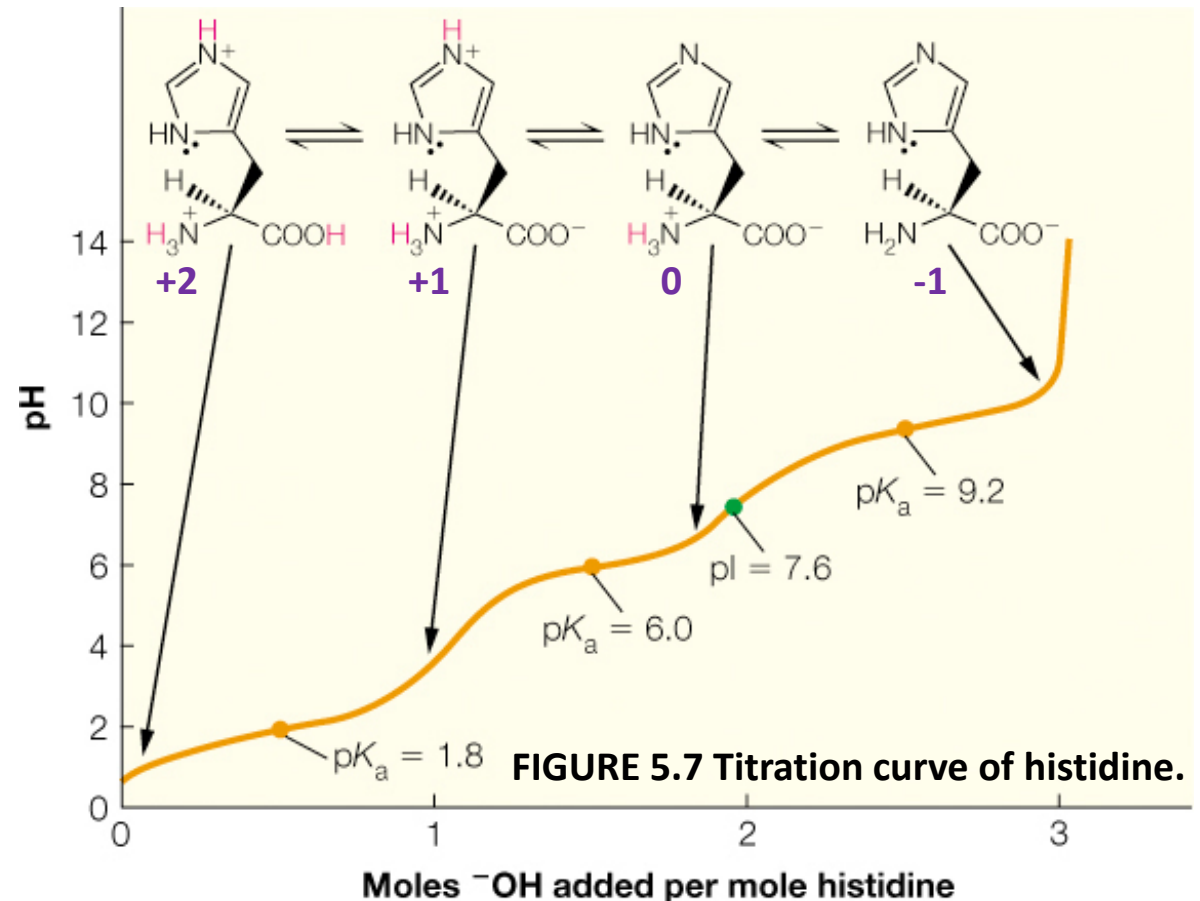


FIGURE 2.22 Titration curve of aspartic acid.

Histidine is a base in neutral pH!

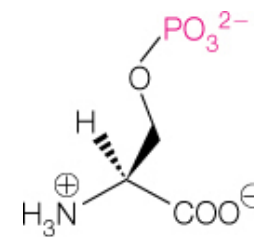
pl = 7.6

- The charge on histidine varies from +2 to -1, depending on pH
- Orange dots correspond to the pK_a values for the red hydrogen atoms
- The green dot corresponds to the pl (net charge on the molecule is zero)

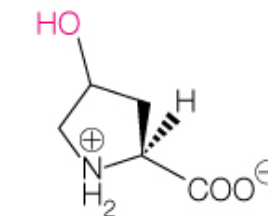


Posttranslational Modification of Amino Acids

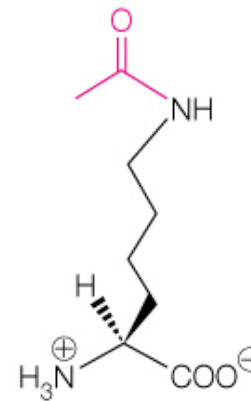
- Posttranslational modifications may function in signaling pathways, calcium binding, stabilizing structures such as collagen, or play roles in gene expression or suppression



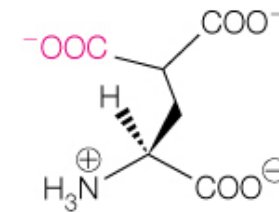
Phosphoserine



4-Hydroxyproline



N-ε-Acetyllysine



γ-Carboxyglutamate

Amino acids - summary

- The 20 standard amino acids share a common structure but differ in their side chains.
- Some amino acid side chains contain ionizable groups whose pK values may vary.
- The charge on amino acid side chains is retained in the proteins they make!

Acid-base properties of amino acids - Summary

- The 5 charged amino acid side chains contain ionizable groups with specific pK values.
 - Acidic side chains (Asp, Glu) have an extra carboxyl group
 - Basic side chains (Arg, Lys) have an extra basic group
- Histidine has a sidechain that can act as a base at physiological pH and is therefore considered a basic amino acid.
- The charges at the ends of the residues in a polypeptide or protein cancel out each other; but the charged side chains are charged at physiological pH.

(Theory for Practical 1)

L2 Spot test

- Now on iLearn
 - See Section 2 (Week 2)
- Available until this weekend (Sunday midnight)
- 2 questions; 5 mins; 1 attempt