Chapter 5: Amino Acids, Peptides & Proteins

- Amino acids share common <u>functional groups</u>
  - Amino, Carboxyl & H bonded to C_α
- <u>Distinct</u> chemistry of αα's result of side chains
- αα's categorized on basis of R group (side chain)
  - Nonpolar, aromatic, polar, (+)-charged, (-)-charged
- αα's can act as both <u>weak acids</u> & <u>weak bases</u>
  - Some R groups can also ionize ($HA \rightleftharpoons H^+ + A^-$)

1

- Proteins & polypeptides are polymers of αα's
  - Peptides < 100 residues, Proteins > 100 residues
- Proteins are studied using variety of methods
  - Solubility, size, shape, charge, binding patterns
- Protein structure is defined at 4 levels
  - Primary (1°) Secondary (2°) Tertiary (3°) Quarternary (4°)
- Proteins with similar functions often have similar αα sequences

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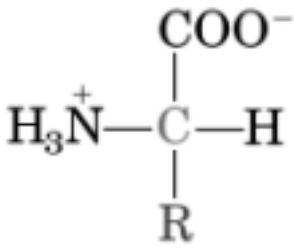
Amino Acids - the alphabet of protein structure



Amino acids share common structural features

Side Chain ("R group") Examples

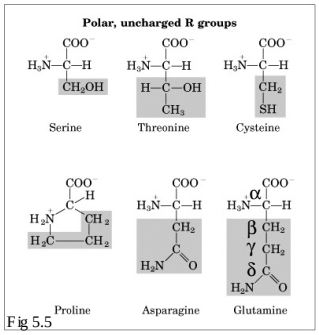


Fig 5.5

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3-letter and 1- letter amino acid abbreviations

table 5–1

Properties and Conventions Associated with the Standard Amino Acids

Amino acid	Abbreviated names		M_r	pK values: pK_1 (—COOH)	pK_2 (—NH_3^+)	pK_R (R group)	pI	Hydropathy index[a]	Occurrence in proteins (%)[b]
Nonpolar, aliphatic R groups									
Glycine	Gly	G	75	2.34	9.60		5.97	−0.4	7.2
Alanine	Ala	A	89	2.34	9.69		6.01	1.8	7.8
Valine	Val	V	117	2.32	9.62		5.97	4.2	6.6
Leucine	Leu	L	131	2.36	9.60		5.98	3.8	9.1
Isoleucine	Ile	I	131	2.36	9.68		6.02	4.5	5.3
Methionine	Met	M	149	2.28	9.21		5.74	1.9	2.3
Aromatic R groups									
Phenylalanine	Phe	F	165	1.83	9.13		5.48	2.8	3.9
Tyrosine	Tyr	Y	181	2.20	9.11	10.07	5.66	−1.3	3.2
Tryptophan	Trp	W	204	2.38	9.39		5.89	−0.9	1.4
Polar, uncharged R groups									
Serine	Ser	S	105	2.21	9.15		5.68	−0.8	6.8
Proline	Pro	P	115	1.99	10.96		6.48	1.6	5.2
Threonine	Thr	T	119	2.11	9.62		5.87	−0.7	5.9
Cysteine	Cys	C	121	1.96	10.28	8.18	5.07	2.5	1.9
Asparagine	Asn	N	132	2.02	8.80		5.41	−3.5	4.3
Glutamine	Gln	Q	146	2.17	9.13		5.65	−3.5	4.2
Positively charged R groups									
Lysine	Lys	K	146	2.18	8.95	10.53	9.74	−3.9	5.9
Histidine	His	H	155	1.82	9.17	6.00	7.59	−3.2	2.3
Arginine	Arg	R	174	2.17	9.04	12.48	10.76	−4.5	5.1
Negatively charged R groups									
Aspartate	Asp	D	133	1.88	9.60	3.65	2.77	−3.5	5.3
Glutamate	Glu	E	147	2.19	9.67	4.25	3.22	−3.5	6.3

[a]A scale combining hydrophobicity and hydrophilicity of R groups; it can be used to measure the tendency of an amino acid to seek an aqueous environment (− values) or a hydrophobic environment (+ values). See Chapter 12. From Kyte, J. & Doolittle, R.F. (1982) *J. Mol. Biol.* **157**, 105–132.

[b]Average occurrence in over 1150 proteins. From Doolittle, R.F. (1989) Redundancies in protein sequences. In *Prediction of Protein Structure and the Principles of Protein Conformation* (Fasman, G.D., ed) Plenum Press, NY, pp. 599–623.

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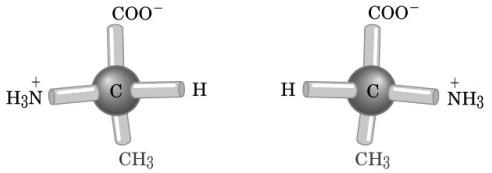
The alpha carbon is a chiral center



(a)

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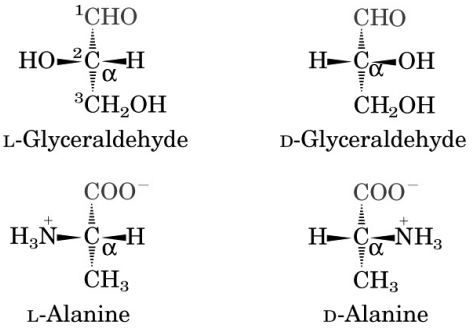
Stereoisomerism in amino acids



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Amino Acids are classified according to their R group

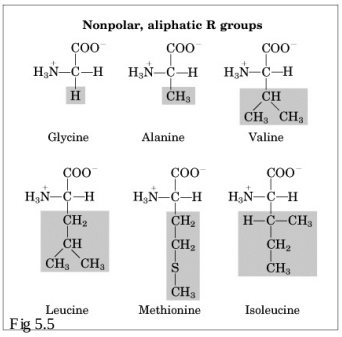

Fig 5.5

Nonpolar, Aliphatic R groups

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Aromatic R groups

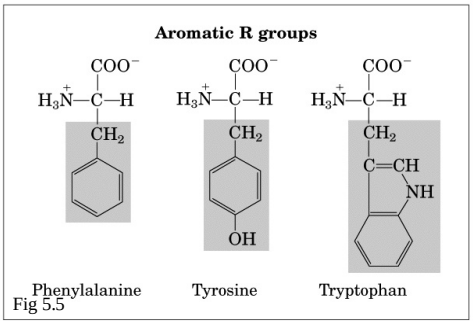

Fig 5.5

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Polar, Uncharged R groups

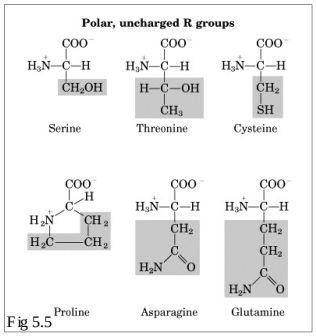

Fig 5.5

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Positively Charged (Basic) R groups

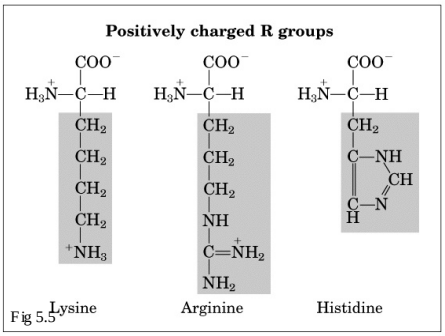

Fig 5.5

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Negatively Charged (Acidic) R groups

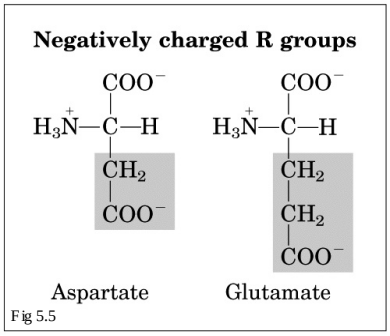

Fig 5.5

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Nonstandard amino acid residues

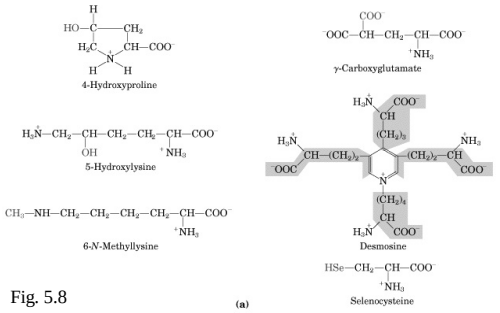


Fig. 5.8 (a)

Created by modification of residues within proteins

Amino acids can act as acids and bases

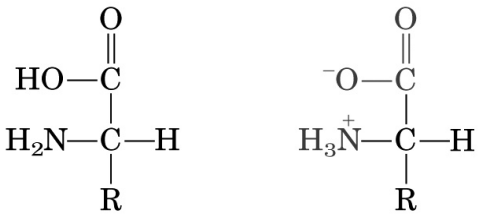


Fig. 5.9

Zwitterions - dipolar ions

Amino Acids have characteristic titration curves

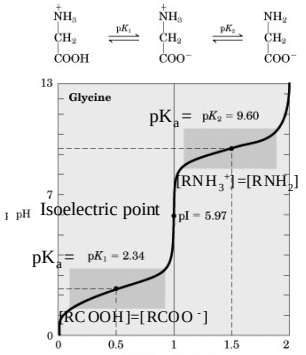


Fig 5.10

Effects of chemical environment on pK_a

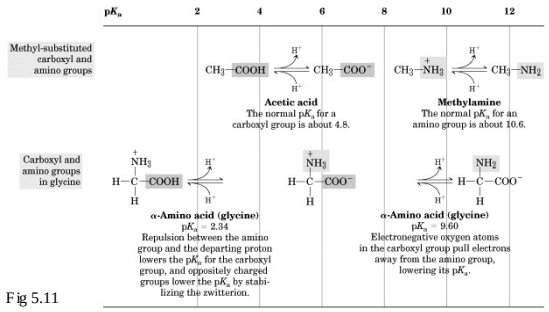


Fig 5.11

pK_a's of functional groups are influenced by neighbors

αα's have at least 2 regions of buffering power

Isoeletric point (pI) = pH
Where net charge on αα = 0

For αα's with 2 ionizable Groups

(ex: R-COOH, R-NH_3^+)

$pI = \frac{pK_1 + pK_2}{2} = \frac{2.3 + 9.6}{2} = 5.97$

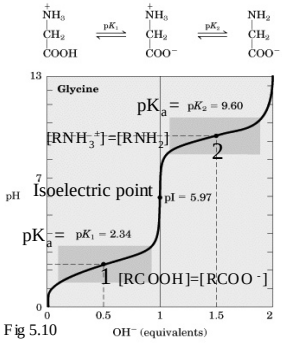


Fig 5.10

Isoelectric point calculations (pI)

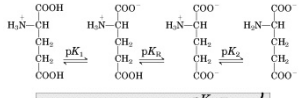
(1) determine the number of ionizable functional groups (-COOH, -NH_3^+, -OH, -SH, etc.)

(2) Draw the series of proton losses from low to high pH

(3)Determine which species has a net charge of 0

(4) Average the pK's which bracket that ionization

αα's with ionizable R groups have more complex titration curves



3 stage titration curve

α-COOH ⟷ α-COO⁻+H⁺

δ-COOH ⟷ δ-COO⁻+H⁺

α-NH_3^+ ⟷ α-NH_2 + H⁺

$$pI = \frac{pK_1 + pK_2}{2} = \frac{2.19 + 4.25}{2} = 3.22$$

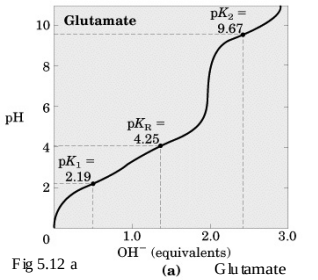


Fig 5.12 a Glutamate

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Histidine - Biological Proton donor/Acceptor

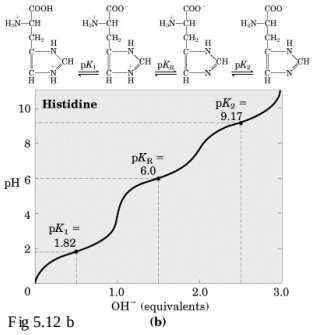


Fig 5.12 b

Only αα with R group pK_a near physiological pH (7.4) 20

Peptides and Proteins

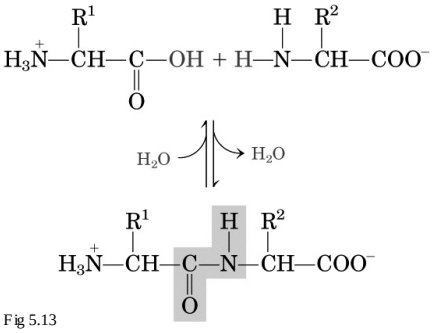
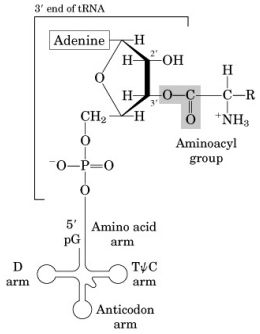


Fig 5.13

αα's are covalently joined through amide linkages

Activation of -COOH by tRNA



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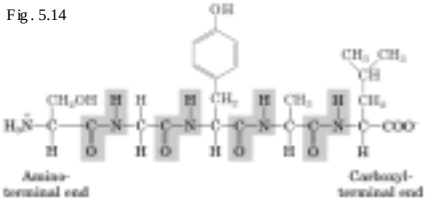
Peptide & Protein Terminology

Fig. 5.14



- Oligopeptide - few αα's
- Polypeptide - > 20 αα's
- Peptides, proteins have amino terminal (*N*-terminal) and carboxyl-terminal (*C*-terminal) ends
- Peptides & proteins also have Isoelectric points (pI's)

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Biologically Active Peptides

- Asapartame (Nutrasweet) 2 αα's
- Oxytocin (uterine contractions) 9 αα's
- Bradykinin (inflammation inhibitor) 9 αα's
- Insulin (sugar uptake) 30/21αα's

Compare to proteins:

- Cytochrome C (energy metabolism) 104 αα's
- Titin (muscle protein) 27000 αα's

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Proteins can have single or multiple polypeptide chains

- Multiple subunits = Multimeric proteins
- Subunits can be same or different
  - Ex: actin (identical subunits)
  - Ex: Hemoglobing (different subunits)
- Dimers, trimers, tetramers, etc. refer to number of subunits in multimeric proteins
- Subunits held together by noncovalent and covalent (disulfide bonds) linkages

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Disulfide bonds

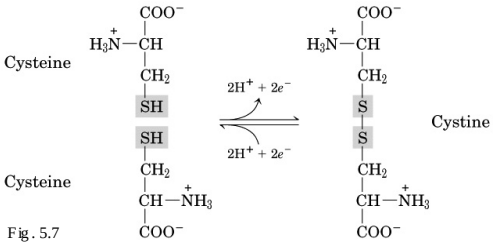

Fig. 5.7

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Polypeptides have characteristic αα compostion

- Hydrolysis yields characteristic proportion of αα's
- The standard 20 αα's almost never occur in equal proportions
- Proteins with different functions will differ significantly in their respective proportions
- Crude yet powerful method of protein identification

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Chemical modification of proteins

table 5–4

Conjugated Proteins

Class	Prosthetic group(s)	Example
Lipoproteins	Lipids	β_1-Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase
Metalloproteins	Iron	Ferritin
	Zinc	Alcohol dehydrogenase
	Calcium	Calmodulin
	Molybdenum	Dinitrogenase
	Copper	Plastocyanin

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Levels of structure in Proteins

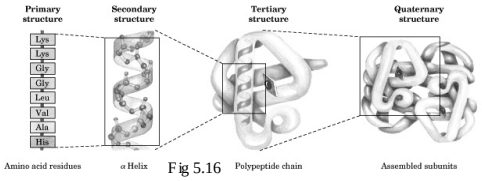

Fig 5.16

1° 2° 3° 4°

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Working with proteins

Proteins must be purified before they can be characterized

- Knowledge of structure and function

Proteins are purified based on their physical and chemical properties

- Size (fractionation)
- Solubility (function of pH, temperature, salt)
- Charge (binding to oppositely charged compounds)
- Binding properties (ligands)

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Chromatography

Takes advantage of differences in:

- Charge
- Size
- Binding affinity

Mobile (liquid) and Stationary (matrix) phases

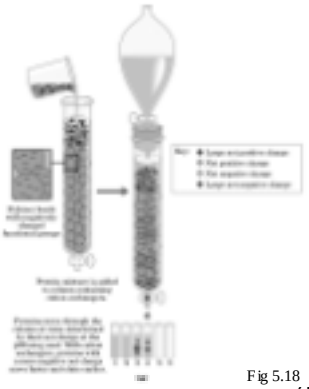

Fig 5.18

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Unseparated proteins can be quantified by measuring catalytic activity

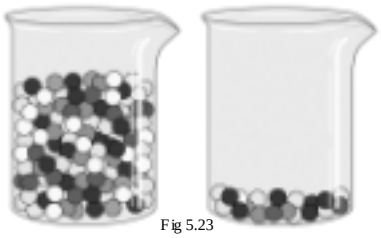

Fig 5.23

Activity = total units of enzyme in solution

Specific Activity = number of units of enzyme per milligram of protein

Proteins can be purified several thousand fold via combination of chromatography steps

table 5–5

A Purification Table for a Hypothetical Enzyme*

Procedure or step	Fraction volume (ml)	Total protein (mg)	Activity (units)	Specific activity (units/mg)
1. Crude cellular extract	1,400	10,000	100,000	10
2. Precipitation with ammonium sulfate	280	3,000	96,000	32
3. Ion-exchange chromatography	90	400	80,000	200
4. Size-exclusion chromatography	80	100	60,000	600
5. Affinity chromatography	6	3	45,000	15,000

*All data represent the status of the sample *after* the designated procedure has been carried out. Activity and specific activity are defined on page 137.

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Electrophoresis: Seperation & Characterization

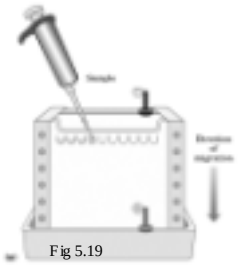

Fig 5.19

Molecular weight

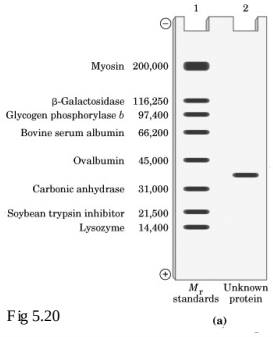

Fig 5.20

Electrophoresis - Isoelectric point (pI)

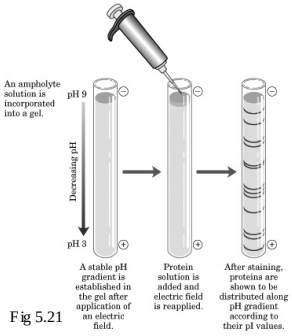

Fig 5.21

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Covalent Structure of Proteins

Purified proteins

- Size (electrophoresis)
- Charge (electrophoresis or chromatography)
- Catalytic activity (enzyme assays)
- Binding properties (ligands)

Detailed Biochemical analysis (Structure/Function)

- 1° - amino acid sequence
- 2 ° - Circular dichroism
- 3 ° - X-ray crystallography, NMR

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1° provides important Biochemical Information

- Insights into 3D structure
  - α-helix, β-sheet, etc
- Cellular location of protein
  - Cytosol, plasma membrane, nucleus, etc.
- Evolutionary relationships
- Genetic disease

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Determining the 1°

(1) Purification of protein

(2) Break disulfide (-S-S-) bonds

(3) Cleave protein into smaller peptide fragments

- Enzymatic digestion

(4) Chemical modification & hydrolysis

(5) Identification of individual amino acids

- Isoelectric point, etc.

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1° provides information on structure & function

- Cellular location or chemical modification
  - N-terminal sequences - signals for export to nucleus, etc.
  - Ser, Thr, Tyr context - phosphorylation signals
  - Asn, Ser, Thr context - glycosylation signals
- Comparison to sequences w/known structure
  - Functional similarity
  - Evolutionary relationship

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1°sequence information (contd.)

- Structural domains
  - Catalytic sites: KTGGL (glucose pocket)
  - LxxxxxxLxxxxxxL : "leucine zipper"
  - NNRKN (Basic (+) residues): DNA binding domain
  - Etc.
- Genetic disease
  - Sickle cell anemia, Cystic Fibrosis, MD
  - Comparison to healthy individuals
  - Single amino acid changes in many cases

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Protein homology & Polymorphism

- Homologous proteins share a significant amount of sequence identity (>25%)
- Evolutionarily related
- Usually perform the same function in different species
- Most proteins are polymorphic - exhibit variation in αα sequence within species

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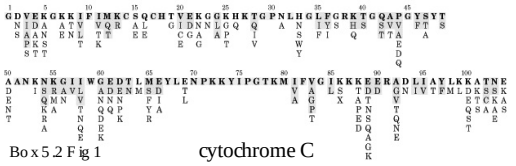
Positions which vary in αα sequence are called variable residues



Box 5.2 Fig 1 cytochrome C

Gradation in variation:

- Conservative (hydrophobic for hydrophobic αα)
- Nonconservative (Polar for hydrophobic, etc.)

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Biomedical & related benefits

- Evolutionary relationships
  - Anthropology
- Forensic Science
  - Identification of individuals, populations
- Proteomics
  - Examination of the expression of all proteins in a cell
  - Comparison of healthy/diseased states - what proteins are (not) expressed?
  - Provide targets for drug development

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Chapter 5 - Summary

- 20 Standard αα's found in proteins
  - α-COOH group
  - α-NH_2 group
  - Distinctive side chain (R group)
- α-carbon (central carbon) is asymmetric in all αα's (except glycine)
  - 2 stereoisomeric forms (D- and L-)
  - Only L- form in proteins

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Summary (contd.)

- αα's classified on the basis of polarity & charge
  - Nonpolar aliphatic
  - Aromatic
  - Polar uncharged
  - Acidic (-) charged
  - Basic (+) charged
- αα's ionize in aqueous solution
  - α-COOH $\rightleftharpoons$ α-COO^- + H^+
  - α-NH_3^+ $\rightleftharpoons$ α-NH_2 + H^+
  - R Group ionization

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Summary (contd.)

- αα's are often characterized by their isoelectric point (pI) - pH where they have no net charge
- αα's are covalently joined through peptide bonds
  - Amino (N-) and Carboxy (C-) terminal ends
- Proteins are often conjugated to other molecules
  - Metal ions
  - Lipids
  - Carbohydrates
  - Etc.

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Summary (contd.)

- 4 levels of protein structure
  - Primary, secondary, tertiary & quarternary
- Protein structural & functional analysis
  - Solubility (Precipitation with salts)
  - Chromatography (Size, Charge, Binding affinity)
  - Electrophoresis (Size and charge)
- 1° provides important Biochemical Information
  - 3D structure, active sites, targeting signals, etc.
  - Protein homology and polymorphism

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