Amino Acids

C483 Spring 2013

Amino Acid Structure

- Alpha carbon
- Sidechain
- Proteins
- Peptides
Stereochemistry

- L-amino acids
  - Glycine
  - R/S vs D/L
  - L-isoleucine
- racemization

Common Amino Acids

Hydrophobic amino acids
- Alanine (Ala, A)
- Valine (Val, V)
- Phenylalanine (Phe, F)
- Tryptophan (Trp, W)

Polar amino acids
- Leucine (Leu, L)
- Isoleucine (Ile, I)
- Methionine (Met, M)
- Proline (Pro, P)
- Serine (Ser, S)
- Threonine (Thr, T)
- Tyrosine (Tyr, Y)
- Cysteine (Cys, C)
- Asparagine (Asn, N)
- Glutamine (Gln, Q)
- Histidine (His, H)
- Glycine (Gly, G)

Charged amino acids
- Aspartate (Asp, D)
- Glutamate (Glu, E)
- Lysine (Lys, K)
- Arginine (Arg, R)
Which amino acid(s)... 

• Is achiral?
• Has a secondary amino group?
• Has a chiral sidechain?
• Form these derivatives:

Acid/base chemistry

• Charged amino acids
• Other amino acids are ionizable

<table>
<thead>
<tr>
<th>TABLE 4-1</th>
<th>pK Values of Ionizable Groups in Polypeptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>pK Value</td>
</tr>
<tr>
<td>C-terminus</td>
<td>COOH</td>
</tr>
<tr>
<td>Asp</td>
<td>CH₂-C-OH</td>
</tr>
<tr>
<td>Glu</td>
<td>CH₂-C-CH₂-C-OH</td>
</tr>
<tr>
<td>His</td>
<td>CH₂-C-NH⁺</td>
</tr>
<tr>
<td>Cys</td>
<td>CH₂-SH</td>
</tr>
<tr>
<td>N-terminus</td>
<td>NH₂⁺</td>
</tr>
<tr>
<td>Tyr</td>
<td>CH₂-OH</td>
</tr>
<tr>
<td>Lys</td>
<td>CH₂-C-CH₂-C-CH₂-C-NH⁺</td>
</tr>
<tr>
<td>Arg</td>
<td>CH₂-C-CH₂-C-CH₂-C-NH⁺</td>
</tr>
</tbody>
</table>

*The pK value is shown in red. All rights reserved.  
*Abbreviation: COOH - carboxylic acid, NH₂⁺ - amino group.
Ionization of Amino Acids

- Polyprotic acids
- Alanine
  - $pK_a_1 = 2.4$
  - $pK_a_2 = 9.9$
- Zwitterion
- Isoelectric point
- More acidic than typical carboxylic acid

Titration Curve
Titration Curve

Which amino acid?

Ionization States Of Amino Acids

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>pK_a values</th>
<th>Carboxyl group</th>
<th>Amino group</th>
<th>Side chain</th>
</tr>
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<tbody>
<tr>
<td>Glycine</td>
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<td>9.4</td>
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<td>Alanine</td>
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<td>Valine</td>
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<td></td>
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<td>Leucine</td>
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<td>9.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
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<td>9.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
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<td>9.5</td>
<td></td>
<td></td>
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<tr>
<td>Proline</td>
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<td>10.6</td>
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<td>Phenylalanine</td>
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<td>9.5</td>
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<td>Tyrosine</td>
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<td>Serine</td>
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<td>Threonine</td>
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<td>Cysteine</td>
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<td>Tryptophan</td>
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<td>Asparagine</td>
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<td></td>
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<td>Glutamine</td>
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<td></td>
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<td>Aspartic acid</td>
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<td>3.9</td>
<td></td>
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<td>Glutamic acid</td>
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<td>4.1</td>
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<td>10.5</td>
<td></td>
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<td>Arginine</td>
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<td>9.0</td>
<td>12.5</td>
<td></td>
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<tr>
<td>Histidine</td>
<td>1.8</td>
<td>9.3</td>
<td>8.0</td>
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</table>
Disulfide bond formation

Peptide bonds

- Amide bond
- Primary structure
- N- and C-terminus
- Condensation and hydrolysis
Peptide bonds

- Amide bond
- Primary structure
- N- and C-terminus
- Condensation and hydrolysis

Polypeptides
Drawing Peptides

- Sidechains
- Stereochemistry
- Ionization states

- Example: Draw the peptide AHSCVE at pH 8.
- Steps
  - Backbone
  - Stereochemistry
  - Sidechains
  - Check ionization

Example: Draw the peptide AHSCVE at pH 8.
Basis of secondary Structure

- Polarity
- Rigidity
- Cis/trans
Conformational Constraint

NOT cis/trans

Ramachandran Plots
Alpha Helix

- Right handed
- Polarity
- $n$ and $n + 4$
- Gly and Pro

Helical Wheel

- Problem 31. The sequence of a domain of the gp160 protein (HIV) is shown below using one-letter codes for the amino acids. Plot this sequence on the helical wheel. What do you notice about the amino acid residues on either side of the wheel?  
  MRVKEKYQHLWRGWGWRWG
Beta Sheets

- Alternating sidechains can lead to amphipathic sheets

Irregular Secondary Structure

- Nonrepeating loops and turns
- Change of direction
- Turns have about 4 residues
- Internal H-bonds
- Gly, Pro
Tertiary Structure

• Too many shapes to memorize
• But not an infinite number of possibilities
• Take away the ability to read a paper
  – Discussions of **motifs** and why important
  – Discussion of **domains** and why important

Motifs
(Super Secondary Structure)

• Recognizable combinations of helices, loops, and sheets
• Match
  – Helix-loop-helix
  – Helix bundle
  – Hairpin
  – β-sandwich
Studying Motifs

• Some Motifs are highly studied
• Know the lingo
  – Leucine zipper
  – Zinc finger
• Often have recurring applications

Domains

• Discrete, independently folded unit (may maintain shape when cleaved on loop)
• May have separate activities: “ATP binding domain” or “catalytic domain”
• Similar activity = similar structure across many proteins
• Binding pockets at interfaces
Common Domains

Major classes of globular proteins

- Mostly $\alpha$
- Mostly $\beta$
- $\alpha/\beta$ combination
- Little secondary
Thermodynamics of protein folding

- ΔG might be 40 kJ/mol for small protein (about 2 H-bonds)
- Hydrophobic effect is important...but the most important?

<table>
<thead>
<tr>
<th>Residue</th>
<th>Scale A^1</th>
<th>Scale B^2</th>
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</thead>
<tbody>
<tr>
<td>Phe</td>
<td>3.8</td>
<td>3.7</td>
</tr>
<tr>
<td>Met</td>
<td>1.9</td>
<td>3.4</td>
</tr>
<tr>
<td>Ile</td>
<td>4.5</td>
<td>3.3</td>
</tr>
<tr>
<td>Leu</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>Val</td>
<td>4.2</td>
<td>2.9</td>
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<tr>
<td>Cys</td>
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<tr>
<td>Tyr</td>
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<td>Ala</td>
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<td>Thr</td>
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<tr>
<td>Gly</td>
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<tr>
<td>Ser</td>
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<td>0.6</td>
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<tr>
<td>Pro</td>
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<td>0.2</td>
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<td>Tyr</td>
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<td>0.7</td>
</tr>
<tr>
<td>His</td>
<td>3.2</td>
<td>3.0</td>
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<tr>
<td>Gln</td>
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<td>4.1</td>
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<tr>
<td>Arg</td>
<td>3.5</td>
<td>8.8</td>
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<tr>
<td>Lys</td>
<td>3.9</td>
<td>8.8</td>
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<tr>
<td>Asp</td>
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<td>9.2</td>
</tr>
<tr>
<td>Arg</td>
<td>4.5</td>
<td>12.3</td>
</tr>
</tbody>
</table>

Thermodynamic Contributions to RNase

**TABLE 4. A rough estimate of the contribution of various forces to the conformational stability of RNase T1**

<table>
<thead>
<tr>
<th>Destabilizing:</th>
<th>destabilizing</th>
<th>value (kcal/mol)</th>
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</thead>
<tbody>
<tr>
<td>Conformational entropy$^a$</td>
<td>$\Delta G$</td>
<td>$-177$</td>
</tr>
<tr>
<td>Peptide groups buried$^b$</td>
<td>$\Delta G$</td>
<td>$-81$</td>
</tr>
<tr>
<td>Polar groups buried$^b$</td>
<td>$\Delta G$</td>
<td>$-25$</td>
</tr>
<tr>
<td>Total destabilizing</td>
<td>$\Delta G$</td>
<td>$-285$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stabilizing:</th>
<th>stabilizing</th>
<th>value (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine ionization$^c$</td>
<td>$\Delta G$</td>
<td>$+4$</td>
</tr>
<tr>
<td>Disulfide bonds$^d$</td>
<td>$\Delta G$</td>
<td>$+7$</td>
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<tr>
<td>Hydrophobic groups buried$^d$</td>
<td>$\Delta G$</td>
<td>$+94$</td>
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<td>Hydrogen bonding$^e$</td>
<td>$\Delta G$</td>
<td>$+166$</td>
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<tr>
<td>Total stabilizing</td>
<td>$\Delta G$</td>
<td>$+271$</td>
</tr>
</tbody>
</table>

$\Delta G$ (Estimated) = $-286 + 271 = -15$ kcal/mol

$\Delta G$ (Experimental) = $+9$

Pace, C.N. Meth. Enz. 1995, 259, 538-554.

Other Stabilization

- Ion pair (salt bridge)
- Zinc finger
- Disulfide bond
Protein Folding

- Denature/renature
- Native structure
- Domains fold separately
- Global vs. local minima
- Molecular chaperones

**Problem 40.** Proteins can be denatured by agents that alter the balance of weak noncovalent forces that maintain the native conformation. How would these agents cause a protein to denature? Heat, pH, detergent, 2-mercaptoethanol

Protein processing

![Chemical structures](image)
Quaternary Structure

- Multiple subunits: Oligomers
- Homodimer, heterotrimer
- Advantages
  - Economy
  - Stability
  - Regulation

Protein Purification

- Chromatography
  - Affinity
  - HPLC
  - Size Exclusion (gel filtration)
  - Ion exchange
- Electrophoresis
  - Isoelectric focusing
  - SDS-PAGE
Size exclusion Chromatography

Ion Exchange Chromatography
Isoelectric Focusing

From Wikipedia

SDS PAGE

- Sodium dodecyl sulfate
- Polyacrylamide gel electrophoresis
- Analysis technique
- Small proteins move faster
Amino Acid Sequencing

- Purify protein
- Break disulfide bonds
- Enzymatic digest (protease)
- Edman degradation
- Overlap fragments

Partial Digestion

**TABLE 4-4**

<table>
<thead>
<tr>
<th>Specificities of Some Proteases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protease</strong></td>
</tr>
<tr>
<td>Chymotrypsin</td>
</tr>
<tr>
<td>Elastase</td>
</tr>
<tr>
<td>Thermolysin</td>
</tr>
<tr>
<td>Trypsin</td>
</tr>
</tbody>
</table>

* Cleavage does not occur if the following residue is Pro.
Partial Digestion

Edman Degradation
Sequencing Oligopeptides

Set 1 (cleaved with trypsin)
Val—Leu—Lys Ser—Phe—Gly—Arg Tyr—Ala—Gln—Thr

Set 2 (cleaved with chymotrypsin)
Val—Leu—Lys—Ser—Phe Gly—Arg—Tyr Ala—Gln—Thr

X-Ray crystallography