Proteins are the “workhorses” of the cell

- Enzymes - catalyze chemical reactions
- Regulatory proteins - control physiological function
- Transport proteins - move substances around/between cells
- Storage proteins - provide a reservoir for a substance
- Motor proteins - endow cells with capability of movement
- Structural proteins - create and maintain biological structure
- Protective proteins - active role in cell defense or protection
- Exotic proteins - have specialized adaptive functions

And all this with just 20 amino acids and some post-translational modifications!

Amino Acids

- Proteins are polymers of amino acids (AAs)
- There are 20 standard AAs
- Four features of AAs structure
  - alpha carbon (H attached)
  - a carboxyl group (acidic)
  - an amino group (basic)
  - R group (variable)
- The R group differentiates 19 of the 20 AAs (Pro is the exception)
Enantiomers

- AAs are chiral (asymmetric; 4 substituents attached to the Cα, except Gly);
- AAs have two possible stereoisomers (enantiomers) that are mirror images of one another and cannot be superimposed on one another;
- Enantiomers are physically and chemically indistinguishable by most techniques.

Characteristics of AAs

- AAs can be either D- or L- enantiomers (except Gly);
- Proteins are made from L-AAs; Not known why;
- D-AAs are found in bacterial cell walls and antibiotics (relatively rare);
- AAs can be either D- or L- enantiomers (except Gly);
- Proteins are made from L-AAs; Not known why;
- D-AAs are found in bacterial cell walls and antibiotics (relatively rare);

Acid-Base Properties of Amino Acids

- All amino acids have at least two ionizable protons (other than R);
- pKa value for:
  - amino group ~ 10
  - carboxyl groups ~ 2
  - Some R groups are ionizable

Zwitterions

AAAs that lack charged R groups at neutral pH

- Carboxyl is deprotonated
- Amino group is protonated
- Charge = 0 (isoelectric pH, pI)
**Gly Titration (R group -H)**

- Similar to the titration of a monoprotic acid;
- Isoelectric point (pI) where net charge = 0; thus, no migration in an electric field;

\[ pI = \frac{(pK_1 + pK_2)}{2} \]

- pI lies midway between the two pKa values that indicate the protonation & deprotonation of the isoionic form.

**His Titration**

- Only AA with physiological buffering power;
- \[ pI = \frac{(6.0 + 9.17)}{2} \]
  \[ = 7.59 \]

Charged R groups are extremely important in the structure and function of proteins.

**Glu Titration (R group: -(CH₂)₂-COO⁻)**

Glutamic acid = Glutamate

\[ pI = \frac{(pK_1 + pK_2)}{2} \]
\[ = \frac{(2.19 + 4.25)}{2} \]
\[ = 3.22 \]

What would be the physiological state?

**AA Classification**

- 20 common amino acids: differ in size, shape, charge, hydrogen bonding capability, hydrophobicity & chemical reactivity
  - **Non-polar** (hydrophobic)
  - **Charged polar**
    - Negative
    - Positive
  - **Uncharged polar**
### Non-polar Amino Acids (hydrophobic)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Three-letter code</th>
<th>One-letter code</th>
<th>R group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>Gly</td>
<td>G</td>
<td>-H</td>
</tr>
<tr>
<td>Alanine</td>
<td>Ala</td>
<td>A</td>
<td>-CH3</td>
</tr>
<tr>
<td>Valine</td>
<td>Val</td>
<td>V</td>
<td>-CH(CH3)2</td>
</tr>
<tr>
<td>Leucine</td>
<td>Leu</td>
<td>L</td>
<td>-CH2-CH(CH3)2</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Ile</td>
<td>I</td>
<td>-CH(CH3)-CH2-CH3</td>
</tr>
<tr>
<td>Methionine</td>
<td>Met</td>
<td>M</td>
<td>-CH2-CH2-S-CH3</td>
</tr>
<tr>
<td>Proline</td>
<td>Pro</td>
<td>P</td>
<td>See below</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Phe</td>
<td>F</td>
<td>-CH2-C6H5</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Trp</td>
<td>W</td>
<td>See below</td>
</tr>
</tbody>
</table>

**You need to know:**

Structure of A.A.

pKa's of C, N, R groups

Name, 3 letter & 1 letter code

Chemical nature of side groups

- **hydrophobic**
- **polar uncharged**
- **charged (acid, basic)**

### Simple Aliphatics

- **Alanine; Ala; A**
- **Valine; Val; V**
- **Leucine; Leu; L**
- **Isoleucine; Ile; I**

- Branched Chain Amino Acids
- Only alkyl chains (i.e. C's and H's);
- Hydrophobic (repel H₂O); Form part of hydrophobic core
- Although no reactivity they promote protein folding (Ala, Leu --> α-helix; Val, Ile --> β-sheet);
- Ala: best substitution for minimal structural and functional impact;
- Staggard rotamer conformation.

### Proline; Pro; P

- Side chain bonded to Cα and N;
- Imposes rigid restraints on the rotation of the N-Cα peptide bond;
- Dramatically influences protein structure;
- Proline specifies bends and turns in polypeptide chains.
Staggered vs. not-staggered

Methionine

- Thioether sidechain
- Little chemical reactivity
- Special in that it is first amino acid in a polypeptide chain

Aromatic Amino Acids

Phenylalanine; Phe; F
Tyrosine; Tyr; Y
Tryptophan; Trp; W

- Less abundant (especially Trp);
- Phe most abundant
- Conjugated π electron clouds confer aromatic character;
- Have ultraviolet; can be used to quantitate proteins (absorption at 280 nm)
- Presence of hydrophilic groups (Tyr O & Trp NH) permits partial solution exposure on protein surface
- Tyr can ionize at high pH (pKa = 10.5) -- therefore its has polar characteristics

Charged Polar Amino Acids

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Three-letter code</th>
<th>One-letter code</th>
<th>R group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>Lys</td>
<td>K</td>
<td>-CH2-CH2-CH2-CH2-NH2</td>
</tr>
<tr>
<td>Arginine</td>
<td>Arg</td>
<td>R</td>
<td>-CH2-CH2-CH2-NH-C(NH2)2</td>
</tr>
<tr>
<td>Histidine</td>
<td>His</td>
<td>H</td>
<td>-See below</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>Asp</td>
<td>D</td>
<td>-CH2-CO-OH</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>Glu</td>
<td>E</td>
<td>-CH2-CH2-CO-OH</td>
</tr>
</tbody>
</table>
Acidic Residues

Glutamic Acid; Glu; E
Aspartic Acid; Asp; D

- Differ by one methylene unit;
- Deprotonated at neutral pH (pKa = 3.9-4.1);
- Can serve as nucleophiles in chemical reactions;
- Love to bind metal ions, especially Ca²⁺ & Mg²⁺;
- H-bond donors and acceptors;
- Chemically reactive (form ester linkages that can be reduced);
- Impart electrostatic properties to proteins: charge charge interactions with substrates, other proteins, etc.

Basic Residues
Lysine; Lys; K and Arginine; Arg; R

- Positively charged at neutral pH; lysine has a pKa ~ 10.5; arg pKa ~12);
- Likes protein surfaces, electrostatic interactions, especially phosphates (nucleic acids);
- Lysines are chemically reactive, NH₂ is a good nucleophile.
- Guanidinium group of arginine has three resonance forms;

Basic Residue
Histidine; His; H

- Only standard amino acid with side chain pKa near neutral pH (pKa ~ 6.0)
- Two resonance forms;
- Non-protonated N is a good nucleophile loves metals especially Zn;
- Histidine containing peptides are important biological buffers
- Involved in the active site of enzymes: can both donate and accept protons (general acid and base);
- The protonation/deprotonation reaction readily occurs at pH 7.0;
- Histidine is also aromatic, uses this with substrates

Uncharged Polar Amino Acids

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Three-letter code</th>
<th>One-letter code</th>
<th>R group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine</td>
<td>Ser</td>
<td>S</td>
<td>-CH₂-OH</td>
</tr>
<tr>
<td>Threonine</td>
<td>Thr</td>
<td>T</td>
<td>-CH(OH)-CH₃</td>
</tr>
<tr>
<td>Asparagine</td>
<td>Asn</td>
<td>N</td>
<td>-CH₂-CO-NH₂</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Gln</td>
<td>Q</td>
<td>-CH₂-CH₂-CO-NH₂</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Tyr</td>
<td>Y</td>
<td>-CH₂-C₆H₄-OH</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Cys</td>
<td>C</td>
<td>-CH₂-SH</td>
</tr>
<tr>
<td>Glycine</td>
<td>Gly</td>
<td>G</td>
<td>-H</td>
</tr>
</tbody>
</table>
Glycine; Gly; G

- The simplest and smallest amino acid (no side chain);
- Not asymmetric (no D-L);
- Can also be considered as a non-polar;
- Three unique properties:
  - Used where backbones closely approach each other
  - Can assume conformations forbidden to others
  - More flexible than others

Hydroxyl Residues
Serine; Ser; S
Threonine; Thr; T

- Chemical reactivity of ethanol (pKa = 14-15);
  - Thus, proton not readily given up,
  - BUT can be activated nonetheless;
- Polarity is contributed by hydroxyl. OH provides H-bonds to solvent and ligand;
- Thr has an additional asymmetric carbon;
- Important in protein surface hydration.

The Amides
Asparagine; Asn; N
Glutamine; Gln; Q

- Hydrogen bonding characteristics resembling the peptide backbone;
- Similar to acid forms;
- Chemically labile at extremes of pH;
- Most likely on protein surfaces

Cysteine; Cys; C

- Most chemically reactive of all amino acid
  - susceptible to oxidation;
  - pKa = 8.5;
- Disulfide bond (cystine) stabilizes long range interactions;
- Adds stability to 3D structure.
**Disulfide Bond**

Formed by oxidation of the thiol groups from two cysteines

**Essential AAs**

- Can not be biosynthesized;
- Must be obtained from diet

- Arg
- His
- Ile
- Lys
- Val
- Met
- Phe
- Thr
- Trp

**Uncommon Amino Acids & Amino Acid Derivatives**

- 4-hydroxyproline
- 5-hydroxylysine
- o-phosphoserine
- phosphotyrosine
- γ-carboxyglutamate
- 3-methylhistidine
- ε-N-acetylylysine
Review

What are the four features of AA structure?

What are the pKa values for:
  amino groups?
  carboxyl groups?
  which R groups are ionizable? What are their pKas?

What is a zwitterion?

How do you calculate the pI of an AA?

What are the structures of the 20 AAs?

How are the 20 AAs classified? Where would you find hydrophobic AAs in a protein? Where would charged groups be found?

Thinking ahead: do you think that there are many free hydrogen bond donors or acceptors in the interior of a protein?

What is an essential AA?