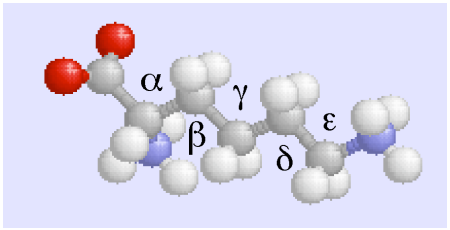
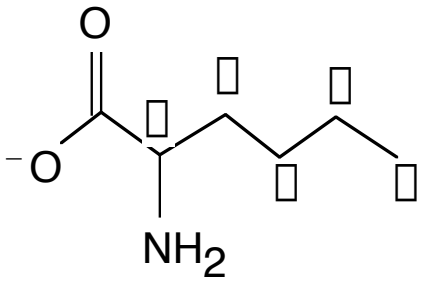
Lecture 5: Amino Acids & Peptides

Assigned reading in Campbell: Chapter 3.1-3.4.



Key Terms:

- **Optical Activity, Chirality**
- **Peptide bond**
- **Condensation reaction**
- **Hydrolysis reaction**
- **Peptide sequence**
- **Amino/carboxy terminus**
- **Cis versus trans**
- **Resonance structures**
- **Polypeptide**
- **Mainchain**
- **Sidechain**



5.1 Structure & Properties of Amino Acids

Expectations:

- Full name of each amino acid
- 3 Letter abbreviation of each amino acid
- Structure of each amino acid
- Functional properties of the side chains:
  1. Ionization (pKa)
  2. H-bonding capability
  3. Solubility properties (polar/nonpolar)
  4. UV absorbance, calculation of protein concentration

Nomenclature:

An amino acid is a carboxylic acid with an amino group. Most biological amino acids are α-amino acids because the amino group is attached to the α-carbon. The side chain carbon atoms are designated with Greek letters as shown in the images of Lys (Lysine contains an amino group attached to its ε-carbon).

Optical activity:

Amino acids have one or more chiral centers. In all amino acids (except glycine) the α-carbon is chiral. In some amino acids, additional chiral centers are present. These are chiral centers because all four groups attached to the carbon are different. Thus, there are two possible configurations (enantiomers) or amino acids. Enantiomers (or stereoisomers) have the following attributes:

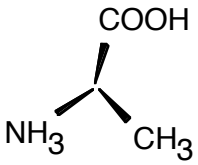
- Identical physical properties
- Opposite rotation of polarized light

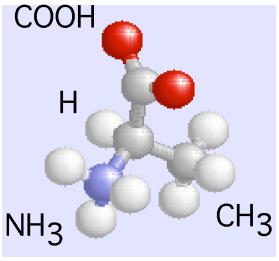
The absolute configuration of amino acids is defined by the Cahn-Ingold-Prelog system.

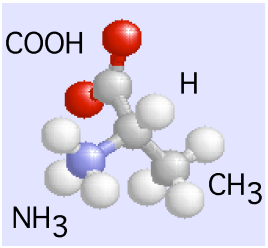
1. Groups attached to the chiral carbon are assigned letters W, X, Y, Z with W being the highest atomic number (the amino group in this case).
2. The molecule is oriented such that the Z group (lowest atomic number, H, the proton in the case of amino acids) is pointing away from the viewer.
3. If WXY describes a counter-clockwise direction, the configuration of the group is (S) (sinister = left). (Point the thumb of your *left* hand in the direction of the Z-atom, your fingers curl in the direction W-X-Y).

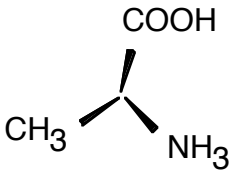
4. If WXY describes a clockwise direction, the configuration of the group is (R). (Point the thumb of your *right* hand in the direction of the Z-atom, your fingers curl in the direction W-X-Y).
5. Most common amino acids have an S configuration. An older, but much used, notation is D(=R) and L(=S). These older definitions are related to the direction of rotation of polarized light. **Most amino acids are L (S)**.

Apply these rules to determine which of the images is L-Ala and which is D-Ala:



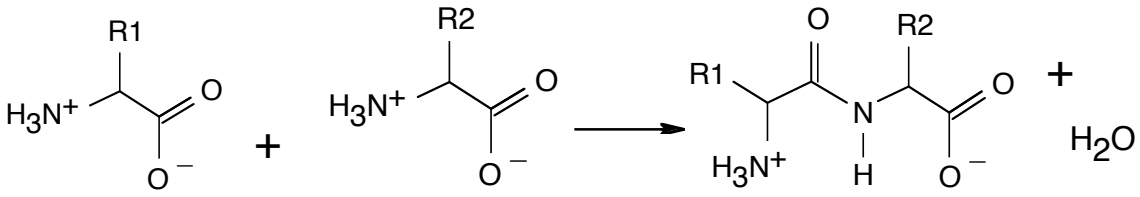


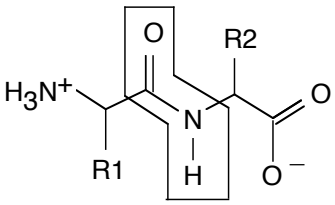




5.2 The Peptide Bond

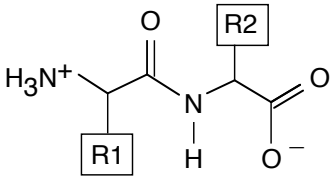
Amino acids are connected together by the formation of a peptide bond (an example of a *condensation* reaction): Protein sequences are written left to right from the N- to the C-terminus.





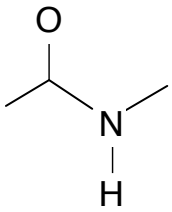
Peptide Bond

Side Chains



Five important features of the peptide bond:

1. The resonance structures that can be drawn for the peptide bond show that the C-N bond has double bond character. Bond length measurements showed that the C=O and C-N bonds were both partial double bonds.
2. All four of the atoms boxed in the above figure lie in a plane. These atoms are planar because of the partial double C-N bond. *It is unfavorable to deviate from planarity.*

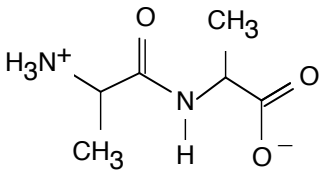


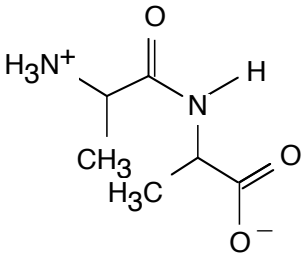
3. The figure below also shows the peptide bond in its highly preferred *trans* configuration, with the C=O across, or trans, from the amide proton.

Rotation of 180°C about the C-N bond would produce the *cis* configuration, but this is rarely observed in proteins. The only exception to this rule occurs for the peptide bond

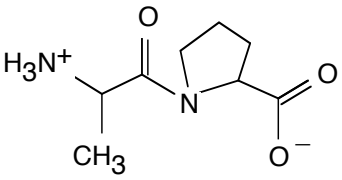
before Pro residues, in which case the *trans* configuration is only slightly more stable than the *cis* configuration. Consider the following two dipeptides: Ala-Ala and Ala-Pro.

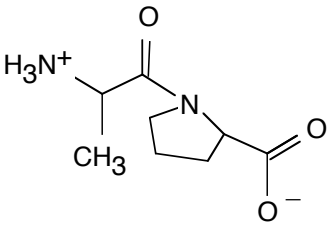
Ala-Ala





Ala-Pro

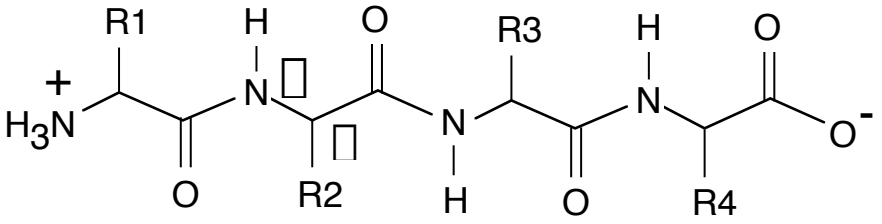


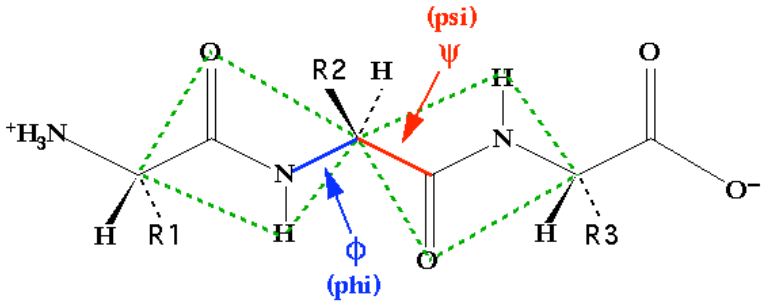


TRANS **CIS**

4. Rotation can and does occur about the two single bonds on either side of the □-carbon.

extended chain conformation (□=180°, □=180°):

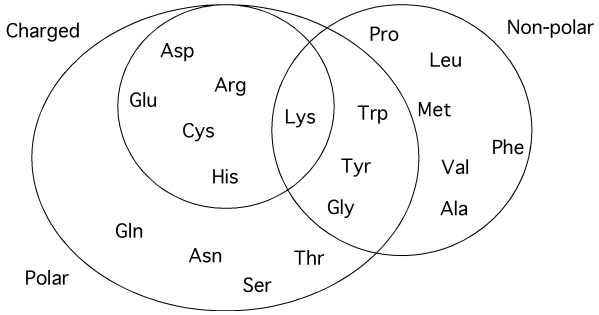




5. The peptide bond is unstable thermodynamically (the equilibrium constant for hydrolysis of the peptide bond favors hydrolysis by 10^3) – but stable kinetically (the half time can be years). Peptide bond hydrolysis is slow in the absence of an enzyme.

5.3 Properties of Amino Acids

Protein structure: Proteins are linear polymers of amino acids (connected by peptide bonds). Most proteins can be characterized as globular (ball-like) with a well defined external surface and a well defined internal core. Just as in micelles we expect to find the exterior to be polar and the interior to be non-polar. The **properties of the side chains determine the 3-dimensional structure of the folded protein**.



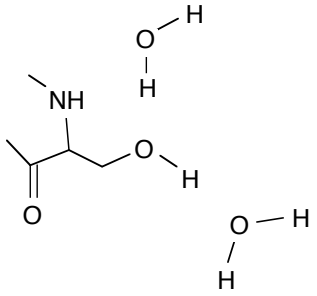
How the properties of amino acids influence protein structure:

Charged Residues: Amino acids that have charged side chains are seldom buried in the interior of a folded protein. They are normally found on the surface of the protein where they interact with water and with other biological molecules (such as other proteins).

The ionizable groups on the side chains of charged amino acids are often involved in biochemical transactions (binding, catalysis). Therefore, pH usually has rather dramatic effects on the function of proteins. The following are pK_a values of potentially charged side chains:

Glutamic Acid (Glu), Aspartic Acid (Asp)	4.0
Histidine (His)	6.0
Lysine (Lys)	10.0
Arginine (Arg)	12.5
Tyrosine (Tyr)	10.0
Cysteine (Cys)	8.0

Polar Residues are both buried as well as on the surface of the protein. They either form hydrogen bonds with other polar residues in the protein or with water. For example, the OH group of Serine can both donate as well as accept a hydrogen bond.



Nonpolar Residues do not interact favorably with water. The central core of most proteins is composed almost exclusively of nonpolar residues, stabilized by numerous van der Waals interactions. However, a significant number of nonpolar residues are also found on the surface of the protein.

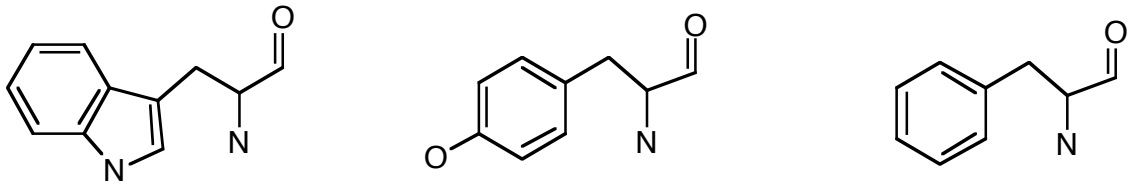
Summary & General Rules Regarding the Distribution of Amino Acids in Proteins:

- Charged residues are hardly ever buried.
- Polar residues are usually found on the surface, but can be buried.
- The inside, or core of a protein contains mostly non-polar residues.
- Non-polar residues are also found on the surface of proteins.

Recognition of one biological molecule by another can utilize charge, polar as well as non-polar interactions.

5.4 Spectral Properties of Amino Acids

Trptophan (Trp), Tyrosine (Tyr) and Phenylalanine (Phe) contain conjugated aromatic rings. Consequently, they absorb light in the ultraviolet (UV) range.



The amount of light absorbed by a solution of concentration [X] is given by the Beer-Lambert Law

$$A = \log \frac{I_o}{I} = \varepsilon [X] l$$

where

A is the absorbance of the sample;
I_o is the intensity of the incident light;
I is the intensity of the light that leaves the sample;
ε is the molar extinction coefficient at a specific wavelength, e.g. at λmax;
[X] is the concentration of the absorbing species; and
l is the path length (usually 1 cm).

Therefore, given a known extinction coefficient it is possible to measure the concentration of a protein.

The extinction coefficients of the above amino acids are:

Amino Acid	**Extinction Coefficient ε (λmax)**
Trp	5,050 $M^{-1}cm^{-1}$ (280 nm)
Tyr	1,440 $M^{-1}cm^{-1}$ (274 nm)
Phe	220 $M^{-1}cm^{-1}$ (257 nm)

- A solution that does not absorb any light ($I=I_o$) has an absorbance of 0.
- A solution that absorbs most of the light that passes through it has a large absorbance. For example, if 90% of the light were absorbed, $I_o/I = 10$, and A = 1.0.
- The above table shows that Trp absorbs UV light the strongest. Furthermore, since both Trp and Tyr show the maximum light absorbance at approximately 280 nm the absorption maximum of most proteins is around 280 nm. In contrast, the absorption maximum for nucleic acids is approximately 260 nm.

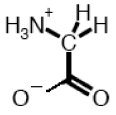
In a mixture of N different chromophores, the absorbance is additive:

$$A = l \sum_{i=1}^{N} \varepsilon_i [X]_i$$

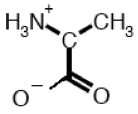
Therefore, if a protein contained 3 Tyrosines and one Tryptophan, its extinction coefficient would be:

$$\varepsilon = 3 \times 1{,}440 + 1 \times 5{,}050$$

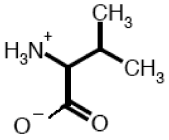
Amino Acid Structures (pH=6.0)



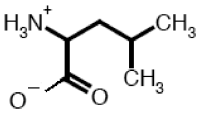
Glycine
Gly



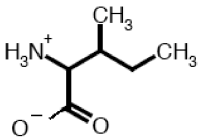
Alanine
Ala



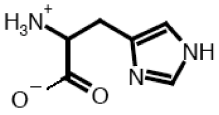
Valine
Val



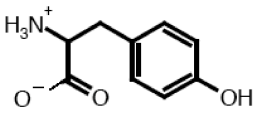
Leucine
Leu



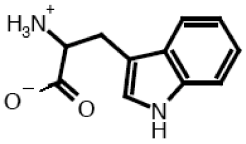
Isoleucine
Ile



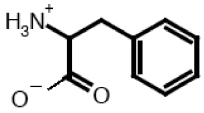
Histidine
His



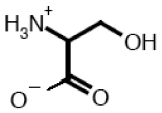
Tyrosine
Tyr



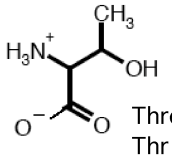
Tryptophan
Trp



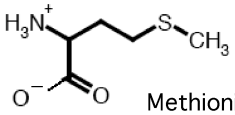
Phenylalanine
Phe



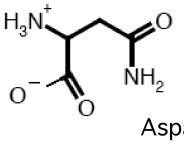
Serine
Ser



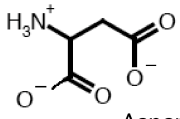
Threonine
Thr



Methionine
Met

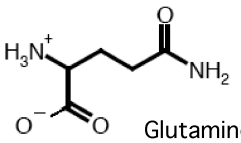


Asparagine
Asn

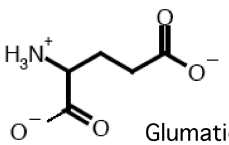


Aspartic Acid
Asp

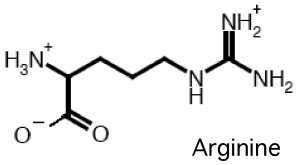
Lysine
Lys



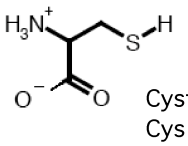
Glutamine
Gln



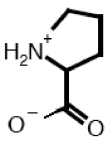
Glumatic Acid
Glu



Arginine
Arg



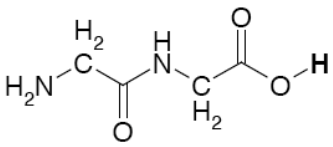
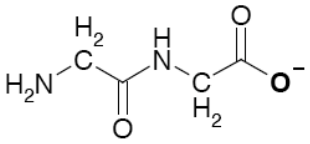
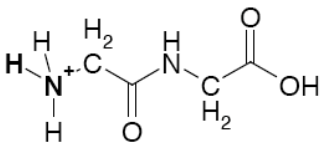
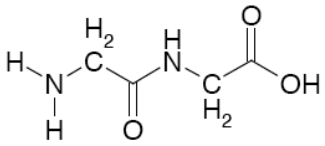
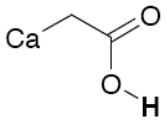
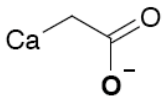
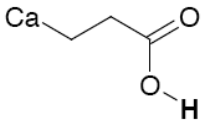
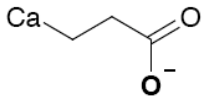
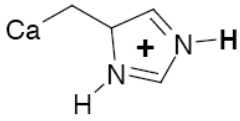
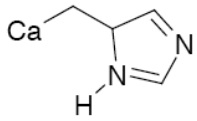
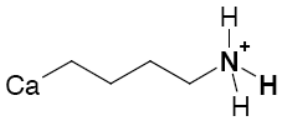
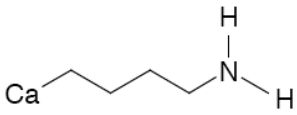
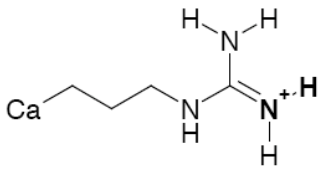
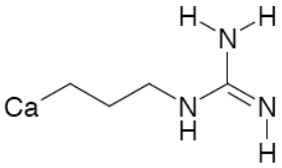
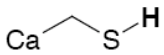
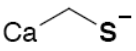
Cysteine
Cys



Proline
Pro

The structure of the 20 common amino acids are shown.
The side chains are shown in their most likely ionization state at pH 6.0
except His, whose pKa is 6.0 and would be 50% protonated.

Ionizable Amino Acids/Protonated - deprotonated forms

	Protonated (pH<<pKa)	Deprotonated (pH>>pKa)
Carboxy-Terminus pKa=2		
Amino-Terminus pka=9		
Aspartic Acid (Asp) pKa=4		
Glutamic Acid (Glu) pKa=4		
Histidine (His) pKa=6		
Lysine (Lys) pKa=9		
Arginine (Arg) pKa=12		
Cysteine (Cys) pKa=8		
Tyrosine (Tyr) pKa=10