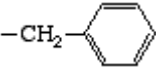
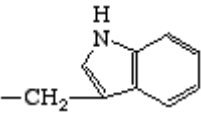
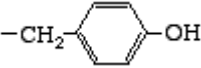
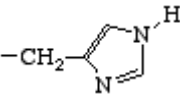
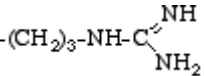
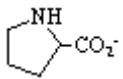


## Chemical Aspects that Influence Protein Structure and Stability

**Goal:** Our goal in this activity is to apply what we learned about amino acids and the primary structure of proteins and begin to consider how proteins fold into the 3-dimensional structures that we observe in our bodies. This activity will specifically investigate the chemistry of amino acid side chains and how the physical and chemical properties of each can lead to the variety of properties that proteins have.

As we saw in the previous activity, amino acids are able to combine together through condensation reactions. The bond that is formed is known as an amide, but in biochemistry it's known as a **peptide bond**. When multiple amino acids combine together, the product is a peptide. Peptides have two ends (or termini), the N-terminus (for amine) and the C-terminus (for carboxylic acid). The physical and chemical properties of peptides are dependent on what types of amino acids are incorporated into the polypeptide chain and also what order they are grouped together.

Amino Acid	R (neutral form)	-NH <sub>3</sub> <sup>+</sup>	-CO <sub>2</sub> H	Side chain
Glycine, Gly	-H	9.78	2.35	
Alanine, Ala	-CH <sub>3</sub>	9.87	2.35	
Valine, Val	-CH(CH <sub>3</sub> ) <sub>2</sub>	9.74	2.29	
Leucine, Leu	CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	9.74	2.33	
Isoleucine, Ile	CH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>3</sub>	9.76	2.32	
Phenylalanine, Phe		9.31	2.20	
Tryptophan, Trp		9.41	2.46	
Tyrosine, Tyr		9.21	2.20	10.46
Histidine, His		9.33	1.80	6.04
Serine, Ser	CH <sub>2</sub> OH	9.21	2.19	
Threonine, Thr	CH(CH <sub>3</sub> )-OH	9.10	2.09	
Methionine, Met	CH <sub>2</sub> CH <sub>2</sub> SCH <sub>3</sub>	9.28	2.13	
Cysteine, Cys	CH <sub>2</sub> SH	10.70	1.92	8.37
Aspartic Acid, Asp	CH <sub>2</sub> CO <sub>2</sub> H	9.90	1.99	3.90
Glutamic Acid, Glu	CH <sub>2</sub> CH <sub>2</sub> CO <sub>2</sub> H	9.47	2.10	4.07
Asparagine, Asn	CH <sub>2</sub> C(O)NH <sub>2</sub>	8.72	2.14	
Glutamine, Gln	CH <sub>2</sub> CH <sub>2</sub> C(O)NH <sub>2</sub>	9.13	2.17	
Lysine, Lys	(CH <sub>2</sub> ) <sub>4</sub> NH <sub>2</sub>	9.06	2.16	10.54
Arginine, Arg		8.99	1.82	12.48
Proline, Pro		10.64	1.95	

Consider the five residue polypeptides below. Predict which of these would be:

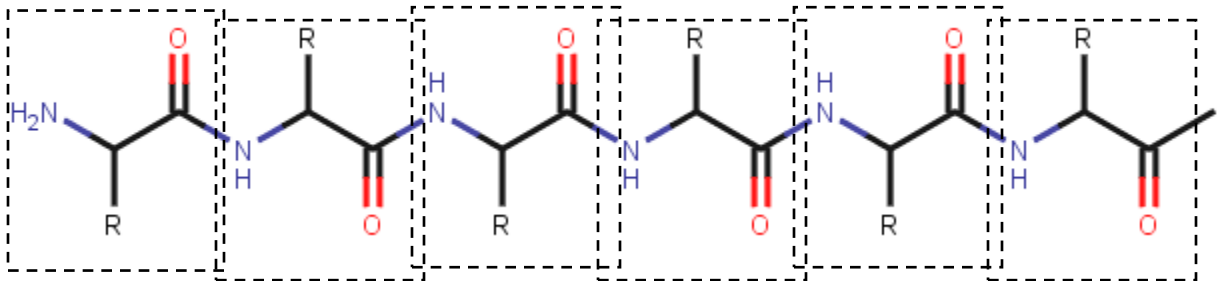
- the most acidic
- the most basic
- the highest logP (remember this means most likely to dissolve in octane)
- the smallest logP (most soluble in water)

Gly-Ala-Lys-Tyr-Gln

Asp-Ser-Cys-Glu-Thr

Phe-Val-Ile-Met-Trp

When the 3D structure of polypeptide backbones is considered, what becomes clear is that the side chains alternate in their orientation; if the first one points up, the next one will point down (see the image below where each box is an amino acid).



As it turns out, this can have a very large influence on the properties of a peptide. The side chains of adjacent amino acids are not in close proximity with each other, but the side chains of alternating amino acids are. For example, consider these two peptides that have the same amino acid composition but different arrangement:

Ala-Asp-Lys-Val-Ala

Ala-Asp-Val-Lys-Ala

Sketch each of these using the model you see above.

Is there anything notable about the orientation of the five amino acids in either of the structures? Hint: think about the ability to interact with water.

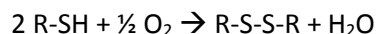
Which of these do you think would be most likely to be a peripheral membrane protein? As a reminder, this is a protein that sits on the surface of a lipid bilayer with one part interacting with the water and one part anchored into the nonpolar region.

The arrangement of amino acids can also have a large impact on the pKa of individual amino acid side chains (and therefore the pI of the peptide).

- Which of these peptides has the acidic and basic side chains oriented on opposite sides?
  - Which of them has these residues on the same side?
  - Which orientation is most likely to have an influence on the pKa of the side chains?
  - Can you explain why? (Hint: Remember that we can think about an acid's strength by considering the reactivity of the base. Also, what does a positive charge ( $R-NH_3^+$ ) in close proximity to the base ( $R-COO^-$ ) do to the stability of the base?)
- 
- Will the pKa of the acid be increased or decreased (remember that the ionic interaction makes the base more stable)?
  - Will the pKa of the base be increased or decreased (here, the acid form is more stable)?

Some amino acids have side chains that can undergo chemical modifications that can increase or decrease the stability of interactions.

- Disulfide bond formation occurs when this reaction below happens:



- Which amino acid can form a disulfide?
- Draw the product of a disulfide between two of the side chains on the amino acid you identified.

- Some amino acids are susceptible to phosphorylation. This is when an **alcohol group** undergoes a condensation reaction with hydrogen phosphate.
  - Which amino acids are subject to phosphorylation?
  - Show the product of this reaction with one of the amino acids you just identified.

Now let's turn our attention to the actual forces that can stabilize interactions between amino acids in proteins.

- For ion-ion interactions, we need to consider charged residues. Which amino acid side chains are likely to be charged at neutral pH?
  - Cations:
  - Anions:
- Dipole-dipole interaction require polar side chains. Which amino acids have polar side chains?
- Identify all amino acid side chains that can participate in H-bonds.
- The amino acids that are left over can only interact through LDF. What are the nonpolar amino acids?
- The most important aspect of protein folding is the burial of hydrophobic residues so that they don't have to interact with water. Think back to our discussion of lipid bilayers forming, what is the main energetic driving force for protein folding?
- How do you think phosphorylation of serine will influence the types of IMF that it can participate in?