The pH of the solution in which the aminoacid is determines whether or not the amino group and carboxyl group are protonated or deprotonated. At a neutral pH of around 7 our aminoacids exist predominantly in their dipolar form. The dipolar form is also know as the **zwitterion form:** The amino group is protonated (it has a full positive charge, NH3+) and the carboxylic group is deprotonated (it has a full negative charge, COO-)

1) At the low pH of 1, the carboxylic acid group is protonated. At this acidic pH, the aminoacids exist as positively-charged species (the aminoacid will have an overall charge of +1 because the amino group is protonated)

2) As the pH increases, we decrease the concentration of H+ ions inside the solution. At around pH=2, the H found in the carboxylic acid group begins to dissociate->deprotonates, loses the H atom and gains that full negative charge. It forms the zwitterion form

3) The zwitterion form usually persists until around a pH of 9. At this basic pH, the low [H+] causes the amino group to deprotonate losing its hydrogen, thereby forming a negatively-charged species (NH2 is neutral and COO- is negative).

The type of bond that holds our amino acids together is a special type of covalent bond known as a **peptide bond** or an **amide bond**. Peptide bond formation is kinetically stable and thermodynamically not favorable. To actually form a peptide bond we have to use ATP molecules as a result of the fact that the reactants are thermodynamically more stable than our products.

peptide bond- between the carboxyl group of one aminoacid and the amino group of the adjacent aminoacid.

One end of the polypeptide chain has a free amino group, while the opposite end has a free carboxyl group. The linear polymer of aminoacids contains polarity.

Even though the reaction from the products to the reactants is thermodynamically favorable it is not kinetically favorable what that means is we

have to input a lot of energy to actually overcome this reverse activation barrier and we simply don't have that much energy under normal conditions inside our cell at a pH of 7 and at the normal body temperature. We would have to increase the temperature to a high temperature or we would have to use an enzyme that decreases that activation energy to actually break the peptide bond and go back to individual constituent amino acids.

There are hydrophobic amino acids which means that their non-polar side chains tend to pack together rather than interact with water. These amino acids tend to display the **hydrophobic effect**.

Phenylalanine contains an unreactive and highly non-polar aromatic ring.

Tyrosine contains an aromatic ring with a hydroxyl group, making it less hydrophobic and more reactive.

Tryptophan contains a NH group, making it slightly reactive.

Serine and **threonine** both have hydroxyl group, which makes them polar (hydrophilic) and reactive.

Cysteine is structurally similar to serine, except it has a thiol group. It is polar and reactive in forming disulfide bridges (covalent bounds that exist within protein structures and play an important role in determining the 3-dimensional structure of the protein).

Asparagine and **glutamine** contain polar carboxamide groups.

Special side chains:

- **Glycine** is the smallest amino acid. It is achiral and can fit into either hydrophobic or hydrophilic environments.
- Although **proline** is hydrophobic, it is special in that it contains a side group that is bound to the alpha carbon and the nitrogen. The five-membered ring of proline makes it structurally restrictive, allowing it to greatly influence the structure of proteins.

of amino acids at a ratio of one attachment per two amino acids.

When the protein is denatured, SDS anions attach onto the side chains of amino acids at a rate of about one SDS anion per two residues. When they attach onto the protein, because these SDS molecules contain a negative charge, they give that protein a negative charge. After we have these many anions attached onto our protein the entire net charge of that protein becomes negative so even if the protein before this process had a native positive charge that protein gains a net negative charge, allowing to move from the top (from the negative side) to the bottom (to the positive side) in our electric field.

If we have a very large protein with many amino acids that means that large protein will contain many more of these SDS anions attachments. Therefore both small and large proteins will have a net negative charge but the larger protein will have a greater net negative charge.

From the equation before velocity is directly proportional to the charge so if the charge is increased then the velocity will increase as well so large proteins should be able to move along the gel with a greater velocity than the smaller molecules. But we know that is not true, in fact the opposite is true: Smaller proteins move along the gel quicker with the greater velocity.

When we increase the size of our protein we increase not only the charge Q but we increase the coefficient of friction f by a greater amount than we increase the Q, decreasing the velocity. Larger proteins will be higher up along that slab than smaller proteins over that same time interval.

What is the difference between gel electrophoresis and gel filtration chromatography?

Both of these techniques separate the proteins based on size.

In gel electrophoresis all of the proteins in the mixture are forced to move through the pores gel but in the case of gel filtration chromatography only the small proteins are actually forced to move through the beads that are composed of that porous gel. In gel filtration chromatography the large proteins are not forced to move through the porous beads, instead they can simply travel through the space around the beads and because of that in gel filtration