

1. Electrophoresis is a technique that uses electricity to "drive" charged molecules through various "supports" such as agarose or acrylamide. DNAs are most simply separated by agarose gel electrophoresis and proteins are most commonly separated using acrylamide electrophoresis.
2. DNA separations rely on the fact that the phosphate backbone is uniformly negatively charged and is repelled a negative electrode and attracted to a positive one. Molecules get separated in the agarose "mesh" by their size - the smallest ones most fastest and the largest ones move slowest.
3. Proteins too are separated using electricity, but to do so requires making them uniformly charged. The detergent known as SDS denatures the proteins and coats them evenly with a negative charge so that they move through a gel support system (acrylamide) much like DNA moves through an agarose support system. The smallest proteins move the fastest and the largest ones move the slowest.
4. The technique of separating proteins in this fashion is often referred to as SDS-PAGE - SDS PolyAcrylamide Gel Electrophoresis
5. 2D gel electrophoresis involves two separations in two dimensions. The first involves separation on the basis of charge. The second involves placing the tube containing the separated charged molecules, adding SDS, and then running the contents on SDS-page. The resulting gel has molecules separated by size in the vertical direction and by charge in the horizontal direction.
6. Proteins can be broken into smaller pieces using enzymes like trypsin (cuts lysine or arginine), chymotrypsin, and cyanogen bromide (breaks at methionines).

Highlights Enzymes I

1. Enzymes catalyze reactions up to many trillion of times faster than the same reactions without any catalysts.
2. Enzymes work by reducing the activation energy required for a reaction to occur. The energy available to cells is called Free Energy (ΔG). Because enzymes lower that "starting" energy requirement, they make the reaction easier to occur and thus speed them up.
3. Note also (important) that enzymes do NOT change the free energy difference between the beginning reactants and the end products. Thus, enzymes do not change the overall energy of a reaction - only the energy required for the transition state.
4. A "substrate" is a molecule bound by an enzyme which it catalyzes a reaction upon. Substrates bind specific binding sites on enzymes that resemble their structure. An "active site" of an enzyme is a site on an enzyme where the reaction it catalyzes occurs.
5. There are two models for enzyme action relevant for our consideration. The "lock and key" model proposes that enzymes act like a "lock" that only certain keys (substrates) fit. This model works well for describing the binding of substrates, but is not helpful (or accurate) for describing the mechanism of catalysis.
6. The "induced fit" model of enzyme action proposes that enzymes change in response to binding of substrate and that change is at least partly responsible for the catalysis that occurs on the substrate. Thus, the induced fit model says that enzymes change substrates (by catalysis) and that substrates change enzymes (enabling catalysis). The upshot of the induced fit model is that the flexibility of enzymes enables them to be

such extraordinary catalysts.