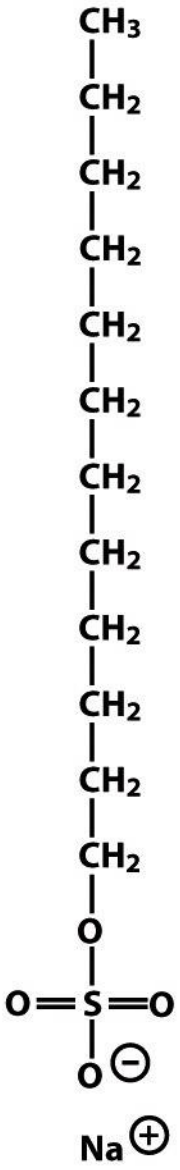
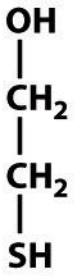
Proteins Can Be Separated by SDS Polyacrylamide-Gel Electrophoresis

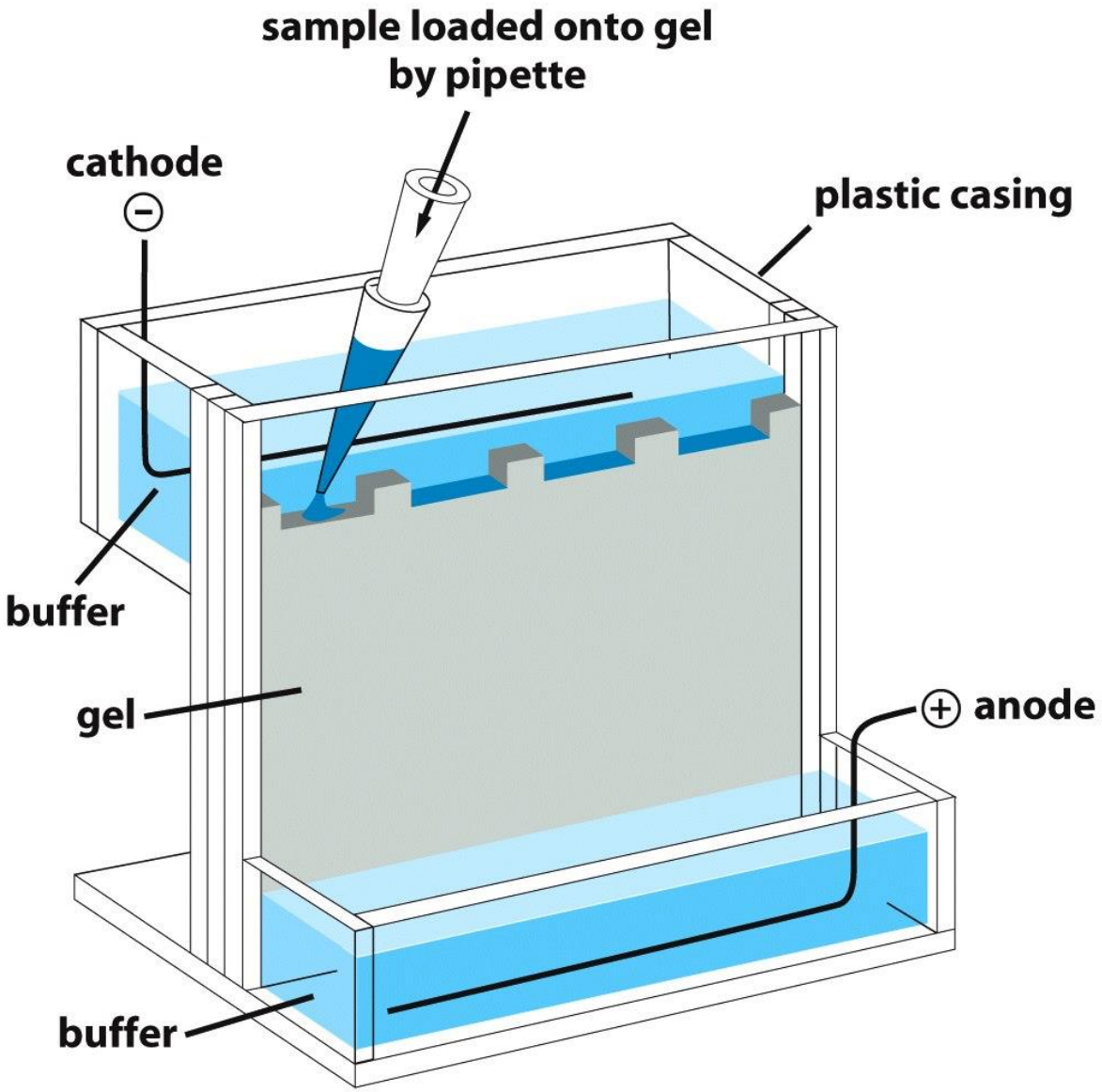


SDS



β-mercaptoethanol

- Proteins usually possess a net positive or negative charge, depending on the mixture of charged amino acids they contain.
- An electric field applied to a solution containing a protein molecule causes the protein to migrate at a rate that depends on its **net charge and on its size and shape.**
- The most popular application of this property is **SDS polyacrylamide-gel electrophoresis (SDS-PAGE).**
- It uses a highly cross-linked **gel of polyacrylamide** as the inert matrix through which the proteins migrate. The gel is prepared by polymerization of monomers.
- The proteins are dissolved in a solution that includes a powerful negatively charged detergent, **sodium dodecyl sulfate, or SDS**. Because this detergent binds to hydrophobic regions of the protein molecules, causing them to **unfold into extended polypeptide chains**, the individual protein molecules are released from their associations with other proteins or lipid molecules and rendered freely soluble in the detergent solution.
- In addition, a reducing agent such as **β-mercaptoethanol** is usually added to break any S–S linkages in the proteins, so that all of the constituent polypeptides in multisubunit proteins can be analyzed separately.

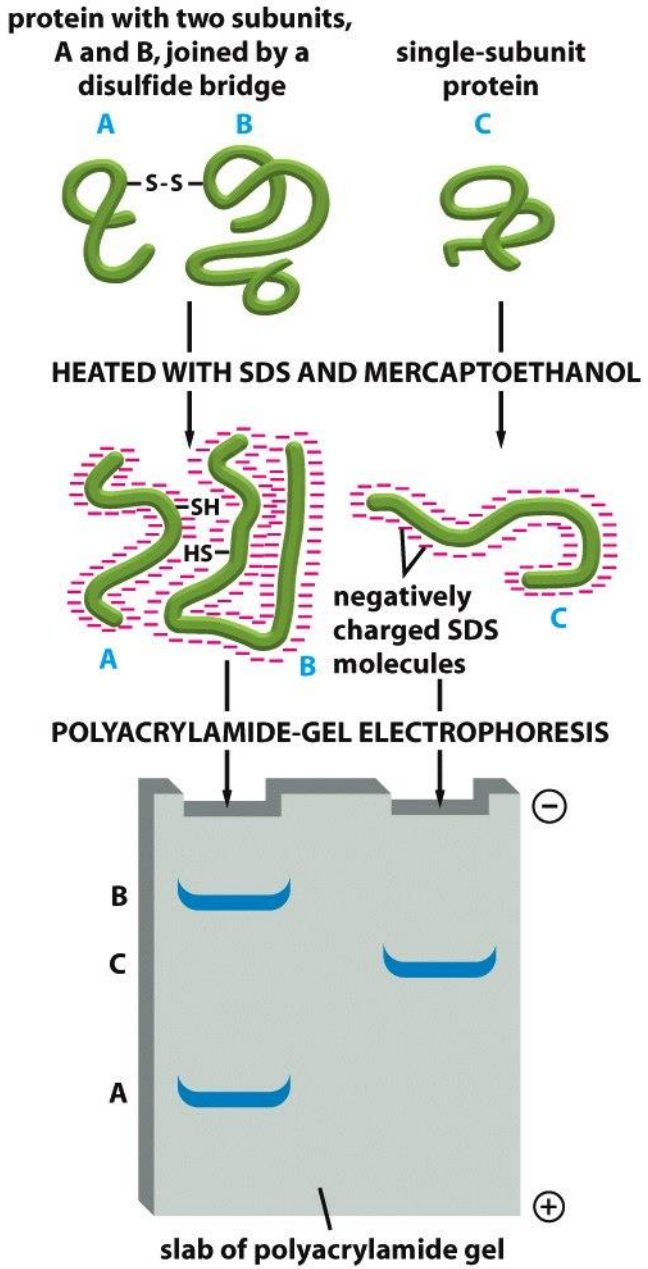


- What happens when a mixture of SDS-solubilized proteins is run through a slab of polyacrylamide gel?
- Each protein molecule binds large numbers of the negatively charged detergent molecules, which **mask the protein's intrinsic charge** and cause it to migrate toward the positive electrode when a voltage is applied.

- Proteins of the same size tend to move through the gel with similar speeds because:

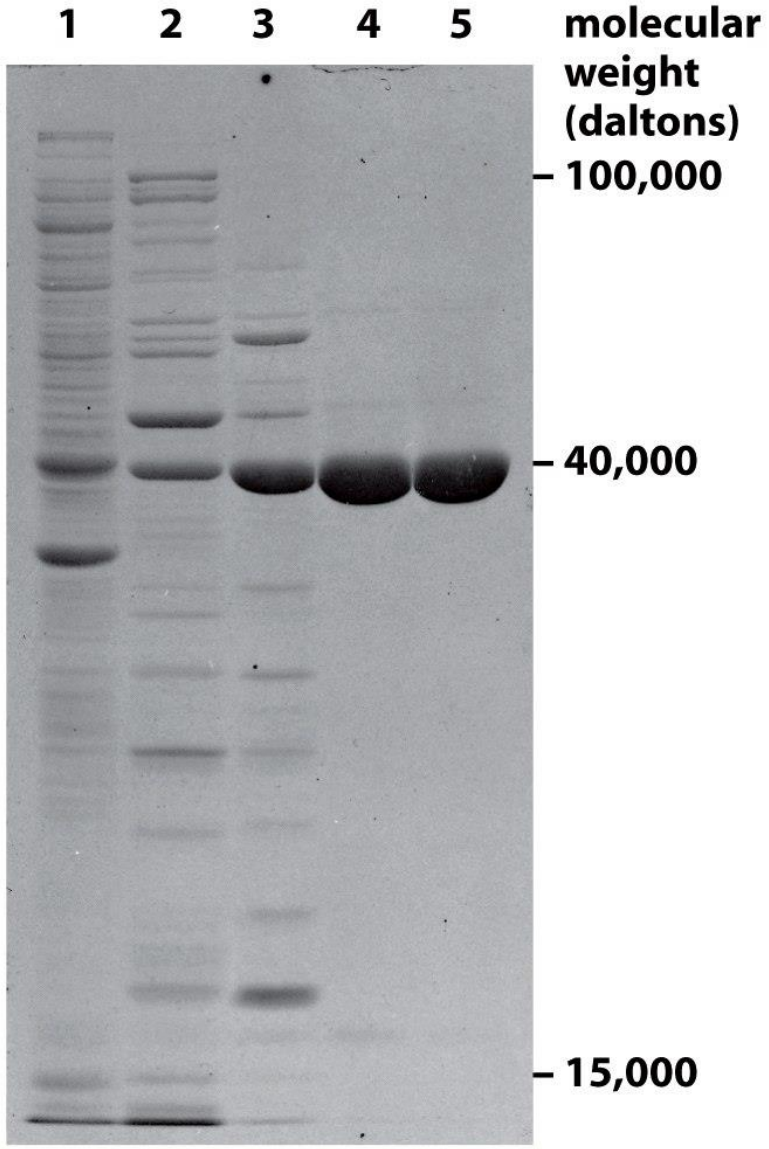
(1) their native structure is completely unfolded by the SDS, so that **their shapes are the same**, and

(2) they bind the same amount of SDS and therefore have the **same amount of negative charge**.



- Larger proteins, with more charge, are subjected to larger electrical forces but also to a larger drag.

- In the mesh of the polyacrylamide gel, which acts as a molecular sieve, large proteins are retarded much more than small ones. As a result, a complex mixture of proteins is fractionated into a series of discrete protein bands arranged **in order of molecular weight.**



A photograph of a gel that has been used to analyze each of the successive stages in the purification of a protein.

- The major proteins are readily detected by staining the proteins in the gel with a dye such as Coomassie blue.
- Even minor proteins are seen in gels treated with a silver stain, so that as little as **10 ng** of protein can be detected in a band.
- SDS-PAGE is widely used because it can separate all types of proteins, including those that are normally insoluble in water—such as the many proteins in membranes.
- And because the method separates polypeptides by size, it provides information about the **molecular weight** and the **subunit composition** of proteins