When an electric field is applied to a solution containing protein molecules, the molecules will migrate in a direction and at a speed that reflects their size and net charge. This forms the basis of the technique called **electrophoresis**.

**GEL ELECTROPHORESIS**

- **Sample loaded onto gel by pipette**
- **Plastic casing**
- **Buffer**
- **Gel**
- **Cathode**
- **Anode**

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**ISOELECTRIC FOCUSING**

For any protein there is a characteristic pH, called the **isoelectric point**, at which the protein has no net charge and therefore will not move in an electric field. In **isoelectric focusing**, proteins are electrophoresed in a narrow tube of polyacrylamide gel in which a pH gradient is established by a mixture of special buffers. Each protein moves to a point in the gradient that corresponds to its isoelectric point and stays there.

**SUPPORTING INFORMATION (Panel 4–5)**

- **Protein with two subunits, A and B, joined by a disulfide bridge**
- **Single subunit protein**
- **HEATED WITH SDS AND MERCAPTOETHANOL**
- **Negatively charged SDS molecules**
- **Slab of polyacrylamide gel**

**TWO-DIMENSIONAL POLYACRYLAMIDE-GEL ELECTROPHORESIS**

Complex mixtures of proteins cannot be resolved well on one-dimensional gels, but **two-dimensional gel electrophoresis**, combining two different separation methods, can be used to resolve more than 1000 proteins in a two-dimensional protein map. In the first step, native proteins are separated in a narrow gel on the basis of their intrinsic charge using isoelectric focusing (see left). In the second step, this gel is placed on top of a gel slab, and the proteins are subjected to SDS-PAGE (see above) in a direction perpendicular to that used in the first step. Each protein migrates to form a discrete spot.

**POLYACRYLAMIDE-GEL ELECTROPHORESIS**

- **Sample loaded onto gel by pipette**
- **Plastic casing**
- **Buffer**
- **Gel**
- **Cathode**
- **Anode**

All the proteins in an *E. coli* bacterial cell are separated in this 2-D gel, in which each spot corresponds to a different polypeptide chain. They are separated according to their isoelectric point from left to right and to their molecular weight from top to bottom. (Courtesy of Patrick O’Farrell.)