**Fish Protein Fingerprinting on Agarose and Polyacrylamide Gels**

**Introduction**

Species that diverged from a common ancestor a long time ago are less similar biochemically than those that diverged more recently. The biochemical composition of organisms includes their protein molecules. Thus, the degree of relatedness of two species can be estimated from the amount of similarity between their protein makeups. To compare protein profiles between organisms, scientists separate the mixture of protein molecules in a particular tissue (such as muscle tissue) by gel electrophoresis. This creates a unique pattern of bands for each organism, called a protein fingerprint. The individual bands correspond to different proteins and may vary in intensity between species. In addition, some bands (i.e., proteins) may be visible in one species fingerprint but not in another. In general, protein fingerprint patterns obtained from different species are more similar when the species are more closely related and less similar when they are more distantly related.

Fish represent a diverse group of organisms that have evolved to live in many different aquatic environments. The evolution of different groups of fish and the varying degrees to which they are related are topics of ongoing study. In this exercise, you will compare the protein fingerprints of seven different types of fish through gel electrophoresis of the fish protein extracts provided. Fish were chosen as the sample sources because there are many different varieties and because protein sources for many fish species are readily available.

The behavior of a molecule during gel electrophoresis depends on its size, shape, and net charge. Linear DNA molecules have uniformly negatively charged backbones and a shape that normally varies only in its length. Therefore, migration of DNA is directly dependent on the size of the DNA fragment. The migration of proteins, however, is affected by multiple factors involving their structural organization.

There are four levels of structural organization in proteins. The primary structure of a protein is its sequence of amino acids. Amino acids can be positively charged, negatively charged, or neutral. This means that proteins can carry either a net positive, net negative, or neutral charge depending on the combination of amino acids they contain.

The shapes of proteins vary widely. The shape of a protein is created by its secondary, tertiary, and quaternary structure. In secondary protein structure, hydrogen bonds form between adjacent parts of the amino acid chain to form folded, coiled, or twisted shapes, including α-helices and β-sheets. Additional interactions, such as hydrogen bonds, hydrophobic interactions, electrostatic interactions, and/or disulfide bonds lead to the tertiary structure of a protein. At the quaternary structural level, several folded amino acid chains associate in unique ways to form a functional protein with a distinctive shape.

Native conformations of proteins (the form in which they are biologically active) vary widely in charge and shape. As such, the molecular weight of proteins cannot be determined by electrophoresis of native proteins. To make protein migration rates a function of molecular weight, it is necessary to impose a uniform shape and charge on all of the proteins in a mixture. This can be primarily achieved by treating the protein mixture with the negatively charged detergent sodium dodecyl sulfate (SDS) and heat. Treatment with SDS and heat disrupts hydrogen bonds and unfolds the protein structure. SDS also binds to and coats the protein backbone, regardless of the amino acid sequence, and imparts a uniform negative charge to all the molecules. Treating protein samples with a reducing agent such as β-mercaptoethanol breaks disulfide bonds and denatures the proteins into linear chains of amino acids (its primary structure).
Under these conditions and for the purpose of electrophoresis, all of the proteins in a mixture assume the same shape and charge. They differ only in molecular weight. Like DNA, they migrate toward the positive electrode during electrophoresis at a rate inversely proportional to the log_{10} of their molecular weights.

The buffer that the fish protein extracts are provided in contains both SDS and \( \beta \)-mercaptoethanol to disrupt the structure of the proteins. To ensure that the proteins are fully denatured, the samples should be boiled immediately before being loaded onto the gels, as described in the procedure. To maintain protein denaturation during electrophoresis, the gels are made with a buffer that contains SDS. The electrophoresis running buffer also contains SDS.