Lecture 12: Two dimensional PAGE for proteome analysis

During last lectures we have studied electrophoretic methods of separation of protein based on mass (PAGE) or isoelectric point (Isoelectric focusing). However, simplest bacteria may have over 4000 individual proteins while eukaryotes the number may be approximately 50,000.

In the large population of protein it may be possible that more than one protein has identical (or close) molecular mass and may not be separated by PAGE. It is also possible that more than one protein has very close isoelectric point (pI) and separation is not possible by isoelectric focusing (IEF). Combination of these two methods may yield better separation.

Two-dimensional gel electrophoresis (2-D electrophoresis) is widely used technique for the analysis of complex protein mixtures. As explained above, this method separate proteins in two steps, the first-dimension is isoelectric focusing, which separates proteins according to their isoelectric points (as we have studied in our previous lectures); the second-dimension is SDS-polyacrylamide gel electrophoresis (SDS-PAGE), which separates proteins according to their molecular weights (as we have studied in our previous lectures). In this way, complex mixtures consisted of thousands of different proteins can be separated.

First dimension electrophoresis (isoelectric focusing) is performed under denaturing conditions which gives the highest resolution and the cleanest results. The IEF is the most critical step of the 2-D electrophoresis process as proteins need to be solubilized without charged detergents like SDS (it can give extra charge on protein and changes isoelectric point). Most common protocol uses high concentration of urea and reducing agents. First dimension separation also requires selecting a 1st dimension pH range (depends on sample) and length of strip (depends on size of second dimension gel). After first dimension run, strip gel is placed on the top of SDS-PAGE gel horizontally (a normal SDS-discontinuous system is used). The protein is already denatured in first dimension (by urea and reducing agents).

Thus, the SDS used in gel running buffers sufficient to bind with already denatured protein maintain the necessary uniform negative charge for SDS-PAGE.

[Recall procedure in normal SDS-PAGE we studied in previous lecture: The process involves boiling of sample with reducing agent and SDS so that protein can denature and bind with SDS to give uniform negative charge). Two dimensions of two-dimensional gel electrophoresis (2-D electrophoresis) is shown in Fig. 1

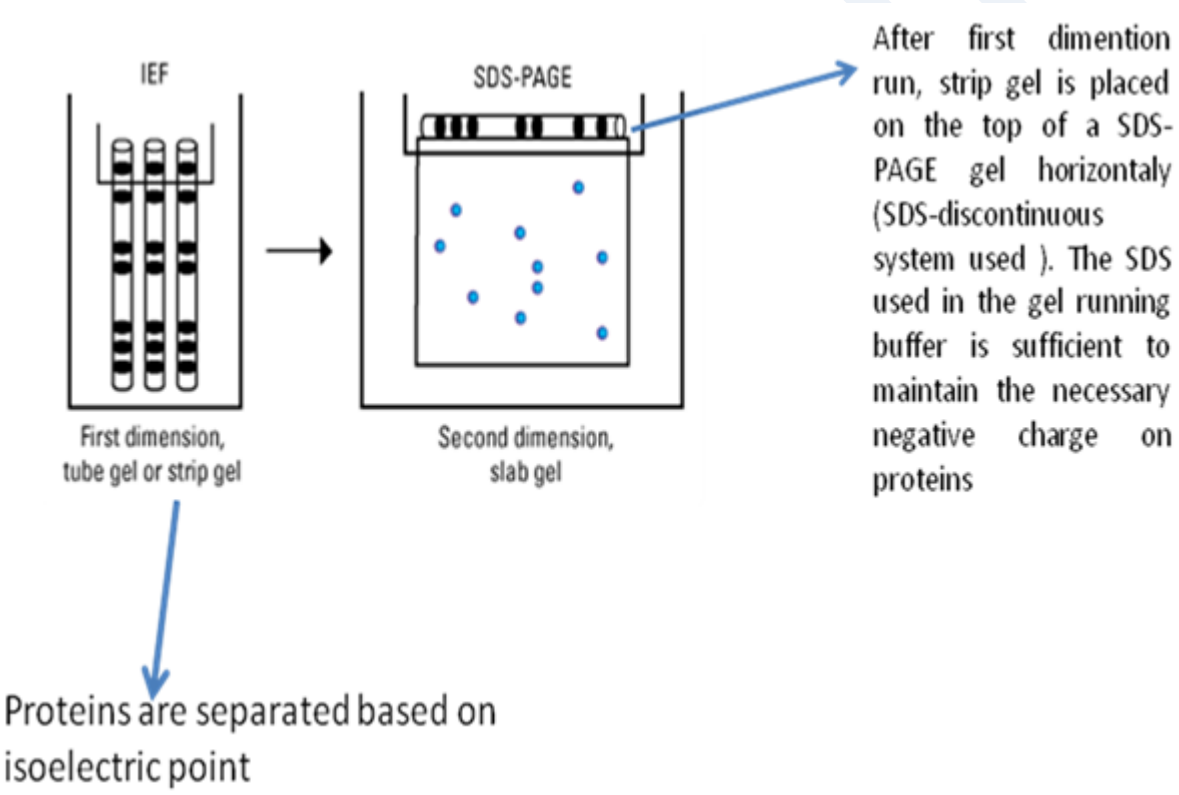

Figure 1: Two dimensions of two-dimensional gel electrophoresis (2-D electrophoresis)

Once the second dimension run is over, gel is separated and stained for protein visualization using methods studied in SDS-PAGE (Coomassie blue staining, Silver staining etc.)

2-Dimential gel electrophoresis is generally undertaken when the global protein expression of an organism or a tissue is being investigated. Comparison of normal and experimental 2D gel data helps is identification of up-regulated or down regulated proteins (Fig. 2.). Once protein band with altered expression is found, the band may be further analyzed for identification of protein by amino acid sequencing or peptide mass fingerprinting [we shall study these methods in coming lectures].

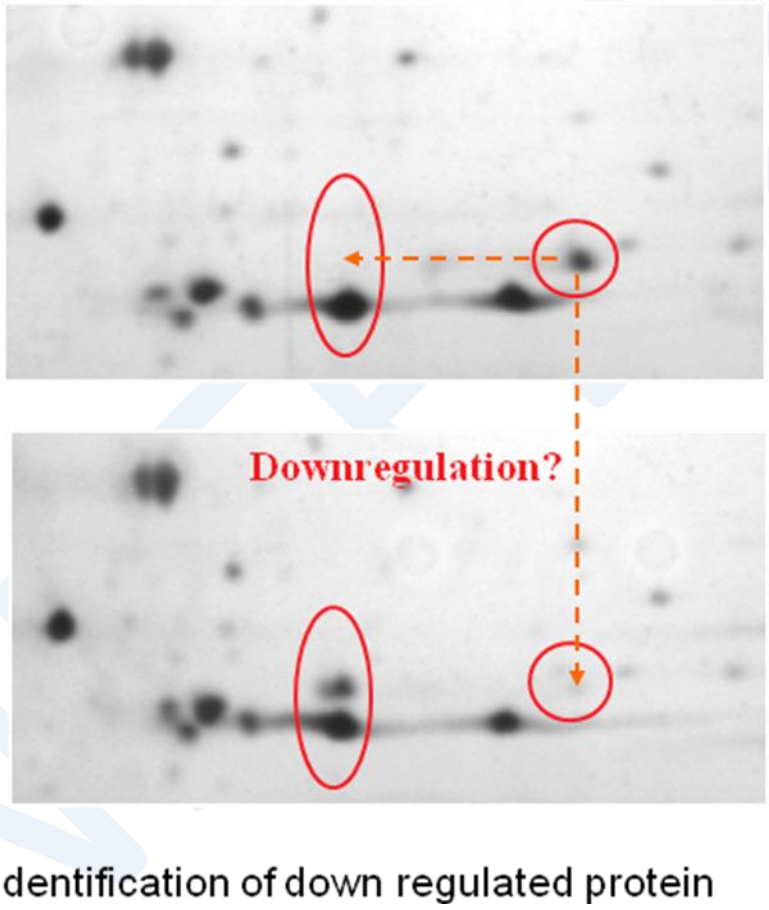

Figure 2: Comparison of normal and experimental 2D gel data for identification of up-regulated or down regulated proteins

Interfering Substances in 2D gel electrophoresis

Lipids: Lipids can bind to proteins changing both their isoelectric point and molecular weight.

Nucleic acids: It can block gel pores and increase sample viscosity, they may also bind proteins, particularly nucleic acid binding proteins changing both their isoelectric point and molecular weight. Thus, sample before loading must be processed to remove nucleic acid. It can be removed by ultracentrifugation method. Higher density of nucleic acids ensures that they are removed without the loss of proteins. Other method include use of nucleases which can digest nucleic acids

Polysaccharides: Uncharged (starch, glycogen) polysaccharides can block gel pores and inhibit migration of sample proteins resulting in poor focusing. These can simply be removed by ultracentrifugation. However, charged (mucins, dextrans) polysaccharides, on the other hand, bind protein due to charge and change isoelectric point and molecular weight. They can also be very difficult to remove. Polysaccharides cause severe smearing in protein bands.

Salts: High concentrations of salt can pose problems in isoelectric focusing process.