Lecture 13: Analysis of 2D gels

A complete proteomic analysis aims at collecting quantitative information about all protein in a sample. A normal 2 Dimensional gel electrophoresis results are analyzed by comparing images of control and treated (experimental sample) as shown in Fig. 1. Primarily we analyze individual proteins spots, and see difference between protein "spots" on a scanned image of 2-DE gels (different spots of same gel image and spots of different gel images).

![Diagram of control and treated samples](image)

**Figure 1:** Comparison of images of control and treated samples

However, the results are always doubtful due to gel to gel variations. Is the minor differences visible is real or due to gel to gel variations? Thus, it is better to analyse different samples in the same gel.

There are several tools available for quantitative analysis of 2 Dimensional gel electrophoresis data very precisely without gel to gel variations. 2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE) is one of the very effective tools. It uses cyanine dye labeling of protein prior to 2-D gel electrophoresis. Three cyanine dyes used in this method are Cy2, Cy3 and Cy5. These dyes contain an N-hydroxysuccinimidyl ester reactive group. When treated with protein sample, it forms a covalent bond with the epsilon amino group of lysine residues to yield an amide linkage. The lysine amino acid in proteins carries a positive charge. When these dyes are coupled to the lysine, replaces the lysine’s single positive charge with its own, ensuring that the pI of the protein does not change (Fig. 2).
Figure 2: Coupling of protein with minimal cyanine dye

These dyes have unique fluorescence properties. Cy2 when excited at 488nm gives fluorescence maxima at 520nm (yellow fluorescence), Cy 3 when excited at 532nm gives fluorescence maxima at 580nm (blue fluorescence) and Cy 5 when excited at 633nm give fluorescence maxima at 670nm (red fluorescence). Thus cyanine dyes labeling gives unique fluorescence property to proteins. When coupled to proteins, these dyes add approximately Mr 500 to the protein’s mass. However, as size and mass of all dyes are matched, a protein labeled by different dye will migrate to the same position. Using these three dye at a time three samples are analyzed.

When dye coupling is done, concentration of dye is kept limiting which leads to 1-2% of lysine residue labeling. In this process only one dye molecule per molecule is labeled. Even if multiples lysine residues of a protein are labeled, percentage of this double labeled species is too small to be visualized. As we have discussed that the binding of these dyes do not effect isoelectric point of protein but adds approximately Mr 500 mass. Unlabeled fraction of
protein corresponding to a given spot resides at Mr 500 difference where fluorescence signal of the spot is seen. Once the spot of a protein of interest is identified by image analysis, recovery of the unlabeled protein by automated spot is achieved for various analysis.

**Steps of the experiment:**

- Control is coupled with Cy3
- Sample 1 is coupled with Cy 5
- Sample 2 is coupled with Cy 2:
  - All three [control coupled with Cy 3, sample 1 coupled with Cy 5 and sample 2 coupled with Cy 5] are mixed and loaded for 2-D electrophoresis.
  - Once electrophoresis is done, the gel is scanned to get:
    - Cy3 fluorescent (580 nm blue fluorescence). This gives control image
    - Cy5 fluorescent (670 nm red fluorescence). This gives sample 1 image
    - Cy2 fluorescent (488 nm yellow fluorescence). This gives sample 2 image

![Figure 3: Rules of color mixing.](image)

Simple rule of color mixing (Fig. 3) is followed in image analysis if expression of protein in control and sample 1 is equal the image overlay of Cy3 (blue) and Cy5 (red) will give purple
spot. If these are difference in expression, image overlay will give different color. Similar control and sample 2 or sample 1 and sample 2 can also be analyzed for any change in proteome expression. This fluorescent method can detect even 125 pg protein [silver staining requires ng protein]. The Cy dyes technology, also called Fluorescence Difference Gel Electrophoresis (DIGE), is summarized in Fig. 4.

**DIGE Technology**

(From http://amershambiosciences.biz/aptrix/upp00919.nsf/Content/Proteomics+DIGE)

**Figure 4:** The Cy dyes technology, also called Fluorescence Difference Gel Electrophoresis (DIGE), is summarized in the figure

**Recommended article for reading:**


**Abstract of the article** (Anal Bioanal Chem., 2005, 382: 669–678)

Two-dimensional (2D) gel electrophoresis is a powerful technique enabling simultaneous visualization of relatively large portions of the proteome. However, the well documented issues of variation and lack of sensitivity and quantitative capabilities of existing labeling reagents, has limited the use of this technique as a quantitative tool. Two-dimensional
difference gel electrophoresis (2D DIGE) builds on this technique by adding a highly accurate quantitative dimension. 2D DIGE enables multiple protein extracts to be separated on the same 2D gel. This is made possible by labeling of each extract using spectrally resolvable, size and charge-matched fluorescent dyes known as CyDye DIGE fluors. 2D DIGE involves use of a reference sample, known as an internal standard, which comprises equal amounts of all biological samples in the experiment. Including the internal standard on each gel in the experiment with the individual biological samples means that the abundance of each protein spot on a gel can be measured relative (i.e. as a ratio) to its corresponding spot in the internal standard present on the same gel. Ettan DIGE is the system of technologies that has been optimized to fully benefit from the advantages provided by 2D DIGE.