2 Dimensional Gel Electrophoresis

First Dimension: pl by Isoelectric Focusing
Second Dimension: MW by standard SDS-PAGE

- First Published in 1975 by Pat O’Farrell

- Can separate at least 1,000 proteins

- Problems with run to run reproducibility limits the ability to easily compare multiple samples.

- Solution to this problem: DIGE (Difference Imaging Gel Electrophoresis)
DIGE experiment

Samples
- control
- treated
- pooled internal standard

Labeled proteins
- label with Cy3
- label with Cy5
- label with Cy2

Mix

Co-migration in 2-D electrophoresis

IPG strip

SDS gel

Slide courtesy of Tracy Andacht
Limitations of DIGE

1. Protein solubility during Isoelectric Focusing.
   - Membrane proteins often lost.

2. Size Limits – difficulty with proteins >100 kD.

3. Identification of the proteins in each spot is tedious and slow.
   - Use of robotics

4. Individual spots typically contain several proteins.
   - Intensity change is therefore the sum of the changes of each individual protein.
Identifying a Protein by Mass Spectrometry on Its Tryptic Peptides

Products from Trypsin digest.

Average length of tryptic peptides = 10 aa residues

Slide courtesy of Andrew Link
The mass difference between the peaks corresponds directly to the amino acid sequence.

**B**-ions contain the N-terminus.
Identifying a Protein by Mass Spectrometry on Its Tryptic Peptides

Y-ions contain the C-terminus
The Hardware for Peptide Mass Spectrometry

Liquid Chromatography

- Pump

Ionization Source → Mass Analyzer → Detector

Different Types:
- Electrospray
- MALDI
- Time of Flight (TOF)
- Quadropole
- Ion Trap
- OrbiTrap
- Ion Cyclotron Resonance (ICR)

Vacuum Pump

Output: Spectra
Limitations and Cautions of Proteomics:
The Range of Protein Concentrations In Human Plasma

Albumin 40 g/l
Complement 0.1 g/l
Myoglobin < 100 µg/l
TNFα < 1 ng/l

Anderson & Anderson, MCP 1:845, 2002
Limitations and Cautions of Proteomics: The Range of Protein Concentrations In Human Plasma

Depletion
Remove abundant proteins that are not of interest to your experiment. Methods: Antibody based depletion, selective lysis technique, subcellular fractionation, etc.

Enrichment
Enrich for the proteins of interest.
Methods – Lysis techniques or subcellular fractionation, affinity-based enrichment (antibodies, resins, etc).

Fractionation
Reduce the complexity of your sample by separating the proteins into different fractions and running these fractions separately.
Protein Quantitation with Mass Spectrometry

1. Stable Isotope Labels based Quantitation

   Examples of Stable Isotopes: $^{13}\text{C}$, $^{15}\text{N}$, $^{2}\text{H}$, $^{18}\text{O}$

   Advantage of Stable Isotopes: They are easy separated and distinguished in the Mass Spec.

   Approach: An internal comparison within one Mass Spec run. Different samples can be “labeled” with different isotopes.

   Advantages: Precision of quantitation, less susceptible to artifacts in Mass Spec runs.

   Limitations: Cost of isotopes. Limited number of isotope combinations are feasible.

2. Label-free Quantitation – No isotopes used.
Please, Consider the Following:

Isotopes of Carbon

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Mass</th>
<th>Abundance in Nature</th>
<th>Half-life</th>
<th>Radioactivity release</th>
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<tbody>
<tr>
<td>$^{12}\text{C}$</td>
<td>12 exactly</td>
<td>98.9%</td>
<td>Stable</td>
<td>None</td>
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<tr>
<td>$^{13}\text{C}$</td>
<td>13.003</td>
<td>1.07%</td>
<td>Stable</td>
<td>None</td>
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<tr>
<td>$^{14}\text{C}$</td>
<td>14.003</td>
<td>Trace</td>
<td>5,700 years</td>
<td>$\beta$ particle</td>
</tr>
<tr>
<td>$^{11}\text{C}$</td>
<td>11.011</td>
<td>Non-natural</td>
<td>20 min</td>
<td>positron</td>
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</tbody>
</table>

Commonly used in Mass Spectrometry for Quantitative Measurements

DO NOT USE IN MASS SPEC.