## 2D Gel Electrophoresis

### Principle:

- The method resolves proteins in a protein cocktail in the form of a two-dimensional protein map based on their size and charge.

### Objective:

- First dimension: Separation according to proteins isoelectric points (pI)
- Second dimension: Separation according to molecular weight by SDS PAGE

### Procedure:

- Proteins are separated by their intrinsic charges in a solution which solubulizes, denatures and dissociates all the polypeptide chains leaving the intrinsic charges unchanged.
    - This solution contains an uncharged detergent, β-mercaptoethanol and denaturing reagent urea.

- Next isoelectric focusing is used to separate polypeptide chains.
    - Isoelectric point (pI) of a protein is the pH at which the protein has a net charge equal to zero. A protein does not move in an electric field when it is at its isoelectric point.

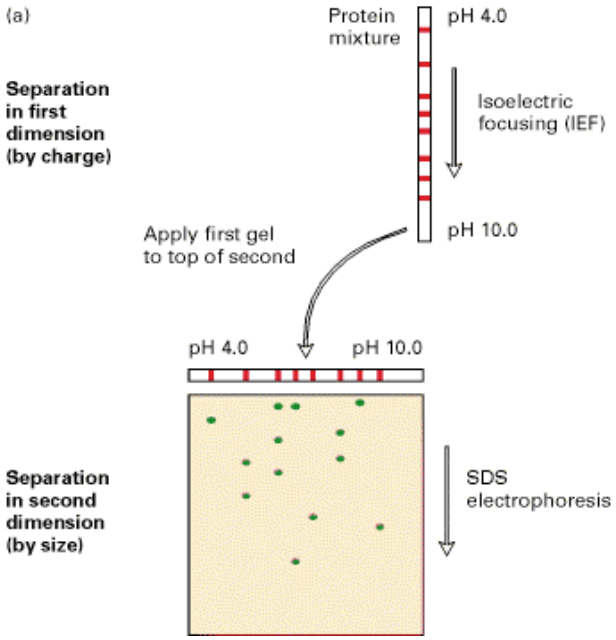


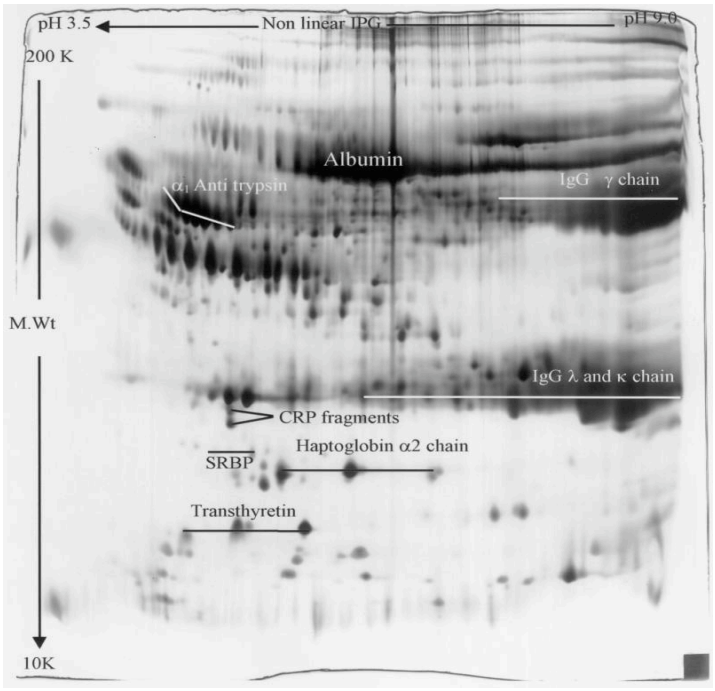

Figure 3-42. Lodish et al. Molecular Cell Biology.

    - The polypeptides are separated electrophoretically in polyacrylamide gel which as a pH gradient.
    - First dimension of the 2D gel electrophoresis is established by the movement of each protein to a position, which corresponds to its isoelectric point in
    - a narrow tube.

- This narrow tube is again subjected to electrophoresis in a direction, which is at right angle to the direction that has been used for isolectric focusing.
    - In the second electrophoresis SDS is used to separate the proteins according to their sizes.
    - The second dimension is established by the migration of the separated protein to its discrete spot on the gel.

- After fractionation a specific protein can be identified on the gel exposing the separated proteins to a specific antibody, which has been coupled to a label (radioactive isotope, a detectable enzyme or fluorescent dye).

**Example:**
From Smith et al., Clinical and Diagnostic Laboratory Immunology **8** (2001), p. 105-111




The proteins of synovial fluid form a patient was subjected to 2D gel electrophoresis. This figure shows the entire gel which were visualized by silver staining. On such a gel around 300 individual proteins with masses ranging from 200 KDa to 10 KDa and isoelectric points between 3.5 and 9.0 can be resolved. Note that in this example albumin and immunoglobulins were the most abundant proteins.

## References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  Dowling, John E. Neurons and Networks 2nd Edition (2001). The Belknap Press of Harward University Press, USA.
- Smith, Marjorie A., Bains, Satbinder K., Betts, Joanna C., Choy, Betts Ernest H.S., Zanders, Edward D. Use of Two-Dimensional Gel Electrophoresis To Measure Changes in Synovial Fluid Proteins from Patients with Rheumatoid Arthritis Treated with Antibody to CD4. 2001. Clinical and Diagnostic Laboratory Immunology 8 (2001), p. 105-111
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