Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2017

Lecture 23:

Some More Variations on Electrophoresis and Introduction to Chromatography

4 April 2017

University of Utah
goldenberg@biology.utah.edu

Public Service Announcement

American Chemical Society Student Chapter
TA of the Semester Award

`https://chem.utah.edu/undergraduate/ta-nomination-form.php`

- Winner gets fame and glory, a $25 gift certificate **and** is eligible for TA of the year award ($1,000!)
- Anyone in Biol 3515 or Chem 3515 can nominate their TA.
- “Please describe how this TA has enriched your experience in chemistry. Please be as specific as possible. The winner will be chosen based on the nomination statements, not a popular vote.”
- Last day for nominations: Thursday, 13 April

Methods for Detecting Proteins in Gels

- To detect all proteins:
  - Staining with coomassie blue:
    Dye binds to proteins and excess is washed away
    Sensitivity: - $\approx$ 0.1–1 μg/band
  - "Silver staining":
    Based on reduction of silver ions to metallic silver in vicinity of protein.
    Much more sensitive than coomassie blue; as little as 10 ng/band
    Messy, finicky and sensitive to artifacts.
  - Staining with fluorescent dyes
    Dye fluorescence is enhanced when bound to proteins
    Much more sensitive than coomassie blue
  - Other dyes and metal ions.
- To detect specific protein classes:
  - Specific dyes for phosphoproteins
  - Specific dyes for glycoproteins

Steinberg, T. H. (2009). Protein gel staining methods: An introduction and overview. *Methods Enzymol.*, 463, 541–563. http://dx.doi.org/10.1016/S0076-6879(09)63031-7

Detection of Gel-fractionated Proteins with Antibodies: Western Blotting

- Allows selective detection of specific proteins: Requires specific antibodies.
- After electrophoresis, proteins are electrophoresed out of the gel and onto a membrane (usually nitrocellulose or polyvinylidene difluoride)

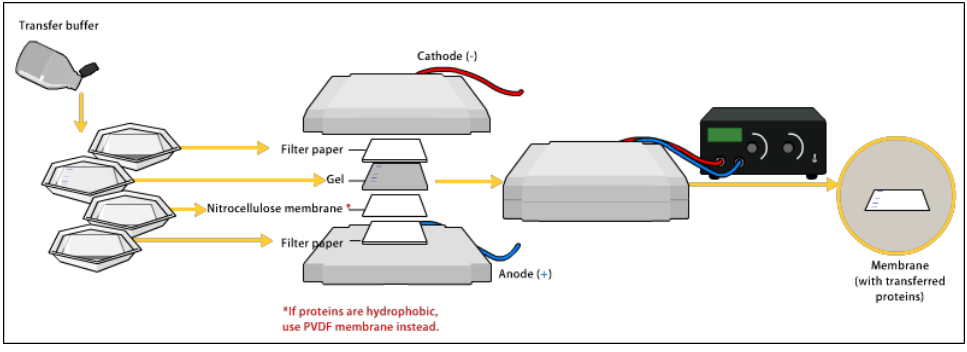
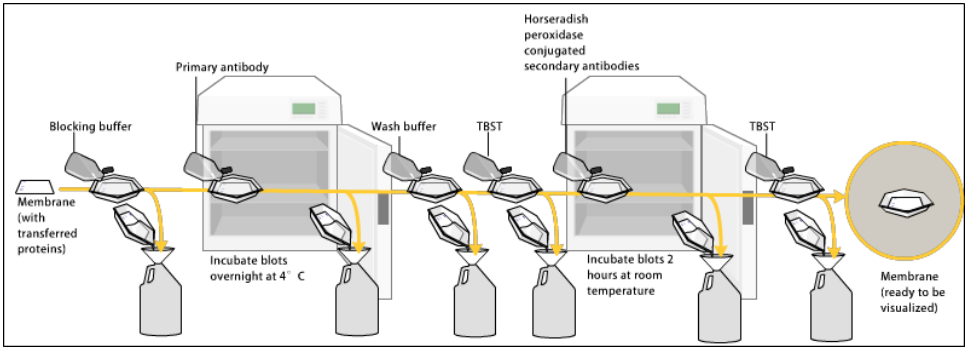


Illustration from `https://en.wikipedia.org/wiki/Western_blot`

Detection of Gel-fractionated Proteins with Antibodies: Western Blotting

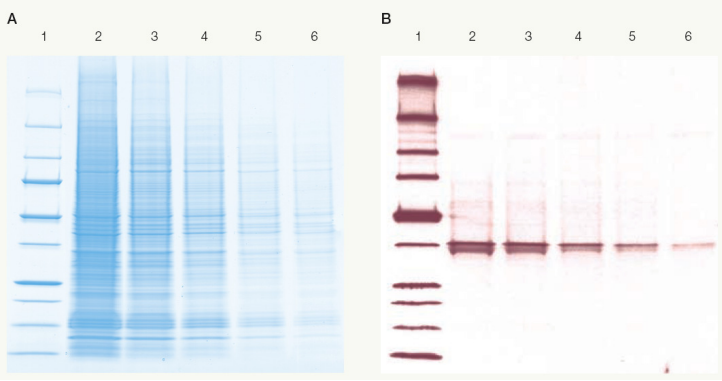
- Multistep treatment of membrane with bound proteins.



  - Generic protein solution to block all protein binding sites.
  - “Primary” antibody specific for protein of interest.
  - “Secondary” antibody, binds to primary antibody and is labeled for detection. Radioactive label or enzyme that generates colored precipitate or chemiluminescence.

Illustration from `https://en.wikipedia.org/wiki/Western_blot`

Western Blot Example



Coomassie blue Western blot

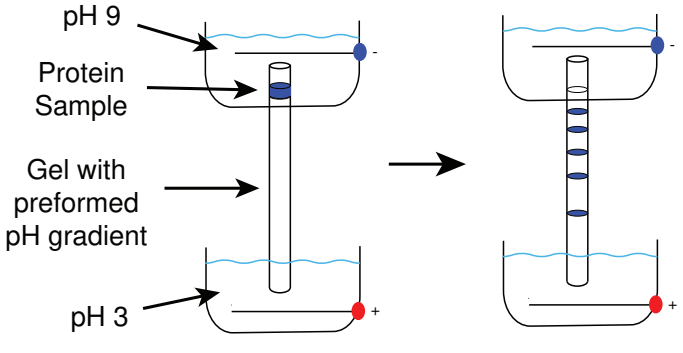
- Sample: HeLa cell lysate, at different dilutions.
- Antibody: Specific for human CDK7 protein. (cyclin-dependent kinase)

Illustration from Bio-Rad Bulletin 2032 Rev. E,
www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_2032.pdf

A Bit More About Western Blots

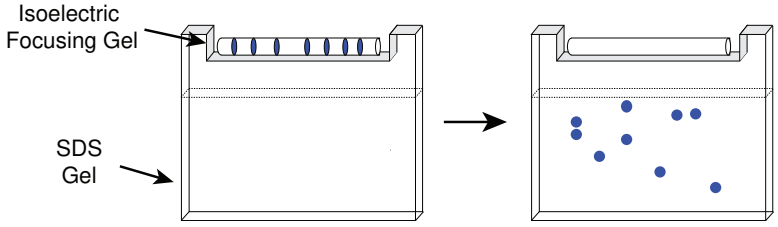
- Applications in clinical diagnostics: High sensitivity and specificity
  Patient serum is used as primary antibody.
  - HIV infection
  - Lime disease
  - Hepatits
  - Other viral and bacterial infections
- Where does the name come from?
  - 1975: DNA blotted from gels and detected by hybridization to radiolabeled DNA. Invented by Edwin Southern.
    https://doi.org/10.1016/S0022-2836(75)80083-0
  - 1977: RNA blotted from gels and detected by hybridization to radiolabeled DNA. Invented by James C. Alwine, David J. Kemp, And George R. Stark and nicknamed by others “northern blotting”.
    https://dx.doi.org/10.1073%2Fpnas.74.12.5350
  - 1981: Proteins plotted from gels and detected by antibody binding. Invented by W.Neal Burnette, who called it “western blotting”.
    https://doi.org/10.1016/0003-2697(81)90281-5

Another Electrophoresis Method: Isoelectric Focusing (IEF



- pH gradient is formed by mixtures of buffering compounds incorporated in the gel.
- Proteins migrate to point where they have no net charge, and then stop.
- Proteins are separated by isoelectric point.
- Usually performed in the absence of detergent, but often with urea present to unfold proteins.

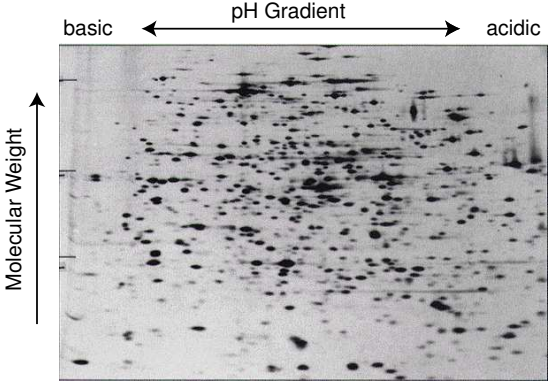
Two-dimensional Gel Electrophoresis



- Isoelectric point and chain molecular weight are largely independent properties.
- Greatly increases ability to resolve proteins in complex mixtures.
- Simultaneously provides information about molecular weight and isoelectric point.
- Other variations are possible (*e.g.,* native gel/SDS gel)

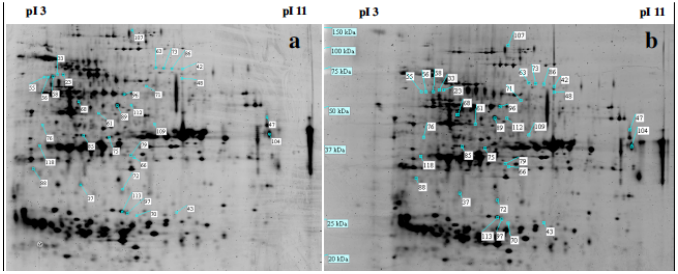
O'Farrell, P. H. (1975). High resolution two dimensional electrophoresis of proteins. *J. Biol. Chem..*, 250, 4007–4021. http://www.jbc.org/content/250/10/4007.abstract

A 2-dimensional Gel of Bacterial Proteins



2-dimensional Gel Electrophoresis in Proteomics

- Proteomics: A (sort of) new discipline focused on analysis of protein compositions of cell, tissues and organisms under different physiological, developmental or environmental conditions. One of many new “omics”!
- An example using 2-d gel electrophoresis:

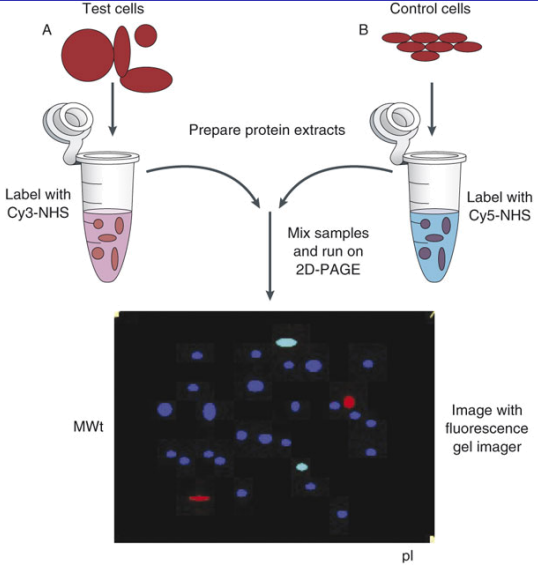


Comparison of cultured tissue (callus) of vanilla orchid under different conditions.

- Spots in gel can be excised and chemically analyzed to identify proteins: Protease digestion, mass spectrometry, sequencing

Palama, T. L., Menard, P., Fock, I., Choi, Y. H., Bourdon, E., Govinden-Soulange, J., Bahut, M., Payet, B., Verpoorte, R. & Kodja, H. (2010). *BMC Plant Biology*, 10, 82.

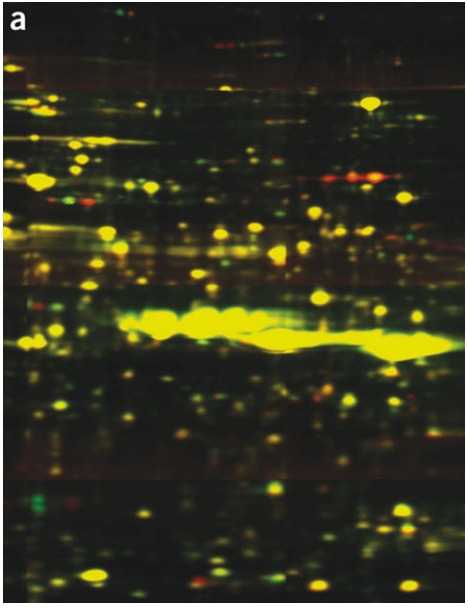
Differential Fluorescent Labeling of Proteins for 2-d Gel Analysis



- 2-d gels are difficult to reproduce precisely.
- Proteins from different samples are labeled with different fluorescent reagents.
- Reagents are matched to have same mass and electric charge.
- Samples are mixed and run on a single 2-d gel.
- Proteins from different samples are distinguished by different colors.

Viswanathan, S., Ŭniŭ, M. & Minden, J. S. (2006). Two-dimensional difference gel electrophoresis. *Nature Protocols*, 1, 1351–1358. http://dx.doi.org/10.1038/nprot.2006.234

An Example of Differential 2-d Gel Analysis



- *Drosophila melanogaster* proteins from different mutants during development, labeled with red and blue fluorescent dyes.
- Yellow indicates proteins present at same levels in both strains.
- Red or green indicates proteins present at higher levels in one or the other strain.

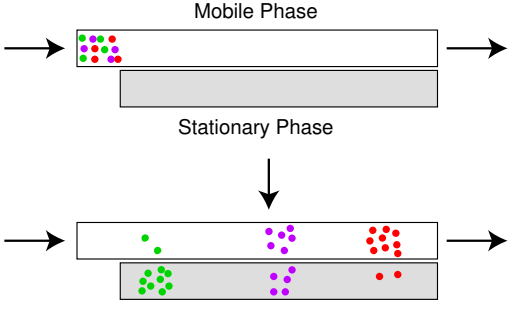
Viswanathan, S., Ŭniŭ, M. & Minden, J. S. (2006). Two-dimensional difference gel electrophoresis. *Nature Protocols*, 1, 1351–1358. http://dx.doi.org/10.1038/nprot.2006.234

Warning!

Direction Change

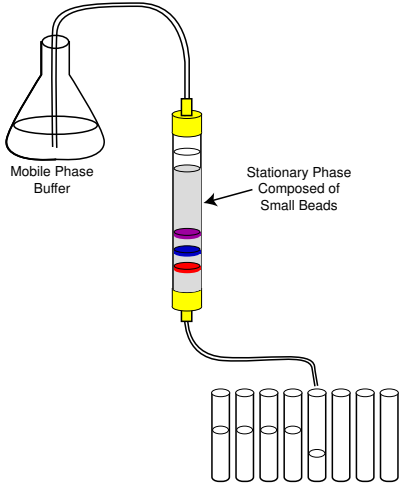
Chromatography

General Description of Chromatography



- Stationary and mobile phases have distinct chemical properties.
- Molecules partition (thermodynamic equilibrium) between two phases.
- Molecules that partition preferentially into mobile phase move more rapidly than molecules that prefer the stationary phase.

Column Chromatography:
The Most Commonly Used Format for Biochemistry



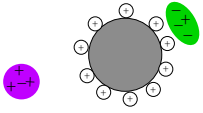
- Sample is placed at top of column.
- Buffer flows through and around beads in stationary phase and caries sample molecules.
- Molecules that interact the least with the beads elute from the column first.
- Eluent is fractionated into tubes as it leaves the column. (automatic fraction collector)
- Eluent can be analyzed as it leaves the column (*e.g.,* spectrophotometer with flow cell) or from fractions.

Chromatography Methods Commonly Used for Biomolecules

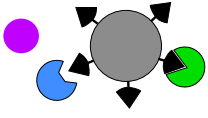
- Gel filtration chromatography - based on molecular size

Form that we will use in lab.

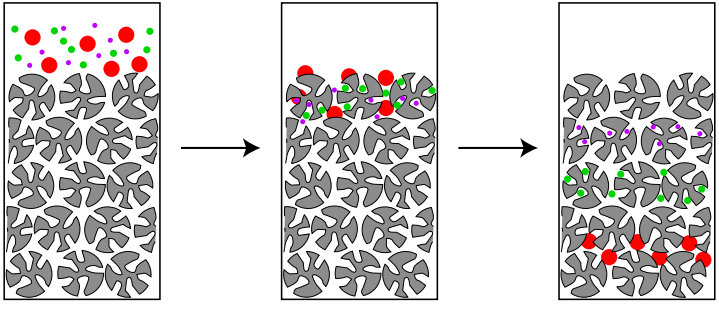
- Ion exchange chromatography - based on electric charge



- Affinity chromatography - based on specific biochemical interactions

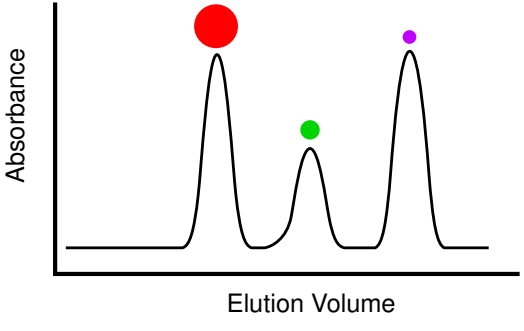


Gel Filtration Chromatography



- Also called “gel permeation”, “size exclusion”, “sizing” or “molecular sieve” chromatography.
- Beads are made of a porous gel. (similar to gels used for electrophoresis)
- Separates molecules on basis of size.
- Larger proteins elute first. (opposite of gel electrophoresis!)
- Beads have a distribution of pore sizes.

Elution Profile for a Gel Filtration Column



- Elution volume is the volume of buffer that flows through the column between when the sample is applied to the top and when a particular protein leaves the column.
- Can be calibrated with proteins of known size in order to estimate molecular weights of other proteins.
- Unless a denaturant is present, elution volume usually reflects molecular weight of native protein, with quaternary and tertiary structure intact, unlike SDS gel electrophoresis.
- Media with different distributions of pore sizes are used to separate molecules of different size ranges.