Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2023

Lecture 28:

The Ultracentrifuge, Crystallography and Some History

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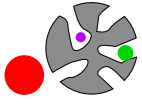
- Calder Lake
- Leon Guerra
- Erik Smith
- Juli Kim
- Adam Rupper

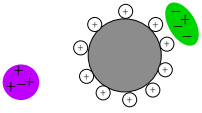
Chromatography Methods Commonly Used for Biomolecules

- Gel filtration chromatography - based on molecular size

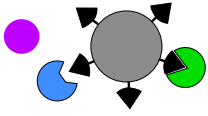


Form that we are using in lab.

- Ion exchange chromatography - based on electric charge



- Affinity chromatography - based on specific biochemical interactions



Clicker Question #1

Suppose that you want to separate the two forms of an enzyme encoded by very closely related genes in the same organism. Which chromatography method might be especially useful?

A) Gel filtration

B) Ion exchange

C) Affinity

Clicker Question #2

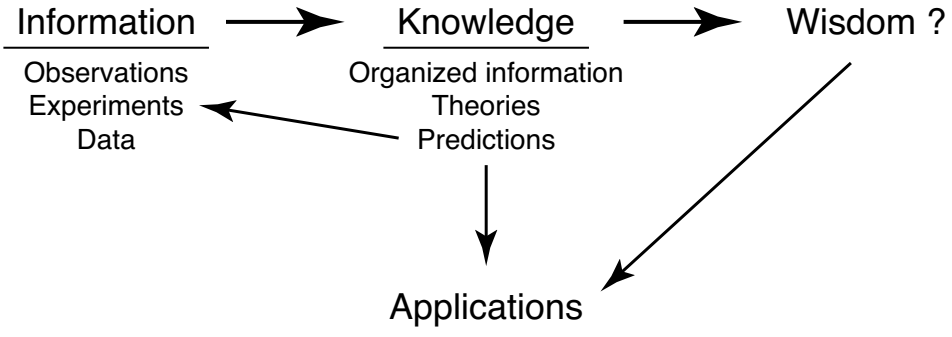
Suppose that you want to separate an enzyme from a DNA-binding protein. Which chromatography method might be especially useful?

A) Gel filtration

B) Ion exchange

C) Affinity

How do we know? What do we do with it?



Forget enlightenment, I want you to concentrate on the structure of the protein molecule.

Sidney Harris, New Yorker Magazine

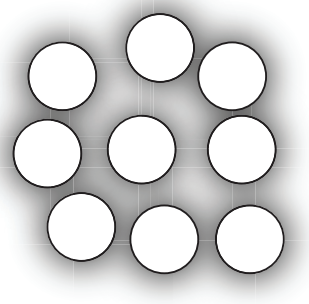
A Major Scientific Question in the 1920s and 30s

What is the nature of proteins?

Molecules?

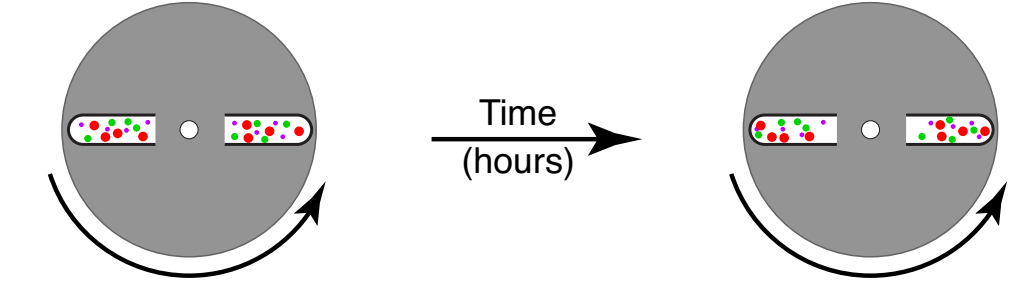
Unique molecular weight.

Colloids?



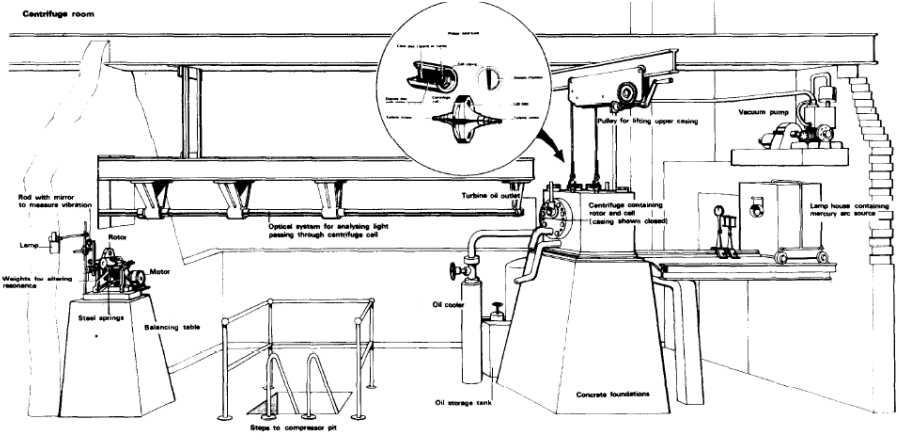
Distribution of molecular weights.

Another Important Separation Method: Centrifugation



- "Centrifugal force" moves molecules outward from the center of the rotor.
- Rate of motion depends on magnitude of centrifugal force and friction between molecules and solvent.
- Larger molecules move faster than smaller ones, allowing them to be separated. (Shape also has an effect.)
- An ultracentrifuge: A centrifuge capable of separating ultra-small particles. Invented by Theodor (The) Svedberg in the 1920s and 30s.

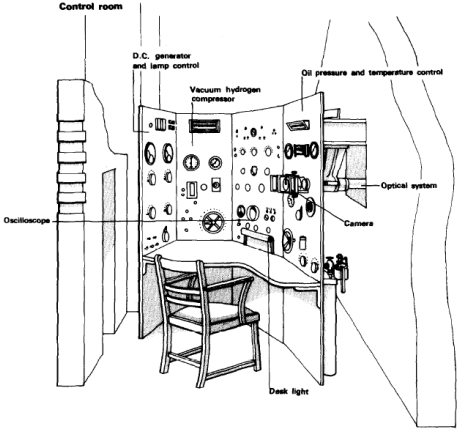
A Svedberg Ultracentrifuge



Centrifuge room

Ogston, A. G. (1977). Life with a Svedberg ultracentrifuge. *Trends Biochem. Sci*, 2, N208–N210. http://dx.doi.org/10.1016/0968-0004(77)90200-6

A Svedberg Ultracentrifuge



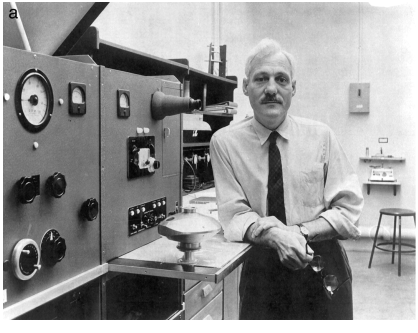
Control room

Ogston, A. G. (1977). Life with a Svedberg ultracentrifuge. *Trends Biochem. Sci*, 2, N208–N210. http://dx.doi.org/10.1016/0968-0004(77)90200-6

Svedberg Centrifuge in the London Science Museum

Link to copyrighted photograph shown in class:
http://www.gettyimages.com/detail/news-photo/svedberg-ultra-centrifuge-1936-with-optical-system-and-news-photo/90738030

Evolution of the Analytical Ultracentrifuge



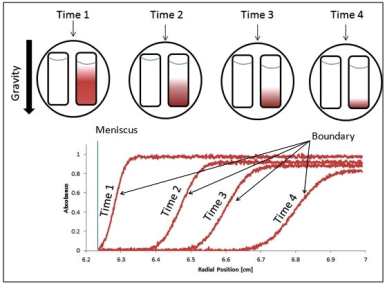
- Spinco (later Beckman) Model E Ultracentrifuge (1950s-70s)
- Jerome Vinograd – Applied ultracentrifuge to analysis of DNA molecules.

Proc. Natl. Acad Sci., USA, 101, 17889–17894.
http://dx.doi.org/10.1073/pnas.0407539101

- Beckman Optima AUC (current model)
- Based on much less expensive models designed for preparative separations.

https://www.beckman.com/centrifuges/analytical-ultracentrifuges

Sedimentation of a Protein Sample



- Sedimentation monitored by UV absorbance.
- "Top" of the sample (closest to rotor center) is depleted as molecules move out.
- Boundary forms and moves outward.
- At relatively low rotor speeds, molecules reach an equilibrium distribution that reflects molecular weight, independent of friction.

Svedberg's big discovery:

- Proteins (of a given type) behave as homogeneous species with discrete molecular weights.
- Contradicted prevailing view in the 1920s that proteins were "colloids", or non-specific aggregates.
- Helped lay the foundation for molecular and structural biology.

Diagram from: https://www.coriolis-pharma.com/analytical-services/aggregate-analytics/analytical-ultracentrifugation-sv-auc-se-auc

Another Landmark Experiment:
First X-ray Diffraction from a Protein Crystal, 1934

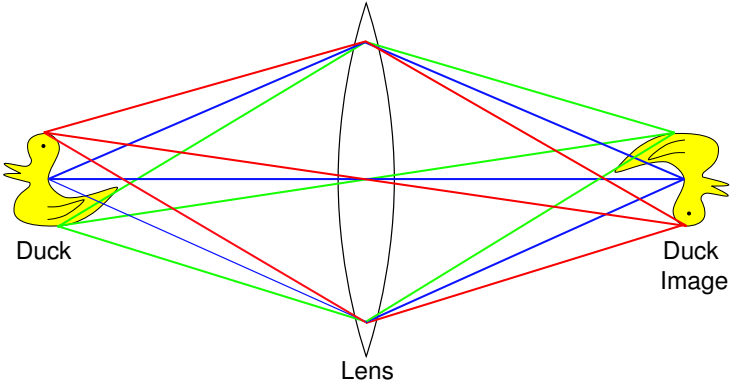
John Desmond Bernal

Dorothy Crowfoot Hodgkin

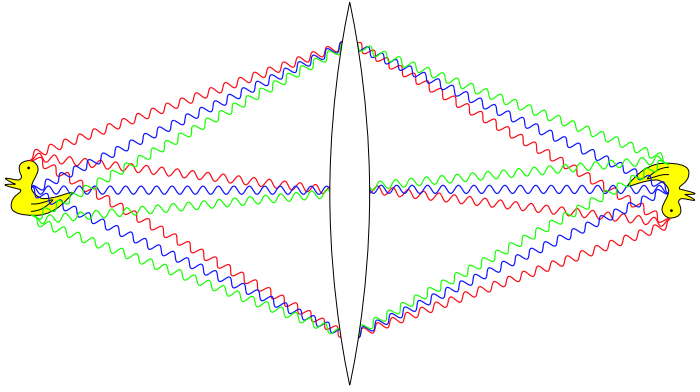
Nobel Prize in Chemistry, 1964

- Ability to generate a diffraction pattern demonstrates that the molecules in a crystal are (nearly) identical in three-dimensional structure.
- Suggested that detailed structures of proteins could be determined by analysis of the diffraction pattern, but how to do it?

Image Formation with a Lens



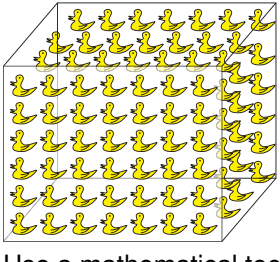
Imaging With a Lens - a Wave Interpretation



- Image is formed at points where waves are brought back in phase.
- Points in the object must be separated by at least $\sim 1/2$ wavelength to give rise to separate points in the image.
- Determining molecular structures at atomic resolution requires very short wavelengths: X-rays (or electrons or neutrons).

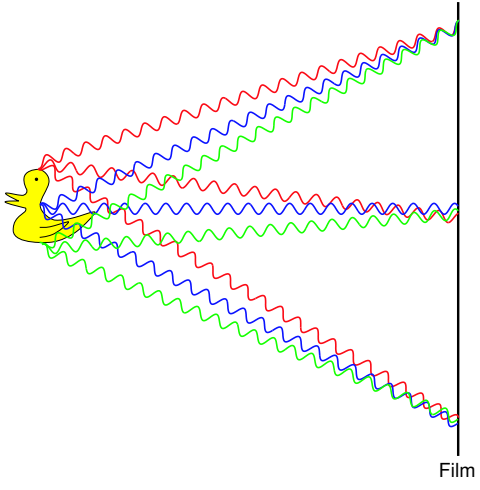
Why Not an X-Ray Microscope?

- Scattering from individual atoms is very weak, especially from elements with low atomic numbers.
- Very difficult to make lenses for X-rays.
- In crystallography:
  - Use crystals to increase the total scattering intensity.

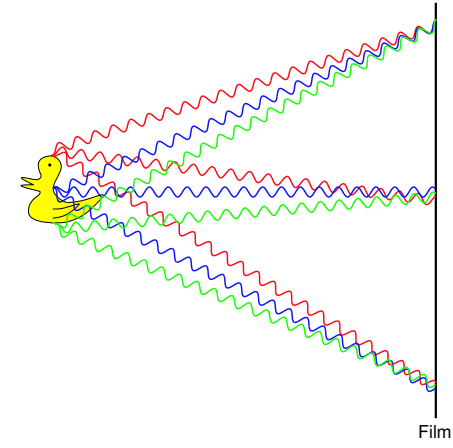


  - Use a mathematical technique, the Fourier transform, to do the job of a lens.

Diffraction from a Duck



The Phase Problem



- Information about the phases of waves is lost when the pattern is recorded.
- This information is essential to calculating the structure.

Methods for solving the phase problem:

- Make a guess at the structure, calculate predicted diffraction pattern and compare. Method used for first structures of salt crystals and small molecules.
- Multiple isomorphous replacement: Modify the crystal by introducing heavy metal ions. Differences between diffraction patterns can be used to calculate phases.
  Most robust and general method for proteins and other large structures.
- Use a structure of a closely related protein to calculate initial phases.
- Other, more exotic computational methods.

First Atomic-resolution Structures of Globular Proteins
Myoglobin and Hemoglobin

Max Perutz and John Kendrew

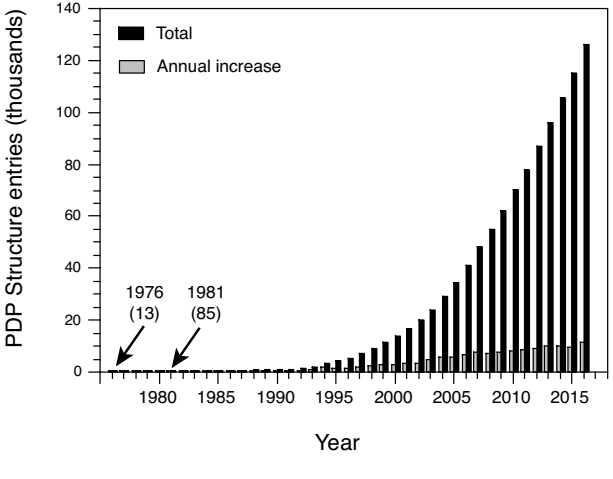
Nobel Prize in Chemistry, 1962.

The Chymotrypsin Group, 1966

Left to right: Jill Collard, Dana Singleton, Paul Siglar, Brian Matthews, David Blow, Sue Simpson, Sue Wickham.

Henderson, R. & Franks, N. P. (2009). David Mervyn Blow. 27 June 1931 – 8 June 2004. *Biogr. Mems. Fell. R. Soc.*, 55, 13–35.
http://dx.doi.org/10.1098/rsbm.2008.0022

The Protein Data Bank Since 1976



What happened in the 1990s?

- Genetic engineering:
  Ability to make large amounts of many proteins.
- Synchrotron X-ray sources:
  Much faster data collection.
- Bigger and faster computers.