Physical Principles in Biology

Biology 3550

Spring 2024

Lecture 39

Rotary Motors: ATP Synthase
and
Cryo Electron Microscopy

Friday, 19 April 2024

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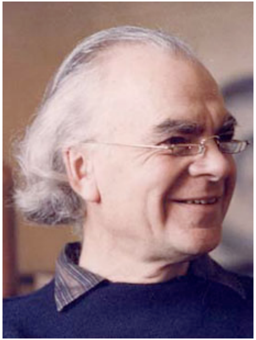
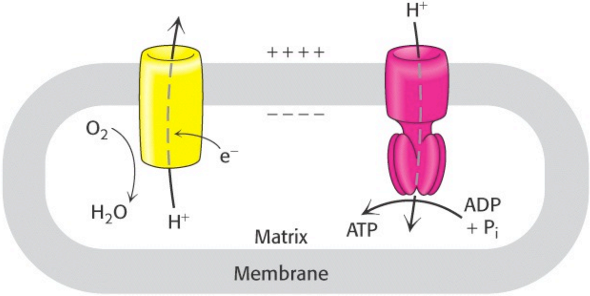
Announcements

- Problem Set 6:
  - Due Monday, 29 April at 11:59 PM
  - Submit pdf file on Gradescope
- Office hours for Tuesday, 23 April:
  - Time changed from 9:00-10:00, to 10:00-11:00 AM (Zoom)
- Final Exam:
  - Thursday, 25 April, 8:00 -10:00 AM
  - HEB 2002

Peter Mitchell and the Chemiosmotic Hypothesis

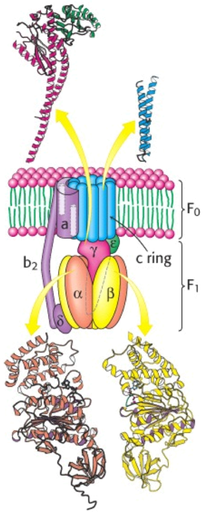




From Berg, Tymoczko & Stryer, Biochemistry, 5th Ed.

- Mitchell, P. (1961). Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature*, 191, 144–148. http://dx.doi.org/10.1038/191144a0
- Nobel Prize in Chemistry, 1978

Proton-Driven ATP Synthase



- F_O component is embedded in membrane.
- F_1 component is in the interior of mitochondria, chloroplasts or bacteria.
- F_1 is held stationary by b2 and δ subunits.
- ATP synthesis (or hydrolysis) is catalyzed by β subunits of F_1 .
- H^+ ions pass through the c ring and a subunit of F_O .
- c ring and γ subunits rotate.

Figure from Berg JM, *et al.* Biochemistry. 5th edition. New York: W H Freeman; 2002. https://www.ncbi.nlm.nih.gov/books/NBK22388/

F1 ATP Synthase as a Motor

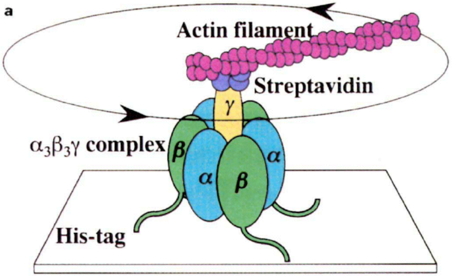


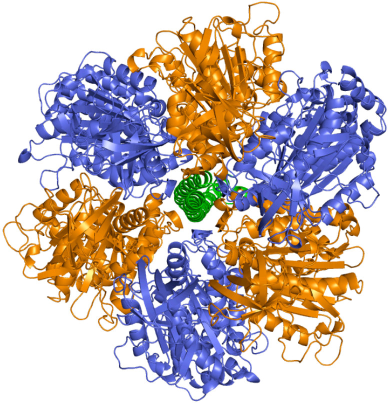
Figure from Berg JM, *et al.* Biochemistry. 5th edition. New York: W H Freeman; 2002.

(ATPase Motor Movie)

Noji, H., Yasuda, R., Yoshida, M. & Kinosita, K. (1997). Direct observation of the rotation of F_1-ATPase. *Nature*, 386, 299–302. http://dx.doi.org/10.1038/386299a0

Movie: http://www.k2.phys.waseda.ac.jp/F1movies/F1Prop.htm

Crystal Structure of the F_1 ATP Synthase



- α-subunits (blue) and β-subunits (orange) are closely related.
- Catalytic sites are shared between the the α- and β-subunits, but mostly are in the β-subunits.
- The 3 β-subunits have distinct conformations.

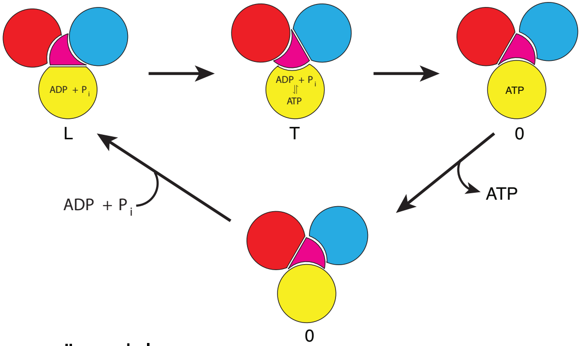
Abrahams, J., Leslie, A., Lutter, R. & Walker, J. (1994). Structure at 2.8 Å resolution of F_1-ATPase from bovine heart mitochondria. *Nature*, 370, 621–628. http://dx.doi.org/10.1038/370621a0

Paul D. Boyer, 1918–2018

- Born and raised in Provo, UT.
- B.S. in chemistry from BYU.
- Ph.D. from University of Wisconsin.
- Many contributions to enzymology, especially the use of isotopes.
- Founding Director of the Molecular Biology Institute at UCLA.
- Shared 1997 Nobel Prize in Chemistry with John Walker and Jens Sko.

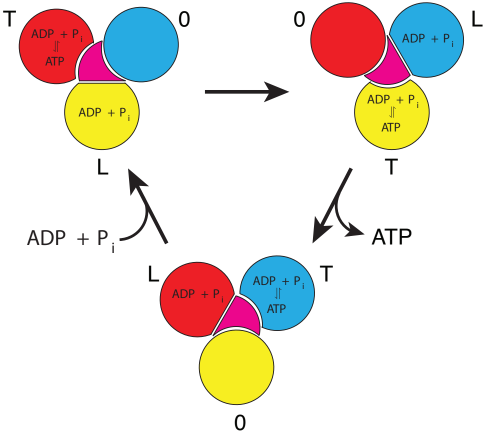
Photograph from UCLA, via:
Kresge, N., Simoni, R. D. & Hill, R. L. (2006). ATP Synthesis and the binding change mechanism: The work of Paul D. Boyer. *J. Biol. Chem.*, 281, E18–E20.
https://doi.org/10.1016/S0021-9258(20)55940-1

Coupling of Motion and ATP Synthesis



- Boyer “binding-change” model.
- Three β-subunits cycle through three conformations:
  - Loose: Binds ADP + P_i, without catalysis.
  - Tight: Promotes reaction, but binds ADP, P_i and ATP very tightly.
  - Open: Releases or binds ATP or ADP + P_i.

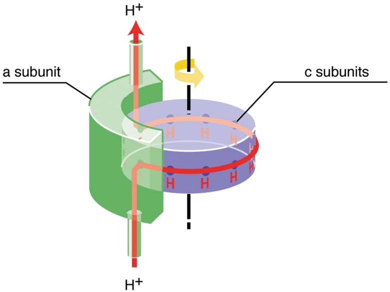
Coupling of Motion and ATP Synthesis

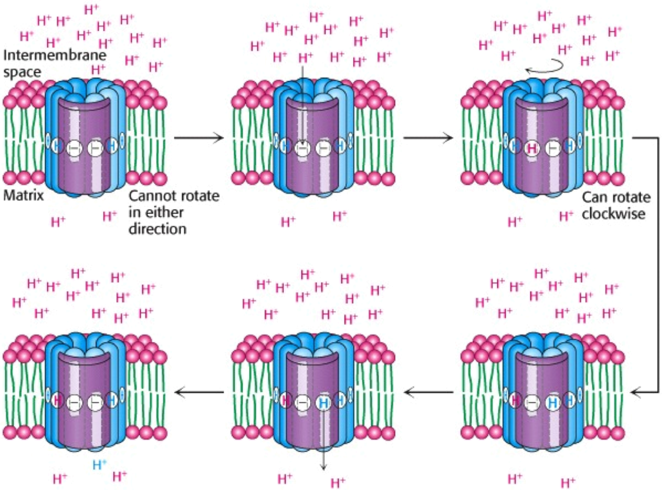


Molecular animations:
https://www.mrc-mbu.cam.ac.uk/research-groups/walker-group/molecular-animations-atp-synthase

Boyer, P., Cross, R. & Momsen, W. (1973). *Proc. Natl. Acad. Sci. USA*, 70, 2837–2839.
http://dx.doi.org/10.1073/pnas.70.10.2837
Figures adapted from Berg JM, *et al.* Biochemistry. 5th edition. New York: W H Freeman; 2002.

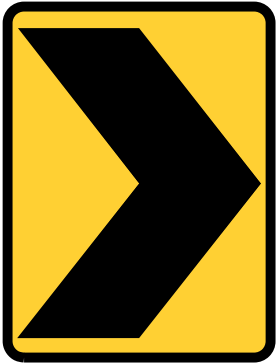
Coupling of H^+ Translocation and Rotation





Left figure from Walker, J. E. (2013). *Biochem. Soc. Trans.*, 41, 1–16. http://dx.doi.org/10.1042/BST20110773
Right figure from Berg JM, *et al.* Biochemistry. 5th edition. New York: W H Freeman; 2002.

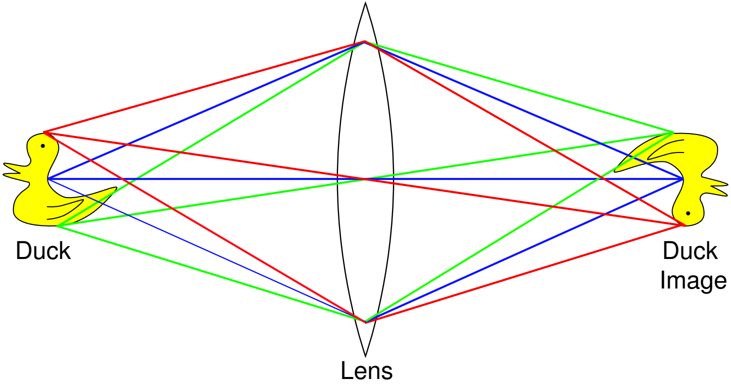
Warning!



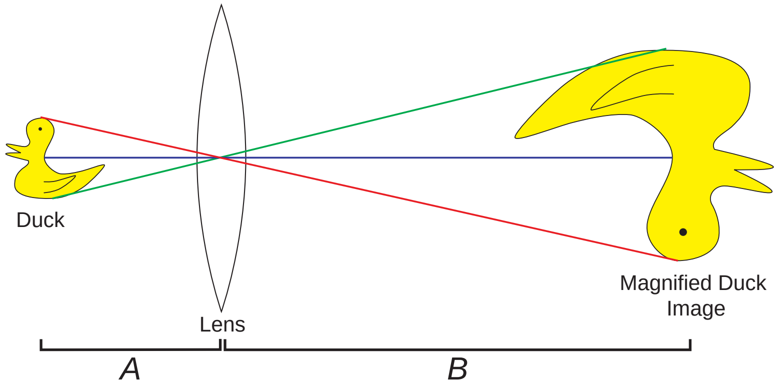
Direction Change

Cryo Electron Microscopy

Image Formation with a Lens



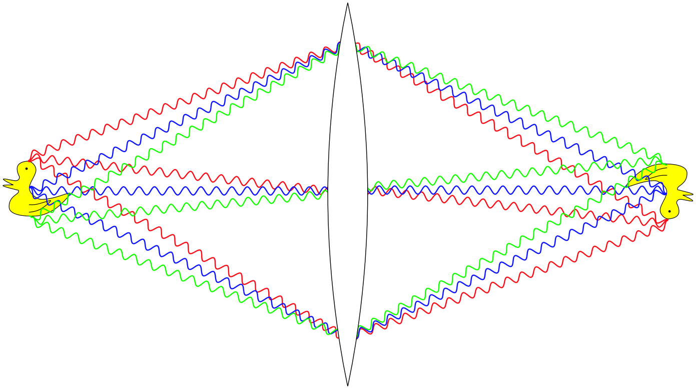
Optical Magnification



As the object is brought closer to the lens:

- Image moves further from the lens and becomes larger.
- Magnification $= B/A$
- Magnification, in principle, is not limited, but resolution is.

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.

The Electromagnetic Spectrum

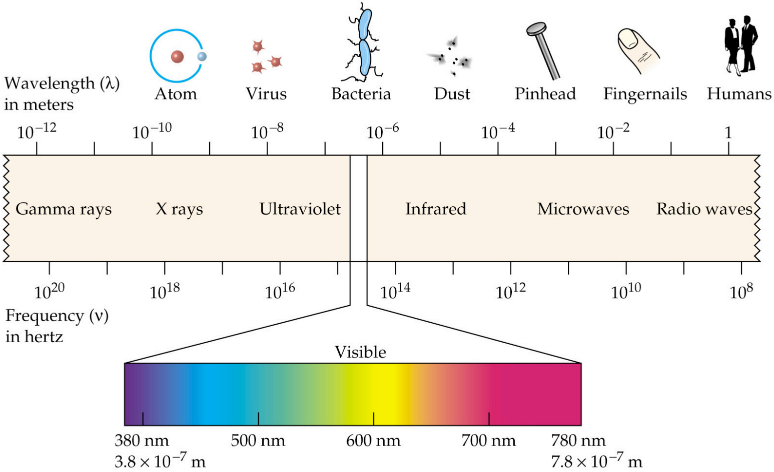
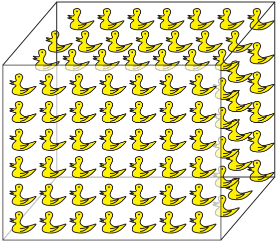


Illustration From: McMurry, J. & Fay, R. (2004). *Chemistry*. Prentice-Hall, 4^{th} edition.

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.

The Transmission Electron Microscope

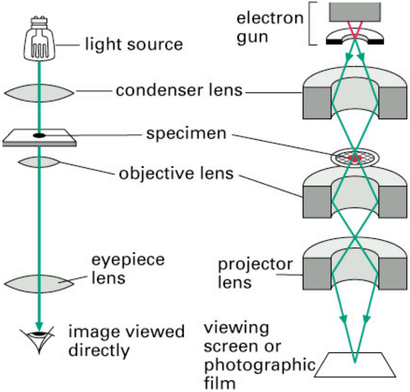


Figure 9–22. Molecular Biology of the Cell, 4th Edition.

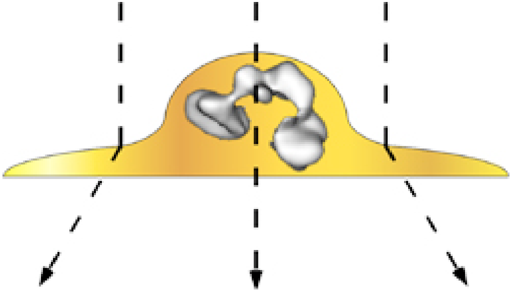
- First electron microscope built by Ernst Ruska in 1930
- 1986 Nobel Prize in Physics

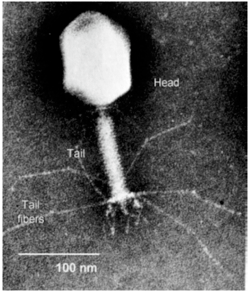
Strengths and Limitations of Electron Microscopy

- Strengths
  - Short wavelengths: $\lambda = 0.025\,\text{Å}$ for 200 kV electron.
  - Electromagnetic lenses can focus electrons and produce images directly.
  - Theoretical resolution $< 1\,\text{Å}$.
- Limitations
  - Vacuum required.
  - Sample damage.
  - Weak signal from light elements.

Negative stain

- Most useful for macromolecular structures such as viruses or protein complexes.
- Particles are embedded in a salt of a heavy metal, *e.g.,* uranyl acetate.

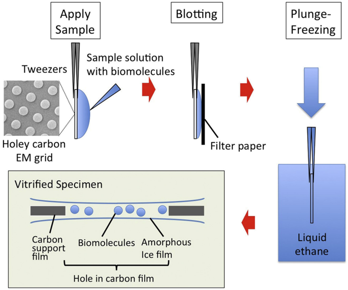


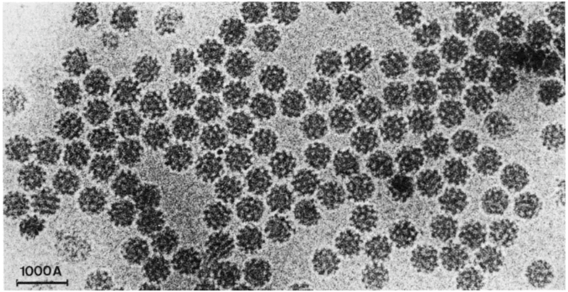


- Image represents a projection of places that are *not* occupied by the protein.
- Relatively high resolution, ≈ 10 Å.
- Only shows surfaces, and structure can be distorted.

Diagram from http://www.snaggledworks.com/em_for_dummies
Electron micrograph of virus particle by by Hans Ackermann.

A Breakthrough: Cryogenic Freezing of Unstained Biological Samples



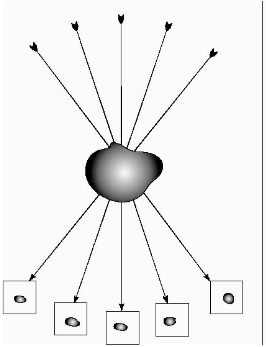


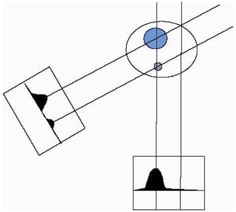
s

- Very rapid freezing - “vitreous ice”
- Low temperature minimizes radiation damage
- Very low contrast

Adrian, M., Dubochet, J., Lepault, J. & McDowall, A. (1984). Cryo-electron microscopy of viruses. *Nature*, 308, 32–36.

Electron Tomography:
3-Dimensional Reconstruction from a Tilt Series

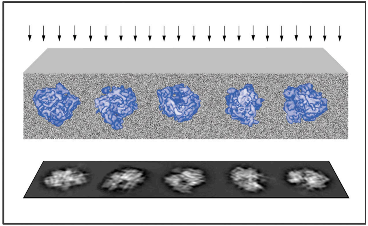


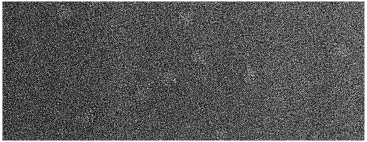


- Each image is like a shadow, *i.e.,* a projection onto two-dimensions.
- 3-dimensional structure is reconstructed from multiple projection views.
- Different views are generated tilting the sample in the microscope.
- Commonly used for cellular structures.
- Similar to an x-ray CAT scan (Computed Axial Tomography)

http://www-vis.lbl.gov/Vignettes/KDowning-Tomo/index.html

Reconstruction from Randomly-Oriented Single Particles

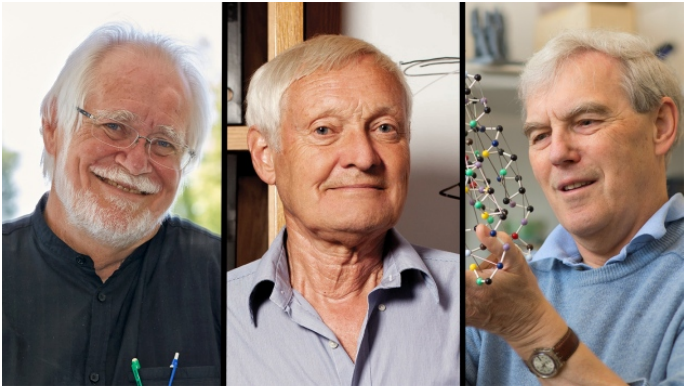




- Low-dose images of 10,000s of individual particles are oriented and averaged.
- Computer program replaces crystallization!

Frank, J. (2002). *Annu. Rev. Biophys. Biomol. Struct.*, 31, 303–319.

2017 Nobel Prize in Chemistry



- Jacque Dubochet
- Joachim Frank
- Richard Henderson

Nature (2017) 550, 167. http://doi.org/10.1038/nature.2017.22738