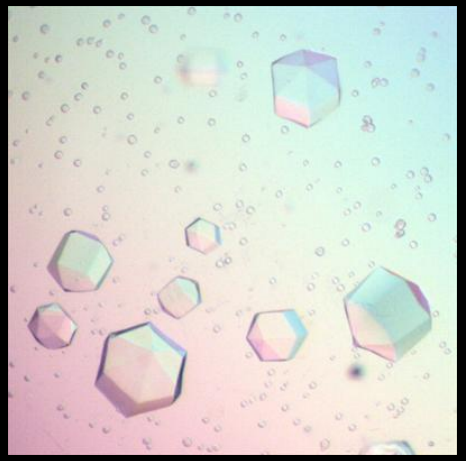
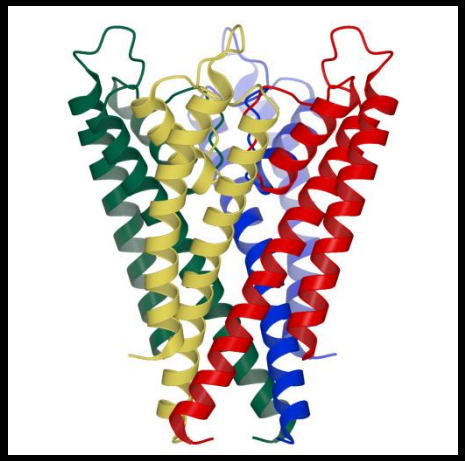
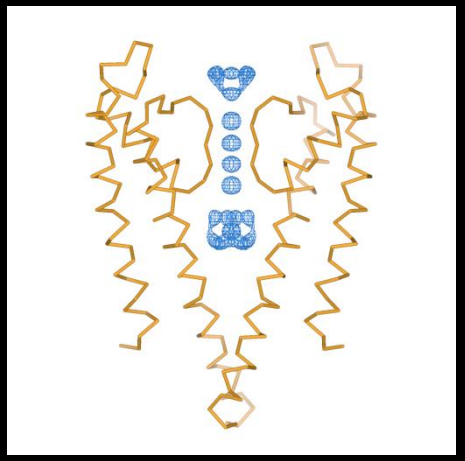




Introduction to X-ray Crystallography

Jesse Rinehart, PhD

Yale Systems Biology Institute

Cellular & Molecular Physiology
Yale University School of Medicine

Than you for contributions to this lecture:

Yong Xiong, PhD
Yale University Department of Molecular Biophysics & Biochemistry

Recommended Course @ Yale: MB&B 720a
Macromolecular Structure and Biophysical Analysis

Additional Resources:

Crystallography Made Crystal Clear: A Guide for Users of Macromolecular Models
by Gale Rhodes (Third Edition, 2006 Elsevier/Academic Press)

CMCC Home Page: http://spdbv.vital-it.ch/TheMolecularLevel/CMCC/index.html

"Crystallography 101" http://www.ruppweb.org/Xray/101index.html

"Introduction to X-ray crystallography" http://vimeo.com/7643687

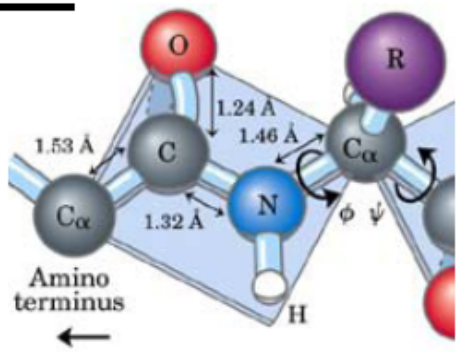
http://ucxray.berkeley.edu/~jamesh/movies/
movies demonstrating diffraction, resolution, data quality, and refinement.

“Just as we see objects around us by interpreting the light reflected from them, x-ray crystallographers "see" molecules by interpreting x-rays diffracted from them.”
- Gale Rhodes

- There's a limit to how small an object can be seen under a light microscope.
- The diffraction limit: you can not image things that are much smaller than the wavelength of the light you are using.
- The wavelength for visible light is measured in hundreds of nanometers, while atoms are separated by distances of the order of 0.1nm, or 1Å.

We need to use x-rays to resolve atomic features.

Distances between atoms are small:
Lab x-ray sources use CuKα radiation. Wavelength = 1.54 Å.
Synchrotron radiation wavelengths in the range 0.5 Å - 2.5 Å.



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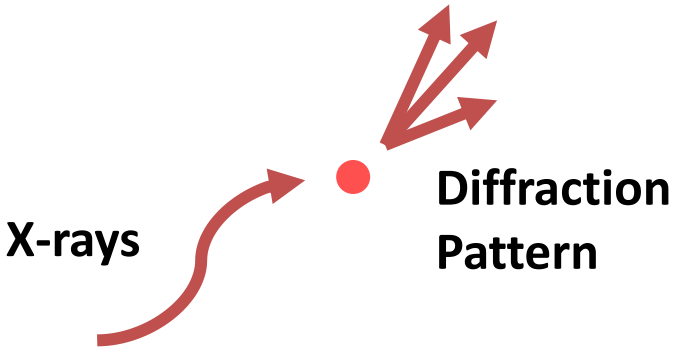
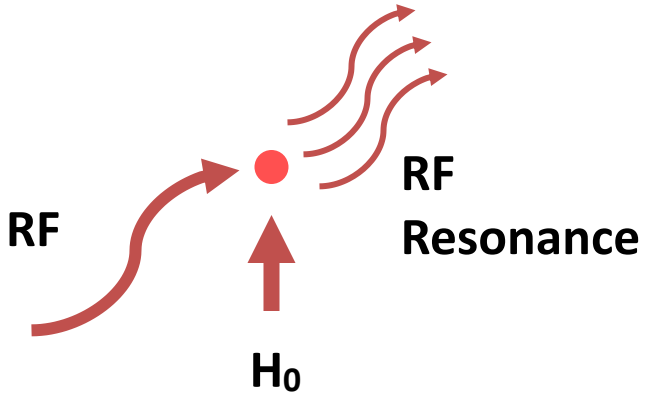
Experimental Determination of Atomic Resolution Structures

X-ray



- Direct detection of atom positions
- Crystals

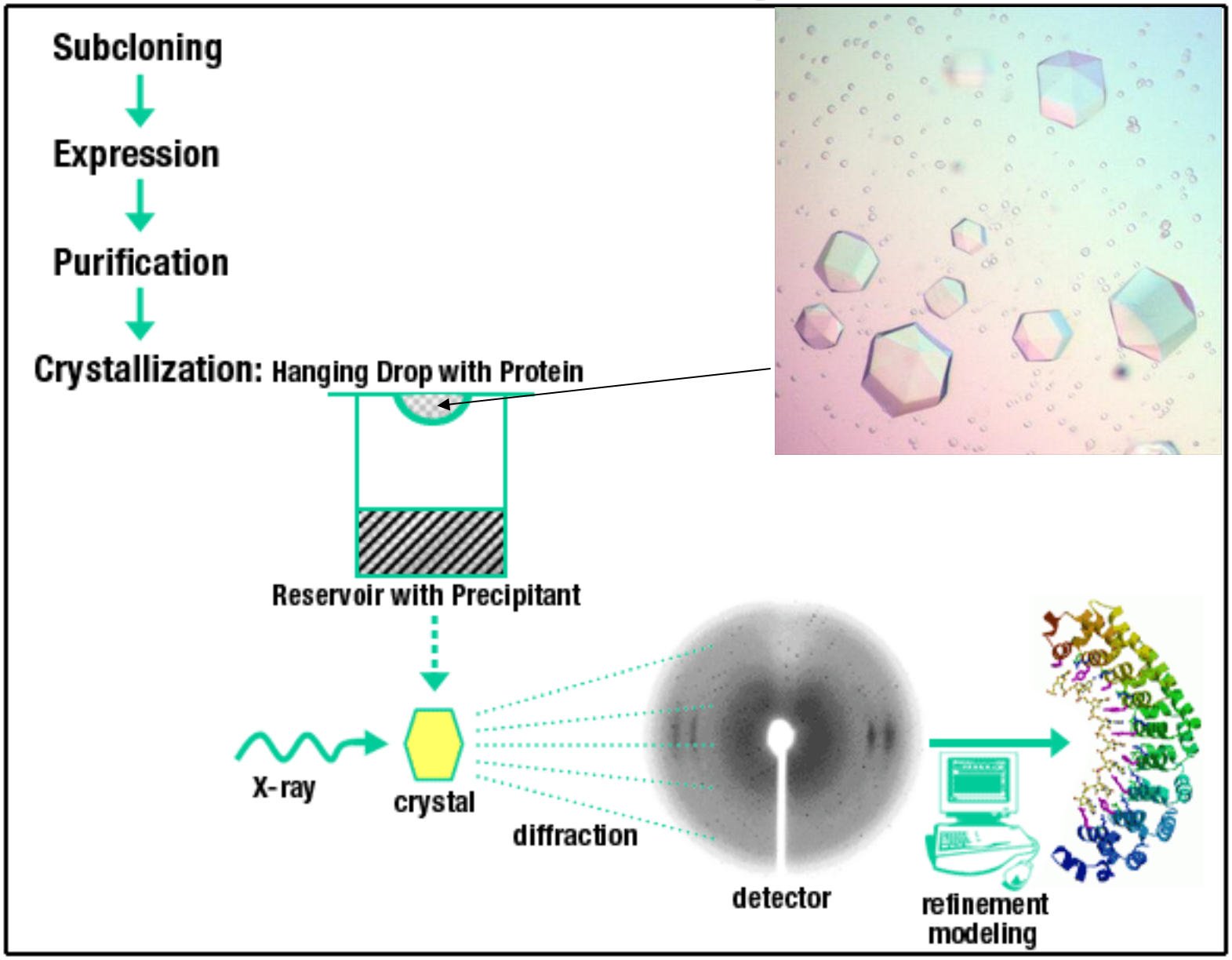
NMR



- Indirect detection of H-H distances
- In solution

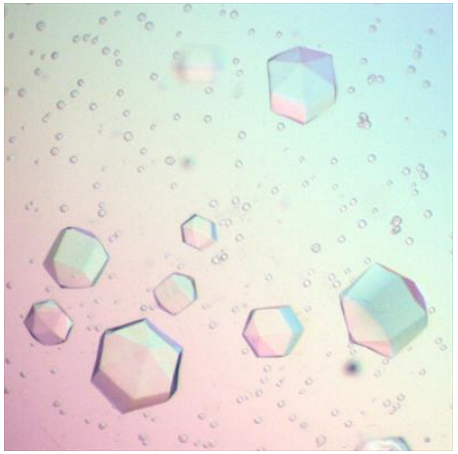
Other methods for determining protein structures:
-EM, Cryo-EM, ESR/Fluorescence

Determination of Protein Crystal Structure



http://www.noble.org/PlantBio/Wang/crystallography.html

Why Crystals?



X-rays are scattered by electrons, too weak to record scattering from a single molecule. Crystals are therefore used because they present many molecules (N) in exactly the same orientation. The scattering from each of the N molecules interferes constructively to give a measurable diffraction pattern (enhanced ~N^2 fold).

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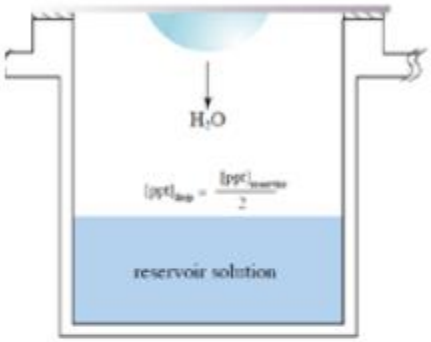
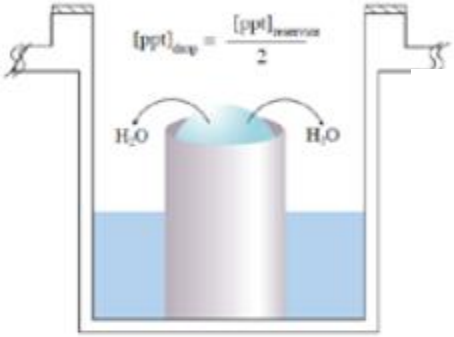
Some Crystallization Methods:

Vapor diffusion

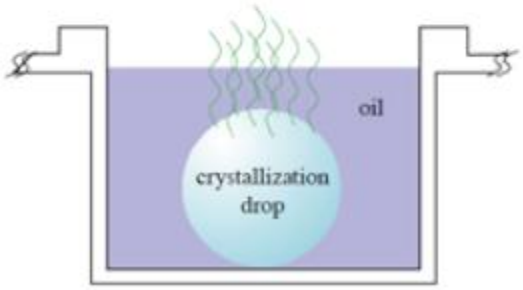
Hanging-drop



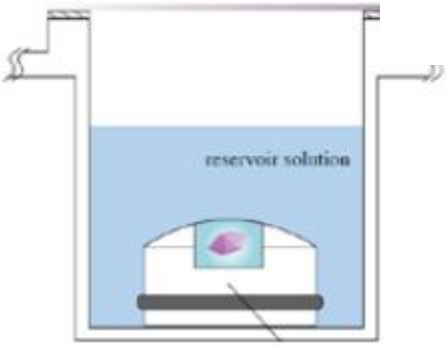
Sitting-drop

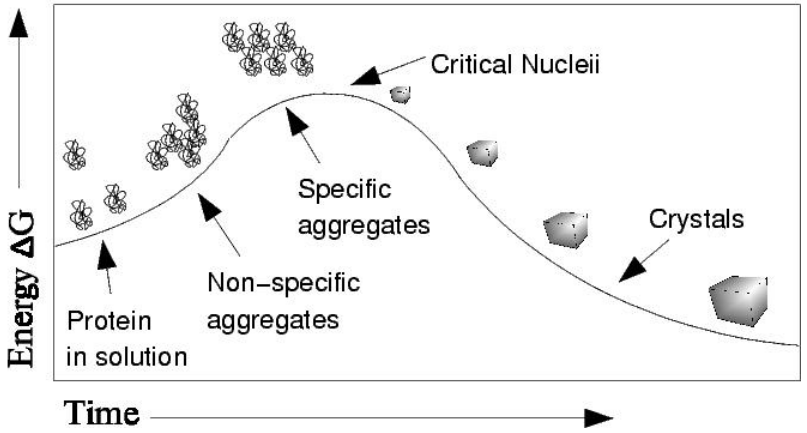


Batch:
micro batch under oil



Dialysis





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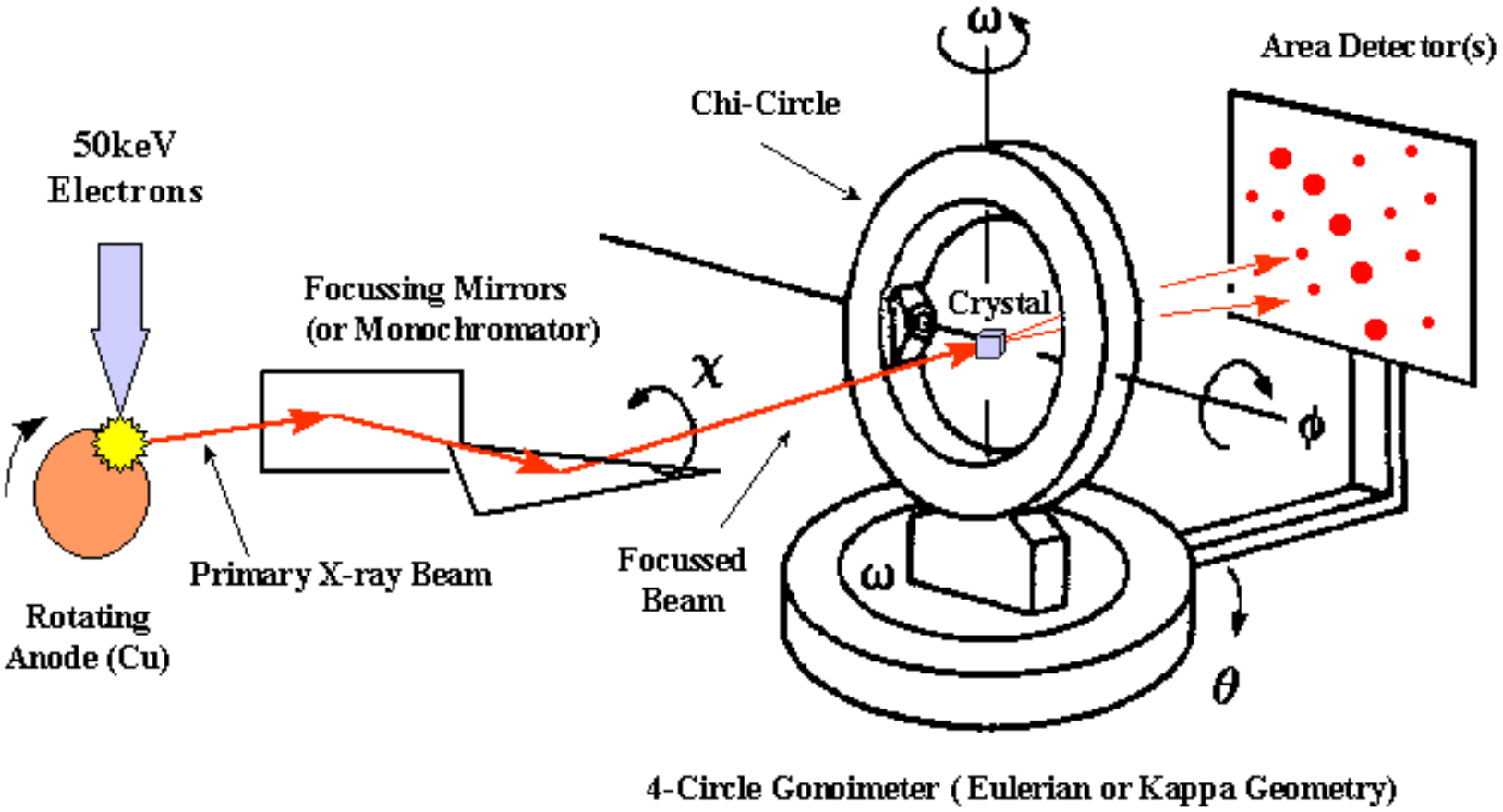
Data Collection



Synchrotron X-ray Sources

Lab x-ray sources @ 1.54 Å VS. Synchrotron @ 0.5 Å - 2.5 Å.

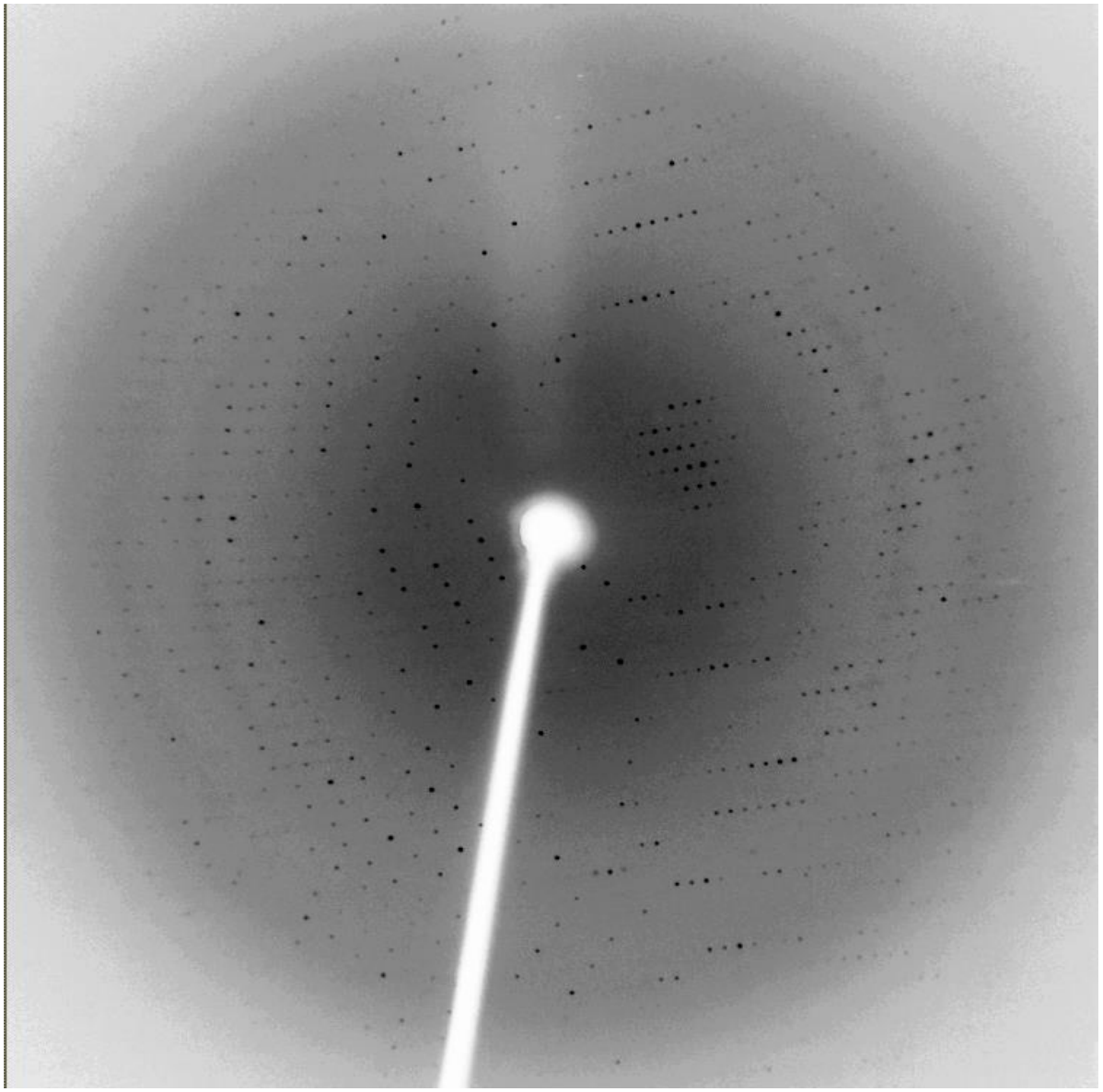
NSLS BNL

ALS Berkeley

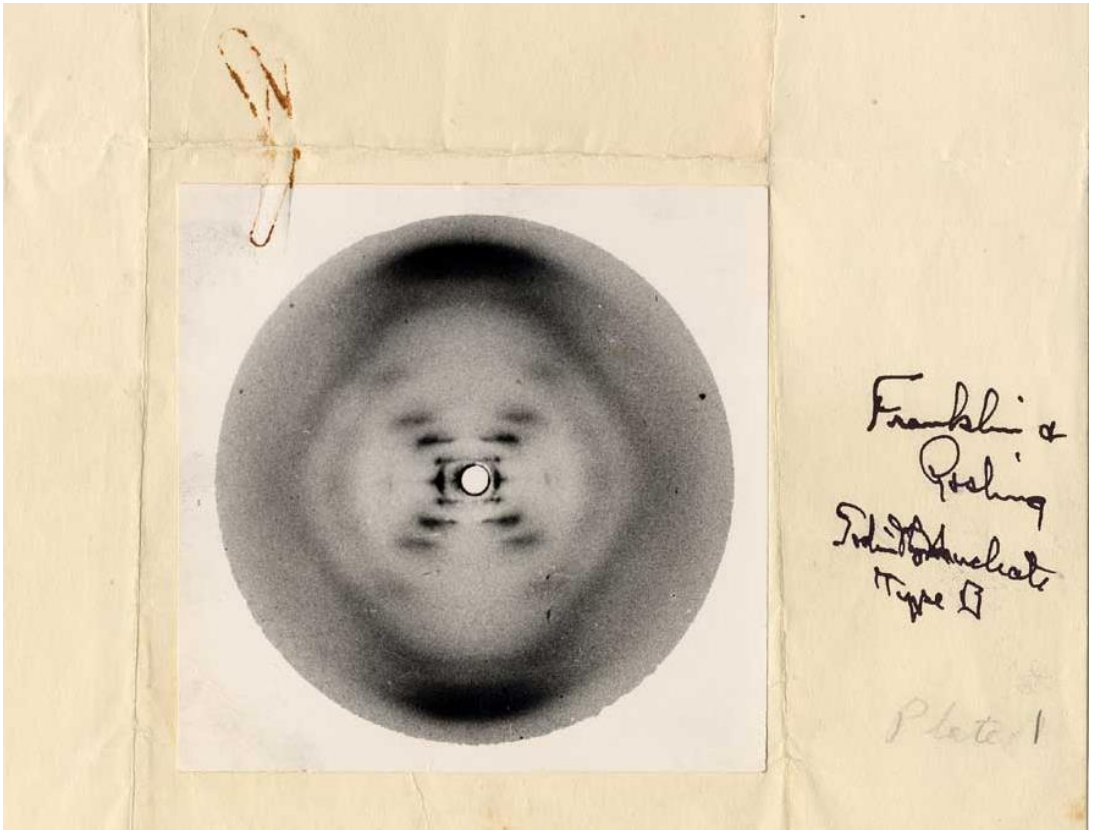
APS Chicago

CHESS Ithaca

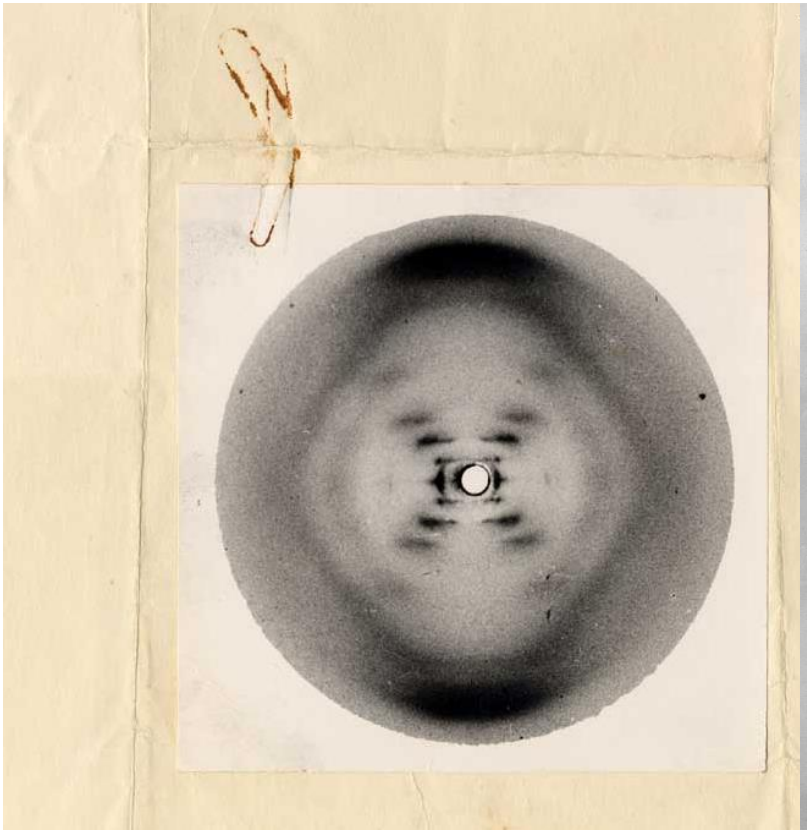
Image of diffraction

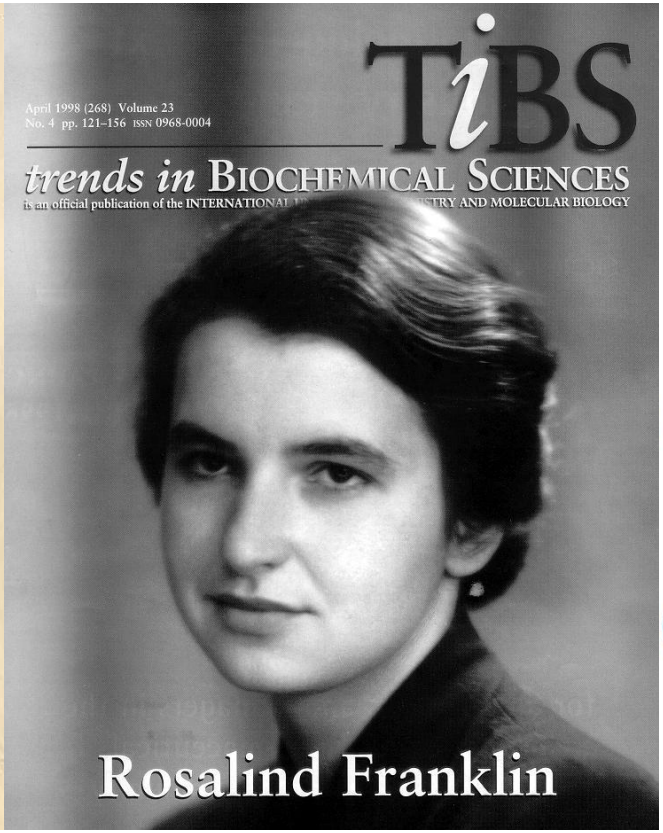


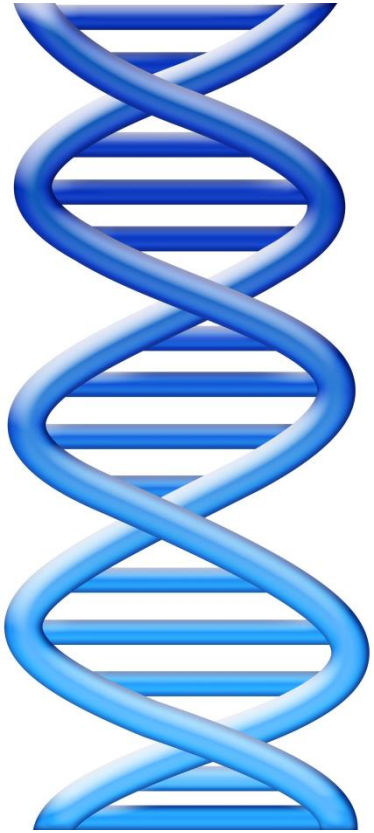
Most famous X-ray diffraction pattern



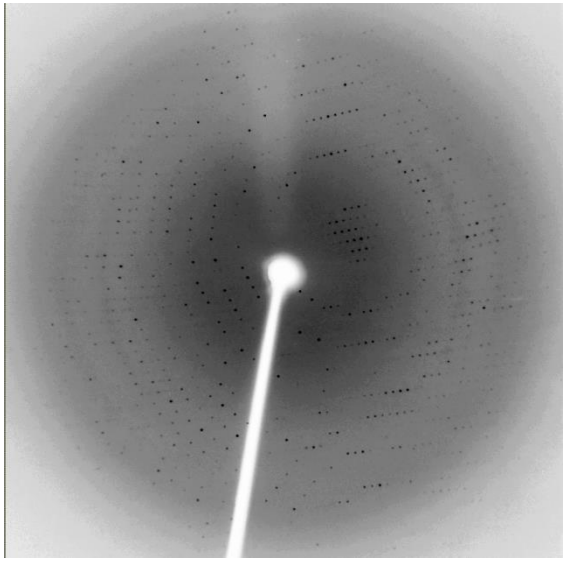
Most famous X-ray diffraction pattern







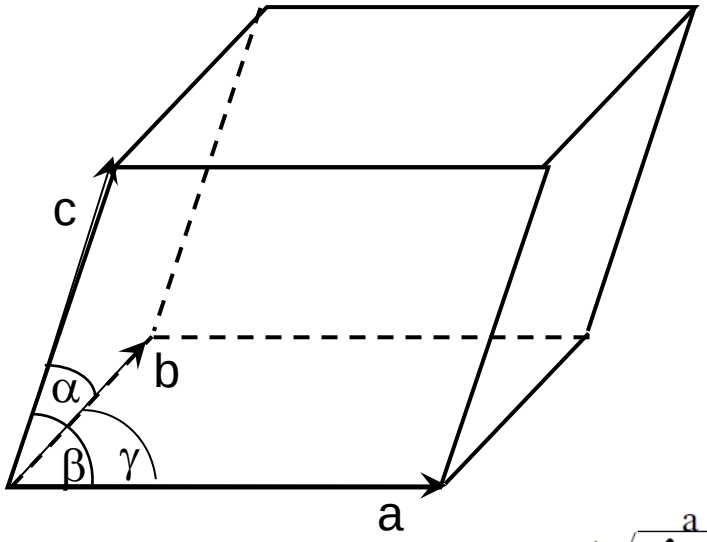
The information we get from a single diffraction experiment



Analyze the pattern of the reflections

(a) space group of the crystal

(b) unit cell dimensions

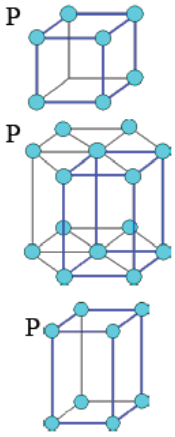


Cubic
a = b = c,
α = β = γ = 90°

Hexagonal
a = b ≠ c,
α = β = 90°, γ = 120°

Trigonal
a = b ≠ c,
α = β = 90°, γ = 120°

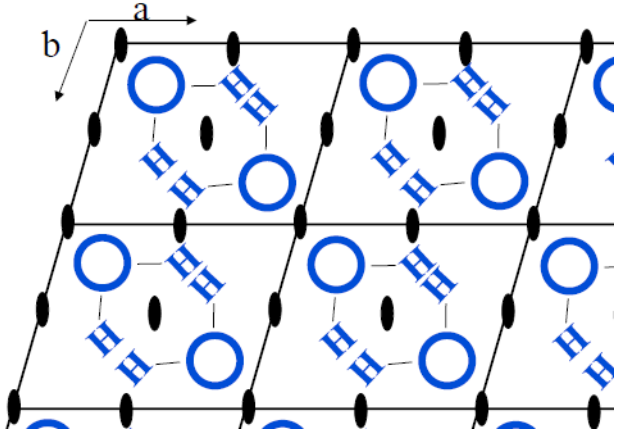
Tetragonal
a = b ≠ c,
α = β = γ = 90°



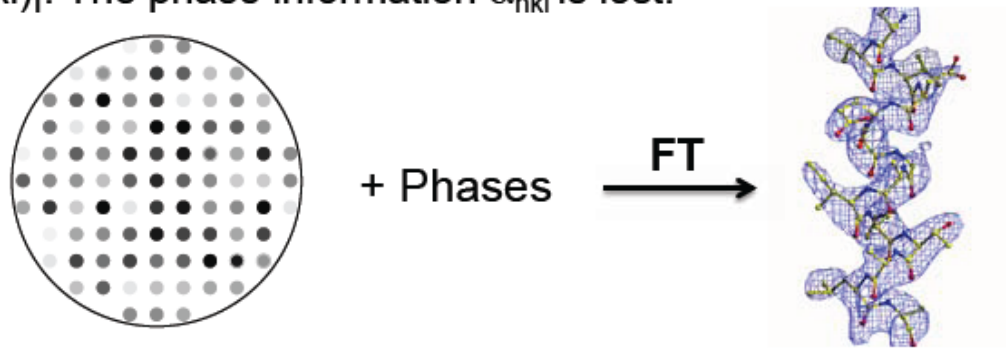
How to understand symmetry?

Crystal = lattice + unit cell content

(asymmetric units (asu) content)

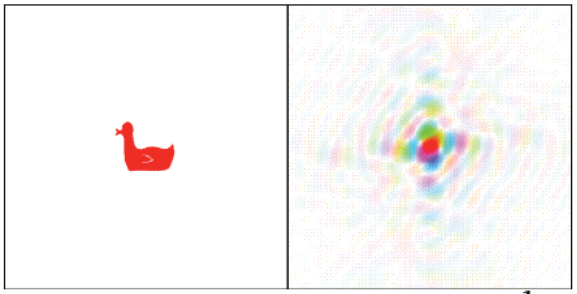


The phase problem: F(hkl) is a complex vector. Measured diffraction data give the amplitude |F(hkl)|. The phase information α_{hkl} is lost!

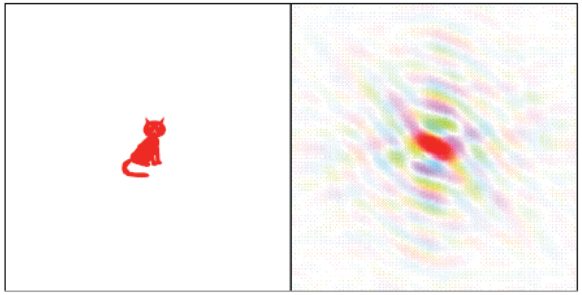


How important are amplitude and phase?

Fourier Duck and his Fourier transform
Phase is color coded

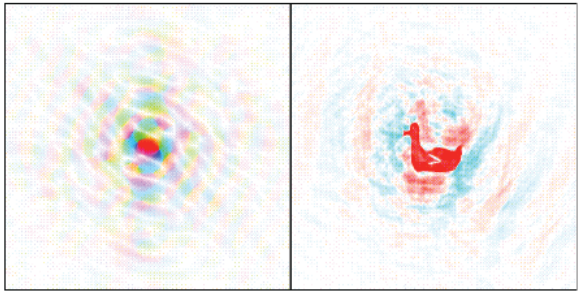


Fourier Cat and his Fourier transform
Phase is color coded

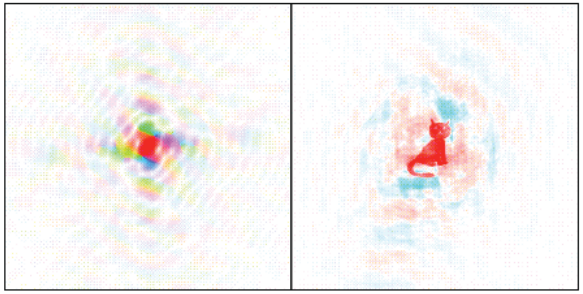


$$\rho(xyz) = \frac{1}{V}\sum_{hkl}|F(hkl)|e^{-2\pi i(hx+ky+lz)+i\alpha_{hkl}}$$

Duck phase and Cat amplitude



Cat phase and Duck amplitude



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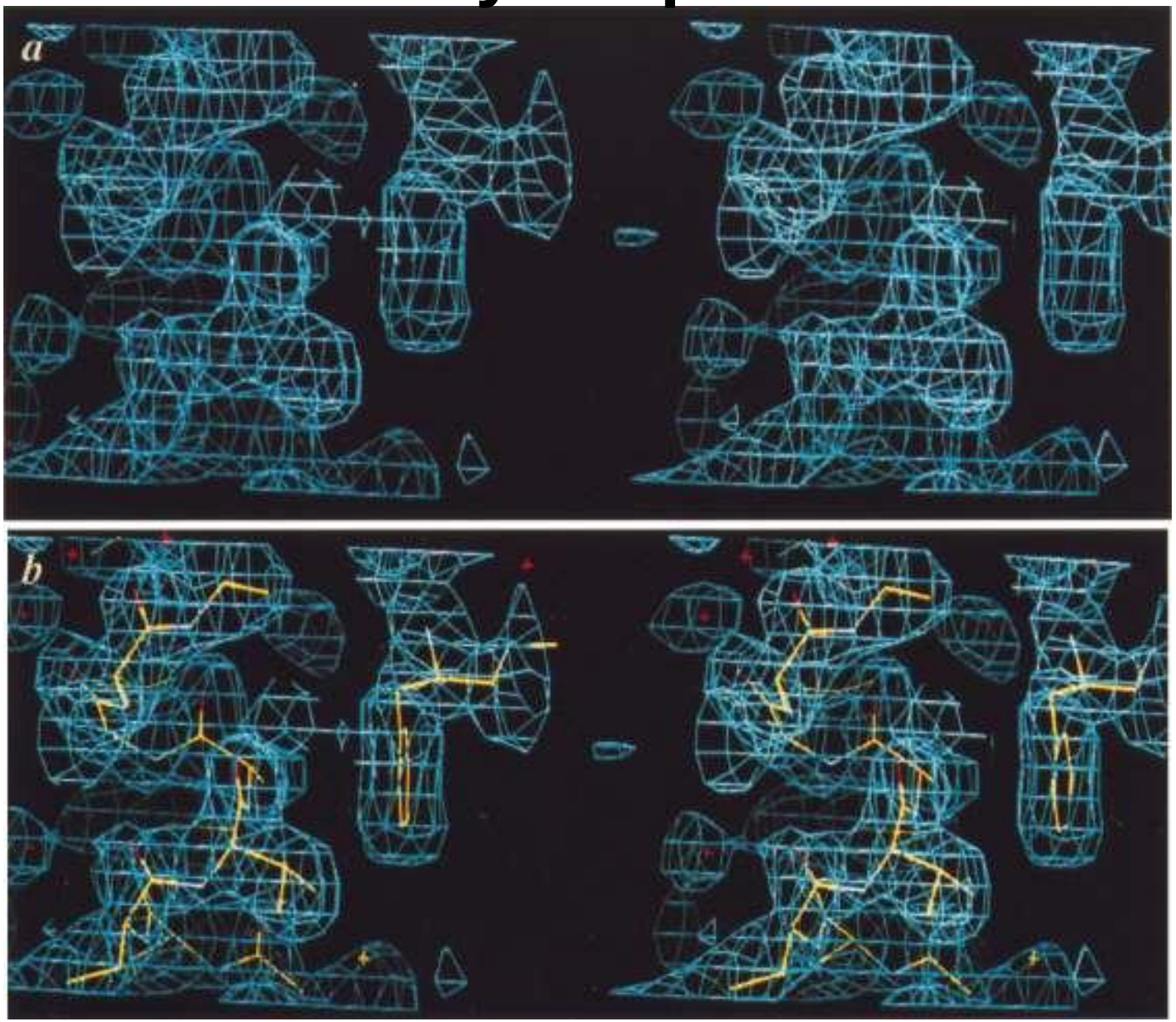
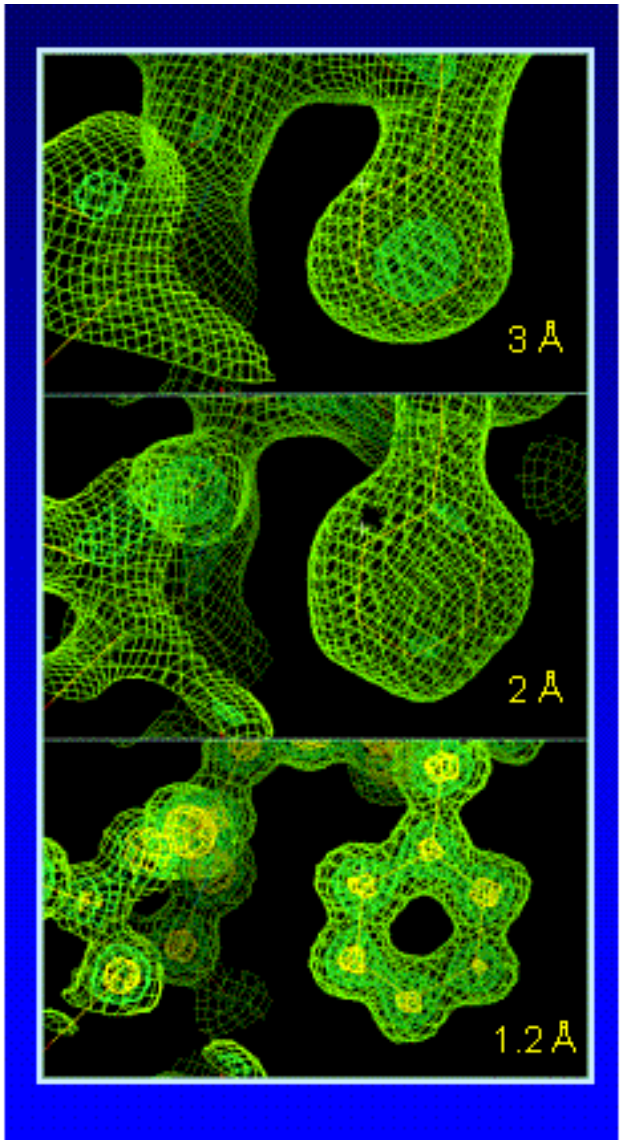
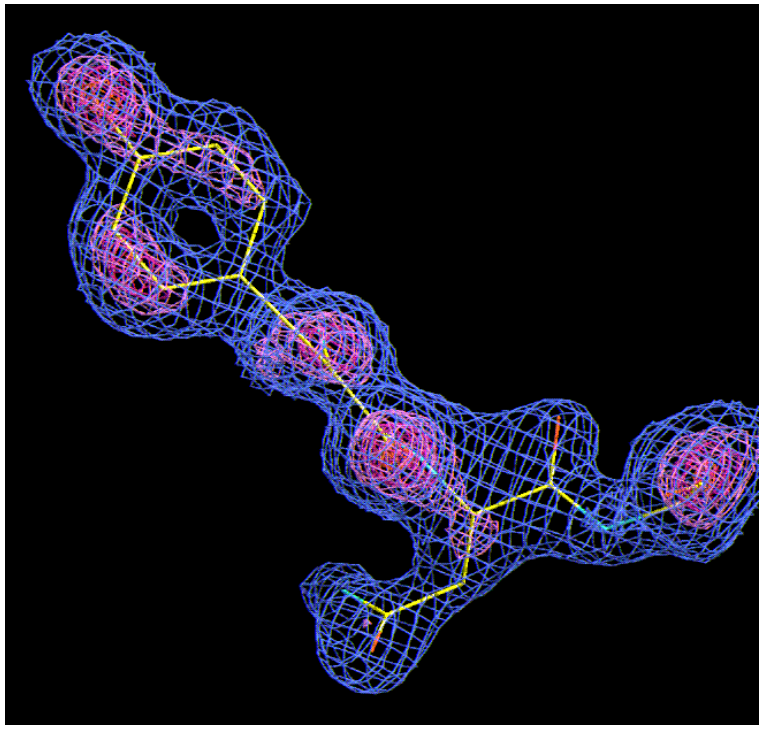
Electron density map



Building a structure model

The importance of resolution





Experimental electron density map created from multi-wavelength data collected at SSRL beam line 1-5 on a Gold derivative of tetanus C fragment.

Example of high quality Experimental data where very little refinement has been applied to fit a tyrosine into the density map.

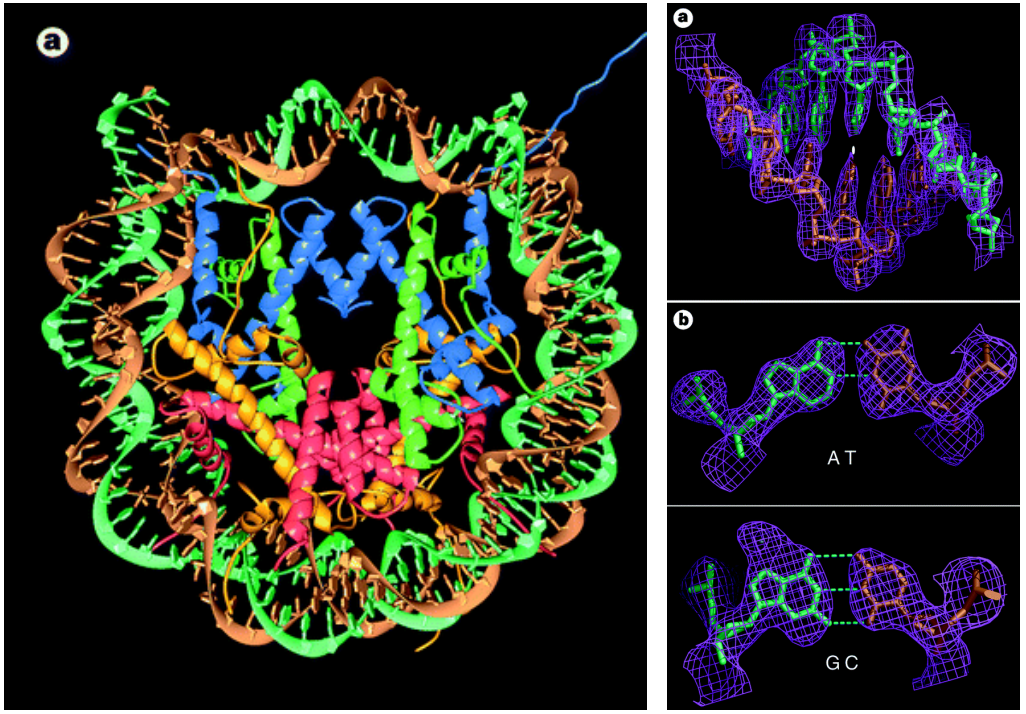
http://www.ruppweb.org/Xray/101index.html

Crystal structure of the nucleosome core particle at 2.8 Å resolution

Karolin Luger, Armin W. Mäder, Robin K. Richmond, David F. Sargent & Timothy J. Richmond

Institut für Molekularbiologie und Biophysik ETHZ, ETH-Hönggerberg, CH-8093 Zürich, Switzerland

The X-ray crystal structure of the nucleosome core particle of chromatin shows in atomic detail how the histone protein octamer is assembled and how 146 base pairs of DNA are organized into a superhelix around it. Both histone/histone and histone/DNA interactions depend on the histone fold domains and additional, well ordered structure elements extending from this motif. Histone amino-terminal tails pass over and between the gyres of the DNA superhelix to contact neighbouring particles. The lack of uniformity between multiple histone/DNA-binding sites causes the DNA to deviate from ideal superhelix geometry.

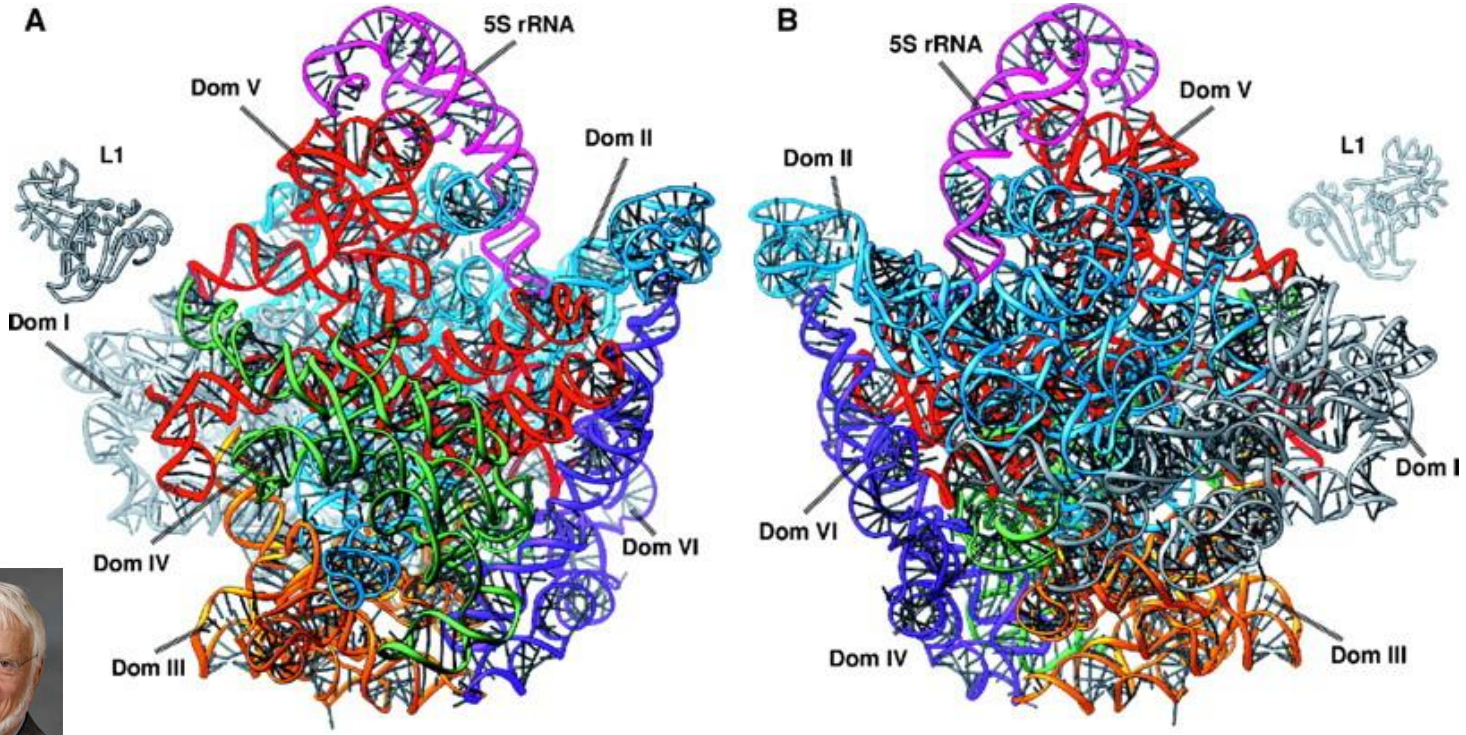


Science
AAAS

RESEARCH ARTICLES

The Complete Atomic Structure of the Large Ribosomal Subunit at 2.4 Å Resolution

Nenad Ban,[1]* Poul Nissen,[1]* Jeffrey Hansen,[1] Peter B. Moore,[1,2]
Thomas A. Steitz[1,2,3]†

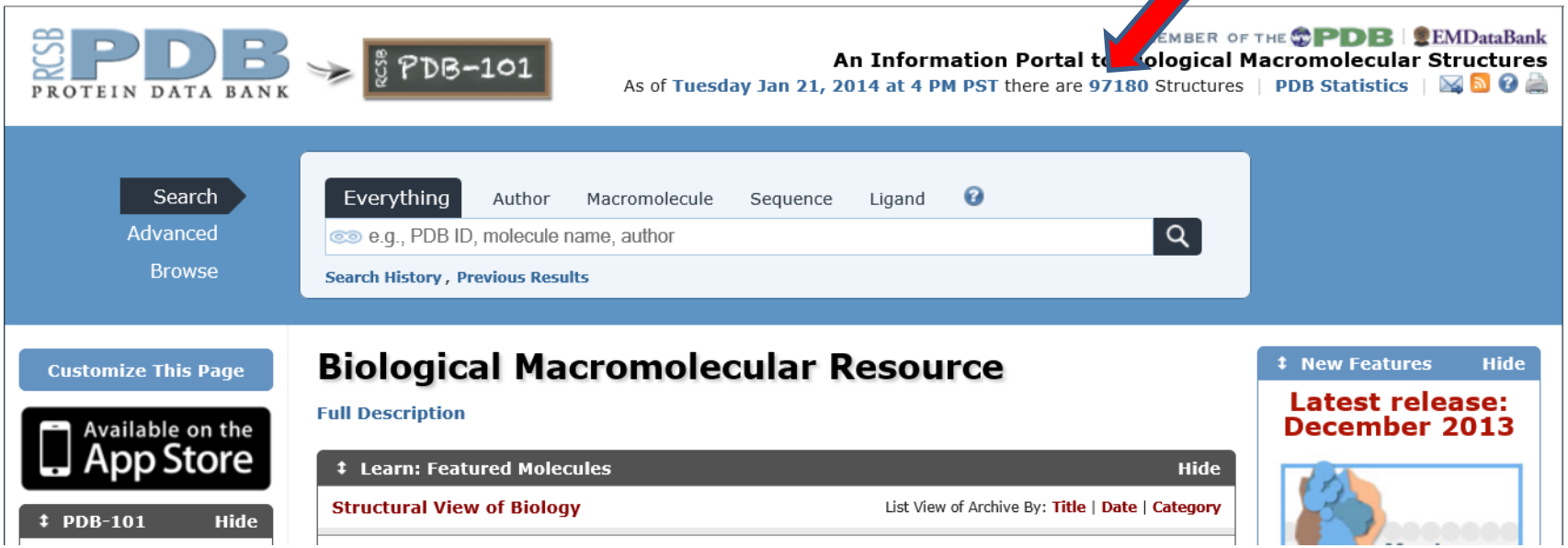


Yale's Thomas Steitz shared 2009 Nobel Prize in Chemistry for this structure

Protein Structure Databases

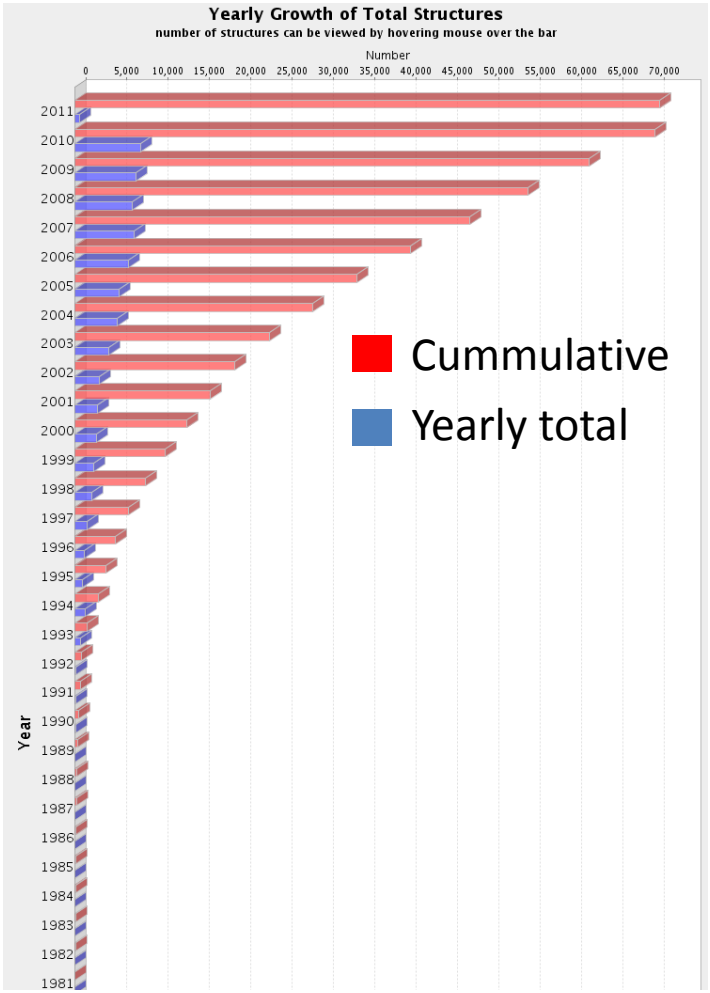
- Where does protein structural information reside?
  - PDB:
    - http://www.rcsb.org/pdb/
  - MMDB:
    - http://www.ncbi.nlm.nih.gov/Structure/
  - FSSP:
    - http://www.ebi.ac.uk/dali/fssp/
  - SCOP:
    - http://scop.mrc-lmb.cam.ac.uk/scop/
  - CATH:
    - http://www.biochem.ucl.ac.uk/bsm/cath_new/

# of structures
2011: 70,813
2014: 97,180



PDB Growth from 2011 to 2014: Δ structures: 26,367

compared to Δ protein interactions: 107,018

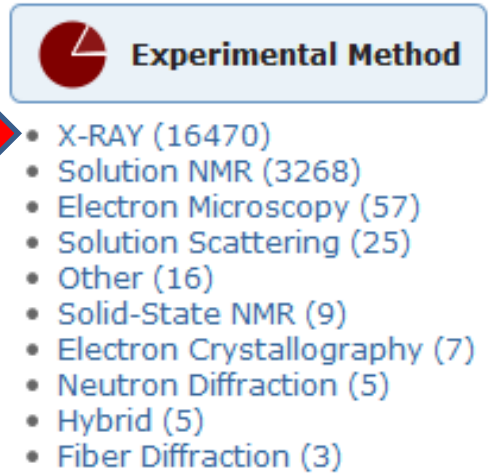


What species are the structures from?

human

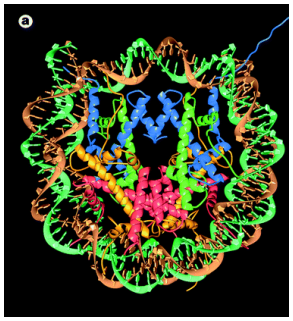
Which methods?

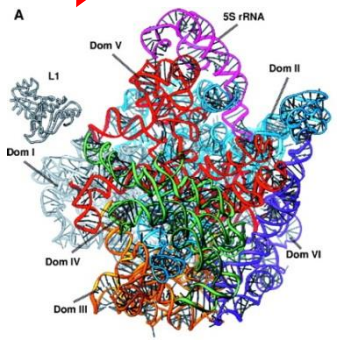
X-ray



PDB Current Holdings Breakdown

Exp.Method	Proteins	Nucleic Acids	Protein/NA Complexes	Other	Total
X-RAY	57513	1256	2761	17	61547
NMR	7632	933	168	7	8740
ELECTRON MICROSCOPY	236	22	85	0	343
HYBRID	28	1	1	1	31
other	130	4	5	13	152
Total	65539	2216	3020	38	70813





Tools for Viewing Structures

- **Jmol**
  - **http://jmol.sourceforge.net**
- **PyMOL**
  - **http://pymol.sourceforge.net**
- **Swiss PDB viewer**
  - **http://www.expasy.ch/spdbv**
- **Mage/KiNG**
  - **http://kinemage.biochem.duke.edu/software/mage.php**
  - **http://kinemage.biochem.duke.edu/software/king.php**
- **Rasmol**
  - **http://www.umass.edu/microbio/rasmol/**