

Introduction to X-ray Crystallography

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Contributions to this lecture:

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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

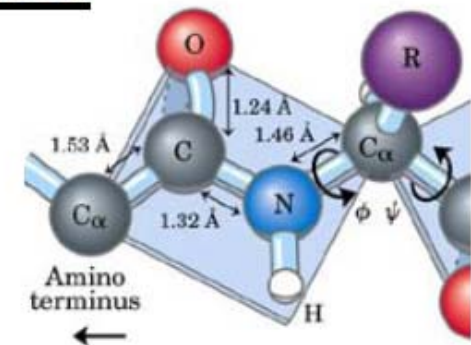
- There's a limit to how small an object can be seen under a light microscope.
- The diffraction limit: you can not image things that are much smaller than the wavelength of the light you are using.
- The wavelength for visible light is measured in hundreds of nanometers, while atoms are separated by 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 $\text{CuK}\alpha$ radiation. Wavelength = 1.54 Å.

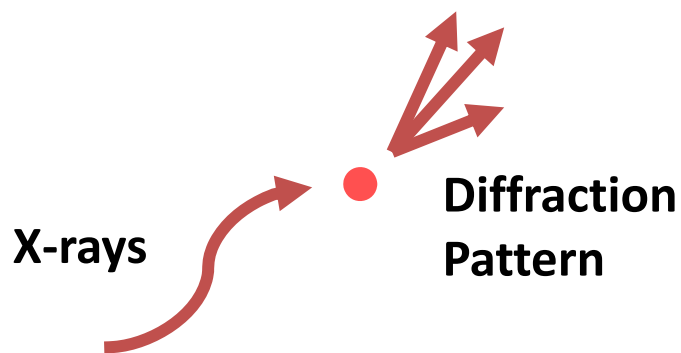
Synchrotron radiation wavelengths in the range 0.5 Å - 2.5 Å.



Yong Xiong

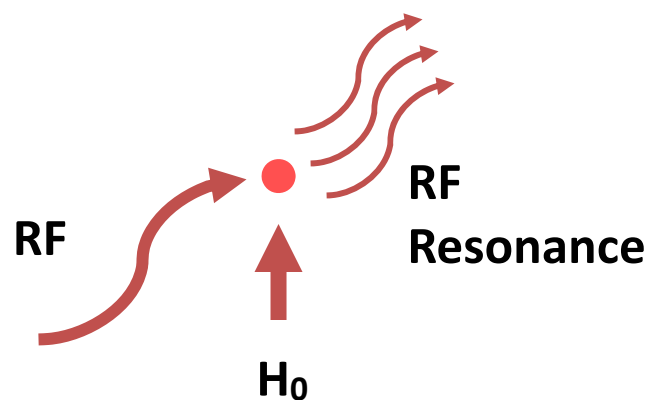
Experimental Determination of Atomic Resolution Structures

X-ray



- Direct detection of atom positions
- Crystals

NMR

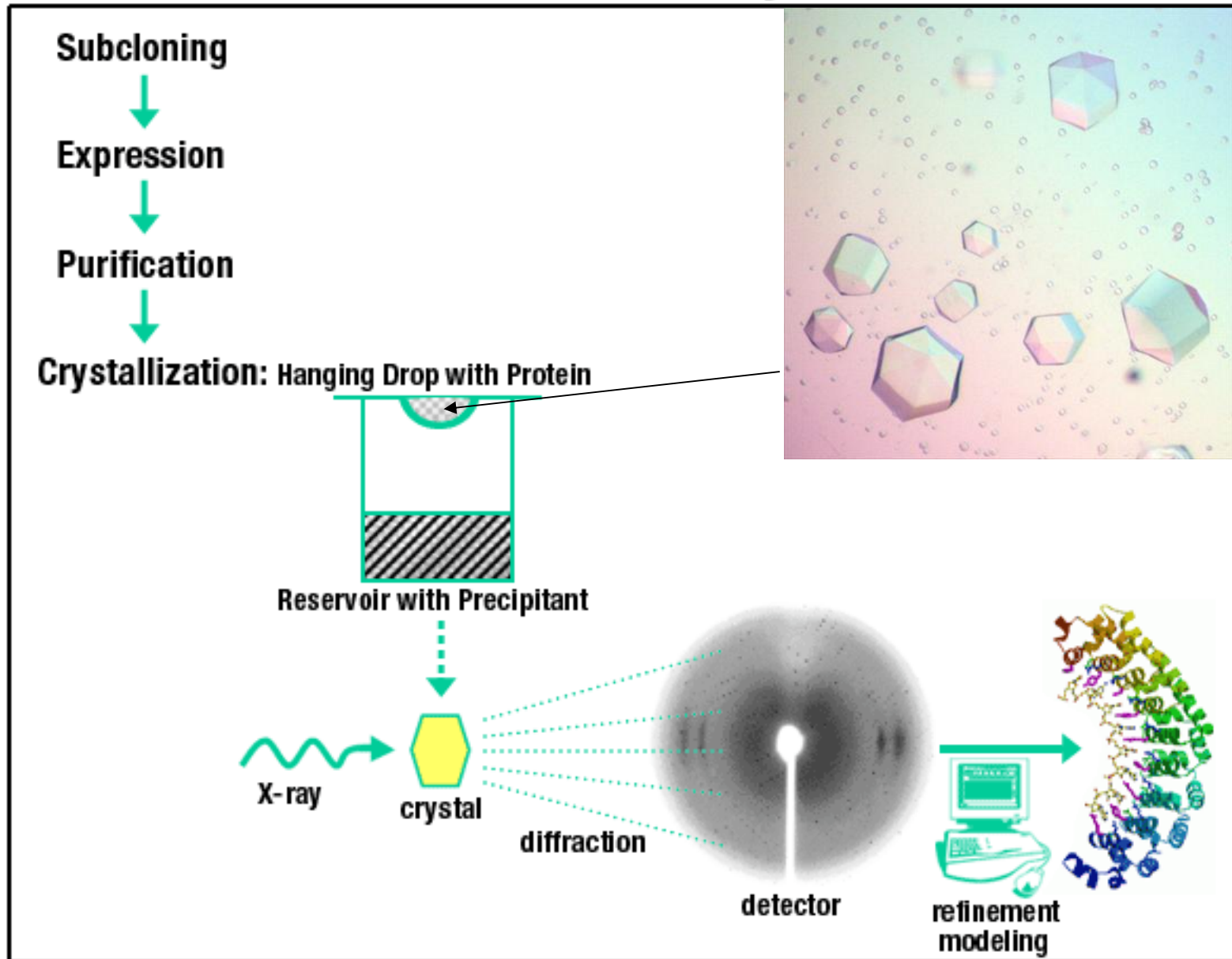


- Indirect detection of H-H distances
- In solution

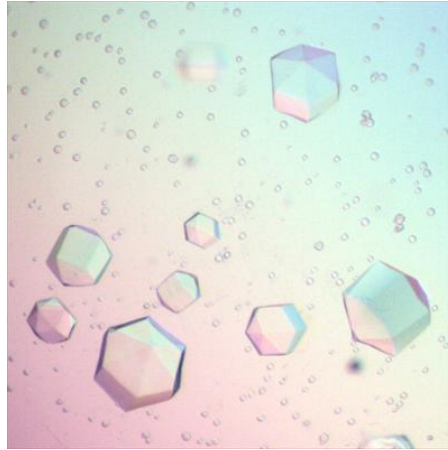
Other methods for determining protein structures:

-EM, Cryo-EM, ESR/Fluorescence

Determination of Protein Crystal Structure



Why Crystals?

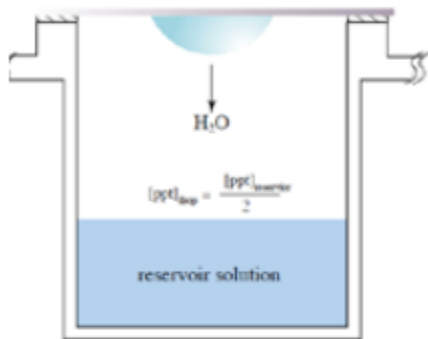


X-rays are scattered by electrons, too weak to record scattering from a single molecule. Crystals are therefore used because they present many molecules (N) in exactly the same orientation. The scattering from each of the N molecules interferes constructively to give a measurable diffraction pattern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

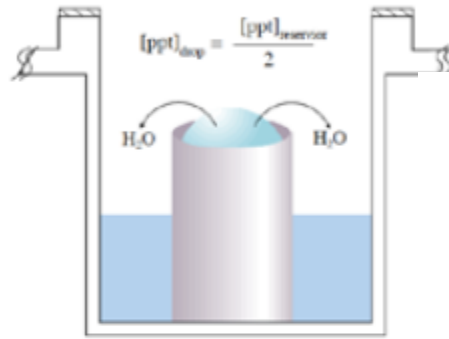
Some Crystallization Methods:

Vapor diffusion

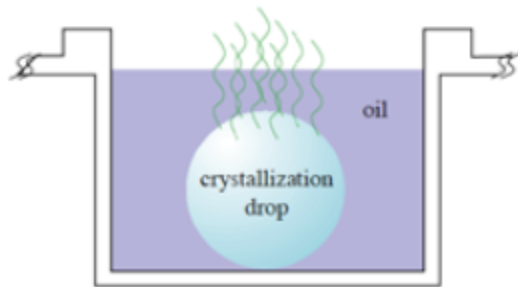
Hanging-drop



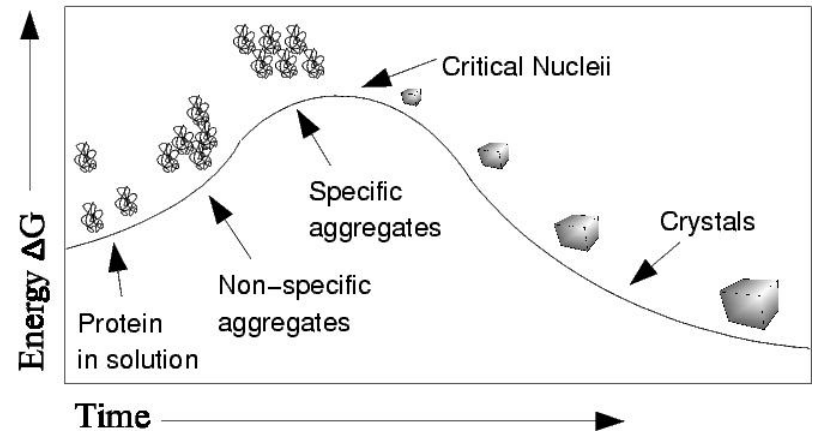
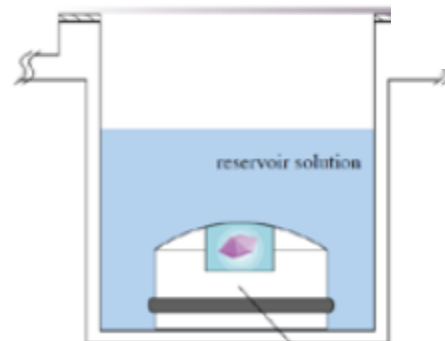
Sitting-drop



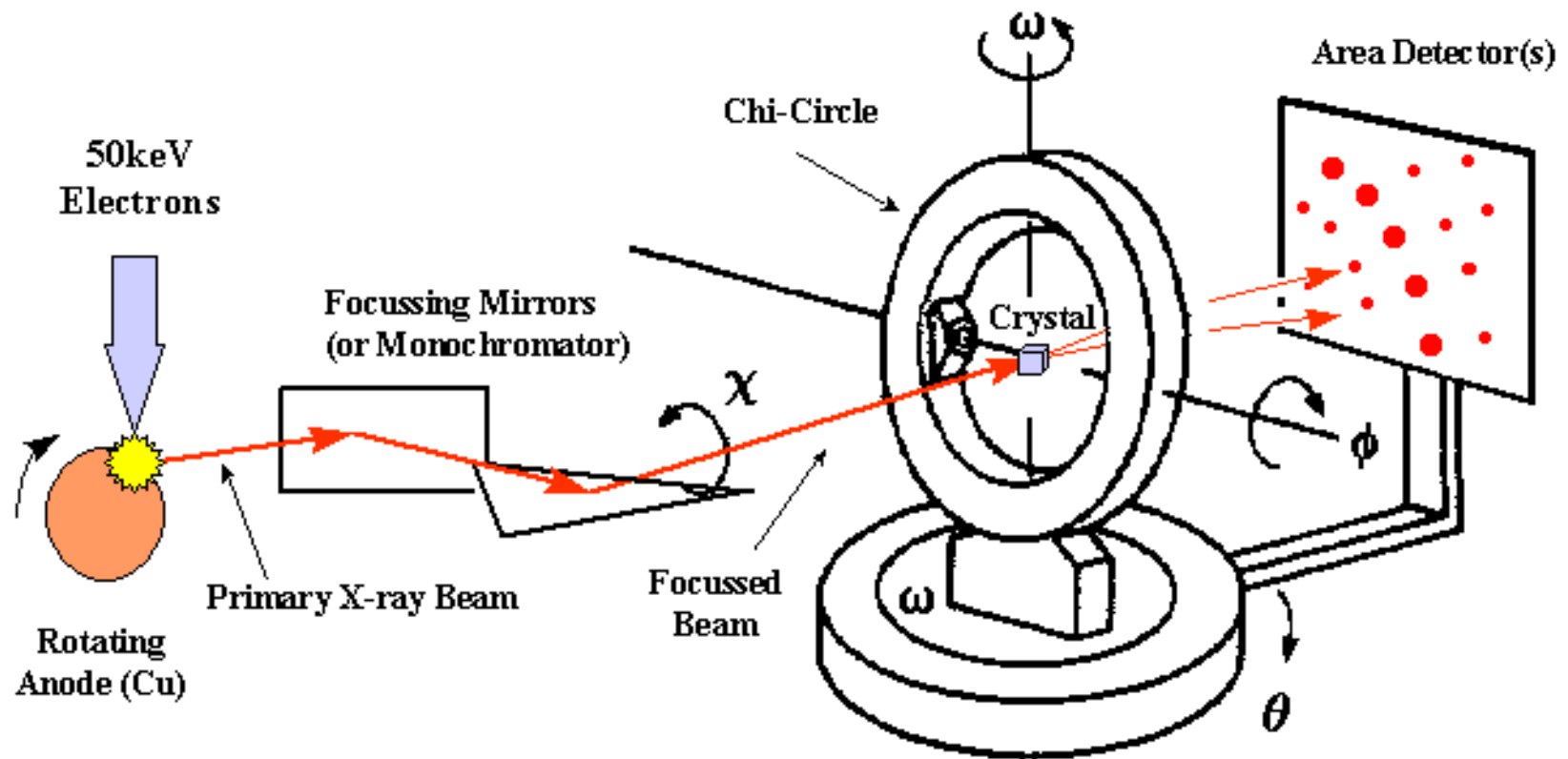
Batch:
micro batch under oil



Dialysis



Data Collection



4-Circle Goniometer (Eulerian or Kappa Geometry)

Synchrotron X-ray Sources

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



NSLS BNL



ALS Berkeley

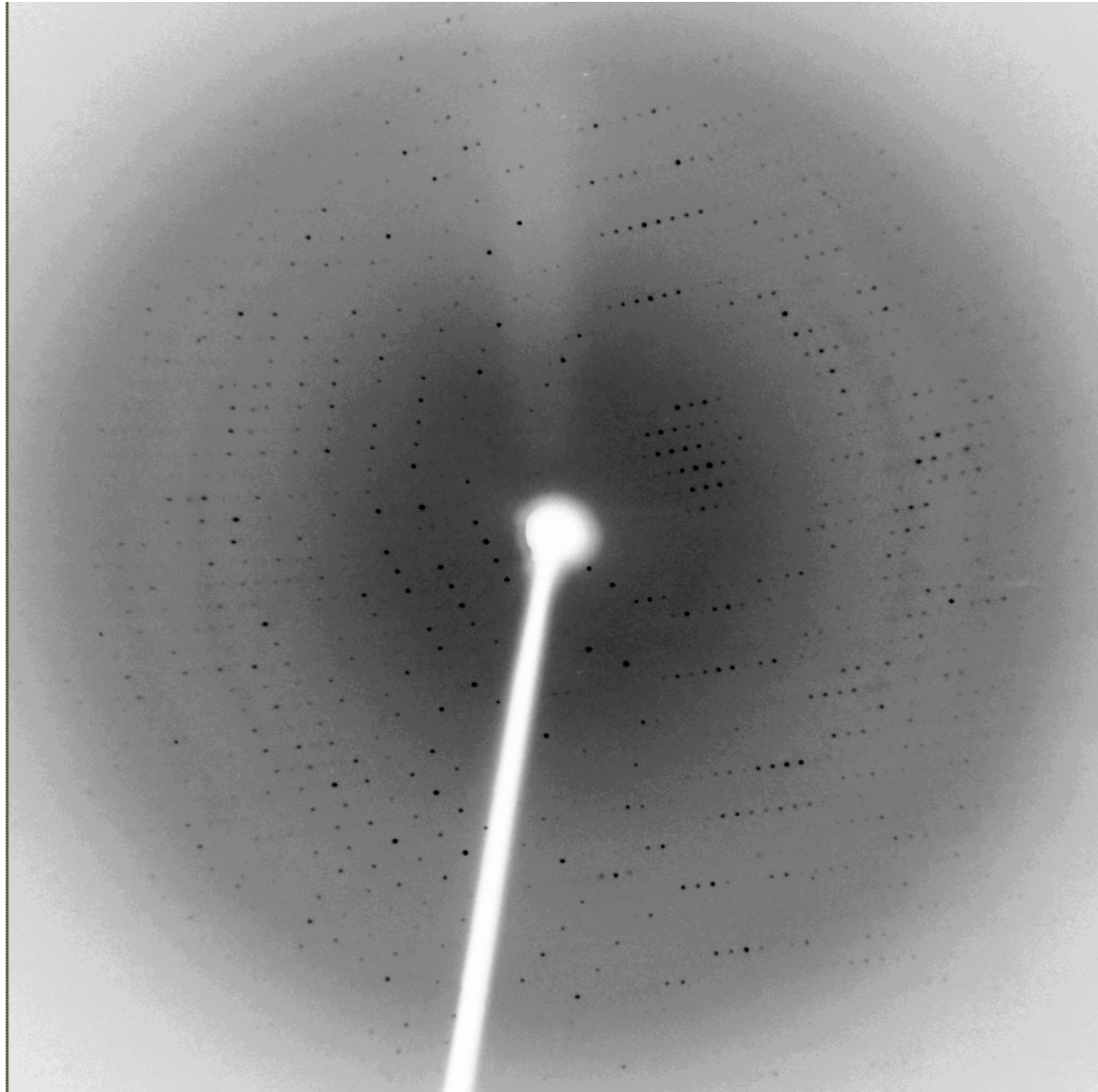


APS Chicago

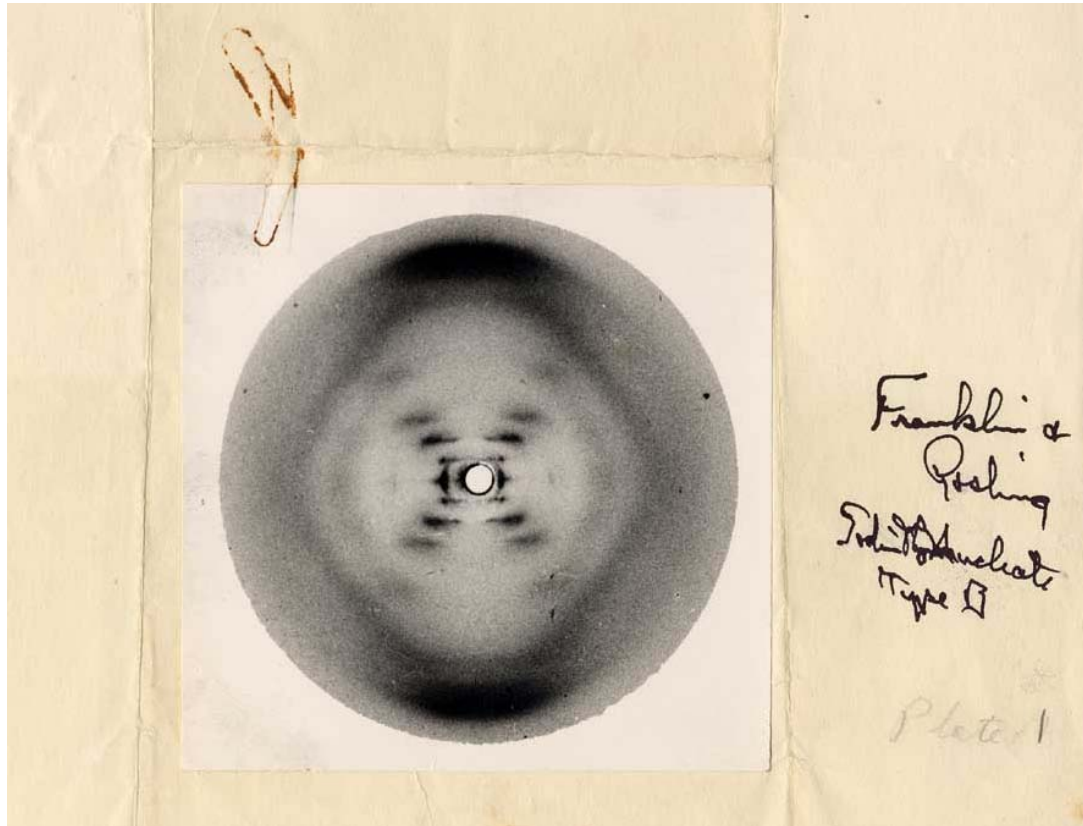


CHESS Ithaca

Image of diffraction

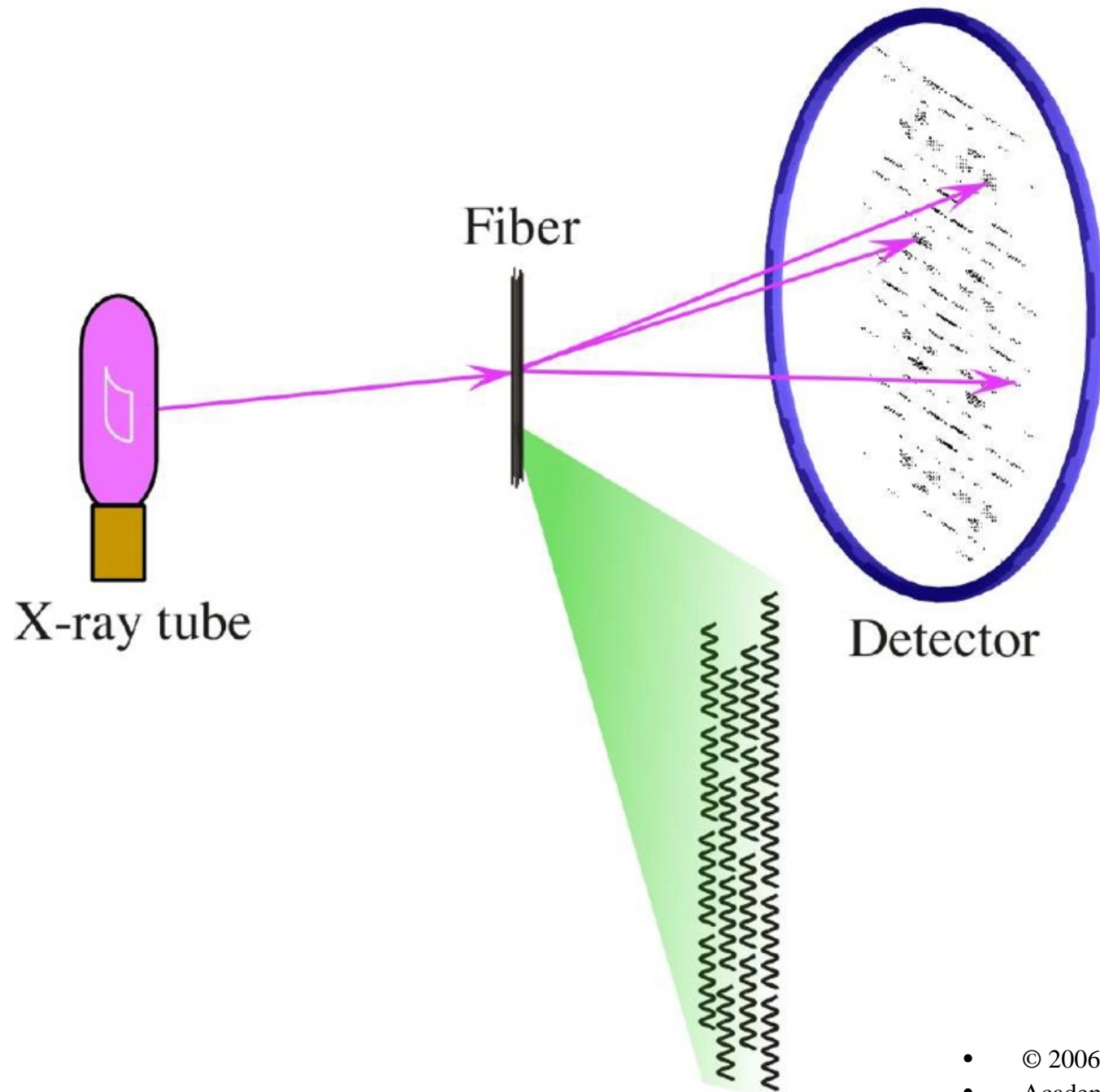


Most famous X-ray diffraction pattern



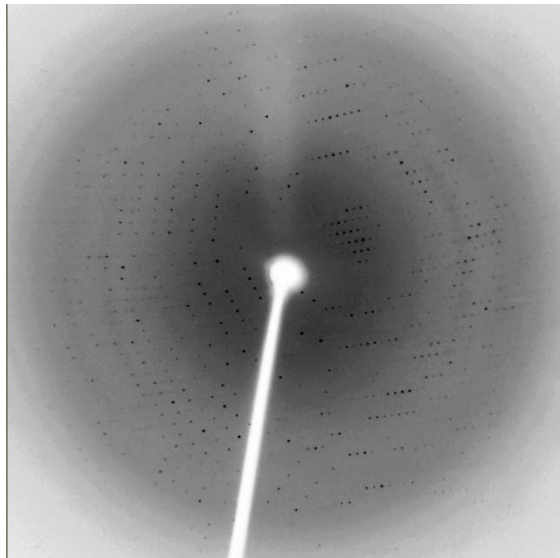
Most famous X-ray diffraction pattern





- © 2006
- Academic Press

The information we get from a single diffraction experiment

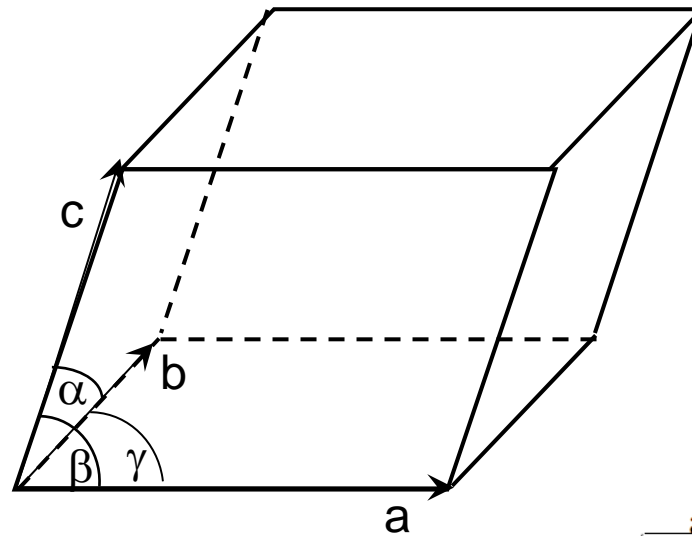


Analyze the pattern
of the reflections

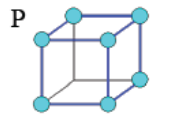


(a) space group of the crystal

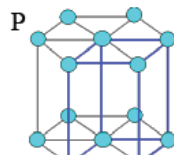
(b) unit cell dimensions



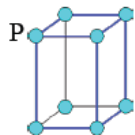
Cubic
 $a = b = c$,
 $\alpha = \beta = \gamma = 90^\circ$



Hexagonal
 $a = b \neq c$,
 $\alpha = \beta = 90^\circ, \gamma = 120^\circ$



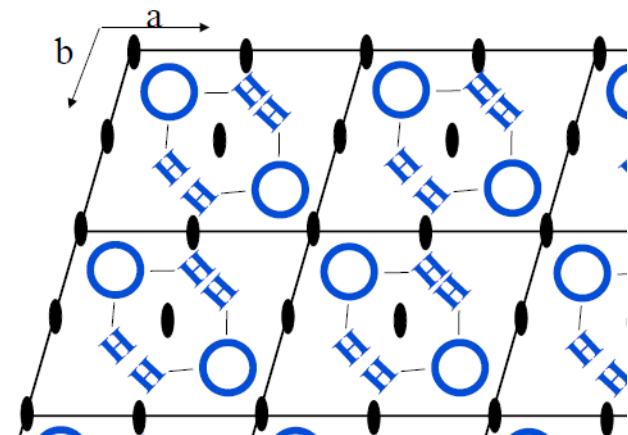
Trigonal
 $a = b \neq c$,
 $\alpha = \beta = 90^\circ, \gamma = 120^\circ$



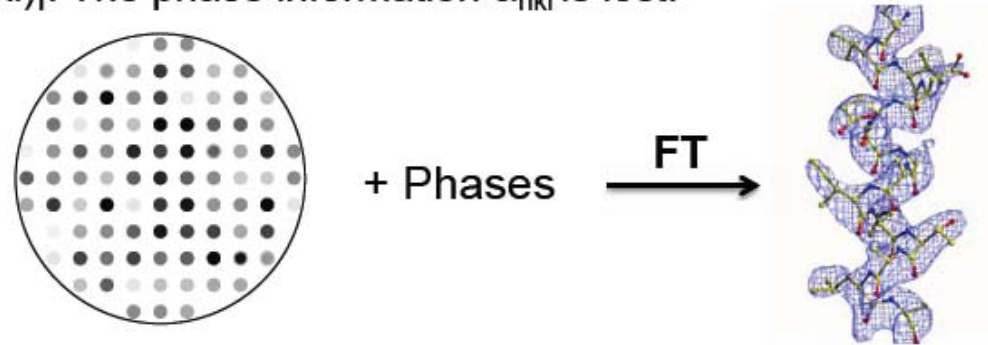
Tetragonal
 $a = b \neq c$,
 $\alpha = \beta = \gamma = 90^\circ$

How to understand symmetry?

Crystal = lattice + unit cell content
(asymmetric units (asu) content)

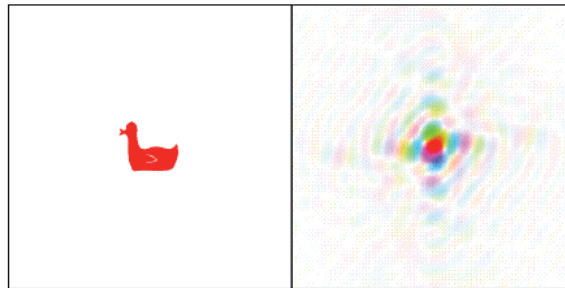


The phase problem: $F(hkl)$ is a complex vector. Measured diffraction data give the amplitude $|F(hkl)|$. The phase information α_{hkl} is lost!

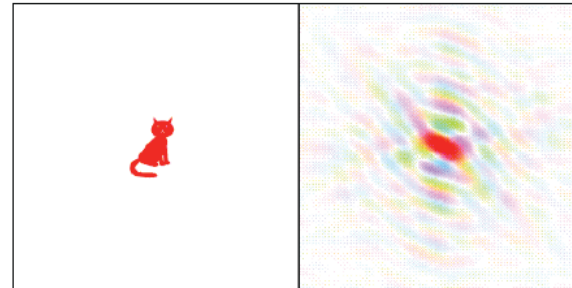


How important are amplitude and phase?

Fourier Duck and his Fourier transform
Phase is color coded

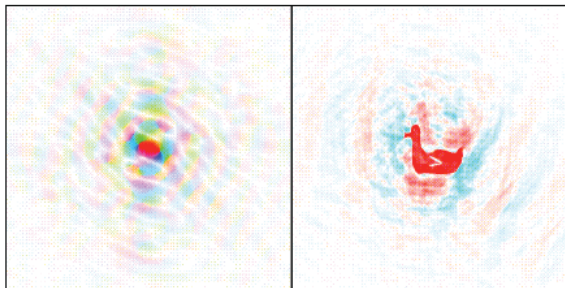


Fourier Cat and his Fourier transform
Phase is color coded

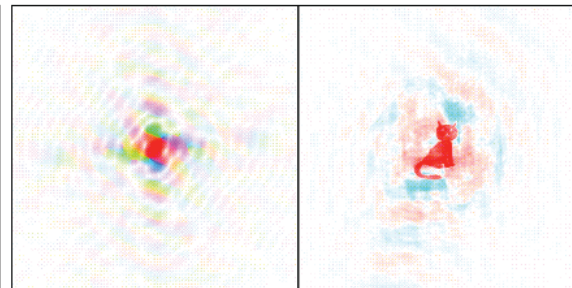


$$\rho(xyz) = \frac{1}{V} \sum_{hkl} |F(hkl)| e^{-2\pi i(hx+ky+lz) + i\alpha_{hkl}}$$

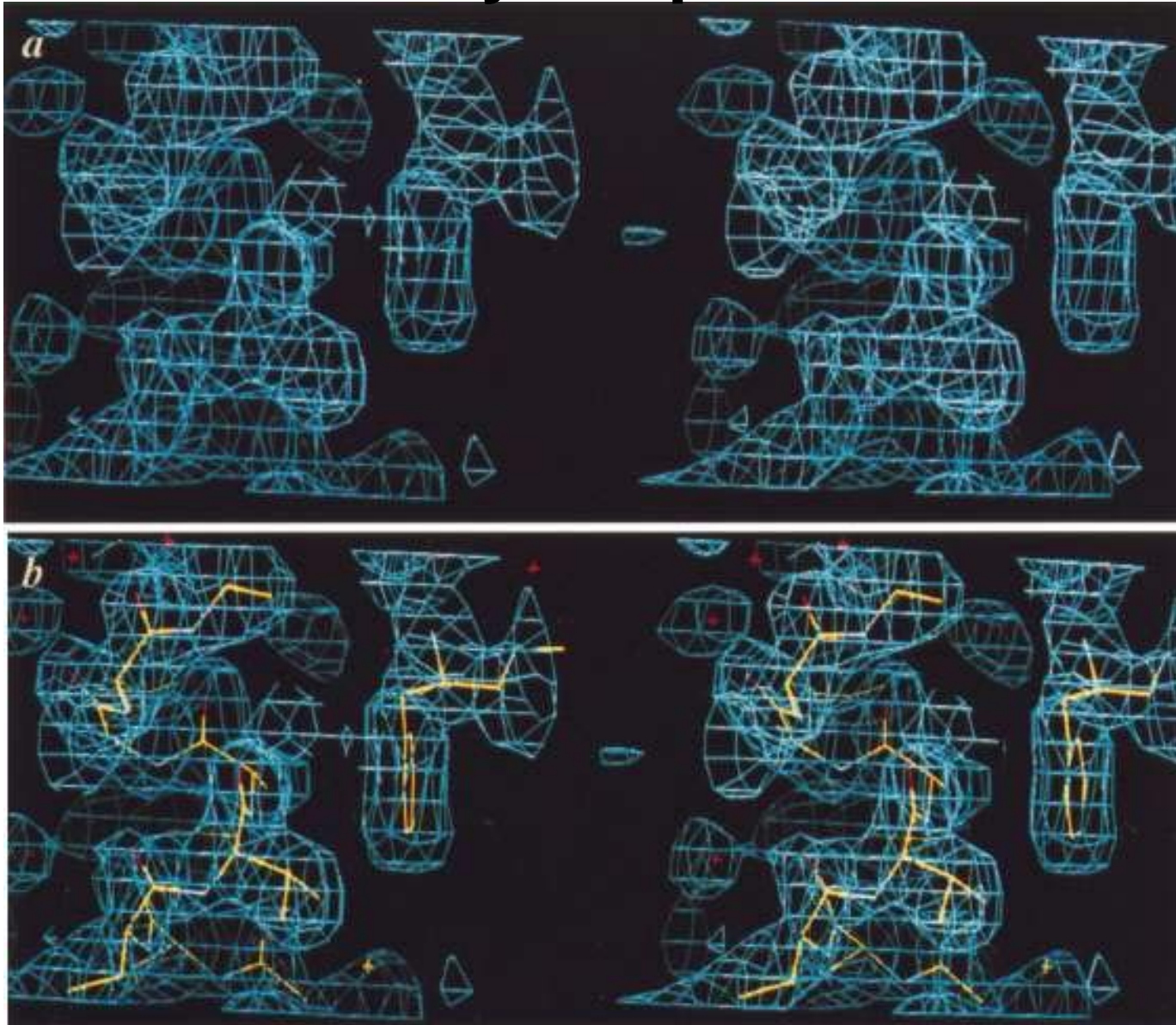
Duck phase and Cat amplitude



Cat phase and Duck amplitude

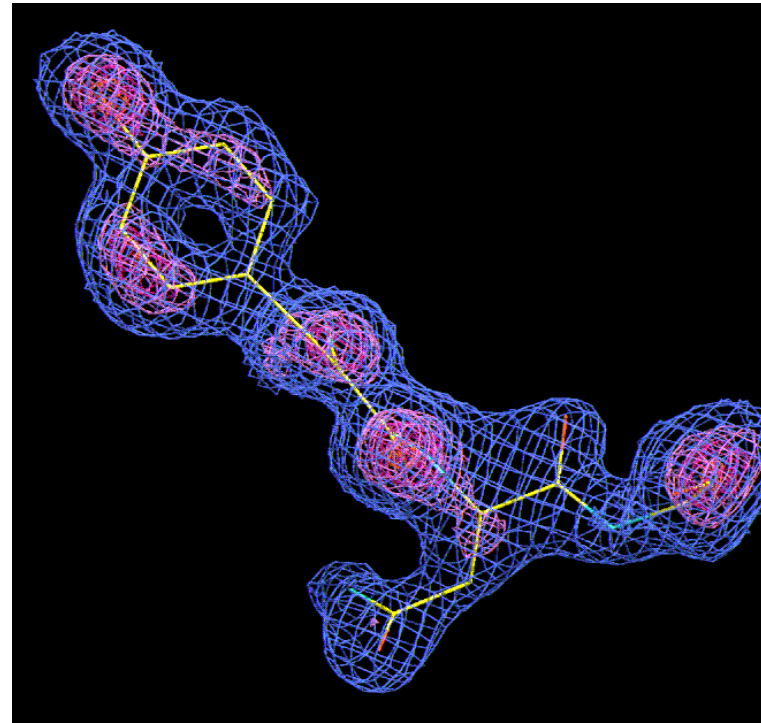
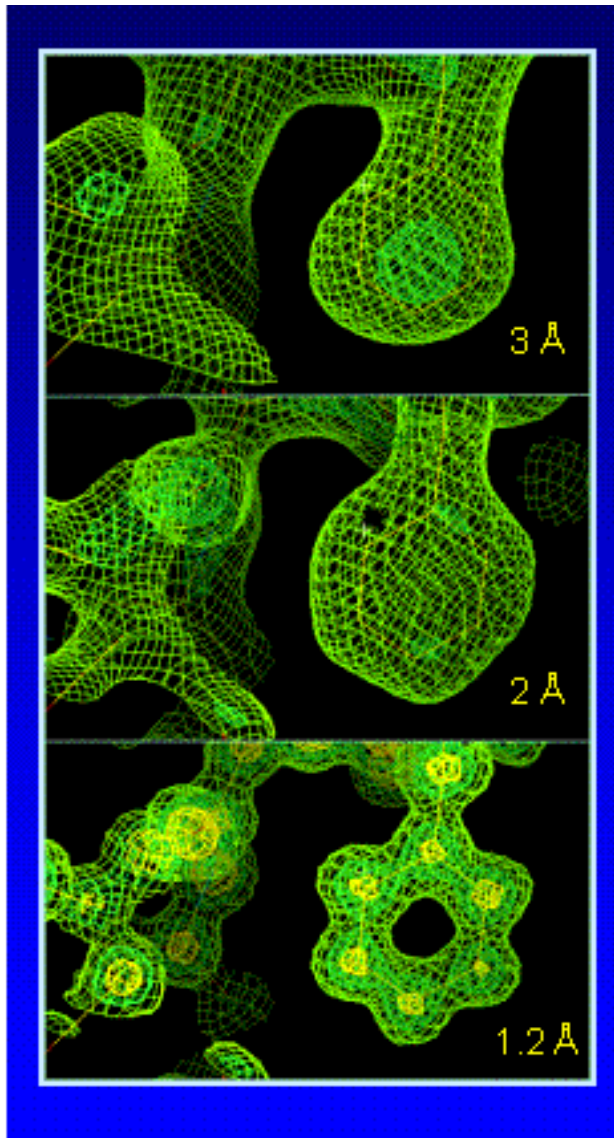


Electron density map



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.

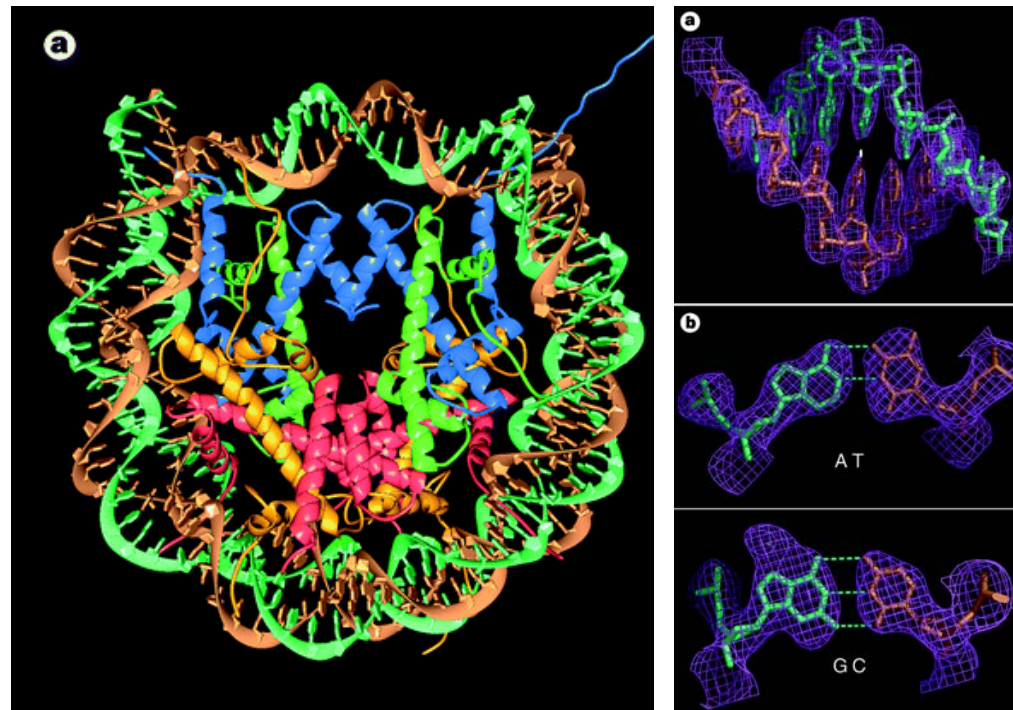
<http://www.ruppweb.org/Xray/101index.html>

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

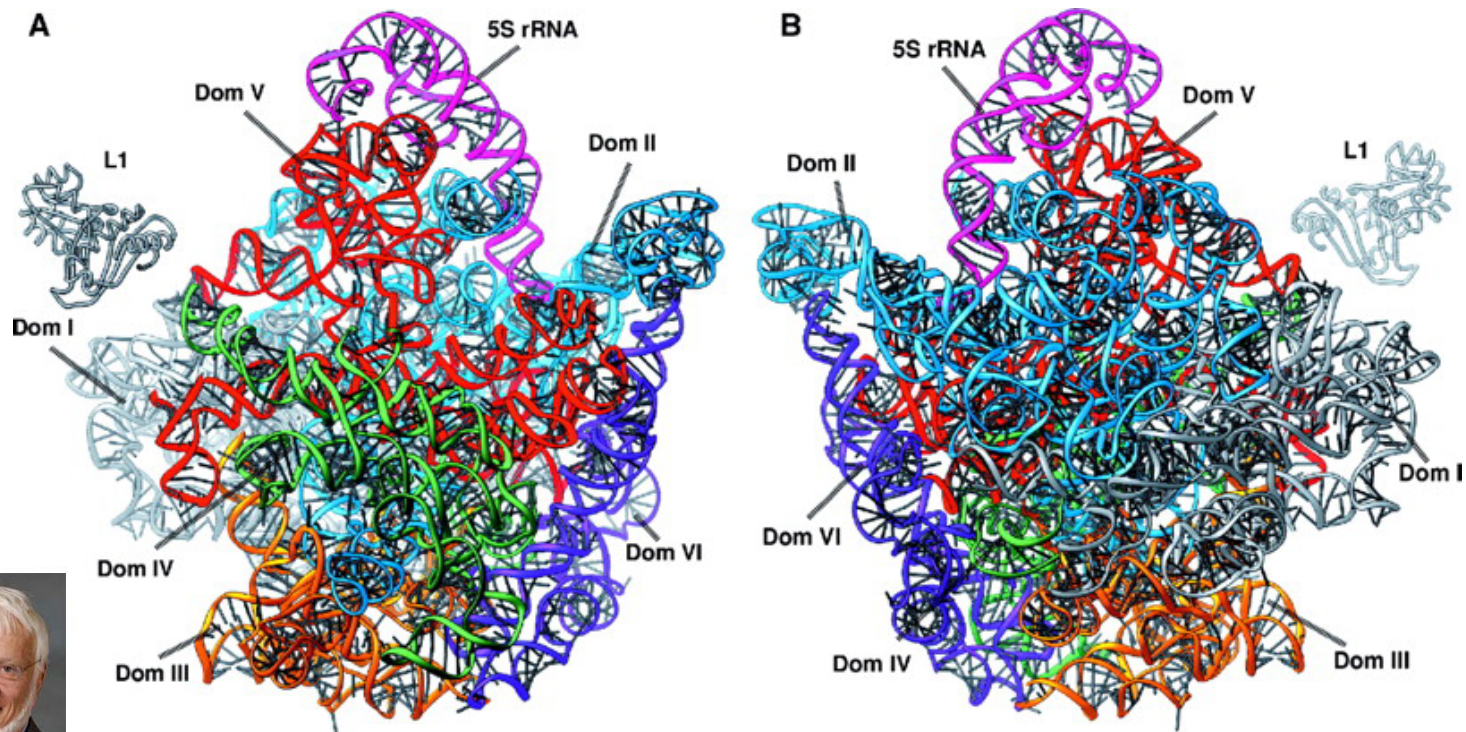
Institut für Molekularbiologie und Biophysik ETHZ, ETH-Hönggerberg, CH-8093 Zürich, Switzerland

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.



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/

The screenshot shows the RCSB PDB website homepage. At the top left is the RCSB PDB logo (Protein Data Bank). At the top right, it states "A MEMBER OF THE PDB" and "An Information Portal to Biological Macromolecular Structures". Below this, it says "As of Tuesday Jan 25, 2011 at 4 PM PST there are 70813 Structures" and provides links for "PDB Statistics". A search bar is located below the header, with a dropdown menu for "PDB ID or Text" and a "Search" button. A sidebar on the left contains navigation links for "MyPDB", "Home", and "Deposition". The main content area features a heading "A Resource for Studying Biological Macromolecules" followed by introductory text. Below this is a "Featured Molecules" section with a "Structural View of Biology" and a "Molecule of the Month" section for "Nitric Oxide Synthase". On the right side, there are sections for "Customize This Page", "New Features", "RCSB PDB News", and "NJ Science Olympiad Protein Modeling Results".

RCSB PDB
PROTEIN DATA BANK

A MEMBER OF THE **PDB**
An Information Portal to Biological Macromolecular Structures
As of Tuesday Jan 25, 2011 at 4 PM PST there are 70813 Structures | PDB Statistics

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Deposition Policies
Website FAQ
Deposition FAQ
Contact Us
About Us
Careers
External Links
Sitemap
New Website Features

Deposition Hide
All Deposit Services
Electron Microscopy
X-ray | NMR
Validation Server
BioSync Beamline
Related Tools

A Resource for Studying Biological Macromolecules

The PDB archive contains information about experimentally-determined structures of proteins, nucleic acids, and complex assemblies. As a member of the **wwPDB**, the RCSB PDB curates and annotates PDB data according to agreed upon standards.

The RCSB PDB also provides a variety of tools and resources. Users can perform simple and advanced searches based on annotations relating to sequence, structure and function. These molecules are visualized, downloaded, and analyzed by users who range from students to specialized scientists.

Hide Welcome Message

Featured Molecules Hide
List View of Archive By: Title | Date | Category

Structural View of Biology

Infrastructure & Communication

Molecule of the Month:
Nitric Oxide Synthase
Nitroglycerin is a powerful explosive, detonating when exposed to heat or pressure. The same molecule, however, can save your life if you're experiencing a heart attack. A small dose of nitroglycerin will slowly break down and release nitric oxide (NO), which then spreads to the muscle cells surrounding blood vessels, telling them to relax.

Customize This Page

New Features Hide
Binding Affinity in Tabular Reports
Latest features released:
Website Release Archive:

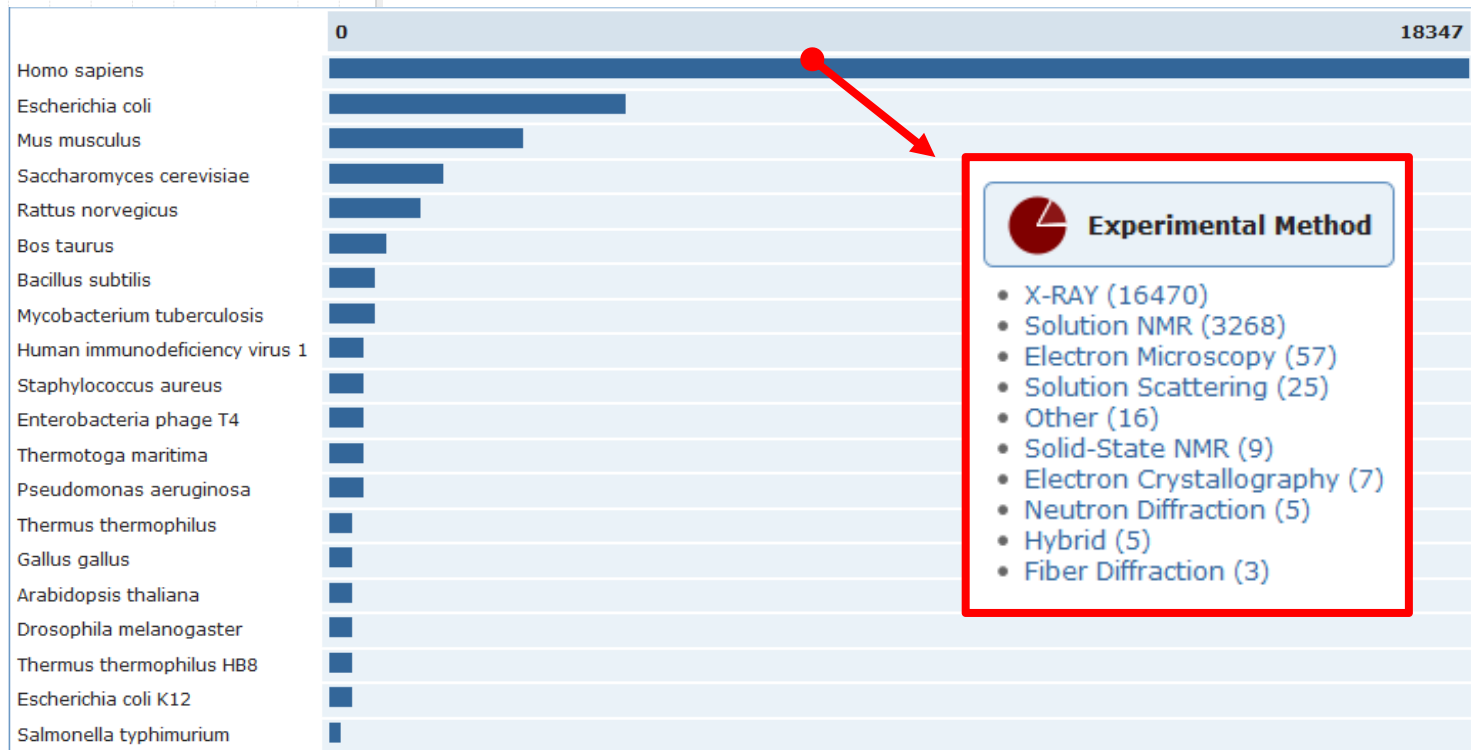
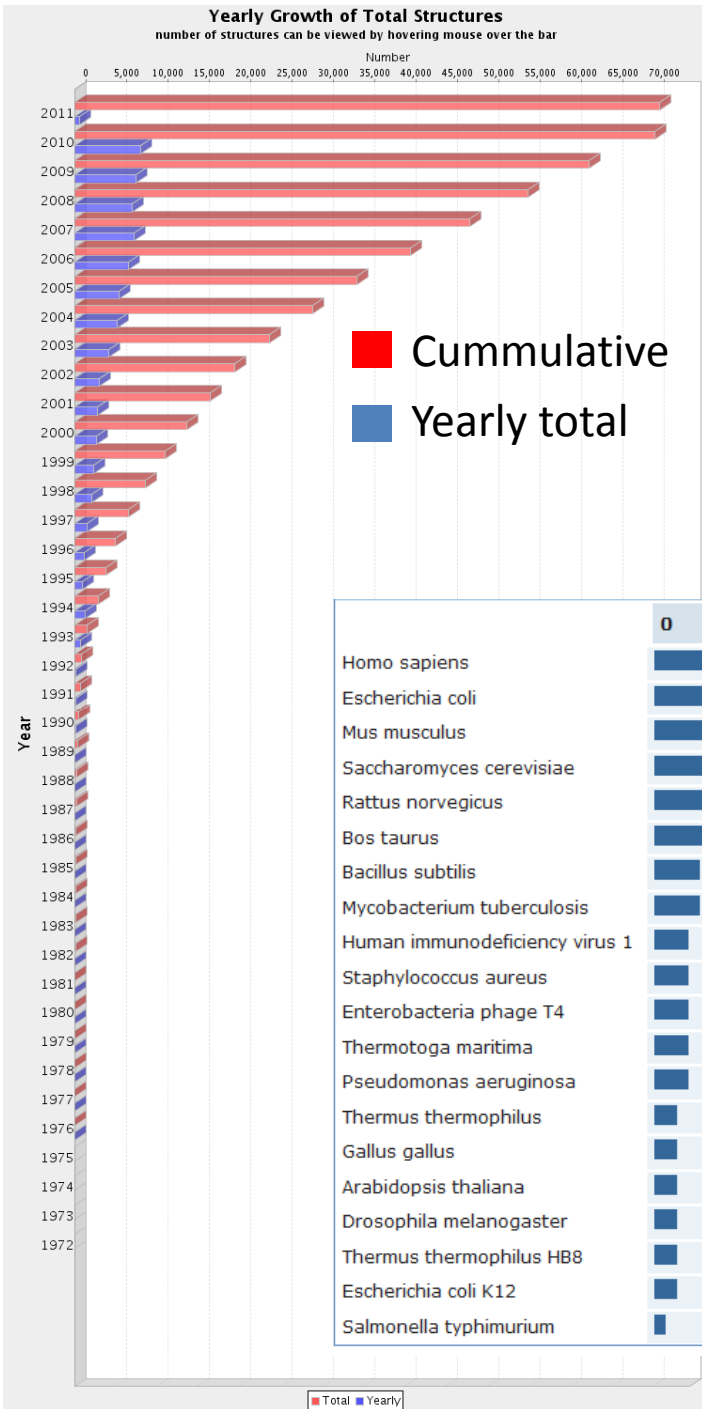
RCSB PDB News Hide
Weekly | Quarterly | Yearly
2011-01-25
NJ Science Olympiad Protein Modeling Results

The RCSB PDB is proud to...

PDB Growth

PDB has
 ~ 70,000 structures
 example:
 ~ 1,000 membrane proteins

What species are the structures from?

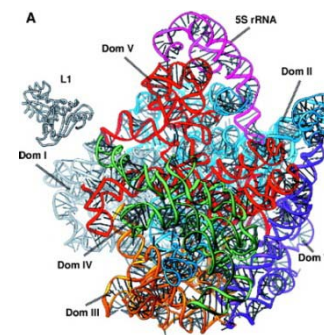
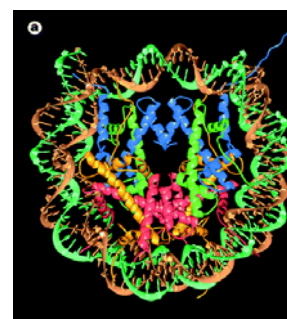


Experimental Method

- X-RAY (16470)
- Solution NMR (3268)
- Electron Microscopy (57)
- Solution Scattering (25)
- Other (16)
- Solid-State NMR (9)
- Electron Crystallography (7)
- Neutron Diffraction (5)
- Hybrid (5)
- Fiber Diffraction (3)

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/>