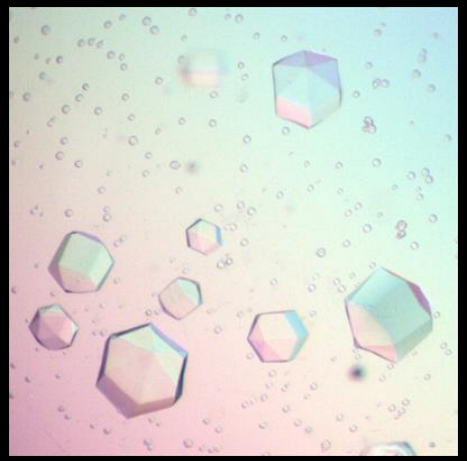
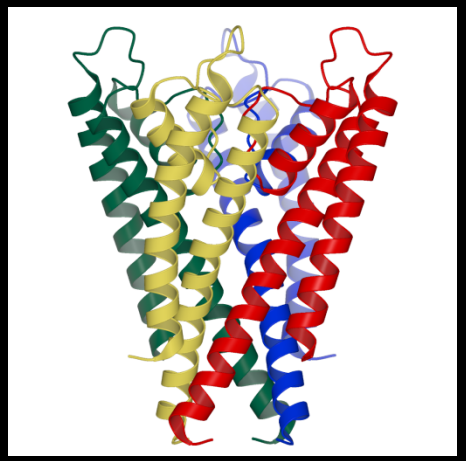
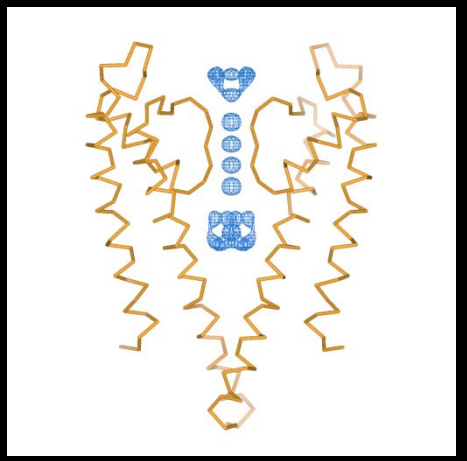




# Introduction to X-ray Crystallography

Jesse Rinehart

Department of Cellular & Molecular Physiology
Systems Biology Institute

Contributions to this lecture:

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

**Yufeng Zhou, PhD**
Yale, Department of Cellular & Molecular Physiology

**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

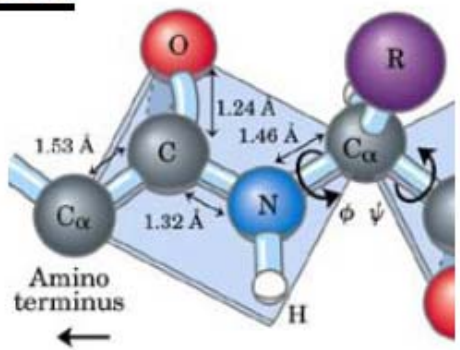
http://ucxray.berkeley.edu/~jamesh/movies/
movies demonstrating diffraction, resolution, data quality, and refinement.

**“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.

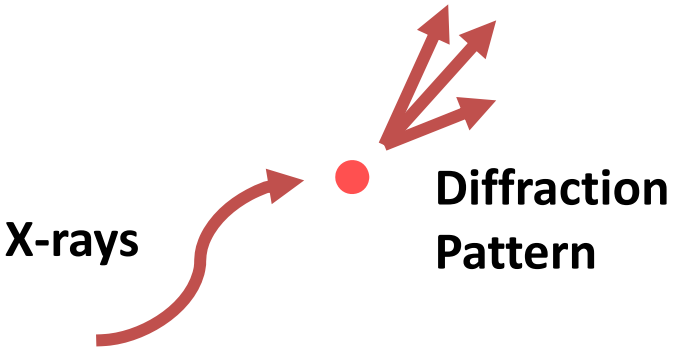
Distances between atoms are small:
Lab x-ray sources use CuK$\alpha$ radiation. Wavelength = 1.54 Å.
Synchrotron radiation wavelengths in the range 0.5 Å - 2.5 Å.




Yong Xiong

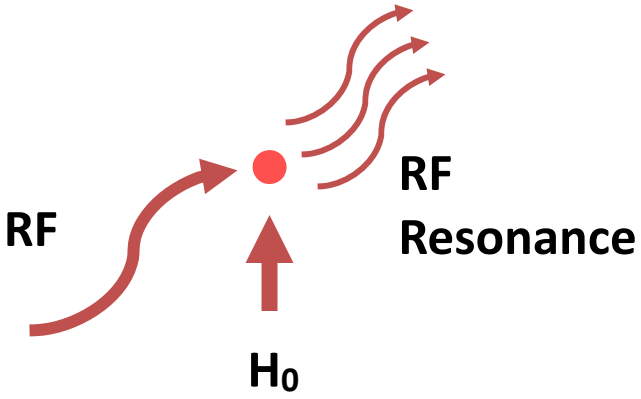
## Experimental Determination of Atomic Resolution Structures

### X-ray




- Direct detection of atom positions
- Crystals

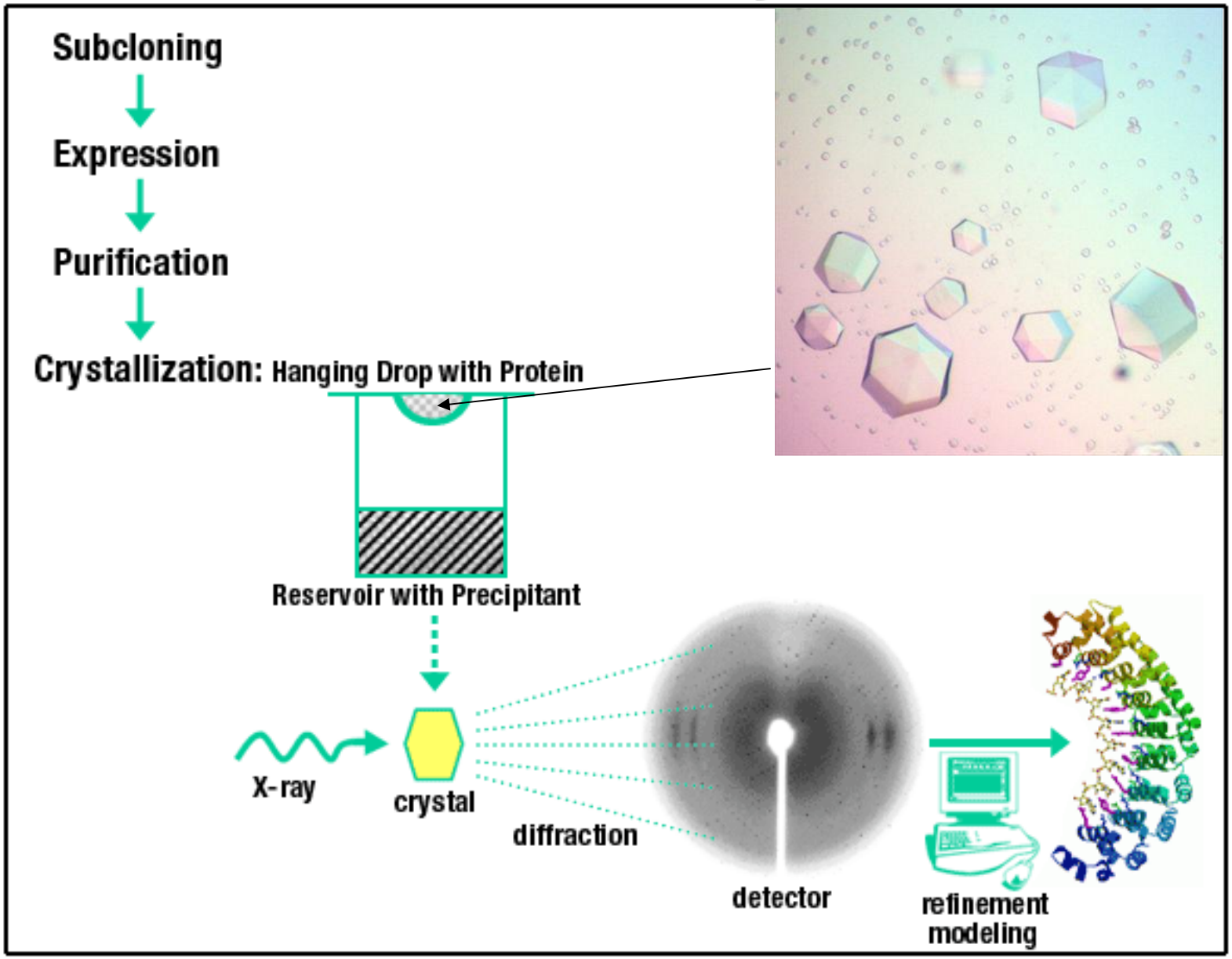
### NMR




- Indirect detection of H-H distances
- In solution

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

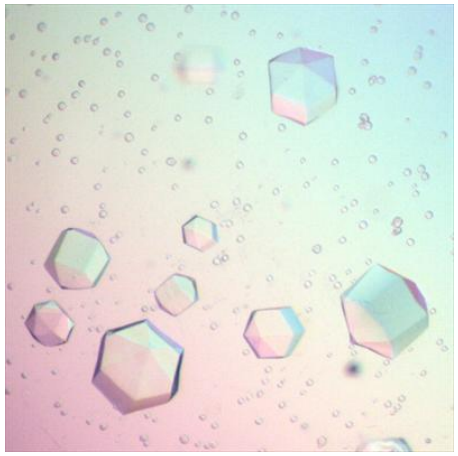
# Determination of Protein Crystal Structure




Yufeng Zhou

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

# Why Crystals?



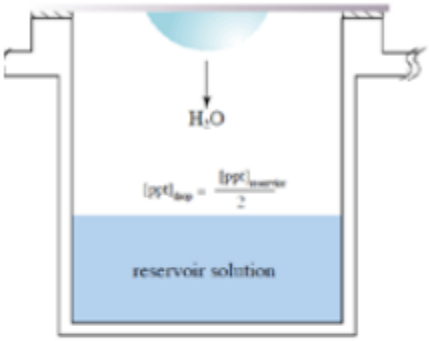
X-rays are scattered by electrons, too weak to record scattering from a single molecule. Crystals are therefore used because they present many molecules (N) in exactly the same orientation. The scattering from each of the N molecules interferes constructively to give a measurable diffraction pattern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

Yong Xiong

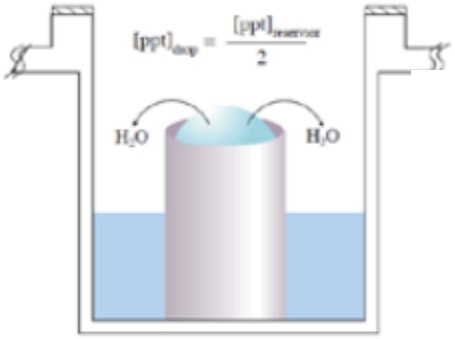
# Some Crystallization Methods:

Vapor diffusion

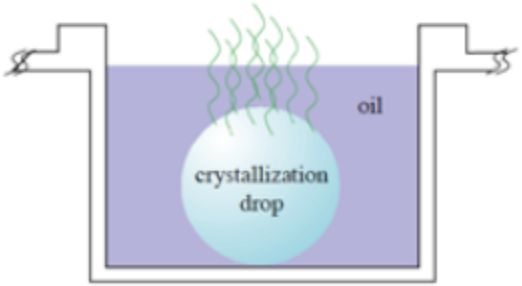
Hanging-drop



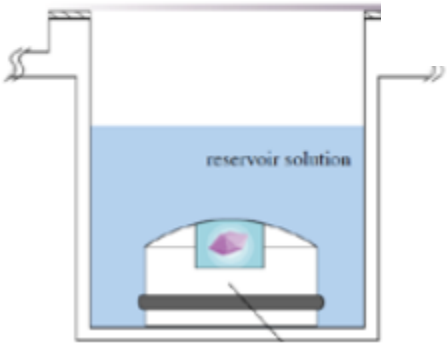

Sitting-drop

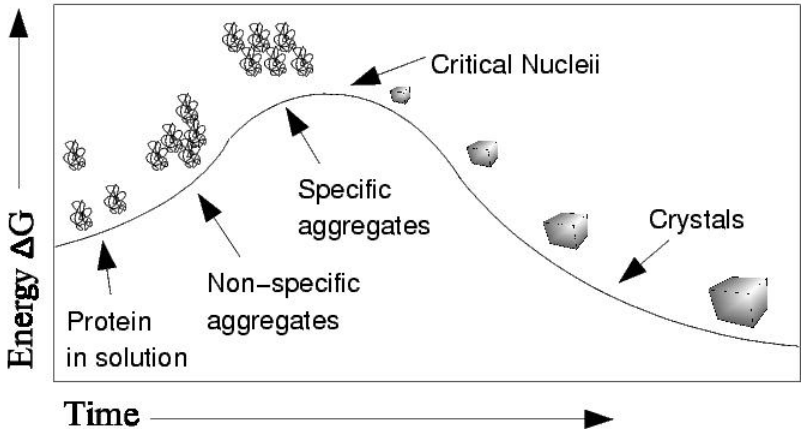



Batch:
micro batch under oil




Dialysis

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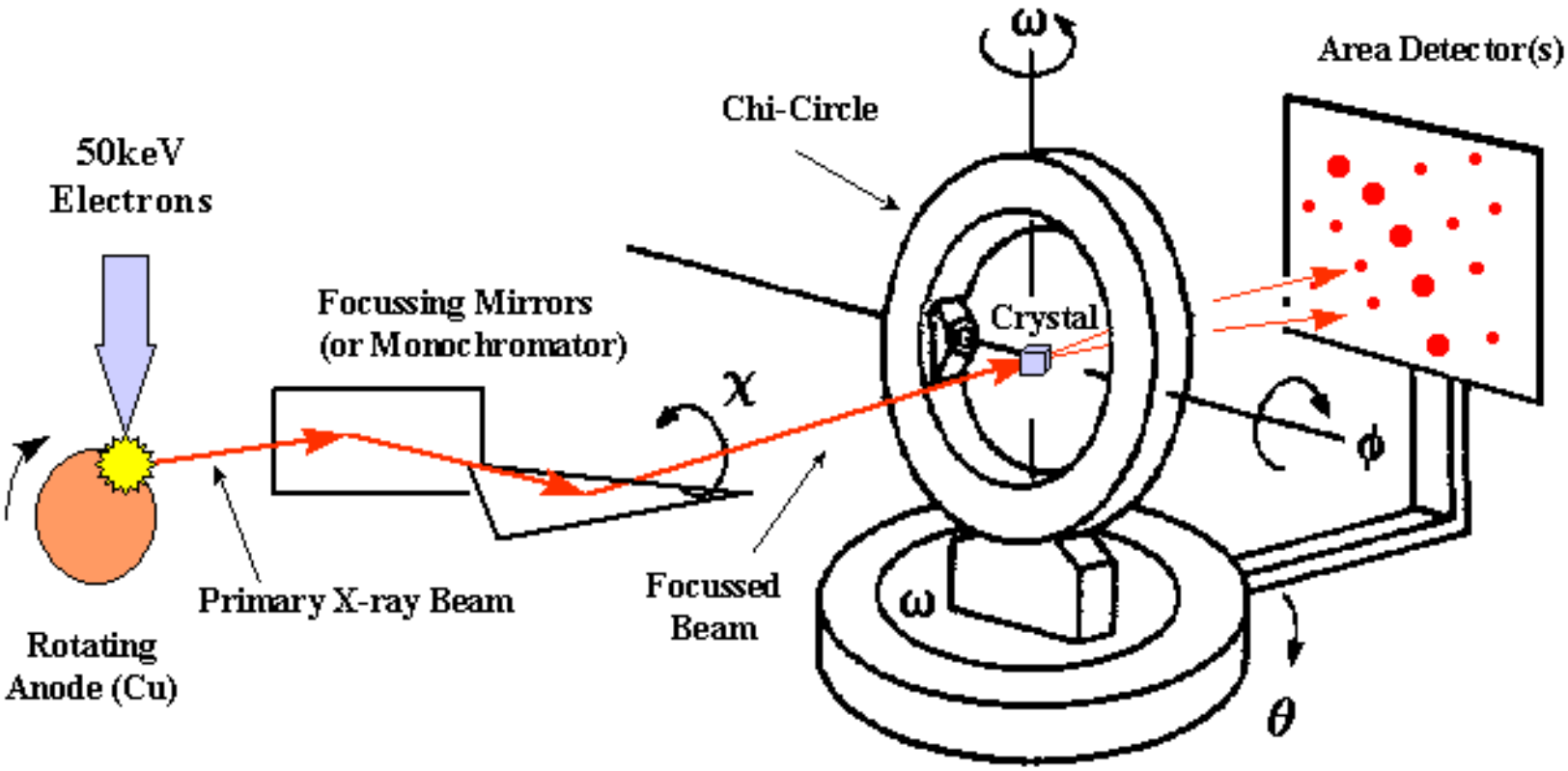
# Data Collection




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.**

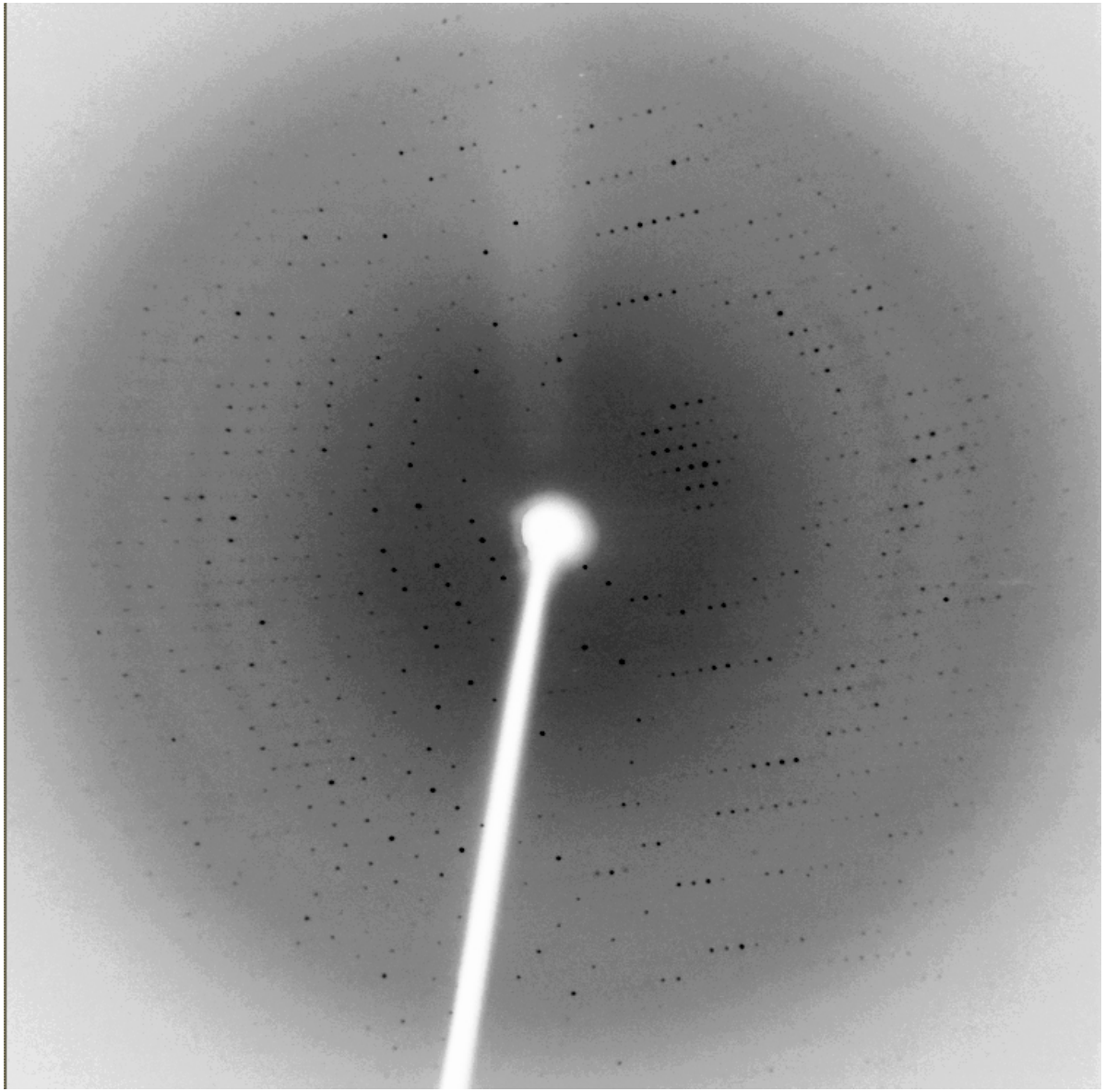
NSLS BNL

ALS Berkeley

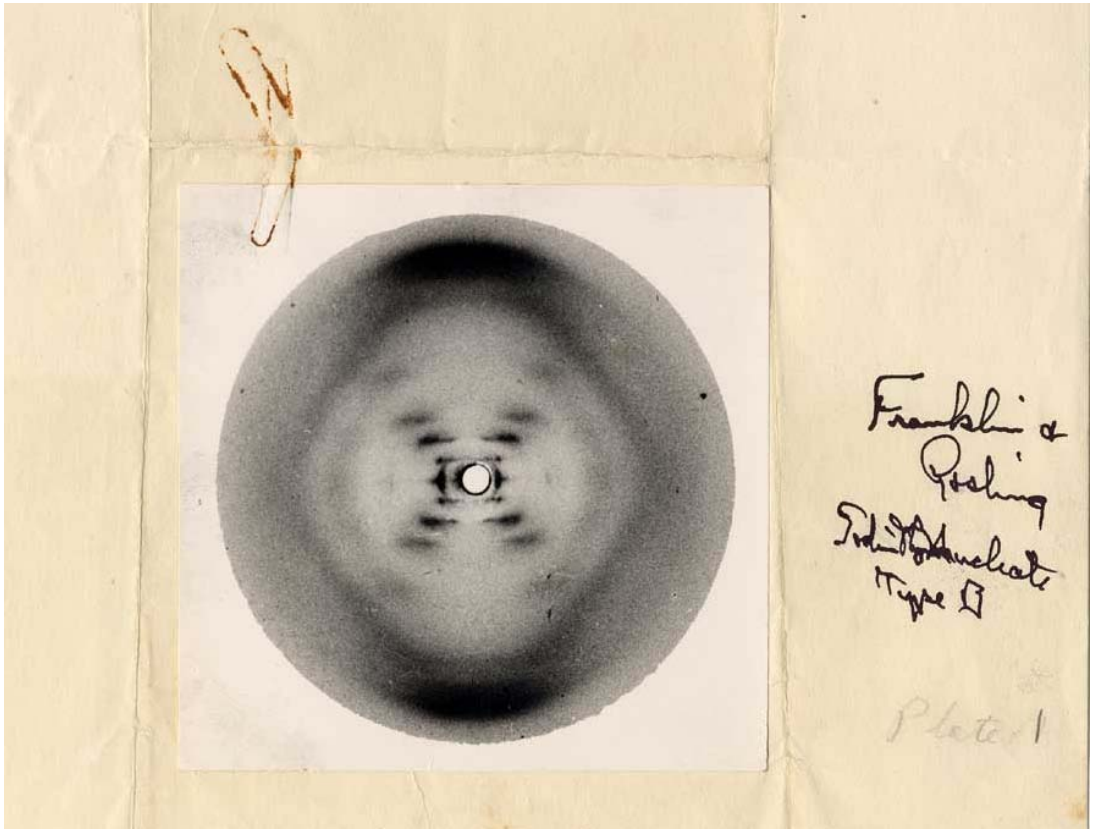
APS Chicago

CHESS Ithaca

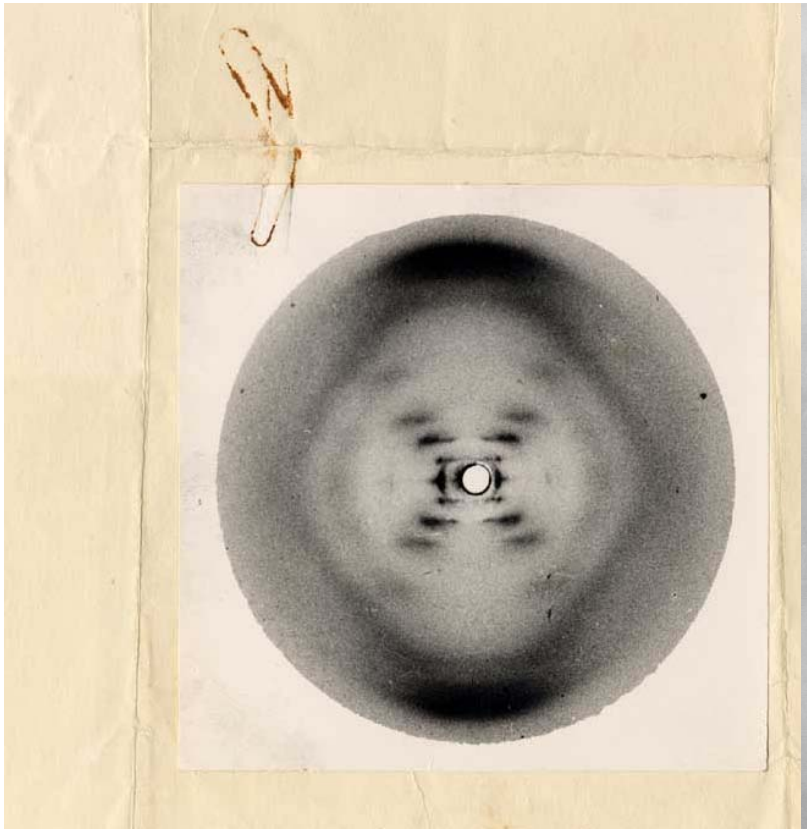
# Image of diffraction

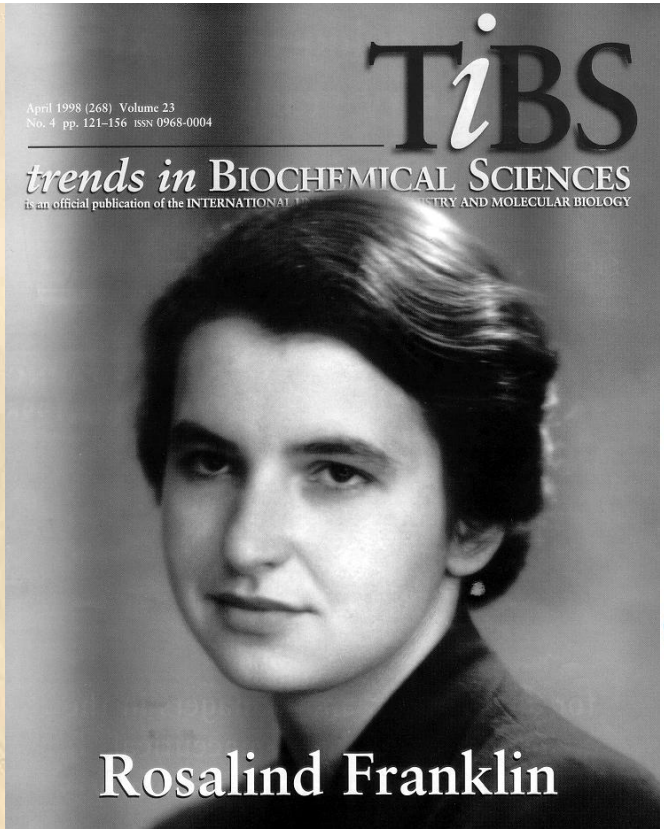


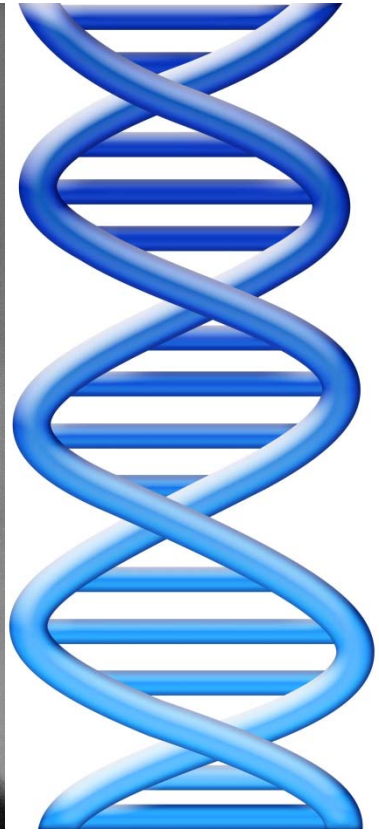
# Most famous X-ray diffraction pattern

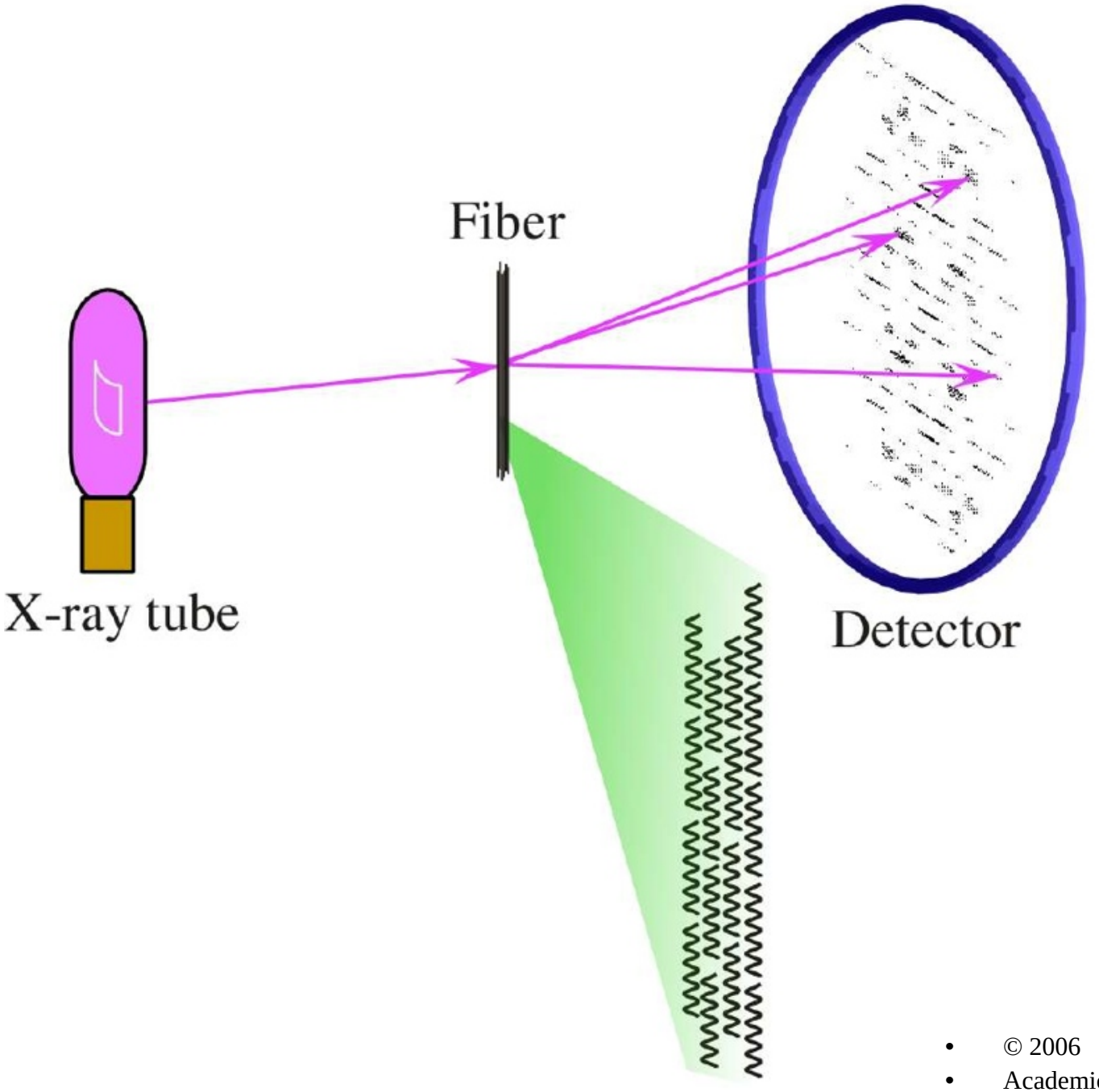


# Most famous X-ray diffraction pattern

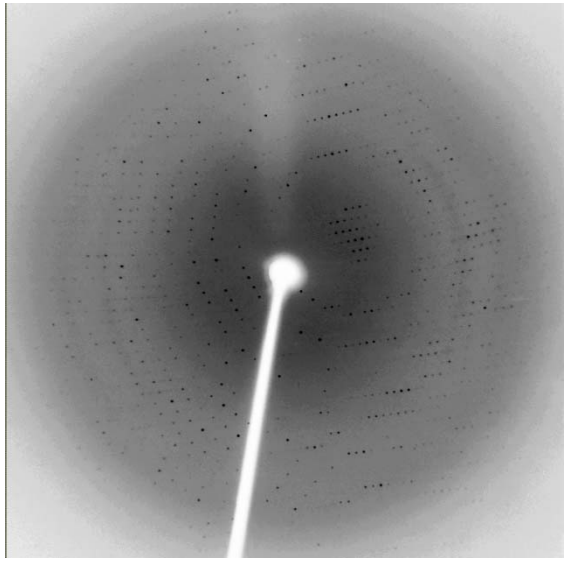






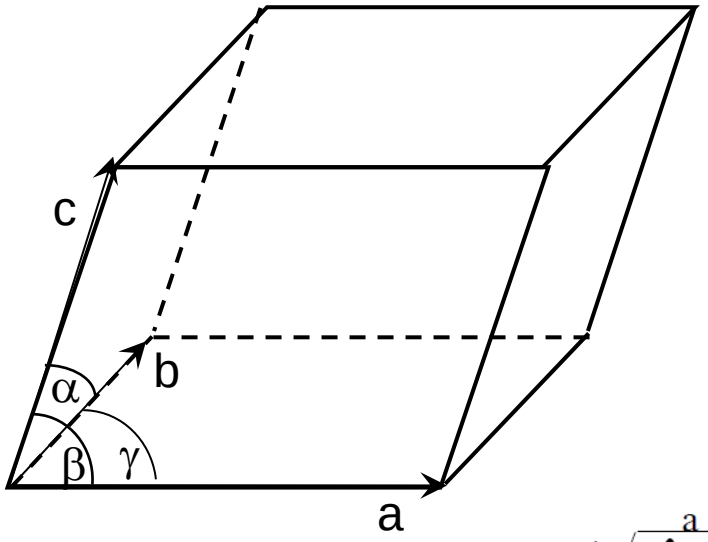
# The information we get from a single diffraction experiment



**Analyze the pattern of the reflections**

(a) space group of the crystal

(b) unit cell dimensions

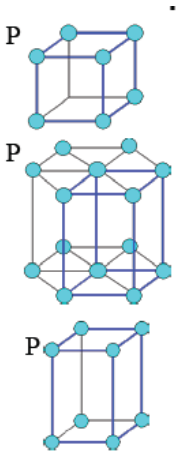



Cubic
a = b = c,
α = β = γ = 90°

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

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

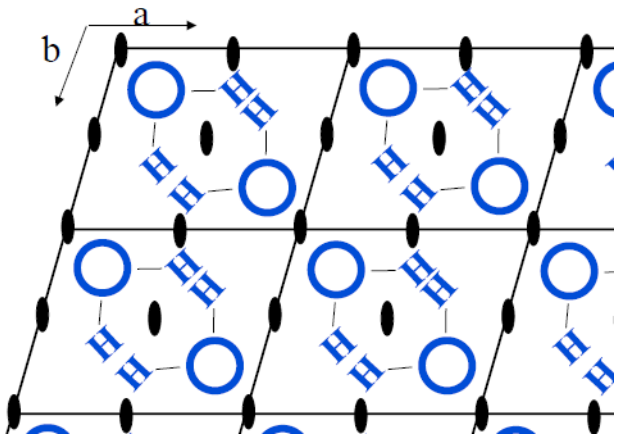
Tetragonal
a = b ≠ c,
α = β = γ = 90°



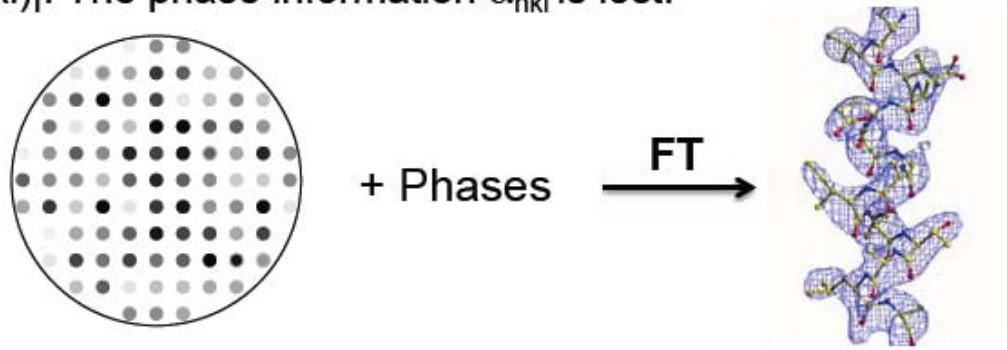

How to understand symmetry?

Crystal = lattice + unit cell content

(asymmetric units (asu) content)



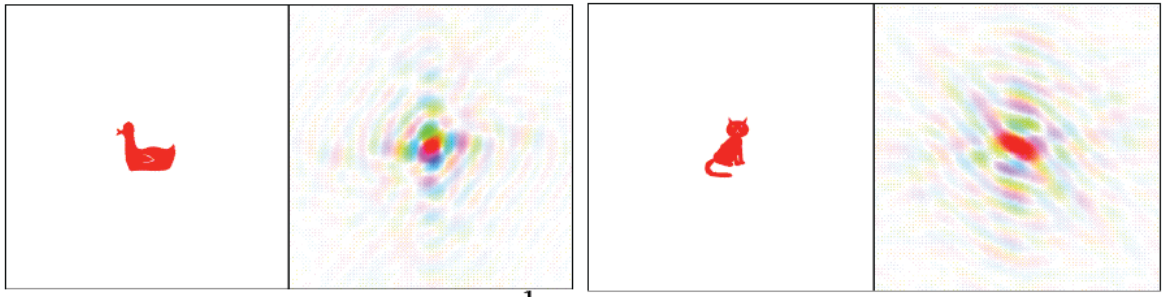
**The phase problem:** F(hkl) is a complex vector. Measured diffraction data give the amplitude |F(hkl)|. The phase information $\alpha_{hkl}$ is lost!




## How important are amplitude and phase?

Fourier Duck and his Fourier transform
Phase is color coded

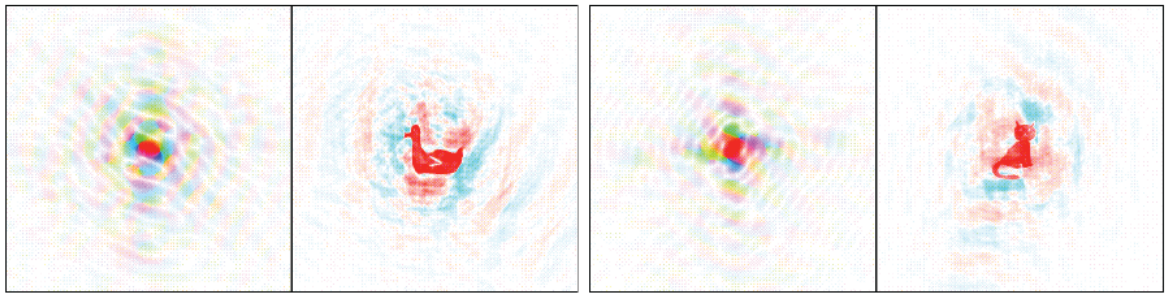
Fourier Cat and his Fourier transform
Phase is color coded



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

Duck phase and Cat amplitude

Cat phase and Duck amplitude



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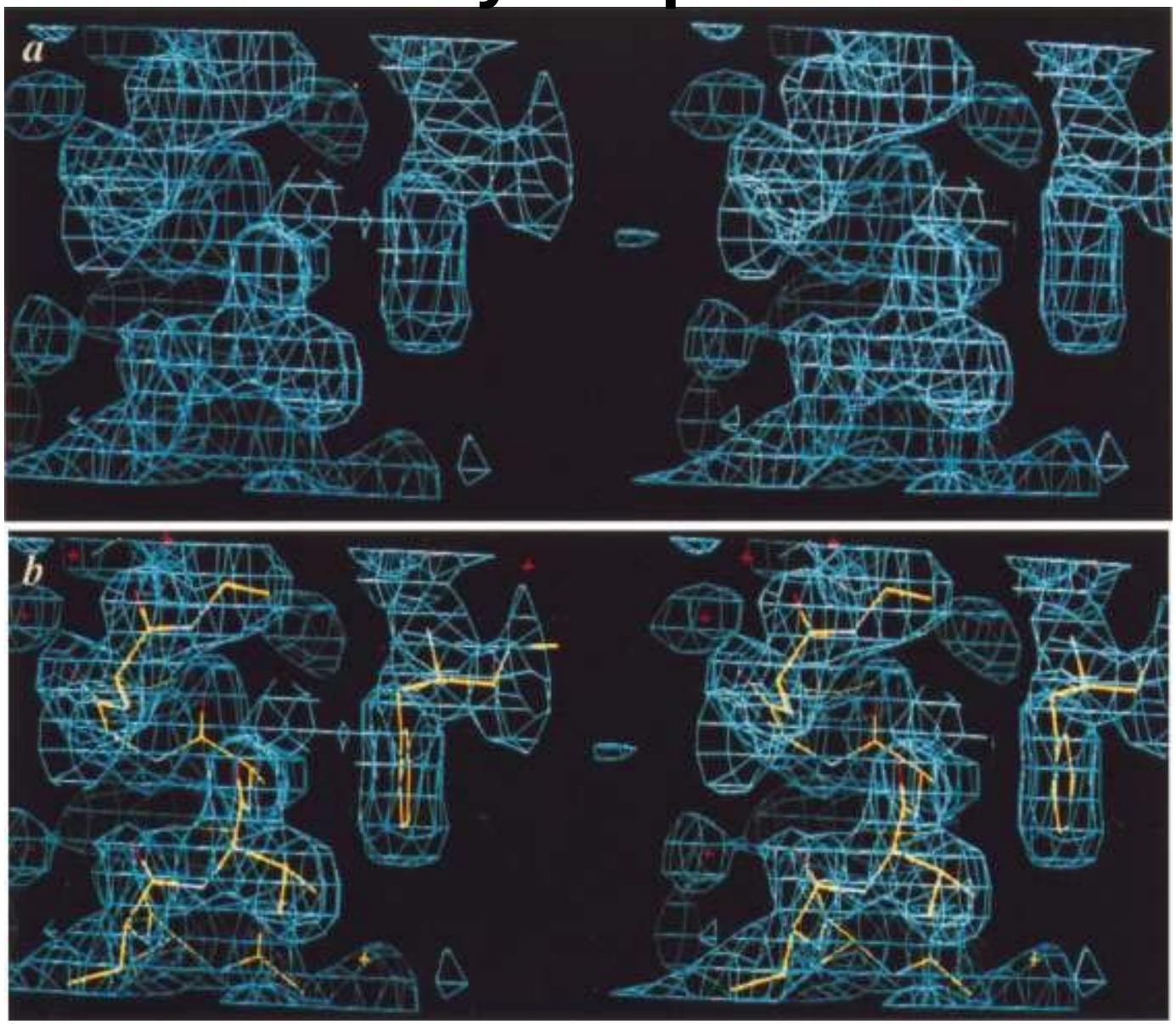
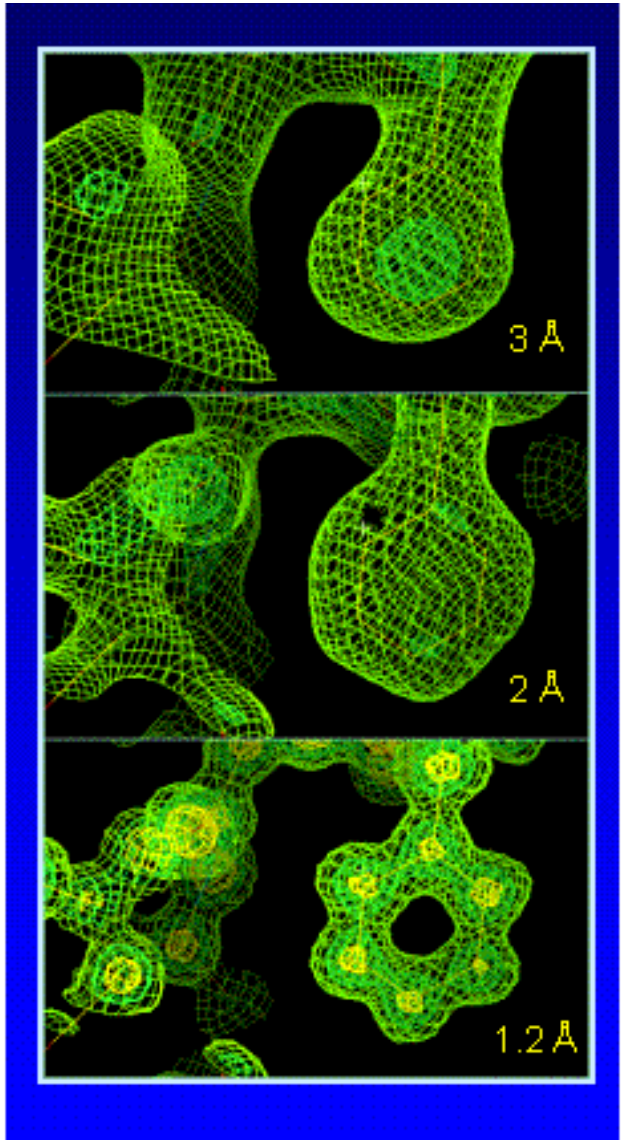
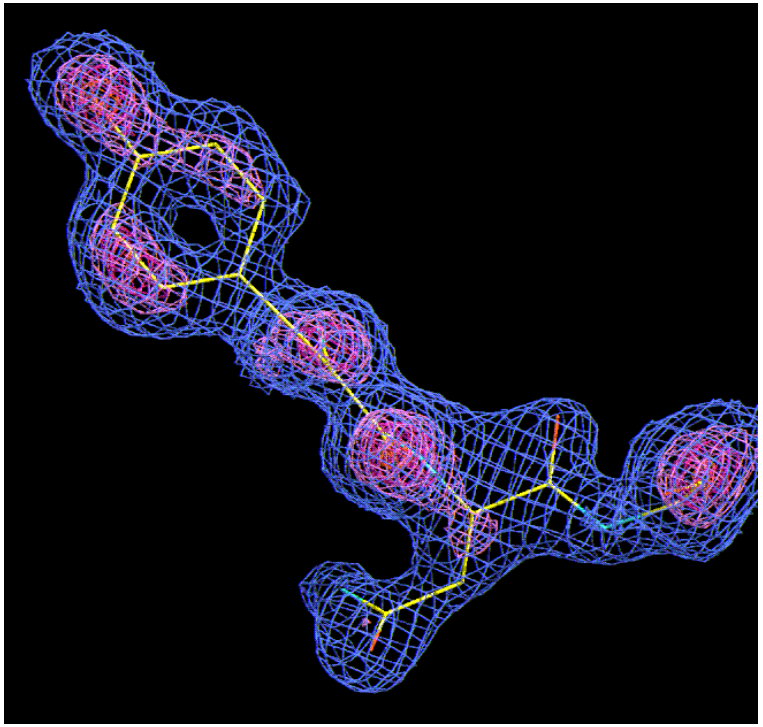
# Electron density map




# 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.

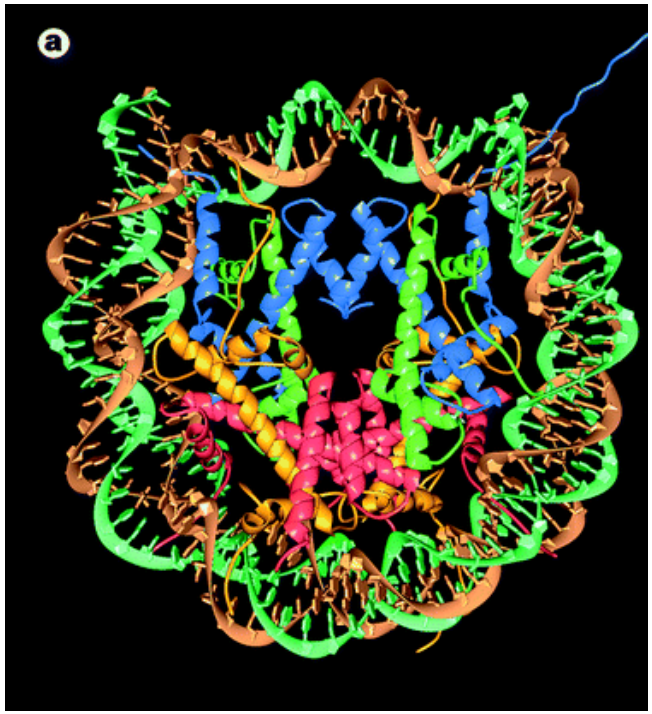
http://www.ruppweb.org/Xray/101index.html

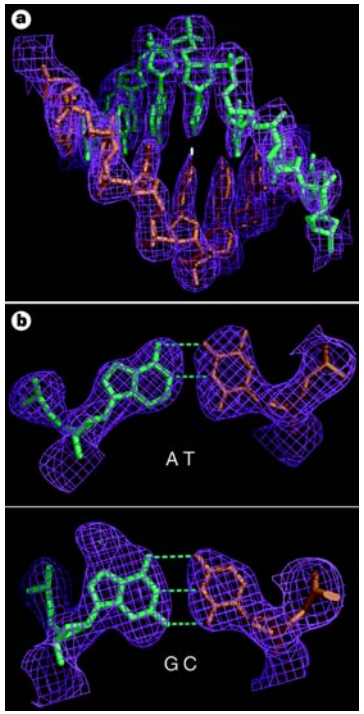
# Crystal structure of the nucleosome core particle at 2.8 Å resolution

**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.**



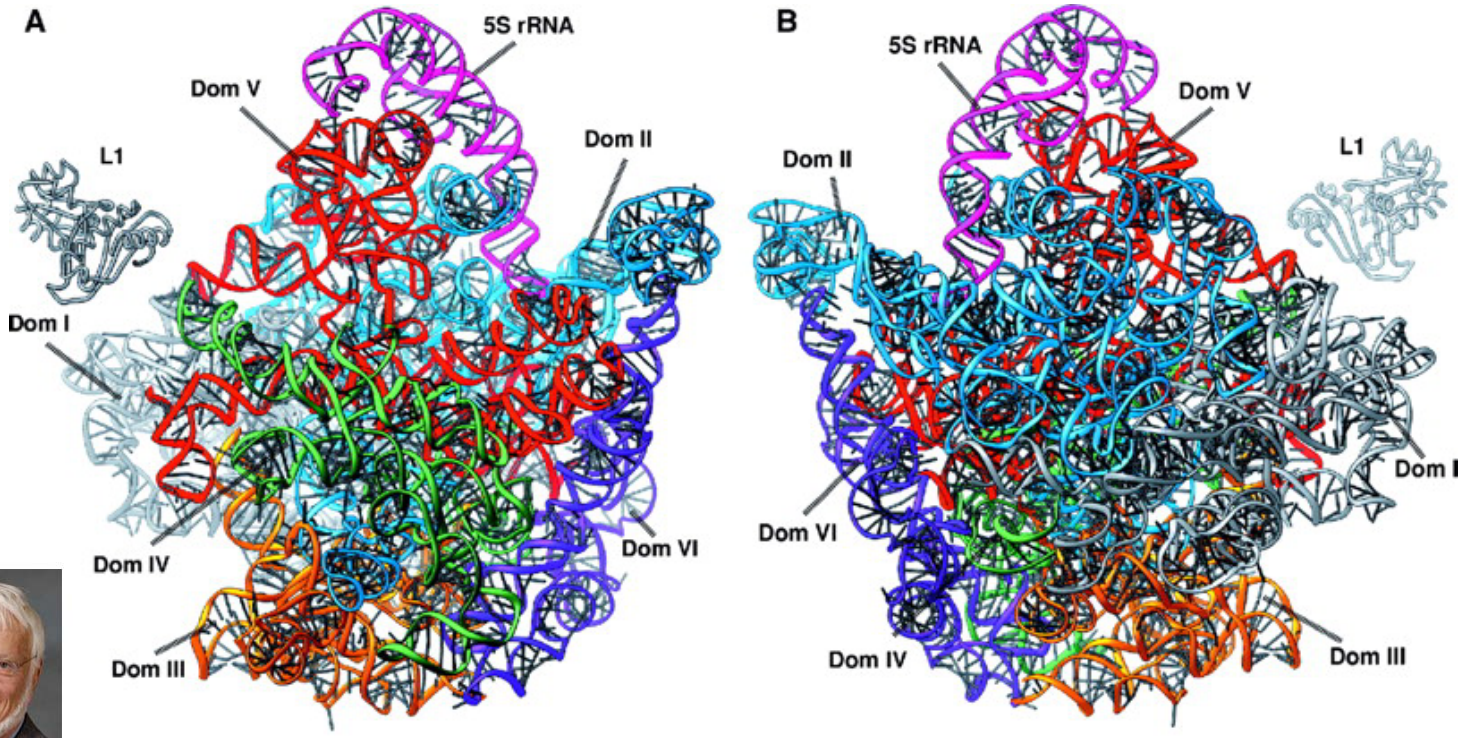



Science
AAAS



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

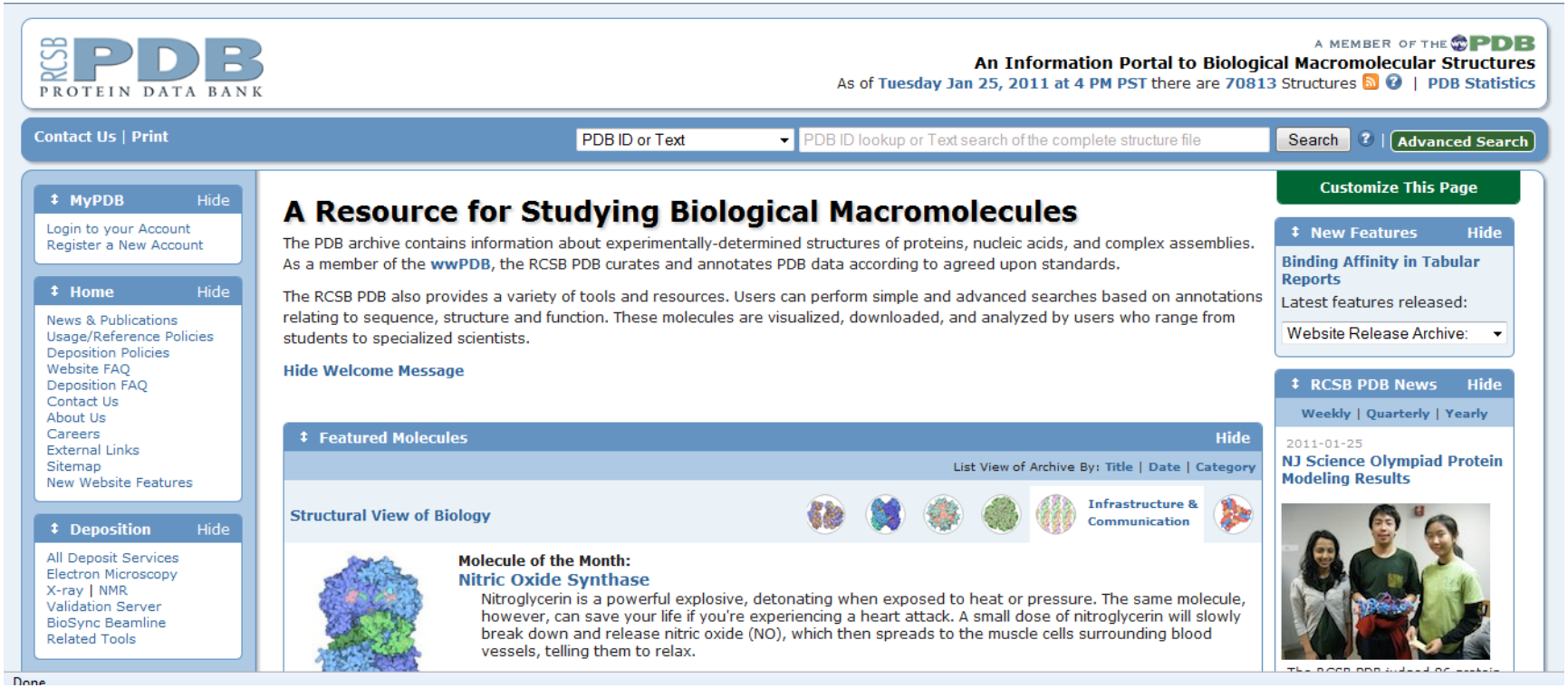
Nenad Ban,[1]* Poul Nissen,[1]* Jeffrey Hansen,[1] Peter B. Moore,[1,2]
Thomas A. Steitz[1,2,3]†



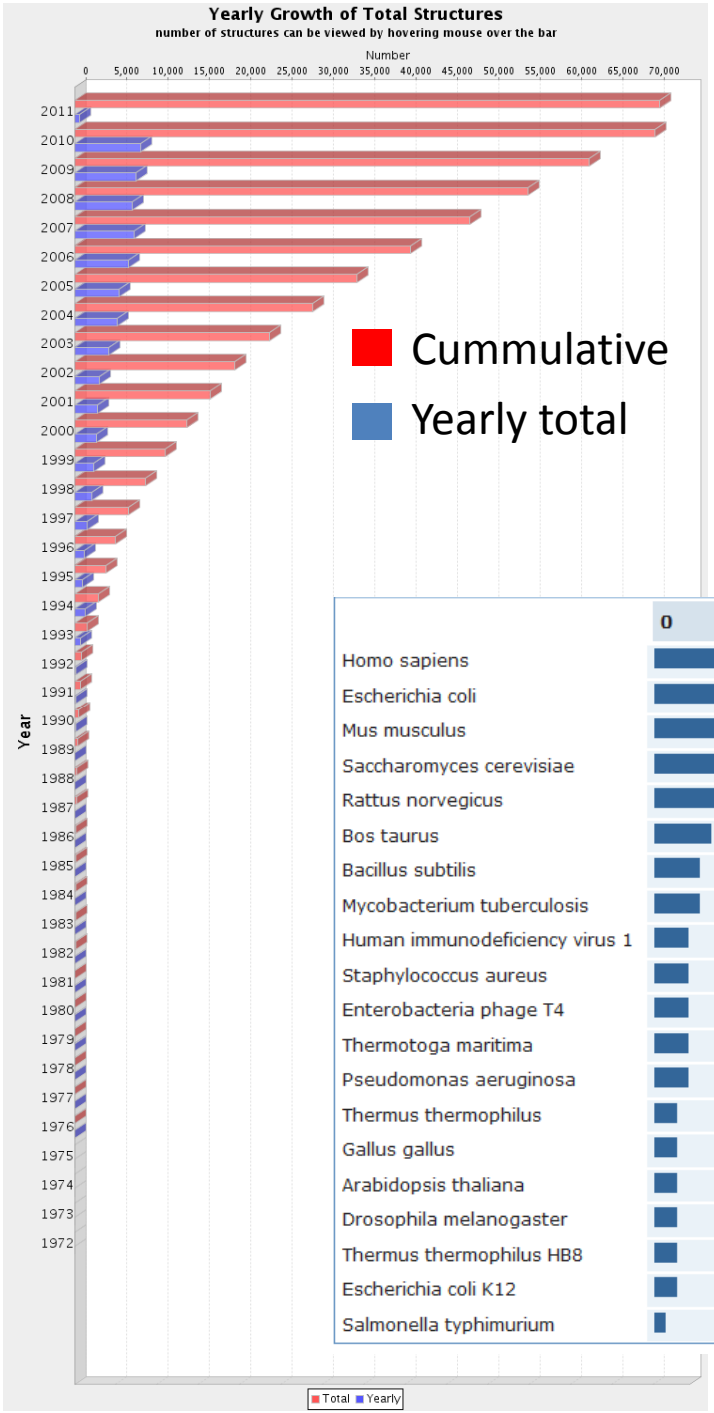

**Yale's Thomas Steitz shared 2009 Nobel Prize in Chemistry for this structure**

# 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/

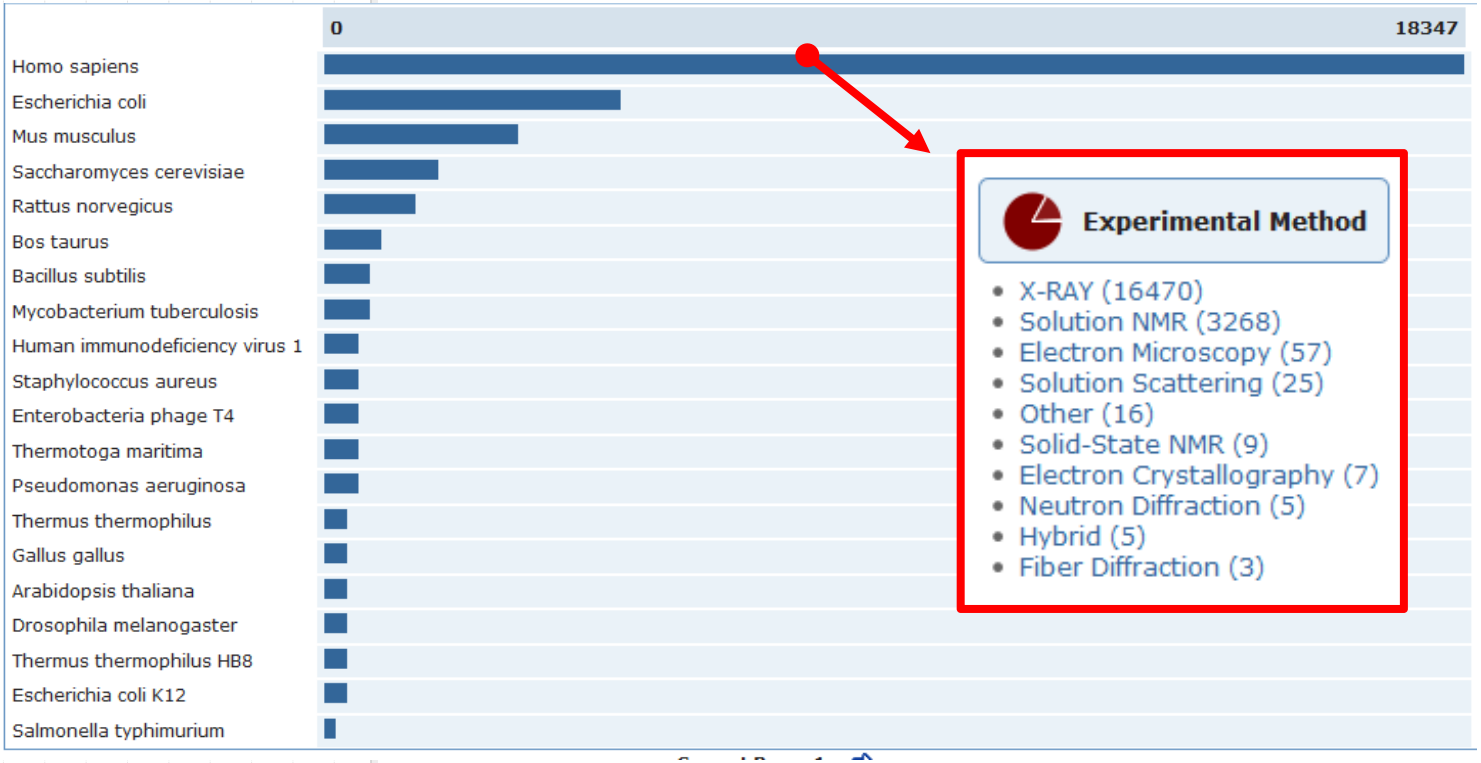


# PDB Growth



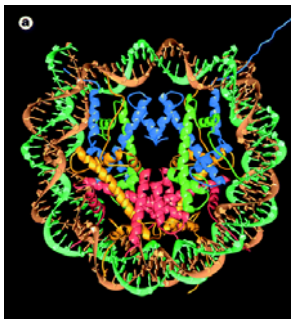

PDB has
~ 70,000 structures
example:
~ 1,000 membrane proteins

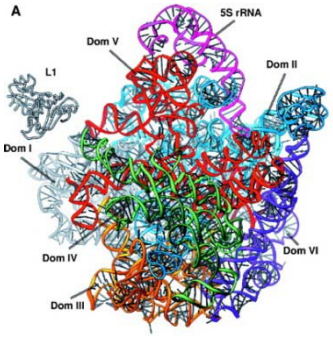
**What species are the structures from?**



## 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/**