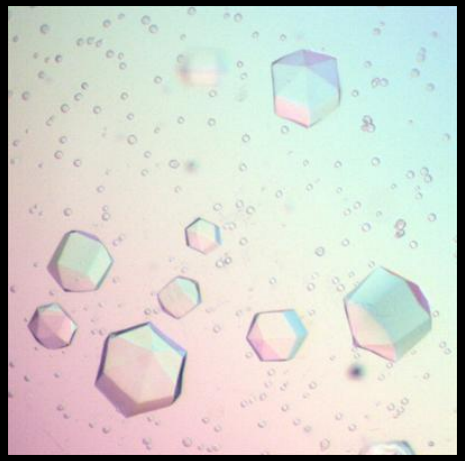
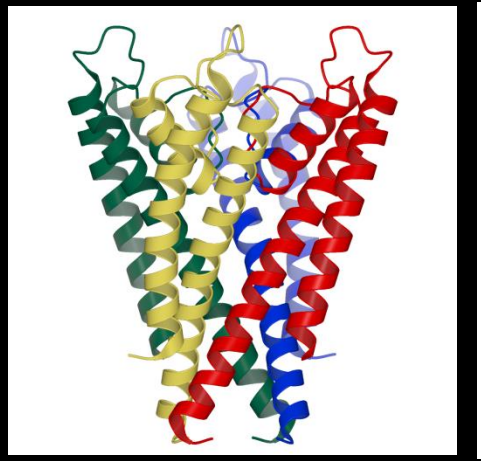
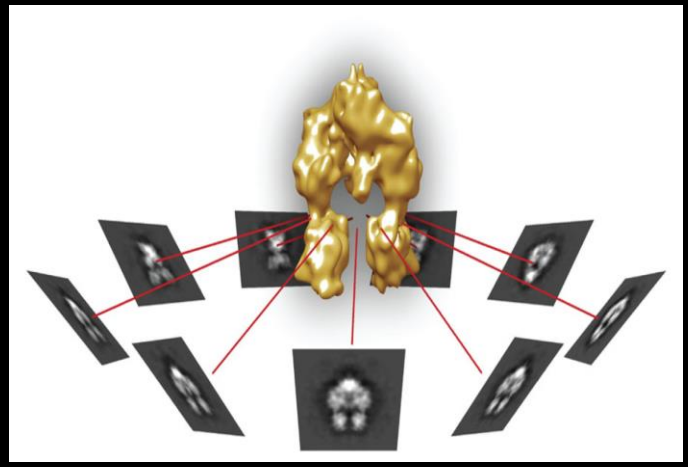




Structural Data:
Introduction to X-ray Crystallography & Cryo-EM

Jesse Rinehart, PhD

CBB 752, Spring 2017

Cellular & Molecular Physiology
Yale University School of Medicine

YALE SYSTEMS BIOLOGY INSTITUTE

Thank you to **Yong Xiong** and **Fred Sigworth** for contributions to this lecture

Yale Structure Courses:

C&MP 710b/MB&B 710b4, Electron Cryo-Microscopy for Protein Structure Determination
Fred J. Sigworth, C.V. Sindelar

MB&B 720a, Macromolecular Structure and Biophysical Analysis
Yong Xiong , Andrew Miranker, Anna Marie Pyle

MB&B 721b, Macromolecular Interactions and Dynamic Properties
Anna Pyle, Donald Engelman, Elizabeth Rhoades, Hongwei Wang

MB&B 760b3: Principles of Macromolecular Crystallography
Thomas Steitz

MB&B 761b4: X-ray Crystallography Workshop
Yong Xiong, Yorgo Modis, and staff

Pharmacology 529b: Structural Pharmacology
Ya Ha, Titus Boggon

Additional Resources:

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

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

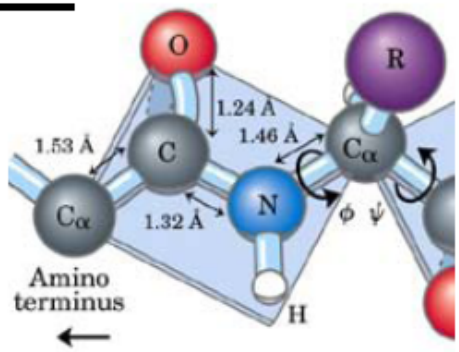
"Single particle electron cryomicroscopy: trends, issues and future perspective."
Vinothkumar KR, Henderson R. Q Rev Biophys. 2016 pubmed:27658821

“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.



Yong Xiong

The 2014 Nobel Prize in Chemistry: Eric Betzig, W.E. Moerner, and Stefan Hell
"The development of super-resolved fluorescence microscopy"

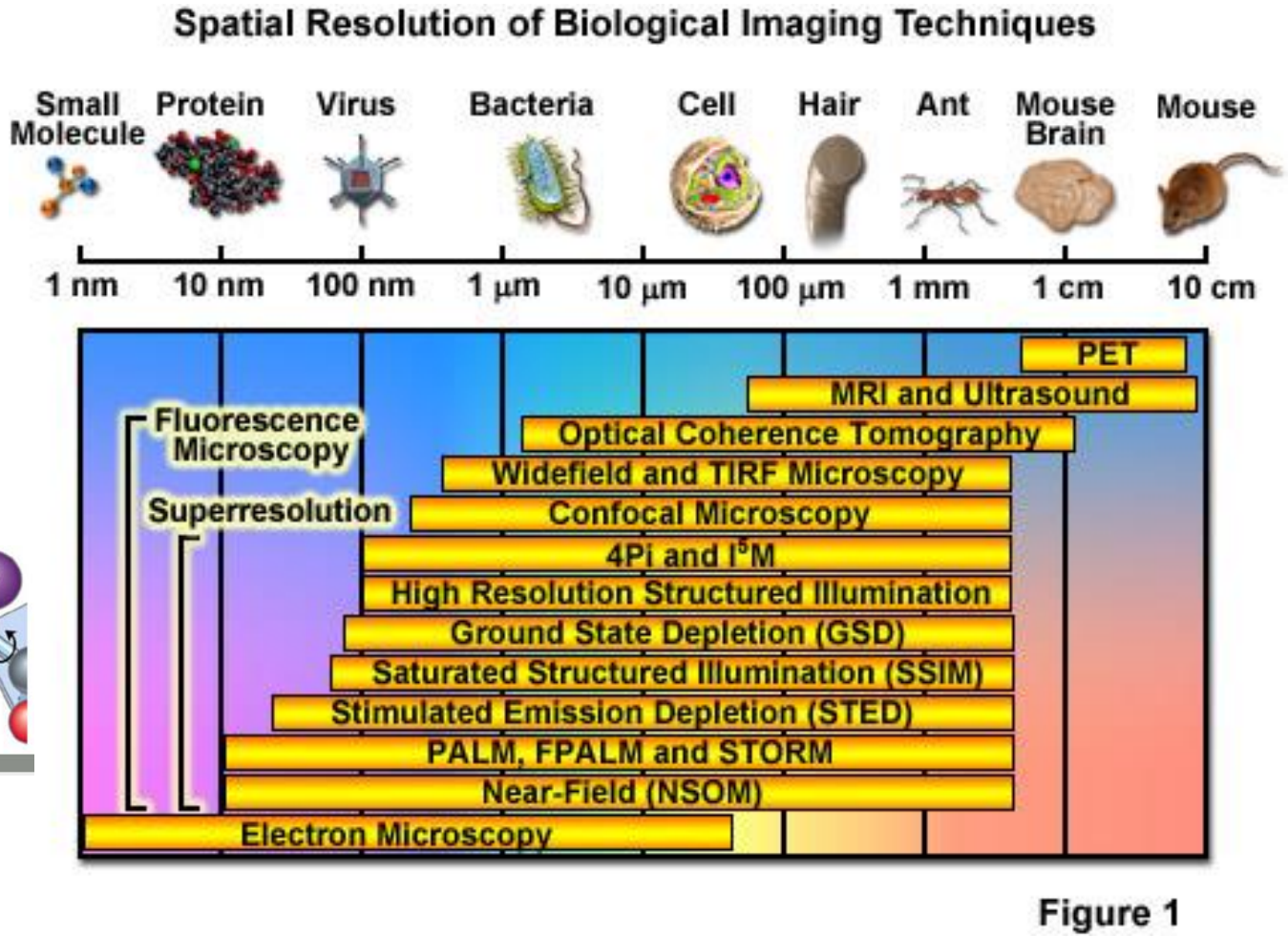
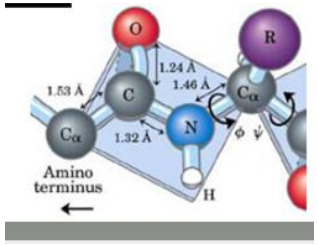


Figure 1

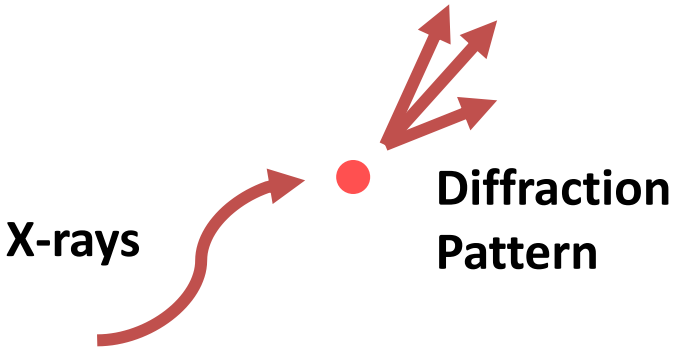
1Å = 0.1nm



http://zeiss-campus.magnet.fsu.edu/articles/superresolution/introduction.html

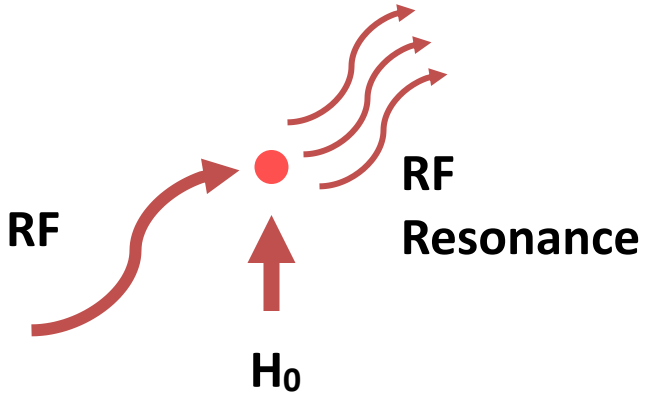
Experimental Determination of Atomic Resolution Structures

X-ray



- Direct detection of atom positions
- Crystals

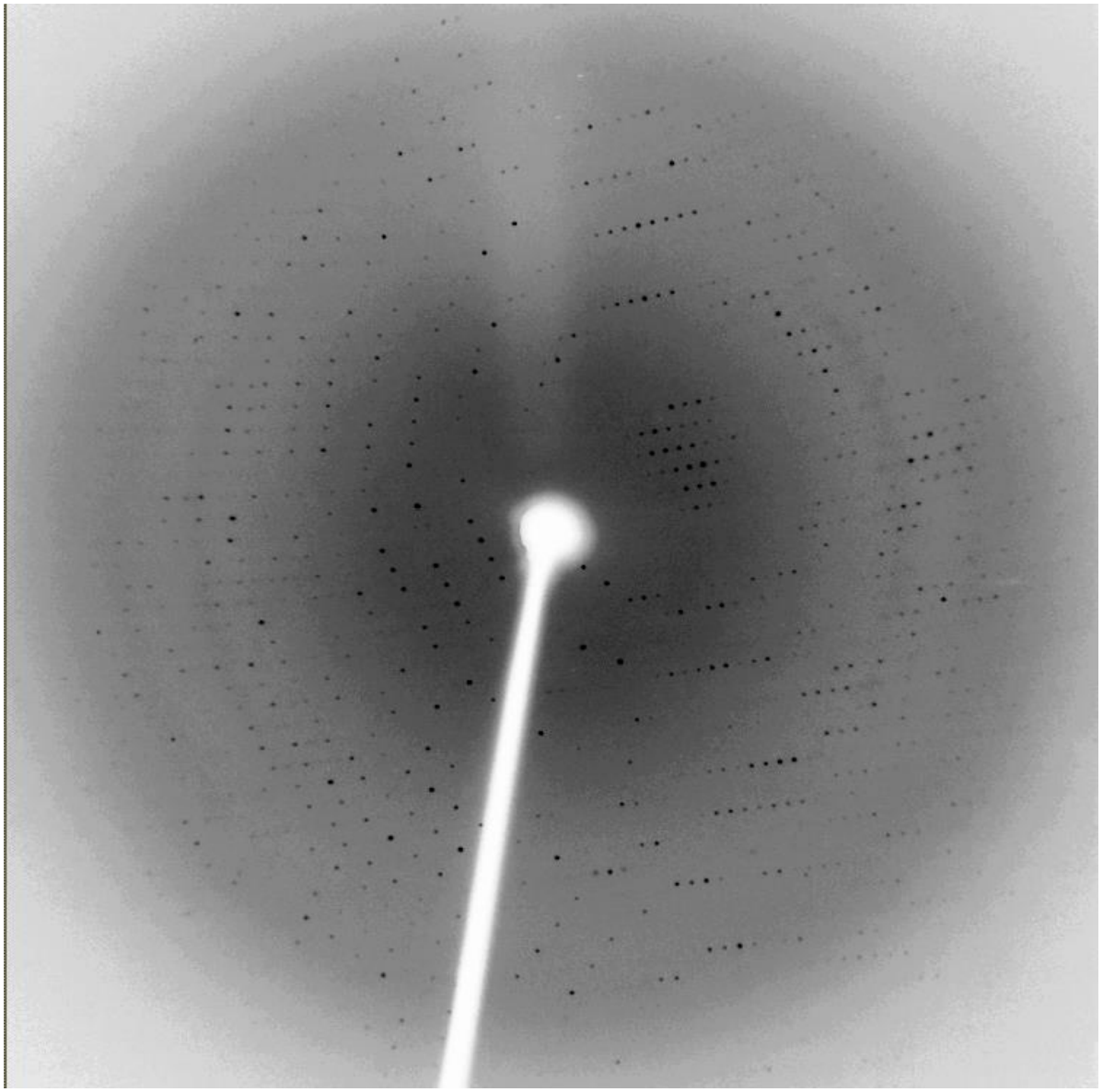
NMR



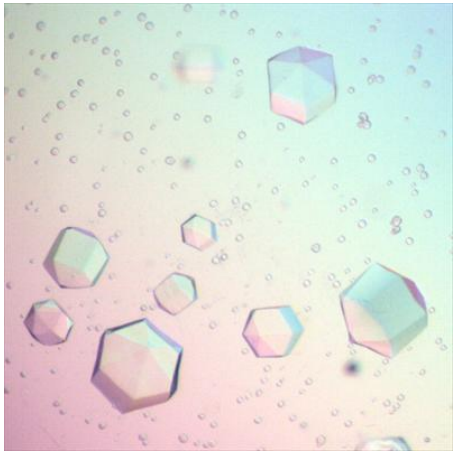
- Indirect detection of H-H distances
- In solution

Other methods for determining protein structures:
-EM (Electron Microscopy), Cryo-EM, ESR/Fluorescence

Image of X-ray diffraction of a protein crystal



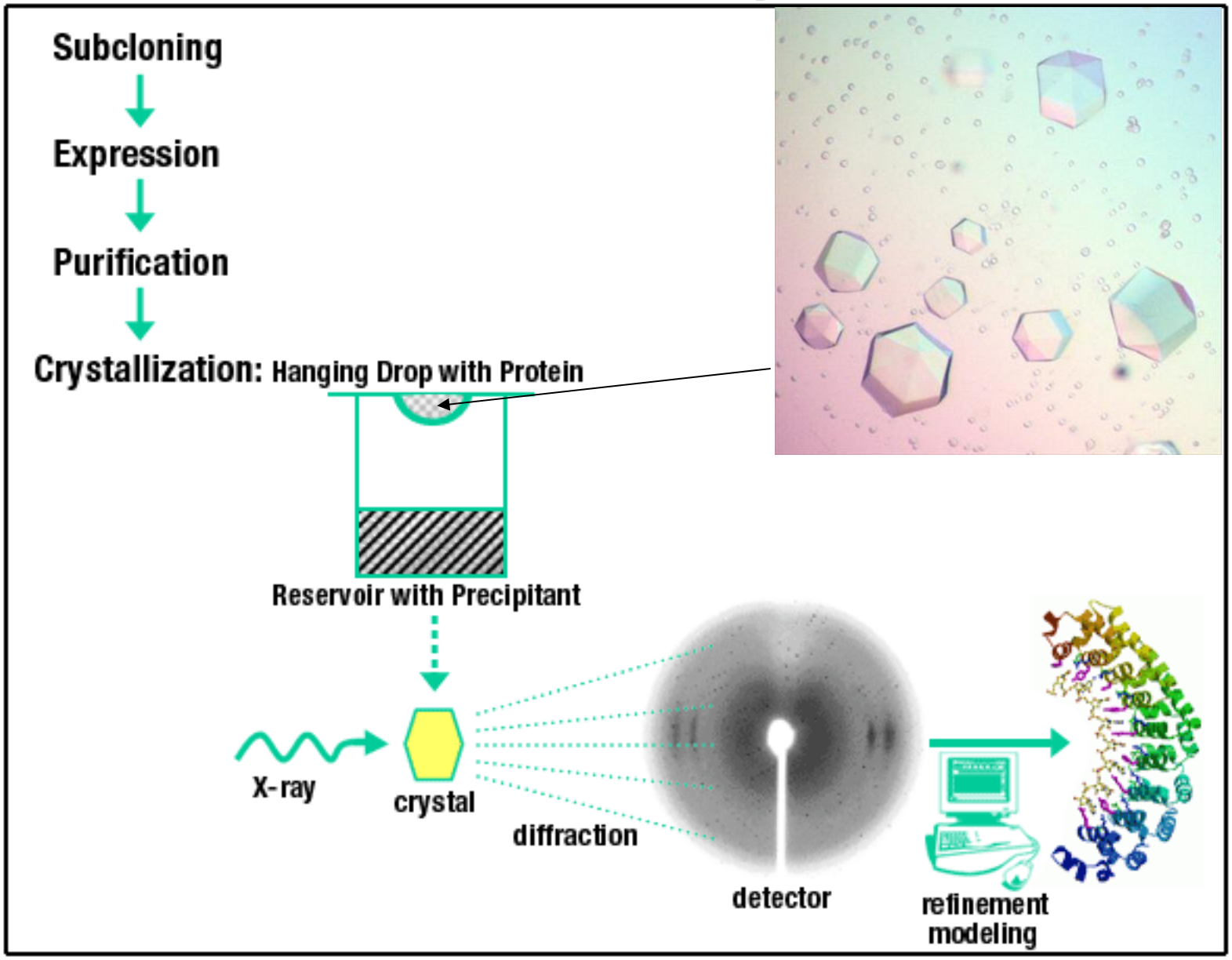
Why Crystals?



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).

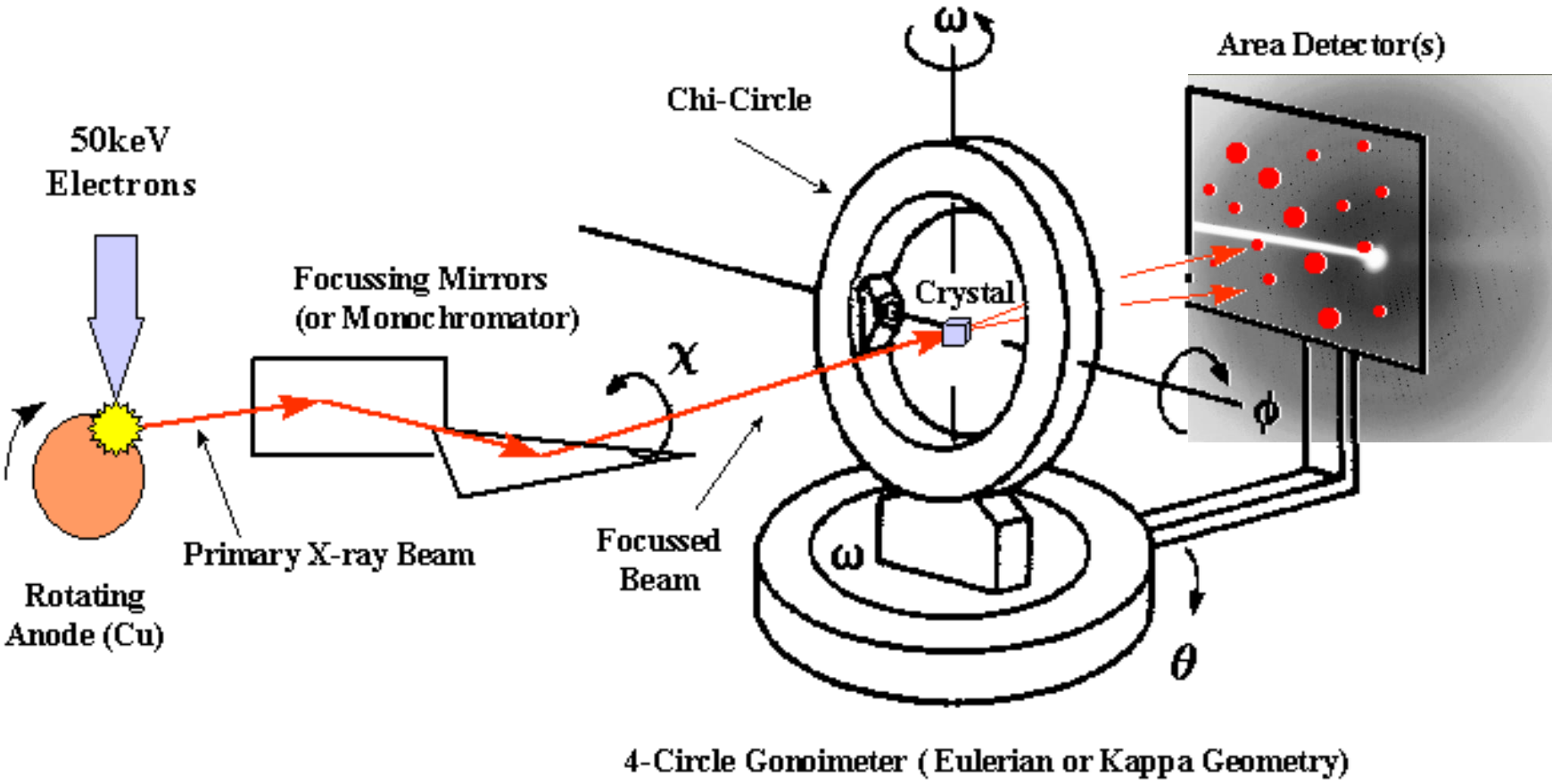
Yong Xiong

Determination of Protein Crystal Structure



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

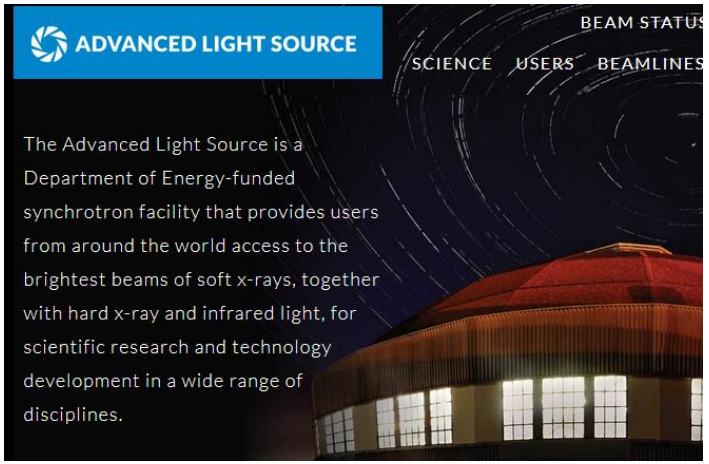
Data Collection



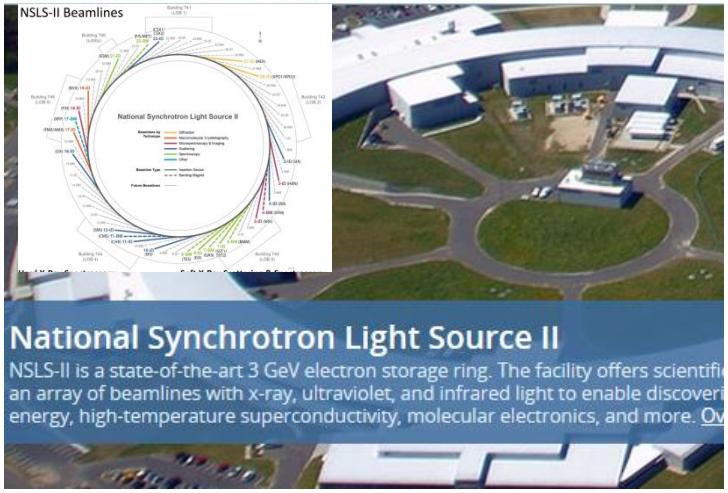
Synchrotron X-ray Sources are the method of choice

Lab x-ray sources @ 1.54 Å compared to Synchrotron X-ray @ 0.5 Å - 2.5 Å.

APS Chicago



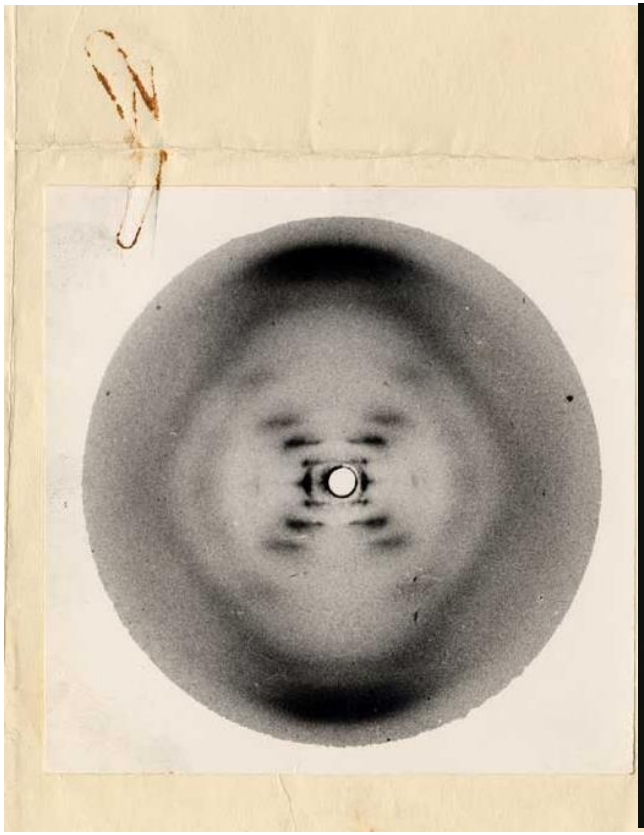
ALS Berkeley

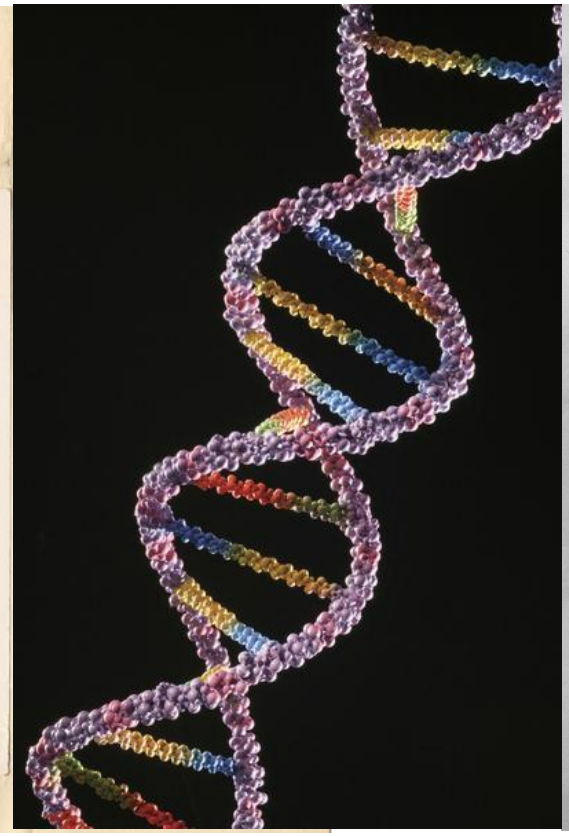


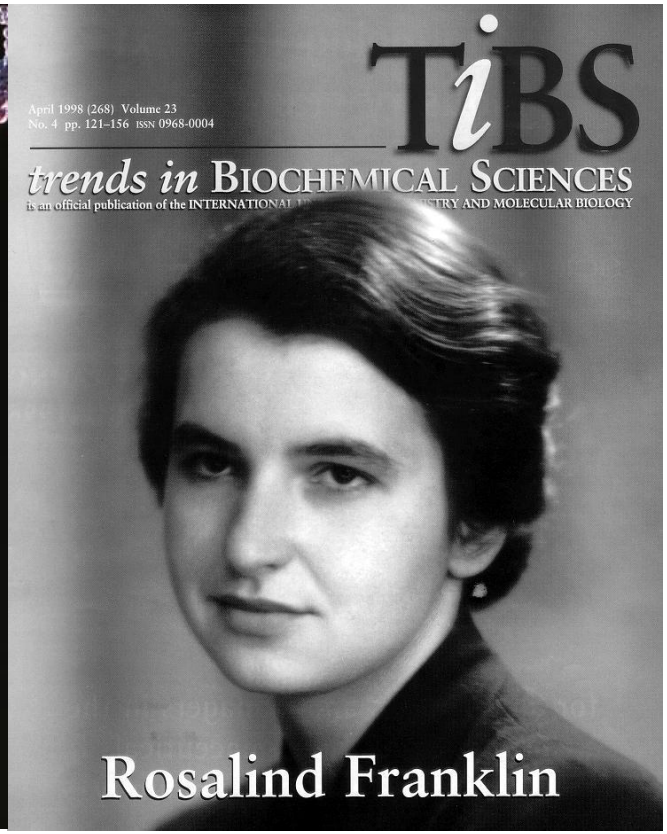
NSLS-II Brookhaven

CHESS Ithaca

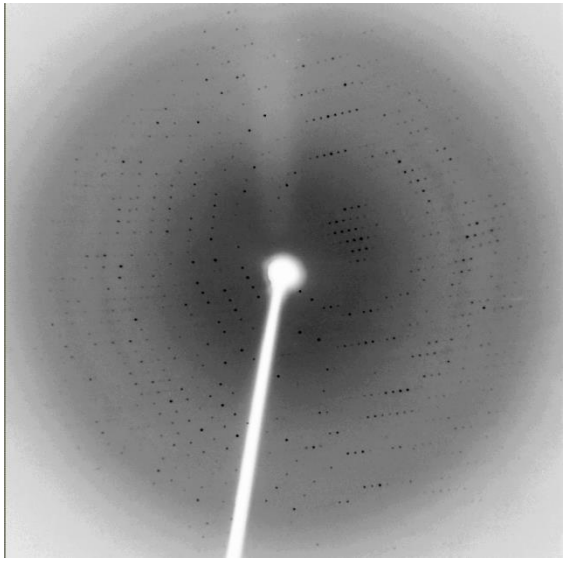
Most famous X-ray diffraction pattern







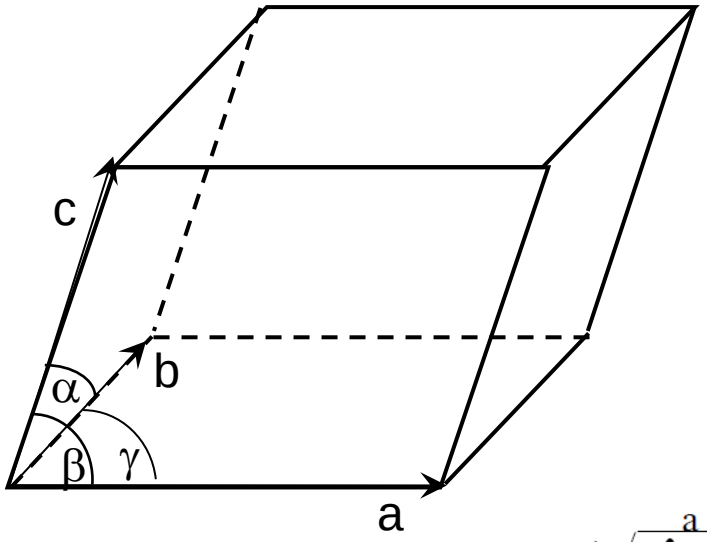
The information we get from a single diffraction experiment



Analyze the pattern of the reflections

(a) space group of the crystal

(b) unit cell dimensions

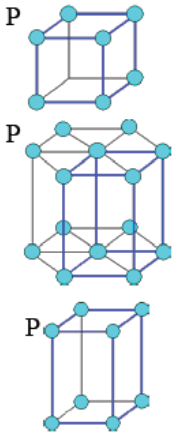


Cubic
a = b = c,
α = β = γ = 90°

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

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

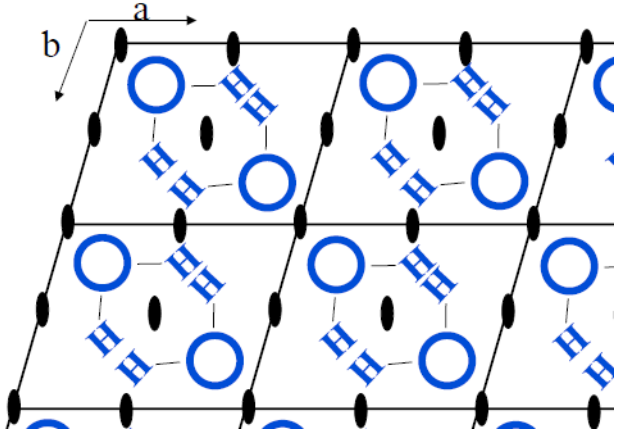
Tetragonal
a = b ≠ c,
α = β = γ = 90°

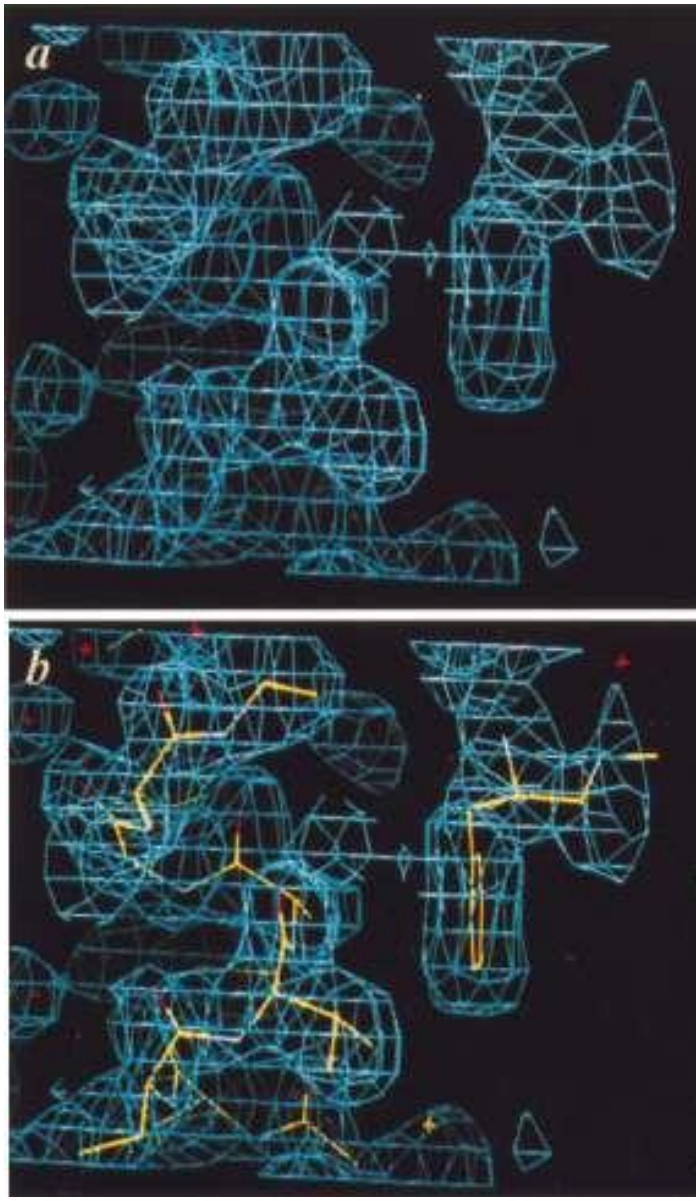


How to understand symmetry?

Crystal = lattice + unit cell content

(asymmetric units (asu) content)

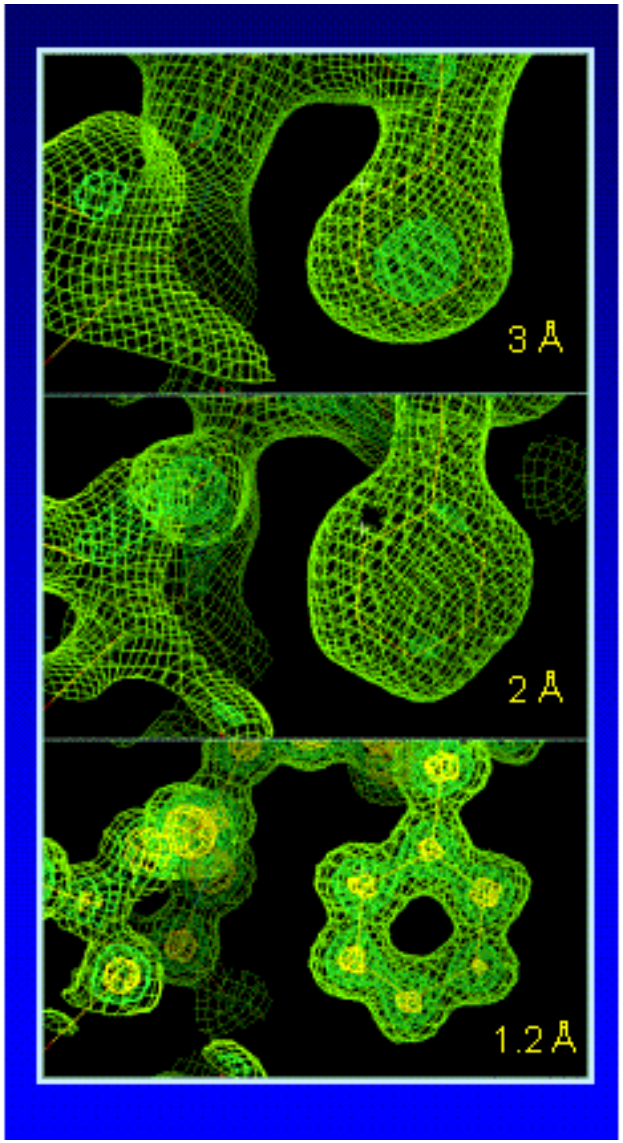


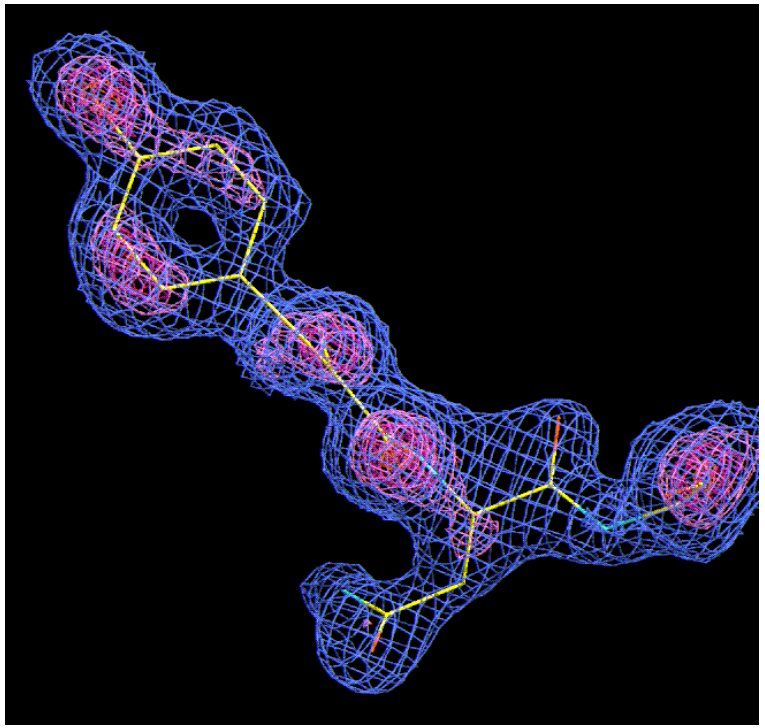


Electron density map

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.

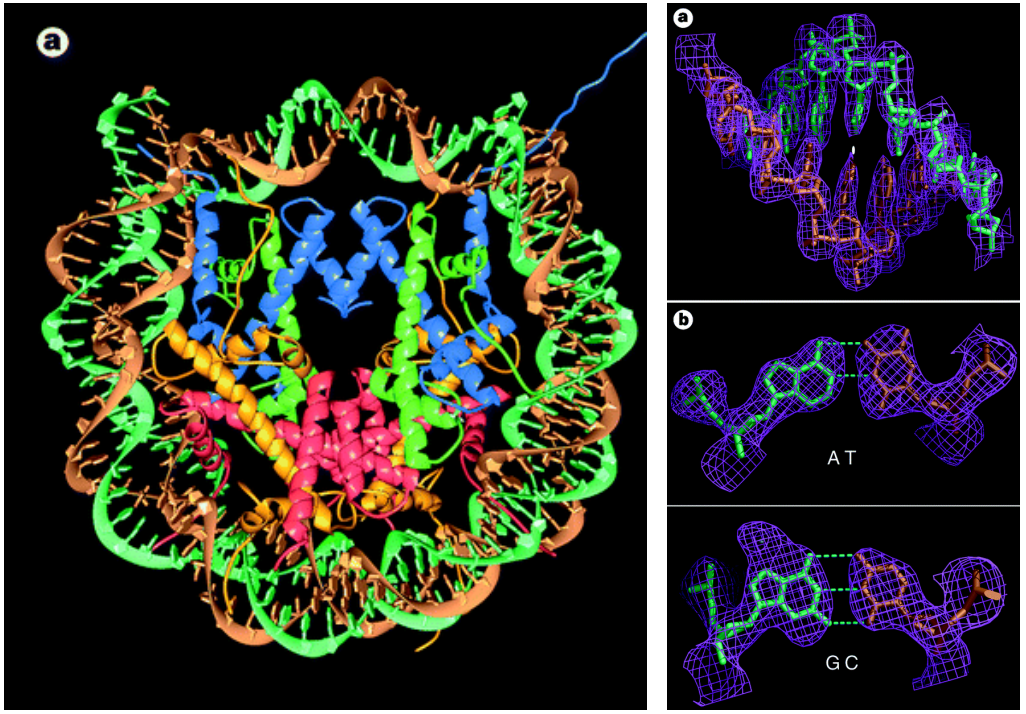
http://www.ruppweb.org/Xray/101index.html

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.

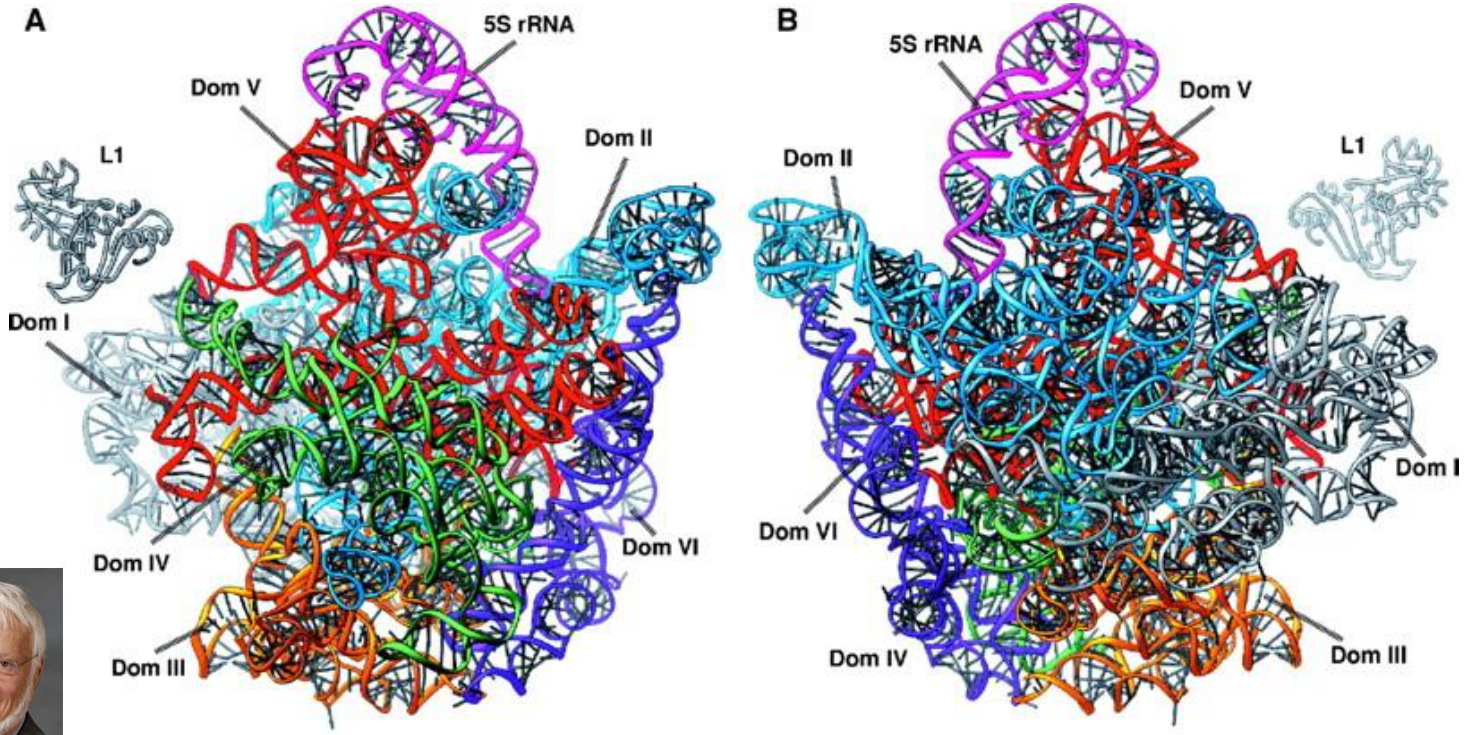


Science
AAAS

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]†



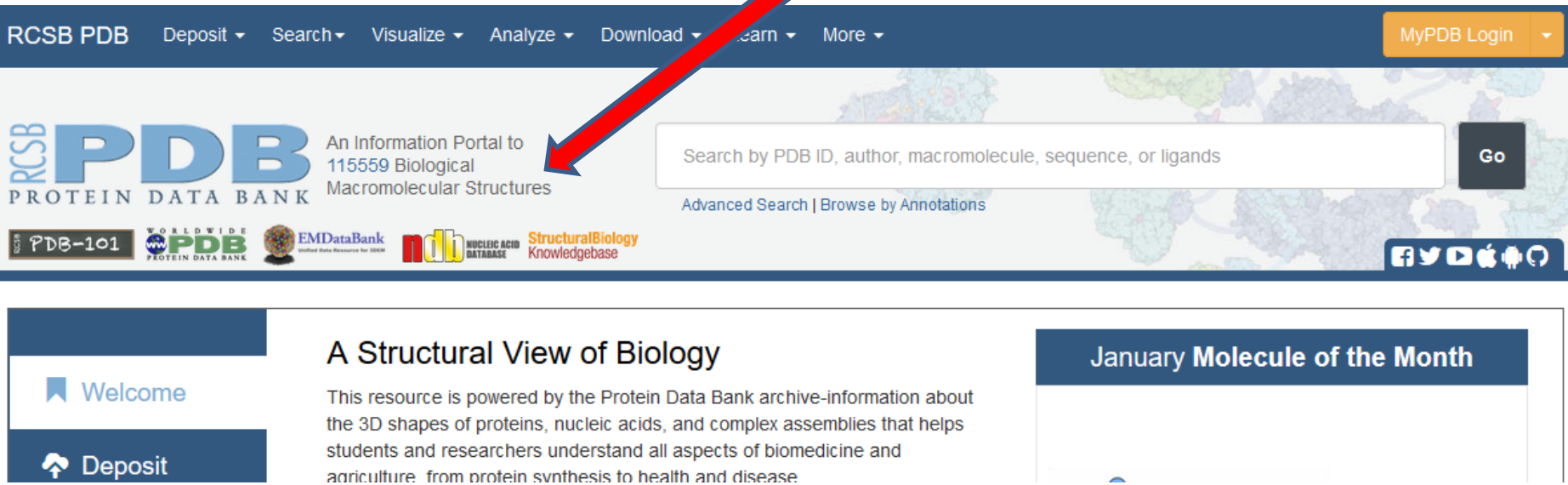
Thomas Steitz shared 2009 Nobel Prize in Chemistry for this structure

Protein Structure Databases

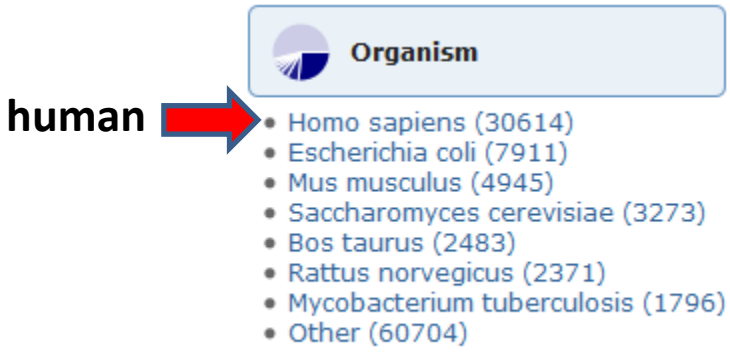
- **Where does protein structural information reside?**
  - **PDB:**
    - http://www.rcsb.org/pdb/
  - **MMDB:**
    - http://www.ncbi.nlm.nih.gov/Structure/
  - **FSSP:**
    - http://www.ebi.ac.uk/dali/fssp/
  - **SCOP:**
    - http://scop.mrc-lmb.cam.ac.uk/scop/
  - **CATH:**
    - http://www.biochem.ucl.ac.uk/bsm/cath_new/

# of structures	
2014:	97,180
2016:	115,559
2017:	117,184

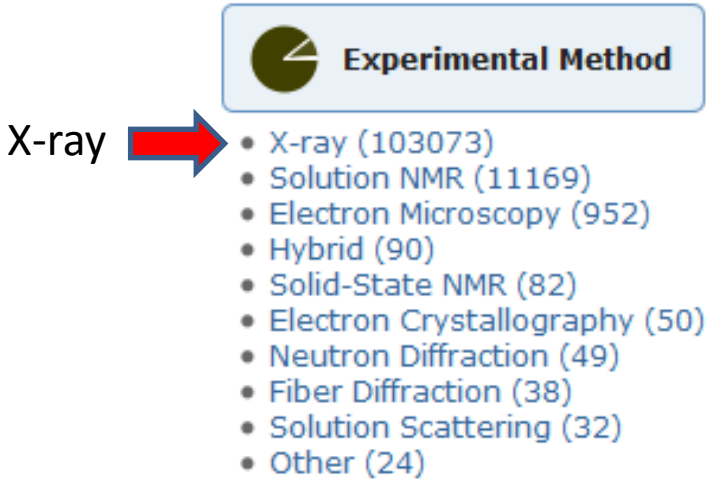
http://www.rcsb.org/pdb/home/home.do



PDB: What species are the structures from?



Which methods?



http://www.rcsb.org/pdb/home/home.do

PDB X-ray Structures:

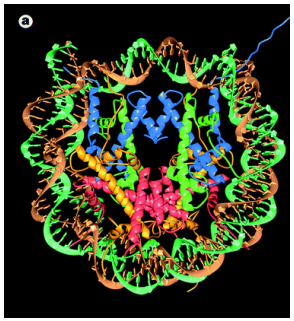
http://www.rcsb.org/pdb/results/results.do?outformat=&qrid=1B04C26E&tabtoshow=Current

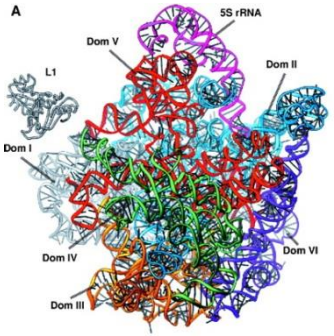
Organism

- Homo sapiens (26612)
- Escherichia coli (7185)
- Mus musculus (4184)
- Saccharomyces cerevisiae (2858)
- Bos taurus (2275)
- Rattus norvegicus (2113)
- Mycobacterium tuberculosis (1745)
- Other (55853)

Polymer Type

- Protein (96499)
- Mixed (4959)
- DNA (973)
- RNA (638)





Membrane Proteins

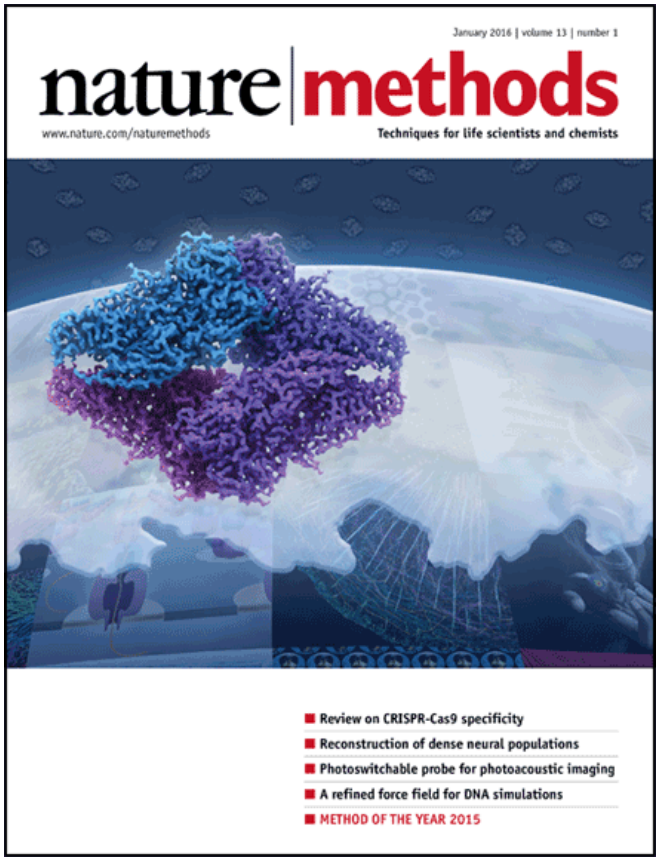
- ALPHA-HELICAL (2100)
- MONOTOPIC MEMBRANE PROTEINS (358)
- BETA-BARREL (352)

Small % of the total x-ray data

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/**

2015 Method of the Year: Single-particle Cryo-EM



NATURE METHODS | VOL.13 NO.1 | JANUARY 2016

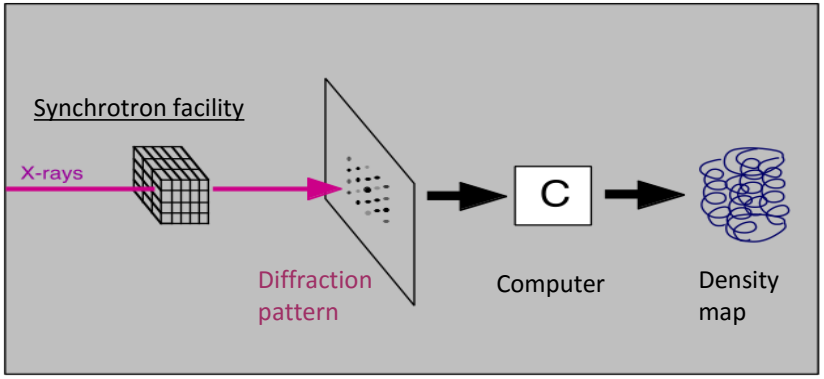
METHOD OF THE YEAR 2015

At *Nature Methods* we are ringing in a new year with our celebration of single-particle cryo-electron microscopy (cryo-EM) as our Method of the Year 2015. Cryo-EM has its roots in work first performed in the 1960s. It has steadily progressed over the past few decades as a medium-resolution structural technique for obtaining information about macromolecular samples that resist analysis by X-ray crystallography. But very recent technical advances, especially the development of direct-detection cameras, have enabled the field to achieve impressive leaps in resolution—even reaching the near-atomic realm of X-ray crystallography—and, by extension, biological applicability. An Editorial, News Feature, Primer, Historical Commentary and Commentary discuss how cryo-EM works, what it is used for, how the field began, why now is such an exhilarating time, and where the field is going in the future. We also cast our predictions about methods with exciting potential in our Methods to Watch section.

Special feature starts on p19

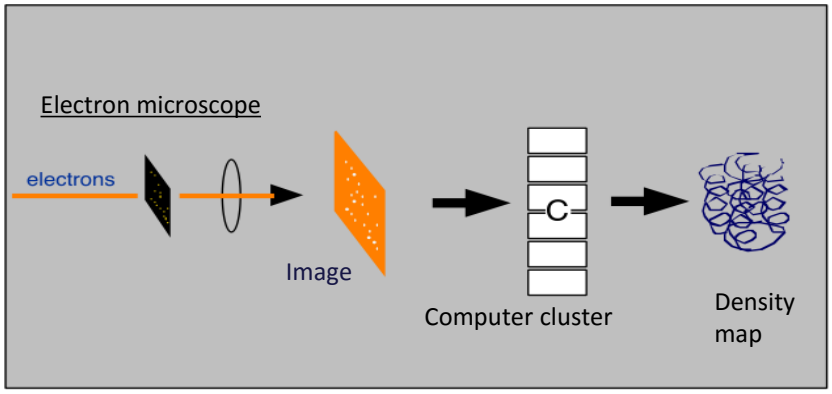
Fred Sigworth

Two methods for structure determination



X-ray crystallography

Well-established (since 1960s)
Requires well-ordered crystals
>10^{12} copies of protein



Single-particle cryo-EM

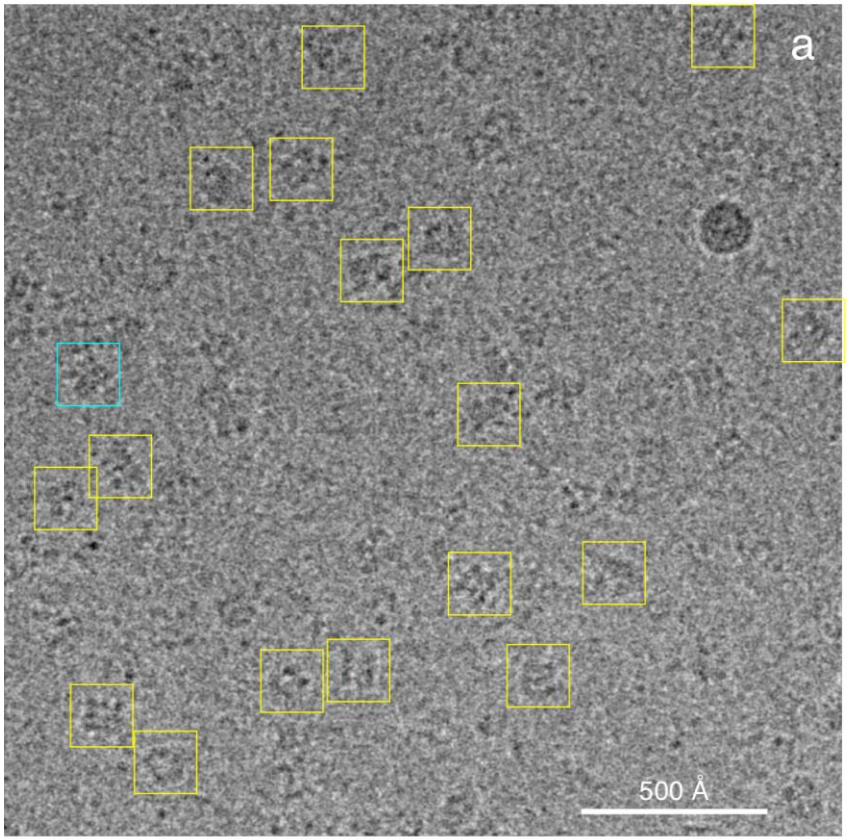
Recent (1990s-present)
No crystals required!
~10^{5} copies of protein

Fred Sigworth

The Cryo-EM specimen gives only a phase contrast image

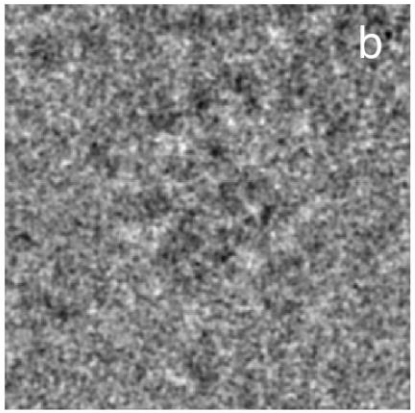
- A constellation of images and data processing are essential.

1/4 of a micrograph, showing some particles

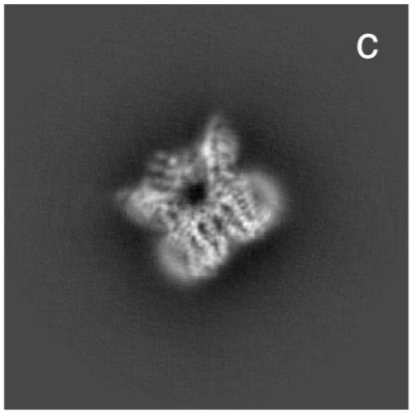


Y. Cheng and D. Julius lab. Nature 2013

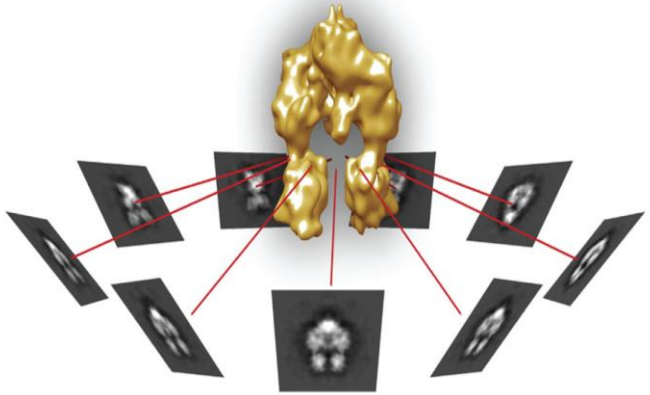
Image



Projection



- orientation assignment and averaging
- 3D reconstruction



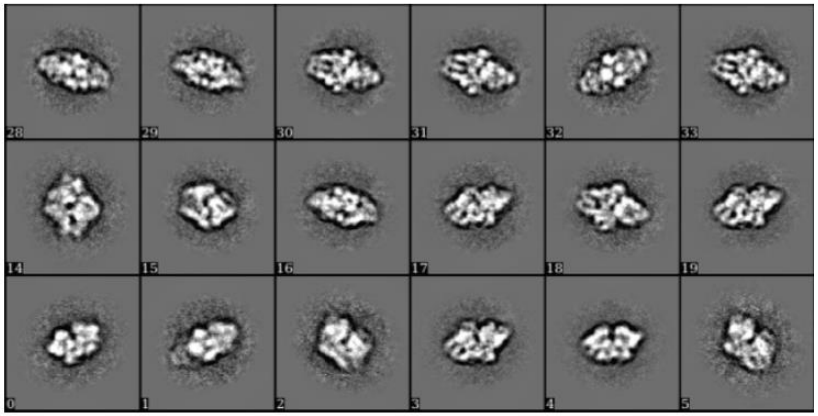
Fred Sigworth

A landmark study for high-resolution single-particle structures

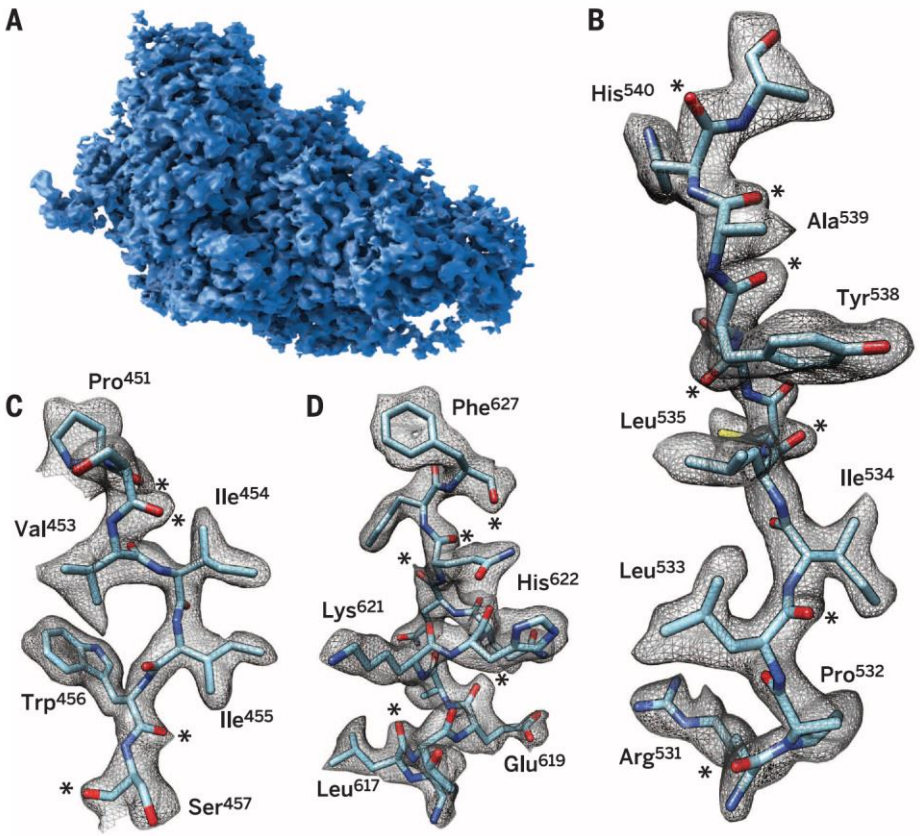
2.2 Å resolution cryo-EM structure of β-galactosidase in complex with a cell-permeant inhibitor

Alberto Bartesaghi,[1]* Alan Merk,[1]* Soojay Banerjee,[1] Doreen Matthies,[1] Xiongwu Wu,[2] Jacqueline L. S. Milne,[1] Sriram Subramaniam[1]†

Science 2015



2D class averages



Fred Sigworth

New Technologies, Automation, & Computation are accelerating the field

Krios at National University of Singapore

Control room at Scripps Research Institute, La Jolla

Krios TEM installation on Yale's West Campus.
Online Summer 2017

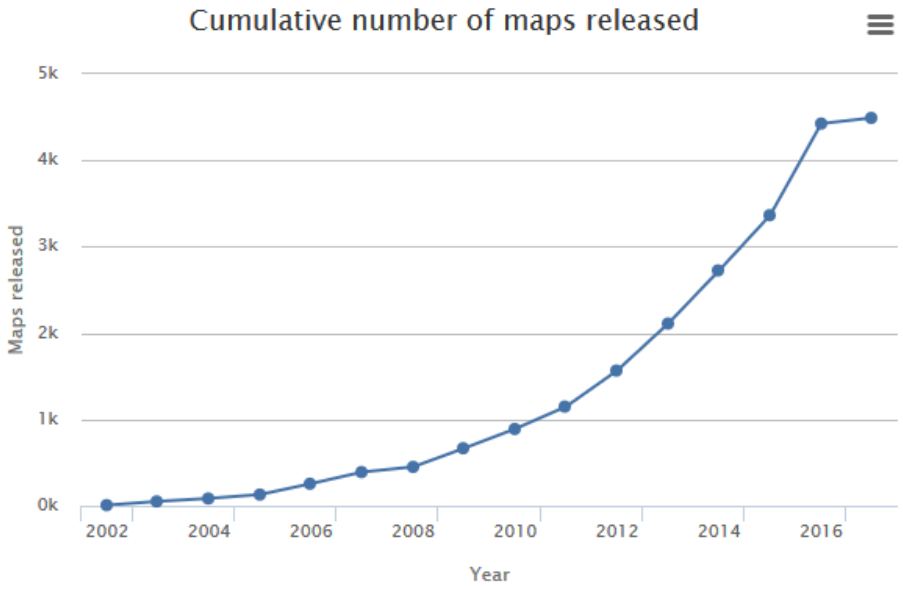
Fred Sigworth

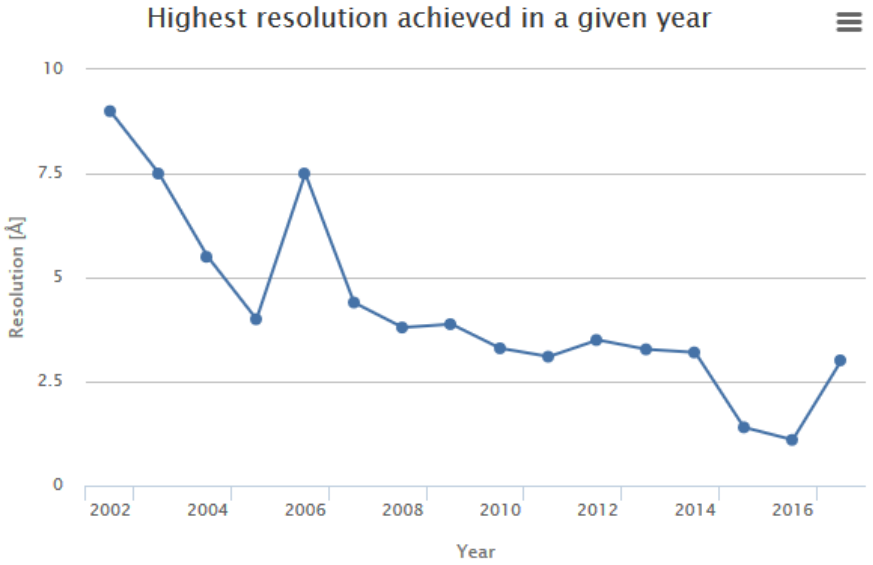
EMBL-EBI

Protein Data Bank in Europe

Bringing Structure to Biology

EMDB statistics





Source: https://www.ebi.ac.uk/pdbe/emdb/statistics_main.html/

EMBL-EBI

Protein Data Bank in Europe

Bringing Structure to Biology

EM resources

https://www.ebi.ac.uk/pdbe/emdb/

Jan 2017: 4,492 total maps
3,614 (~77% single particle)

EM method
Single-particle (3614 / 3614)

Component type
Protein (2603 / 2603)
Virus (761 / 761)
Nucleic acid (415 / 415)
Ligand (274 / 274)
Prokaryotic ribosome (264 / 264)
Eukaryotic ribosome (217 / 217)
Cell component (113 / 113)
EM label (2 / 2)

Less than 5Å resolution

Filter results
748 entries

EM method
Single-particle (661 / 661)
Helical (63 / 63)
2D crystallography (21 / 21)
Subtomogram averaging (3 / 3)

Component type
Protein (601 / 601)
Ligand (178 / 178)
Nucleic acid (140 / 140)
Virus (134 / 134)
Eukaryotic ribosome (69 / 69)
Cell component (44 / 44)
Prokaryotic ribosome (22 / 22)