



Universidad
de Concepción

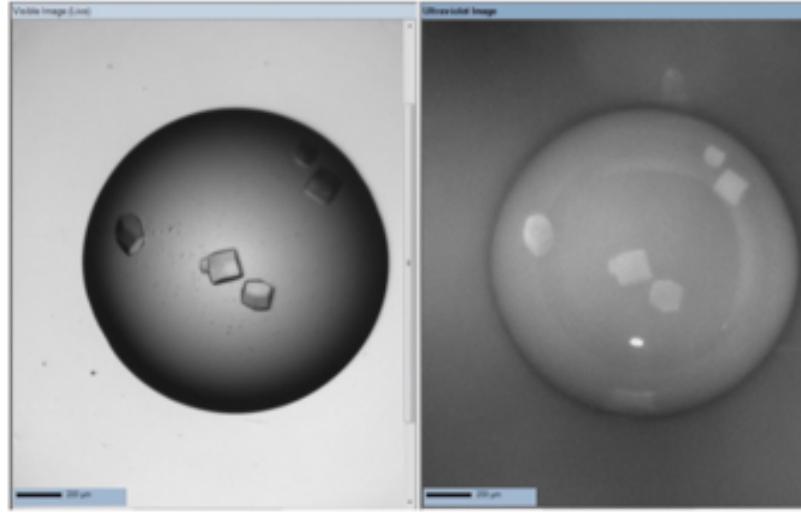


X-ray crystallography - CryoEM

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X-ray crystallography:

What do I need to obtain the structure of a protein?

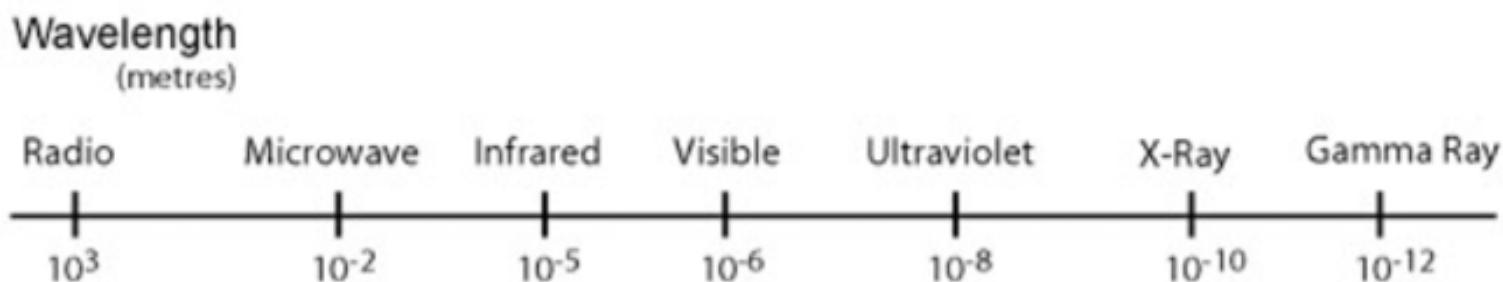


rotating anode X-ray generator

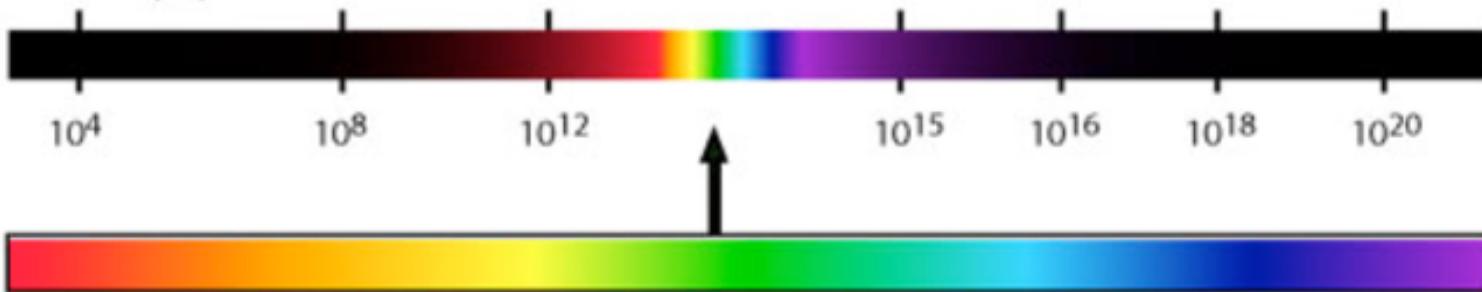


synchrotron X-ray beamline

THE ELECTRO MAGNETIC SPECTRUM



Frequency (ν) is defined as the number of wave cycles passing a fixed reference point in one second (Hz)

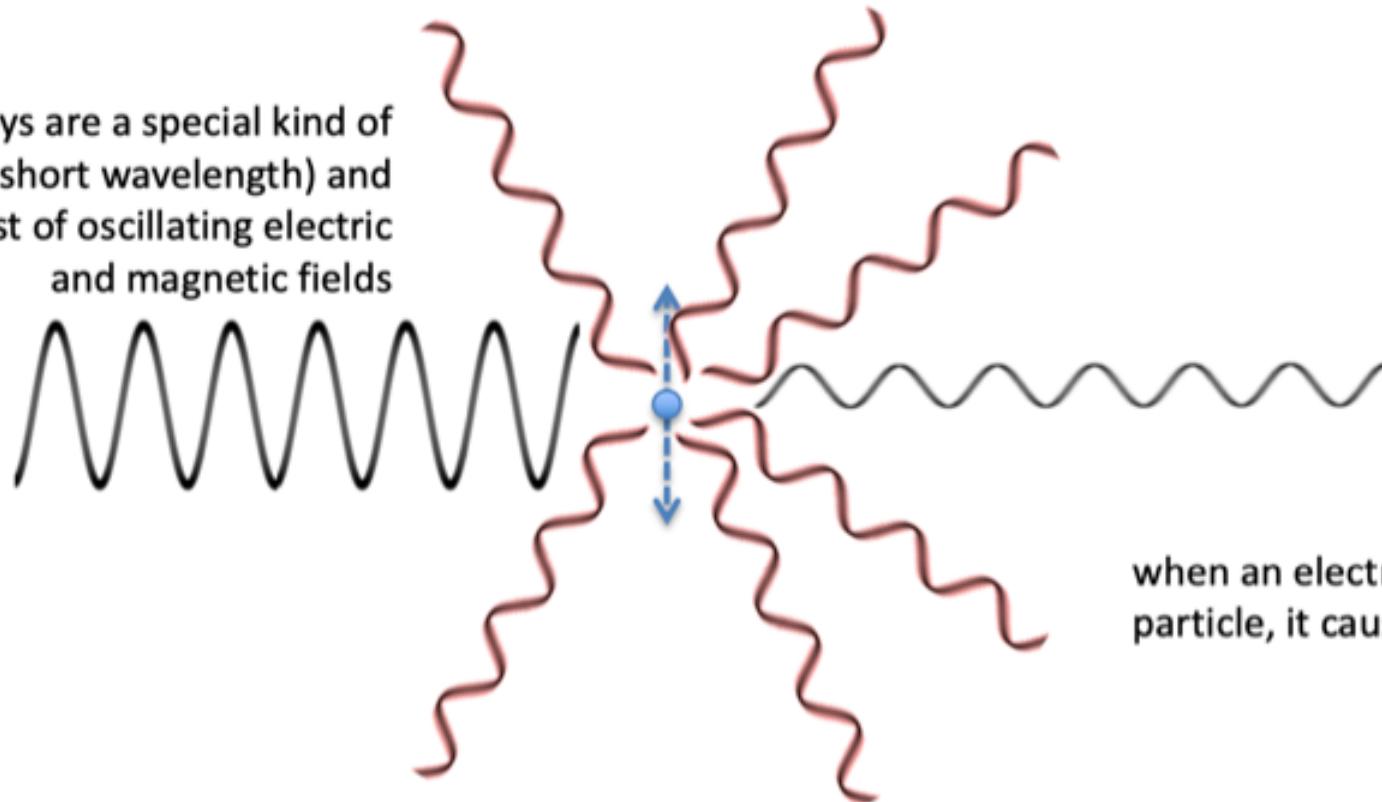


- The higher the frequency, the shorter the wavelength, according to:
$$\nu = c / \lambda$$

- The energy is proportional to the frequency:
$$E = h\nu$$

X-ray scattering

X-rays are a special kind of light (short wavelength) and consist of oscillating electric and magnetic fields



when an electric field hits an electron, a charged particle, it causes the electron to oscillate

History

1840 - proteins can cristalize! → 1957

Protein	Investigator	Date
Hemoglobin	Hünefeld	1840
	Reichert	1849
	Leydig	1849
	Kölliker	1849
	Budge	1850
	Fünke	1851
	Lehmann	1853
	Pasteur	1863

Hünefeld accidentally discovered the formation of **crystalline material** in samples of earthworm blood held under two glass slides and occasionally observed small plate-like crystals in desiccated swine or human blood samples

Fünke described the first **use of organic solvents** in protein crystallization, by crystallizing human haemoglobin with solvents such as alcohol or ether, followed by slow evaporation of the solvent from the protein solution

History

Wilhelm Conrad Röntgen

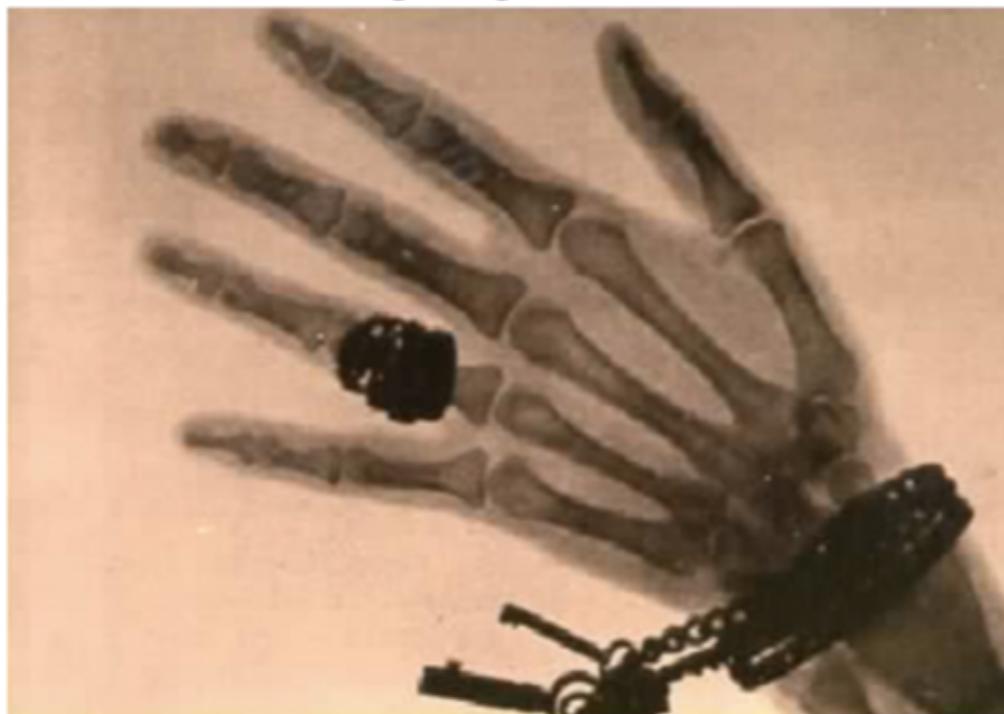
1895

1840 - proteins can cristalize!

1957

- the X-ray is penetrating and sampling the structure of the matter

When we take a medical X-ray, we are looking at the transmitted beam and ignoring the scattered radiation



The hand of Mrs. Roentgen, 1896

History

Wilhelm Conrad Röntgen

1895

1840 - proteins can cristalize!

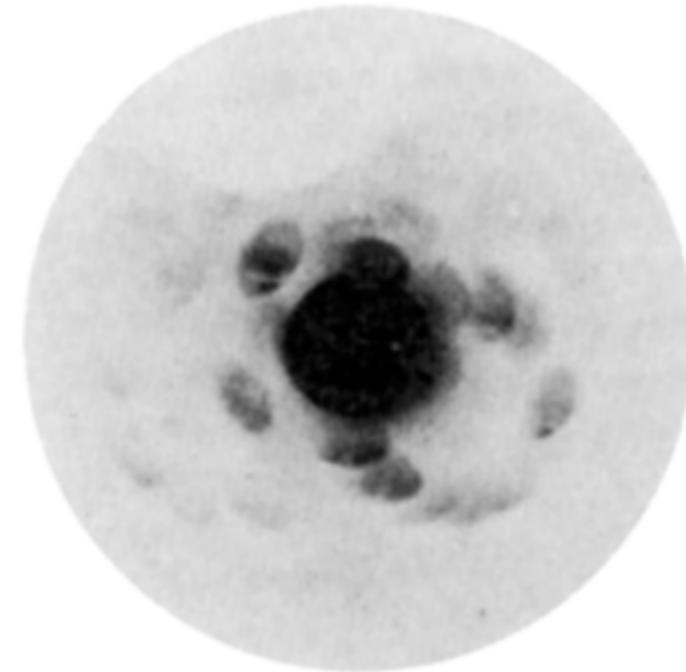
1957

1912 von Laue, Friedrich and Knipping

The first **diffraction pattern**, from copper sulfate pentahydrate,
taken by Laue's group

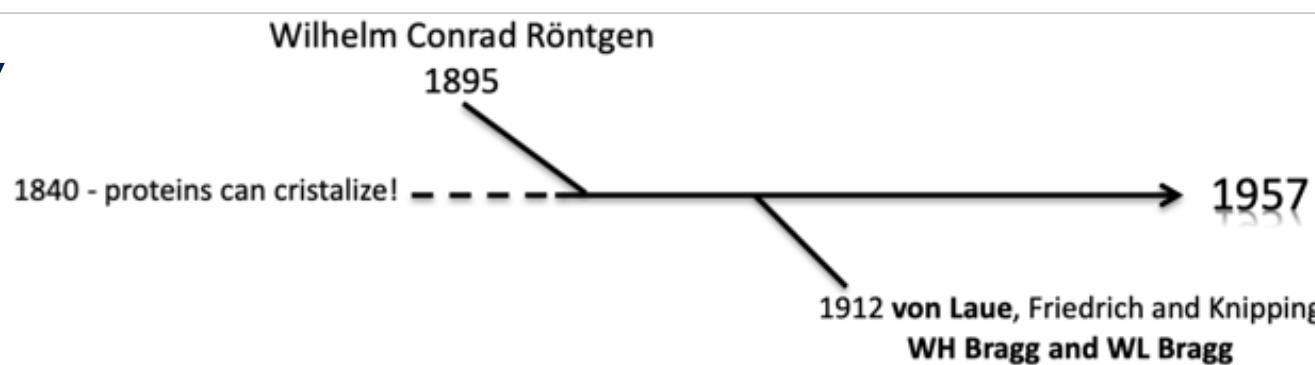


crystals could be used as a grating
to diffract X-ray beams

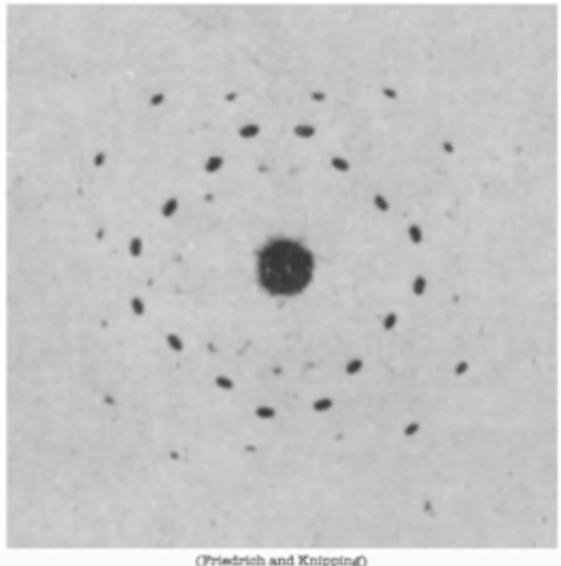


evidence for scattering and interference...
X-rays **are waves**

History

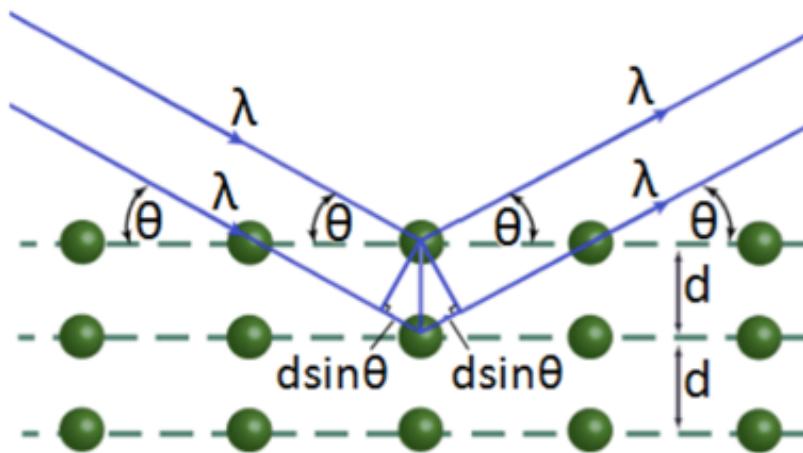


Diffraction from ZnS



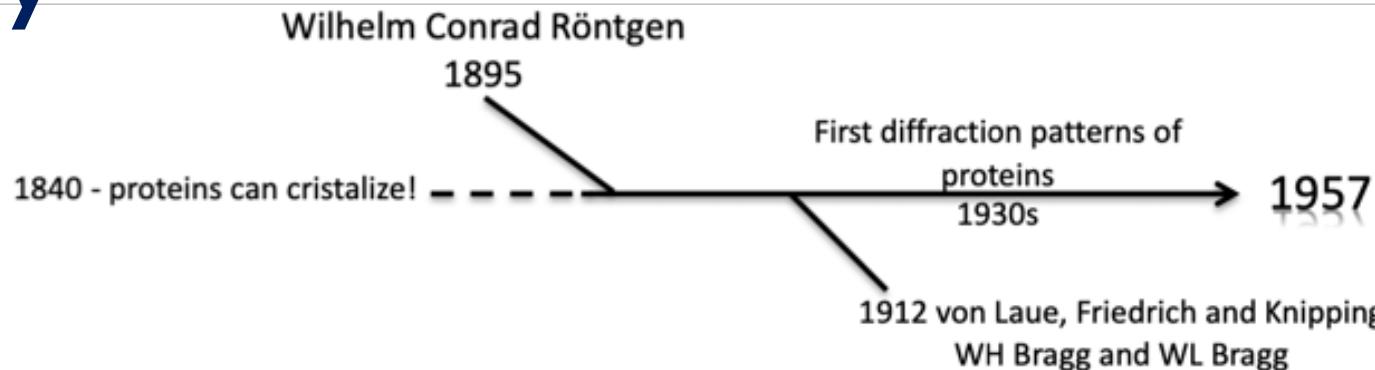
Proc. Camb Phil Soc., Vol 17, Part 1, pp. 43-57, 1912

The X-rays are reflected from planes of atoms



As a result, the scattering of X-radiation is enhanced enormously in selected directions and extinguished completely in others

History



794 NATURE MAY 26, 1934

X-Ray Photographs of Crystalline Pepsin

At this stage, such ideas are merely speculative, but now that a crystalline protein has been made to give X-ray photographs, it is clear that we have the means of checking them and, by examining the structure of all crystalline proteins, arriving at far more detailed conclusions about protein structure than previous physical or chemical methods have been able to give.

J. D. BERNAL.
D. CROWFOOT.

662 NATURE March 8, 1958 VOL. 181

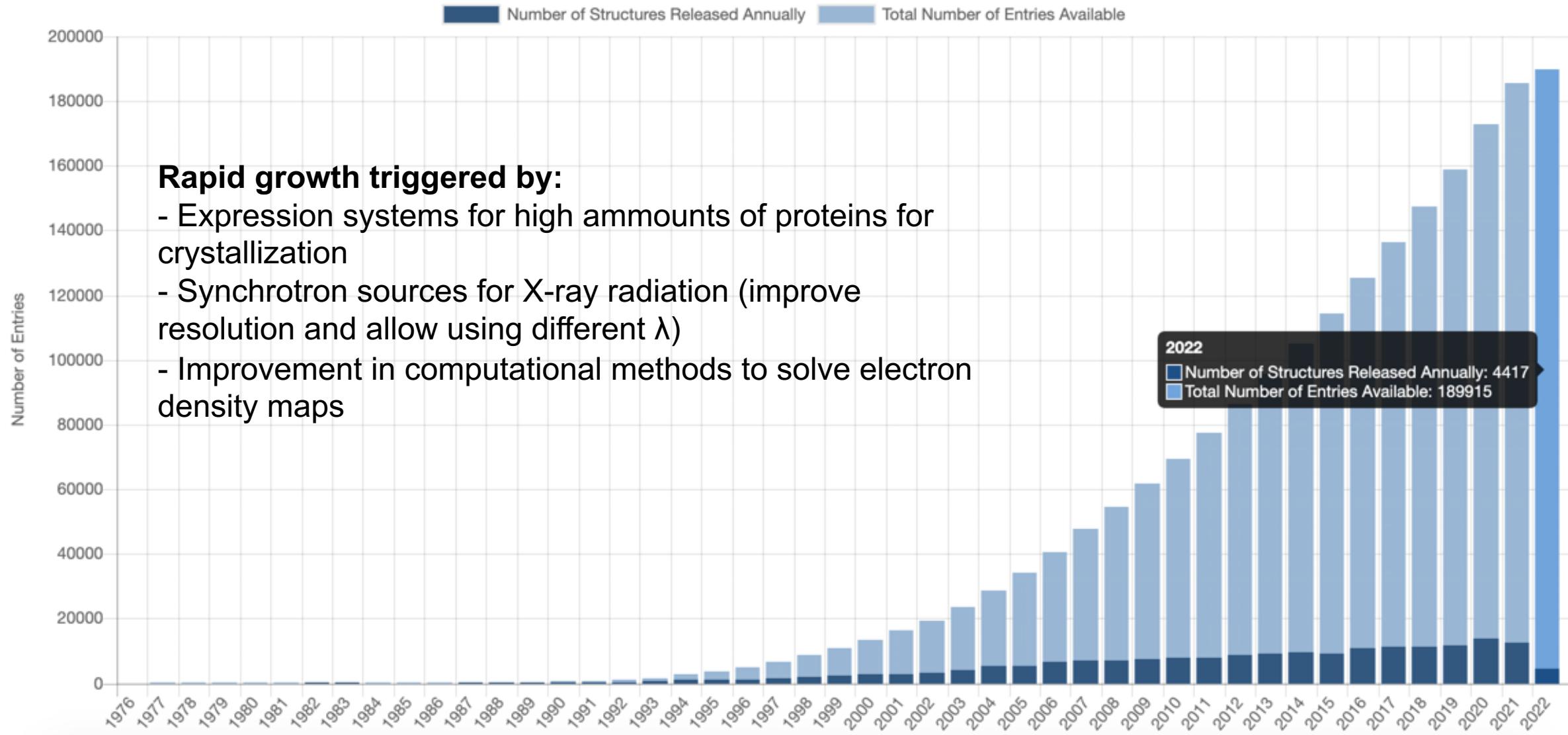
A THREE-DIMENSIONAL MODEL OF THE MYOGLOBIN MOLECULE OBTAINED BY X-RAY ANALYSIS

By Drs. J. C. KENDREW, G. BODO, H. M. DINTZIS, R. G. PARRISH and H. WYCKOFF
Medical Research Council Unit for Molecular Biology, Cavendish Laboratory, Cambridge
AND
D. C. PHILLIPS
Davy Faraday Laboratory, The Royal Institution, London

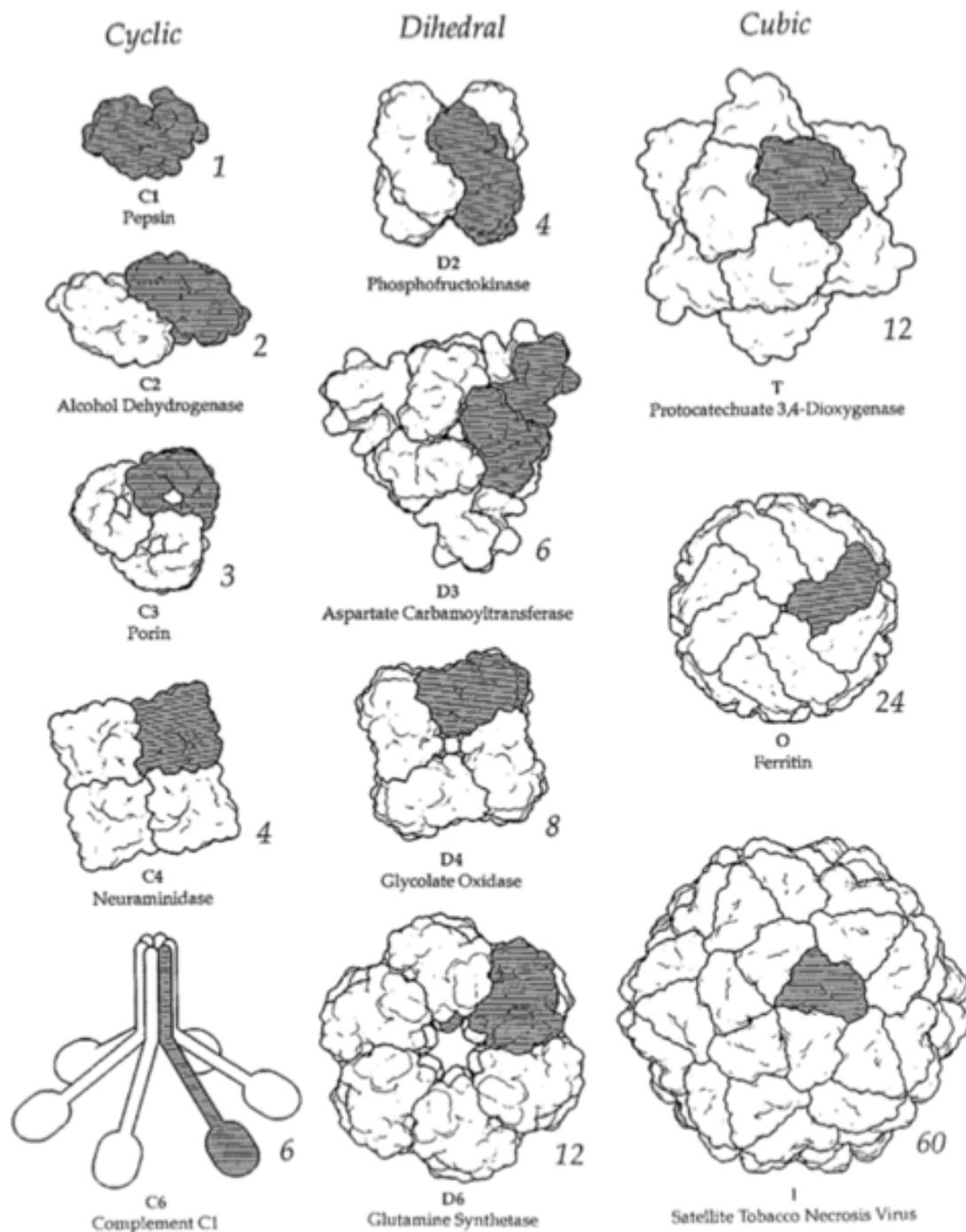
This situation was transformed by the discovery, made by Perutz and his colleagues², that heavy atoms could be attached to protein molecules in specific sites and that the resulting complexes gave diffraction patterns sufficiently different from normal to enable a classical method of structure analysis, the so-called 'method of isomorphous replacement', to be used to determine the relative phases of the reflexions.

PDB Statistics: Overall Growth of Released Structures Per Year

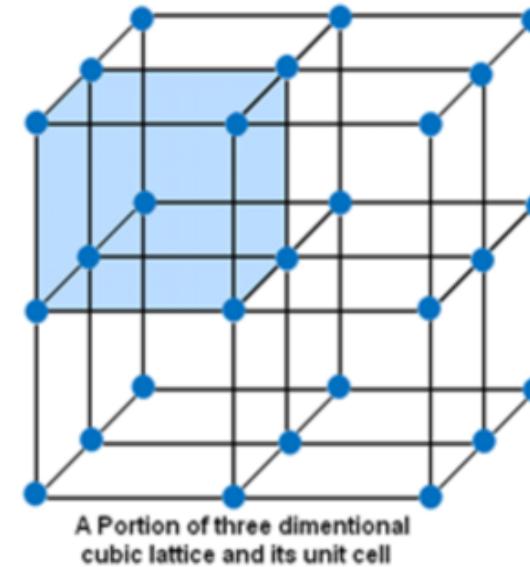
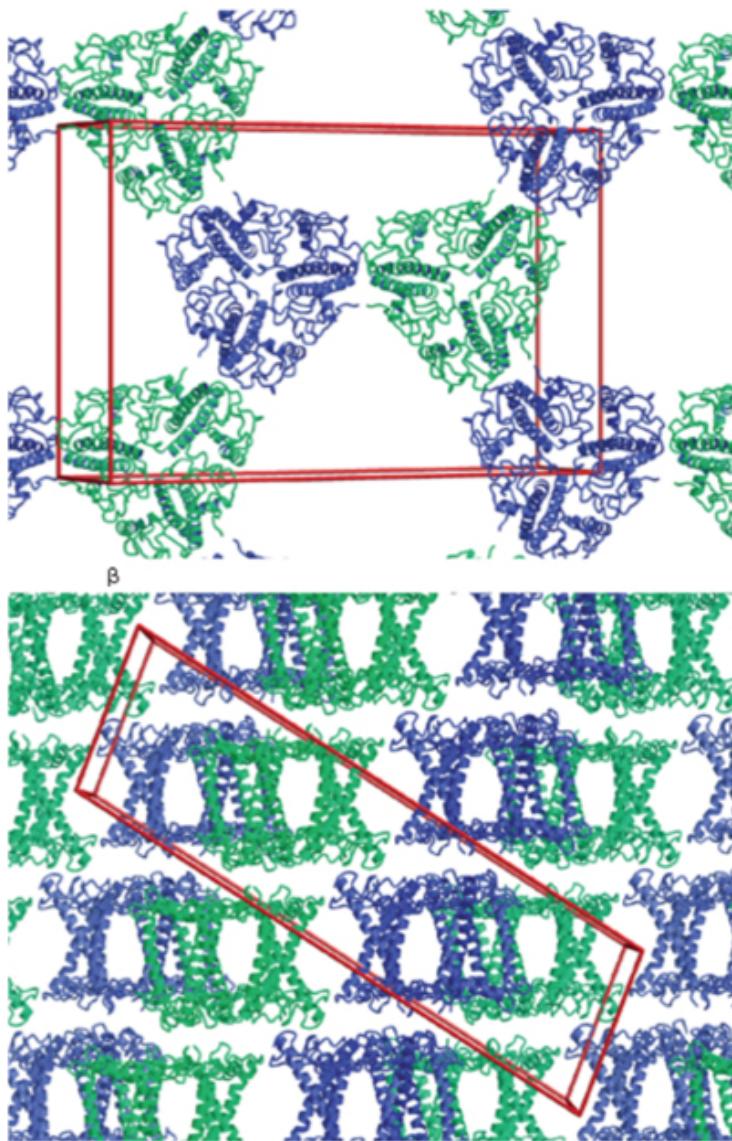
2022-04-26



In their natural environment, proteins form symmetrical associations

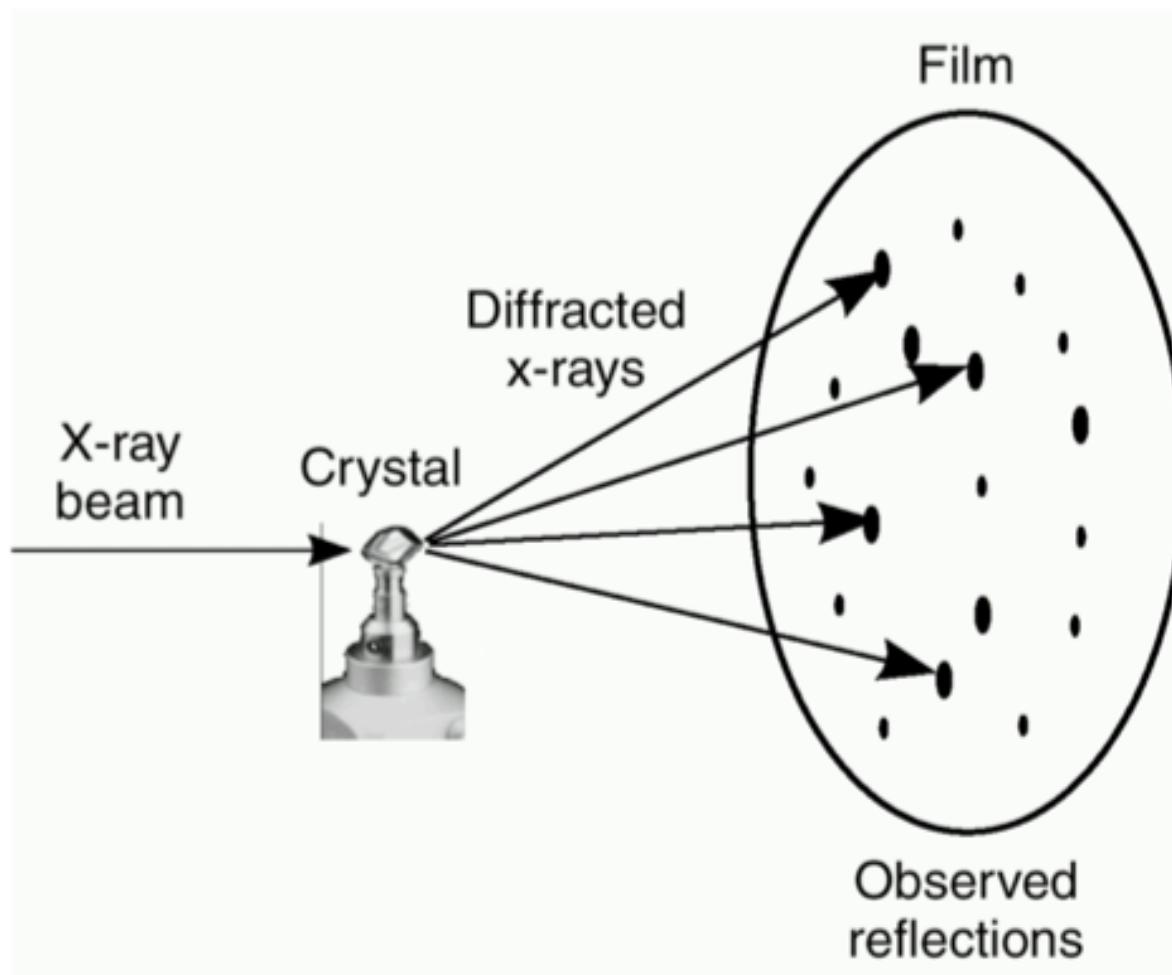


Symmetrical associations of proteins lead to crystal formation



Methods for Determining Atomic Structures

The diffraction pattern

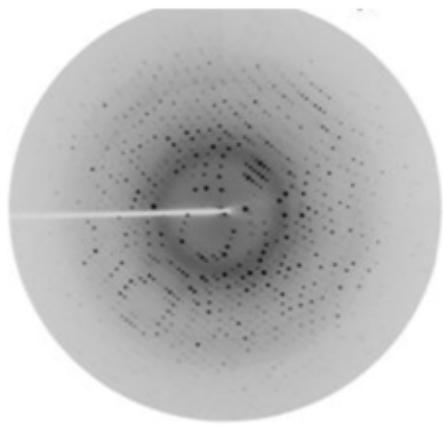


2D grid of pixels
containing intensities of
the individual reflections

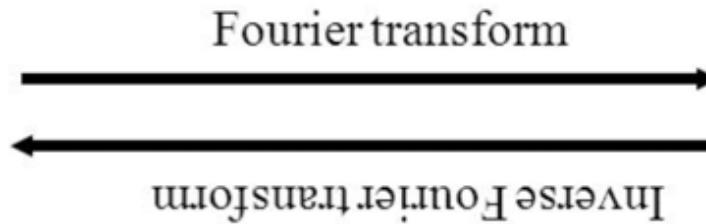
The diffraction pattern do not provide a direct image of the molecular structure

However, there is a relationship
between the electron density and the
diffraction pattern

The pattern that we see in these experiments can be calculated as the Fourier transform of the structure in the material (electron density)

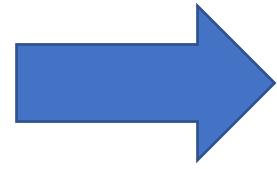
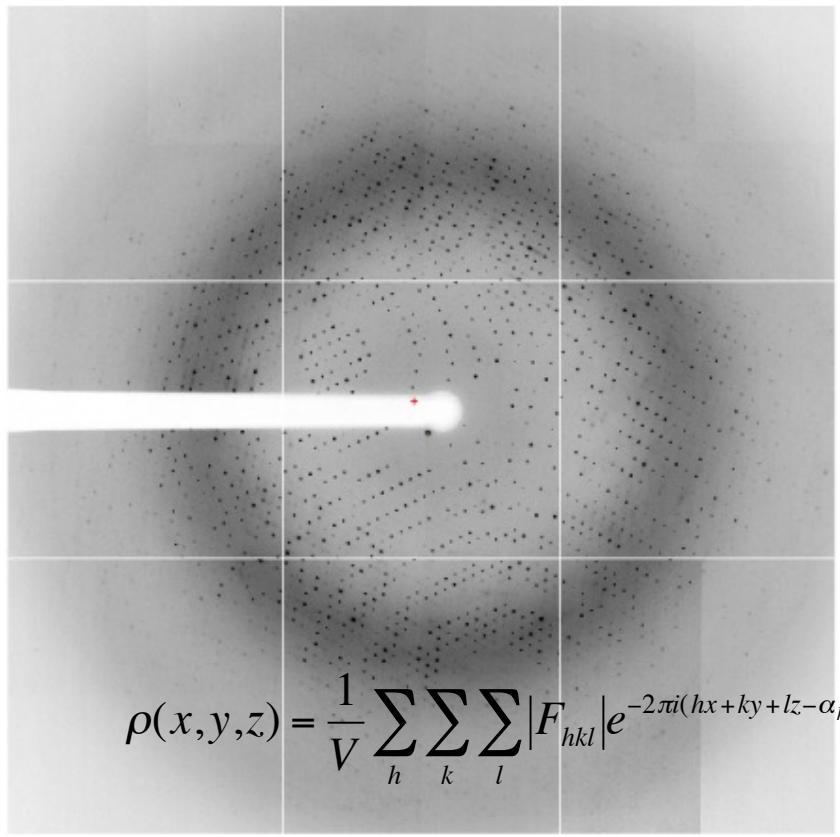


Structure Factor

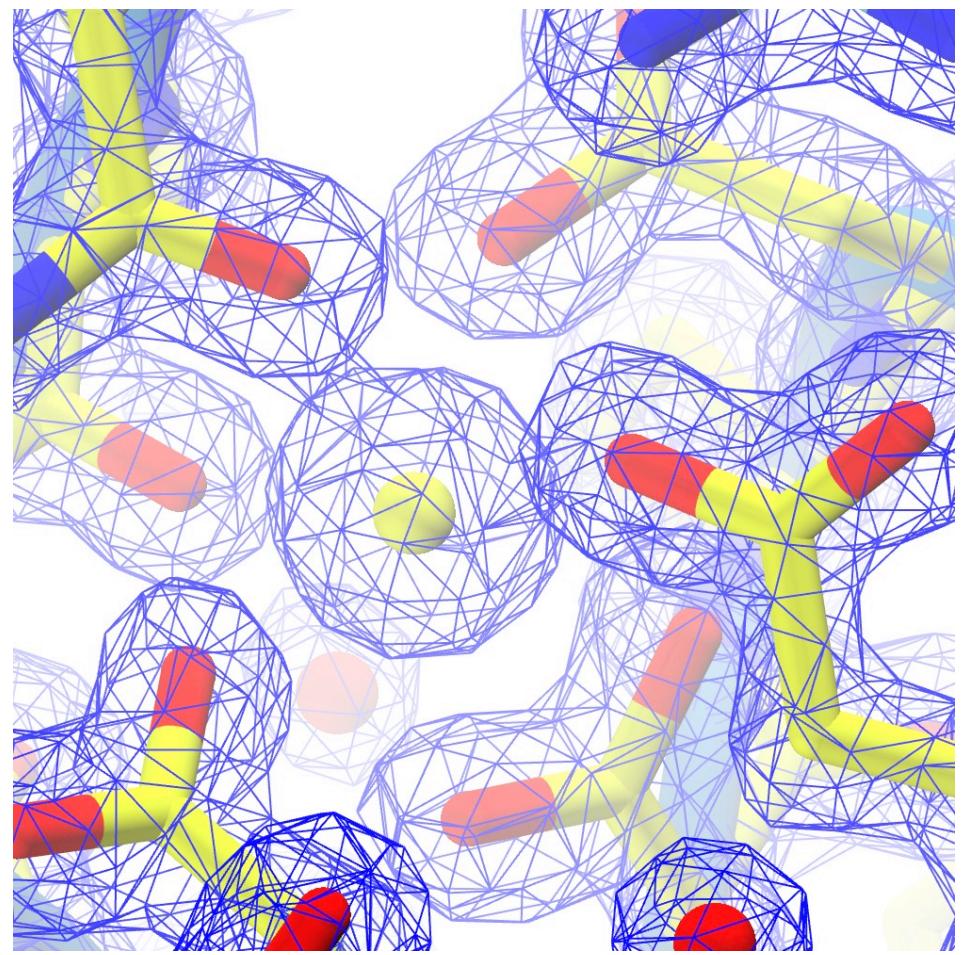


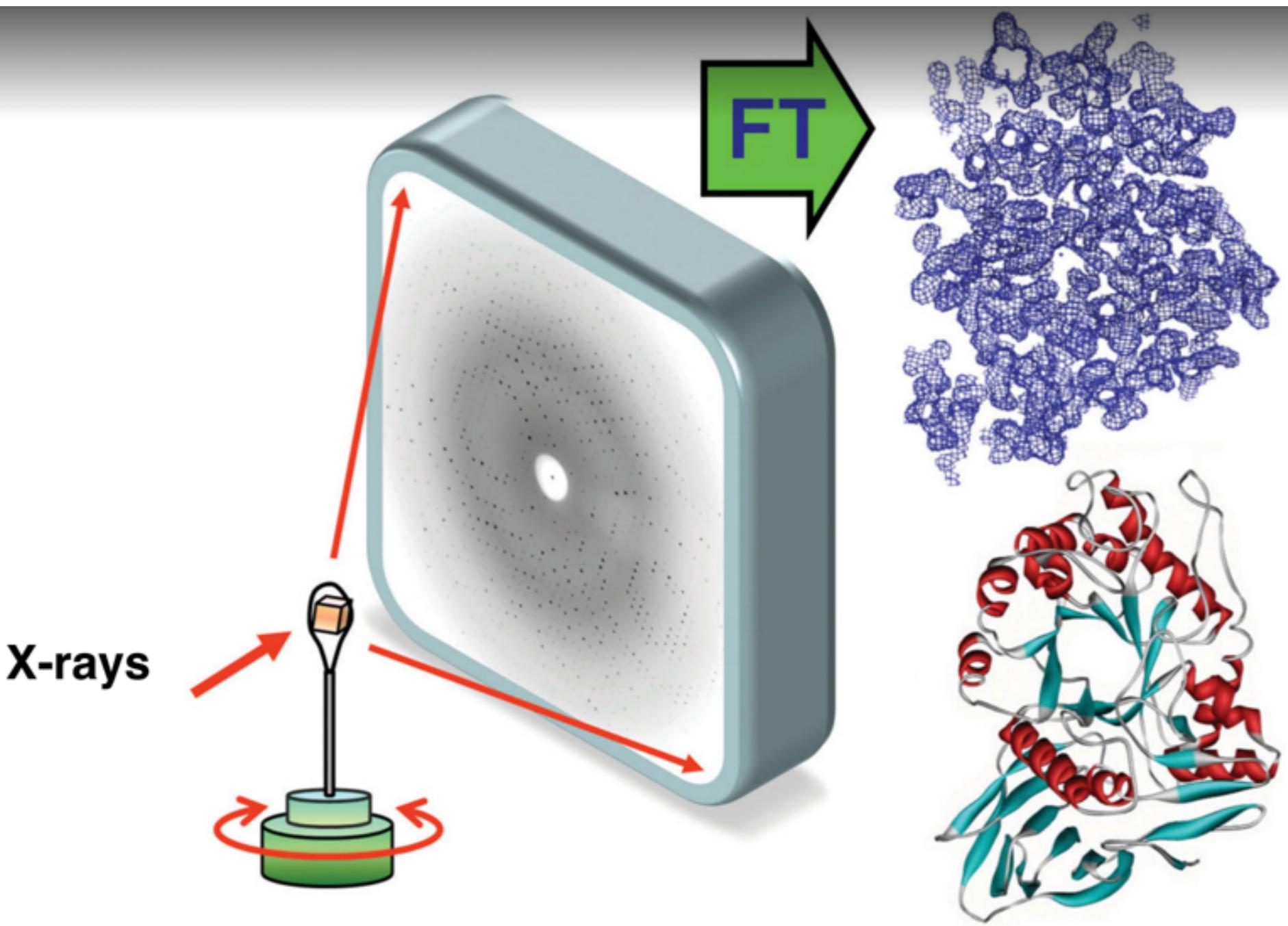
Electron Density

In order to reconstruct the molecular image (electron density) from its diffraction pattern both the intensity and phase, which can assume any value from 0 to 2π , of each of the thousands of measured reflections must be known.

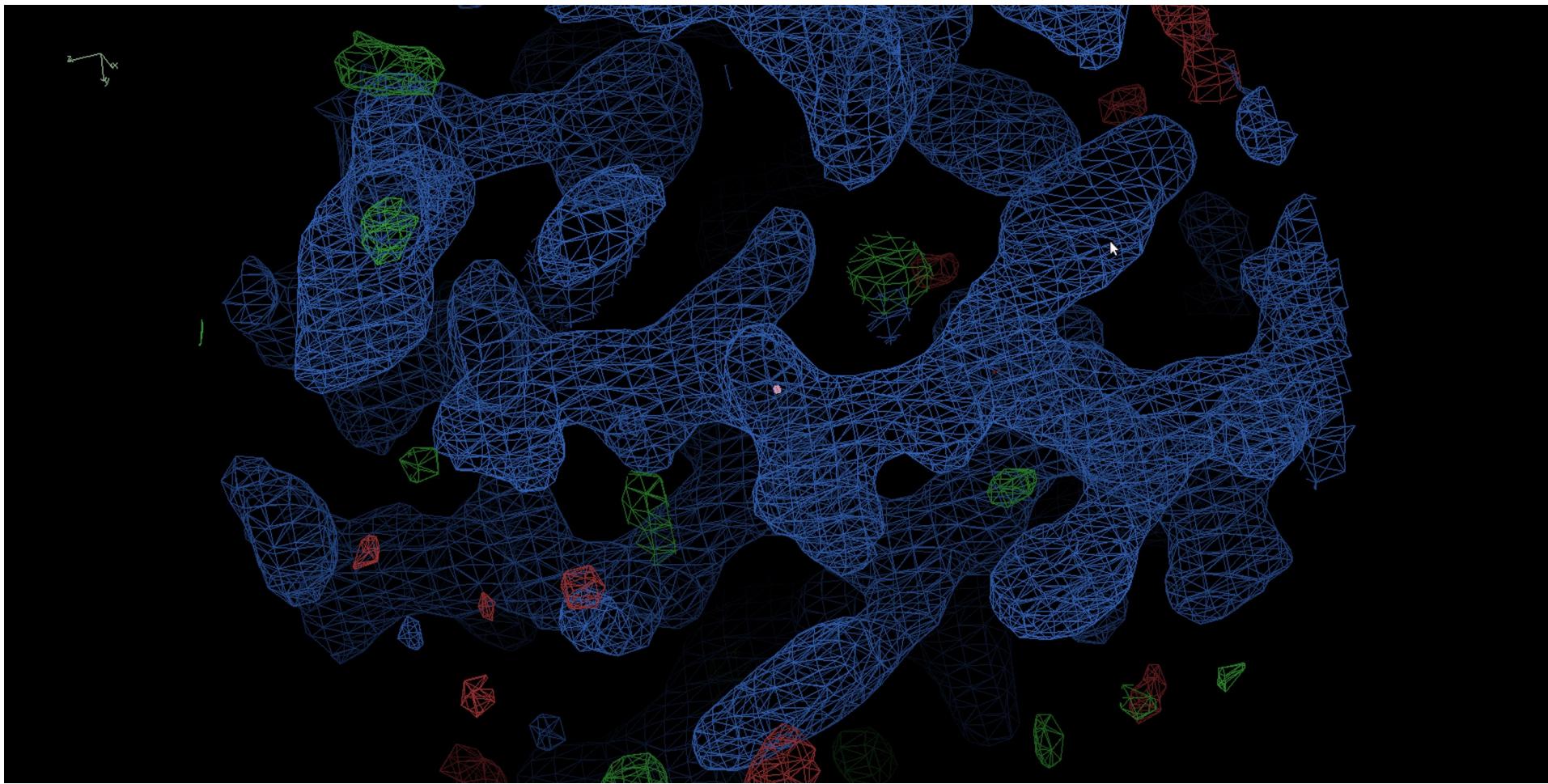


$$R = \frac{\sum |\vec{F}_{obs}| - |\vec{F}_{calc}|}{\sum |\vec{F}_{obs}|}$$

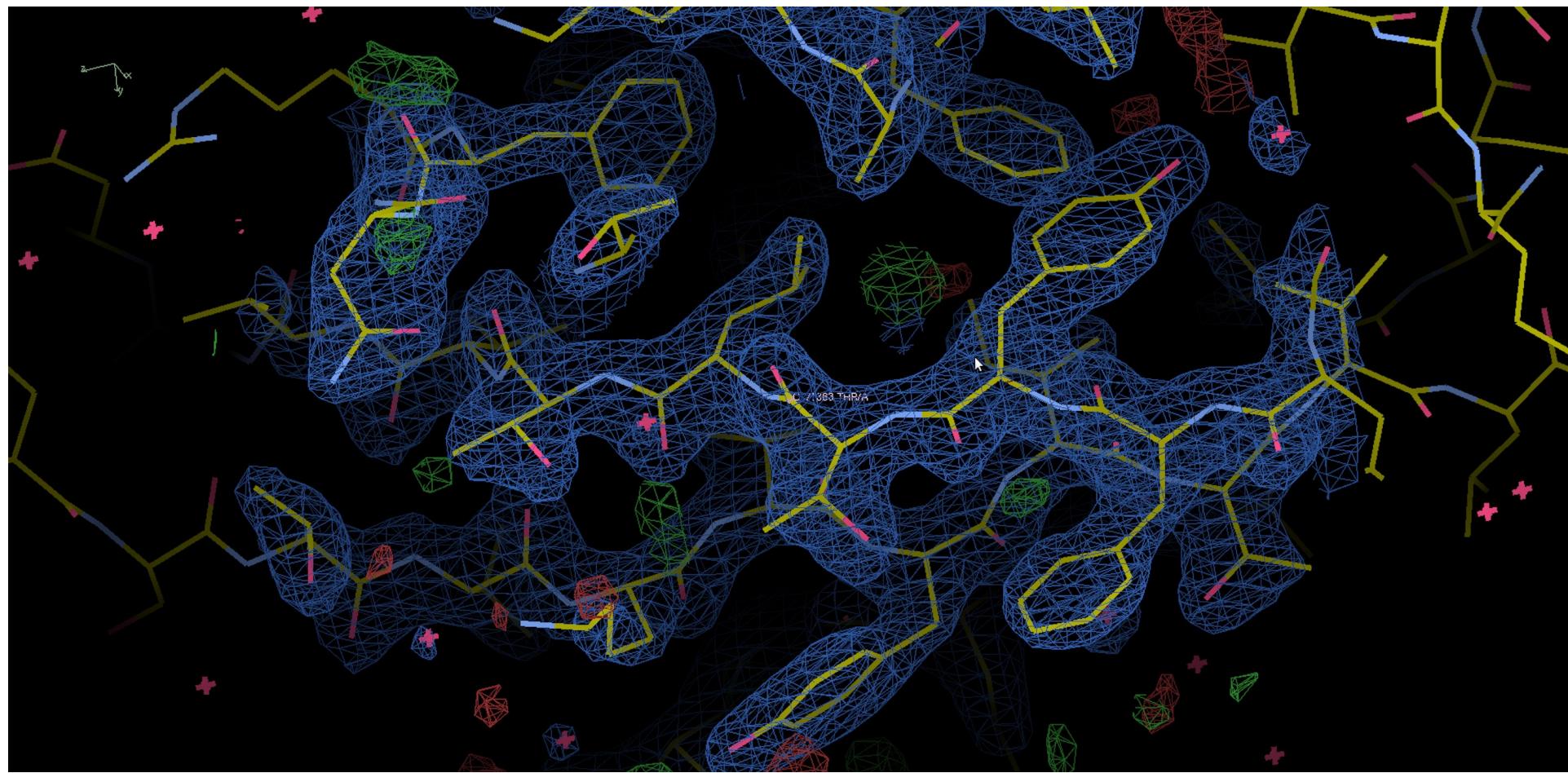




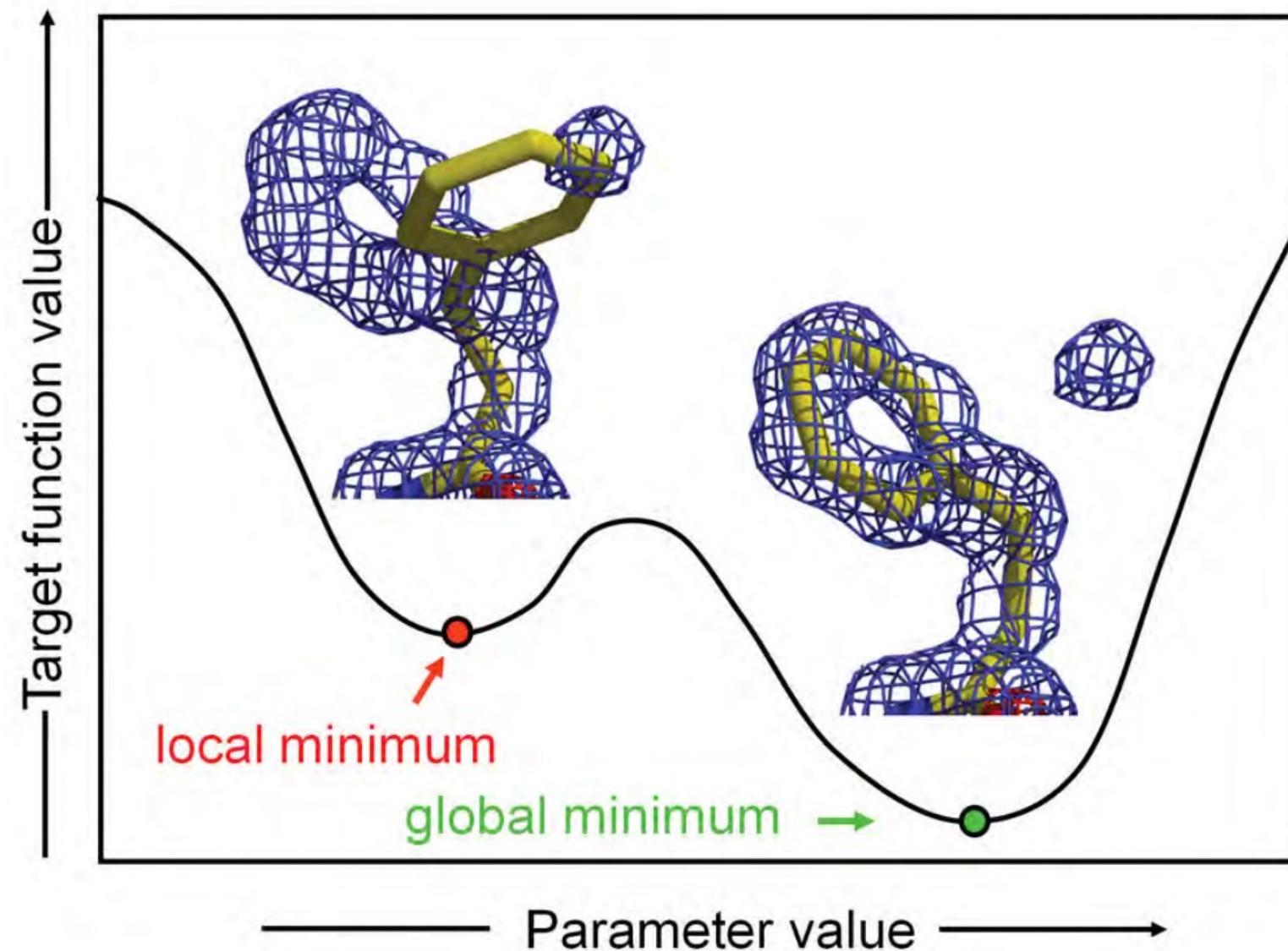
Electron density



3D - Model



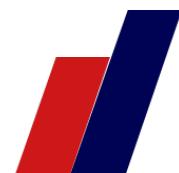
From Electron density to a 3D-model



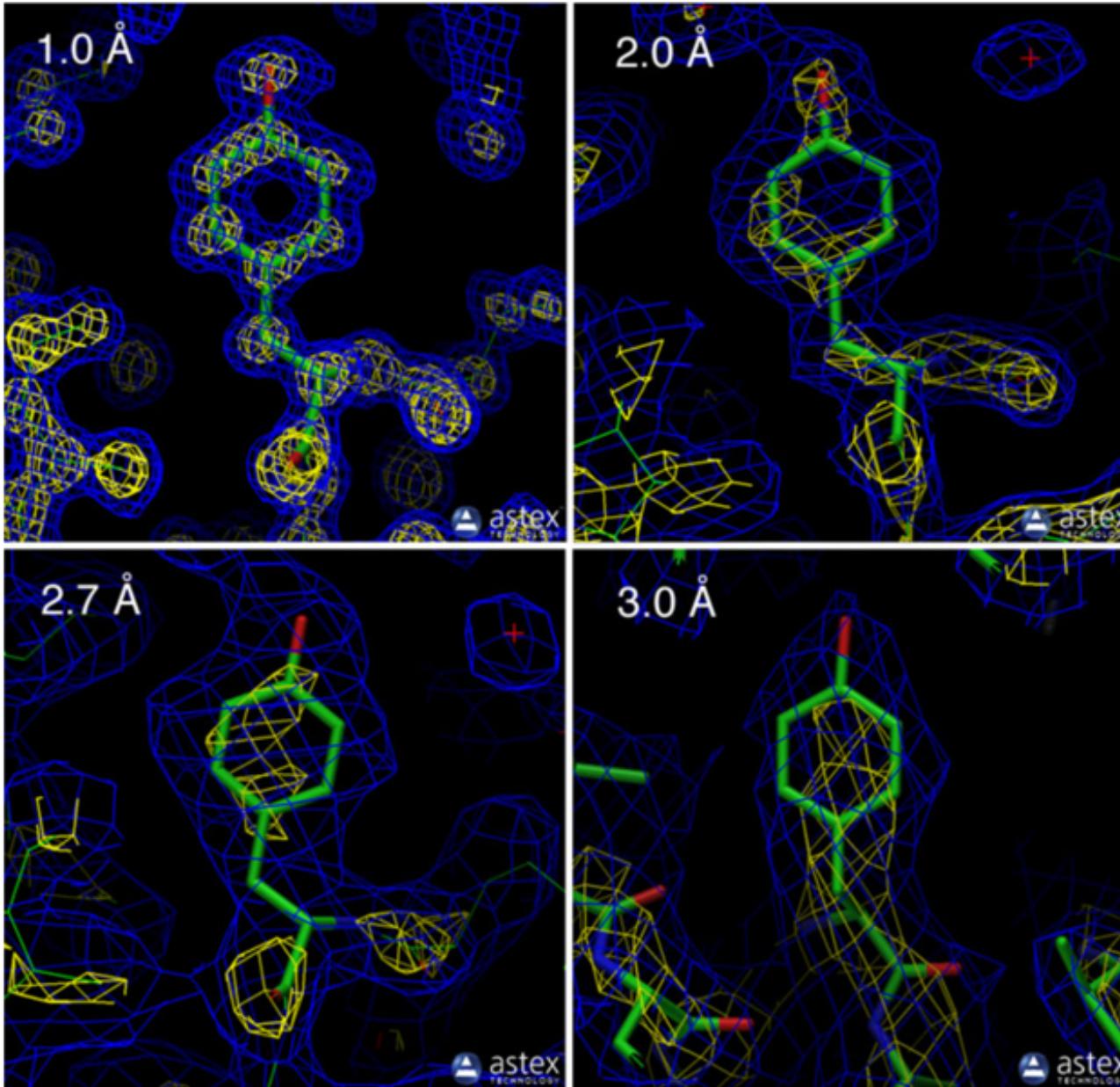
Resolution

Resolution is a measure of the quality of the data that has been collected on the crystal containing the protein or nucleic acid. If all of the proteins in the crystal are aligned in an identical way, forming a very perfect crystal, then all of the proteins will scatter X-rays the same way, and the diffraction pattern will show the fine details of crystal. On the other hand, if the proteins in the crystal are all slightly different, due to local flexibility or motion, the diffraction pattern will not contain as much fine information. So resolution is a measure of the level of detail present in the diffraction pattern and the level of detail that will be seen when the electron density map is calculated. High-resolution structures, with resolution values of 1 Å or so, are highly ordered and it is easy to see every atom in the electron density map. Lower resolution structures, with resolution of 3 Å or higher, show only the basic contours of the protein chain, and the atomic structure must be inferred. Most crystallographic-defined structures of proteins fall in between these two extremes. As a general rule of thumb, we have more confidence in the location of atoms in structures with resolution values that are small, called "high-resolution structures".

Resolution



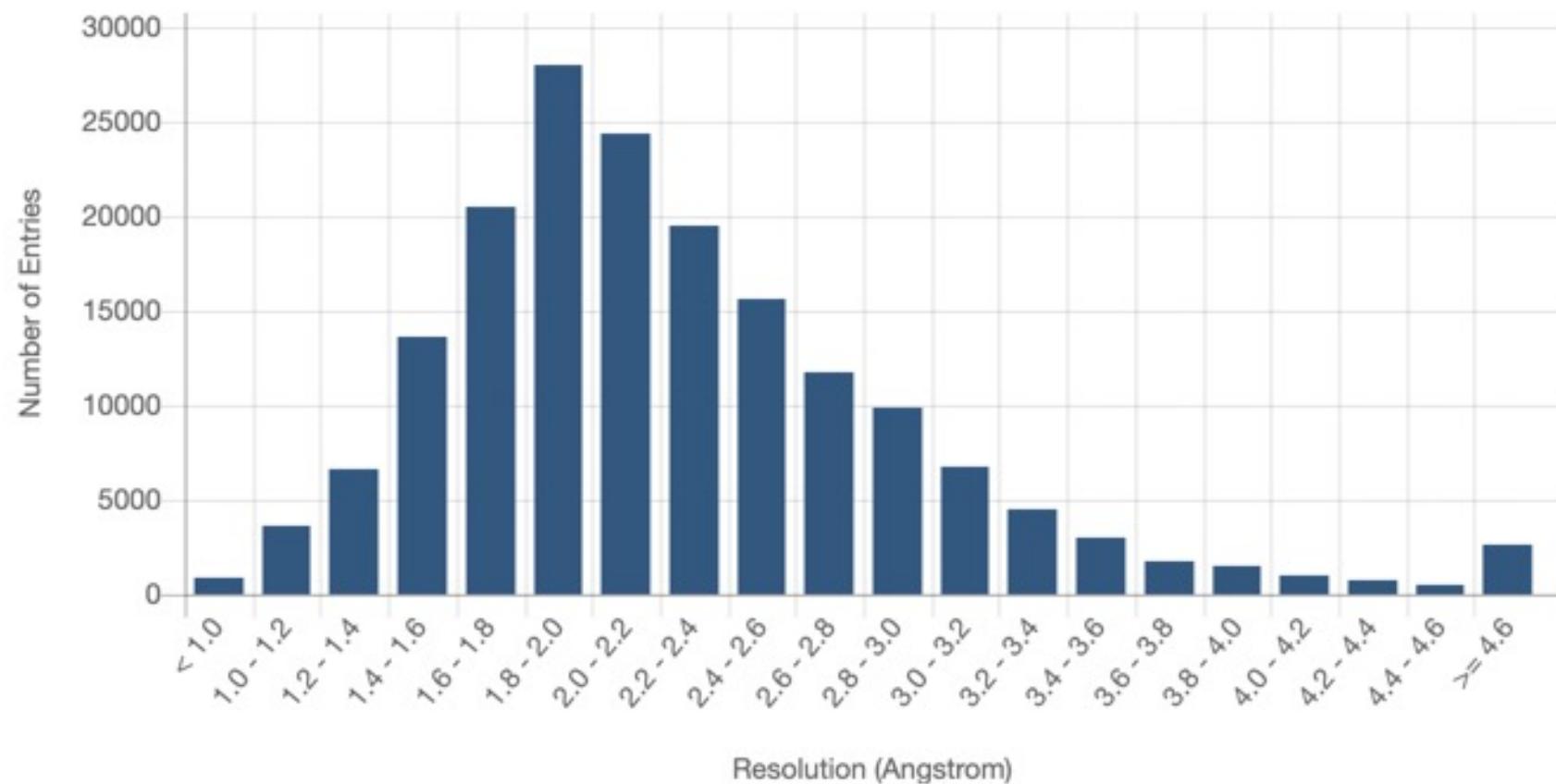
Resolution



PDB Statistics: PDB Data Distribution by Resolution

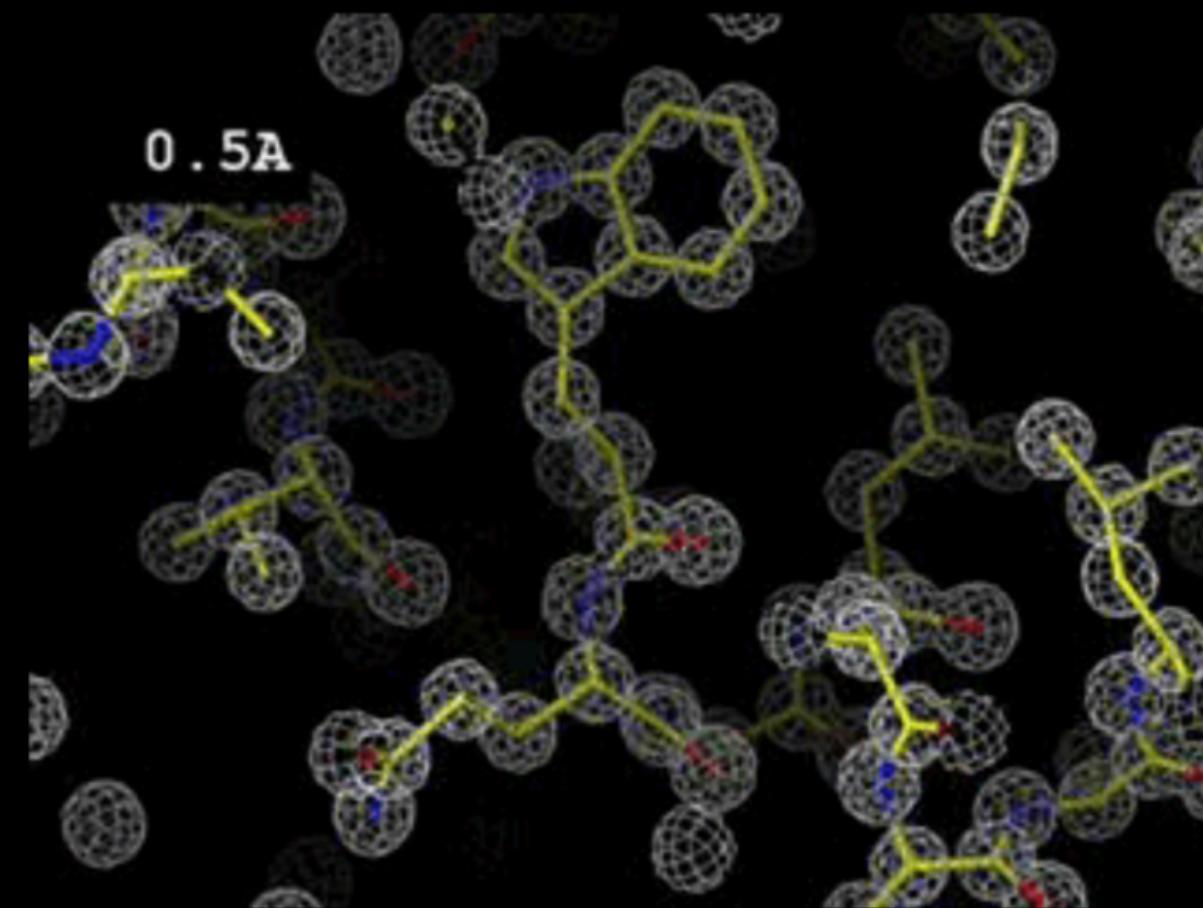
Distribution by structure [resolution](#). Data shown include structures solved by X-ray crystallography or electron microscopy.

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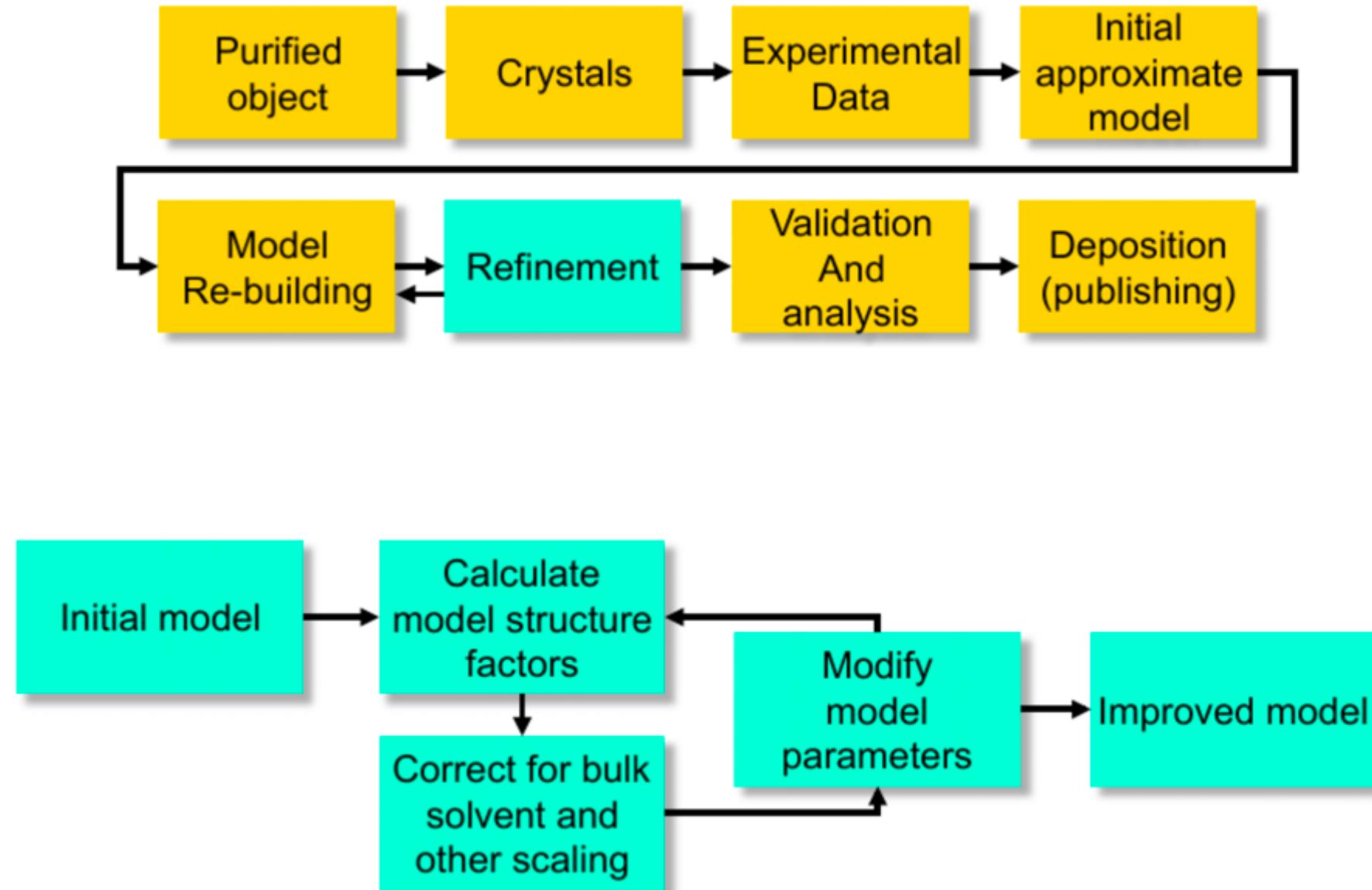


Resolution

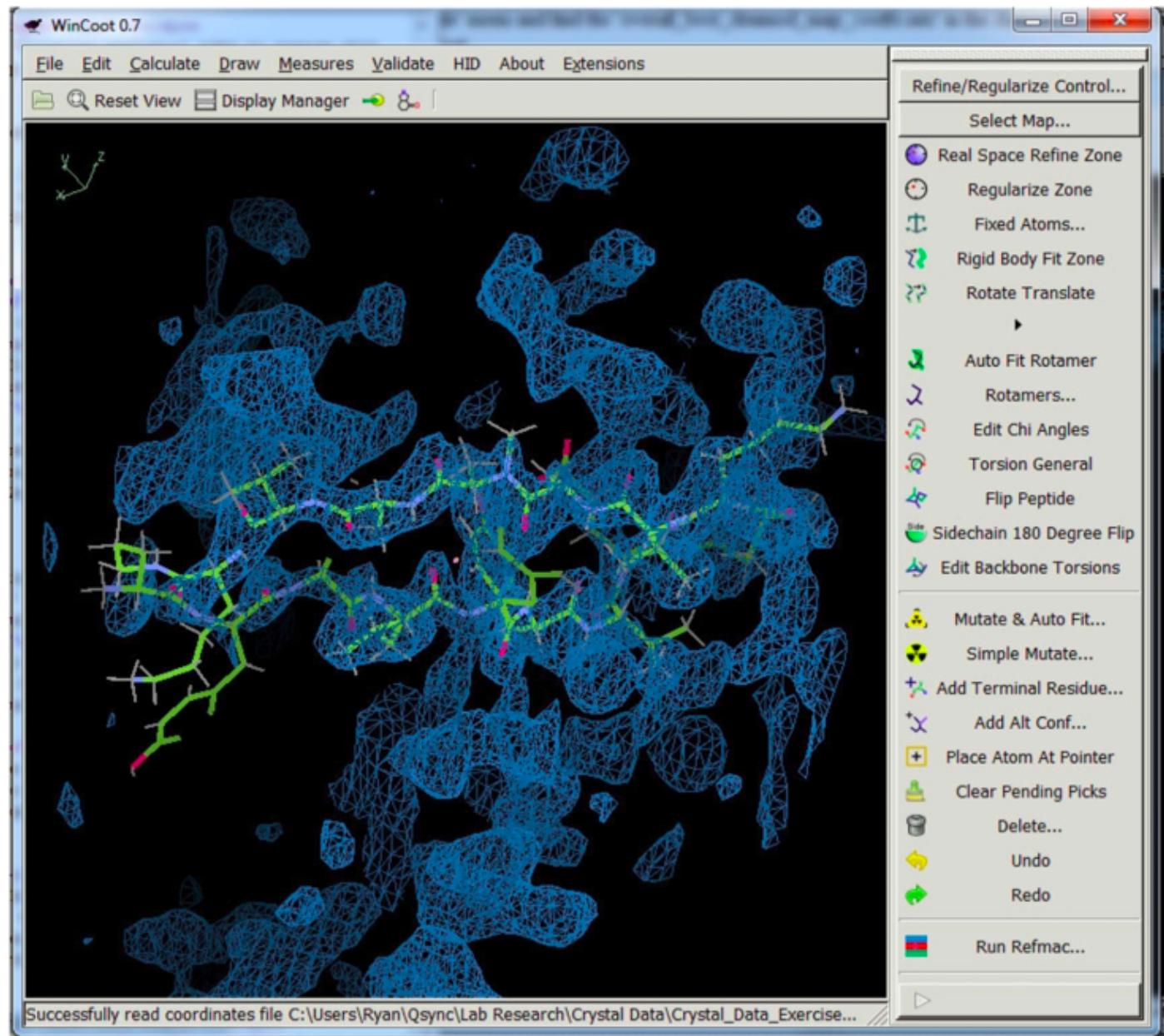
The meaning of resolution

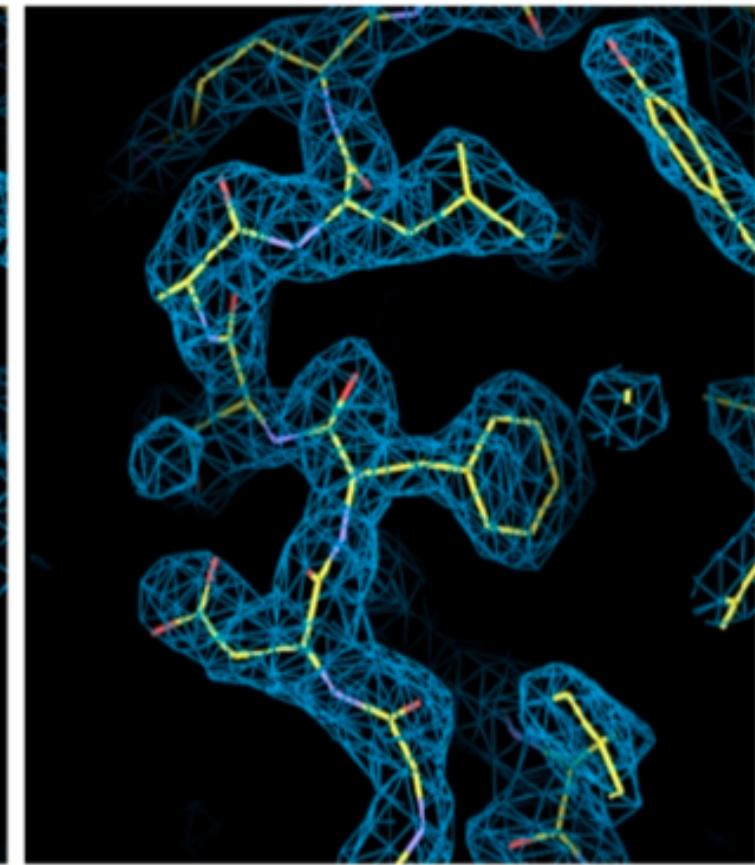
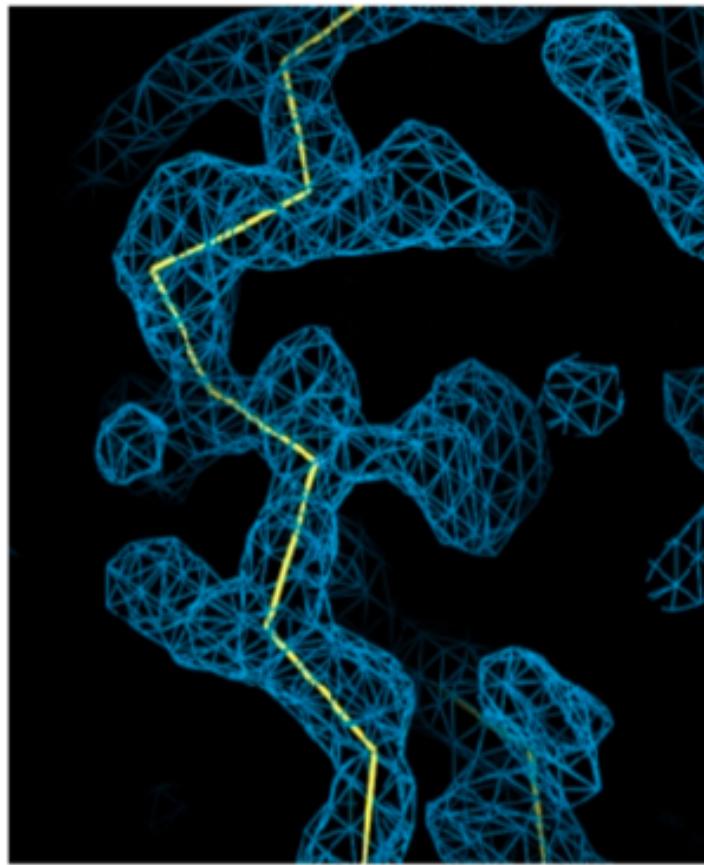
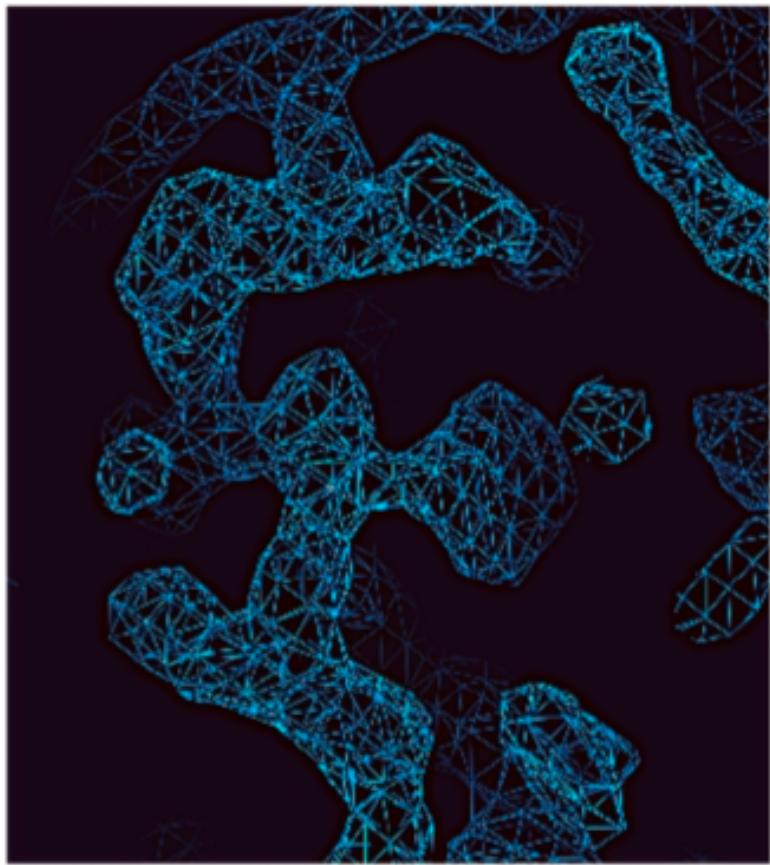


From experimental data to a 3D model

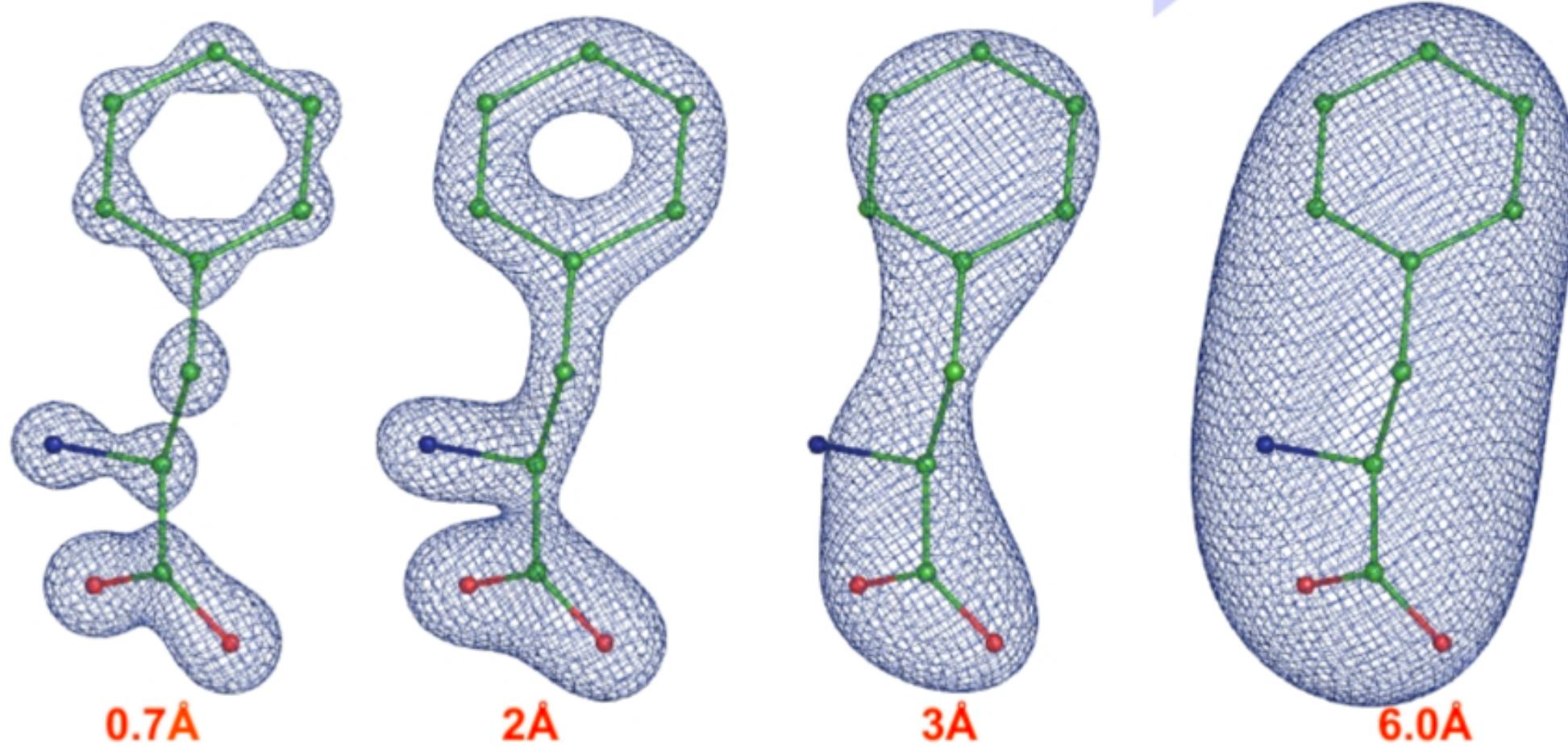


Software -> Coot

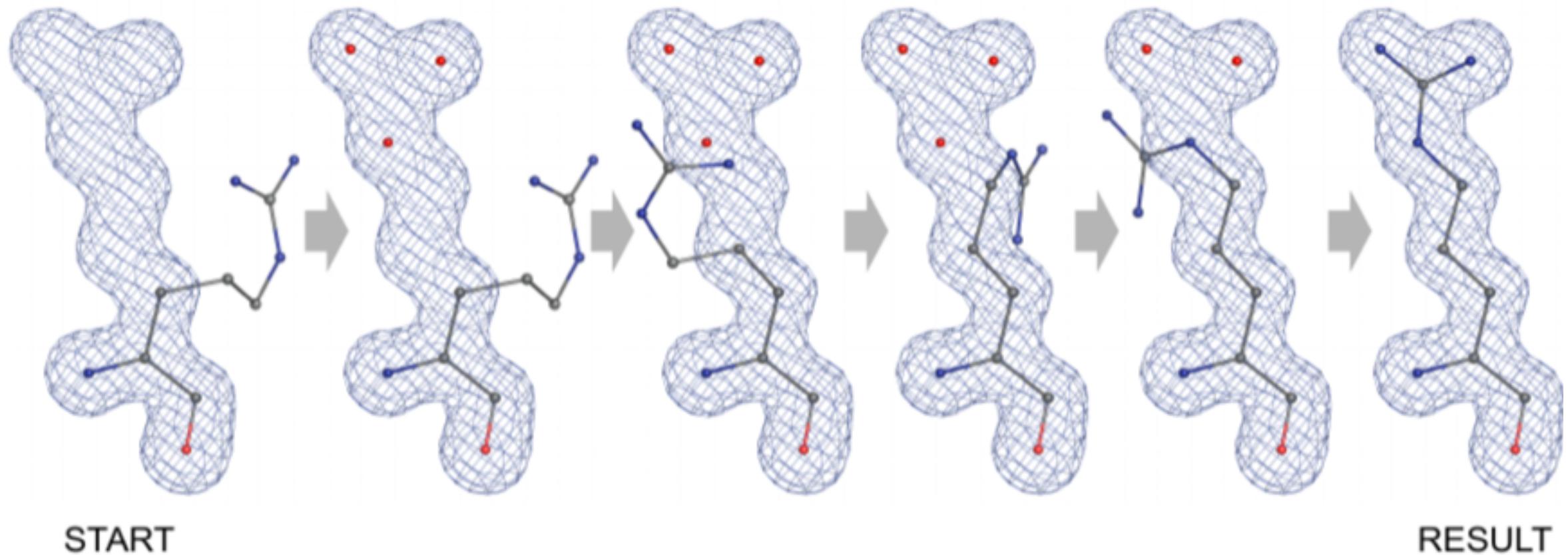




High Resolution → Low



Refinement



The Nobel Prize in Physics 1914



Photo from the Nobel Foundation archive.

Max von Laue

Prize share: 1/1

for his discovery of the diffraction of X-rays by crystals.

The Nobel Prize in Physics 1915

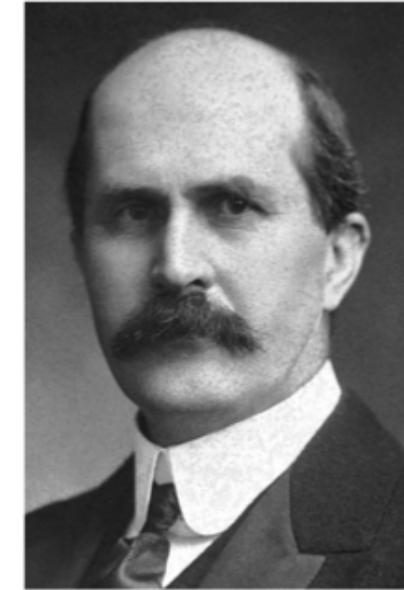


Photo from the Nobel Foundation archive.

Sir William Henry Bragg

Prize share: 1/2



Photo from the Nobel Foundation archive.

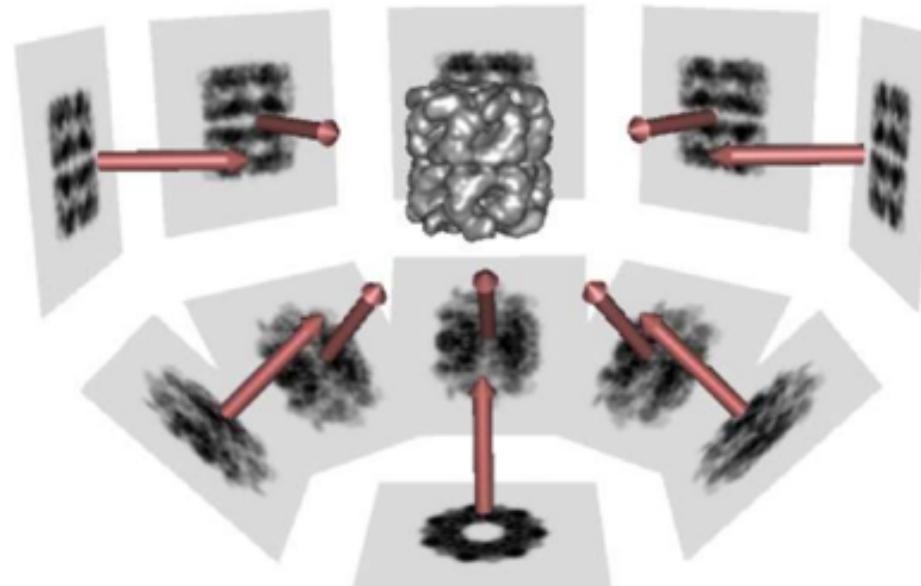
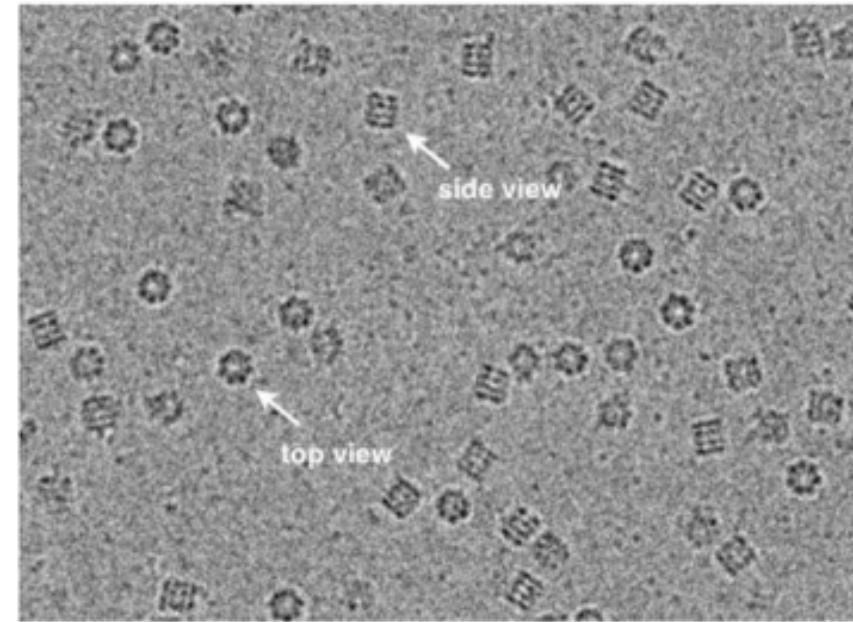
William Lawrence Bragg

Prize share: 1/2

For their services in the analysis of crystal structure by means of X-rays.

Cryo-EM – 3D Electron Microscopy

- Collect images of macromolecules frozen in ice
- Extract and orient particle images
- Reconstruct 3D volume
- In general, more particles => higher resolution (< 3 Å)



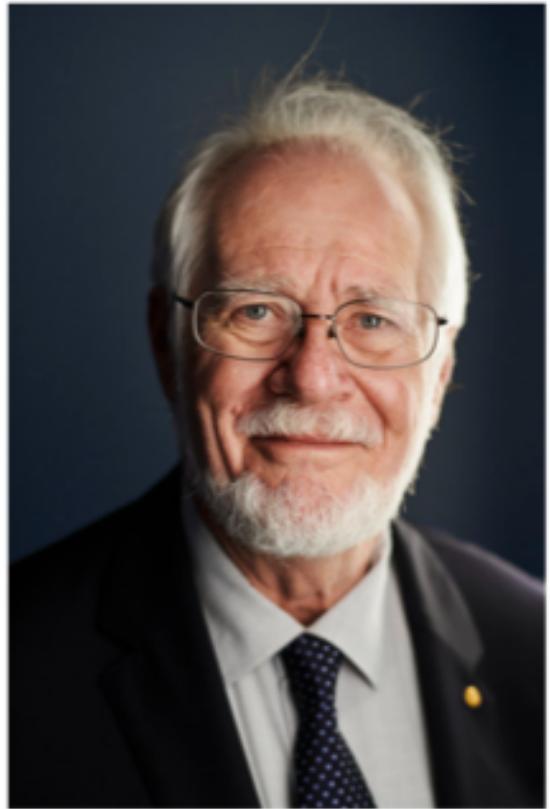
Methods for Determining Atomic Structures

Transmission Cryo-Electron Microscopy

A tool used by structural biologists to study
molecular nanomachines

Gabriel Lander, Thesis Defense 2009

The Nobel Prize in Chemistry 2017



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Mahmoud
Jacques Dubochet
Prize share: 1/3



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Richard Henderson
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For developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution





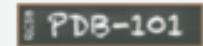
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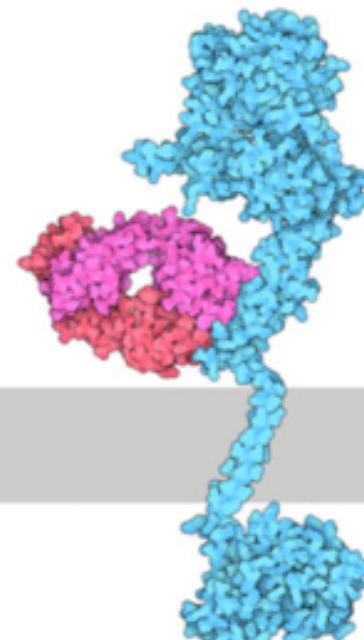
This resource is powered by the Protein Data Bank archive-information about the 3D shapes of proteins, nucleic acids, and complex assemblies that helps students and researchers understand all aspects of biomedicine and agriculture, from protein synthesis to health and disease.

As a member of the wwPDB, the RCSB PDB curates and annotates PDB data.

The RCSB PDB builds upon the data by creating tools and resources for research and education in molecular biology, structural biology, computational biology, and beyond.



April Molecule of the Month



HER2/neu and Trastuzumab



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