

X-ray crystallography

CS/CME/BioE/Biophys/BMI 279

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Outline

- What is x-ray crystallography and why
- Crystals
- Diffraction basics: from electromagnetic waves to density
- The phase problem
- Methods for determining structure from the diffraction pattern

What is x-ray crystallography and why

From the previous “microscopy” lecture....

Recap: One way to beat the diffraction limit for light microscopy is to choose a wave with a lower wavelength. X-rays have very short wavelengths ~ 1 Angstrom.

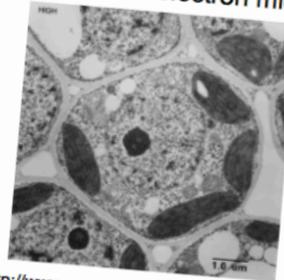
Resolution limits
Beating the diffraction limit

28

Option 1: Decrease the wavelength

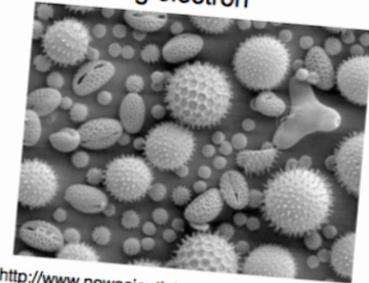
- Higher-frequency radiation (e.g., x-rays) has shorter wavelengths and thus allows higher resolution
 - It also damages the sample more
- It's possible to image with electrons, which have a *much* shorter wavelength (~.1 nm)
 - Electron microscopy can thus achieve much higher resolution
 - Disadvantages: can't use living cells, and molecules of interest won't glow

Transmission electron microscopy



http://www.cas.miamioh.edu/~meicenrd/ANATOMY/Ch2_Ultrastructure/Tempcell.htm

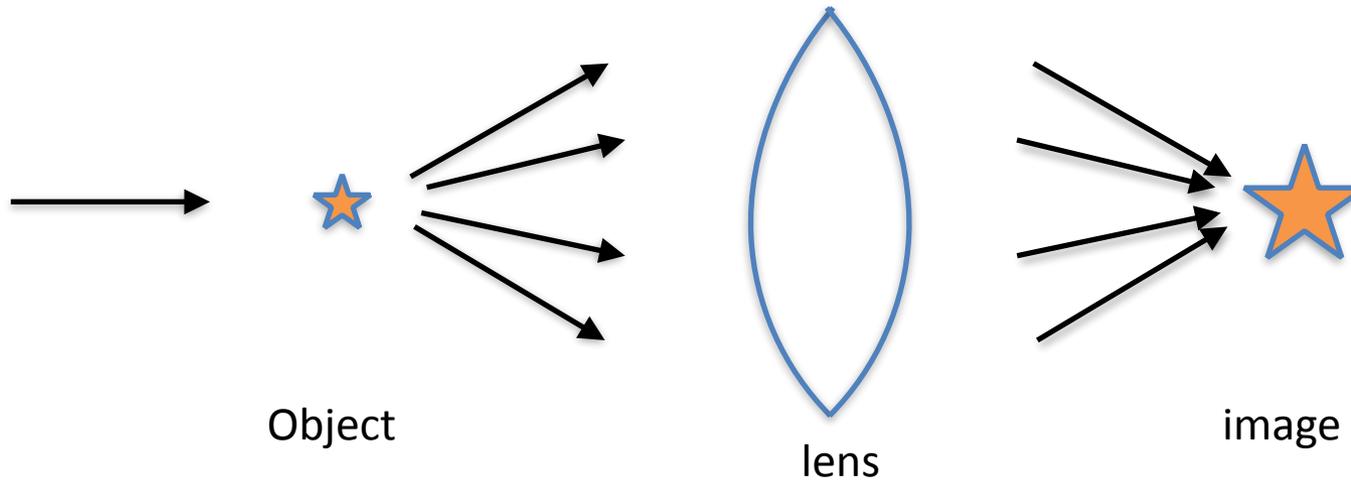
Scanning electron



http://www.newscientist.com/data/images/ns/cms/dn14136/dn14136-1_788.jpg

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microscopy through lens



To see atomic-level resolution, needs lights with wavelength around 1 \AA (x-ray) **Problem solved! (?)**

But unfortunately there are is no lens to focus x-ray!

So perhaps we can collect the scattering information and use computation to create the image without the lens

(you'll see why we need crystals later)

X-ray crystallography is the most common way to determine 3D molecular structures

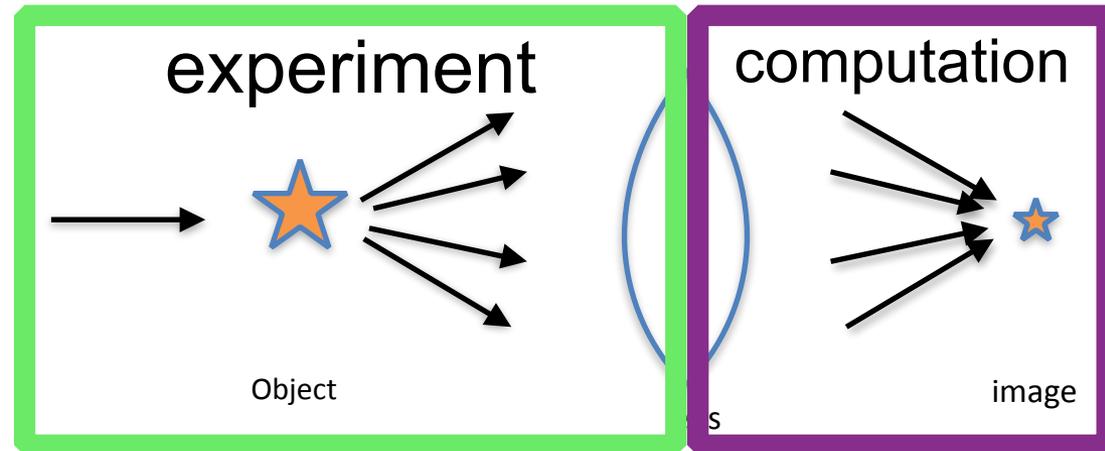
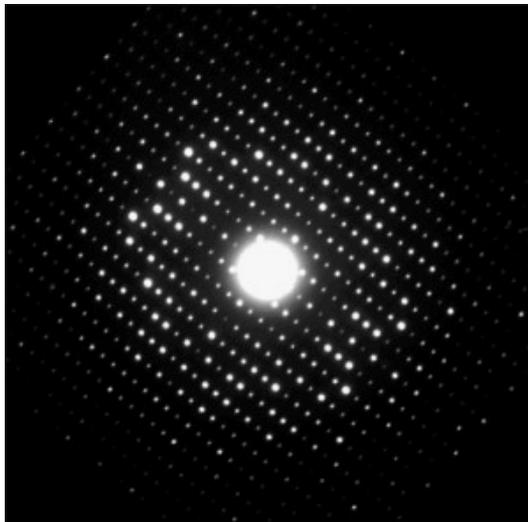
Of the pdb structures, structures resolved from crystallography tend to be better than those resolved from CryoEM or NMR.

- 90% of the structures in the PDB were determined through x-ray crystallography
- X-ray crystallography is also frequently used to determine structures of other biomolecules (e.g, RNA) or of small molecules (including drugs)
- Why are we covering it in this course?
 - So you know where biomolecular structures come from
 - Because determining a structure this way involves solving a challenging computational problem

The basic idea

We can now obtain higher-resolution information about the structure, but we need to reconstruct this information via computation; this is because we can't focus x-rays like we do light waves.

- Get the molecule whose structure you want to determine to form a crystal
- Shine an intense beam of x-rays through the crystal, giving rise to a “diffraction pattern” (a pattern of spots of varying brightnesses)



<http://lacasadeloscristales.trianatech.com/wp-content/uploads/2014/09/image005-300x300.jpg>

Crystals

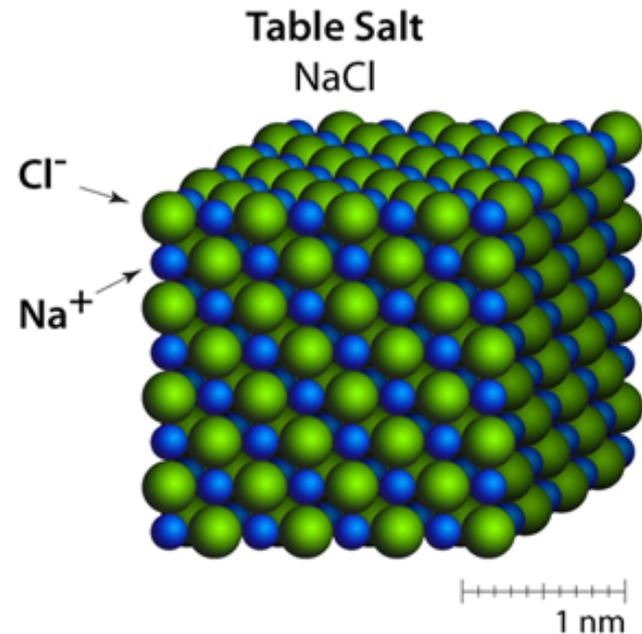
Why do we use protein crystals for x-ray diffraction?

What's a crystal?

- Under certain conditions, molecules line up into a regular grid (a “lattice”).
 - Example: table salt

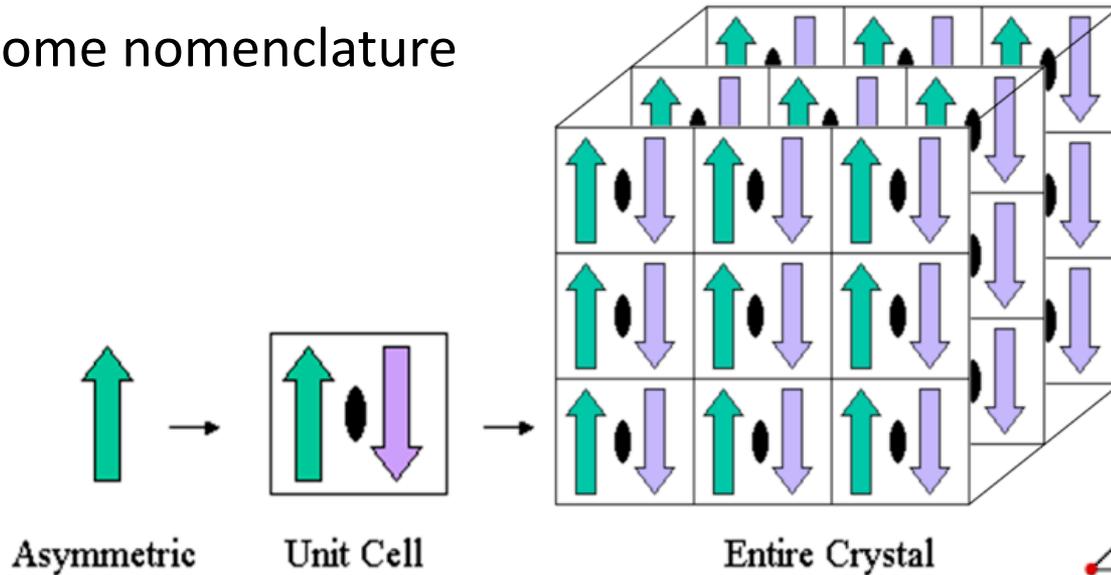


<http://www.bigfoto.com/miscellaneous/photos-16/salt-crystals-94jf.jpg>



<http://www.atomsinmotion.com/book/chapter4/rockSalt.png>

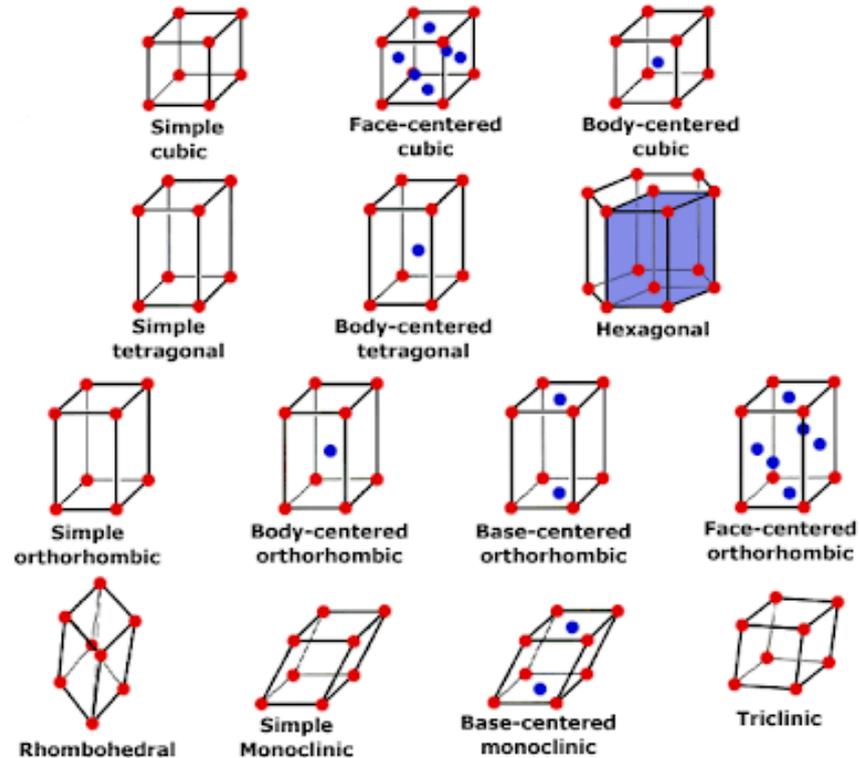
Some nomenclature



smallest symmetrically related unit of a crystal

From the Educational Resource of the IUCr, 'Looking at Structures: Introduction to Biological Assemblies and the PDB Archive', <http://www.iucr.org/>

There are many different kinds of lattices with various sizes and shapes

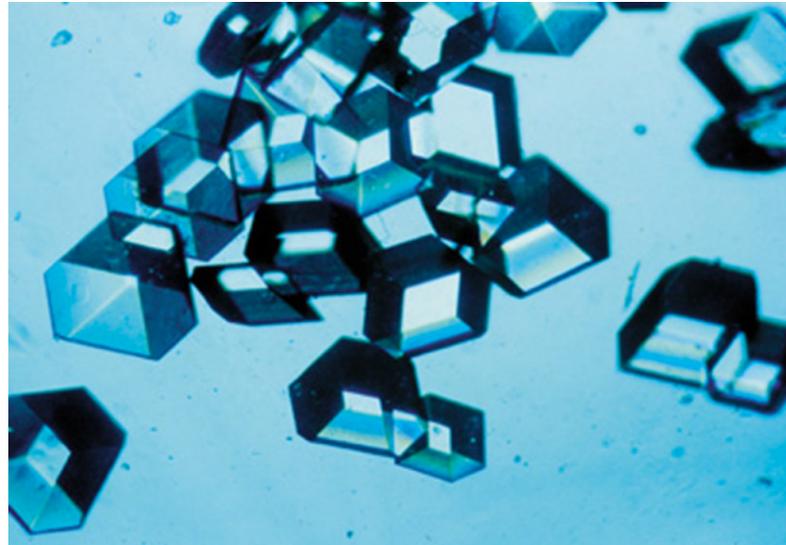


Proteins can also form crystals

- Under certain conditions, entire proteins will pack into a regular grid (a lattice)

Proteins provide the lattice for the crystal

Insulin crystals



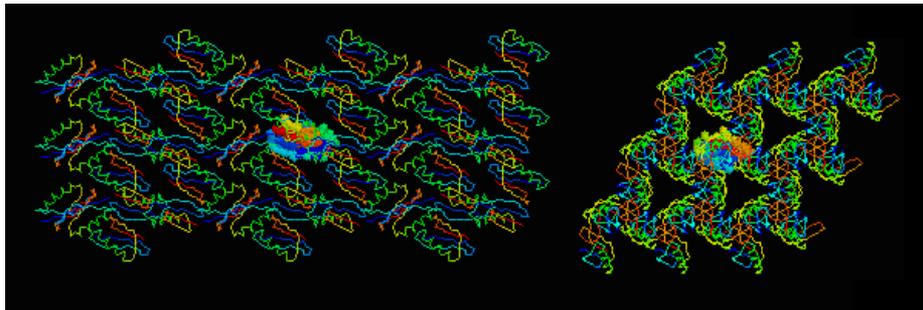
Note: even crystals without large regular edges can contain very regular internal structure

http://science.nasa.gov/media/medialibrary/1999/09/10/msad20sep99_1_resources/9901879.jpg

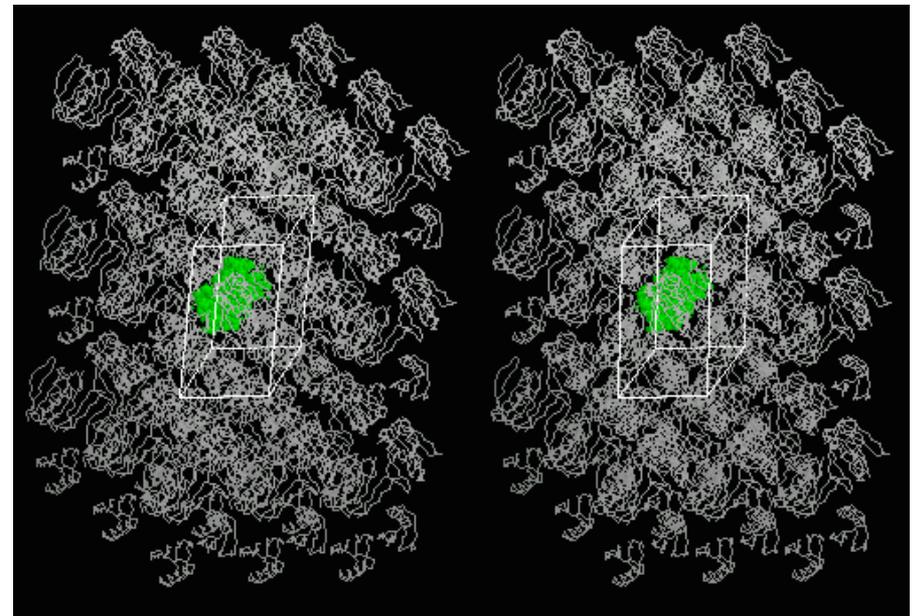
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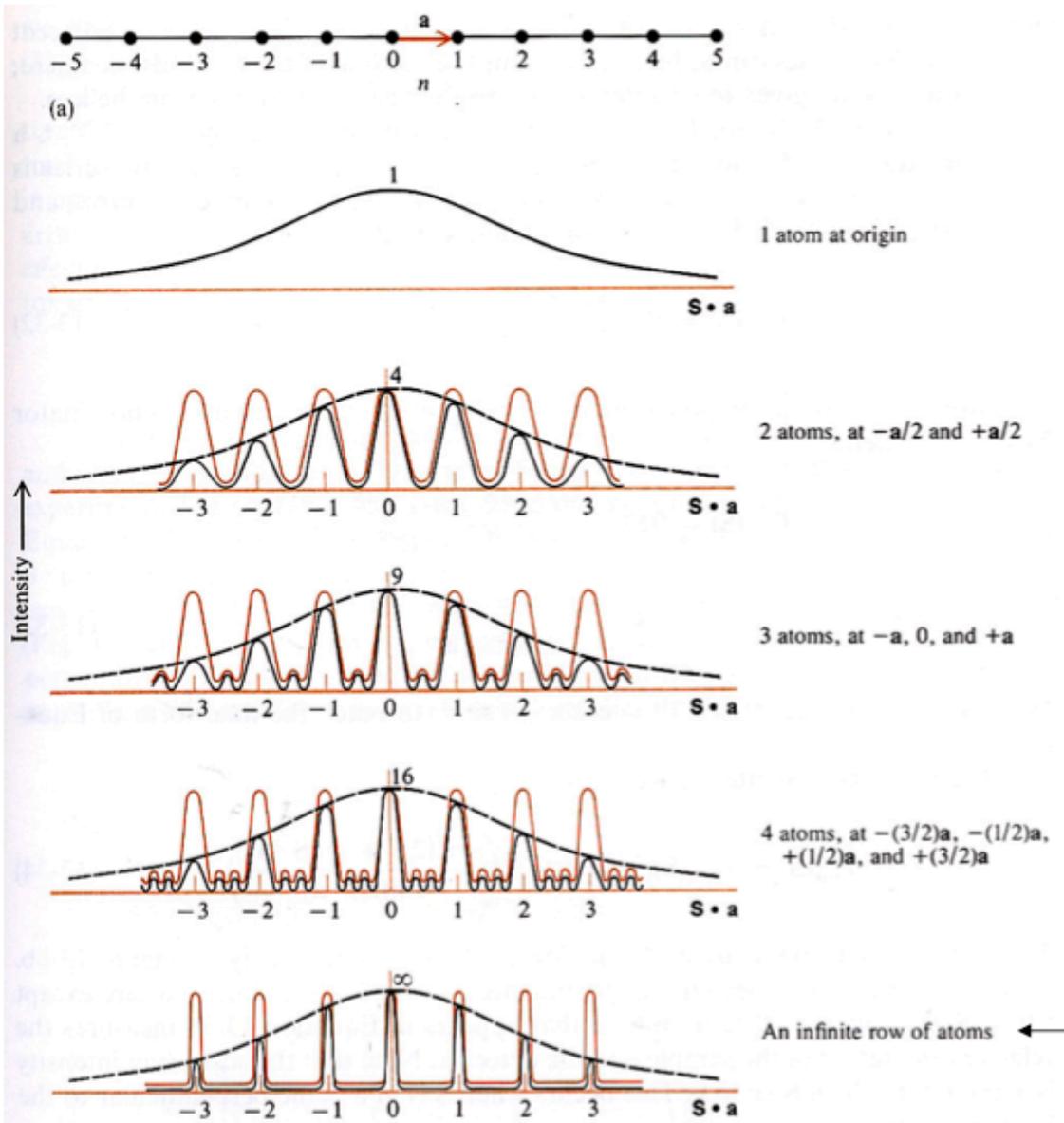
Multiple views of the crystal formed by an immunoglobulin-binding domain (PDB entry 1PGB)



Multiple asymmetric units (each a protein) form the entire protein crystal. The interior or "open" space between proteins is water and ions



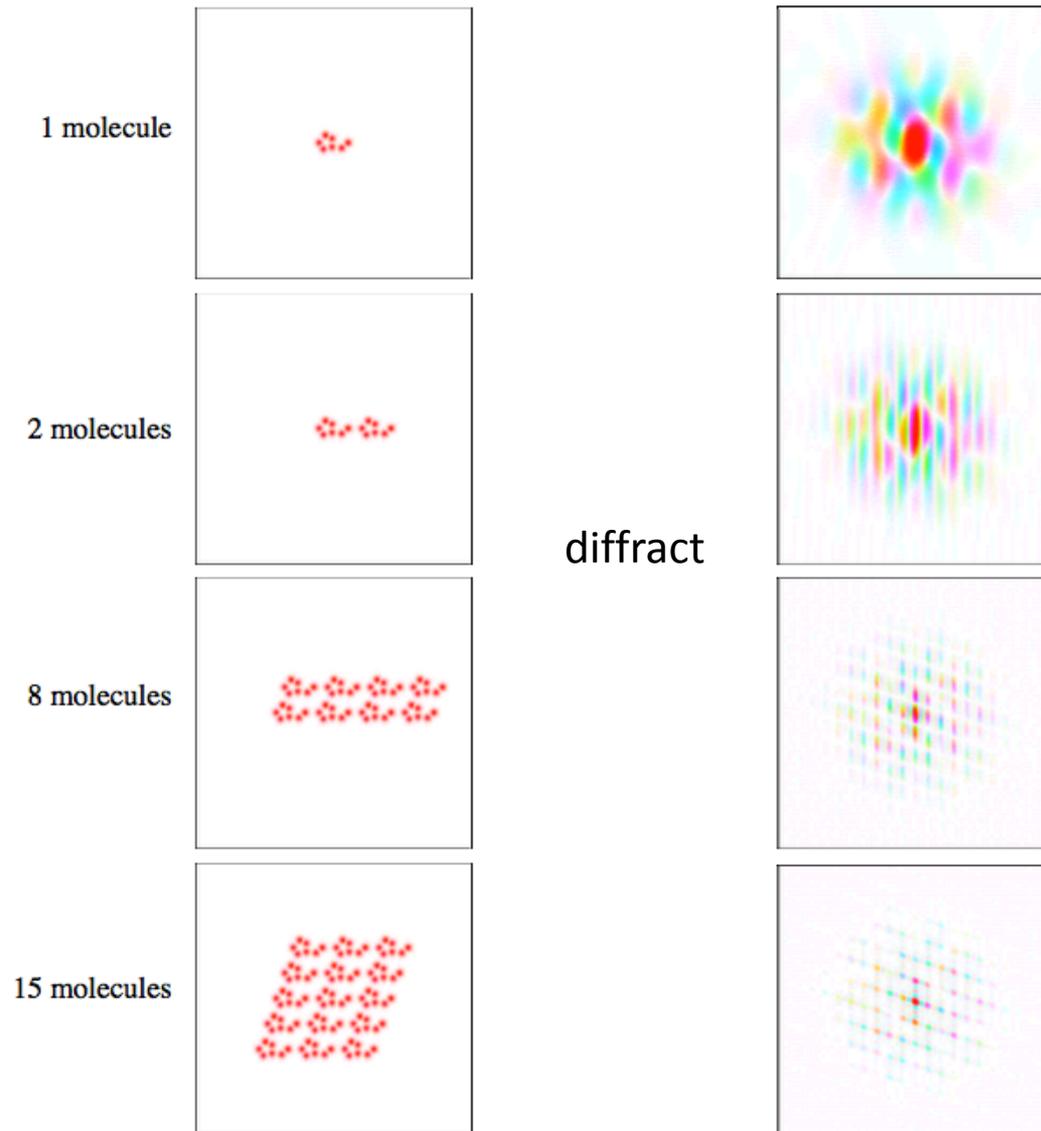
Why we use crystals



Diffraction of x-rays from a single protein unit crystal will form grating patterns. These patterns are amplified by multiple proteins in the same orientation. Thus crystals are important for diffraction, because specific orientations of the proteins are enforced through the crystal structure, amplifying the grating patterns for a given conformation.

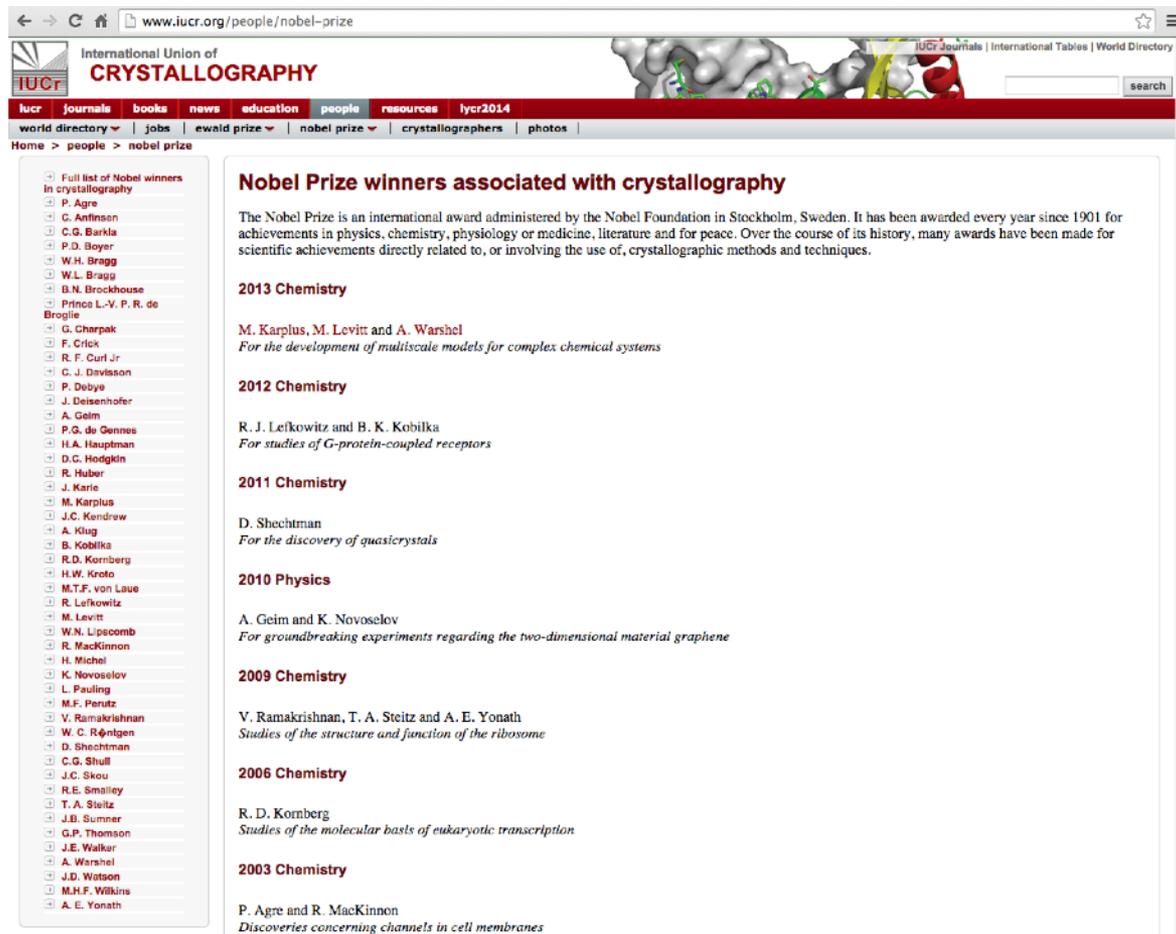
The repeating crystal lattice is used to amplify the signal

Single object diffracts weakly



Caveats

- Getting proteins to form crystals can be hard
 - Crystallographers sometimes work for decades to get good crystals of a particular protein



The screenshot shows the IUCr website page titled "Nobel Prize winners associated with crystallography". The page features a navigation menu with options like "lucr", "journals", "books", "news", "education", "people", "resources", and "lycr2014". Below the menu, there is a search bar and a list of categories including "world directory", "jobs", "ewald prize", "nobel prize", "crystallographers", and "photos". The main content area is titled "Nobel Prize winners associated with crystallography" and includes a brief introduction to the Nobel Prize. It lists winners from 2003 to 2013, each with their names and a short description of their work. A sidebar on the left provides a full list of Nobel winners in crystallography, including names like P. Agre, C. Anfinsen, C.G. Barkla, P.D. Boyer, W.H. Bragg, W.L. Bragg, B.N. Brockhouse, Prince L.-V. P. R. de Broglie, G. Cherpak, F. Crick, R. F. Curl Jr, C. J. Davison, P. Debye, J. Deisenhofer, A. Geim, P.G. de Gennes, H.A. Hauptman, D.G. Hodgkin, R. Huber, J. Karle, M. Karplus, J.C. Kendrew, A. Klug, B. Kobilka, R.D. Kornberg, H.W. Kroto, M.T.F. von Laue, R. Lefkowitz, M. Levitt, W.N. Lipscomb, R. MacKinnon, H. Michel, K. Novoselov, L. Pauling, M.F. Perutz, V. Ramakrishnan, W. C. Röntgen, D. Suckman, C.G. Shull, J.C. Skou, R.E. Smalley, T. A. Steltz, J.B. Sumner, G.P. Thomson, J.E. Walker, A. Warshel, J.D. Watson, M.H.F. Wilkins, and A. E. Yonath.

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Nobel Prize winners associated with crystallography

The Nobel Prize is an international award administered by the Nobel Foundation in Stockholm, Sweden. It has been awarded every year since 1901 for achievements in physics, chemistry, physiology or medicine, literature and for peace. Over the course of its history, many awards have been made for scientific achievements directly related to, or involving the use of, crystallographic methods and techniques.

2013 Chemistry

M. Karplus, M. Levitt and A. Warshel
For the development of multiscale models for complex chemical systems

2012 Chemistry

R. J. Lefkowitz and B. K. Kobilka
For studies of G-protein-coupled receptors

2011 Chemistry

D. Shechtman
For the discovery of quasicrystals

2010 Physics

A. Geim and K. Novoselov
For groundbreaking experiments regarding the two-dimensional material graphene

2009 Chemistry

V. Ramakrishnan, T. A. Steitz and A. E. Yonath
Studies of the structure and function of the ribosome

2006 Chemistry

R. D. Kornberg
Studies of the molecular basis of eukaryotic transcription

2003 Chemistry

P. Agre and R. MacKinnon
Discoveries concerning channels in cell membranes

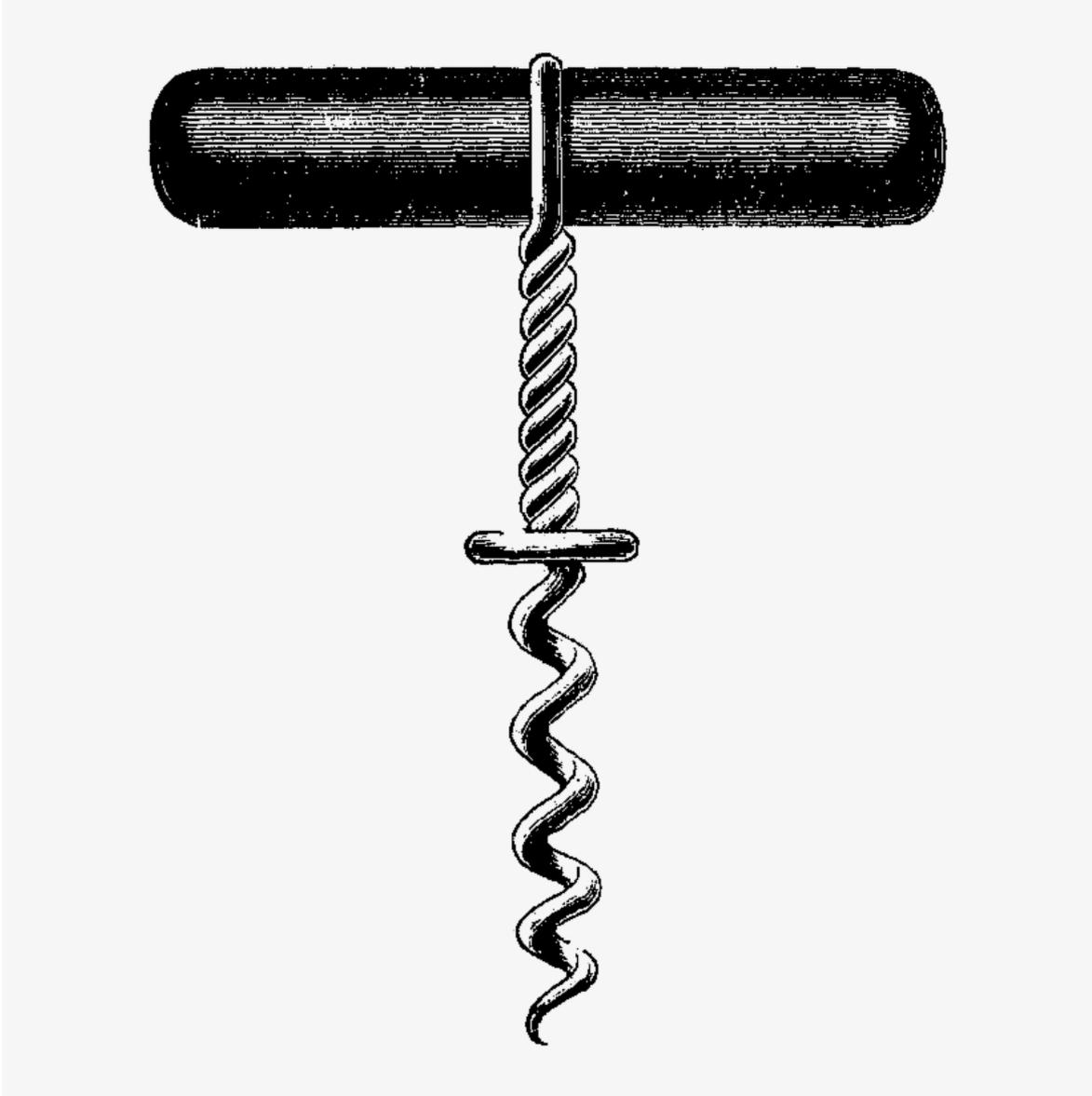
- Full list of Nobel winners in crystallography
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- R. F. Curl Jr
- C. J. Davison
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- H.A. Hauptman
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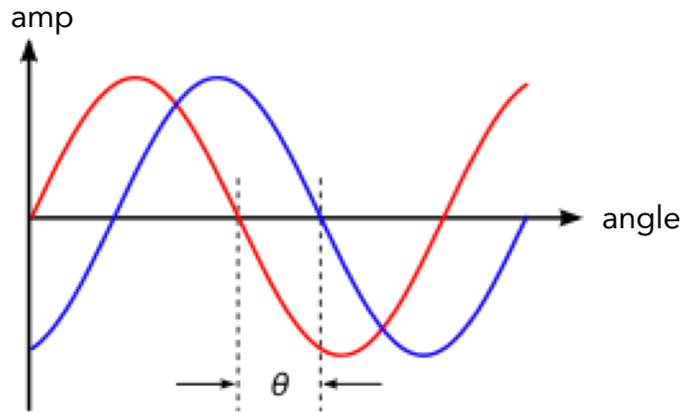
Caveats

- Sometimes a protein will adopt a different structure in a crystal than it does in its natural environment
- Crystallography gives you a static snapshot of a protein's structure
 - Usually (but not always) this snapshot corresponds to the protein's “average” structure

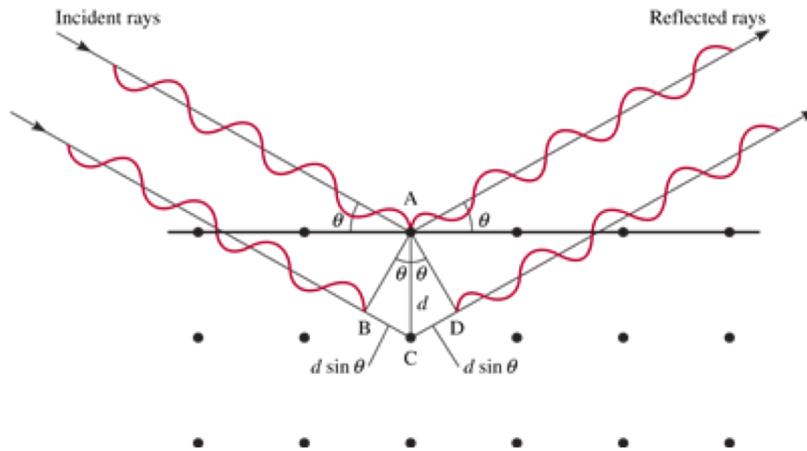
Diffraction basics: from electromagnetic waves to density

(whiteboard notes will be provided later)





For a given wave with offset x (in distance), we can represent the offset as a function of the waves angle and wavelength. Note that every 360 degrees or 2π radians, the wave repeats itself, or travels its wavelength λ . So if a given wave (RED) is shifted by a distance x to form the new wave (BLUE), we can represent this shift as an angular shift as follows $\theta = x * \frac{2\pi}{\lambda}$. If our original function is of the form $\cos(\text{angle})$, then our new shifted function will be of the form $\cos(\text{angle} + x * \frac{2\pi}{\lambda})$



In X-ray diffraction, x-rays will reflect off of proteins within the crystal. Because most of the x-rays will be entering the crystal as a parallel beam, and because there is physical distance between adjacent proteins within the crystal, the reflected x-rays will be offset. We can define the amount of offset between the waves based on the incoming angle of the x-rays as well as the distance between two proteins in the crystal. What this means is we can calculate the distance between two proteins in space, given the angle of the incoming x-ray beam (more on this in the next lecture).