# 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

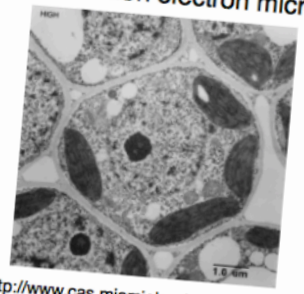
## Option 1: Decrease the wavelength

- 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)

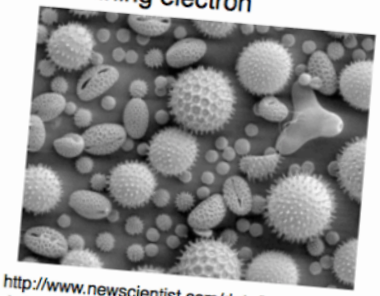
- Electron microscopy can thus achieve much higher resolution
- Disadvantages: can't use living cells, and molecules of interest won't glow

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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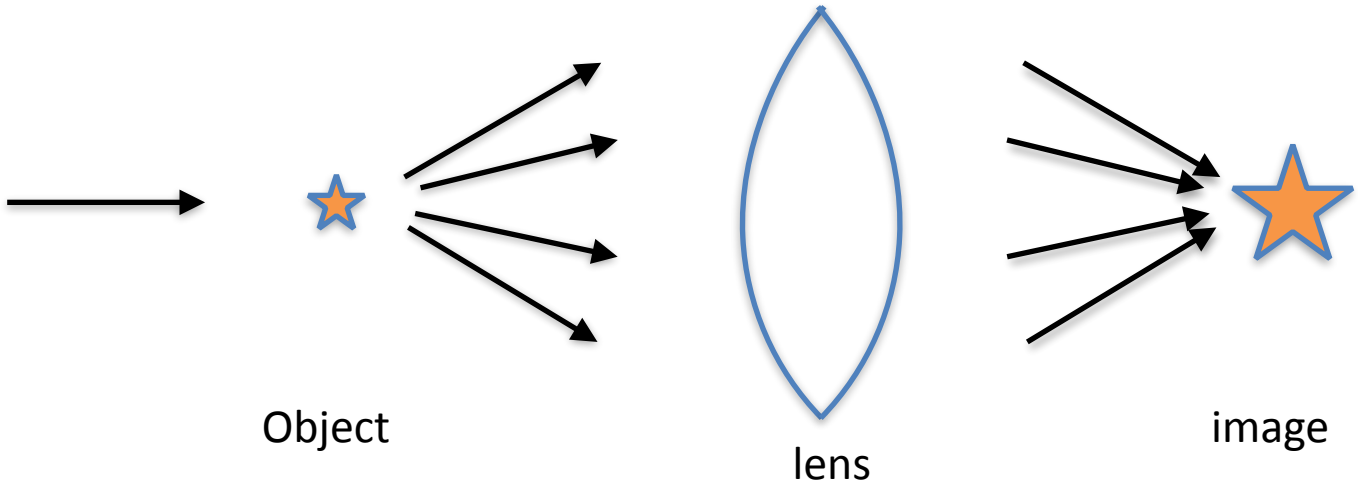
# microscopy through lens




To see atomic-level resolution, needs lights with wavelength around 1 Å (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

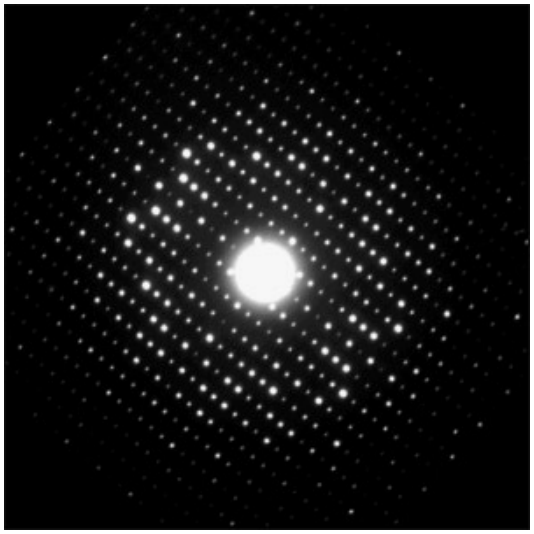
Of the pdb structures, structures resolved from crystallography tend to be better than those resolved from CryoEM or NMR.

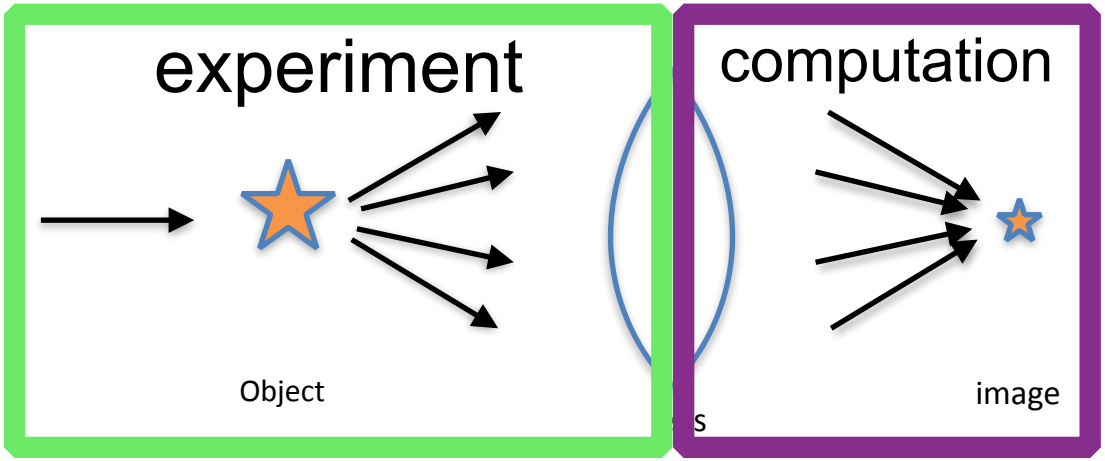
- 90% of the structures in the PDB were determined through x-ray crystallography
- X-ray crystallography is also frequently used to determine structures of other biomolecules (e.g, RNA) or of small molecules (including drugs)
- Why are we covering it in this course?
  - So you know where biomolecular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

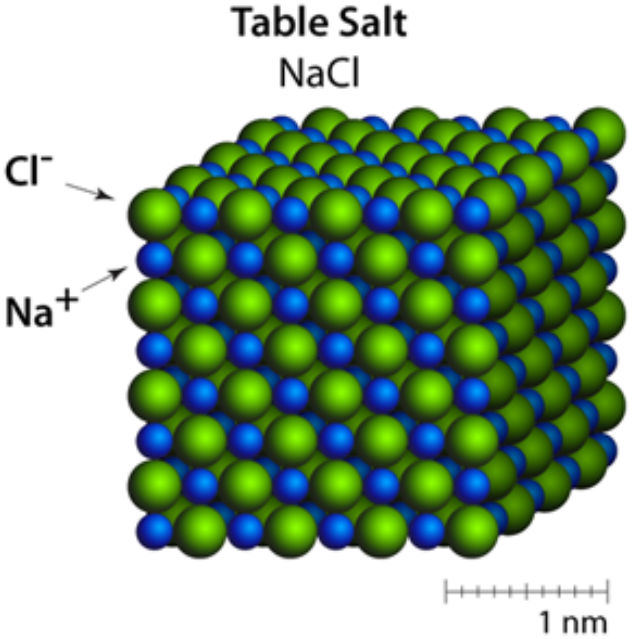
# Crystals

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

# What's a crystal?

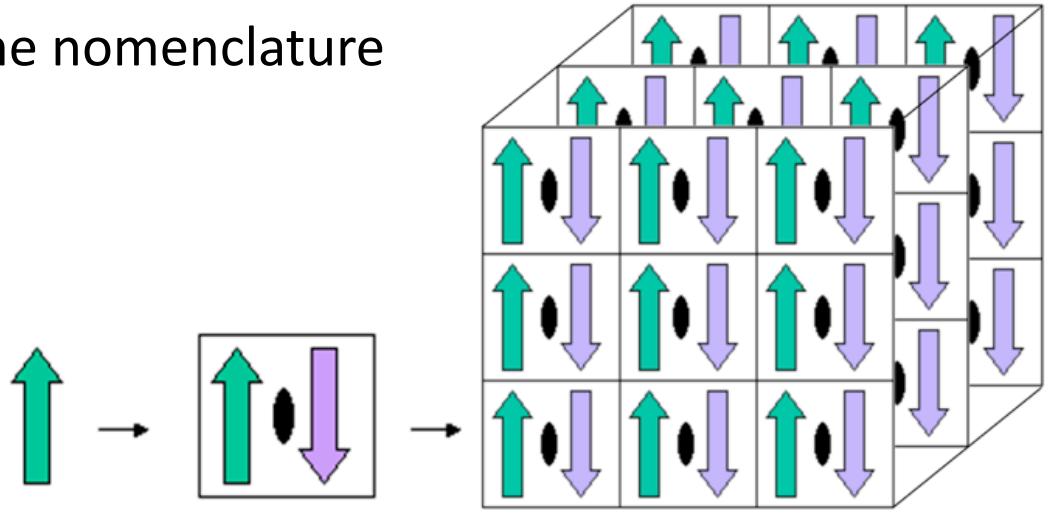
- Under certain conditions, molecules line up into a regular grid (a "lattice").
  - Example: table salt

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




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

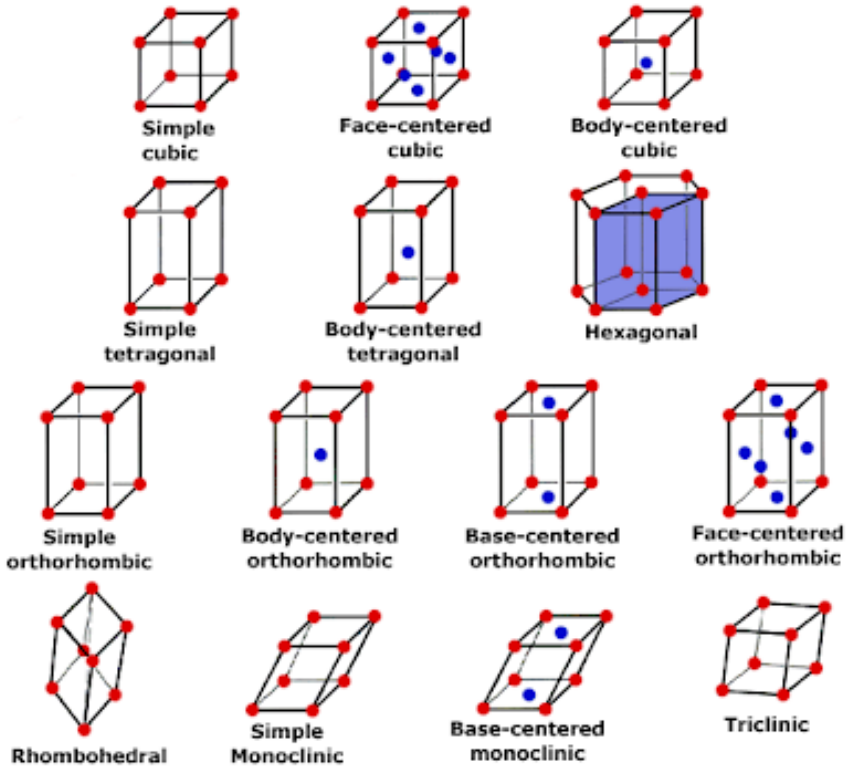
# Some nomenclature




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

smallest symmetrically related unit of a crystal

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



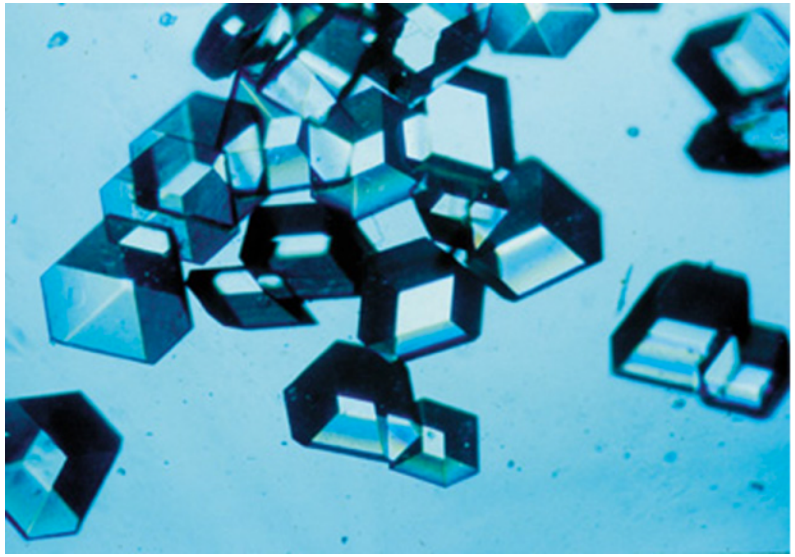

Bravais lattices. Image from http://www.seas.upenn.edu/~chem101/sschem/solidstatechem.html

# 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



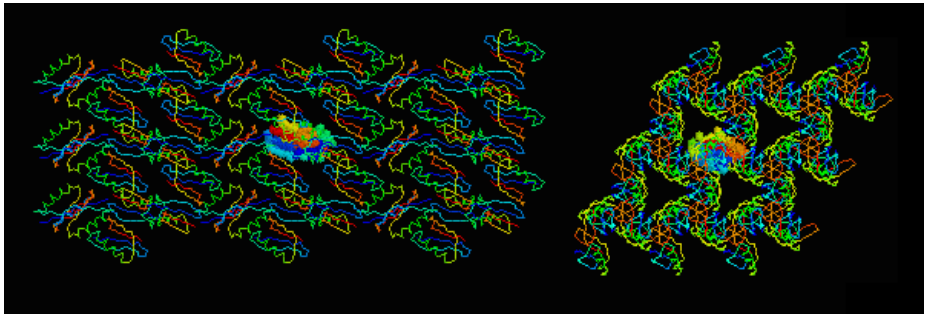
Note: even crystals without large regular edges can contain very regular internal structure

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

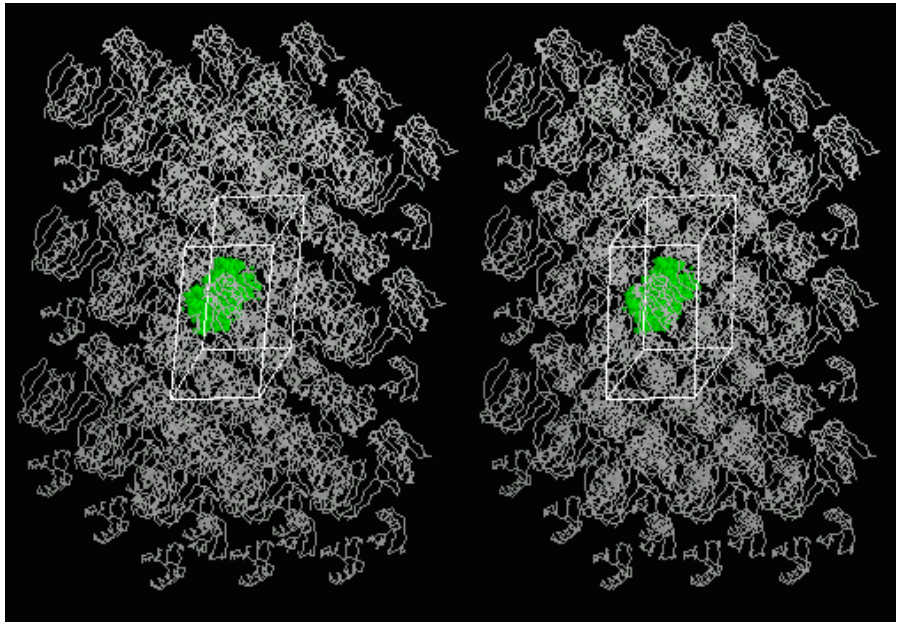
# Proteins can also form crystals

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

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



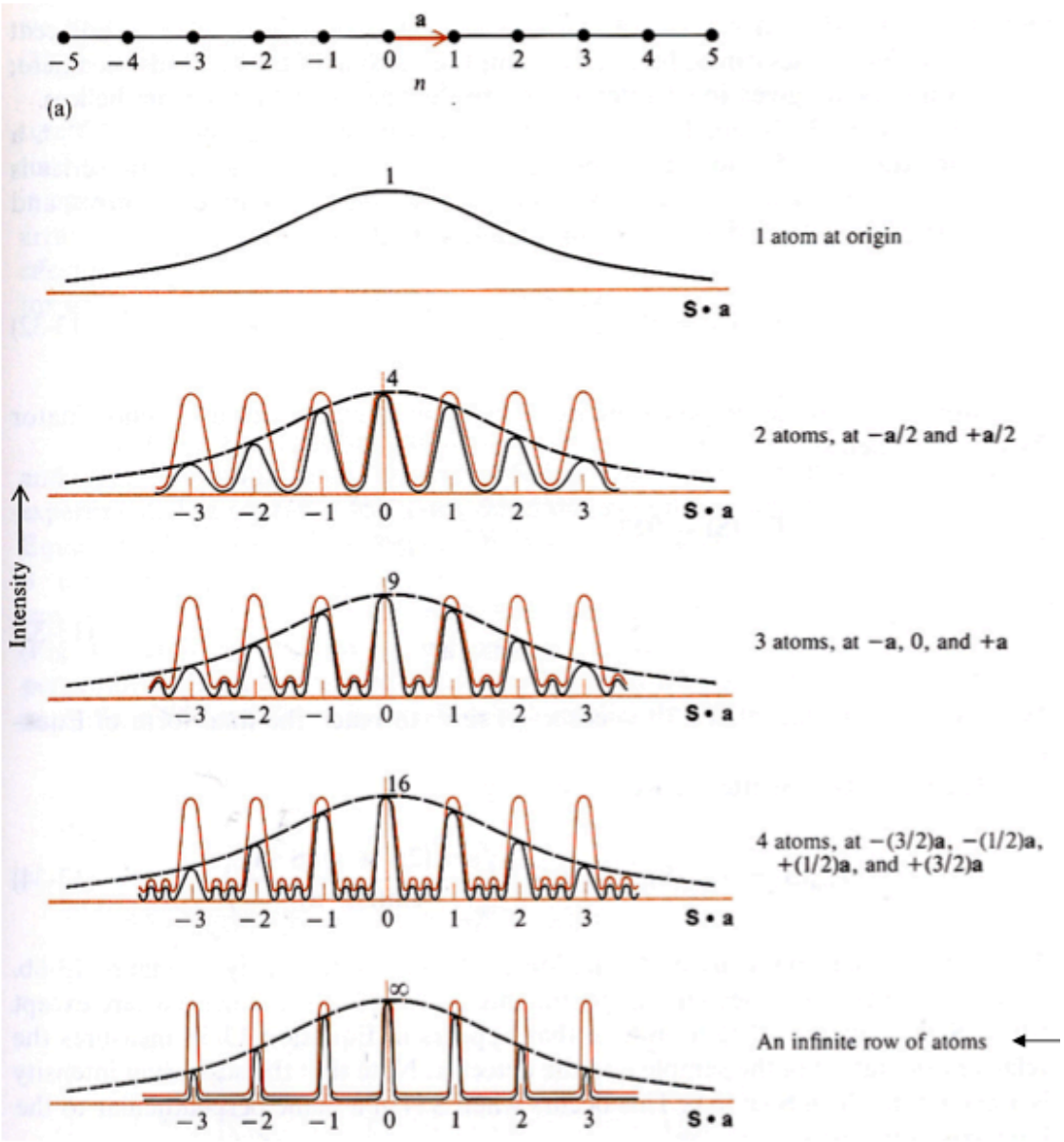
Multiple asymmetric units (each a protein) form the entire protein crystal. The interior or "open" space between proteins is water and ions





http://www.umass.edu/molvis/decatur/pe2.727/protexpl/xtlcon.htm

# Why we use crystals

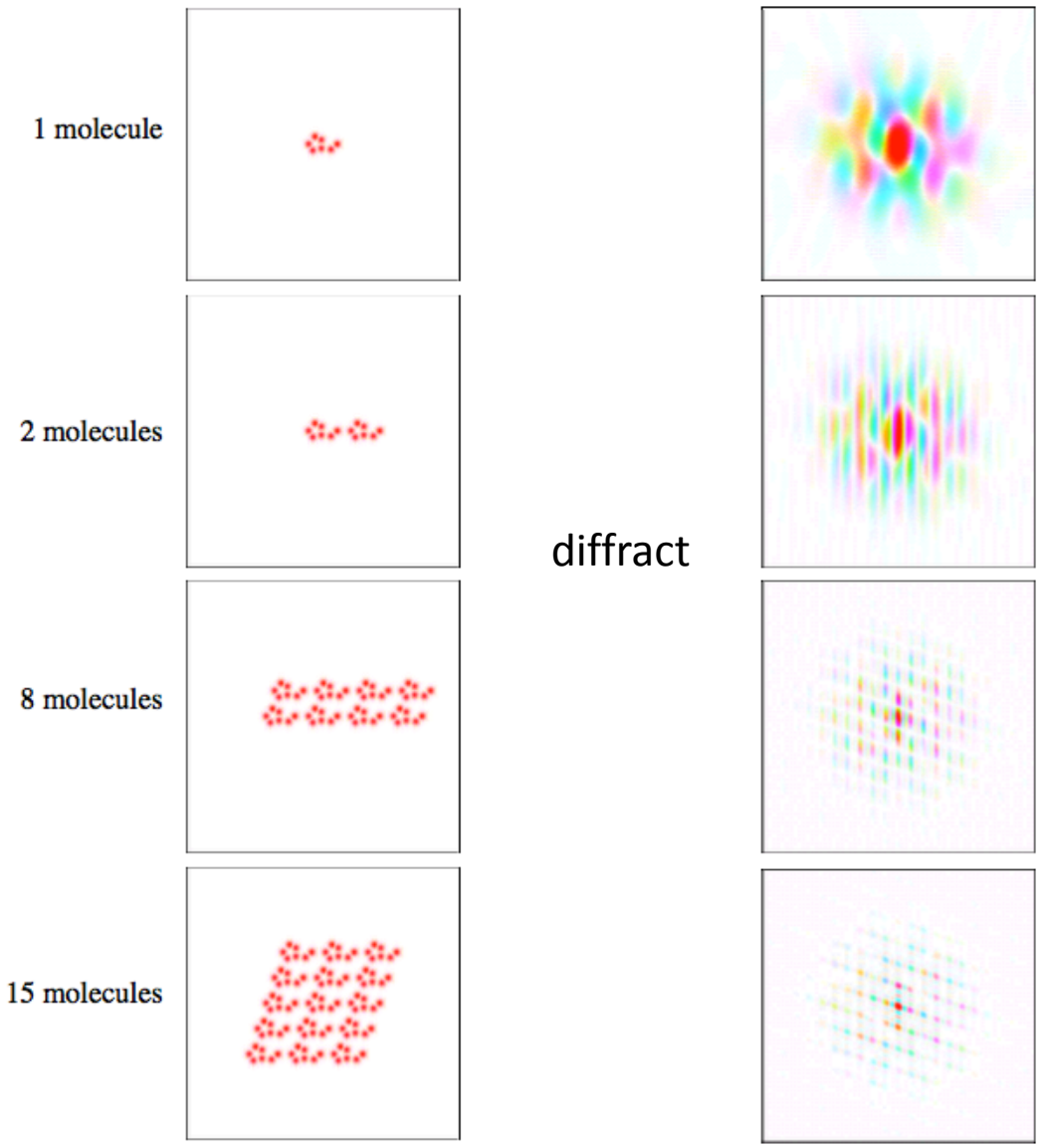



**Diffraction of x-rays from a single protein unit crystal will form grating patterns. These patterns are amplified by multiple proteins in the same orientation. Thus crystals are important for diffraction, because specific orientations of the proteins are enforced through the crystal structure, amplifying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

The repeating crystal lattice is used to amplify the signal

Single object diffracts weakly

From Bill Weiss lecture notes

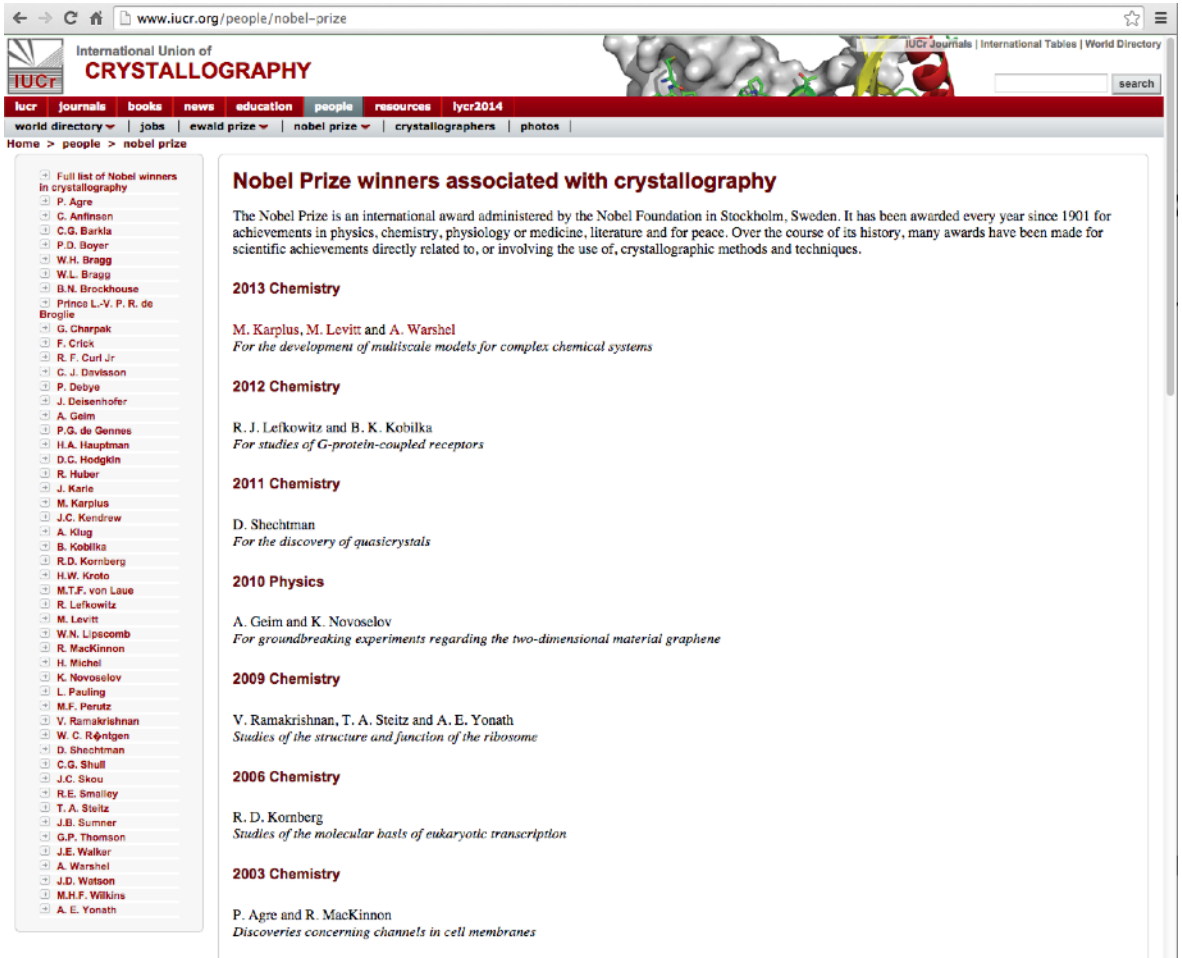





http://www.ysbl.york.ac.uk/~cowtan/fourier/gallery.html

# Caveats

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

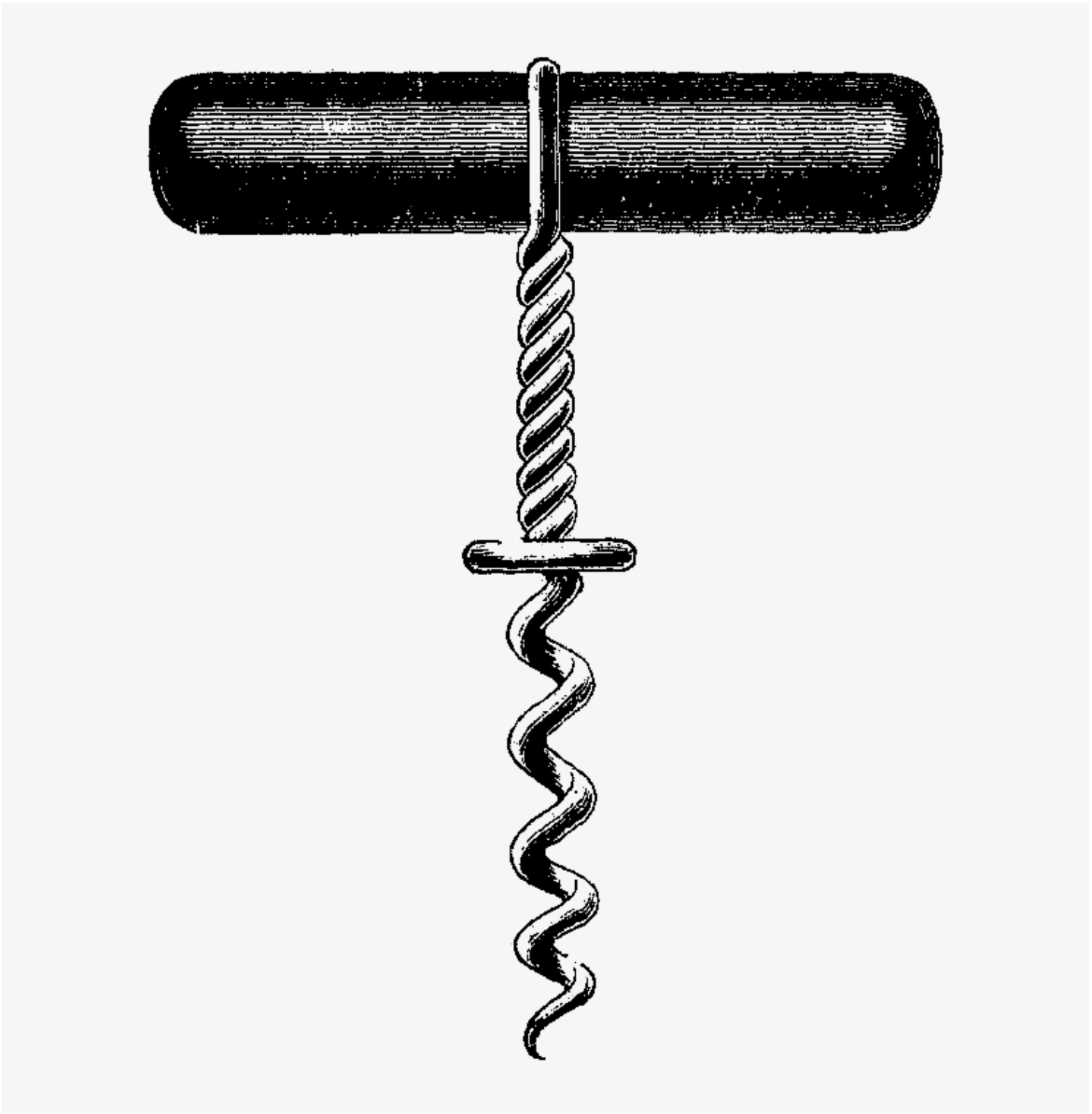


# Caveats

- 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

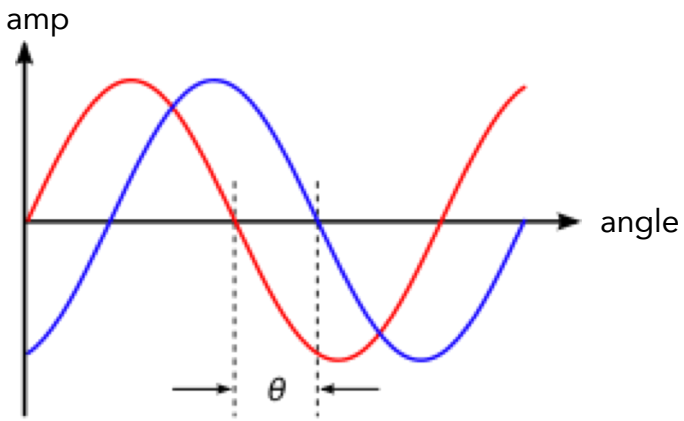
Diffraction basics: from electromagnetic waves to density

(whiteboard notes will be provided later)

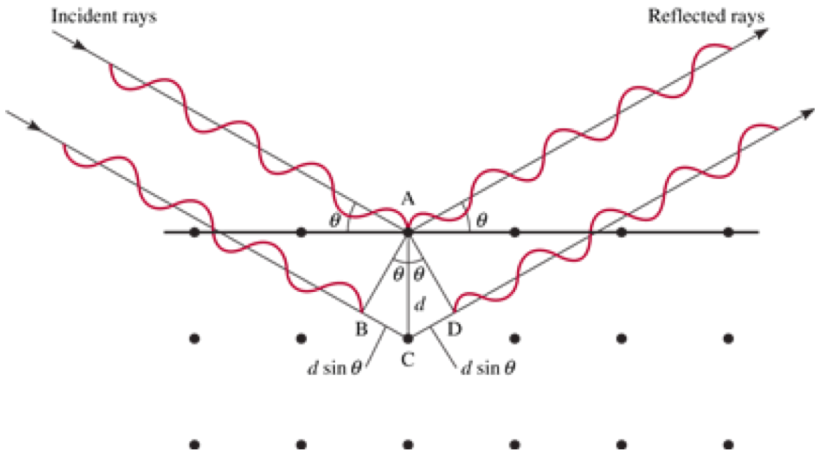




https://www.pngkey.com/detail/u2e6u2y3w7w7u2i1_svg-royalty-free-stock-cork-screw-clipart-vintage/




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 2*pi radians, the wave repeats itself, or travels its wavelength $\lambda$. 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(angle), than our new shifted function will be of the form cos(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).