X-ray crystallography

CS/CME/BioE/Biophys/BMI 279

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Outline

- Overview of x-ray crystallography
- Crystals
- Electron density
- Diffraction patterns
- The computational problem: determining structure from the diffraction pattern

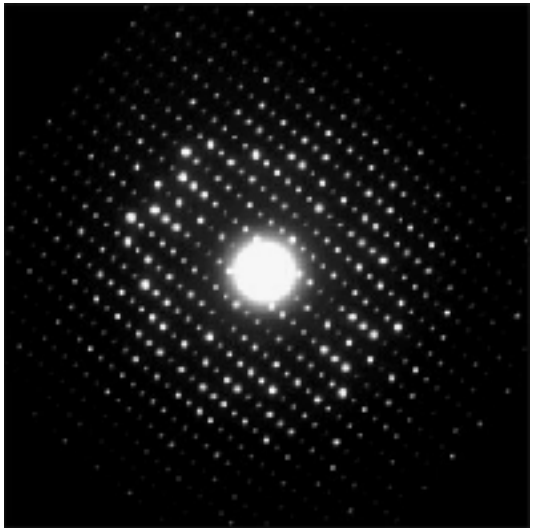
Overview of x-ray crystallography

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

- 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

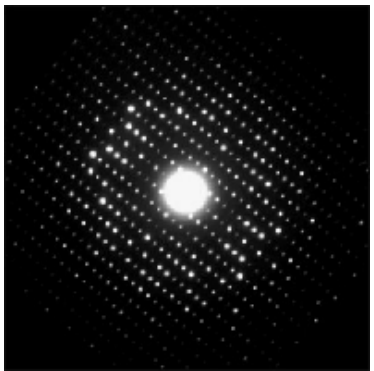
- Get the molecule whose structure you want to determine to form a crystal
- Shine an intense beam of x-rays through the crystal, giving rise to a “diffraction pattern” (a pattern of spots of varying brightnesses)



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

The basic idea

- From that pattern, infer the 3D structure of the molecule
  - In fact, one uses multiple images, with the x-rays shining through the crystal at different angles
- This is a challenging computational problem!
- It turns out the diffraction pattern is closely related to the *Fourier transform* of the electron density of the molecule that was crystallized
  - Before we even worry about what that means, let's go back and discuss what a crystal is and what electron density is



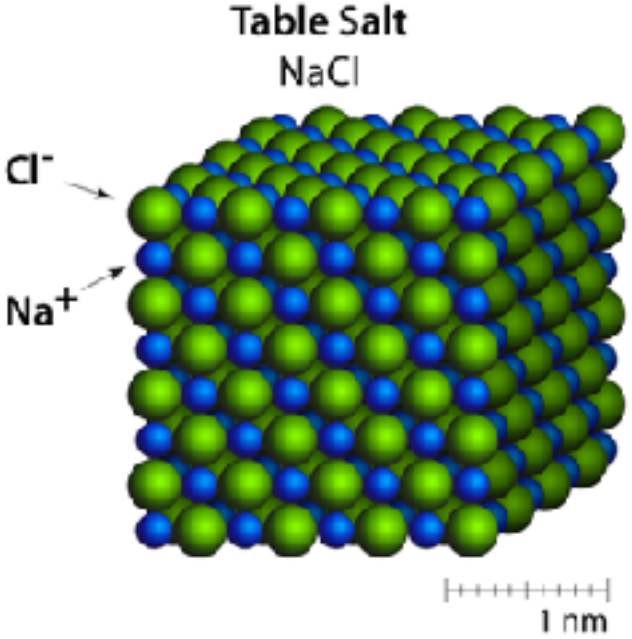
http://lacasadeloscristales.trianatech.com/wp-content/uploads/2014/09/image005-300x300.jpg

Crystals

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

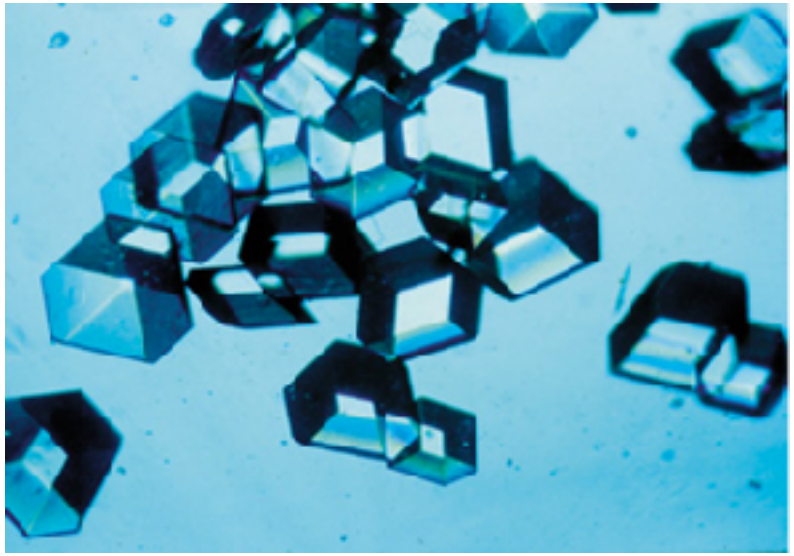


http://www.atomsinmotion.com/book/chapter4/rockSalt.png

Proteins can also form crystals

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

Insulin crystals

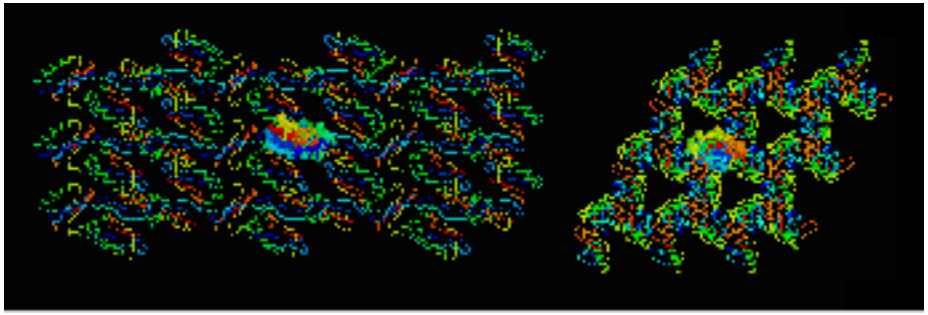


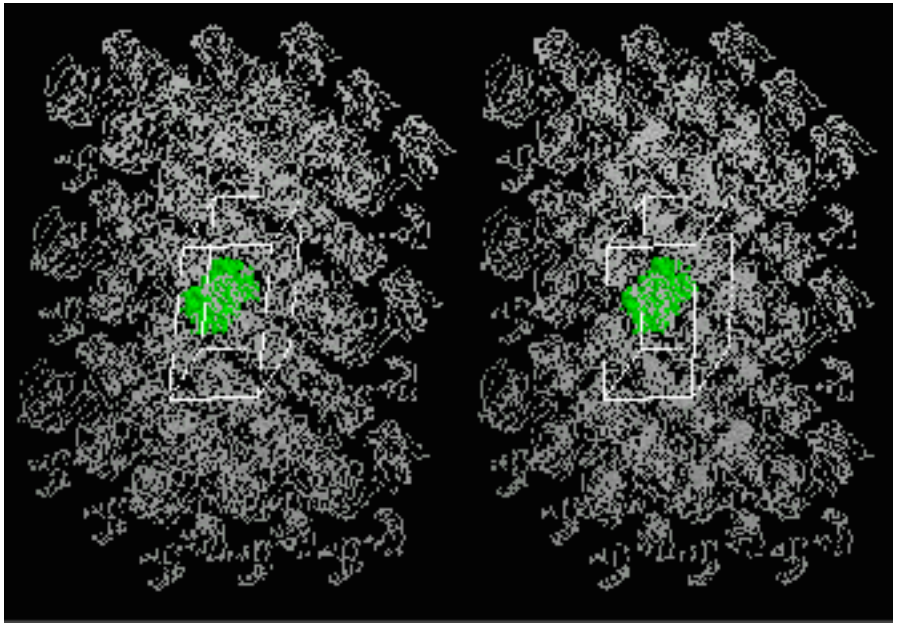
http://science.nasa.gov/media/medialibrary/1999/09/10/msad20sep99_1_resources/9901879.jpg

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)





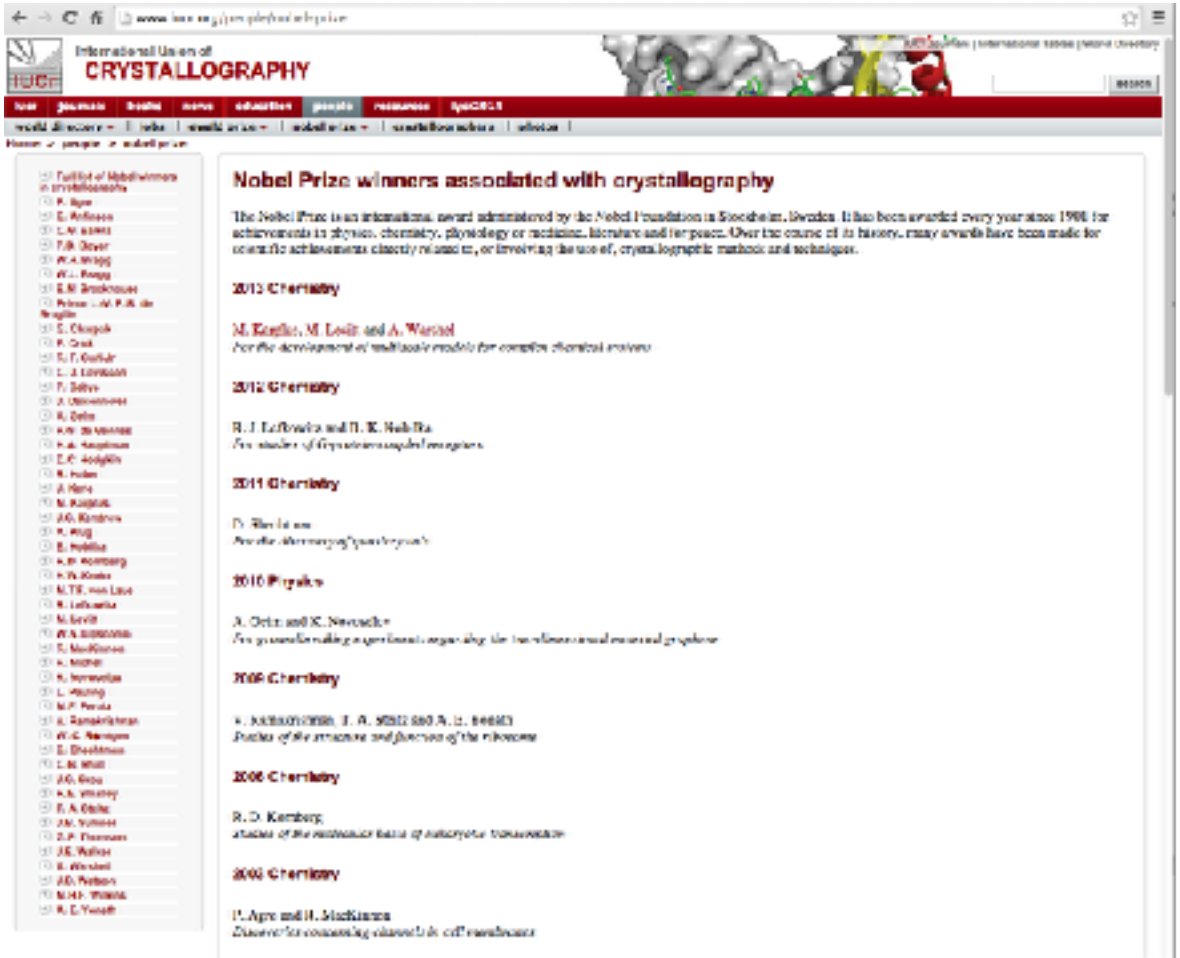
Note:

1. The protein forms a regular pattern as with the able salt crystal.
2. There's a lot of "open space" not filled by the protein, which is instead filled with water. Crystals are often ~50% water. The exact position of water molecules will be different across the crystal, so their positions will get averaged out.
3. Sometimes you'll have an "asymmetric unit," where multiple proteins are packed together in a non-symmetric way, and that configuration is repeated to form the crystal.

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

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

  For example, you can compare structures determined by crystallography to structures determined by NMR or electron microscopy. Or even compare structures determined from two crystals with different crystal lattice configurations.

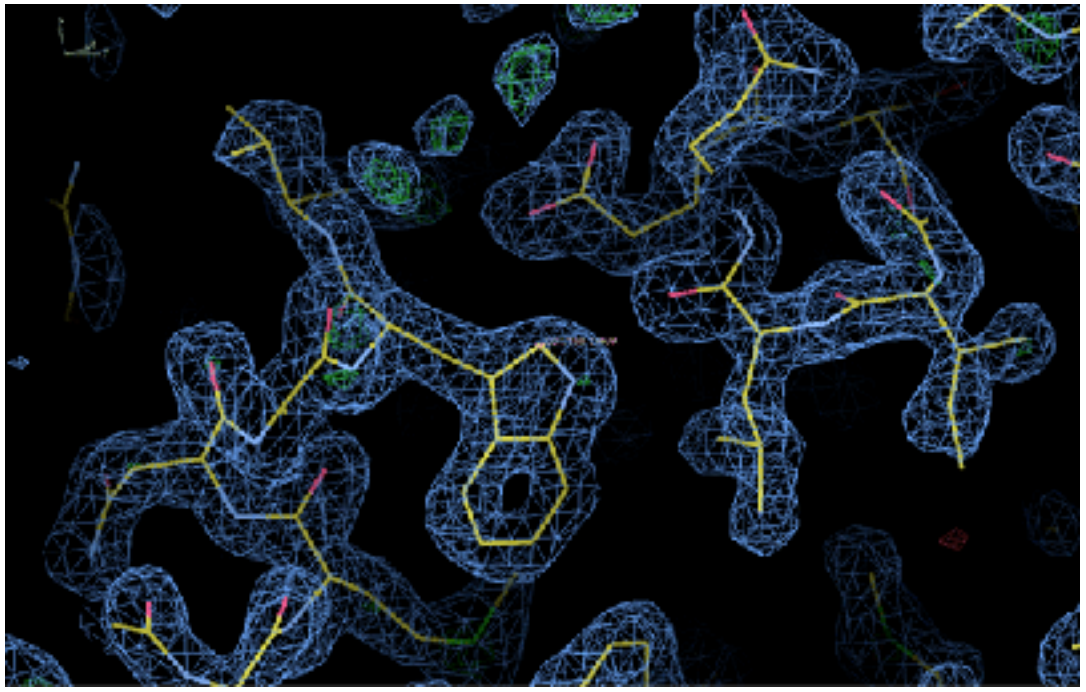
- Crystallography gives you a static snapshot of a protein's structure
  - Usually (but not always) this snapshot corresponds to the protein's "average" structure

Electron density

Electron density of a molecule

- The *electron density* corresponding to the 3D structure of a molecule gives the probability of finding an electron at each point in space
- X-rays bounce off electrons they hit

v These disconnected densities could be water molecules



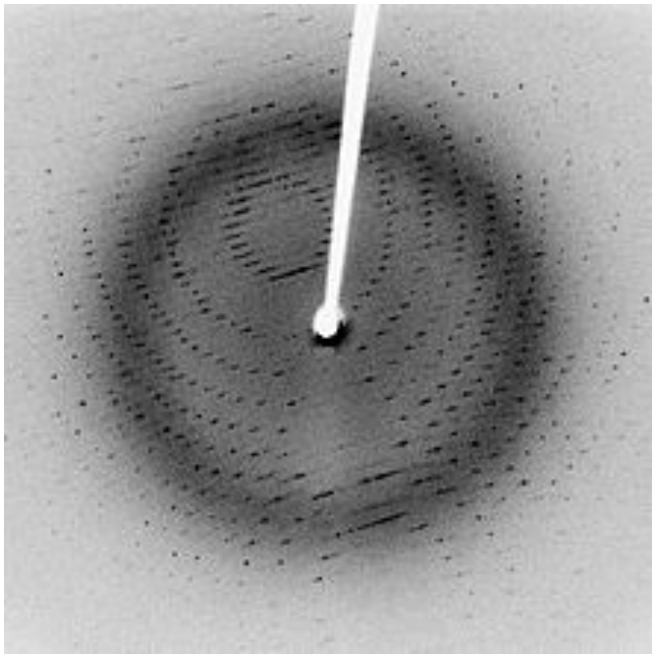
http://www.lynceantech.com/images/electron_density_map.png

Diffraction patterns

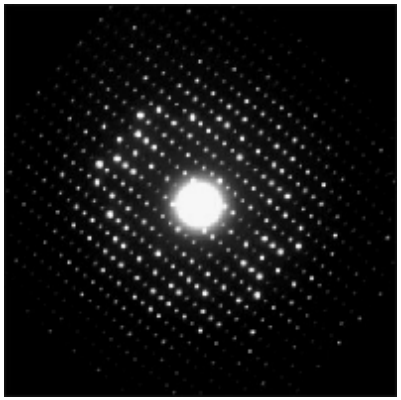
Diffraction patterns

- When you shine a light beam through a crystal, you get a distinctive pattern of bright spots called a diffraction pattern

The white line is the rod that holds the crystal in place, and it blocks the X-ray beam.



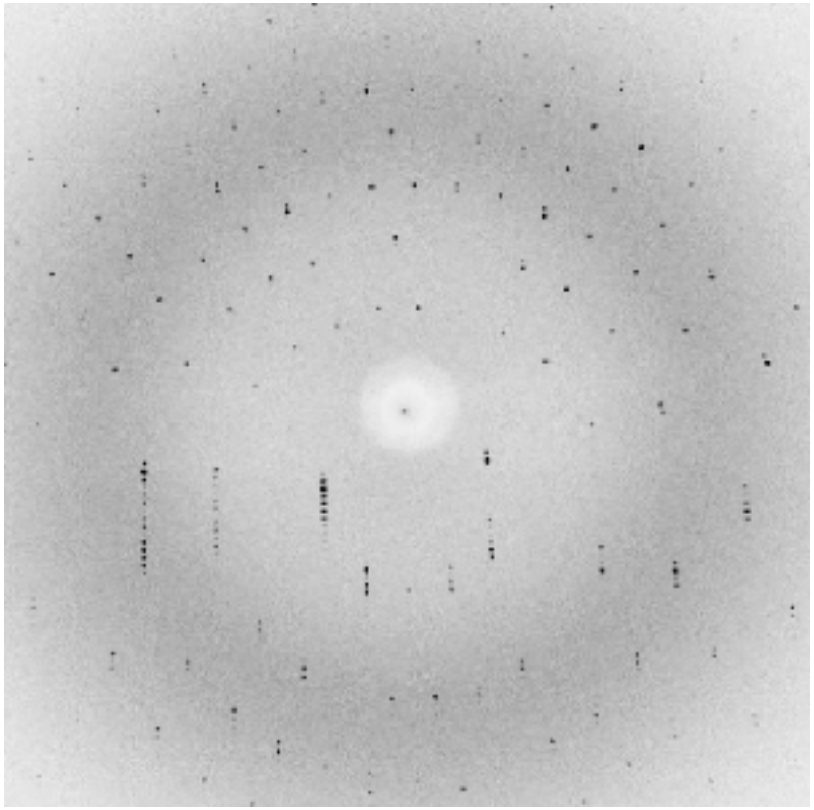
Bright spot in the middle is the original X-ray beam!



Note that the bright spots are sometimes pictured in light/white shades (left) and sometimes in dark/black shades (right)

Diffraction patterns

- This pattern is actually three dimensional.
  - If you move the imaging plane (or rotate the crystal), you see different parts of it

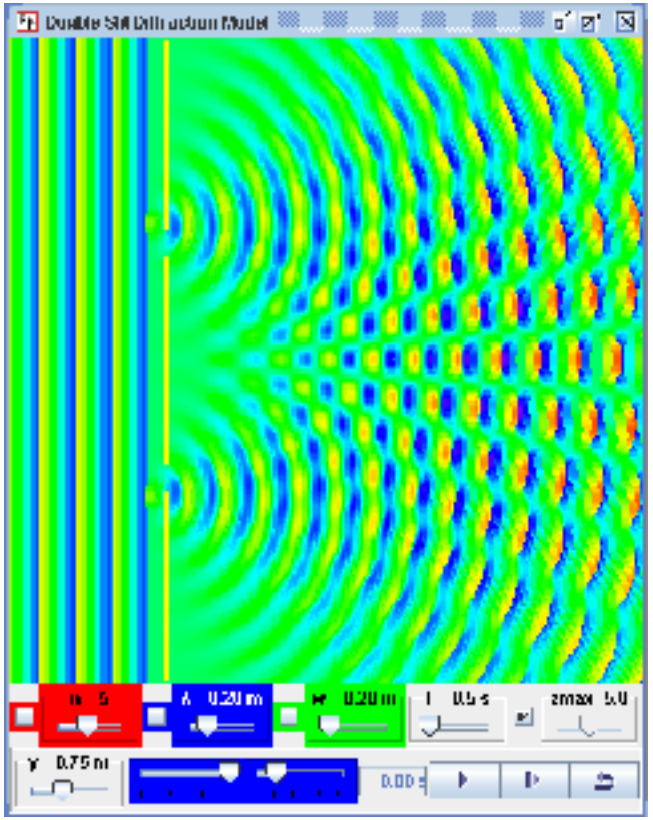


What causes diffraction patterns?

- Short answer: interference of light
  - The bright spots are places where light interferes constructively. Elsewhere it tends to interfere destructively (cancel out).

e.g. you might have done this experiment in high school with waves of water. With two slits, you get patterns with alternating strong signal (constructive interference) and no signal (destructive interference). If you have a pattern with many, many slits, it turns out that most positions will be canceled out and you'll get specific bright spots.

Think of crystal as blob of electrons. When a photon hits an electron, a circular wave emanates at the same wave length as the original photon. Since you have a lattice of proteins. You get interference from each unit, leading to strong signal at a few points, but elsewhere the waves cancel each other.

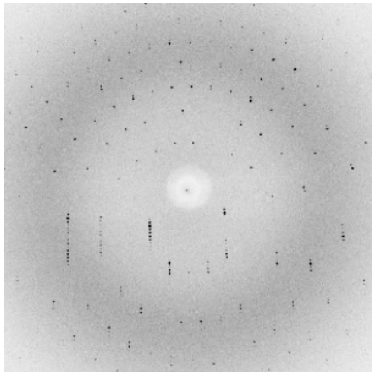


You're not responsible for this

http://weelookang.blogspot.com/2011/10/ejs-open-source-double-slit-diffraction.html

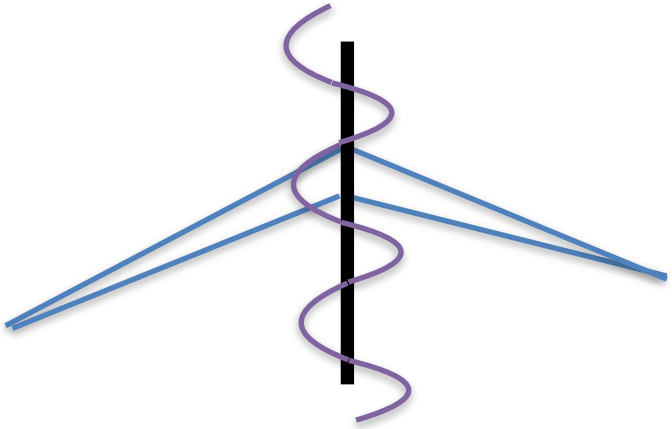
Relationship between diffraction pattern and electron density

- It turns out that the diffraction pattern is the *Fourier transform* of the electron density
  - Both the electron density and the diffraction pattern are functions of three dimensions (i.e., defined at every point in a 3D volume)
  - Each bright spot in the diffraction pattern corresponds to one sinusoidal component of the electron density
  - The Fourier transform gives a magnitude and a phase for each sinusoid, but it's only practical to measure the magnitude, not the phase
    - Brightness of the spot gives the magnitude



You need to understand this relationship, but not exactly *why* it holds

Light hits the line at different points in the wave. If light hits at the midpoint of the wave, little happens, however, if it hits the at the trough or peak, it gives a large response. The result is a sinusoidal pattern along the axis of the electrons. This is related to the light calculating a Fourier transform of the electron density.



The computational problem: determining structure from the diffraction pattern

The challenge

- Given a diffraction pattern, determine the electron density and/or the position of each atom
- If we had a magnitude and a phase associated with each spot in the diffraction pattern—and thus with each 3D sinusoid—then we could just sum up appropriately scaled and shifted 3D sinusoids to recover the electron density
- But we don't have the phases
  - This makes the problem "underdetermined"—in principle, multiple electron densities could give rise to the same set of diffraction pattern magnitudes
  - **But the vast majority of those won't correspond to reasonable 3D structures of the protein**

General approach to solution

- Step 1: *Initial phasing*
  - Come up with an approximate solution for the structure (and thus an approximate set of phases)
- Step 2: *Phase refinement*
  - Then consider perturbations to the structure
  - Search for perturbations that improve the fit to the experimental data (the diffraction pattern)

Initial phasing

An iterative procedure is needed because there are too many potential structures of any given primary sequence

- The most common method for initial phasing is *molecular replacement*
  - Start with a computational model of the protein structure (often the structure of a homologous protein)
  - Search over the possible ways that a protein with this structure could be packed into a crystal, and find the one that gives the best fit to the data
- If one can't build a good computational model of the protein, then one can try various experimental methods to help determine phases
  - Example: *isomorphous replacement*, where one replaces several atoms of the protein with heavier atoms (usually metals), and then uses the *change* in the diffraction pattern to solve for the phases
    - You're not responsible for this

Phase refinement

- Once we have an initial model, we can search for perturbations to that model that improve the fit to the experimental data
  - This is usually done through a Monte Carlo search (via simulated annealing)
  - One usually restrains the search to “realistic” molecular structures using a molecular mechanics force field
    - This dramatically improves the accuracy of the results
    - The idea was introduced by Axel Brunger, now on the Stanford faculty

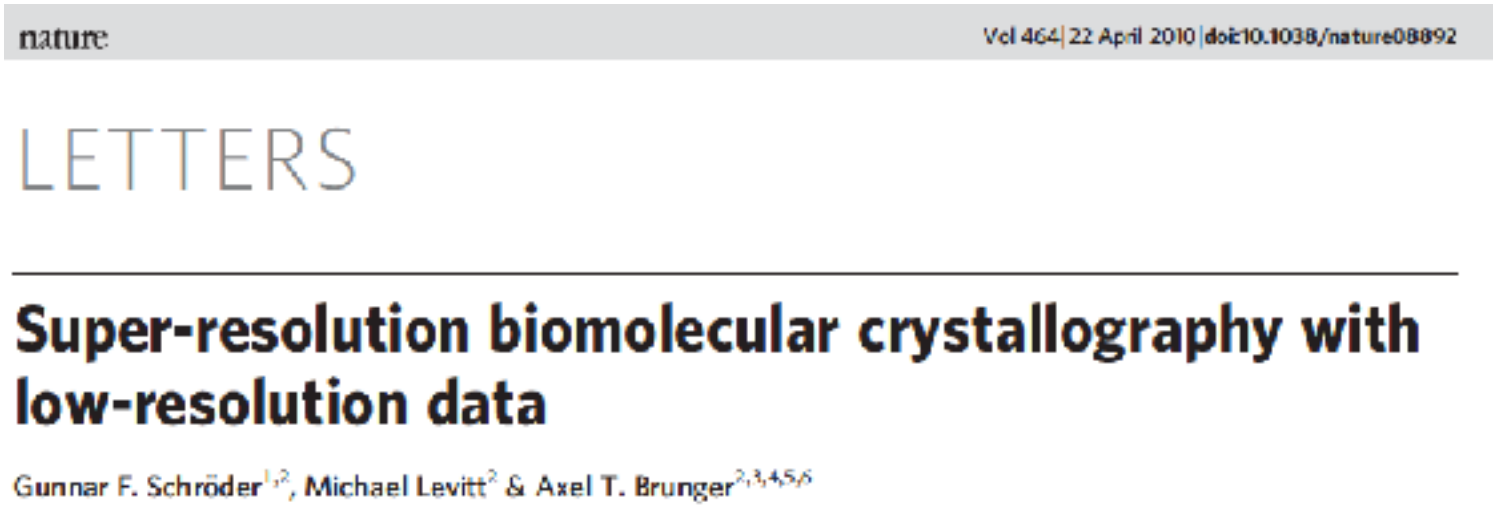
Phase refinement

- A major challenge in the phase refinement process is to avoid overfitting—i.e., fitting to the noise in the experimental measurements
- To avoids this, one generally ignores a small subset of the experimental data during the refinement process, then sees how well one can predict it at the end
  - Just like cross-validation in machine learning
  - This idea also came from Brunger (who termed it R_{free})

R_free term is the current standard for evaluating the quality of a crystal structure

Computational methods continue to improve

- Although the phasing problem is decades old, researchers are still inventing better solutions



nature Vol 464 | 22 April 2010 | doi:10.1038/nature08892

LETTERS

Super-resolution biomolecular crystallography with low-resolution data

Gunnar F. Schröder[1,2], Michael Levitt[2] & Axel T. Brunger[2,3,4,5,6]

A few additional notes

- Protein crystals contain water
  - Often half the crystal is water (filling all the empty spaces between copies of the protein)
  - Usually only a few water molecules are visible in the structure, because the rest are too mobile
- One usually can't determine hydrogen positions by x-ray crystallography
  - But one can model them in computationally
- Some high-profile, published crystal structures have turned out to be completely incorrect, due to computational problems/errors