X-ray crystallography

CS/CME/BioE/Biophys/BMI 279

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No class on Tuesday (election day)

Outline

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

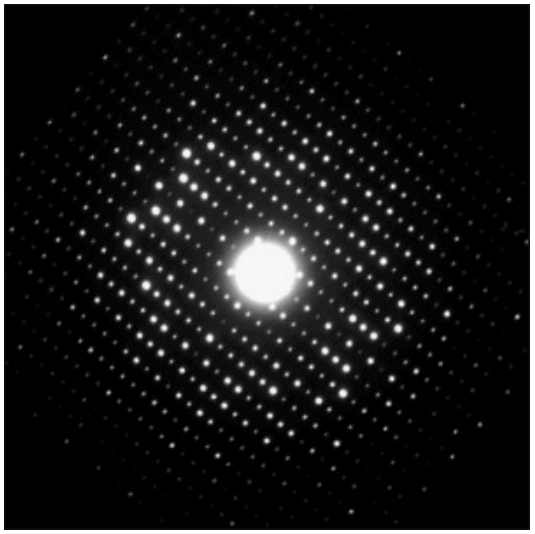
Overview of x-ray crystallography

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

- Nearly 90% of the structures in the PDB were determined through x-ray crystallography
- X-ray crystallography is also frequently used to determine structures 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

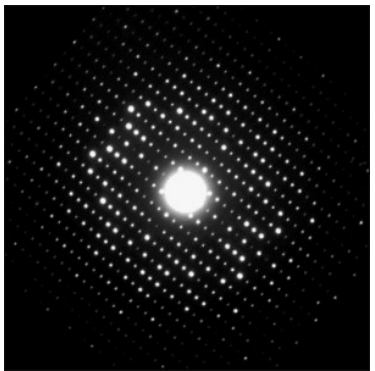
- Get the molecule whose structure you want to determine to form a crystal
- Shine an intense beam of x-rays through the crystal, giving rise to a “diffraction pattern” (a pattern of spots of varying brightnesses)



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



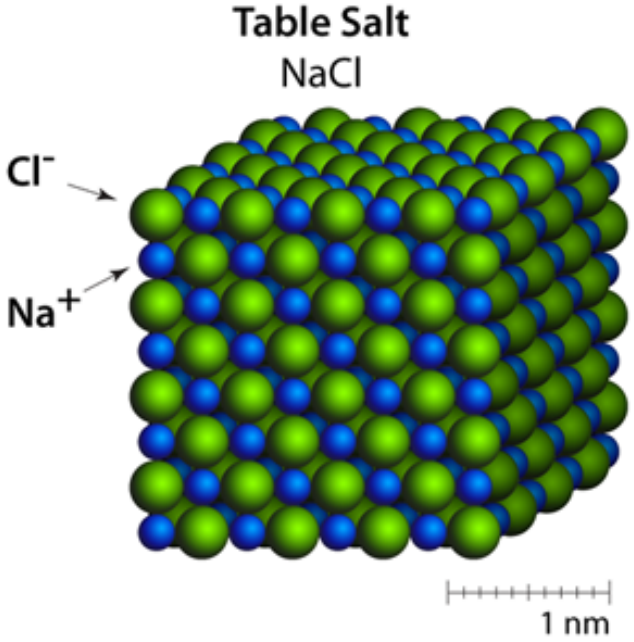
http://lacasadeloscristales.trianatech.com/wp-content/uploads/2014/09/image005-300x300.jpg

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



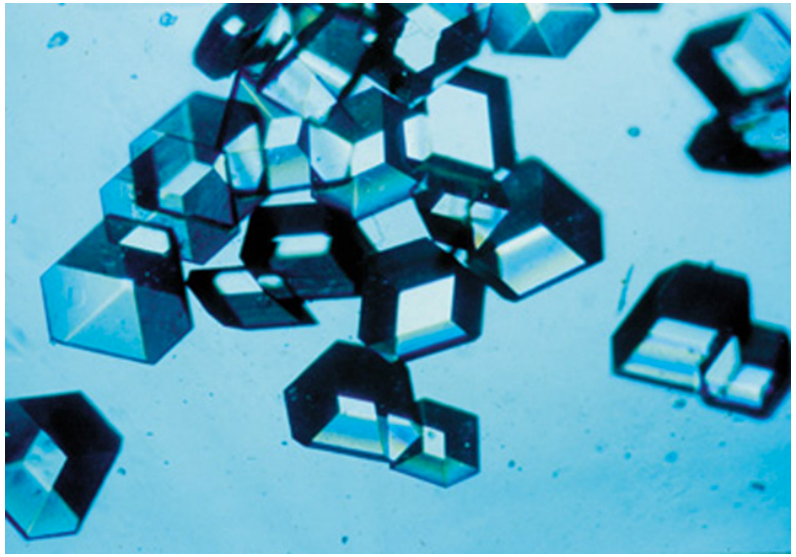
http://www.atomsinmotion.com/book/chapter4/rockSalt.png

Macromolecules can also form crystals

Macromolecules including protein, DNA, RNA, etc.

- Under certain conditions, proteins and other macromolecules will pack into a regular grid (a lattice)

Insulin crystals

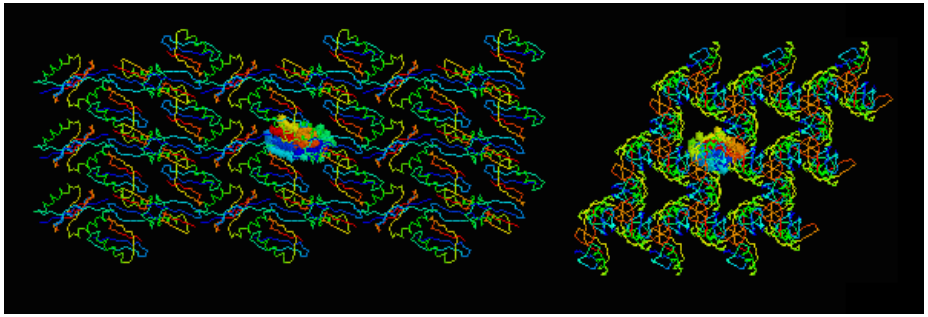


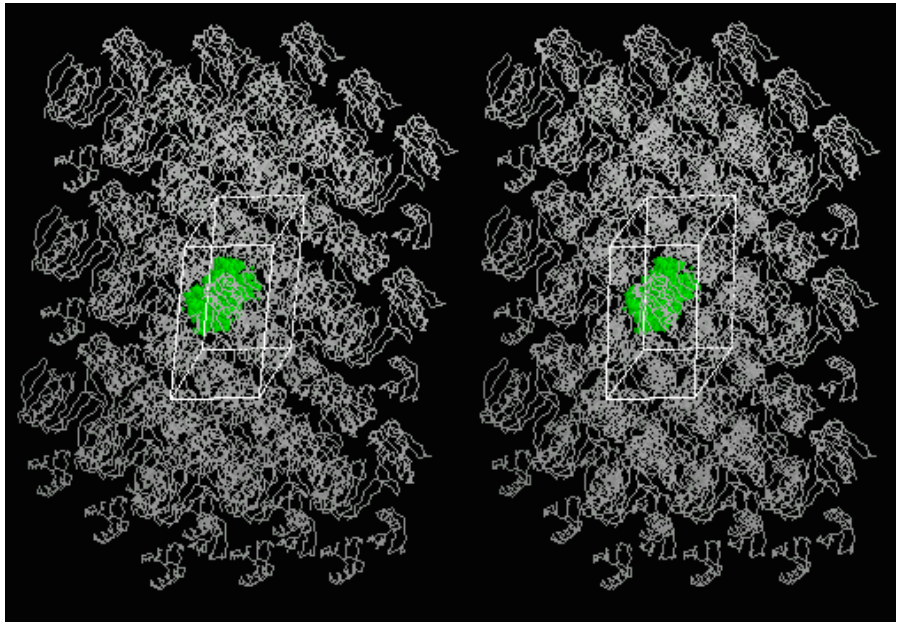
http://science.nasa.gov/media/medialibrary/1999/09/10/msad20sep99_1_resources/9901879.jpg

Macromolecules can also form crystals

Under certain conditions, entire proteins and even protein complexes will pack into a regular grid (a lattice)

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

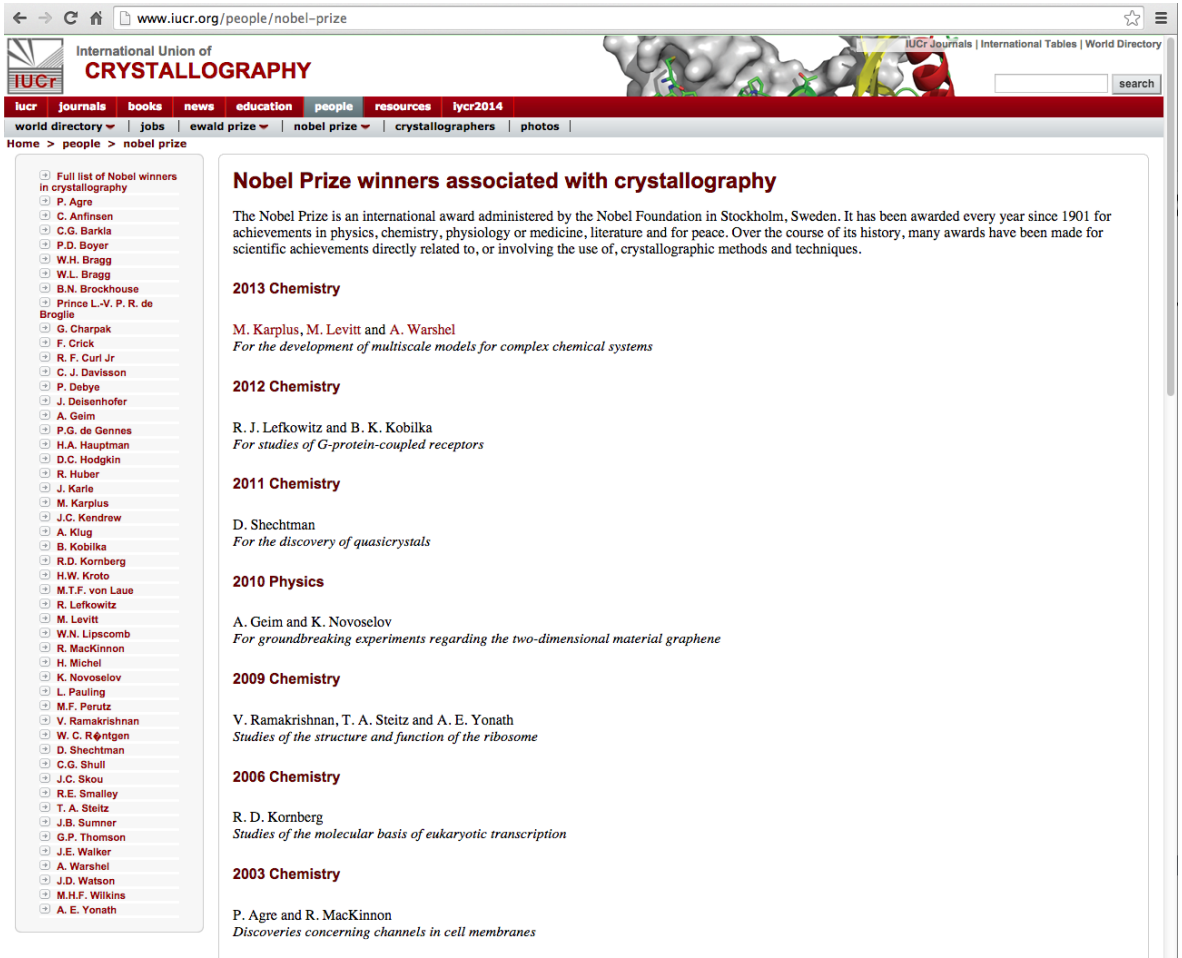




http://www.umass.edu/molvis/decatur/pe2.727/protexpl/xtlcon.htm

Caveats

- Getting macromolecules to form crystals can be hard
  - Crystallographers have sometimes worked for decades to get good crystals of a particular protein



Caveats

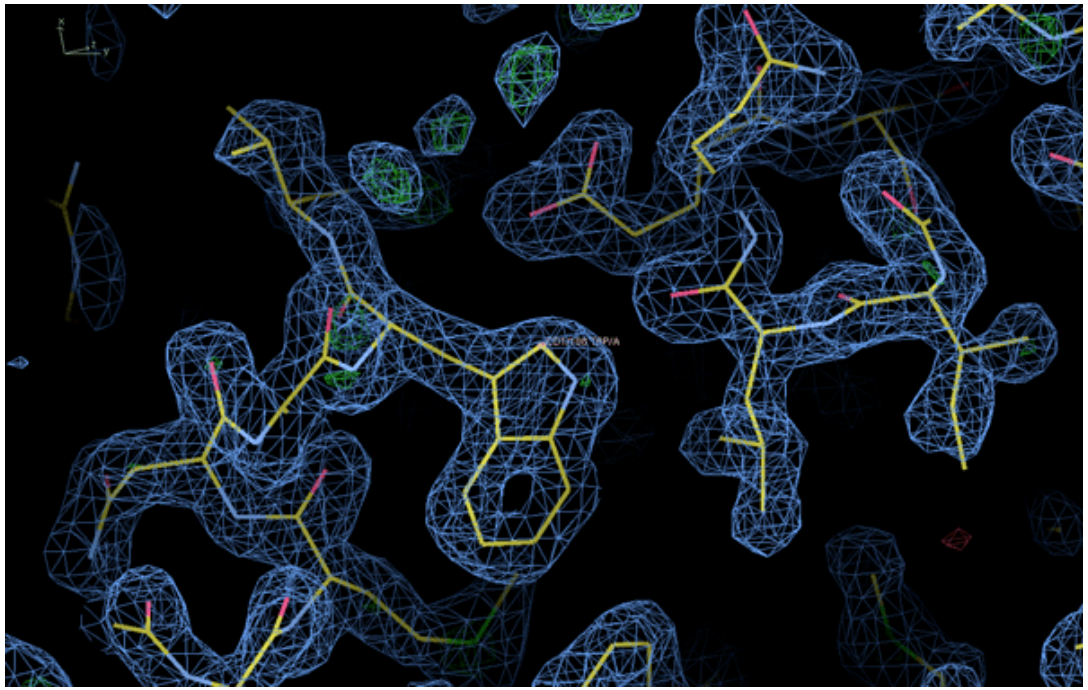
- Sometimes a molecule will adopt a different structure in a crystal than it does in its natural environment
- Crystallography gives you a static snapshot of a molecule's structure Remember molecule is not static!
  - Usually (but not always) this snapshot corresponds to the molecule's "average" structure

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

The blue mesh represents the electron density (which corresponds to the probability of finding electrons in that area)



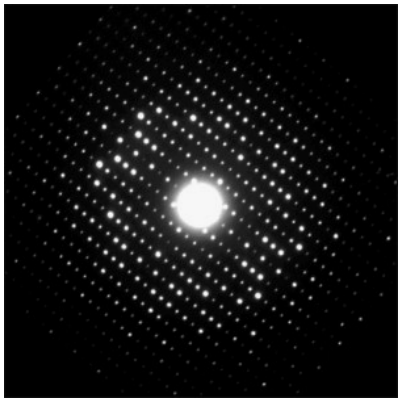
http://www.lynceantech.com/images/electron_density_map.png

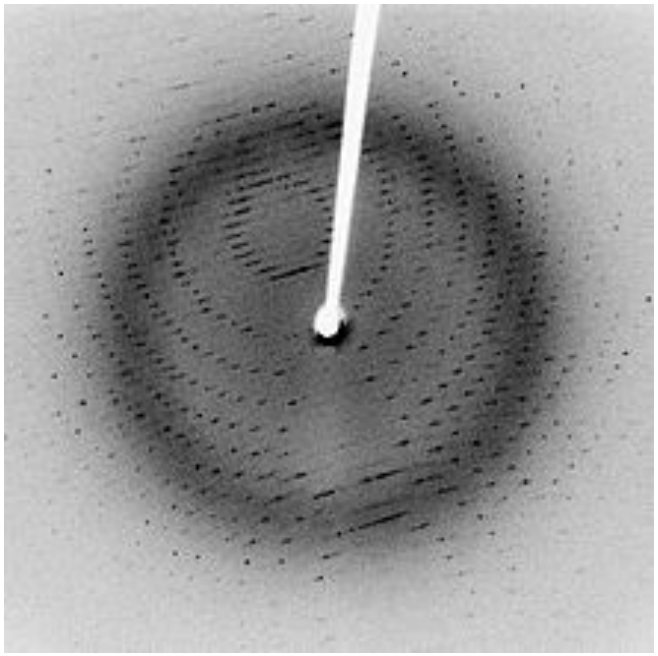
Diffraction patterns

Diffraction patterns

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

X-ray is used in crystallography because the wavelength of X-ray is roughly the same as the radius of an atom (on the order of 1Å/ 10^{-10}m)

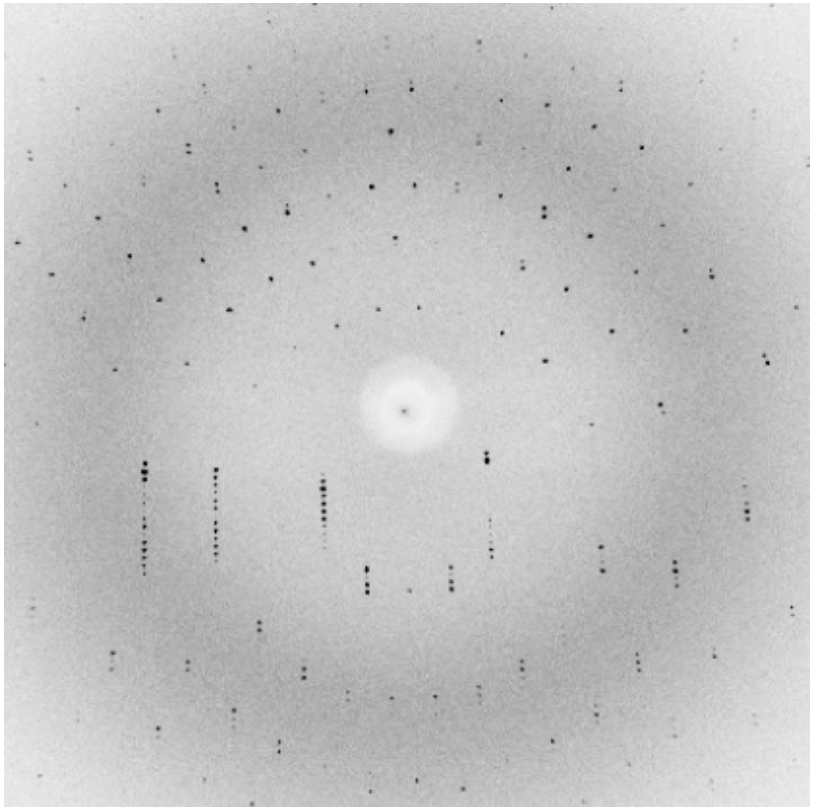




Note that the bright spots are sometimes pictured in light/white shades (left) and sometimes in dark/black shades (right)

Diffraction patterns

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

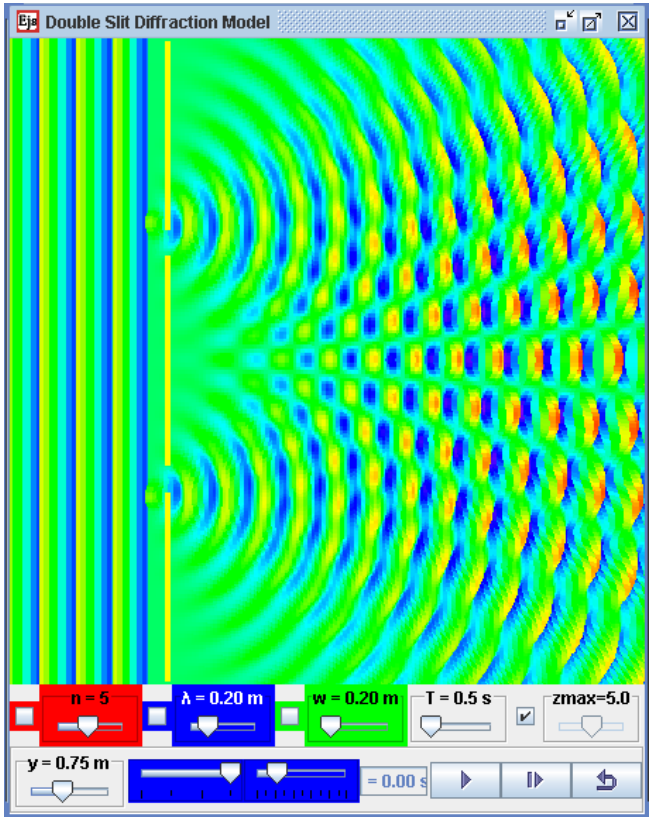


What causes diffraction patterns?

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

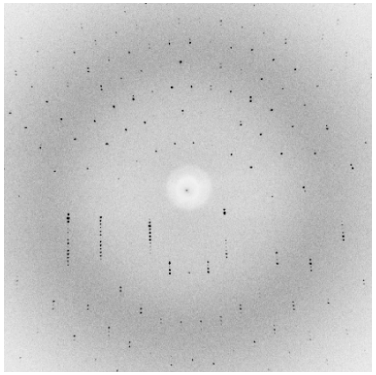
You're not responsible for this

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



The brightness of the spot gives the magnitude of the sinusoid

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

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* Solving for the shift of each of the sinusoids
  - 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

- 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

Mercury, lead, uranium are often used for isomorphous replacement

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 Including a term that corresponds to agreement with the diffraction pattern
    - 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 avoid 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 introduced the term R_{free} to quantify the error in the prediction)

Lower R_{free} is better (i.e. your prediction is more accurate)

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

Super-resolution biomolecular crystallography with low-resolution data

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

NOVEMBER 2013 | NATURE METHODS

Improved low-resolution crystallographic refinement with Phenix and Rosetta

Frank DiMaio[1,6], Nathaniel Echols[2,6], Jeffrey J Headd[2], Thomas C Terwilliger[3], Paul D Adams[2,4] & David Baker[1,5]

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