Topic: Practical Aspects of Single Crystal X-ray Crystallography, Part 1

(Administrative)
Next Tuesday: Guest lecturer on NMR.
Next Thursday: Guest lecturer on other characterizations methods.
Following Tuesday: Institute holiday!
Take-home exams handed out next week.
The course text has a short but excellent section on NMR. Also consider the text
"Crystallography Made Crystal-Clear."

New tools are emerging in X-ray crystallography. In is believed that within 20 years, we will be able to observe single molecules using crystallography!
>   Demonstration of modern crystallography tools:
>>   *Crystallization: Research Tools*. Aliso Viejo, CA: Hampton Research, 2005.
>>   *Crystallization: Research Tools*. Aliso Viejo, CA: Hampton Research, Volume 14, Number 1, 2004.

Two pioneers in X-ray crystallography:

>   **Lawrence Bragg**, Nobel Prize winner at age 25, invented X-ray diffraction for the study of molecules, and is known for the famous Bragg's Law relation for crystallography. He focuses on small-molecule crystallography of substances like salt and sucrose.

>   **Linus Pauling**, the only winner of two unshared Novel prizes, observed larger molecules, like peptide, dipeptides, tripeptides, etc. From this data, he proposed the structures of alpha-helices and beta-sheets, which we have seen are ubiquitous patterns in proteins.

Ways to observe objects:
>   large: telescope
>   macroscopic: naked eye
>   microscopic: microscope
>   too small to be resolved by visible light: x-ray diffraction

The W. L. **Bragg's Law** condition for diffraction:

$$n\lambda = 2d\sin\theta$$

>   Constructive interference between parallel incident beams creates "peaks" of intensirt in the diffraction patterns, indicating reflection off a crystallographic plane.
>>   *d* is the distance between planes in the crystal structure.
>> θ is the angle of the incident beam relative to the plane.
>> λ is the wavelength of the light used for the incident beam.

With a smaller wavelength (λ), you can observe smaller objects. For atoms, typical x-ray wavelengths range from 0.1 to 100 angstroms.
The larger the crystal, the more intense the diffracted beam, allowing for shorter collection times.

Problems with X-ray crystallography include:

> X-rays cannot be bent with a lens like visible light. Must use a computer as a "pseudo-lens."
>
> X-ray scattering from a single molecule is weak! In order to measure a detectable pattern, the molecules must all be aligned in a crystallographic pattern, so that the sum of their diffraction pattern "amplifies" the signal.

What information can X-ray crystallography give about atomic structure:

> Structure may show how atoms interact.
> Knowing the molecular structure, one might guess the chemistry of enzymatic catalysis.
> Structure can also provide a clue as to the function of the molecule.
>> **Structural genomics**, for example, is the determination of the structures of proteins with unknown functions and comparing them to a database of known structures in order to hypothesize about the function. Many genes, for example, have unknown function.
> Caveats:
>> Crystal form is sometimes hard to obtain.
>> Structures are subject to interpretation.
>> More data is necessary to support hypotheses derived from structural studies.

Electron density maps:

> The quality of an electron density maps depends on the resolution of the image. A resolution as low as 1.2 angstroms can show the positions of single atoms. With current technology, a crystal of only 10 microns in size can be imaged. Many companies have dedicated themselves to X-ray diffraction data collection, sample preparation, and data interpretation. Example: http://www.emeraldbiostructures.com/

Sample preparation:

> Isolate protein from cells and purify to over 95% using:
>> Chromatography (e.g., affinity chromatography)
>> Separate from other proteins based on
>>> Charge
>>> Solubility
>>> Functional groups

> Crystallize the sample using:
>> Crystallization robots can work with samples as small as 0.1 microliters! A machine made in Cambridge, England called a mosquito uses a technique inspired by the injection device of mosquitoes to work with small volumes

of a sample. A tiny seed crystal grows to provide a sample for diffraction. (In the recent past, machines required 10 mg of a protein—the requirement is now much smaller.)

The hanging drop method is a preferred method to cause crystallization. The tiny 1-microliter droplet can easily evaporate, so the dish must be carefully sealed with vacuum grease, since crystallization can take months!

The sitting drop method seals a Petri dish full of droplets sitting in a siliconized Pyrex 9-well glass plate.

Protein crystals can be created from toxins, enzymes, and even viruses. Some of the most difficult proteins to purify with high yield are membrane proteins, some of which still have unknown structure.

Once the sample has been crystallized, it must be mounted using glass tubes, fibers, or loops. The crystal can be protected using a small drop of glycerol or sucrose to add density and immobilize the crystal. The crystal is cooled in liquid nitrogen (-170°C) to enhance its lifetime and reduce damage due to X-ray radiation.

X-rays are generated by acceleration of electrons at a rotating (typically copper) anode. The X-ray beam is focused using mirrors, and the focused beam is directed at the sample, which can be rotated so that the x-ray pattern can be detected from all angles. Synchrotron radiation (e.g., Advances Photon Source (APS) at Argonne National Laboratory; Brookhaven Labs in New York; SSRL at SLAC in Stanford, CA, etc.) can be used to obtain extremely high-resolution information about molecules. A synchrotron can provide in minutes the information that would take weeks to obtain otherwise (weeks during which the sample could degrade!).

Emergeng technology and entrepreneurship:

Scientists from SLAC at Stanford and CERN in Geneva, Switzerland came together to found Lyncean Technologies, Inc., a company attempting to make a tabletop synchrotron (Compact Light Source) that can fit into a regular room. This $4 million dollar machine is expected to come out in summer 2006! Maybe this would break the pattern of scientists having to travel far to spend sleepless nights taking advantage of precious time on the world's few synchrotrons!

A typical diffraction frame displays reflections as visible spots. Diffraction peaks far from the center of the frame have higher resolution. The crystal properties and energy source determine the maximum resolution that can be achieved.

Phase determination methods:

Multiple Isomorphous Replacement (MIR)

This commonly used method was invented in 1960's by Perutz and Crick, based on the principle of "soaking" the sample with heavier atoms in order to replace certain atoms with heavier ones. Structures can often be solved using a home X-

ray source by this method. However, the method requires multiple heavy atoms, which may be toxic, etc.

Molecular Replacement (MR)

This technology appeared relatively late because of the large database of information. Can only be done with homologous (similar) proteins of which the structure is already known. (E.g., for understanding the change in the structure when the protein binds to a small molecule.) Due to "phase bias" toward the model structure used, the method may not give accurate data, and is more appropriate for simpler molecules (e.g., rhodopsin).

Multi-wavelength Anomalous Diffraction (MAD)

This method emerged only 10 years ago, and looks at multiple sites on the protein at once to simultaneously give a single image. It compares a regular molecule with a sample of the molecule in which atoms have been replace with selenium. The only heavy atom needed is selenium, and the electron density maps are cleaner from this method. However, the method requires a synchrotron radiation source.

Direct Methods (MD)

This method is still emerging, and thus far can only be used for small molecules, such as sucrose. It uses the fact that certain intensities can be generated by particular combinations of phases, due to semi-random arrangement of atoms in the unit cell. The method itself is cheap, but computationally expensive.

The molecular model is iteratively improved and compared with the calculated phases. It uses a least-squares refinement of atomic coordinates, flattening to account for the solvent, molecular averaging, and an additional weighting factor.

The structural refinement gives an R-factor, which measures the deviation between the observed and calculated values for structural factors. A small R-factor (e.g., 10%), gives essentially full confidence for the structural model. Other factors in quality control include:
> The resolution: it's easier to make mistakes tracing a protein chain in lower-resolution maps.
> The RMS deviations of the bond lengths and angles from standard values: deviations should be below 0.02 angstroms and 3 degrees, respectively
> Temperature factors: The B-factors greater that 50 angstroms indicate weak electron density.

Although still in its infancy, it may be the method of the future, since computational power is increasing, and expense is falling.

Analysis of a large, complex structure: e.g., ribosome.

In 1999-2000, the structures of some enormous ribosomal components were determined. E.g., unit 50S has a molecular mass of 1.7 MDa, and is just one component of an enormous machine!

Once these high-resolution structures were discovered with atomic detail, models for the peptide bond forming mechanism could be proposed. Current models propose that adenine performs the actual catalysis in forming a peptide bond for an amino acid brought in by tRNA.

Structural biology: Past, present, and future:

Past: Insufficient computing power, slow methodological advances, and esoteric academic pursuit led to slow development from the 1960s to the 1990s.

Present: Synchrotron radiation sources, recombinant expression of gene products, investment from biotech companies, and automation of genomics processes helped speed up development. The Protein Data Bank has grown exponentially due to the combination of corporate investment and availability of synchrotron sources.

Future: New horizons include X-ray free electron lasers, structural determination for single molecules or whole cells, and dynamic studies of macromolecular assembles.