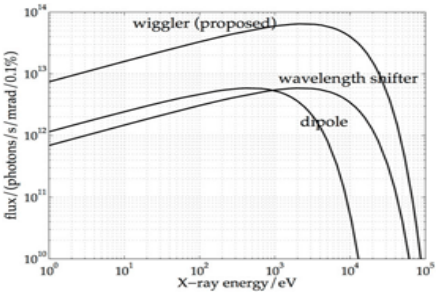
BC530 2015 X-ray Crystallography Unit Homework

More information about X-ray sources

As discussed in class, each element in the periodic table has a characteristic set of energy states associated with its electron orbitals. X-ray photons can be selectively absorbed or emitted by an atom if the photon energy matches a transition between two of its characteristic states. See http://skuld.bmsc.washington.edu/scatter/AS_chart.html.

X-rays produced by an in-house source (X-ray tube or rotating anode generator) are essentially monochromatic (all the photons have the same energy). This is because they are produced by exciting electrons from some particular metal, generally copper, chosen as the anode material. The energy of photons from a copper X-ray source is 8.98 keV. This corresponds to a wavelength of 1.54Å.

In contrast to this, synchrotron radiation is more like a white light bulb. The photon energy distribution is smooth, although not uniform. See figure at right. For most diffraction experiments we filter this "white light" by inserting mirrors, monochromators, and other gizmos so that only a very narrow range of X-ray energies hits the sample crystal. The off-energy photons are deflected or absorbed elsewhere. This is analogous to creating a beam of red light by placing a piece of red plastic in front of a white light bulb; only the red photons (wavelength ~780Å) pass through.



Question 1: Polychromatic X-ray diffraction

Usually the apparatus at a synchrotron X-ray source is set to illuminate your crystal with nearly monochromatic photons with wavelength near 0.979 Å.

(**a) Why 0.979 Å?**

This wavelength is good for the two most common types of experiment:

1. In general we would like to use a wavelength near 1Å. Anyone expecting to solve a new structure using molecular replacement does not care very much what the exact wavelength is, so they are probably happy with this value.

2. If you need something other than molecular replacement, the most common experimental phasing technique is currently SAD using the signal from Se atoms in proteins engineered to contain selenomethionine rather than normal methionine. The maximum signal from anomalous scattering is just above the Se K-absorption edge (energy slightly higher than 12.6578 keV = 0.9795 Å). So this is a very good wavelength for anyone who plans to use selenomethionine substitution to solve their structure.

(**b) If you collect diffraction images from the same crystal using a typical home X-ray source and using a typical synchrotron X-ray source, how will the images differ?**

To keep things simple, suppose that geometry of crystal+camera+detector is the same for both experiments. Now consider some arbitrary "spot" in a diffraction image. It is a solution of the Bragg equation for one particular set of indices [h,k,l]. The planes of atoms described by that set of indices have spacing (resolution) d_{hkl}. The Bragg equation tells us that $\lambda = 2d_{hkl}sin(\theta)$. From the information above, let's say that for the home source λ = 1.54 Å and for the synchrotron source λ = 0.98 Å. Since d_{hkl} remains the same, that means the value of $sin(\theta)$ for each spot in the pictures from the synchrotron will be smaller by a factor of 0.98/1.54 than it is in pictures from the home sources. I.e. all the spots in the images from the synchrotron are closer to the center of the image.

If you remove some of the optical elements that filter the X-rays, you can instead illuminate your crystal with polychromatic (as opposed to monochromatic) photons. Usually there is still a low-energy cutoff and a high-energy cutoff so that the photon energies cover a range of roughly a factor of two. I.e. the photon energies are, say, 6 keV to 12 keV and the corresponding range of wavelengths is roughly 1Å to 2Å.

(**c) What will happen to the diffraction pattern from your crystal?**

1. For every wavelength in that range, some different set of Bragg planes in the crystal will satisfy the Bragg equation. All of these will appear at the same time on your recorded images, so they will have a *lot* of spots.
2. Even though the wavelengths come from a continuous spectrum, this does not elongate the individual spots into lines. For each set of Bragg planes there are only a discrete set of wavelengths that satisfy the Bragg equation.

A diffraction experiment carried out in this way is often called a Laue diffraction experiment. There are a few specialized synchrotron beamlines that are specifically designed for this kind of data collection.

(**d) Why might this be a good idea (i.e. why does anyone do this)?**

Here is a link to a simulated diffraction image from a Laue experiment, with each spot colored to indicate which wavelength[s] produced it.
http://skuld.bmsc.washington.edu/~merritt/bc530/laue_falsecolor_image.jpg

1. Because there are so many spots in each diffraction image, you can collect a full data set in many fewer pictures. In extreme cases you may need fewer than 10 images rather than hundreds of images. This is important if for some reason you can only expose the crystal once (or a very few times).
2. In practice this is only the case for time-resolved experiments like the one shown at the end of Wednesday's class. You illuminate the crystal with a laser (or some other triggering event) to initiate a reaction, then measure the X-ray diffraction after some specific time interval. But unless you can "undo" the reaction somehow, you can only take one image at each time point. To get complete data you need to repeat the experiment many times using a new crystal each time.

(**e) Why might this be a bad idea (i.e. why doesn't everyone do this)?**

1. For normal (not time-resolved) experiments the larger number of spots is a disadvantage rather than an advantage. Many will overlap with each other and thus their intensities cannot be easily separated.
2. We didn't discuss this in class, but as part of preparing your data for refinement you need to bring all the measured intensities onto a common scale. This becomes much harder if the X-ray wavelength is different for each measurement, since (as mentioned in the explanation of anomalous scattering) the effective optical density of a material is different for different wavelengths of light.
3. The total dose of X-rays hitting the crystal is very large, so X-ray damage becomes a major problem.

Question 2: Strange crystal additive?

Some protein structures in the PDB contain tungstate (WO_4). Tungstate is not a common metal in biological systems, so why would there be tungstates in crystal structures like this one?

http://www.rcsb.org/pdb/explore/explore.do?structureId=1OHD

1. Tungsten has many electrons (atomic number 74) so it diffracts very strongly. It also has a very large anomalous scattering signal at wavelengths near 1 Å. (Click on element "W" in the periodic table here: http://skuld.bmsc.washington.edu/scatter/AS_periodic.html). So having tungsten atoms in the crystal is good for either MIR or SAD phasing.

2. But why tungstate rather than a Pt, Hg, or Pb compound? Because tungstate WO_4 mimics phosphate PO_4, and phosphate-binding proteins may be happy to bind tungstate instead. This is a very mild and reproducible perturbation of the structure as compared to, say, covalent attack on random Cys residues by mercury compounds.

From the Methods section of the primary citation for this structure (follow the doi link on the PDB page): *"Determination of the structure of wild-type apo Cdc14B was performed using the single anomalous dispersion method utilizing tungstate, a phosphate mimic and catalytic site inhibitor, as a heavy atom derivative. The concentration of tungstate used to derivatize Cdc14B was estimated from the concentration required to inhibit the Cdc14 catalytic activity towards p-nitrophenol-phosphate (pNPP; data not shown)."*

Question 3: Evaluate a structure

New PDB structures are released every Tuesday. One of the structures released yesterday was entry 4RWB "Racemic influenza M2-TM crystallized from monoolein lipidic cubic phase". Here is a link to its PDB page

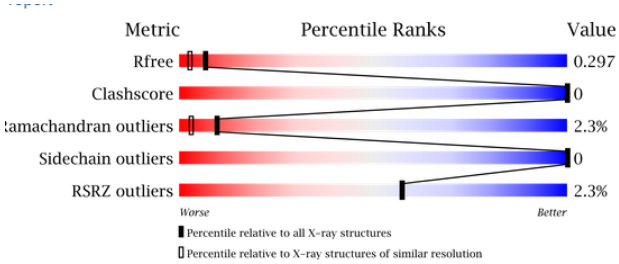
http://www.rcsb.org/pdb/explore/explore.do?structureId=4RWB

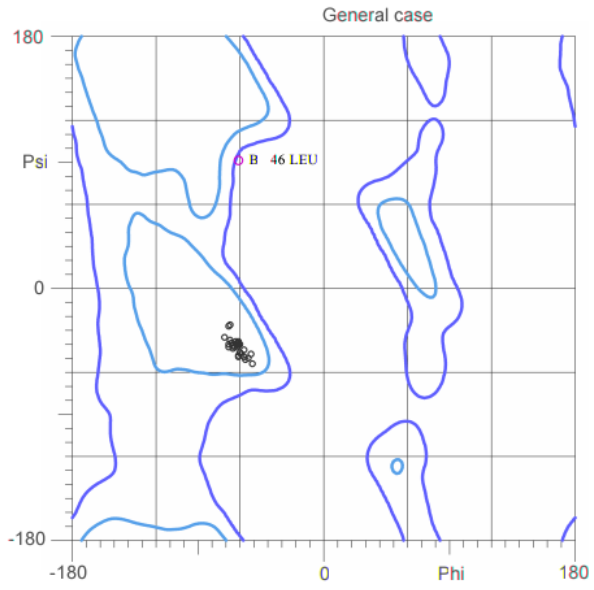
(a) What can you say about the quality of this structure?

1. The PDB validation summary gives it poor marks for both R_{free} and the presence of Ramachandran outliers (i.e. residues whose φ/ψ angles are energetically disfavored).

2. ***However***, this is a really unusual structure. Notice the title. The structure contains both D- and L- peptides! The normal Ramachandran diagram of favorable φ/ψ values only applies to L-peptides. It turns out that both the D- and L-copies of the peptides in the structure have reasonable energetics; the poor validation score may arise partly because the validation tools were not expecting D-peptides. But that doesn't explain the poor R_{free}, and residue Leu 46 really is a Ramachandran outlier so maybe the poor score is justified.

3. The structure contains lipid molecules (1-monooleoyl-rac-glycerol, residue code MPG). If you drill down to the end of the "full validation report" you will find that the LLDF validation score for all copies of the lipid is highlighted as being poor. The values are 7.9, 5.4, and 5.4 where a good score would be 0. If you cared about the precise conformation of the lipid, this would be a concern and you should inspect the electron density maps yourself to see how much you trust the model that was deposited.





Here is another new one released this week. It is interesting because the data was collected using the X-ray free electron laser (XFEL) mentioned in class.

http://www.rcsb.org/pdb/explore/explore.do?structureId=4YUP

It also is an example of one thing about XFEL data that I forgot to point out explicitly.

(**b) Can you spot what it is?**

Primary Citation: "*Mapping the conformational landscape of a dynamic enzyme by multitemperature and XFEL crystallography*" Keedy et al [2015].

Standard procedure for most crystallography is to freeze the crystals in liquid nitrogen and keep them frozen during data collection. This keeps them from drying out, reduces the spread of free radicals caused by oxidation or X-ray damage, and slow the rate at which crystals are melted or burned by the heat of the X-ray beam. It also reduces the thermal vibration of all atoms in the crystal. But the "diffract and destroy" method introduces crystals into the XFEL beam while they are still in solution at room temperature. The title of the PDB deposition and the associated publication gives a huge clue that the reason for the XFEL experiment was partly to compare dynamic motion in room temperature crystals and frozen crystals.