Question Topics:

- Books or Internet Resources
- Course Content
- Diffraction from a Point Scatterer versus many Scatters
- Crystallographic Refinement
- The R-free or Free R Factor
- The Multiple Isomorphous Replacement (MIR) Method
- MAD Phasing
- Choosing a Method for Phasing

Books or Internet Resources

Q: Are there any reliable internet resources besides wikipedia that can put x-ray crystallography in laymen terms? I am a little lost and would really like another resource because there is nothing in the book. Thanks so much!

A1: The National Institute of General Medical Sciences has a page about X-ray crystallography (Science Marries Art).

http://publications.nigms.nih.gov/structlife/chapter2.html

A2: My lectures in Crystallography are now complete. If you require a more authoritative description of X-ray crystallography beyond my lecture notes, web links and suggested reading, I recommend the following book:

"Crystallography Made Crystal Clear" 3rd Ed. by Prof. Gale Rhodes
You can purchase it new from Amazon.com for $41.38 or used for $24.24.

I really would not recommend buying the book unless you have more than a casual interest. However, the discussion is quite rudimentary.

As I said in workshop, I will be interested to know specifically your ability to assess the quality of a structure given the Quality Control Indicators I described in your notes. Second, you should also be able to articulate a suitable method for phasing a structure when presented with the appropriate facts. You must be able to justify your answer. See my previous post on Sept 13 about phasing. Finally, you should be familiar with the five steps involved in solving a crystal structure.

Course Content

The lectures for this class are completely over my head. From the first slide each day there are many words and acronyms that are not defined and might as well be another language. Because of this I don’t get anything from them. So basically I will do all of the learning on my own, outside of class. Am I the only one that feels this way? Should I bring my computer and switch between wikipedia and facebook like that curly haired guy who sits in the front and asks lots of questions?

A3: I would recommend that if you cannot follow that you make an appointment to see me. The only way I know whether people follow is for them to communicate with me. You are the
only person that has mentioned that you cannot follow the lectures in an apparently extreme manner -- again I encourage you to speak to the TAs or contact me or other lecturers about your perceived difficulties.

The internet is not the answer to learning this material.

My email address is:

joseph.wedekind@rochester.edu

My office phone number is 273-4516.

Q: Why do I need to learn crystallography? I am not even studying biophysics and don’t plan to.

The simple answer is that crystallography is the most widely used experimental method to visualize structures at the atomic level. For example, Watson and Crick used Rosalind Franklin's X-ray diffraction data to generate a model of the DNA double helix. Molecular machines such as myosin, the ribosome, RNA polymerase, topoisomerases, ion channels, G-protein signal transduction, RISC and many more are known because of crystallography. Structures have proven essential to develop drugs (e.g. HIV-1 protease). The basis for antigen recognition by and antibody, and MHC complex displays of self and foreign peptides are known from crystallography. The structure of histones are known from crystallography. Imagine a scientific world in which you couldn't visualize these things ... It would have a deep impact on the basic foundation of biology and our modern understanding of life.

Significantly, the pervasive nature of crystallography requires that you understand its fundamental principles. Examine the covers of Cell, Nature and Science to observe what an impact crystallography has on the field. Even if you don't plan to use it as a technique, you should be familiar with its use and limitations. This is important for your career and training as a scientist.

Also, to clarify, each of the persons awarded the 2009 Nobel Prize in Chemistry were classically trained X-ray crystallographers. Apparently the Royal Swedish Society appreciates the importance of visualizing structures at the near atomic level. This serves as an essential platform upon which to base and interpret experiments, but coordinates alone have little importance if no one looks at them, or understand what they signify...

Q: One question on the learning objectives of biophysical techniques is "what are the capabilities and limitations of electron microscopy for high resolution imaging?" I would have said that EM gives only a general outline of a structure, except that in one of the slides the resolution is 1.9 Angstroms for aquaporin. Can someone explain how EM could have this resolution, and why we don't use it all the time?

A1: This is a good question. Single particle EM, with or without heavy metal staining generally only gives a general idea of the envelope of the overall protein shape. However, by using image processing or diffraction approaches on a crystalline array of proteins, it is possible to get around the radiation damage problems that are inherent in the technique and obtain a high resolution atomic structure as for the aquaporin.

A2: Dr. Wedekind discussed this point with a student in his office. In general EM as a high-resolution technique is limited because crystals are 2-D rather than 3-D. Data must be collected by tilting the sample stage (rather than rotating the crystal on a reticulated goniometer head).
Consequently, this reduces the completeness of the data used in the Fourier transform, which can lead to series truncation errors. [Recall the square wave example – suppose we removed some of the low frequency or high frequency waves – would we get the square wave then?] In the case of aquaporin (Nature) 80% of the total possible diffraction data was recorded, with only 70% in the 2.0 to 1.9 Angstrom resolution shell. This will have the effect of making round objects look elliptical in the electron density. Thus, although this approach is technologically innovative, it has limitations with regard to providing high-quality electron density maps (and complete data sets), which are the basis for visualizing, and hence building the model, as well as performing the model refinement.

**Diffraction from a Point Scatterer versus many Scatters**

Can anyone please explain what slides 31-34 in the 1st crystallography lecture are trying to portray? I don't understand what they are talking about at all with two, four, and infinite point scatters.

**A1:** The slide 30-34 want to say "diffraction pattern is inversely related to the dimension of the diffracted objects". That is, if the diffracting objects are separated more, the diffraction pattern becomes closer together. So if you observe two large objects in real space, you are actually seeing very spots very close together in the diffraction pattern (reciprocal space).

**A2:** There is a reciprocal relationship between the spacing of objects in direct (real) space and the observed diffraction pattern. Another concept is that increasing the number of point scatterers sharpens the diffraction pattern. An infinite number of scatters will result in "fringes" with sharp intensities (i.e. a delta function). We can image that if the point scatterers are replaced by actual proteins in a 3-D array that you would require a large number of molecules to actually elicit a diffraction pattern that is sharp enough to measure the intensities of individual reflections (Ihkl).

Another point of the slides is based in Young's two slit experiment. In this experiment, the spacing between slits (similar to point scatterers) is responsible for the constructive and destructive interference of the incident wave that leads to the observed interference pattern. If these point scatters are repeated many times, the constructive interference sharpens and the backgrounds drops (destructive interference). What is left is the 3-D reciprocal lattice.

**Q:** So I am slightly confused. With x-ray diffraction you are collecting the patterns from many different proteins, and taking little differences in intensity collected from each one to compile one structure, or are there many structures created then compiled?

**A1:** In class we discussed Young's two-slit experiment in which waves produce a pattern based on constructive or destructive interference. We then turned to a 1-D array of point scatters and demonstrated that more points produced a sharper diffraction pattern. We generalized this to a 3-D lattice, and then populated each point with a protein.

**Q:** I don't understand how if you have so many space groups contributing, how do you have one structure outcome?

**A1:** In this case, each protein can be surrounded by a simple unit cell. We defined the unit cell as being able to generate the crystal by pure translational operations (i.e. imagine an infinite array of boxes with a shoe inside each). We said that the diffraction from the infinite array approximates the scattering from molecules in a crystal. The diffraction pattern that arises is due to constructive and destructive interference that is based on whether or not planes of atoms in
the unit cell diffract such that the path length traveled by the incident radiation is an integral number of wavelengths on successive planes (i.e. Bragg's Law). So, in fact you are recording a 3-D diffraction pattern that results from sampling many identical (or nearly identical) unit cells in the crystal. If the contents of the unit cell were not identical, there would be destructive interference that would smear the diffraction pattern (diminish the intensities) depending on the nature of and extend of the differences among unit cells. Suppose the shoes placed in each box were different. Some had laces, some did not. As long as the laced shoes were all identical, we would see a shoe structure whose electron density comprises an apparently laced state, although the laces themselves might have weak electron density proportional to the actual population of laced versus non-lace containing shoes. So, whatever is in the electron density must be the result of the average of the contents of all the billions of proteins (or shoes) in the unit cells (shoe boxes). At present, it is not possible to collect diffraction data from one protein sample. Since there is no periodicity, there would be no diffraction pattern (recall that the scattering from a single point is a smear not a sharp pattern).

Crystallographic Refinement

Q: I'm looking at slide 19 from crystallography lecture 2. It says that computational refinement methods are supposed to minimize the agreement between the model and an ideal target geometry. This seems counterintuitive: should it just say 'maximize,' or am I totally missing the concept?

A1: So in other words, you want to maximize the agreement of the Fobs with Fcalc, so that would result in the minimization of the parameters that Dr. Wedekind listed (bond angles, bond lengths, etc).

Q: Could you explain what exactly structural refinement of the X ray structure mean? The flow chart outlining the different steps in obtaining the structure show a step where in calculation of new phases is indicated. Does that mean from each model we calculate new phases and then again construct a refined model?

Yes, each new structure resulting from refinement has changed the positions of the atoms. It has improved geometry to match ideal values. Possibly, new details have become clearer as the electron density maps improve. The new model IS the source of the new phase information (or alpha-calc). This process of using the new model to phase the experimental Fobs is iterative. When the Rwork no longer improves, one can assume refinement has converged.

Q: On slide 19 of the second x-ray crystallography packet ("Goal of Structural Refinement") it says "Most common computational refinement methods are designed to minimize the agreement between the model and an ideal target geometry". This did not make sense when I read it, and conflicts with what you said in your prior answer: "It has improved geometry to match ideal values." I am assuming that the packet has a typo, or that I am missing a major point here. Could you tell me which it is?

The programs employed are often referred to as "minimization" programs. Here I use minimize to describe that the refinement aims to provide the best agreement between the model and the ideal values. In “minimizing” we aspire to improve the agreement.

Both statements are correct as written in the notes. The goal is to obtain model values close to those of the target (ideal) values.
Q: What's the difference between the B-factor and the RMS deviations? The acceptable values for each (for deviations in bond and angles) are the same for both...are they the same thing?

I am not sure why you think they are the same?

RMS is the root mean square deviation from ideality. It is a term based on least squares minimization. Values for bonds are <0.01 Angstrom. Values for angles are 1.5 degrees approximately. See your lecture notes.

The B-factor is an indicator of the static or thermal disorder of an atom. Higher values mean it is more difficult to localize the atom in the electron density map. (This might be caused by the mixture of laced versus unlaced shoes in the description of crystals given above).

**The R-free or Free R Factor**

Q: Isn't the Rfree factor dependent on the Rwork? The Rwork should be between 20-25%, and the Rfree should not be no more than 8-10%. How does 40% work as a general indication? Thanks.

A1: 8-10% more than the Rwork* for the Rfree as a quality indicator.

A3: Yup! Rfree comes from taking out random reflections before refinement and using those values to calculate the Rfree value during the entire course of the refinement (until you are done). If you search "Free R Factor" in google and click on the first link (Online Dictionary of Crystallography), it provides a good explanation of how Rwork and Rfree are related to each other. Specifically, the last paragraph is particularly useful:

After each cycle of refinement, the free R factor and the R factor (Rwork) for the working set of reflections are both calculated. However, as the refinement of the model converges (stops changing), the working and free R factors both approach stable values. It is common practice, particularly in structures at high resolution, to stop monitoring $R_{free}$ at this point and to include all the reflections in the final rounds of refinement (this give you the $R_{cryst}$ or $R_{factor}$).

Also, Dr. Wedekind used 40% as an example of a model that may have serious errors. A test case showed that such a model would have a reasonable Rwork but be traced backwards in the electron density. Generally, you want to know that a model may be incorrect if the Rfree is significantly larger than the R-factor. I hope this helps!

A4: I think this problem has been satisfactorily addressed. The important thing is that minimization requires that you satisfy good stereochemistry (i.e. ideal values), but also that the model still fits in the electron density map ($F_o - F_c$) goes to zero. In NMR, one calculates structures based on the distance restraints derived from NOEs (for example). There is no electron density, so any model calculated that is consistent with the distance restraints will potentially describe the structure. The model is also subjected to energy minimization to make sure that ideal geometry is observed.

Q: My second question is on R free values. Is it that each time we refine a structure we keep aside a fraction of reflections to determine this value? I was reading somewhere that it refers to the redundant data set. Could you please put this into context?
The Rfree or test set is set aside prior to the start of refinement. The test should must remain excluded from the entire refinement so that this subset of reflections is not biased by the refinement process.

The Multiple Isomorphous Replacement (MIR) Method

Q: Would someone please clarify what is meant by "the relationship F(PH) = FP + FH"? What is this triangle describing? (I'm not a math person).

A1: Here F(PH) is the intensity of the protein with the heavy atom, FP is the intensity of the protein by itself, and FH is the intensity of the heavy atom.

In isomorphous replacement, the F(PH) is simply the sum of the FH and FP since you're simply adding a heavy atom to the crystal. To find the intensity for the protein, you just rearrange the equation F(PH) = FH + FP to solve for FP: FP = F(PH) - FH. So you just need to subtract the intensity of the heavy atom from the intensity of the protein with the heavy atom F(PH). (Recall that the values of F(PH) and FP each come from X-ray data sets. So, you have intensities from these data sets, which can be converted to amplitudes and then used in the subtraction to give FH.

In non-isomorphous replacement (probably not a useful technique), FP is NOT equal to F(PH)-FH. This is because adding the heavy metal changes the conformation of the protein. Therefore you can find FP easily enough and you can find the apparent FH too. Unfortunately since the protein changed conformation from adding the heavy metal you cannot assume that FP = F(PH) - FH. This is because some of the light atoms moved when the heavy atom was added (i.e. C,N,O,S) and they also contribute to the change in FH (as compared to the heavy atom alone).

Q: I don't quite understand why you're trying to find the intensity of the native protein. I thought it was the phase of the protein we were trying to find, the intensity is already known from the diffraction data.

A1: Yes, we do have the intensities (and hence amplitudes of the native protein), but not their phases. We need to find the phases, and this cannot be done directly, which is why we need the heavy atom. The problem of locating the heavy atom in the MIR method relies on the difference between F(PH) - F(P) = fH. It is this difference in the intensities that greatly simplifies the structure determination since essentially you are subtracting away all the scattering contribution from the protein atoms (C, N, O, S) and leaving only the heavy atom. Consequently, it is possible to locate the heavy atom using (difference) Patterson methods (by simple algebra). If you know the heavy atom position you know its phase, and this allows the phases for the light atoms to be calculated (i.e. C, N, O, P, S etc). [See the explanation in the 2010 questions as well].

MAD Phasing

Q: In the first x-ray crystallography slides, the section on solving the phase problem with MAD refers to using the x-ray "absorption edge" of a heavy atom. What does this mean? Thanks!

A1: The absorption edge refers to a specific wavelength of energy required to elicit an electron orbital transition in an element of interest. Rather than ejecting the electron, it moves from a lower to a higher energy state. Excitation can be accompanied by fluorescent emission. The elemental absorption edge also happens to be where the corrections to the elemental scattering factor change the most due to the so called "anomalous diffraction" effect. By collecting X-ray...
data from the same crystal on or near the X-ray absorption edge, one can change the values of
the dispersive difference corrections to the intensities. By examining the differences in
|Ihkl(\lambda_1) - Ihkl(\lambda_2)| one can locate the anomalously diffracting atom in a Patterson
map. What you need to know is that multiple wavelengths are involved. The method requires
a single crystal, and that it must have a natural (i.e. Zn^{2+}) or artificially introduced heavy atom with
an accessible absorption edge. For example, Carbon, Nitrogen, Oxygen, Sulfur and Phosphorus
do not have accessible X-ray absorption edges -- the correct energy needed for these
experiments in unavailable given modern instrumentation and other physical limitations. This
method is significant because it is the most widely used method to solve the experimental phase
problem.

Q: Could someone please explain the graph which is showing the anomalous and dispersive
differences in the absorption on slide 56 of lecture held on 9/09/09?

A1: I believe you referring to the wavelength dependent corrections near the X-ray absorption
edge. The slide shows what are the corrections to the atomic scattering factor of the
anomalously diffracting atom as a function of energy change or wavelength. So, the corrected
scattering factor fc = fo + f' + if'' where fo is the scattering factor in the absence of anomalous
diffraction (i.e. far away from the absorption edge). OK?

Q: I have three questions on Multiwavelength Anomalous Diffraction:
1) What is anomalous diffraction?
2) Am I correct in saying that the reason we do not need the F(P) data set (and thus can
   be non-isomorphous) is that we collect data sets at multiple wavelengths? And that we
can use f prime and f prime prime to restore dispersive and anomalous differences,
respectively?
3) What are dispersive and anomalous differences?

A1: This is in your notes, but I'll try to explain it here. Anomalous diffraction is an energy
dependent change in the scattering factor of an atom due to resonant absorption and emission
of X-ray radiation from a particular atomic orbital. The result is a change in the atomic scattering
factor for the anomalously diffracting atom such that f(corrected) = f(uncorrected) + f' +if''.
Here the corrections to the scattering factor are f' (the dispersive correction, which is greatest at
the inflection point of the fluorescence spectrum) and f'' (the anomalous correction, which is
greatest at the peak of the fluorescence scan).

The dispersive correction is measured between wavelengths. For example, I told you about the
"inflection", "peak" and "remote" wavelengths. These each represent complete data sets that we
collect from one crystal containing an anomalously diffracting atom with an accessible
absorption edge (i.e. we need to be able to change the energy to scan through the edge, but we
are limited by our instrumentation to a range between 6KeV and 20KeV; elements like sulfur
have absorption edges of course, but this is around 5 Angstroms, which is not available on our
cyclotron, not to mention that such a low energy would not diffract well and likely heat our
crystal). Getting back to the question, we need to collect each data set (I1, I2, I3 for inflection,
peak and remote) and then take the differences for reflections of the same index between data
sets:

|F(\lambda_1)hkl - F(\lambda_2)hkl| = F(\lambda_1-I2)hkl (which is some difference)

We do this for every index (i.e. F(I1)111 - F(I2)111). Remember that each reflection has a
unique address in reciprocal space. OK, you can imagine other combinations of differences:
\[ |F(\lambda_1)_{hkl} - F(\lambda_3)_{hkl}| = F(\lambda_1 - \lambda_3)_{hkl} \]
\[ |F(\lambda_2)_{hkl} - F(\lambda_3)_{hkl}| = F(\lambda_2 - \lambda_3)_{hkl} \]

What you need to realize is that the dispersive correction is energy (wavelength)-dependent. Very close to the absorption edge, it will be large (i.e. the inflection is maximum), but far away it will be smaller, like the remote. This difference in the correction results in changes in the Fhkl at each wavelength. This gives rise to the differences.

Importantly, these differences can be used in a so-called Patterson Map to locate the positions of the anomalously diffracting atom. If you know the position of the atom you know its phase (roughly speaking). The more wavelengths you collect, the more potential differences you get. The differences should be in agreement in terms of their ability to locate the anomalously diffracting atom (i.e. redundancy).

Q: Now, what about the anomalous component \( f' \)?

A1: This correction is recorded within a single wavelength. The maximum correction (\( f'' \)) will be recorded at the peak energy. Like the dispersive "difference", we need to record an anomalous "difference". Where does the difference come from? It can be obtained by recording Fhkl for a crystal, then rotating the crystal by 180 degrees. This results in F-h-k-l (which is the opposite side of the same Bragg plane). In the presence of anomalous scattering Fhkl does not equal \( (\neq) F-h-k-l \), but without anomalous scattering these amplitudes should be the same. By subtracting \( |Fhkl - F-h-k-l| \) we get the anomalous difference. Again, we can use this in a Patterson Map to locate the anomalously diffracting atom. This approach is the basis for SAD phasing, but is not as powerful as using the dispersive differences from MAD because the magnitude of \( f'' \) is not very large (i.e. poor signal). When the dispersive and anomalous differences are used, you can see there would be redundancy in the data, which helps to locate the anomalously diffracting atom with greater confidence.

Q: Also, how do we know that the crystal structure we get would be the same as one without our heavy atom substitution?

A1: This is a good point. In practice, it depends on the nature of the atom used for phasing. In the case I described for class, the enzyme already has Zn(II) present in the active site, which is necessary for biological function. Substitution of Met for Se-Met replaces the sulfur with selenium. Se has a slightly larger ionic radius than S, and it is also more susceptible to oxidation. However, few substantive differences can be detected in practice.

Another point, which we discussed in the workshop, is the crystallographer almost always collects a "native" data set devoid of a non-natural anomalously diffracting atom. It is quite easy to phase this structure since the atomic coordinates derived from the MAD-phased electron density map should provide an excellent source of phases for the Native Fhkl data set. It is rare that the anomalously diffracting atom would create a conformational change in the protein large enough that the fold would be altered. Any local changes would be resolved by refining the MAD coordinates against the Native data, just in case.

Q: I was just curious about the process of analyzing crystals in a synchrotron at another institution. When remotely crystals somewhere else (like California for instance) are crystals sealed in some sort of container and shipped or are the parameters for forming those particular crystals sent to the institution so they can grow the crystals on site?
A1: This is a good question, but is beyond the scope of the course. If you really want to know, ask me in the hallwaway some time (or take my classes BPH 411/CHM 402 or BCH 412).

Choosing a Method for Phasing

Q: I'm a bit confused as to how one would choose the correct method for phasing. How would you know whether to introduce a heavy metal to phase or to use SAD or molecular replacement?

1). Use MR if you have a related structure. It must be:
   a). Available from the PDB.
   b). >30% identical in sequence to the unknown protein (greater identity means a better chance for success).
   c). The search model cannot be significantly different in overall conformation relative to the unknown. Recall the example of the Fab fragment I gave in class in which the two domains are related by a flexible hinge. In this case, the known and unknown might not have the same conformation.

2). Multiple Isomorphous Replacement (MIR). Use this method if you cannot use molecular replacement. It requires:
   a). Many crystals in order to identify the correct heavy atom that actually binds. For example, if you have a cysteine, soak in Hg(II). If you soak in too much, your crystal may crack (start again). If you soak in too little, it may not react. So, there is a trial and error process to this. If you are lucky, you can collect the F(PH) data and then subtract the F(P) data set. \(|F(PH) - F(P)| = F(H)\). That is, the resulting structure factor for each Fhkl should give the change in amplitudes caused only from Hg(II) binding. One can then use these F(H) values to locate the Hg(II) position. If you know the Hg(II) position in the unit cell, you know its phase. This can be used to find the phases of all the other light atoms, C, N, O, S in the protein.
   b). The method must be "isomorphous". If you soak in too much Hg(II), the difference in \(|F(PH) - F(P)|\) will be too large on average. Ideally it will be a 20% change. Larger values indicate perhaps the heavy atom caused an undesired conformational change in the protein. Now you are observing differences in your data sets caused by changes in the protein atoms, not solely from the heavy atom. Therefore the F(P) or native data will no longer be isomorphous with the heavy atom derivatized data set or F(PH).
   c). Because there is an ambiguity in the phase calculation resulting from a single heavy atom, two possible phase values are possible.
   d). Therefore, you must repeat the process again with another crystal and another heavy atom that binds in a new location. If successful, the second heavy atom will produce one of its two solutions for a given Fhkl that overlaps with the first derivative. The incorrect solution is neglected and the unique phase can be applied.
   e). The more heavy atoms you have, the better chance you have of converging on the most probable phase.
   f). This method can be conducted at home where your X-ray source only makes one wavelength.
3). Use MAD phasing if you can't use MR and you have access to a cyclotron that produces tunable X-ray energies. MAD is then a first choice if MR is not possible and you have:
   a). >1 Met per 80 amino acids in your protein sequence (excluding the N-terminus or C-terminus, which are usually not well ordered).

   b). You have a naturally occurring metal such as Zn(II) in your protein. Remember your metal must have an accessible K or L absorption edge at the cyclotron source. Sulfur will not work since its edge is near 5 Angstroms.

   c). Or you may have soaked your crystal in a heavy atom, found it reacted, but was non-isomorphous. Since the MAD (or SAD) approaches do not require the F(P) data set, one collects only data from the F(PH) data set in this case. Changing the energy will change the contribution to scattering for the heavy atom, just like the MIR method: |F(\lambda_1) - F(\lambda_2)| = F_{heavy atom}, which is then used to locate the heavy atom position. The goal of the experiment is to optimize these intensity differences (i.e. the dispersive correction to the scattering factor f-prime), which is done by collecting data sets at the inflection and some other remote points.

   d). MAD gives high quality phases from only one crystal. If you have a heavy atom, it is better than 'soaking and praying' like the MIR approach, especially if you can substitute Met for Se-Met and grow crystals.

   e). SAD requires one wavelength. We did not really discuss it, but all the data are collected from one crystal again. Of course you need a heavy atom with an absorption edge. Here, you collect only at the peak wavelength, only where f-prime-prime (f''') is maximum. You collect data for Fhkl and F-h-k-l, which are derived from opposite sides of the same Bragg plane. This can be accomplished by collecting data to get Fhkl, then rotating the crystal by 180 degrees. The result is to make an anomalous difference Patterson Map using |Fhkl - F-h-k-l| = |F(\text{anom})|. One can again locate the position of the anomalously diffracting atom to gain phase information. As you can tell, it has advantages in that you only need one wavelength and it does not require isomorphism. In practice, the data must be very redundant to record the anomalous signal. More so than MAD. Your crystal might die from radiation damage in the meantime. SAD also needs to be coupled with another method to break the bimodal phase problem. Usually this is done by solvent flattening, which we did not talk about. I will not ask you about SAD on an exam, but now you have some idea what it is.