

WHAT WE NEED TO KNOW

Spots on the diffraction pattern come from electron density in atoms. Atoms that don't have many electrons (like hydrogens) don't give diffraction spots. Heavier atoms (especially metals) do.

We record a large number of two-dimensional diffraction patterns. The position and intensity of the spots tell us the size of the unit cell and also where the atoms lie with respect to each other in three-dimensional space (after we do a lot of calculations).

Remember, each spot is the result of interference of all x-rays with the same diffraction angle from all atoms.

So – we're dealing with waves that constructively and destructively interfere to produce the diffraction spots.

It's no surprise that we lose phase information of the diffracted x-rays, since we are only working with two-dimensional images. We need this phase information to ultimately get our structural information.

THE PHASE PROBLEM – PHASE DETERMINATION

Go back to the wet-lab

Basic idea – we are going to introduce heavy metals into and around the protein. These electron rich, good diffractors will cause the intensity of the diffraction patterns to change. We can then use simple differences in intensities and easy simultaneous equations to estimate the phase of the diffraction spots in the protein spectra.

Generally: heavy metals in – intensity changes – do a comparison – get the phase

Multiple isomorphous replacement (MIR)

We add a few heavy metals to the protein (their position can easily be located) and they do not change the structure of the protein – this means the crystals are isomorphous.

So-called isomorphous replacement is done by diffusing heavy metal complexes into the solvent channels of preformed protein crystals. The exposed sidechains that stick out into the solvent channels can grab these metals.

For metalloproteins that naturally want light metals in there (like Zn), we can simply replace those with heavier ones (like Hg).

There are few metals so we can straightforwardly get their 3D positions and if we know that we know their absolute phase. We can then use that information to estimate the phase of JUST the protein in a protein+metal pattern.

Again – we are getting this information because of intensity changes in the diffraction patterns between protein only and protein+metal data.

MIR though is not the most accurate approach. In fact we can really only estimate the phase to within about 40° (out of 180°).

So – what we have to do is.....another metal another experiment.

Normally 2 or more metals are needed to get the phase right for all reflections.

Important points: MIR – usually monochromatic X-ray source (rotating anode generator) – do more than one metal to get the protein phase. Multiple experiments

ANOMALOUS SCATTERING

Another method – but again based on introducing heavy metals and looking for intensity changes.

In this method we usually put selenium in there as the metal of choice. Normally recombinant DNA methods allow the introduction of selenomethionine in place of regular methionine.

Here we use X-rays of different wavelengths – so we need a synchrotron. We call the method **MULTIWAVELENGTH ANOMALOUS DIFFRACTION (MAD)**. Very popular.

In this case, we record one set of diffraction data (but from multiple wavelengths). Within this one data set, we see intensity differences between symmetry related diffraction spots. Without the metal – the intensities are the same – with the metal they are different. We can then translate these intensity differences into determining the phase of the protein.