What is the resolution?

The figure to the right shows a simple diffraction experiment. A collimated beam of X-rays comes in from the lower left and passes through a crystal on its way to hitting the center of a circular detector. The crystal is in a cubic space group, which means that the unit cell is a cube edges $a=b=c$. Assuming that the crystal is well-ordered and diffracts X-rays very well, what is the resolution of the data that you can measure?

How much can we learn about a protein from [only] a structure?

You have determined the structure of a 40kD metalloprotein at 2.2Å resolution. The structure was reported to have $R = 0.240, R_{free} = 0.280$, and all but 2 of the protein residues lie in the “favored” or “allowed” regions of the Ramachandran plot. There are two copies of the peptide chain in the asymmetric unit of the crystal, and they share an extensive interface. There are 200 water molecules in the crystallographic model in addition to the protein itself.

1. This protein was cloned from genomic DNA extracted from a different strain of bacteria than the reference strain whose sequence is in GeneDB. Therefore it is at least possible that some residues in the protein that was crystallized are different from those in the GeneDB sequence. You can of course ask for the expression construct to be re-sequenced, but first let’s look at the X-ray structure.

   (a) This is a 408 residue protein, but you can only find residues 2-380 in the electron density map. What does this suggest?

   (b) Residue 27 is supposedly a Lysine, but it looks rather like a Phenylalanine in the structure. Similarly, it is not clear whether residue 345 is a Valine or a Threonine. To what extent can you be confident that the X-ray structure correctly distinguishes the two possibilities in each case?

   (c) It’s a metalloprotein, and there are two clear metal sites. Can the X-ray structure tell you what metal[s] these sites might be?

2. What about those two residues with “disallowed” phi/psi angles? Do you go back and try to re-build the model at those locations? Do you attribute it poor resolution or other experimental artifact? What would you say about these in a paper reporting the structure.

3. Can you conclude that the protein is a dimer? Why? Consider all the possibilities you can think of.

4. We have a rule-of-thumb that the $R$ factor of a well-refined structure is expected to be roughly 1/10 of the resolution in Å. In this case the $R$ factor is 0.24 and the resolution is 2.2Å. What might be contributing to the higher-than-expected $R$ factor?