

Bioc 585 Final Exam – X-ray portion (2006)

The following questions concern the paper by Schumacher et al (2001) *Nature* 410:1120, general crystallographic theory and equation 1:

$$\text{eq. 1} \quad F_{hkl} = \sum f_i \cos [2\pi(hx_i+ky_i+lz_i)] + f_i \sin [2\pi(hx_i+ky_i+lz_i)]$$

(1) (10 pts.) Describe the material used to obtain the crystal structure. In other words, what was crystallized?

Full length rat calmodulin (CaM), expressed in E. coli, and the calmodulin binding domain (CaMBD) from rat SK channel 2 (residues 395-490), with a His-tag on the C-terminus, also expressed in E. coli.

(2) (10 pts.) What roles did the PbCl_2 and Selenomethionine derivatives play in the structure solution?

The PbCl_2 was used for phase determination by "MIR" (note this cannot be entirely correct since there is only one derivative described). This yielded an interpretable map that allowed placement of CaM and CaMBD, but for which the CaMBD sequence could not be unambiguously identified. To overcome this problem, the SeMet derivative of CaMBD was made, which allowed for the correct sequence alignment.

(3) (10 pts.) The crystals were in space group C2. What do the C and the 2 refer to?

The C refers to a centered space group with 2 origins related by translation, one at x,y,z and the other at $x+1/2, y+1/2, z$. The 2 refers to a crystallographic 2-fold rotation axis of symmetry that runs through the entire crystal.

(4) (10 pts.) Define the term “asymmetric unit”. For a crystallized homodimeric protein, what are the possible contents of the asymmetric unit?

The asymmetric unit is the unique part of the crystal. For a homodimer, the asymmetric unit could contain a monomer, a dimer or multiple dimers.

(5) (20 pts.) What is in the asymmetric unit of the structure determined by Schumacher *et al.*? Do the crystal and solution complexes contain the same oligomer? Be sure to support your conclusions.

The ASU contains one CaM and one CaMBD. The biological unit, formed via the crystallographic 2-fold axis of symmetry, is dimeric (2 CaM, 2 CaMBD). Solution studies (DSL, centrifugation) yield values consistent with a dimeric complex (Supplementary Table 2). The dimer interface in the crystal is extensive, suggesting this is the correct oligomer.

(6) (10 pts). What is the quality of the structure determination by Schumacher *et al.*? Be sure to support your conclusions.

The structure is fine, but not outstanding. The structure is to 1.6 Å (excellent), with $R_{cryst} = 0.228$ and $R_{free} = 0.252$ (acceptable, not great). $R_{sym} = 0.289$ and $I/\sigma I = 2.5$ in the highest resolution bin (good). Stereochemistry is fine (RMSD bonds = 0.015 Å, angles = 1.6 degrees), although Ramachandran values are not included in the paper. Completeness and multiplicity are fine overall, but values in the highest resolution bin were not given.

(7) (10 pts). Solvent flattening (leveling), a form of density modification, was used by Schumacher *et al.* What is solvent flattening and how is it useful?

Solvent flattening is used to improve phases for MIR and MAD experiments. The solvent portion of an electron density map is set to a constant value for solvent, which removes errors in the map. The resulting map is Fourier transformed to give new phases, which are combined with the experimental phases, yielding better overall phase estimates. This is an extremely powerful method for phase improvement.

(8) (10 pts). What is an “Omit map” and how was it used in the paper by Schumacher *et al.*?

Omit maps are calculated using a model where atoms of interest have been omitted and phases calculated by Fourier transform. The resulting map, generally calculated with $F_o - F_c$, αc values for the structure factor, yields an unbiased look at the omitted atoms. In the paper, this was used to examine the calcium binding sites, ensuring that not all sites were filled.

(9) (10 pts). Define f_i in equation 1.

This is the atomic scattering factor, which describes scattering by a particular atom type as a function of diffraction angle.