

## 18. Frequently Asked Questions

**Q1:** Please send me a copy of SHELX-76. I am afraid that I cannot use the new version because **my diffractometer measures  $F$ -values, not intensities.**

**A:** Buy a CCD detector. They measure intensities! [In fact, diffractometers measure intensities too. You just need the right data reduction program. If you are desperate you can even feed SHELXL with  $F$ -values using HKLF 3.]

**Q2:** When I start SHELXL on my PC the disk rattles loudly for several hours and smoke comes out of the back. Is this a bug?

**A:** You must be trying to run SHELX under some version of **WINDOWS!** The best solution is to reformat the hard disk and install LINUX. If you are running WINDOWS-95 an inferior alternative is to 'Reboot to DOS' (as recommended for games programs).

**Q3:** The **referee rejected my paper** because the weighted  $R$ -factor was too high and because the stupid program had forgotten to fix the  $y$  coordinate of one atom to fix the origin in space group  $P2_1$ . What should I do?

**A:** Try another journal; if you emphasize the 'biological relevance' enough, they may not notice the  $R$ -factor! Note that  $wR2$  (based on intensities and all data) is of necessity 2 to 3 times higher than  $wR1$  (based on  $F$  and leaving out reflections with say  $F < 4\sigma$ ). Unfortunately SHELXL cannot work out  $wR1$ , because the weighting scheme for intensities does not apply to  $F$ -values. It is better to quote the *unweighted*  $R1$  (with or without a  $4\sigma$  threshold) anyway, because it is too easy to cheat on  $wR2$  by modifying the weights!

It is no longer necessary or desirable to fix the origin by fixing coordinates, the program applies appropriate *floating origin restraints* automatically when they are needed.

**Q4:** The program tells me to refine **extinction**, this does reduce the  $R$ -factor but the extinction parameter becomes very large although my crystal could hardly be described as 'perfect'. Is this reasonable?

**A:** No. The most likely causes of large apparent extinction are: (a) you have input  $F$  with HKLF 4, (b) A few reflections that should be very strong have been measured as weak because they were cut off by the beam-stop, (c) your counter was saturating and an inadequate dead-time correction was made (in the case of an image plate this is an 'overload'), or (d) your counter was defective or the energy discrimination was set wrongly. Overloads may be eliminated by 'OMIT  $h k l$ ' if necessary.

**Q5:** The structure could only be solved in **P1**, not  $P\bar{1}$ , but on refinement some of the bond lengths and U-values are wildly different in the two molecules. If I use SAME the geometries of the two molecules become very similar but how do I restrain the  $U_{ij}$  components of equivalent atoms to be the same?

**A:** You could use EADP, but it might be better to look for the inversion center instead, otherwise you will probably be **'marshded'**.

**Q6:** I included batch numbers in the *.hkl* file and BASF parameters in the *.ins* file, but the stupid program still **didn't refine the batch scale factors!**?

**A:** You need MERG 0 (the default MERG 2 will average the batch numbers).

**Q7:** How do I obtain the molecular replacement program **PATSEE**?

**A:** PATSEE has been maintained by its author, Ernst Egert, since he moved from Göttingen to the University of Frankfurt. He can be contacted by fax (+49-69-7982-9128) or email (bolte@chemie.uni-frankfurt.d400.de).

**Q8:** What should I do about **'may be split'** warnings?

**A:** Probably nothing. The program prints out this warning whenever it might be possible to interpret the anisotropic displacement of an atom in terms of two discrete sites. Such atoms should be checked (e.g. with the help of an ORTEP plot) but in many cases the single-site anisotropic description is still eminently suitable.

**Q9:** I get the message ' **\*\* UNSET FREE VARIABLE FOR ATOM ... \*\***' but I haven't used any 'free variables'!?

**A:** There is a typo in your atom coordinates, e.g. a decimal point missing or replaced by a comma.

**Q10:** After using SHELXPRO to prepare the *.ins* file from a PDB file and then running SHELXL, I get the message: ' **\*\* No match for 2 atoms in DFIX \*\*** '!?

**A:** This message probably refers to the fact that SHELXPRO labels the oxygens of the carboxy-terminus OT1 and OT2 so that special restraints can be applied, so there is no atom called 'O' in this residue. This is normal and can be safely ignored. Other similar messages, also messages about bad CHIV or AFIX connectivity, should be investigated (by checking the extra information, including the connectivity table, given in the *.lst* file) to see if they can be ignored safely or not. If the initial geometry is poor, it may be necessary to edit the automatically generated connectivity table with BIND and FREE.

**Q11:** The program prints out a **Flack x parameter** of 0.3 with an esd of 0.05. Is the crystal racemically twinned?

**A:** Not necessarily! The Flack parameter estimated by the program in the final structure factor calculation ignores correlations with all other parameters (except the overall scale factor). Since these parameters may have refined so as best to fit a wrong absolute structure, it is quite possible to get an estimate of about 0.3 for the Flack parameter when the true value is 1, i.e. the structure needs to be inverted and is not racemically twinned. On the other hand a value close to zero with a small esd is a strong indication that the absolute structure is correct. If there is any doubt the Flack parameter should be refined together with all the other parameters using TWIN and BASF.

**Q12:** Neither direct methods nor Patterson interpretation in **SHELXS** can find the 24 selenium atoms from the **MAD data** of my selenomethionine labeled protein.

**A:** I'm not surprised.

**Q13:** How does one set up **restraints for a non-standard residue** in a protein for SHELXL?

**A:** First find a suitable fragment in a database such as the CSD, then calculate all 1,2- and 1,3-distances and turn them into DFIX and DANG instructions resp. FLAT and (zero chiral volume) CHIV restraints can easily be added by hand. If the structure contains a number of identical units such as sulfate ions, SADI or SAME can be used instead, then it is not necessary to invent any target values.

**Q14:** What is the **worst resolution** that is acceptable for: (a) solution of a structure by direct methods using SHELXS, (b) refinement with SHELXL?

**A:** Direct methods assume randomly distributed resolved atoms. Direct methods are crucially dependent on having atomic resolution data, say better than 1.2Å. A good rule of thumb is that a least one half of the theoretically possible number of reflections between 1.1 and 1.2Å should have been measured with  $I > 2\sigma$  for direct methods to be successful, though this rule can be relaxed somewhat for centrosymmetric structures and structures containing heavier atoms. In particular the resolution is not so critical for the location of heavy atoms from  $\Delta F$ -data, provided that the minimum distance between heavy atoms is much greater than the resolution.

SHELXL lacks the energy terms used by e.g. X-PLOR for refinement against low-resolution data. This imposes an effective limit of about 2.5Å, but this limit may be extended a little to lower resolution if NCS restraints can be used.