Shelx C/D/E Tutorial, CCP4 Workshop, Chicago 2008

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1 General Notes

There are several test data sets available through /opt/workshop/data/shelxcde/. This tutorial uses two of them.

You are welcome to use your own data instead, either from the beginning or after working through these pages.

The tutorial first shows how to use shelxcde from the command line, by a MAD example. It then switches to the hkl2map GUI by Thomas Schneider. An Elastase structure is solved once with Sulphur-SAD, once with SIRAS from an Iodine soak.

All software used in this tutorial is available for free for academic users.

2 Gere: MAD from command line

Ducros et al., Acta Cryst. D54 (1998) 1453. Standard CCP4 test structure. PDBID 1FSE, C2, 6x

This section provides an example of how to call shelx c/d/e in order. The cell dimensions of this project are

2.1 shelxc

At a command prompt, in a new directory, type

```
shelxc gere << eof
CELL 109.02   61.75   71.74   90.00   97.08   90.0
SPAG P2
PEAK  /opt/workshop/data/shelxcde/gere/gere_peak.sca
INFL  /opt/workshop/data/shelxcde/gere/gere_infl.sca
HREM  /opt/workshop/data/shelxcde/gere/gere_hrem.sca
LREM  /opt/workshop/data/shelxcde/gere/gere_lrem.sca
FIND  12
eof
```

The last table printed to the terminal,

```
Correlation coefficients (%) between signed anomalous differences
Resl.   Inf - 8.0 - 6.0 - 5.0 - 4.2 - 4.0 - 3.8 - 3.6 - 3.4 - 3.2 - 3.0 - 2.75
HREM/PEAK  89.5  80.0  68.2  58.5  51.9  50.0  44.5  34.6  24.3  23.2  17.2
HREM/INFL  87.3  75.8  64.2  54.5  40.7  40.4  34.9  21.8  16.1  17.7  19.2
PEAK/INFL  95.4  91.1  84.1  77.8  67.0  68.4  58.0  52.8  38.1  26.3  32.0
```

suggests that a resolution cut-off at 3.1Åis a reasonable choice ($CC > 30\%$). Since shelxc does not make sophisticated guesses but simply adds 0.5Å to the high resolution limit, you have to edit the file **gere_fa.ins** and correct the `SHEL` card accordingly.

2.2 shelxd

Now run shelxd by typing

```
shelxd gere_fa
```

at the command prompt. Observe how the figures CCall/CCweak quickly go up to around 45%/30%. With MAD, a value of CCall between 40% and 50% is a good sign the correct solution was found.

2.3 shelxe

shelxe does not require an instruction file but takes all options from the command line:

```
shelxe gere gere_fa -s0.48 -m20 -b
shelxe gere gere_fa -s0.48 -m20 -b -i
```

Both runs are necessary to tell the correct hand for the substructure solution. The '-b' option writes the files `gere.pha` with the anomalous signal only map and `gere.hat` with improved heavy atom positions that could be recycled into shelxe.

Compare the values for 'mapCC' and the 'Contrast' and judge which hand is correct.

You can load the '.res' file and the '.phs'/ '.pha' files into coot to check visually. In order to calculate the map from the '.phs'-file, coot needs to know the cell and space group. Therefore it is best to read in the '.res'-file before the '.phs'/'.pha'-files.

3 Elas: SAD using hkl2map

Elastase, collected by Marta Vuckovic as a rotation project, supervised by José Cuesta. In-house CuKα native to 1.37Å, iodide soak to 1.85Å.

At the command prompt type

```
hkl2map
```

in order to open the shelx c/d/e gui by Thomas Pape/ Thomas Schneider.

3.1 shelxc

Enter 'elastase' or similar as project name, hit 'Enter' and find the file 'elastase.sca' through the 'Browse' button. With a '.sca' file as input, cell and spacegroup are filled in automatically. You will need to click the 'confirmed' button, though.

Run 'shelxc' and click on 'View Graphics' in order to graphically check the tables shelxc provides.

3.2 shelxd

Since the data are already merged, we do, unfortunately, do not get helpful statistics to judge the resolution cut-off and therefore go with the suggested 1.9Å.

You should ask for 12 S-atoms to search for and run 'shelxd'.

It generally takes a couple of tries before the CCall value goes to above 30%.

Check out the graphics, especially the 'Site Occupancy'- was the estimated number of heavy atoms correct?

3.3 shelxe

Elastase consists of 240 amino acids per asymmetric unit which hkl2map calculates to correspond to a solvent content of 0.37.

shelxe automatically runs both hands at the same time and finishes the wrong one after a few cycles of density modification.

Untick the corresponding box in order to compare the two resulting density maps with coot.

4 Elastase: SIRAS with Iodine soak

In this section we are going to use the very same data set that in the previous section was sufficient for S-SAD as **native** data set for a SIRAS case.

4.1 shelxc

The derivative data is elas-iod.sca, an Iodine soak collected inhouse.

4.2 shelxd

The data are of high quality and the whole resolution range can be included in the shelxd-search.

Start looking for 17 Iodines. While running shelxd, watch the graph "Site Occupancy vs. Peak Number": There is no clear cut-off. Iodines can have a smoothly varying occupancy, so there is no clear break in this graph.

As a rule of thumb an occupancy above 30% can be used as cut-off. In the Elastase case you should see about 21 Iodines.

4.3 shelxe

hkl2map runs shelxe automatically with the '-b' option which will cause shelxe to write the anomalous density map and the refined heavy atom position.

Load the '.pha'-file and the '.phs'-file and find out which of the peaks might correspond to real Iodines.

You should also leave the 'Phase Extension' button checked and look at the quality of the "invented" phases!