COMPUTER INTRODUCTION

For the method course, there are two different kinds of computers: External ones for internet usage and internal ones for everything else. We have different types of Linux installed on our machines and also slightly different versions of programs like coot.

LINUX

We will be working with LINUX. LINUX can be used like Windows in many respectives, but it has also some unique features. Two will be discussed here, namely the **shell** and the **mouse copy/paste** function. Please play around a little with the possibilities listed here to get in touch.

MOUSE COPY/PASTE FUNCTION

Highlight a text fragment to copy. Then move the mouse cursor to any input line, and click the mouse middle button. The highlighted text is pasted into the input line. The input place can be anything, from a browser navigator bar to a text editor or a shell command line.

THE SHELL

The shell is a way to give commands to the computer trough with the keyboard only, and if mastered, it can be very fast and exact. To start a new shell window, you double click the shell icon on the shortcut bar at the lower left of your LINUX desktop. A window comes up, in which you can type commands and execute them by hitting “Enter”.

LINUX COMMAND LIST

ls	What is in the folder I am right now? Give a list.
ls -lisa	What is in the folder I am right now? Give a list with details.
pwd	Where am I? Please give me the path. Example: user@computer:~/praktikum> **pwd [Enter]** /net/home/user/praktikum
more [file]	Show me this file in the shell. (Escape with “Q”.)
kate [file]	Open this file in the Editor. If no file name is given, a new file is generated. Only works if kate is installed.
cd [path]	Changes your folder to the given path.
mv [file1] [file2]	Rename file1 to file2.
cp [file1] [file2]	Copy file1 to file2.
mkdir [path]	Make a new folder with this name.
rm [file]	Delete file.
rmdir [path]	Delete empty folder.
firefox	Opens a firefox browser window. Alternatively, use conqueror, which also serves as file browser.

OTHER IMPORTANT FUNCTIONALITIES

[TAB]	The TAB key lets you automatically complete your input. If there is several possibilities, the first TAB hit will yield no reaction, the second will show all available options. Example: user@computer:~/praktikum> **ls [Enter]** my.pdb my.hkl another.pdb user@computer:~/praktikum> **more a [TAB]** becomes automatically: user@computer:~/praktikum> more another.pdb but: user@computer:~/praktikum> **more m [TAB]** yields nothing. user@computer:~/praktikum> **more m [TAB] [TAB]** gives: my.pdb my.hkl user@computer:~/praktikum> more m
<Up>	Select previously executed commands and edit them.
[Ctrl] + [C]	Stops the command you execute. For example, when viewing a file with "more".
*****	Replaces in file names a random string. Example: user@computer:~/praktikum> **ls *.pdb [Enter]** my.pdb another.pdb
~	Means your home folder in a path. Example: user@computer:/media> **ls ~/praktikum** my.pdb my.hkl another.pdb
/media/	In this folder, you can find a USB stick you've attached to your computer. The name of your USB stick depends. Example: user@computer:~/praktikum> **cd /media/ [TAB]** USBstick/ user@computer:~/praktikum> **cd /media/USBstick/ [Enter]**
[file]	If the file is in a certain folder you are not in, you can also type [path/file]. Example: user@computer:~/praktikum> **cp meine.pdb /media/USBstick/deine.pdb**
..	.. refers to the folder above the one you're actually in and can be used in any [file] part of a command. Most often, this is used to go up a directory as "**cd ..**". Example: user@computer:~/praktikum/1mbn/> **cp meine.pdb ../deine.pdb [Enter]**
&	If & is attached to a random command, that command is executed in the background, so the shell stays available for typing another command. This is especially useful in connection with a text editor. Example: user@computer:~/praktikum> **kate meine.pdb &**

Basic SHELXC/D/E tutorial

Andrea Thorn

Overview

In this tutorial, SHELX will be used for experimental phasing. Two cases will be processed: An easy S-SAD case and a MAD case. Several additional tasks are given in the end. We will use the command line and COOT for this. I am very grateful to Tim Gruene and George Sheldrick for their kind advice!
An online version of this tutorial hand-out can be found at: http://shelx.uni-ac.gwdg.de/~athorn

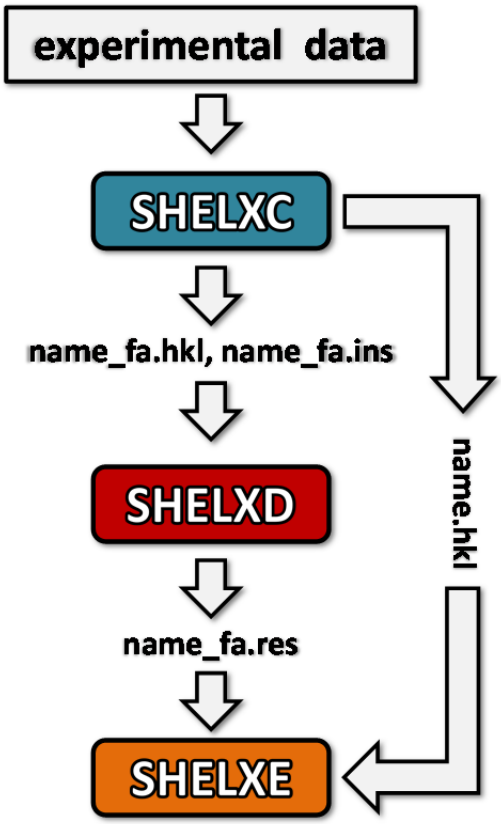
Program availability

SHELX is available from http://shelx.uni-ac.gwdg.de/SHELX/ and free for academic use.
SHELX beta versions can be obtained from Prof. George Sheldrick, gsheldr@shelx.uni-ac.gwdg.de
MAC versions are only compatible with an Intel CPU and 64-bit OS, not with PPC or 32 bit.

Installation

To install SHELXC/D/E on your computer put the executable files into a directory in the 'PATH'.

General data flow



SHELXC sets up files and calculates the estimated substructure structure factor amplitude $|F_A|$ and the phase shift α from input data.

name_fa.ins	cell, symmetry and instructions for SHELXD
name_fa.hkl	h, k, l, $|F_A|$, σ($|F_A|$) and the estimated α angles
name.hkl	h, k, l, $|F_{obs}|^2$, σ($|F_{obs}|^2$)

SHELXD finds the marker atom substructure.

name_fa.res	potential marker atom positions

SHELXE applies density modification to establish the correct handedness, if necessary, and can autotrace the poly-Ala backbone given good resolution native data.

name.phs	contains information for map generation
name.hat	improved marker atom positions
name.pdb	if auto-tracing has been used
name.pha	phases for marker atom substructure

S-SAD Phasing

Molecule:	Viscotoxin-A1
Number of animo acids	92
Resolution	1.25 Å
Space group	$P4_32_12$
Cell	a=b=65.73Å c=47.16Å α=90° β=90° γ=90°
Strategy	In-house CuK_α (λ=1.54178 Å) for S-SAD

Pal A, Debreczeni JE, Sevvana M, Gruene T, Kahle B, Zeeck A, Sheldrick GM. Acta Crystallogr. D64 (2008), 985-92.

These data were collected by Aritra Pal on a Cu home source. The solution of this structure is also discussed in Bernhard Rupp's book "Biomolecular Crystallography", Garland Science 2010. pp. 530-535.

Data preparation with SHELXC

We start in the directory which contains the data, which is named visc-a1 or something similar. The data are contained in the file anom_data.hkl. SHELXC can read data from XDS (XDS_ASCII.HKL), sca files and SHELX hkl files. It is best to read in XDS_ASCII.HKL directly rather than processing it with other programs, so that the data are *unmerged*, which is very desirable. You should create a new file, which could have really any name (and file extension); here it is 'visc-a1.txt'. It has to be in text format and should contain the following lines:

```
CELL 65.73 65.73 47.16 90 90 90
SPAG P43212
FIND 12
NTRY 100
SAD anom_data.hkl
SFAC S
```

Cell parameters
Space group
Number of marker atoms*
Number of tries (100 is sufficient in this case)
Filename of data set
Marker atom type; only for naming

* NOTE: Disulfide bridges are 2.03 Å long, so if we cut the resolution to 2.1 or less, we have to change this number: The disulfide peaks should then fuse into one anomalous density peak!

```
shelxc visc-a1 < visc-a1.txt
```

Note that the first 'visc-a1' is the project name you want to assign and is used to generate the names of the files that are written by SHELXC. The second is the name of the input file. Having the same name makes it easier to find the input file later, but is not required!

Look at your screen output! There is a table like this:

```
Resl.    Inf.  9.11  5.61  4.22  3.45  2.95  2.60  2.33  2.12  1.96  1.82  1.70
 N(data)     143   288   476   669   867  1047  1296  1490  1592  1880  2096
 Chi-sq     1.09  0.94  0.91  0.93  0.89  0.94  1.02  1.13  1.14  1.15  0.96
 <I/sig>    88.4 125.0 138.2 130.7 105.6  72.6  58.5  39.1  19.5   8.0   3.8
 %Complete 127.7  98.6  99.0  99.4  99.7  99.6  99.8  99.7  99.7  99.8  99.2
 <d"/sig>   1.76  3.50  2.41  2.01  1.52  1.35  1.25  1.12  0.91  0.81  0.74
 CC(1/2)    89.0  86.7  77.3  61.2  48.2  37.1  29.7  22.6   6.0  -1.1  -1.9
```

It gives against resolution:

- The number of reflections read in
- χ^2 which should be about 1 if the agreement of equivalents agrees with their estimated standard deviations.
- The average intensity divided by its standard deviation σ
- Completeness (in %)

- d" divided its estimated standard deviation σ, giving you a good indication for the strength of the anomalous signal, which should asymptote to 0.8 in the outer shell, if the data are processed well. If this line is missing, you should check if you gave an input data set in which the Friedel opposites have been merged.
- The self-correlation coefficient for the anomalous signal. It should be above 25% for a significant signal.

In SHELXD, the anomalous differences will be used to find the marker atom positions. Where should you cut the data for SHELXD? This value should be varied for more difficult cases, as it is often crucial!

Output files from SHELXC are:

visc-a1_fa.hkl contains **||F_{hkl}|-|F_{-h-k-l}||** (Bijvoet differences) and estimates for the angles α
visc-a1_fa.ins contains instructions for SHELXD
visc-a1.hkl contains intensity data for SHELXE

You can look at these files with a text editor. Where is the resolution cut-off for SHELXD?

Finding the marker atom substructure with SHELXD

Just run from the command line:

```
shelxd visc-a1_fa
```

Output files from SHELXD are:

visc-a1_fa.res marker atom solution with the best CFOM; this file is updated while SHELXC runs
visc-a1_fa.lst log file

Did it work?

The **best CFOM value** gives an initial indicator on solutions. It should be at least over 35 a good S-SAD solution. For a good solution, CC_{weak}, CC_{all} and CFOM should be high!

Have a look to the file visc-a1_fa.res! What are the different columns?

SHELXD always tries to find more marker atoms than requested in the FIND instruction. This is why you can get more sites in the output. A sharp drop in occupancy between sites can indicate a good solution. However, it can be difficult to discern correct solutions at this stage. You should process the best solution (in terms of CFOM) to SHELXE.

How could you get even more high-occupancy marker atom positions than you expected in S-SAD?

Density modification and auto-tracing with SHELXE

SHELXE carries out density modification and auto-tracing of the protein backbone. With these good data, all other parameters are sufficient at default values. Even the solvent content defaults to 45%, but here we expect 50%.

(The volume of the rectangular unit cell is approximately 66 Å x 66 Å x 47 Å = 204732 $Å^3$. There are 8 asymmetric units per unit cell, which can be looked up in the International Tables A. An average amino acid in a crystal occupies 140 $Å^3$ of space. We expect two chains à 46 amino acids in the asymmetric unit, 92 in total. They would hence occupy 92 x 140 $Å^3$ x 8 = 103040 $Å^3$. 103040 $Å^3$ divided by 204732 $Å^3$ is approximately 0.50, which can then be the protein content. 1 - 0.5 is 0.5 which is the solvent content.)

Please use these two commands in separate windows:

```
shelxe visc-a1 visc-a1_fa –a5 –h –s0.5
shelxe visc-a1 visc-a1_fa –a5 –h –s0.5 -i
```

This will read in

visc-a1.hkl native data

visc-a1_fa.hkl anomalous differences, α
visc-a1_fa.res marker atom substructure

At this stage, we cannot know if the substructure or its mirror image is the correct solution. The **`-i`** option ensures that both enantiomorphs are tested.

NOTE: SHELXE input follows the general form

```
shelxe XX YY <options>
```

XX is the native data set
YY is the experimental data file prepared by SHELXC or XPREP.

The output files are:
visc-a1.lst Log file
visc-a1.pha Anomalous density map
visc-a1.pdb Backbone trace with 92 traced residues (out of 92!)
visc-a1.hat Improved substructure coordinates (hat stands for heavy atom solution)
visc-a1.phs hkl file with phases to display electron density

Running SHELXE will take some time. Please take care to study the output! What information is in there?

Selected SHELXE options

-h	Use this option if the marker atoms are part of the native structure and data. If their number is known, append it to h, e.g. –h12.
-a[N]	Do N cycles of auto-tracing of the protein's backbone.
-q	Explicitly search for helices.
- i	Invert the enantiomorph of the substructure!
-s	Solvent content, set to 45% by default.
-e	free lunch, e.g. –e1.2 (particularly useful when data are missing, or when auto-tracing is not feasible – low resolution and nucleic acids)
-z	optimize marker atom substructure before starting density modification

Did it work?

If the auto-tracing actually worked can be judged by the '**CC for partial structure against native data**' in SHELXE. In several rounds of auto-tracing, it should vary at a low value, increase suddenly and than vary at a higher value, which is higher than 25% for a successful trace at resolutions up to 2.2Å. Here, it should rise over 25% in the fourth cycle of auto-tracing.

How do we discern the correct hand after running SHELXE? Look at the values for the CC for the partial structure against native data. If you did not autotrace the structure, the correct hand is the one with the higher contrast which is printed to the screen at each cycle of density modification. This contrast can also be given graphically by HKL2MAP, as well as the less reliable map correlation coefficient. If in doubt: The correct map should look less ragged after some cycles of density modification. Clear side chains protruding from a traced backbone are an unambiguous sign of right hand!

You should use **COOT** to look at your results: Open the PDB file in COOT and then open the pha file. Therefore you have to use the option 'Open MTZ, mmCIF, fcf or phs…'. You will be asked if the cell from the PDB file should be used for the map, and you confirm this.To see if the anomalous density confirms the positions from the hat (and PDB) file, heighten the sigma value for the map. You can also look at the autotraced structure in the PDB file or at the normal electron density in the phs file. Which elements/which residues may account for the marker positions? This density is a good, mostly model-bias free starting point for building a model as complete as possible before your first REFMAC refinement. It can be enhanced a little by having a free lunch in SHELXE.

MAD

Molecule:	Concanavalin A
Number of animo acids	237
Best resolution	1.68 Å
Space group	I222
Cell	a=61.90Å b=87.06Å c=88.92Å α=90° β=90° γ=90°
Strategy	Three-wavelengths MAD data set

Data measured by Marianna Biadene and Ina Dix at DESY.
See also Hardman K.D., Ainsworth C.F. Journal: Biochemistry 11 (1972) 4910-4919

Data preparation with SHELXC

The input text file should be given as:

```
CELL 61.90 87.06 88.92 90 90 90
SPAG I222
FIND 1
NTRY 100
PEAK conca_peak.sca
INFL conca_infl.sca
HREM conca_hrem.sca
```

Cell parameters
Space group
Marker atom number
Number of tries (Good data, 100 sufficient)
Filenames of data sets; **`NAT filename.sca`** could be a native data set with better resolution; LREM is also possible.

If you are using XDS format, just give the files from different integrations different names – SHELXC does not rely on the filename extension to determine the format!

```
shelxc conca_mn < yourinputfile.inp | tee shelxc.log
```

(The command 'tee' prints the output both to the terminal and to the file shelxc.log, so that the program output can be kept for further reference. '`| tee shelxc.log`' can be omitted if you run SHELX on a Windows machine.)
The last table in the log file gives correlation coefficients (%) between signed anomalous differences. As the data sets have to have consistent relative Bijvoet differences, this is an indicator of real anomalous signal or noise:

```
Correlation coefficients (%) between signed anomalous differences
Resl.    Inf - 8.0 - 6.0 - 5.0 - 4.0 - 3.5 - 3.0 - 2.8 - 2.6 - 2.4 - 2.2 - 1.96
HREM/PEAK  72.7  74.8  68.8  54.4  59.6  60.2  55.5  45.1  47.4  36.2  34.5
HREM/INFL  72.4  74.3  67.1  48.9  60.0  55.8  49.7  42.8  38.6  29.6  34.5
PEAK/INFL  94.5  91.3  90.6  84.8  84.6  79.0  70.7  63.5  53.9  50.8
```

This correlation is so high that it is not necessary to truncate the data. If the data are of lower quality, they should be truncated where best correlation coefficient drops below 25% To do so you have to change the **SHEL** line in the file name_fa.ins correspondingly, which was written by SHELXC.

SHELXD

```
shelxd conca_mn_fa
```

In the output, you can see that the best CFOM (which is CC_{All} + CC_{Weak}) quickly rises above 60%, above one can be rather confident that the MAD solution in SHELXD is correct, so continue to SHELXE.

> **While SHELXC runs, try to estimate the solvent content.** (Hint: In I222, there are 8 ASUs per unit cell.)

SHELXE

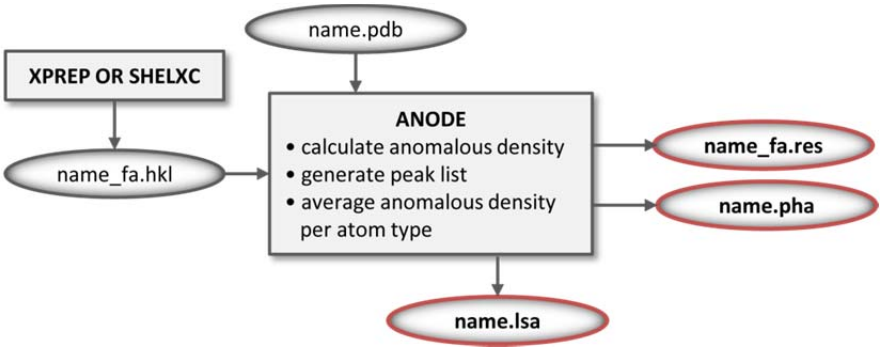
```
shelxe conca_mn conca_mn_fa –a
shelxe conca_mn conca_mn_fa -a -i
```

Here, no other options are needed. (The solvent content is 36% which is close enough to the default.) Again, evaluate your results in COOT. Can you optimize the last SHELXE steps with other options? (A list of options is given on top of the output when SHELXE is run!)

Extra tasks

a) The folder co-conca contains Cu in-house data for SAD phasing of Concanavalin A with Cobalt. Try to solve the structure!

b) Validation with anomalous density in ANODE: To validate the anomalous scatterer positions in your structure, and to find anomalously scattering ions in the hydration shell of your protein, you can use the tool ANODE. It will calculate the anomalous density for a PDB file.



Try it! In the folder visc-a1 is a final model of visc-a1 from the PDB and the file visc-a1_fa.hkl you generated. Now rename 3C8P.pdb to visc-a1.pdb and give:

```
anode  visc-a1
```

You get an output on screen and can read in vsc-a1.pha to COOT, just as the pha file from SHELXE, but now it's based on the phases from the final refined structure. Look at the sulfates.

c) Try having a free lunch with Concanavalin A, expanding the data to 1.2 Å. Compare the maps in COOT. Which one would you take to start building a model, and why?

d) Try solving both structures with HKL2MAP and make use of the graphical output of HKL2MAP!

e) What happens if you give a better resolution by the SHEL command in SHELXD and also heighten the number of NTRY, for example NTRY 1000? Do the positions of the anomalous scatterers improve? Do solutions occur more frequently? (Hint: This is most comfortably done with the shelxd_mp, the multi-processor version of SHELXD, as 1000 tries require some time.) Does this lead to better phases?

f) Try the difficult SAD Tutorial!

A difficult SAD case with SHELXC/D/E

Andrea Thorn

Overview

The prerequisite for this tutorial is the basic SHELXC/D/E tutorial.
In this tutorial, we will solve a relatively difficult SAD case, fibronectin.

Molecule:	Fibronectin
Number of animo acids	90
Resolution	1.51 Å
Space group	$P4_12_12$ or $P4_32_12$
Cell	a=b=37.86Å c=108.42Å α=90° β=90° γ=90°

Rudino-Pinera, Ravelli, Sheldrick, Nanao, Korostelev, Werner, Schwarz-Linek, Potts & Garman, JMB 368 (2007), 833-844.

Data preparation with SHELXC

We start with two files: fibro-nat.sca contains high-resolution data; fibro-sad.sca contains long-wavelength high-multiplicity data but to lower resolution. The input file for SHELXC should use all available data, e.g. both files. The native high-resolution data will be useful in SHELXE density modification. The protein contains 4 disulfide bridges, 2 methionine residues and the buffer contained sulfate. We do not know how many sulfates may have bound to the protein. In addition, we do not know whether the correct space group is $P4_12_12$ or $P4_32_12$. For the marker atom search, it will not play a role if the symmetry is inverted, as it does not matter for the substructure itself. SHELXE, if run with the –i option, can be used to discern between the hand, and if necessary, will put the symmetry operators and the space group to the correct enentomorph. So, you do not have to worry and can just use $P4_32_12$.

```
CELL 37.86 37.86 108.42 90 90 90
SPAG P43212
FIND 10
NTRY 100
SAD fibro-sad.sca
NAT fibro-nat.sca
SFAC S
```

Cell parameters
Space group
Number of marker atoms: 8 CYS + 2 MET
Number of tries (100 is here sufficient)
Filename of data set

Marker atom type; only for naming

We then start SHELXC:

```
shelxc fibro < myinputfile
```

The screen output gives:

```
Resl.   Inf - 8.0 - 6.0 - 5.0 - 4.0 - 3.5 - 3.0 - 2.8 - 2.6 - 2.4 - 2.2 - 2.00
 N(data)     117   141   175   366   353   625   386   511   685   954  1333
 Chi-sq     1.05  0.66  0.76  1.10  1.92  3.55  2.49  2.27  1.93  2.06  3.65
 <I/sig>   142.7 158.5 163.9 154.9 142.8 115.7  95.4  79.6  59.6  40.2  18.9
 %Complete  87.3  96.6  98.3  94.8  97.0  95.6  97.0  97.3  97.2  96.4  94.5
 <d"/sig>   7.45  6.85  4.49  3.31  2.63  2.03  2.05  1.80  1.57  1.26  1.07
 CC(1/2)    96.9  97.9  94.6  90.6  78.1  61.7  53.0  53.9  36.9  20.3  12.6
```

If **SHEL <lower limit> <higher limit>** has not been specified in the SHELXC input, the program automatically includes a resolution cut-off that is 0.5Å from the resolution limit of the data (**SHEL 999 2.5**). A better guide is where CC(1/2) drops below 25%, hence a cut-off between 2.4 and 2.2 would be also feasible. As disulfide bridges are 2.03 Å long and the resolution would be less than this, the number of peaks to search for (FIND) has to be changed in any case. We are searching for 4 disulfide bridges and 2 methionines instead of 8 cysteine and 2 methionine residues – hence you should replace “FIND 10” with “FIND 6” in either the SHELXC input (and run SHELXC again) or in SHELXD.

Output files from SHELXC are:

fibro_fa.hkl contains $||F_{hkl}|-|F_{-h-k-l}||$ (Bijvoet differences) and estimates for the phase shifts α
fibro_fa.ins contains instructions for SHELXD
fibro.hkl contains intensity data for SHELXE

Finding the marker atom substructure with SHELXD

Run
`shelxd fibro_fa`
or
`shelxd_mp fibro_fa`

Output files from SHELXD are:

fibro_fa.res marker atom solution with the best CFOM; this file is updated while SHELXC runs
fibro_fa.lst log file

Repeat this with the other space group. The best CFOM-values are between 35% and 42%, which might be a solution, but not a good one – however, we can try to get a better CFOM by using DSUL in the .ins file (the instructions file for SHELXD), which will explicitly search for elongated peaks. Just include DSUL 4 to search for 4 disulfide bridges and make sure MIND is set to MIND -3.5. We will also heighten the number of tries by setting "NTRY 1500".
(If does not give you a significant higher CFOM value, you can also change the resolution limit by using, for example SHEL 999 2.4 in the .ins file.) This is an exemplary instructions file:

```
TITL fibro_p43212_fa.ins SAD in P43212          Title. Irrelevant to SHELX.
CELL  0.98000 37.86 37.86 108.42 90.00 90.00 90.00   Cell parameters
LATT  -1                                        Lattice type
SYMM 1/2-Y, 1/2+X, 3/4+Z                        Symmetry operators
SYMM -X, -Y, 1/2+Z
SYMM 1/2+Y, 1/2-X, 1/4+Z
SYMM 1/2-X, 1/2+Y, 3/4-Z
SYMM Y, X, -Z
SYMM 1/2+X, 1/2-Y, 1/4-Z
SYMM -Y, -X, 1/2-Z
SFAC S                                          Marker atom type; only for naming
UNIT   192                                      Number of atoms in the unit cell. Irrelevant to SHELXC/D/E.
SHEL 999 2.5                                    Resolution cut-offs for SHELXD.
PATS                                            Use Patterson seeding.
DSUL 4                                          Find 4 disulfide bridges.
FIND 6                                          Find 6 anomalous density peaks (4 disulfide bridges, 2 MET residues).
MIND -3.5                                       The minimum distance between peaks is 3.5Å.
NTRY 1500                                       Do 1500 tries.
SEED 1                                          Use 1 as a seed (this ensures the same starting point if repeated).
HKLF 3                                          Input format is F_A values, hence HKLF 3. (HKLF 4 would be intensities.)
END                                             End of file.
```

You should now be able to get best CFOM values over 60, almost certainly a good solution.

Density modification and auto-tracing with SHELXE

First, we need to calculate the solvent content. The volume of the rectangular unit cell is approximately 38 Å x 38 Å x 108 Å = 155952 $Å^3$. There are 8 asymmetric units per unit cell, which can be looked up in the International Tables A. An average amino acid in a crystal occupies 140 $Å^3$ of space. We expect 90 amino acid residues in the asymmetric unit. They would hence occupy 90 x 140 $Å^3$ x 8 = 100800 $Å^3$. 100800 $Å^3$ divided by 155952 $Å^3$ is approximately 0.65, which can then be the protein content. The solvent content is hence 0.35, rather low and hence unfavorable for density modification.

Please use these two commands in two windows, but in the same folder:

```
shelxe fibro fibro_fa -a –h –s0.35
shelxe fibro fibro_fa -a –h –s0.35 -i
```

This will read in

fibro.hkl native data
fibro_fa.hkl anomalous differences, α
fibro_fa.res marker atom substructure

A CC of partial structure against native data over 25% indicates a correct solution (and consequently, the correct space group choice).

The output files are:

fibro.lst Log file
fibro.pha Anomalous density map
fibro.pdb Backbone trace
fibro.hat Improved substructure coordinates (hat stands for heavy atom solution)
fibro.phs hkl file with phases to display electron density

The files from the run with the inverted substructure will have "_i" attached, for example "fibro_i.pha".

If you find that the structure cannot be traced at your first try, you should try one of the following options (or a combination thereof):

- Use **–z** to optimize the marker atom substructure before density modification and auto-tracing
- Use **–q** to search for helices.
- Use more auto-tracing macrocycles (e.g. **–a20**)

If the structure is not immediately solved, this might be due to not all marker atom positions being correct, a high variance in B factors, the wrong hand is used or the structure is not containing any helices. (Pure beta sheet structures are more difficult to trace.)

After you have solved the structure, have a look at the output coordinates and maps in COOT!