SAD experiment on a lysozyme Gd derivative

Data collection on beamline FIP-BM30A

The fluorescence of Gd was measured with a Roentec MCA at the Gd LIII edge. Raw data are in

```
Edge/lyso_1_Gd1 (columns 5 and 7)
```

and the plot vs Energy in

```
Edge/lyso_1_Gd1.jpg
```

The spectrum was processed with Chooch. Final drawing of calculated f' and f'' is in

```
Edge/final.jpg
```

Based on that, beam energy was tuned to 7242.6 eV, and a single-wavelength dataset was collected (300 frames, 1 deg each). Frames (compressed with bzip2) are named

```
img/e000_prefix_1_00xxx.img.bz2
```

Data reduction with XDS package

The different steps are presented in a series of directories, for sake of clarity. In practice, they can be performed in a single directory by successive modification of the input files and running the XDS package programs at the command line.

xds_step0

You will find there the initial parameter file for xds, as automatically created by the beamline control software

```
xds_step0/XDS.INP
```

as well as a fully commented one

```
xds_step0/XDS.INP_sav
```

xds_step1

As we expect anomalous signal, the Friedel mates will differ. So uncomment the line

```
FRIEDEL'S_LAW=FALSE
```

in XDS.INP. Then, run

```
xds
```

at the command line (or `xds_par` for the paralleled version).

xds_step2

At the previous step, xds automaticaly figures out the Bravais symmetry, and picked up space group P422 as a representative. To check for extinctions (helices), just select space group 96 (P4(3)2(1)2) and enter refined cell parameters in XDS.INP

```
SPACE_GROUP_NUMBER=96
UNIT_CELL_CONSTANTS= 77.268   77.268   38.704  90.000  90.000  90.000
```

and select only the final scaling step of the processing (CORRECT)

```
JOB= CORRECT
```

Then run

```
xds
```

In CORRECT.LP, check for low intensity of reflections that should be absent (marked with "*") in the list above lines

```
AVERAGE INTENSITY FOR     207 REFLECTIONS WHICH SHOULD
BE SYSTEMATICALLY ABSENT IS     0.2% OF MEAN INTENSITY
```

xds_step3

Optional: use XSCALE for final scaling, merging of several dataset. Create the XSCALE.INP input file with the following lines

```
OUTPUT_FILE=XSCALE.HKL
INPUT_FILE= XDS_ASCII.HKL
```

and run

```
xscale
```

at the command line.

Use XDSCONV to generate reflection files in CCP4 FP/DANO format:

```
INPUT_FILE=XSCALE.HKL  XDS_ASCII
OUTPUT_FILE=temp_ccp4.hkl  CCP4
FRIEDEL'S_LAW=FALSE
```

and run

```
xdsconv
```

again at the command line.

XDSCONV generates the input file F2MTZ.INP needed by f2mtz (CCP4 package) for the final conversion to binary mtz format. To run the CCP4 programs f2mtz just type the command:

```
f2mtz HKLOUT temp_ccp4.mtz < F2MTZ.INP
```

Use XDSCONV again to generate reflection files in CCP4 F+/F- format:

```
INPUT_FILE=XSCALE.HKL  XDS_ASCII
OUTPUT_FILE=temp_ccp4_f.hkl CCP4_F
FRIEDEL'S_LAW= FALSE
GENERATE_FRACTION_OF_TEST_REFLECTIONS=0.05
```

and run again

```
xdsconv
```

at the command line

Then run CCP4 program f2mtz

```
f2mtz HKLOUT temp_ccp4_f.mtz < F2MTZ.INP
```

Then, to run cad (to convert indices to the CCP4-asymmetric unit),

```
cad HKLIN1 temp_ccp4.mtz HKLIN2 temp_ccp4_f.mtz HKLOUT Lyso-
Gd_SAD.mtz <<EOF
  LABIN FILE 1 E1=FP   E2=SIGFP   E3=DANO E4=SIGDANO E5=ISYM
  LABIN FILE 2 E1=F(+) E2=SIGF(+) E3=F(-) E4=SIGF(-) E5=FreeRflag
  END
EOF
```

Quick SAD phasing

Launch

```
ccp4i
```

and define a new project with

`lyso-Gd_SAD/ccp4_SAD`
as working directory

Run Phaser SAD Pipeline (button highlighted in blue in Figure 1).
Then enter the following parameters:
- reflexion file: `xds_step3/Lyso-Gd_SAD.mtz` (field highlighted in blue in Figure 2)
- sequence in fasta format directory ccp4_MR (field highlighted in green in Figure 2)
- heavy atom type: GD (field highlighted in red in Figure 2)
- wavelength: 1.7119 (field highlighted in orange in Figure 2)

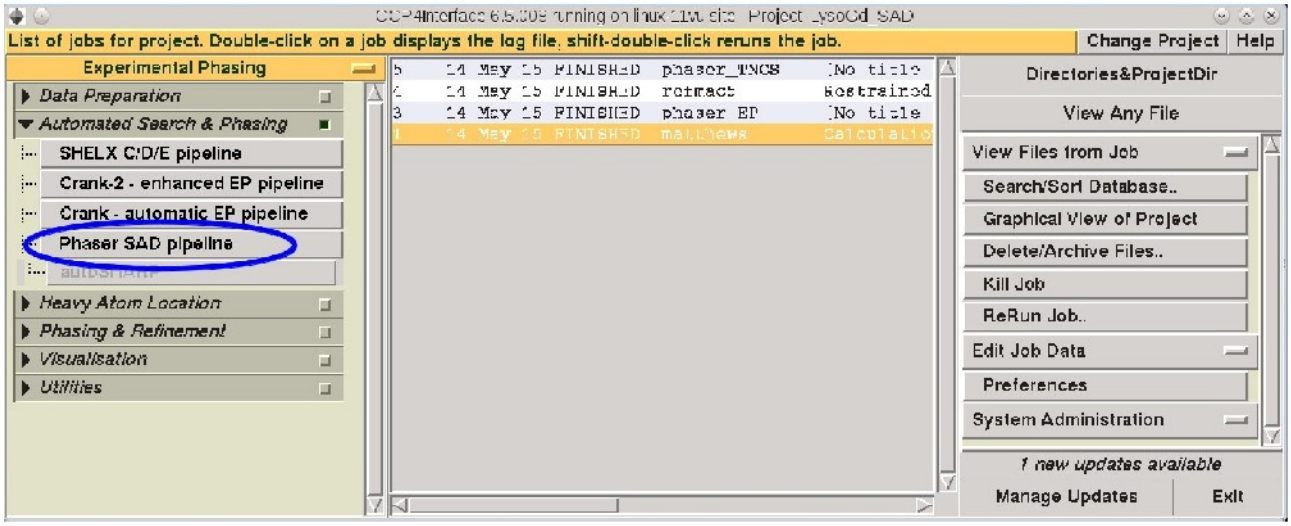

Figure 1

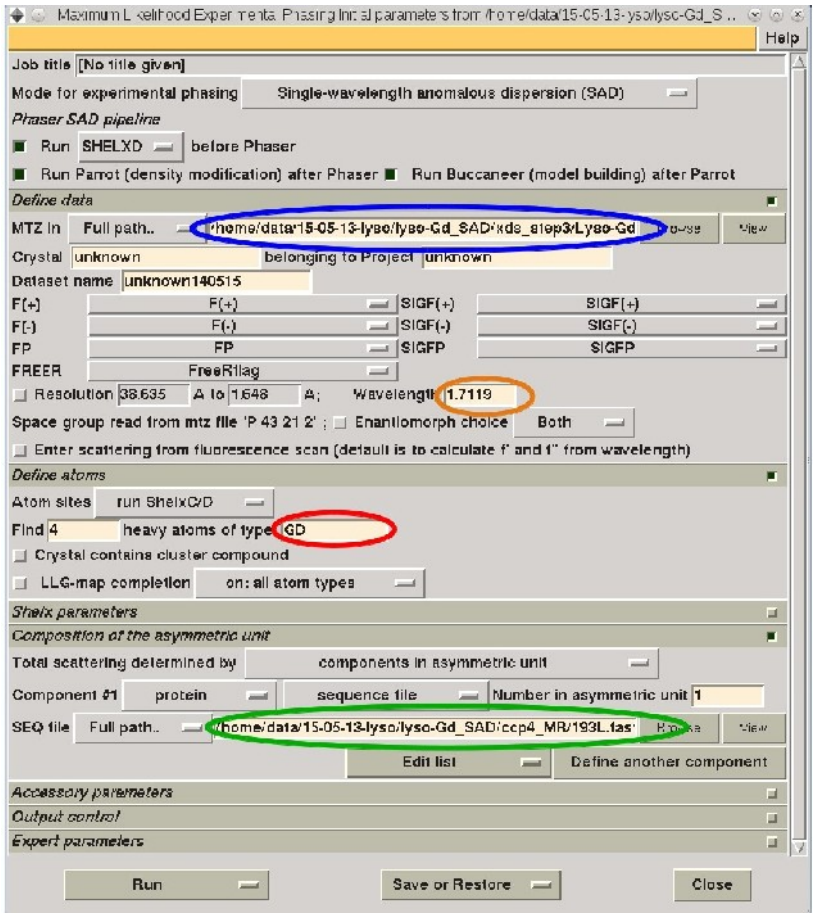

Figure 2

=> ~80% of residues built automatically
Upon completion of the job, and to analyze the log file, select the “Phaser_EP” job in the list (button highlighted in blue in Figure 3). Then, from the “View Files from Job”, select “View Job Results (new style)” (button highlighted in red in Figure 3)

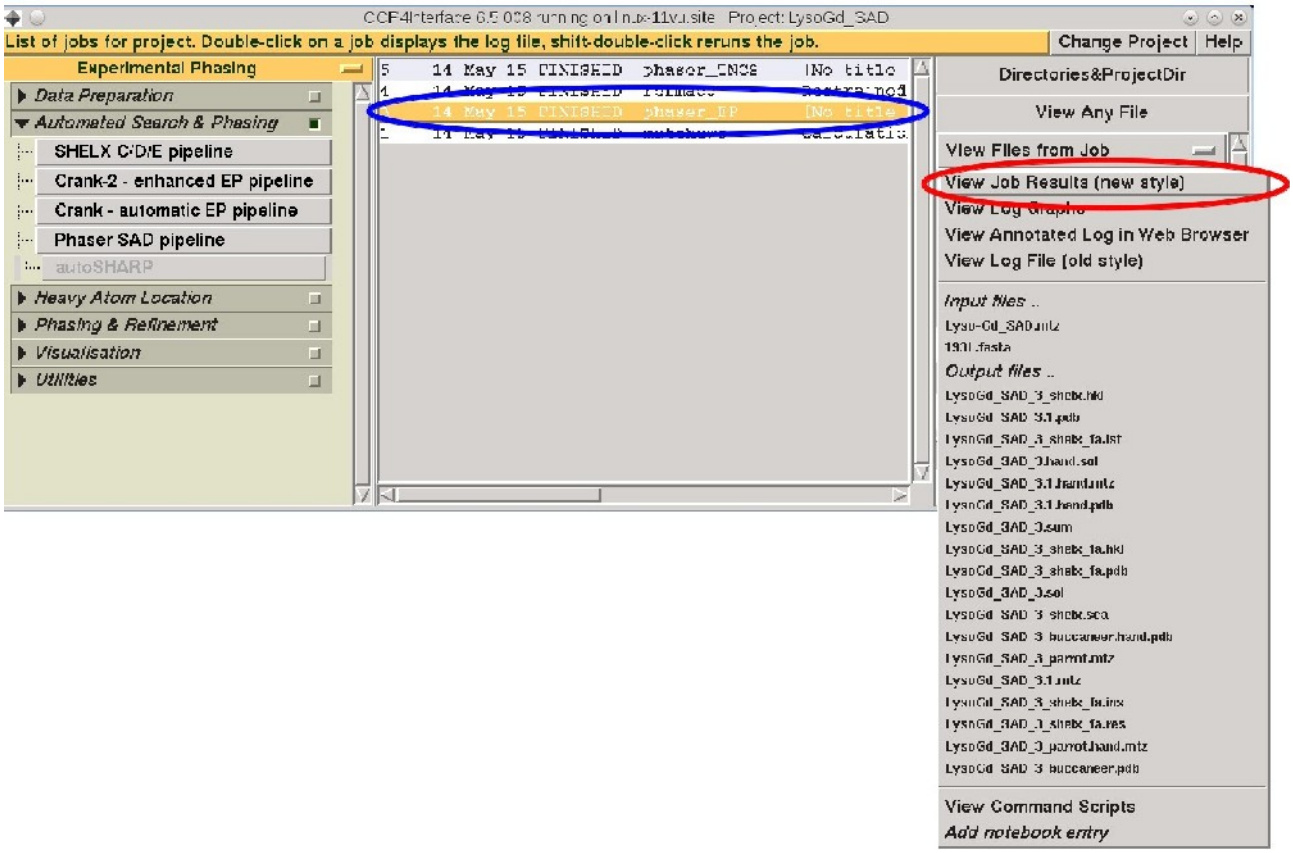

Figure 3

Experimental map, sub-structure of anomalous atoms and model can be displayed with Coot:
Run

```
coot
```

at the command line, and load pdb files (button highlighted in blue in Figure 4) and mtz files (button highlighted in red in Figure 4) as listed below:

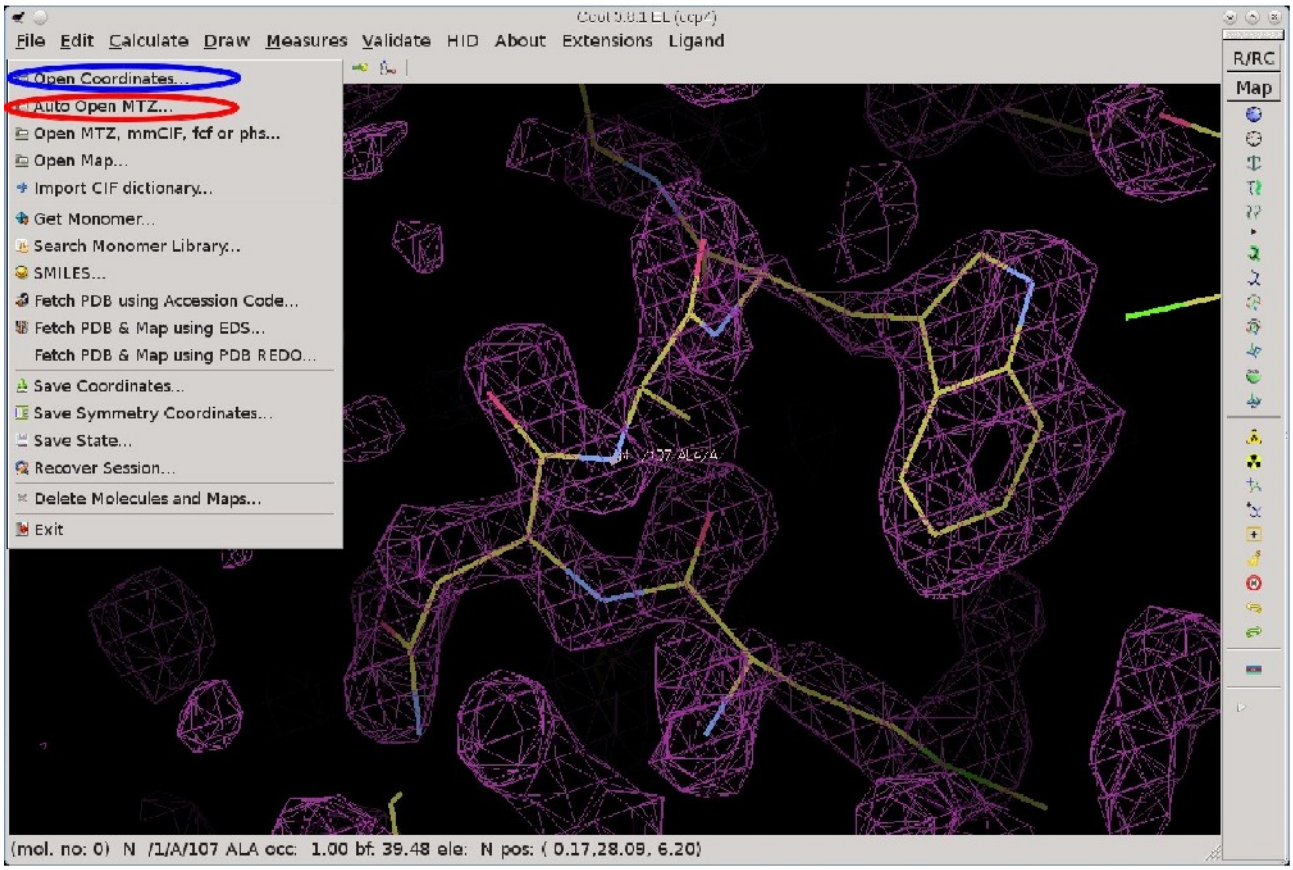

Figure 4

Sub structure of Gd atoms is in

`lyso-Gd_SAD/LysoGd_SAD_3.1.pdb`

Experimental map is in

`lyso-Gd_SAD/LysoGd_SAD_3.1.mtz`

and after automated density modification with `parrot`

`lyso-Gd_SAD/LysoGd_SAD_3_parrot.mtz`

The model built automatically (80% of the residues) is available in

`lyso-Gd_SAD/LysoGd_SAD_3_buccaneer.pdb`

This model and the experimental map are good enough to start manual building.

Alternatively, run Refmac for a first refinement and manual rebuilt with

`LysoGd_SAD_3_buccaneer.pdb`

as pdb input file.