# 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 automatically 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 FOR207 REFLECTIONS WHICH SHOULDBE SYSTEMATICALLY ABSENT IS0.2% 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</pre>

```
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)





Job title [No title given]		
Mode for experimental phasing	Single-wavelength anomalous dispersi	on (SAD) 🔤
Phaser SAD pipeline		
🔳 Run SHELXD 🚐 before P	haser	
Run Parrot (density modificat	tion) after Phaser 🔳 Run Buccaneer (mo	del building) after Parrot
Define data		
MTZ in Full path Thoma	o/data15-05-13-lyso/lyso-Gd_SAD/xds_step	3/Lyso-Gd > o-se View
Crystal unknown	belonging to Project unknown	
Dataset name unknown140515		
F(+) F(+)	SIGF(+)	SIGF(+)
F(-) F(-)	SIGF(-)	SIGF(•)
FP FP	SIGFP	SIGFP
REER FreeKillag	g	
A TO THE	48 A; Wavelengtr 1.7119	
Space group read from mtz file 'F	243 21 2' ; 🔲 Enantiomorph choice 🛛 Bo	th 💻
Space group read from mtz file 'F Enter scattering from fluoresc	2 43 21 2' ; □ Enantiomorph choiceBo ence scan (default is to calculate f and f"	th from wavelength)
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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:





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.