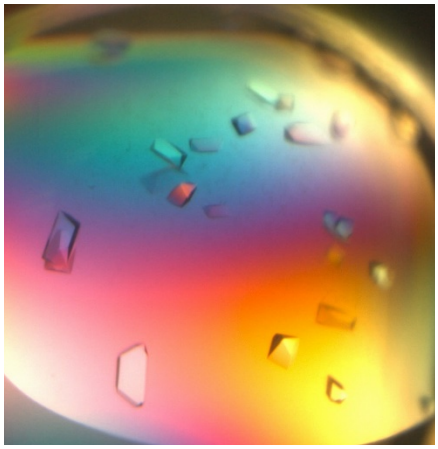
Structure solution of USP5 zinc-finger ubiquitin binding domain - 2018-03-29

Recap of previous work https://zenodo.org/record/1164793#.Wr0kD2aZPOQ

Crystallisation:



USP5 Zf-UBD aa. 171-290 from pET28-MHL: 8.3 mg/mL in 50 mM Tris pH8, 150 mM NaCl, 1 mM TCEP

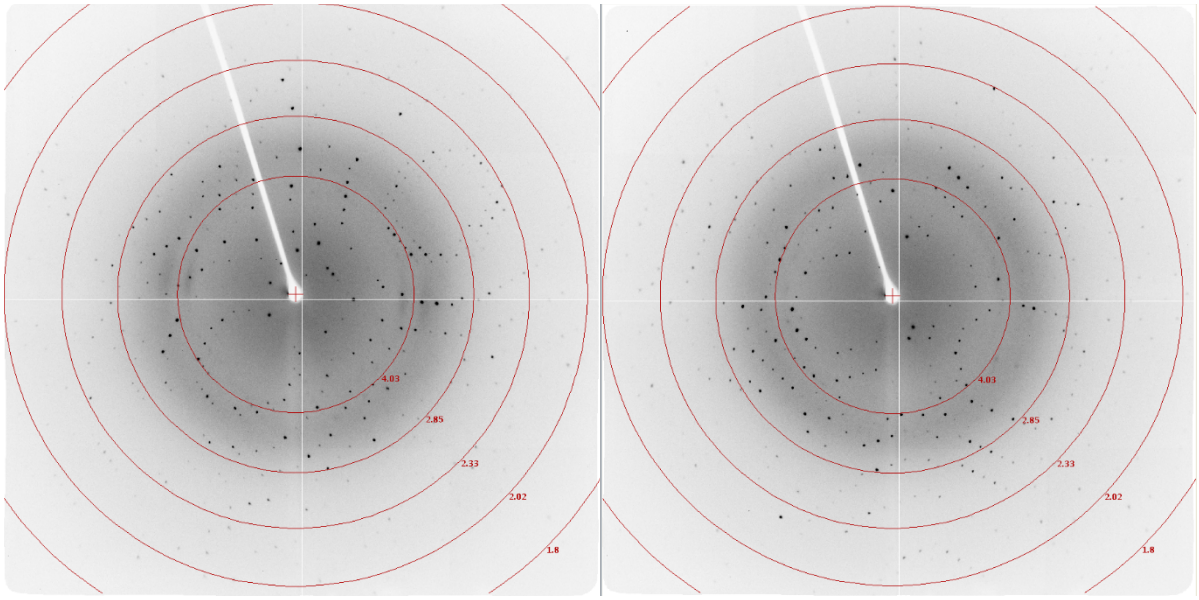
SGC-I screen, condition G3: 1.8 M ammonium sulphate, 0.1 M sodium cacodylate pH 5.5, 0.2 M sodium acetate https://www.thesgc.org/science/high-throughput-crystallization

Hampton additive screen D12: 0.01 M GSH (L-glutathione reduced) and GSGG (L-glutathione oxidized) https://hamptonresearch.com/product_detail.aspx?sid=36&pid=27

Samples were cryo-protected with 25% EG and mounted onto Hampton nylon loops before being flash cooled in liquid nitrogen.

Screening:

Mounted crystals were screened in house using RIGAKU FR-E SUPERBRIGHT at 1.54178 Å wavelength. Images were collected using RIGAKU SATURN A200 CCD detector. Experiments were conducted at 100 K. 2 images were collected with 20 seconds exposure and 0.5 degrees oscillation at 0 and 90 degrees phi. No ice rings are visible and diffraction goes beyond ~2 Å resolution (resolution limit at CCD set up of 100 mm crystal-detector distance).



Large scale data collection:

The crystal screened previously was set up for large scale data collection under the following conditions:

Crystal-detector distance: 60 mm
Number of images: 180
Oscillation: 1 degree
Exposure time: 10 seconds

The images are compressed in file USP5_GTT.tar.gz

Data processing and structure solution:

NB: critical input and output files are provided alongside this post and are highlighted below in red

Images were initially processed using Xia2 using the following command line:

Xia2 pipeline=3d
image=home/disk2/tempcollect/Mandeep/20180129a/AV5_USP5a_2_1_01/images/AV5_USP5a_screen_0001.img:1:180

See attached document xia2.txt for details as well log files AUTOMATIC_DEFAULT_aimless.log and AUTOMATIC_DEFAULT_NATIVE_SWEEP1_INDEX.log.

An indexing solution was found to be:
Spacegroup: $P2_12_12_1$
abc = 48.55, 49.90, 56.22
αβγ = 90.00, 90.00, 90.00

This differs from the USP5 apo structure PDB: 2G43 which is C_12_1 and abc = 61.38, 86.18, 59.90 αβγ = 90.00, 99.29, 90.00 suggesting a completely different crystal packing.

Data were autoprocessed by the Xia2 pipeline to ~1.45 Å resolution. This is outside the limits of the CCD at 60 mm detector distance.

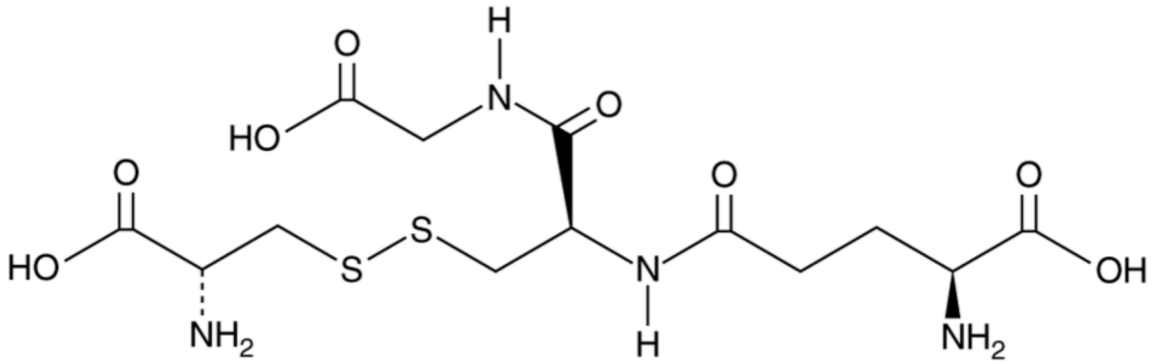
Therefore, the data were reprocessed using Xia2 output file AUTOMATIC_DEFAULT_scaled_unmerged.sca in Aimless. Resolution cut offs of 30-1.6 Å resolution were assigned and 5 % of reflections were added to the FreeR column. The output file was USP5A_1-180_3d_1-6.mtz.

Next, Phaser was used to solve the structure with model 2g43_simple.pdb which has the coordinates for 1 chain of USP5 with no heterogens. Results can be seen in 9_phaser_MR.log and in the pdb/mtz files USP5_9.1.mtz and USP5_9.1.pdb.

Model and maps for this solution looked sensible and the log file revealed that the solution was highly likely with high LLG scores.

This model was used to refine the structure, with iterative builds in Coot and refinement in Refmac. Clear difference density was visible from Cys195 and omit map calculation revealed density which correlated with the shape of a glutathione molecule conjugated to this cysteine residue.

A ligand composed of $_{L}$-cysteine-glutathione disulphide (see below) was built using JLigand and exported as file GSC.cif



Following deletion of the Cys195, the ligand was modelled into the resulting density and real-space refined in Coot.

The resulting pdb and mtz files are included in this upload: USP5_refine18.pdb and USP5_refine18.mtz

The final structure solution has R/Rfree 18.6/21.9

NB: This structure is not processed or refined to the point of being ready for PDB deposition and has not undergone stringent validation checks so please interpret with caution.