Structure solution of apo USP5 zinc-finger ubiquitin binding domain - 2018-03-30

Recap of previous work:

1. https://zenodo.org/record/1164793

Crystallisation of sample in 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 with 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

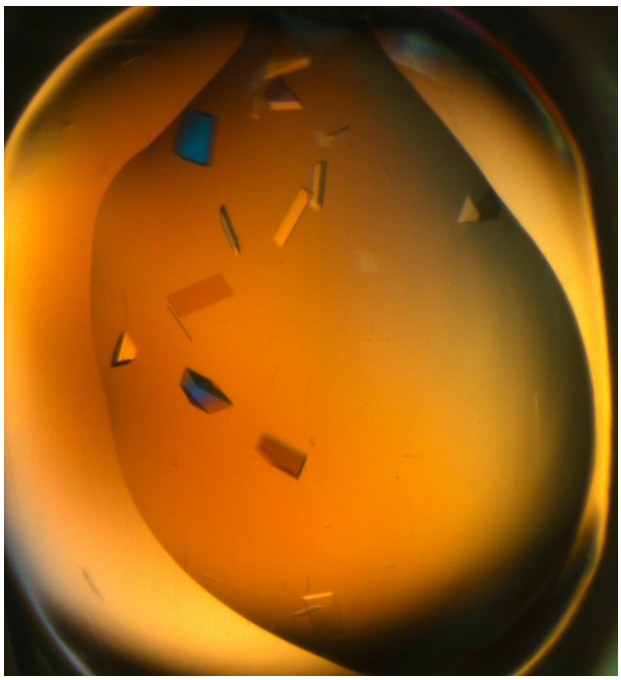
2. https://zenodo.org/record/1209986

USP5 zinc-finger ubiquitin binding domain with L-cysteine(residue195)-glutathione disulphide structure solution

Objective:

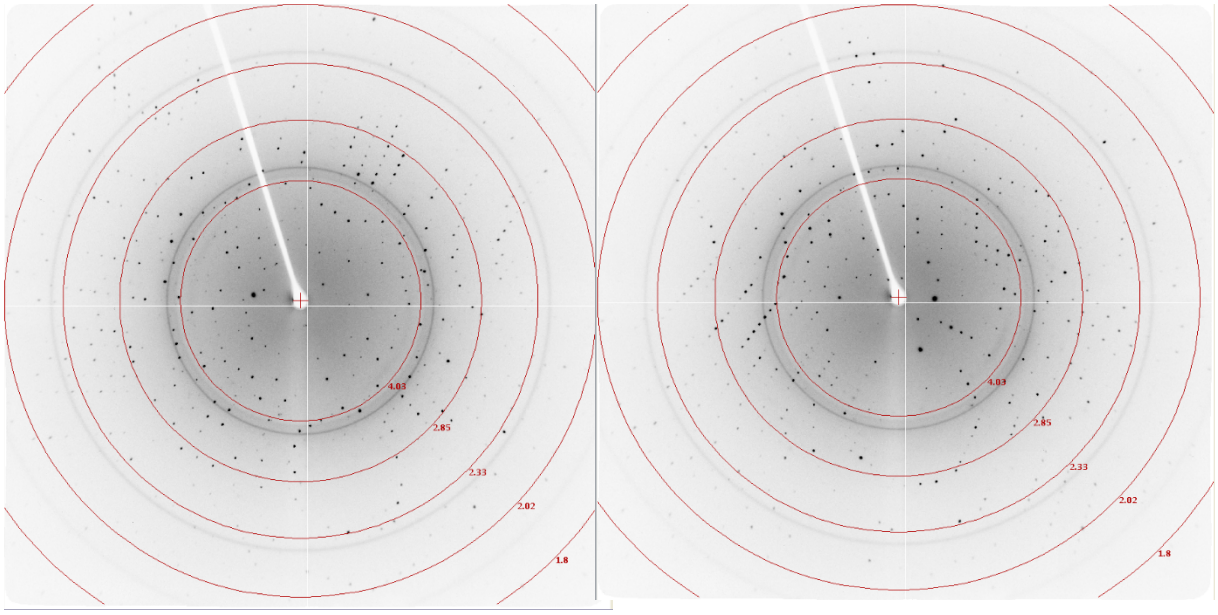
To find crystallisation conditions which allow growth of well-diffracting apo crystals of the USP5 zinc-finger ubiquitin binding domain (Zf-UBD) permissible to soaking with small molecule ligands. The crystals grown to date reveal the formation of a disulphide bond at Cys195 with glutathione additive which occupies the ubiquitin binding pocket and therefore is not suitable for ligand soaking.

Screening of crystals:

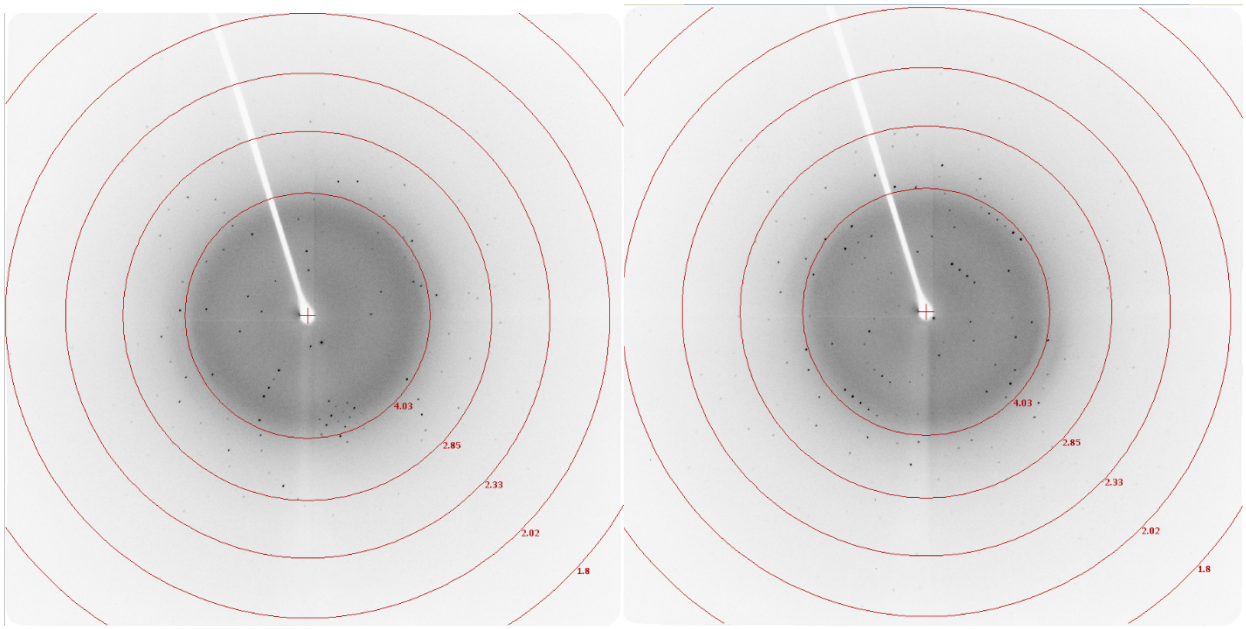


Crystals were grown in an optimisation screen condition, based on the SGC-I G03 condition described above. The crystals pictured were grown with well solution consisting of 1.4 M ammonium sulphate, 0.2 M Na-acetate, 0.1 M Na-cacodylate pH 6.8.

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.



Crystal mounted with no cryo shows strong diffraction beyond ~2 Å resolution (resolution limit at CCD set up of 100 mm crystal-detector distance). However, ice rings are clearly visible.



Crystal mounted with paratone cryo show no ice rings but the diffraction is much weaker with reflections to ~2.3 Å resolution.

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

Large scale data collection:

The strongest diffracting crystal screened (no cryo) was set up for large scale data collection under the following conditions:

Crystal-detector distance: 60 mm
Number of images: 360
Oscillation: 0.5 degree
Exposure time: 10 seconds

The images are compressed in file USP5_apo.tar.gz

Data processing and structure solution:

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

Xia2 pipeline=3dii
image=home/disk2/tempcollect/Mandeep/20180220a/AB6_RH_USP5_2_1_02/images/AB6_RH_USP5_screen_0001.img:1:360

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: C121
abc = 61.23, 85.52, 59.52
αβγ = 90.00, 100.00, 90.00

This is highly similar to the USP5 apo structure PDB: 2G43 which is C121 and abc = 61.38, 86.18, 59.90 and αβγ = 90.00, 99.29, 90.00 suggesting a similar 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 40-1.55 Å resolution were assigned and 5 % of reflections were added to the FreeR column. The output file was USP5_IR_aimless_1-55.mtz and the log file is 53_aimless.log

Using 2g43.pdb (unmodified PDB file of previously deposited structure) with the aimless output file were refined in Refmac. Output model and maps looked sensible and the 56_refmac5.log file revealed that the refinement was running successfully with R/Rfree dropping from 40.65/41.11 to 24.86/26.92.

This model was used to refine the structure, with iterative builds in Coot and refinement in Refmac.

The resulting pdb and mtz files are included in this upload: USP5_IR_refine3.pdb and USP5_IR_refine3.mtz

The final structure solution has R/Rfree 19.51/22.84

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.

Concluding remarks:

This crystal form of USP5 has an apo zinc-finger ubiquitin binding domain which is connected to a nearby solvent channel suggesting that the crystal form may be amenable to soaking with small ligands. Next these crystals should be optimised for cryo-protection to prevent ice rings and to assess DMSO tolerance.