Mandeep Mann
2018/02/02

Crystallization of USP5 Zf-UBD

Objective: To find crystallization 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.

Experiment & Results:

Using SGC standard crystallization protocols, SGC and RW screens were used to prepare a crystal screen in 96-well intelli plates (Art Robbins Instruments). 70 µL of each condition was dispensed into the well of the plate and the 0.5 µL well solution was dispensed to the crystal plate by a liquid handling robot, Phoenix followed by 0.5 µL of 8.3 mg/mL USP5 Zf-UBD in 50 mM Tris pH 8, 150 mM NaCl, 1 mM TCEP. Crystal plates were sealed and stored at 18˚C.

Following the initial crystal screen, the best condition was 1.8 M ammonium sulfate, 0.1 M sodium cacodylate pH 5.5, 0.2 M sodium acetate (SGC condition-G03); however crystals were too small to mount for a diffraction screen. A 24-well sitting drop crystallization plate was then used to manually set up a crystal plate with 1:1, 2:1, 2.5:1 ratios of protein: mother liquor for a total volume of 4 µL. Various morphologies of crystal structures was observed: clustered and plates (Figure 1).

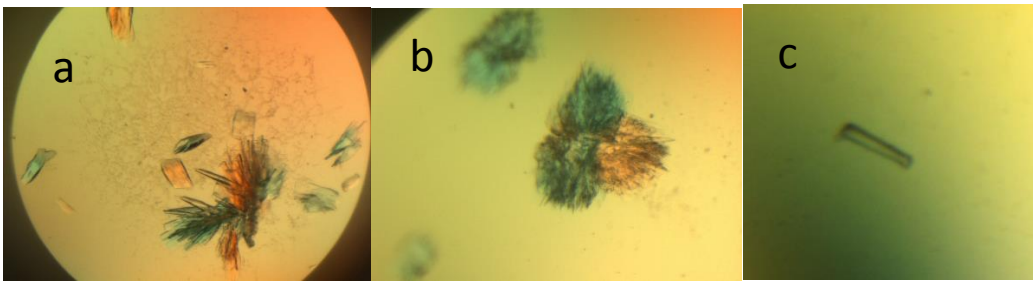

Figure 1. Crystals of USP5 Zf-UBD in 1.8 M ammonium sulfate, 0.1 M sodium cacodylate pH 5.5, 0.2 M sodium acetate (a) clustered plates, 3D crystals & microcrystals (b) clustered crystals (c) crystal plate

25% ethylene glycol mixed with well solution was used to cryo-protect the crystals. A 3D crystal grown in a 1:1 ratio of mother liquor: protein was mounted using a nylon loop and cryo-cooled in liquid nitrogen. The crystal was screened using our in house diffractometer collecting 2 images at 90 deg with a 0.5 deg oscillation, 20 s exposure and 100 mm crystal-detector distance at a wavelength of 1.54178 Å.

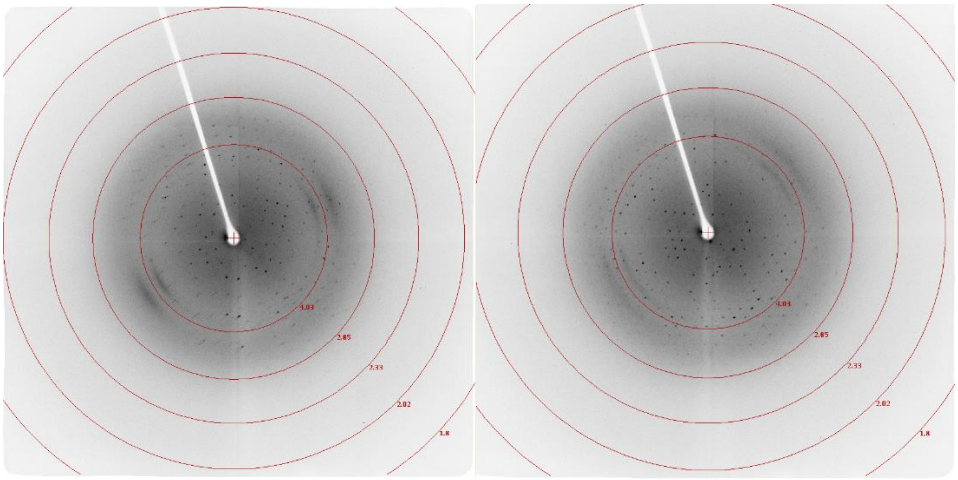

Figure 2. Diffraction images with USP5 Zf-UBD crystal in 1.8 M ammonium sulfate, 0.1 sodium cacodylate pH 5.5, 0.2 M sodium acetate

The diffraction images with the crystal showed good diffraction, where it looks like the crystal is packing in a hexagonal manner; however, as only two diffraction images were collected we cannot be sure of the packing symmetry until crystals have been reproduced and screened. This crystal was shipped to the CLS for large scale data collection.

To optimize the screening condition for USP5 Zf-UBD, a Hampton additive screen was done using the crystallization condition: 1.8 M ammonium sulfate, 0.1 M sodium cacodylate pH 5.5, 0.2 M sodium acetate. In a 96-well intelli plate (Art Robbins Instruments), 0.5 µL of mother liquor, 0.5 µL of 8.3 mg/mL protein in 50 mM Tris pH 8, 150 mM NaCl, 1 mM TCEP and 0.2 µL of additive condition was added to the well by the Phoenix robot. Crystal plates were sealed and stored at 18˚C.

4 days after plating, 3D crystals formed in the buffer condition with the additive 0.01 M L-glutathione reduced and 0.01 M L-glutathione oxidized, where the final concentration of the additives is 1.7 mM (Figure 3).

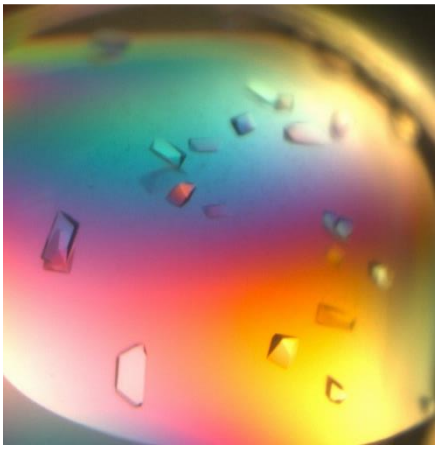

Figure 3. 3D crystals of USP5 Zf-UBD in 1.8 M ammonium sulfate, 0.1 M sodium cacodylate pH 5.5, 0.2 M sodium acetate, 1.7 mM L-glutathione reduced, L-glutathion-oxidized

25% ethylene glycol mixed with well solution was used to cryo-protect the crystals. The larger 3D crystals remained intact. Smaller planar crystals dissolved in the cryo-solution likely due to the high salt concentration in the buffer. The larger 3D crystals were mounted using a nylon loop and cryo-cooled in liquid nitrogen. The crystal was screened using our in house diffractometer collecting 2 images at 90 deg with a 0.5 deg oscillation, 20 s exposure and 100 mm crystal-detector distance at a wavelength of 1.54178 Å, and reflections were observed to 1.8 Å resolution. Data collection for these crystals will be done in-house at SGC due to the larger size of crystals as well as stronger diffraction (Figure 4).

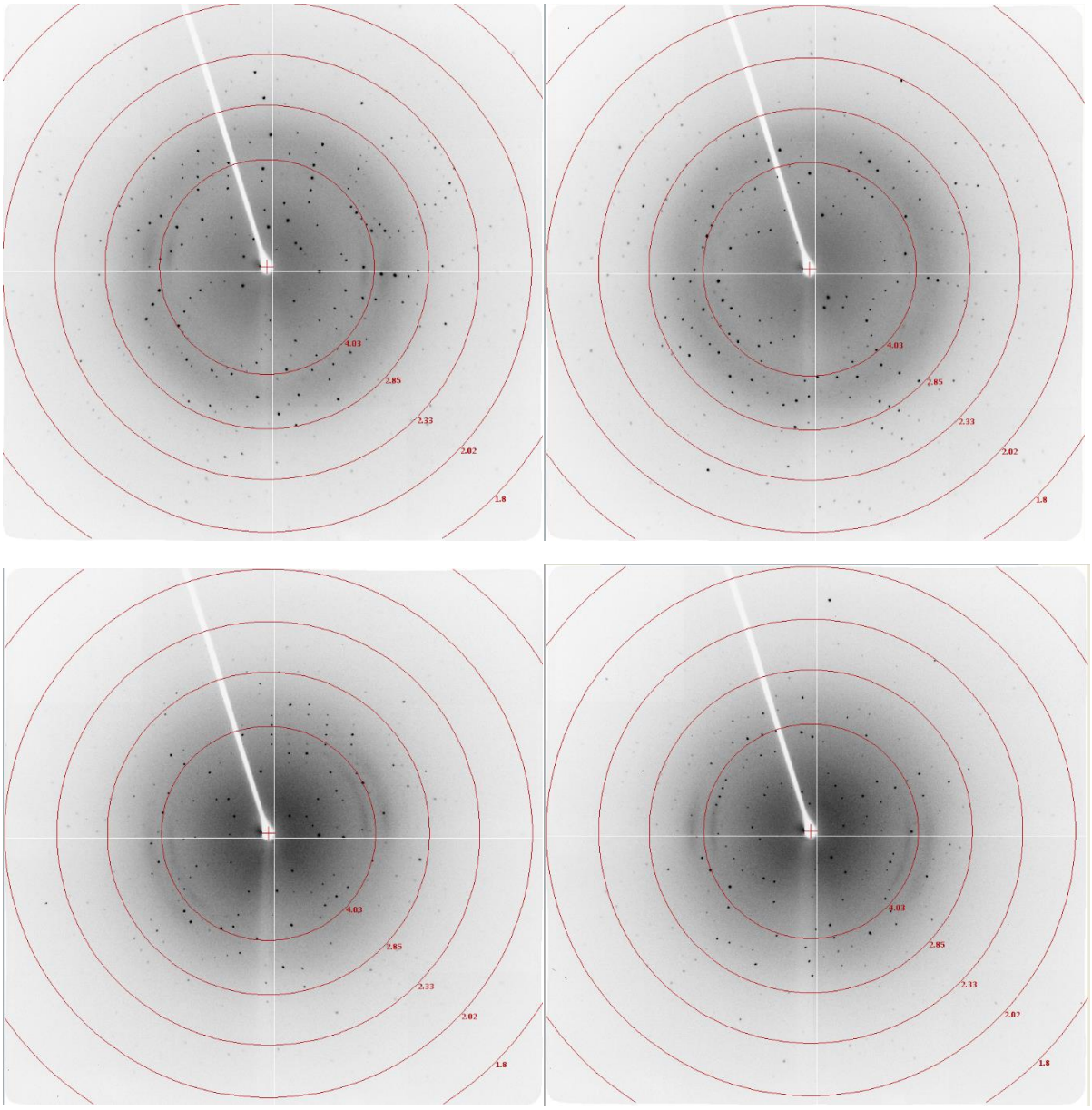

Figure 4. Diffraction images with USP5 Zf-UBD crystals in 1.8 M ammonium sulfate, 0.1 M sodium cacodylate pH 5.5, 0.2 M sodium acetate, 1.7 mM L-glutathione reduced, L-glutathione oxidized

Mandeep Mann
2018/02/02

Table 1. Comparison of solved 2G43 structure vs. USP5 Zf-UBD

	Sequence	**Crystallization condition**
USP5 Zf-UBD	GGEVRQVSKHAFSLKQLDNPARIPPCGWKCS KCDMRENLWLNLTDGSILCGRRYFDGSGGN NHAVEHYRETGYPLAVKLGTITPDGADVYSYD EDDMVLDP SLAEHLSHFGIDMLKMQKTD	1.8 M ammonium sulfate, 0.1 M sodium cacodylate pH 5.5, 0.2 M sodium acetate, 1.7 mM L-glutathione reduced,L-glutathione oxidized Cryo-protection with 25% EG [USP5 Zf-UBD]=8.3 mg/mL in 50 mM Tris pH 8, 150 mM NaCl, 1 mM TCEP
PDB: 2G43	KQEVQAWDGEVRQVSKHAFSLKQLDNPARI PPCGWKCSKCDMRENLWLNLTDGSILCGRRY FDGSGGNNHAVEHYRETGY PLAVKLGTITPDGADVYSYDEDDMVLDPSLAE HLSHFGIDMLKMQKTDK	16% polyethylene glycol 8000, 0.8 M sodium cacodylate pH 6.5, 0.16 M calcium acetate, 20% glycerol