Mandeep Mann
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**USP5 ZnF-UBD Co-Crystal Structure with Compound UBTR012574a**

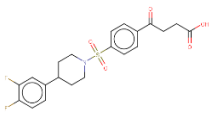
Objective: To grow well-diffracting co-crystals of USP5 zinc finger ubiquitin binding domain (ZnF-UBD) to solve the co-crystal structures to determine ligand interactions in the binding pocket and to determine if the predicted binding pose is similar to the experimental binding pose.

Experiment & Results:

A. Co-crystal structure determination

Based on previous displacement and SPR data, compound UBTR012574a ($K_D$= 10 µM) (Table 1) was used to set up co-crystallization screens with $USP5^{171-290}$. 180 µL of 12 mg/mL $USP5^{171-290}$ and 2.2 mM compound UBTR012574a solution (1:2.5 protein:compound) was prepared in 50 mM Tris pH 8, 150 mM NaCl, 1 mM TCEP.

**Table 1**. UBTR012574a

| Compound Name | Compound Structure | SMILES |
|---|---|---|
| UBTR012574a |  | C1CN(CCC1c1ccc(c(c1)F)F)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O |

Using standard crystallization protocols, SGC and RW screens were used to prepare a crystal screen in 96-well Intelli plates (Art Robbins Instruments). Please find screens attached as .xls files. 70 µL of each condition was dispensed into the well of the plate and then 0.5 µL of the well solution was dispensed to the bottom drop of the plate by a liquid handling robot (Phoenix) followed by 0.5 µL of 1:2.5 protein:compound solution. Crystal plates were sealed and stored at 18°C.

One week after preparing the crystal trays, crystals formed in some conditions in the RW-plate: RWD04 (20% PEG 3350, 0.2 M calcium acetate), RWF09 (18% PEK 8K, 0.2 M calcium acetate, 0.1 M sodium cacodylate pH 6.5), RW G06 (1 M sodium/potassium phosphate pH 6.9).

The largest and most 3D crystals for each compound were mounted using a nylon loop then transferred to a 2 µL drop of well solution supplemented with 25% ethylene glycol (v/v) and submerged for approximately 30 seconds, and then cryo-cooled in liquid nitrogen. The crystal was screened using our in-house diffractometer, RIGAKU FR-E SUPERBRIGHT at 1.54178 Å. 2 images at 90 degrees with a 0.5 degrees oscillation, 20 s exposure and 100 mm crystal-detector distance were collected with a RIGAKU SATURN A200 CCD detector at 100 K. All crystal conditions had an oily skin on the top of the drop which needed to be removed prior to crystal mounting. Figures 1-3 are diffraction images with the crystals.

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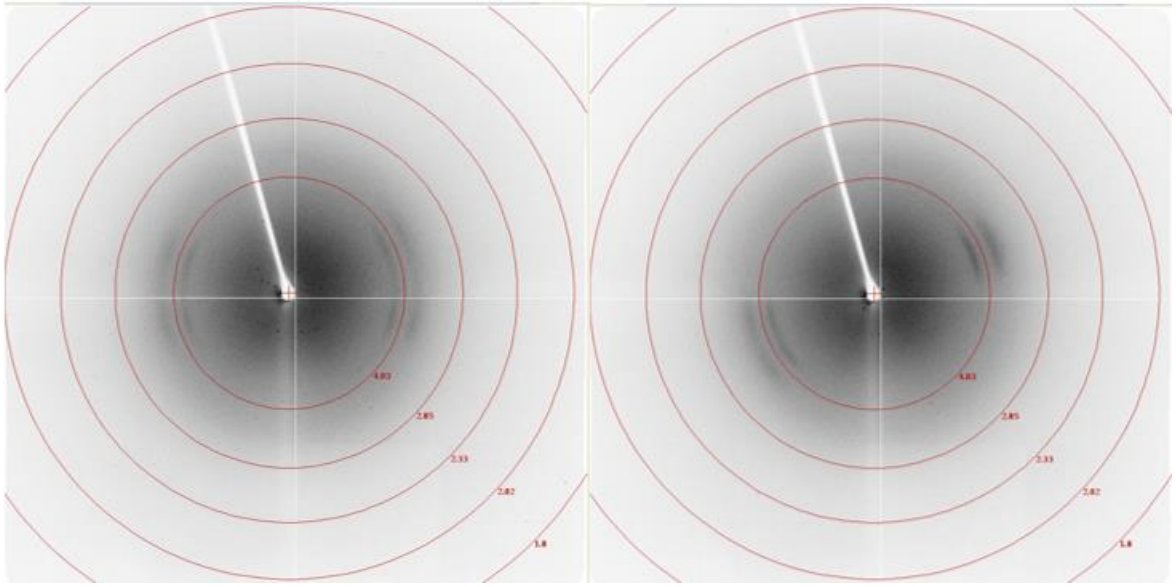

**Figure 1**. Diffraction images with USP5$^{171-290}$ and UBTR012574a in condition RWD04 (20% PEG 3350, 0.2 M calcium acetate, 1.1% DMSO (v/v), 25% ethylene glycol (v/v))

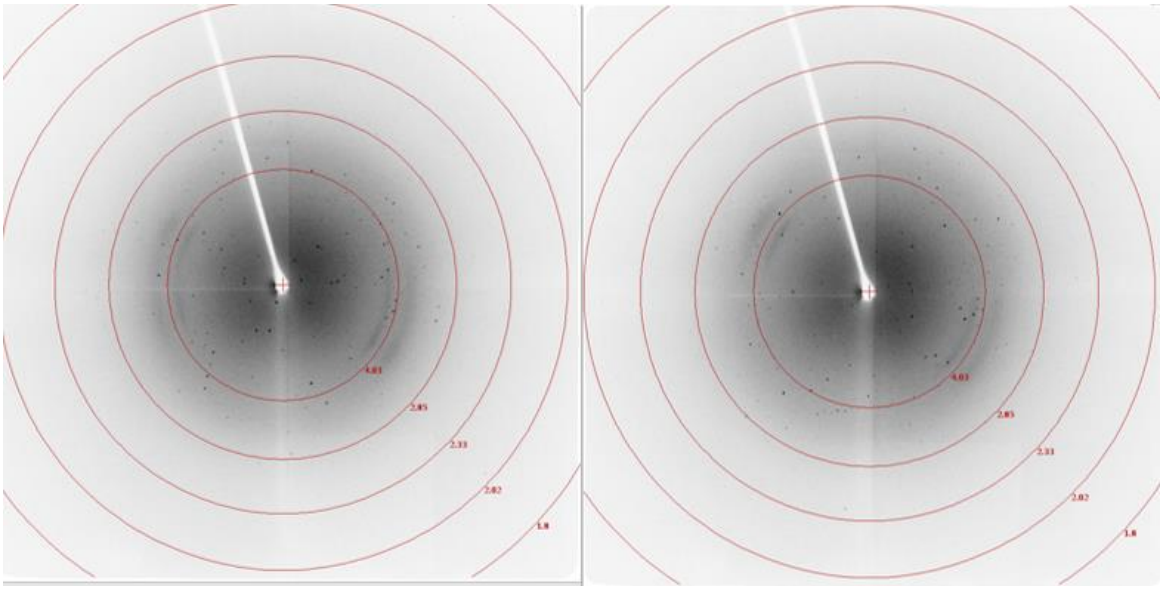

**Figure 2.** Diffraction images with USP5$^{171-290}$ and UBTR012574a in condition RWF09 (18% PEK 8K, 0.2 M calcium acetate, 0.1 M sodium cacodylate pH 6.5, 1.1% DMSO (v/v), 25% ethylene glycol (v/v))

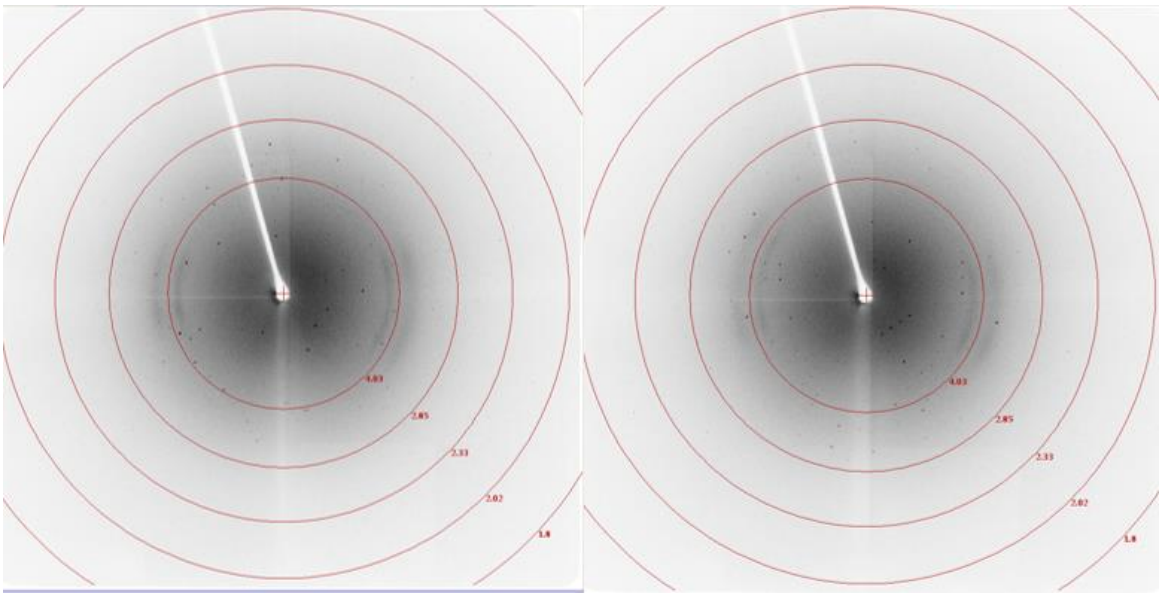

Figure 3. Diffraction images with USP5$^{171-290}$ and UBTR012574a in condition RWG06 (1 M sodium/potassium phosphate pH 6.9, 1.1% DMSO (v/v), 25% ethylene glycol (v/v)).

Large scale data collection of the crystal from Figure 3 were collected in-house on the RIGAKU FR-E X-ray generator with a RIGAKU Saturn A200 CCD detector with the following conditions: crystal detector distance: 100 mm, 400 images, 200 degree oscillation and exposure time of 60 seconds.

The images were processed with HKL3000 and scaled with AIMLESS by Dr. Aiping Dong. The structure was then refined with iterative builds in Coot and refinement in Refmac. Clear electron density of UBTR012574a was seen in the binding pocket for the dataset. The resulting pdb, aimless mtz files are attached. Table 2 summarizes the co-crystal structure. It should be noted the structure is not processed or refined to the point of being ready for PDB deposition at this point and should be interpreted with caution.

**Table 2**. Summary of co-crystal structure with compound UBTR01257a

| **Compound Name** | **Rfactor/Rfree** | **Symmetry: Space Group** | **Unit Cell** | | **Resolution(Å)** |
|---|---|---|---|---|---|
| | | | **Length (Å)** | **Angle (º)** | |
| UBTR012574a | 0.18/0.22 | C121 | a=61.697<br>b=84.494<br>c=59.642 | α=90.00<br>β=98.27<br>γ=90.00 | 1.98 |

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Conclusions & Future Directions

The experimental binding pose turned out to very similar to the predicted/docked one, except for the difluoro phenyl group, which is flipped out of the pocket compared to the predicted docked pose (Figure 4).

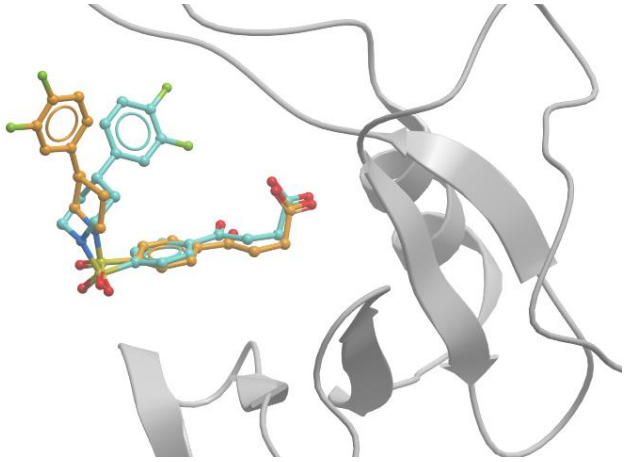

**Figure 4**. Ribbon diagram of USP5 ZnF-UBD (grey) with UBTR012574; predicted docked pose (cyan) and experimental pose (orange)

The addition of the extended sulfonamide moiety, outlined in red in Figure 3, increased potency 60-fold from the parent compound (outlined in green in Figure 3). There are some pi-interactions with Y223 in the binding pocket that could explain this increase in potency. The good news is there are several areas of improvement in this compound based on the co-crystal structure. Potential improvements may be to replace the piperidine with an aromatic ring to improve pi-interactions with Y223. Decorating the outer phenyl ring with polar groups may lend to hydrogen bond interactions with D225.

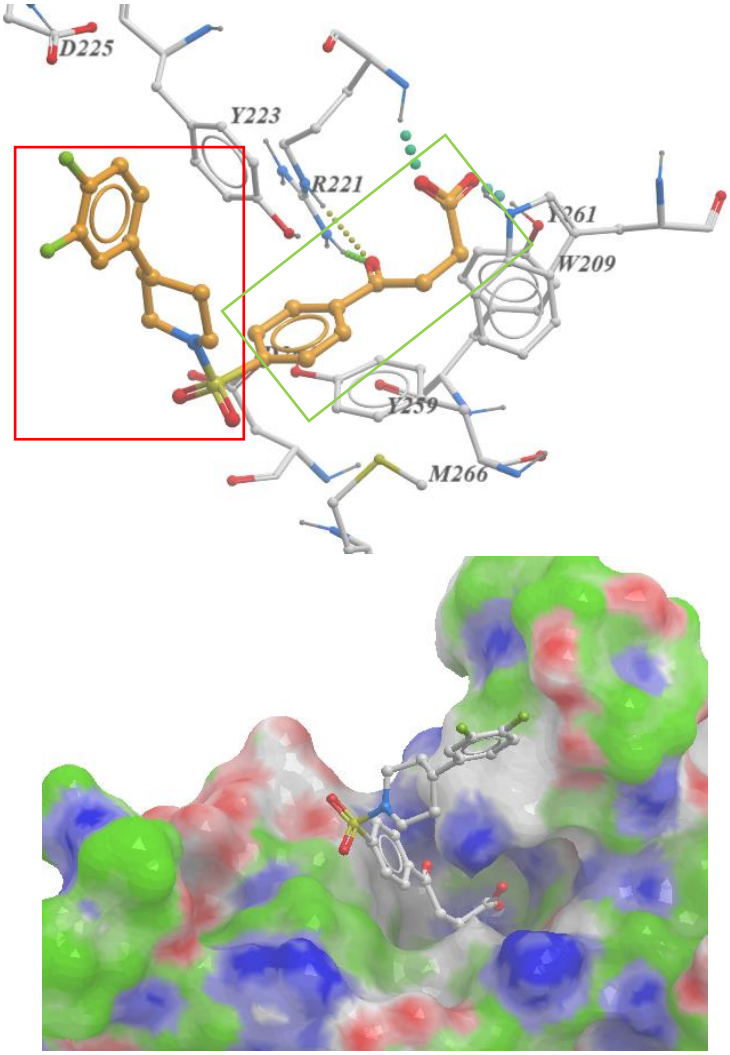


**Figure 5**. Co-crystal structure of USP5 ZnF-UBD and UBTR012574a

Next, I'll be looking for commercial analogues of UBTR012574a to get a better understanding of the structure activity relationship of the extended sulfonamide group at the para position of the benzene ring. We also are hoping to have some chemistry resources soon, so I'll be designing some analogues of interest based on the co-crystal structure of USP5 ZnF-UBD and UBTR012574a.