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Structural Analysis of USP5 Zf-UBD

Objective: To determine if the solved structure of USP5 zinc finger ubiquitin binding domain (Zf-UBD) in previously determined crystal conditions allows for the ubiquitin binding pocket to be accessed through solvent channels for inhibitor screening against the Zf-UBD.

The structure solution and how it was solved can be found here.

Analysis:

Cys195 is a surface residue that is not involved in Zn-coordination but forms disulphide bonds in all known USP5 structures (Figure 1).

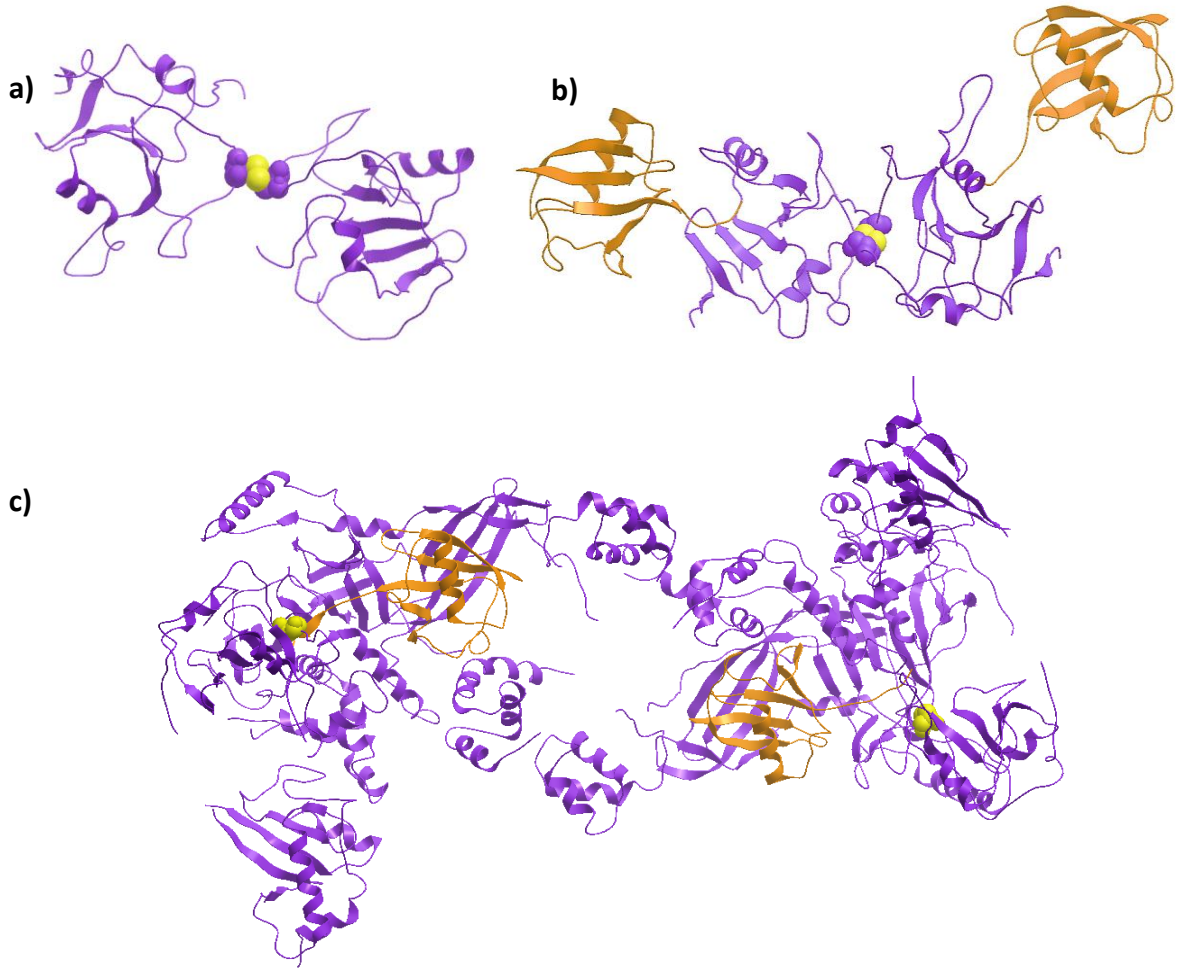

Figure 1. Standard cartoon ribbon diagrams of USP5 structures with disulphide bonds (yellow) a) apo USP5 Zf-UBD (PDB: 2G43) b) ubiquitin (orange) bound USP5 Zf-UBD (PDB: 2G45) c) full length USP5 with bound ubiquitin (orange) (PDB: 3IHP)

Ubiquitin-bound USP5 Zf-UBD structure (Figure 1b) shows a dimer formed through C195 residue in each molecule and a domain swap of the N-terminal strand. Alternatively, apo USP5 Zf-UBD (Figure 1a) structure forms a dimer through the disulphide formed by the C195 in neighboring molecules

but does not have the domain swap of the N-terminus. This suggests that ubiquitin binding causes the N-terminal domain swap in the USP5 Zf-UBD structure. Full length USP5 with bound ubiquitin (Figure 1c) has an intramolecular disulphide bond that holds the Zf-UBD domain in place relative to the rest of the USP5 structure. In a previous experiment, I showed that USP5 Zf-UBD is a monomer in solution. The labile cysteine residue may form a disulphide bond for structural stabilization or perhaps is an artefact of crystallography conditions.

Similarly, a disulphide bond formation is seen at Cys195 in our novel structure of USP5 Zf-UBD in crystallization conditions of 1.8 M ammonium sulfate, 0.2 M sodium acetate, 0.1 M sodium cacodylate pH 5.5, 1.7 mM l-glutathione reduced, l-glutathione-oxidized. This time, a glutathione molecule forms a disulphide with C195 (Figure 2, 3).

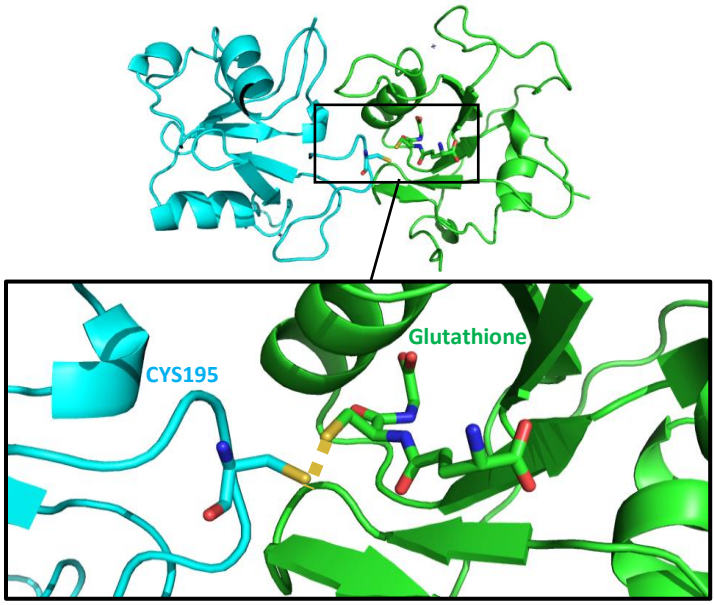

Figure 2. Ribbon diagram of two adjacent molecules of USP5 Zf-UBD in the crystal lattice where glutathione forms a disulphide bond with C195

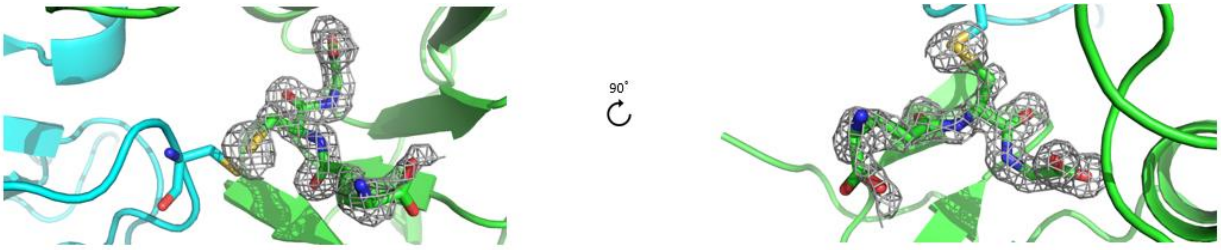

Figure 3. Omit map of glutathione in USP5 Zf-UBD calculated using Fast Fourier Transform (FFT) displayed in pymol to 2 sigma within 1.6 Å of atoms

Our structure of USP5 Zf-UBD and PDB 2G45 were superimposed using SSM in COOT to align Chain A of both molecules over residues 200-280 to determine how glutathione binding compares to ubiquitin binding for USP5 Zf-UBD. Glutathione binds the ubiquitin binding pocket in a similar manner to the C-terminus of ubiquitin. The carboxyl group of the ubiquitin C-terminus and the glutathione occupy the same position in the ubiquitin binding pocket (Figure 4).

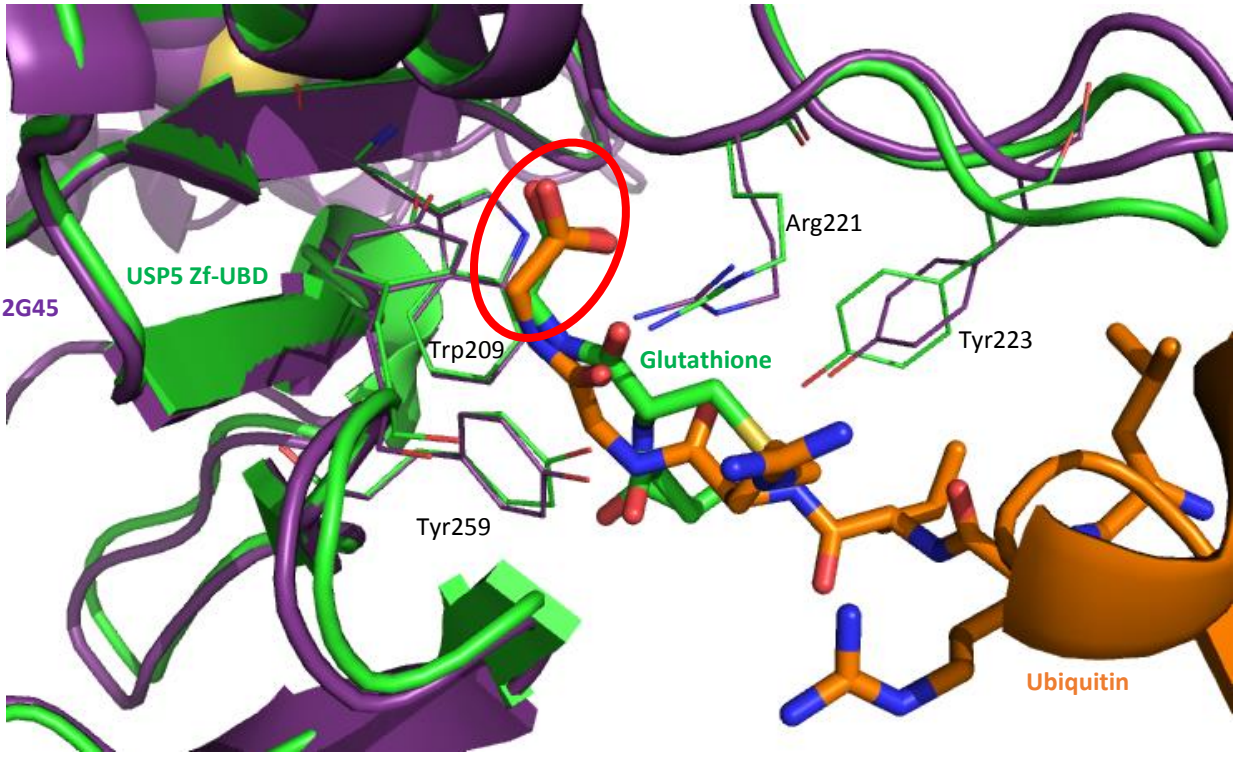

Figure 4. Ribbon diagram of superimposed USP5 Zf-UBD structures: 2G45 (purple) and our structure (green) showing the C-terminal carboxylate of ubiquitin (orange) and glutathione (green) overlap in the ubiquitin binding pocket

Conclusions & Future Directions:

In conclusion, the crystallization condition: 1.8 M ammonium sulfate, 0.1 M sodium cacodylate pH 5.5, 0.2 M sodium acetate , 1.7 mM l-glutathione reduced/oxidized is not an ideal crystallization condition for USP5 Zf-UBD as the crystals will not be amenable to soaking with inhibitor compounds due to the carboxyl group of glutathione binding in the pocket. However, the solved structure did reveal common binding orientations of ubiquitin and glutathione in the ubiquitin binding pocket and confirms the labile nature of Cys195 in USP5 structures.

Next, I will continue to optimize crystallization conditions so the binding pocket of USP5 Zf-UBD is accessible to solvent channels. This will allow for the USP5 Zf-UBD crystals to be soaked with compounds for screening.