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Is USP5 Zf-UBD a dimer?

Objective: To determine if the USP5 zinc finger ubiquitin binding domain (Zf-UBD) is a dimer in solution to elucidate the binding properties of ligands against USP5 Zf-UBD for future inhibitor screening studies.

Reasoning: USP5 structures have shown that there is a cysteine surface residue that is not involved in Zn-coordination but forms disulfide bonds (2G43, 2G45). The disulfide bond links two neighboring USP5 Zf-UBD molecules.

Experiments & Results:

1 mL of 0.2 mg/mL of USP5 Zf-UBD$^{171-290}$ (MW=13.5 kDa) was prepared in 50 mM Tris pH 8, 150 mM NaCl. 500 µL of the sample was injected though a S75 10/300 GL analytical column (GE Healthcare Life Sciences) with 1) Buffer A: 50 mM Tris pH 8, 150 mM NaCl and 2) Buffer B: 50 mM Tris pH 8, 150 mM NaCl, 5 mM DTT, where DTT was added to reduce disulfide linkages in the structure.

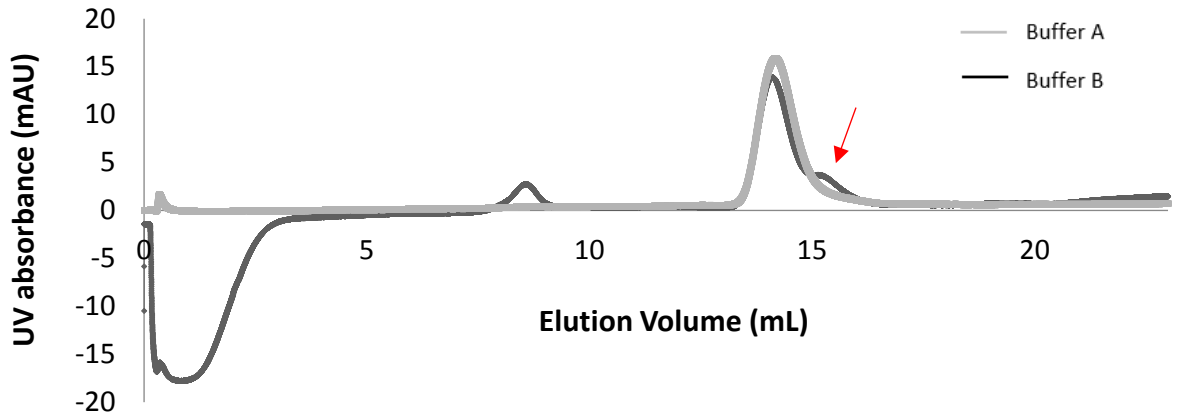

Figure 1. Elution chromatogram of USP5 Zf-UBD in Buffer A and B

Elution with the addition of 5 mM DTT resulted in a very small peak at approximately 8 mL; this is likely degraded protein or contaminant. Elution with 5 mM DTT also resulted in a shouldered peak at approximately 15 mL as indicated by the arrow in Figure 1. This shouldered peak was not seen in the elution peak with Buffer A that contained no reducing agent. It is likely, this peak occurs due to lack of zinc coordination by the cysteine residue. To further investigate if USP5 Zf-UBD is a dimer, size exclusion chromatography with multiple angle light scattering analysis (SEC-MALS) was done.

1 mL of 5 mg/mL of USP5 Zf-UBD$^{171-290}$ was prepared in 50 mM Tris pH 8, 150 mM NaCl. Sample was injected though a S12 10/300 GL column (GE Healthcare Life Sciences) followed by a Dawn Heleos-II light scattering detector (Wyatt Technologies). Molecular mass calculations were performed using ASTRA 6 assuming a dn/dc of 0.1850 mL/g (Wyatt Technologies).

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Table 1. Average molar mass moments of USP5 Zf-UBD$^{171-290}$ from SEC-MALS

	Molar Mass Moments (g/mol)
Mn	19.9 x 10^3 (±9.6%)
Mp	16.7 x 10^3 (± 8.4%)

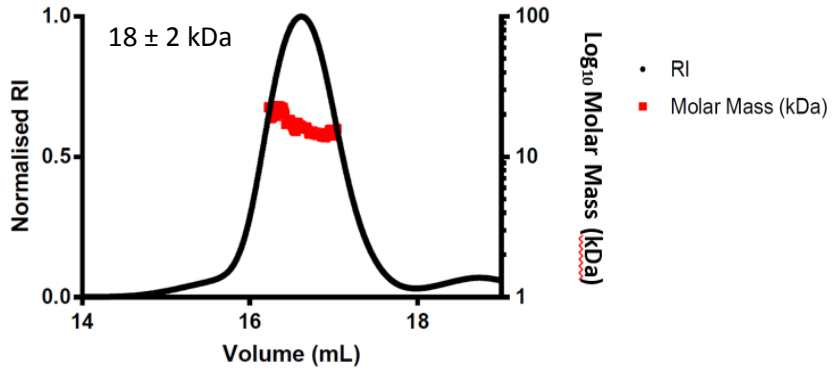

Figure 2. SEC-MALS of 5 mg/mL USP5 Zf-UBD$^{171-290}$

Conclusions:

Light scattering studies show that USP5 Zf-UBD has an average measured mass of approximately 18 ± 2 kDa (Figure 2). The mass of USP5 Zf-UBD monomer is approximately 13.5 kDa. The signal to noise ratio for the scattering was high due to the small size of the USP5 zinc finger domain and the limited dynamic range of the MALS detector. The MALS instrument is optimized for larger proteins that have a higher intensity of scattering. The scatter signal: noise ratio increases for higher concentrations of proteins, but because the size of USP5 Zf-UBD is small, in order to achieve the highest possible scattering from the USP5 sample, the sample concentration was increased so that it was measurable by the refractive index (RI) detector. Due to the high signal: noise ratio, the calculated average molar mass is higher than expected at ~18 kDa; however, it is important to note that this is an average of the scattering across the USP5 Zf-UBD elution peak, where scattering is variable at the ends of the peak. It is the centroid molar mass of USP5 Zf-UBD that we are interested in. Visual inspection of the scattering at the centre of the USP5 Zf-UBD elution peak, where it is most consistent and has less signal: noise shows a molar mass of ~15 kDa (Figure 3) which is close to the expected molar mass of the USP5 Zf-UBD monomer.

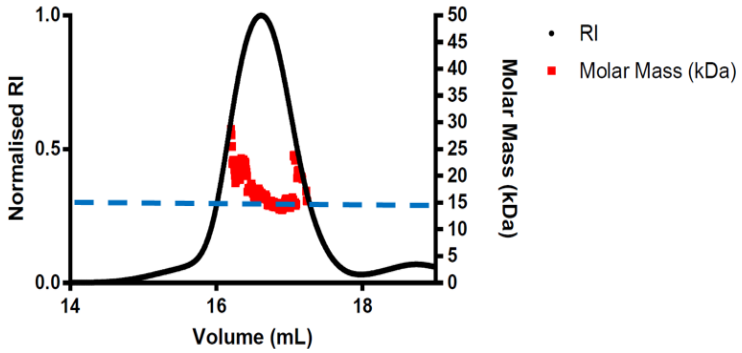

Figure 3. SEC-MALS of USP5 Zf-UBD$^{171-290}$ scaled to see differences in scattering signal across the elution peak

Therefore, USP5 Zf-UBD is a monomer in solution, despite the disulfide bond that forms between cysteine residues in neighboring molecules in the crystal lattice.