= Title =

Crystal structure of a highly specific and potent USP7 ubiquitin variant inhibitor

= Keywords =

Ubiquitin, inhibitor

= PDB Code =

N/A

= Ligands =

N/A

= Entry clone accession =

Ubv.7.2

= Entry clone source =

Sachdev Sidhu lab

== SGC clone accession ==

YTC016E10

= Tag =

N-terminal His6-tag, removed

= Construct comments =

UBC:M1-P78:vu7.2

= Construct sequence =

gMPIFVKTLTGKNITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFTGKKLEDGRTLSDYNIKFASTLHLVIRLRGARP

= Vector =

pET28-MHL

= Expression host =

BL21(DE3)

= Growth method =

LEX Bubbling. The target protein was expressed in E. coli by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 2 L of Terrific Broth medium in the presence of 50 mg/mL kanamycin, 600 uLantifoam at 37 degree. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 degree and the culutre was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before harvested by centrifugation (12,227xg 20min) and flash frozen in liquid nitrogen and stored at -80 degree.

= Extraction buffers =

25mM Tris, pH 7.5, 500mM NaCl, 5% Glycerol

= Extraction procedure =

Frozen cells from 4L TB culture were thawed in water bath at r.t. for about 30 mins and resuspended in 200 mL extraction buffer, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 uL benzonase (Sigma Catalog #E1014, 250U/uL), and lysed using sonication for 10 min (duty cycle 5s on and 7s off)

= Purification buffers =

Washing Buffer: 25mM Tris, pH 8.0, 250mM NaCl, 1mM TCEP, 20 mM imidazole

Elution Buffer: 25mM Tris, pH 8.0, 250mM NaCl, 1mM TCEP, 250mM imidazole

Ion Exchange buffer: 25mM HEPES pH7.5, 0-1 M NaCl,1mM DTT

Gel Filtration Buffer: 25mM HEPES pH 7.5, 150m M NaCl,1mM DTT

= Purification procedure =

The lysate was centrifuged at 15,000 rpm for 60 minutes and the supernatants were mixed with 4 mL Ni-NTA (50% flurry), supplemented with 5uM imidazole (final concentration) and incubated at 4 degree for 1 hour. The beads were collected by centrifugation at 1500 rpm for 5 min at 4 degree. The supernatant

went through another batch absorption with 4 mL Ni-NTA. The beads were pooled and loaded onto an open column, washed with 30 mL washing buffer and the target protein was elud with 10 mL elution buffer twice. The elute was added TEV protease at (15:1 protein:TEV m/m) ratio and dialyzed overnight to remove His6-tag. Uncut sample and TEV proteas was removed by another pass through the Ni-NTA beads. Pooled flow-through was loaded onto a Source 30Q column followed by a Superdex 75 size exclusion column. Target protein was collected and concentrated using Amicon Ultra-15 centrifugal filter (mwco 3 kDa). The purity of the preparation is tested by SDS-PAGE to be greater than 95%.

= Protein stock concentration =

6-10 mg/mL using nanodrop based on calculated absorbance coefficient.

= Mass spec =

Cut versioin measured 8862.5 Da, expected 8862.3 Da.

= Functional muiltmerization =

Monomer

= Crystallization =

Crystal was obtained from SGC-II screen condition B03 (1.2M NaCit 0.1M Tris 8.5) Well solution supplemented with 10% EG was used as cryoprotectant.