PDB Code5VBDEntry clone accessionSGC cDNA collection: 38-B3BC172429.1Entry clone sourceMGC collection: OHS5893-202503866SGC clone accessionYTC046E04TagN-terminal His6-tag, not removedConstruct commentsUSP9X:A880-S970Construct sequencegAFRGKHLSFVVRFPNQGRQVDDLEVWSHTNDTIGSVRRCILNRIKANVAHTKIELFVGGELIDPADDRKLIGQLNLKDK
SLITAKLTQISSVectorpET28-MHLExpression hostBL21(DE3)Growth methodLEX 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 OD_{600} reached ~3.0, the temperature of the medium was lowered to 15 degree and the culutre was induced with 0.1 mM IPTG. The cells were allowed to grow overnight before harvested by centrifugation (12,227g 20min) and flash frozen in liquid nitrogen and stored at -80 degree.Extraction buffers25mM Tris, pH 7.5, 500mM NaCl, 5% GlycerolExtraction procedureFrozen cells from 4L TB culture were thawed in water bath at r.t. for about 30mins and resuspended in 200 mL extraction buffer, and supplemented with proteaseinhibitor cocktail (SIGMA Catalog # P8849), and 3 uL benzonase (Sigma Catalog #E1014, 250U/uL), and lysed using sonication for 10 min (duty cycle 5s on and7s off)Purification buffersWashing Buffer: 25mM Tris, pH 7.5, 500mM NaCl, 5% Glycerol, 20 mM imidazole - Elution Buffer: 25mM Tris, pH 7.5, 150mM NaCl, 250mM imidazolePurification procedureThe lysate was centrifuged at 15,000 rpm for 60 minutes and the supernatantswere mixed with 4 mL Ni-NTA (50% flurry), supplemented with 5uM imidazole(final concentration) and incubated at 4 degree for 1 hour. The beads werecollected by centrifugation at 1500 rpm for 5 min at 4 degree. The supernatantwent through another batch absorption with 4 mL Ni-NTA. The beads were pooledand loaded onto an open column, washed with 30 mL washing buffer and thetarget protein was elud with 10 mL elution buffer twice. The elute was addedTEV protease at (15:1 protein:TEV m/m) ratio and dialyzed overnight to removeHis6-tag. Uncut sample and TEV proteas was removed by another pass through theNi-NTA beads. Pooled flow-through was loaded onto an ion exchange column(Source 15S). Two peaks were observed on the elution profile and both containsthe target protein with the correct mass, suggesting the existance of twospecies of probably different oligomeric status. The two peaks were collectedseparately, 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 concentration28-30 mg/mL using nanodrop based on calculated absorbance coefficient.Mass specCut versioin measured 10282.1 Da, expected 10281 DaCrystallizationCrystal was obtained from SGC-II screen condition E10 for the lower chargedspecies from the ion exchange elution profile. Crystals were also observed inmultiple different conditions with different diffraction power.Crystal used for structure determination was grown in 20% PEG 8000, 0.2M NaCl,0.1M HEPES, pH 7.5, 5% MPD using an 0.5 uL protein, 0.5uL well solution,sitting drop vaporization setup. The crystals are rod-shaped and grown to amountable size within 2 days.N-paratone was used as cryoprotectant.