TBC1D22B

PDB:3DZX

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:CDV:C6orf197
Entry Clone Source:SGC:19-E2
SGC Clone Accession:HPC074-C10
Tag: Tag not removed, Tev-tag mhhhhhhssgrenlyfq*g
Host:BL21-CodonPlus(DE3)-RIL

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgMTVREKTRLEKFRQLLSSQNTDLDELRKCSWPGVPREVRPITWRLLSGYLPANTERRKLTLQRKREEYF
GFIEQYYDSRNEEHHQDTYRQIHIDIPRTNPLIPLFQQPLVQEIFERILFIWAIRHPASGYVQGINDLVTPFFVVFLSEYVEEDVEN
FDVTNLSQDMLRSIEADSFWCMSKLLDGIQDNYTFAQPGIQKKVKALEELVSRIDEQVHNHFRRYEVEYLQFAFRWMNNLLMRELPL
RCTIRLWDTYQSEPEGFSHFHLYVCAAFLIKWRKEILDEEDFQGLLMLLQNLPTIHWGNEEIGLLLAEAYRLKYMFADAPNHYRR

Vector:pET28-mhl (GI:134105571)

Growth

Medium: Terrific Broth
Antibiotics:
Procedure:LEX Bubbling. The target protein was expressed in E. coli by inoculating 60 mL of overnight culture grown in Luria-Bertani medium into a 1.8 L of Terrific Broth medium in the presence of 50 µg/mL kanamycin and 25 µg/mL chloramphenicol at 37 °reeC. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 µC and the culture was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before they were harvested and flash frozen in liquid nitrogen and stored at -80 °C.

Purification

Procedure
The lysate was centrigued at 15,000 rpm for 45 minutes and the supernatants were mixed with 3 mL 50% Ni-NTA beads, and incubated at 4 °reeC for 1 hours. The supernatant was then passed through a gravity column (Poly-Prep, Bio-Rad, Catalog #731-1550) and the beads were washed using 15 mL washing buffers(contains 5mM, 30mM or 75 mM Imidazole separately). The protein bound to beads were eluted using 15 mL elution buffer once. The flow-through fractions washed

using buffer containing 30mM, 75mM, 300mM Imidazole were collected and loaded onto Supderdex-75 gel filtration column. Eluted fractions were pooled and concentrated using amicon centrifugal filter (m.w. cut-off 10,000). The purity of the proteins was higher than 93% judged by SDS-PAGE.

Extraction

Procedure
Frozen cells from 1.8L TB culture were thawed and resuspended in 150 mL extraction buffer with freshly added 0.5% CHAPS and 2mM BME, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 µL benzonase (Sigma Catalog # E1014, 250U/µL), and lysed using microfluidizer.
Concentration:21.6 mg/mL
Ligand
N/A**MassSpec:**Native: 41716.23, expected 41715.54.
Crystallization: Crystallization was setup using sitting drops with Red Wings and SGC-I screen kits and also with in situ proteolytic treatment. Initial hits include RW-C06, RW-F05, RW-G06 (1:100 Subtilisin), RW-B01 (1:100 Trypsin), RW-C01(1:100 Trypsin), RW-C01(1:100 Chymotrypsin). Crystals grown without protease treatment generally adopt cone shape, long and fragile.
The crystal used for data collection was grown in 1.4M (NH4)2SO4, 0.1M Bis-Tris pH 6.3, 1mM DTT and in the presence of 1:100 Chymotrypsin. The crystals grow to a mountable size within two days, and show 3D-bipyramid shape. Paratone-N were used as cryoprotectant.
Last updated by ytong 20080731
NMR Spectroscopy:
Data Collection:
Data Processing: