Molecular Biology

Entry Clone Accession: BC101628
Entry Clone Source: MGC
SGC Construct ID: FAM83BA-c004
Protein Region: G117-V294
Vector: pNIC28-Bsa4
Tag: N-6HIS;N-TEV
Host: BL21(DE3)-R3-pRARE2

Sequence (with tag(s)):
MHHHHHHSSGVDLGTENLYFQSMGGTHIDLLFHPPRAHLLTIKETIRKMIKEARKVIALV
MDIFTDVDIFKEIVEASTRGVSVYILLDESNFNHFLNMTEKQGCSVQRLRNIRVRTVKGQ
DYLSKTGAKFHGKMEQKFLLVDCQKVMYGSYSYMWSFEKAHLSMVQIITGQLVESFDE
EFRTLYARSCVPSSFAQEESARV
Sequence after tag cleavage:
SMGGTHIDLLFHPPRAHLLTIKETIRKMIKEARKVIALVMDIFTDVDIFKEIVEASTRGVSV
YILLDESNFNHFLNMTEKQGCSVQRLRNIRVRTVKGQDYLSKTGAKFHGKMEQKFLLVD
CQKVMYGSYSYMWSFEKAHLSMVQIITGQLVESFDEEFRTLYARSCVPSSFAQEESARV
DNA Sequence:
CATATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTGTA
CTTCCAATCCATGGGGGGCACCCATATAGATCTCCTTTTTCATCCACCAAGAGCACATC
TACTTACGATAAAAGAAACTATTCGGAAGATGATAAAAGAAGCAAGAAAGGTCATTG
CTTTAGTGATGGATATATTTACAGATGTGGACATTTTCAAAGAAATCGTTGAGGCATCA
ACTCGAGGAGTATCTGTTTACATTCTGCTTGATGAGTCCAATTTTAATCATTTTCTAAAT
ATGACTGAGAAACAAGGTTGTTCAGTTCAGCGTCTCAGGAATATTCGAGTGCGAACA
GTAAAAGGCCAAGATTATCTTTCAAAAACAGGGGCAAAATTCCATGGAAAAATGGAA
CAGAAATTTTTGTTAGTTGACTGCCAGAAAGTGATGTACGGTTCTTACAGTTATATGTG
GTCATTTGAGAAAGCTCACCTCAGCATGGTTCAGATAATTACAGGACAACTTGTTGAG
TCCTTTGATGAAGAATTTAGAACTCTCTATGCCAGATCCTGTGTCCCTAGTTCATTTGC
TCAGGAAGAATCAGCAAGGGTGTGACAGTAAAGGTGGATACGGATCCGAA

Protein Expression

Medium: LB
Antibiotics: Kanamycin/Chloramphenicol
Procedure: A glycerol stock was used to inoculate a starter culture which was supplemented with Kanamycin (50 µg/ml)/ Chloramphenicol (34 µg/ml). These were then grown overnight at 37 °C with shaking at 240 RPM. The starter culture was used to inoculate 1 litre LB flasks supplemented with Kanamycin/Chloramphenicol. The cultures were then grown at 37 °C with shaking at 160 RPM to an OD_{600} of between 0.6-0.8. The cultures were then cooled to 18 °C, induced with 0.35 mM IPTG and the protein was expressed overnight at 18 °C /160 RPM. Cultures were harvested, pellets made up to 40 ml with Nickel affinity binding buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM Imidazole, 5% Glycerol) and 40 ul of SET III protease inhibitors (Merck) added before storage at -20 °C.

Protein Purification

Procedure: Cells were lysed by sonication, 0.125% polyethyleneimine added, and lysates were clarified by centrifugation at 50,000G. The supernatant was then applied to a Nickel-NTA gravity column and washed and eluted with 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, and 10-300mM imidazole. Protein containing fractions were then treated with TEV protease overnight at 4 °C prior to gel filtration on an S200 gel filtration column using 50 mM HEPES pH 7.5, 150 mM NaCl, 1 mM TCEP as the running buffer. The protein was concentrated to 13.2 mg/ml using a spin concentrator.

Columns: Column 1: Ni-NTA; Column 2: S200;
Concentration: 13.2 mg/ml
Mass-spec Verification: Intact mass correct

Structure Determination

Crystallization: Crystals were grown using the sitting drop vapor diffusion method at 20˚C. Crystals were grown in 600 nl drops consisting of 360 nl of mother liquor (27.3% tacsimate -- 0.15M sodium chloride) and 240 nl of protein (12.0 mg/ml). To the crystallization drop, 10% of the crystallization drop volume (60 nl) of 500 mM fragment (in this case N13362a a.k.a. H27173 a.k.a. FMOPL000010a) in DMSO was added, 20% Ethylene glycol was then added as a cryoprotectant and the crystal was flash frozen in liquid nitrogen.

Data Collection: Data was collected at beamline I04-1 at 100K.
Data Processing: Data was processed to a resolution of approximately 1.7 Å using XDS and either xia2 or autoPROC, or DIALS.