Molecular Biology

Entry Clone Accession: BC132743
Entry Clone Source: MGC
SGC Construct ID: KCTD8A-c016
Protein Region: A201-P322
Vector: pNIC28-Bsa4
Tag: N-6HIS;N-TEV
Host: BL21(DE3)-R3-pRARE2

Sequence (with tag(s)):
MHHHHHHSSGVDLGTENLYFQSMAQDKRSGFLTLGYRGSYTTVRDNQADAKFRRVARI
MVCGRIALAKEVFGDTLNESRDPDRQPEKYTSRFYLKFTYLEQAFDRLSEAGFHMVACN
SSGTAAFVNQYRDDKIWSSYTEYIFFRP
Sequence after tag cleavage:
SMAQDKRSGFLTLGYRGSYTTVRDNQADAKFRRVARIMVCGRIALAKEVFGDTLNESRD
PDRQPEKYTSRFYLKFTYLEQAFDRLSEAGFHMVACNSSGTAAFVNQYRDDKIWSSYTE
YIFFRP
DNA Sequence:
CATATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTGTA
CTTCCAATCCATGGCGCAGGACAAGCGCTCGGGCTTCCTCACGCTGGGCTACCGGGG
CTCCTACACCACCGTGCGCGACAACCAGGCCGACGCCAAATTCCGGCGTGTGGCGCG
CATCATGGTGTGCGGGCGCATCGCGCTGGCCAAGGAGGTCTTCGGGGACACGCTCAA
CGAGAGCCGCGACCCCGACCGGCAGCCGGAGAAGTACACGTCCCGCTTCTACCTCAA
GTTCACCTACTTGGAGCAGGCCTTTGATCGCCTGTCCGAGGCCGGCTTCCACATGGTG
GCGTGTAACTCCTCGGGCACCGCCGCCTTCGTCAACCAGTACCGCGACGACAAGATC
TGGAGCAGCTACACCGAGTACATTTTCTTCCGACCATGACAGTAAAGGTGGATACGGA
TCCGAA

Protein Expression

Medium: LB
Antibiotics: Kanamycin and Chloramphenicol
Procedure: A glycerol stock was used to inoculate a 15ml starter culture of LB with 50ug/ml Kanamycin and 34 ug/ml Chloramphenicol. The starter culture was grown overnight at 37ºC, shaking at 220rpm. The starter culture was used to inoculate 1l cultures supplemented with Kanamycin and Chloramphenicol at 5ml/l which were then grown at 37ºC, shaking at 180rpm, until reaching an optical density of 0.7-0.8. Cultures were induced with 0.4mM Isopropyl β-D-1-thiogalactopyranoside and cooled to 18ºC and left shaking overnight at 180rpm. Cultures were harvested by centrifugation at 5000g for 15 mins. Cell pellets were resuspended in 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM Imidazole, 5 % glycerol plus Merck Set III protease inhibitor and stored at -20°C.

Protein Purification

Procedure: Cells were lysed by sonication and lysates clarified by centrifugation at 21,000rpm following the addition of 0.125% polyethyleneimine. The resulting supernatant was incubated with Ni-IMAC resin (equilibrated in 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM Imidazole, 5 % glycerol), mixing by inversion, at 4ºC for 1 hour. This was centrifuged at 700g for 5mins, the supernatant removed and resin resuspended in 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM Imidazole, 5 % glycerol. This was applied to a gravity column and washed and eluted with 50

mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, and 30-250mM imidazole. Fractions containing protein (as seen by SDS-PAGE) were treated with TEV protease and 10mM DTT overnight at 4ºC prior to gel filtration on an S200 gel filtration column using 50 mM HEPES pH 7.5, 300 mM NaCl and 1mM TCEP as the running buffer. Fractions containing purified protein (verified by SDS-PAGE) were concentrated to 12.0 mg/ml using a centrifugal concentrator.
Columns: Column 1: Ni-IMAC; Column 2: S200 Gel Filtration;
Concentration: 12.0 mg/ml
Mass-spec Verification: Intact mass confirmed by LC-MS as 14417.8 Da

Structure Determination

Crystallization: The protein sample was filtered using a 0.22um spin filtration unit. Crystals were grown using the sitting drop vapor diffusion method at 20ºC. Crystals were grown in 150 nl drops consisting of 1:1 mother liquor (50% PEG200, 0.2M sodium chloride, 0.1M sodium/potassium phosphate pH 7.5) to protein (12.0 mg/ml). 25% ethylene glycol was added to the crystal as a cryoprotectant and the crystal was mounted and 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 2.75 Å using XDS and either xia2 or autoPROC, or DIALS.