Molecular Biology

Entry Clone Accession: IMAGE:4132071
Entry Clone Source: MGC
SGC Construct ID: FDPSA-c003
Protein Region: M67-K419
Vector: p11
Tag: N-6HIS;N-TEV
Host: BL21

Sequence (with tag(s)):
MGSSHHHHHHSSGRENLYFQGHMMNGDQNSDVYAQEKQDFVQHFSQIVRVLTEDEMG
HPEIGDAIARLKEVLEYNAIGGKYNRGLTVVVAFRELVEPRKQDADSLQRAWTVGWCVE
LLQAFFLVADDIMDSSLTRRGQICWYQKPGVGLDAINDANLLEACIYRLLKLYCREQPYY
LNLIELFLQSSYQTEIGQTLDLLTAPQGNVDLVRFTEKRYKSIVKYKTAFYSFYLPIAAAM
YMAGIDGEKEHANAKKILLEMGEFFQIQDDYLDLFGDPSVTGKIGTDIQDNKCSWLVVQ
CLQRATPEQYQILKENYGQKEAEKVARVKALYEELDLPAVFLQYEEDSYSHIMALIEQYA
APLPPAVFLGLARKIYKRRK
Sequence after tag cleavage:
GHMMNGDQNSDVYAQEKQDFVQHFSQIVRVLTEDEMGHPEIGDAIARLKEVLEYNAIG
GKYNRGLTVVVAFRELVEPRKQDADSLQRAWTVGWCVELLQAFFLVADDIMDSSLTRRG
QICWYQKPGVGLDAINDANLLEACIYRLLKLYCREQPYYLNLIELFLQSSYQTEIGQTLDL
LTAPQGNVDLVRFTEKRYKSIVKYKTAFYSFYLPIAAAMYMAGIDGEKEHANAKKILLE
MGEFFQIQDDYLDLFGDPSVTGKIGTDIQDNKCSWLVVQCLQRATPEQYQILKENYGQK
EAEKVARVKALYEELDLPAVFLQYEEDSYSHIMALIEQYAAPLPPAVFLGLARKIYKRRK
DNA Sequence:
ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCAGAGAAAACTTGTATTTCC
AGGGCCATATGATGAACGGAGACCAGAATTCAGATGTTTATGCCCAAGAAAAGCAGG
ATTTCGTTCAGCACTTCTCCCAGATCGTTAGGGTGCTGACTGAGGATGAGATGGGGCA
CCCAGAGATAGGAGATGCTATTGCCCGGCTCAAGGAGGTCCTGGAGTACAATGCCATT
GGAGGCAAGTATAACCGGGGTTTGACGGTGGTAGTAGCATTCCGGGAGCTGGTGGAG
CCAAGGAAACAGGATGCTGATAGTCTCCAGCGGGCCTGGACTGTGGGCTGGTGTGTG
GAACTGCTGCAAGCTTTCTTCCTGGTGGCAGATGACATCATGGATTCATCCCTTACCCG
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GCTAACCTCCTGGAAGCATGTATCTACCGCCTGCTGAAGCTCTATTGCCGGGAGCAGC
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CAGACCCTGGACCTCCTCACAGCCCCCCAGGGCAATGTGGATCTTGTCAGATTCACTG
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ACATTATGGCTCTCATTGAACAGTACGCAGCACCCCTGCCCCCAGCCGTCTTTCTGGG
GCTTGCGCGCAAAATCTACAAGCGGAGAAAGTAGGGATCCTAA

Protein Expression

Procedure: Overnight cultures in TB (10 ml with100 µg/ml ampicillin) were used to inoculate 1 litre of TB medium containing 100 µg/ml ampicillin. Cultures were grown at 37oC until they reached an OD600 of 0.65 and then induced with 1 mM IPTG. The temperature was adjusted to

18oC and expression was allowed to continue overnight. The cells were collected by centrifugation, transferred to 50 ml tubes, resuspended in 25 ml binding buffer, and frozen at -80°C.

Protein Purification

Ni-affinity Ni-NTA (Qiagen), 4 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.
Binding buffer: 50 mM HEPES pH 7.5, 5 mM imidazole, 500 mM NaCl, 5% glycerol. Wash buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM imidazole, 5% glycerol. Elution buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM imidazole, 5% glycerol.
The supernatant was applied by gravity flow onto the Ni-NTA column. The column was sequentially washed with 30 ml binding buffer and 12.5 ml wash buffer. The protein was eluted by applying 12.5 ml of elution buffer and the eluate was collected in 1.5 ml fractions. The fractions were analyzed by SDS-PAGE gel, pooled and concentrated. FDPS was exchanged into crystallization buffer (10 mM Hepes, 0.5 M NaCl, 5% glycerol, pH 7.5) and concentrated to 13 mg/mL using a Millipore centrifugal concentrator with a 10 kDa MW cutoff.

Concentration: 13 mg/ml
Mass-spec Verification: Yes

Structure Determination

Crystallization: 2mM Mgcl2 and 2mM zoledronic acid were added to the protein prior to crystallization.

Crystals were grown at 20^{o}C in 150 nl sitting drops by mixing 100 nl of protein solution and 50 nl of precipitant consisting of 40% PEG 300 and 0.1 M phosphate-citrate buffer pH 4.2.
Data Collection: *Beamline:* SLS-X10; *Resolution:* 1.98 Å