Molecular Biology

Entry Clone Accession:
Entry Clone Source: Site-directed mutagenesis
SGC Construct ID: FDPSA-c021
Protein Region: M67-K419 (Y204A point mutation)
Vector: p11
Tag: N-6HIS;N-TEV
Host: BL21(DE3)-R3-pRARE2

Sequence (with tag(s)):
MGSSHHHHHHSSGRENLYFQGHMNGDQNSDVYAQEKQDFVQHFSQIVRVLTEDEMGHP
EIGDAIARLKEVLEYNAIGGKYNRGLTVVVAFRELVEPRKQDADSLQRAWTVGWCVELL
QAFFLVADDIMDSSLTRRGQICWYQKPGVGLDAINDANLLEACIYRLLKLYCREQPYYLN
LIELFLQSSYQTEIGQTLDLLTAPQGNVDLVRFTEKRYKSIVKYKTAFASFYLPIAAAMYM
AGIDGEKEHANAKKILLEMGEFFQIQDDYLDLFGDPSVTGKIGTDIQDNKCSWLVVQCL
QRATPEQYQILKENYGQKEAEKVARVKALYEELDLPAVFLQYEEDSYSHIMALIEQYAAP
LPPAVFLGLARKIYKRRK
Sequence after tag cleavage:
GHMNGDQNSDVYAQEKQDFVQHFSQIVRVLTEDEMGHPEIGDAIARLKEVLEYNAIGGK
YNRGLTVVVAFRELVEPRKQDADSLQRAWTVGWCVELLQAFFLVADDIMDSSLTRRGQI
CWYQKPGVGLDAINDANLLEACIYRLLKLYCREQPYYLNLIELFLQSSYQTEIGQTLDLL
TAPQGNVDLVRFTEKRYKSIVKYKTAFASFYLPIAAAMYMAGIDGEKEHANAKKILLEM
GEFFQIQDDYLDLFGDPSVTGKIGTDIQDNKCSWLVVQCLQRATPEQYQILKENYGQKEA
EKVARVKALYEELDLPAVFLQYEEDSYSHIMALIEQYAAPLPPAVFLGLARKIYKRRK
DNA Sequence:
ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCAGAGAAAACTTGTATTTCC
AGGGCCATATGATGCAGCACTTCTCCCAGATCGTTAGGGTGCTGACTGAGGATGAGAT
GGGGCACCCAGAGATAGGAGATGCTATTGCCCGGCTCAAGGAGGTCCTGGAGTACAA
TGCCATTGGAGGCAAGTATAACCGGGGTTTGACGGTGGTAGTAGCATTCCGGGAGCTG
GTGGAGCCAAGGAAACAGGATGCTGATAGTCTCCAGCGGGCCTGGACTGTGGGCTGG
TGTGTGGAACTGCTGCAAGCTTTCTTCCTGGTGGCAGATGACATCATGGATTCATCCCT
TACCCGCCGGGGACAGATCTGCTGGTATCAGAAGCCGGGCGTGGGTTTGGATGCCATC
AATGATGCTAACCTCCTGGAAGCATGTATCTACCGCCTGCTGAAGCTCTATTGCCGGGA
GCAGCCCTATTACCTGAACCTGATCGAGCTCTTCCTGCAGAGTTCCTATCAGACTGAG
ATTGGGCAGACCCTGGACCTCCTCACAGCCCCCCAGGGCAATGTGGATCTTGTCAGAT
TCACTGAAAAGAGGTACAAATCTATTGTCAAGTACAAGACAGCTTTCGCCTCCTTCTA
CCTTCCTATAGCTGCAGCCATGTACATGGCAGGAATTGATGGCGAGAAGGAGCACGCC
AATGCCAAGAAGATCCTGCTGGAGATGGGGGAGTTCTTTCAGATTCAGGATGATTACC
TTGACCTCTTTGGGGACCCCAGTGTGACCGGCAAAATTGGCACTGACATCCAGGACA
ACAAATGCAGCTGGCTGGTGGTTCAGTGTCTGCAACGGGCCACTCCAGAACAGTACC
AGATCCTGAAGGAAAATTACGGGCAGAAGGAGGCTGAGAAAGTGGCCCGGGTGAAG
GCGCTATATGAGGAGCTGGATCTGCCAGCAGTGTTCTTGCAATATGAGGAAGACAGTT
ACAGCCACATTATGGCTCTCATTGAACAGTACGCAGCACCCCTGCCCCCAGCCGTCTT
TCTGGGGCTTGCGCGCAAAATCTACAAGCGGAGAAAGTAGGGATCCTAA

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: 15.53 mg/ml
Mass-spec Verification: Yes

Structure Determination

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:* Dmnd I02; *Resolution:* 1.95 Å