Molecular Biology

Entry Clone Accession: IMAGE:4340013
Entry Clone Source: MGC
SGC Construct ID: FLJ21802A-c102
Protein Region: A167-N641
Vector: pNIC-CTHF
Tag: C-TEV;C-6HIS;C-FLAG
Host: BL21(DE3)-R3-pRARE2

Sequence (with tag(s)):
MASGPQVDNTGGEPAWDSPLRRVLAELNRIPSSRRRAARLFEWLIAPMPPDHFYRRLWE
REAVLVRRQDHTYYQGLFSTADLDSMLRNEEVQFGQHLDAARYINGRRETLNPPGRALP
AAAWSLYQAGCSLRLLCPQAFSTTVWQFLAVLQEQFGSMAGSNVYLTPPNSQGFAPHYD
DIEAFVLQLEGRKLWRVYRPRAPTEELALTSSPNFSQDDLGEPVLQTVLEPGDLLYFPRGF
IHQAECQDGVHSLHLTLSTYQRNTWGDFLEAILPLAVQAAMEENVEFRRGLPRDFMDY
MGAQHSDSKDPRRTAFMEKVRVLVARLGHFAPVDAVADQRAKDFIHDSLPPVLTDRERA
LSVYGLPIRWEAGEPVNVGAQLTTETEVHMLQDGIARLVGEGGHLFLYYTVENSRVYHL
EEPKCLEIYPQQADAMELLLGSYPEFVRVGDLPCDSVEDQLSLATTLYDKGLLLTKMPLA
LNAENLYFQSHHHHHHDYKDDDDK
Sequence after tag cleavage:
MASGPQVDNTGGEPAWDSPLRRVLAELNRIPSSRRRAARLFEWLIAPMPPDHFYRRLWE
REAVLVRRQDHTYYQGLFSTADLDSMLRNEEVQFGQHLDAARYINGRRETLNPPGRALP
AAAWSLYQAGCSLRLLCPQAFSTTVWQFLAVLQEQFGSMAGSNVYLTPPNSQGFAPHYD
DIEAFVLQLEGRKLWRVYRPRAPTEELALTSSPNFSQDDLGEPVLQTVLEPGDLLYFPRGF
IHQAECQDGVHSLHLTLSTYQRNTWGDFLEAILPLAVQAAMEENVEFRRGLPRDFMDY
MGAQHSDSKDPRRTAFMEKVRVLVARLGHFAPVDAVADQRAKDFIHDSLPPVLTDRERA
LSVYGLPIRWEAGEPVNVGAQLTTETEVHMLQDGIARLVGEGGHLFLYYTVENSRVYHL
EEPKCLEIYPQQADAMELLLGSYPEFVRVGDLPCDSVEDQLSLATTLYDKGLLLTKMPLA
LNAENLYFQ
DNA Sequence:
CTTAAGAAGGAGATATACTATGGCGTCGGGGCCGCAGGTGGATAACACGGGTGGGGA
GCCGGCCTGGGACTCCCCGCTGCGGCGCGTCTTGGCCGAGCTGAACCGCATCCCCAG
CAGCCGGCGGCGAGCGGCCCGCCTCTTTGAGTGGCTCATCGCGCCCATGCCGCCAGA
TCACTTCTACCGGCGCCTATGGGAGCGCGAGGCGGTGCTGGTGCGGCGGCAGGACCA
CACCTACTACCAGGGACTTTTCTCTACCGCTGACCTGGATTCGATGCTGCGCAACGAG
GAGGTGCAGTTCGGCCAGCATTTGGACGCCGCTCGCTACATCAACGGACGACGCGAG
ACCCTGAACCCACCCGGCCGCGCGCTGCCCGCCGCCGCGTGGTCCCTGTACCAGGCC
GGCTGCTCCCTGCGTCTCCTCTGTCCGCAGGCTTTCTCTACTACTGTGTGGCAGTTTTT
GGCTGTGCTTCAAGAGCAGTTTGGAAGCATGGCAGGCTCCAACGTTTACCTCACGCC
CCCTAACTCGCAGGGCTTTGCCCCCCACTACGACGACATCGAGGCCTTCGTGCTGCAG
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GCTCTGACATCCAGCCCCAACTTCAGTCAGGACGACCTCGGTGAGCCGGTGCTGCAG
ACCGTGCTGGAACCTGGAGATTTGCTGTATTTTCCTCGGGGCTTCATTCACCAAGCTG
AATGCCAGGATGGAGTCCACTCTCTGCACCTCACCTTGTCCACGTACCAGCGCAATAC
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AAATGTGGAGTTTCGGAGGGGTCTGCCCCGAGACTTCATGGATTACATGGGGGCCCA
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TTTACGGGCTTCCAATTCGCTGGGAGGCTGGAGAACCTGTAAACGTGGGGGCCCAGT
TGACAACAGAAACAGAAGTCCATATGCTTCAGGATGGGATAGCTCGGCTGGTGGGTG

AGGGGGGCCATTTGTTTCTCTATTACACAGTGGAAAACTCCCGTGTGTATCATCTGGA
AGAACCCAAGTGCTTGGAAATATACCCCCAGCAAGCTGATGCCATGGAACTGTTGCTT
GGTTCTTATCCAGAGTTTGTGAGAGTGGGGGACCTGCCCTGTGACAGTGTGGAGGAC
CAGCTGTCCTTGGCAACCACGTTGTATGATAAGGGGCTGCTGCTCACTAAGATGCCTC
TAGCCCTAAATGCAGAGAACCTCTACTTCCAATCGCACCATCATCACCACCATGATTAC
AAGGATGACGACGATAAGTGAGGATCC

Protein Expression

Medium: LB
Antibiotics: Kanamycin
Procedure: Cells were grown in 2L LB medium supplemented with kanamycin (30 μg ml^{-1}) at 37 °C (while shaking at 200 r.p.m.). Protein expression was induced with 0.5 mM (isopropyl-β-d-thiogalactoside (IPTG) and allowed to continue for 18 h at 18 °C.

Protein Purification

Procedure: Protein was purified from cell lysates using immobilized Ni^{2+} affinity chromatography with gradient elution using imidazole and/or ion-exchange chromatography. His_6 tag was removed by incubation with TEV protease followed by a final-step purification using size-exclusion chromatography in 50 mM HEPES-Na pH 7.5, 500 mM NaCl, 5% (v/v) glycerol, 0.5 mM tris(2-carboxyethyl)phosphine (TCEP). Proteins were concentrated to 10 mg ml^{-1} and were of >95% purity, as determined by SDS–PAGE.
Concentration: 9.74 mg/ml
Mass-spec Verification: Yes

Structure Determination

Crystallization: Crystals were grown by vapour diffusion in 300nl sitting drops containing 200nl protein (pre-incubated with 1mM Pyridine-2,4-dicarboxylic acid) and 100nl reservoir solution composed of 0.05M (NH4)2SO4, 0.05M BIS-TRIS pH 6.5, 30% pentaerythritol ethoxylate.

Data Collection: *Beamline:* SLS-X10; *Resolution:* 2.4 Å
Data Processing: An N-terminally truncated form of MINA53 (amino acids 30–260), comprising the JmjC domain, was used as a search model for MR using PHASER. The two molecules in the asymmetric unit of FLJ21802 were readily located, but the electron density away from the JmjC core of FLJ21802 was ambiguous. Density modification with RESOLVE, as implemented in PHENIX, which took advantage of the two-fold non-crystallographic symmetry (in a $P2_12_12$ space group), led to a marked map improvement and allowed automated model building with BUCCANEER. Refinement was carried out with REFMAC; after several cycles of manual rebuilding with COOT.