Structure    MBD1

PDB Code    5W9Q

Entry clone accession   AF078830

Entry clone source    AU94-F10

Tag    N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgrenlyfqg.

Construct sequence    Mhhhhhhssgrenlyfqg TNRRQNRKCGACAACLRRMDCGRCDFCCDKPKFGGSNQKRQKCRWRQCLQFAMKRLL PS

Vector  pET28-MHL

Expression host E. coli BL21(DE3)-V2R-pRARE2

Growth medium   TB

Growth method   A fresh transformation was used to inoculate 50 mL LB media containing 50 µg/mL kanamycin and 30 µg/mL chloramphenicol  The culture was grown overnight at 37ºC with shaking. The next day this starter culture was used to innoculate 2L of TB growth medium. The culture was grown in LEX at 37ºC to OD600 of 3.1. IPTG-based induction was carried out according to the manufacturer's protocol. The temperature was reduced to 16ºC and the culture was incubated for a further 18 hours before harvesting the cells.

Extraction buffers    Lysis buffer: 20mM,Tris pH 7.5, 500mM NaCl, 5% Glycerol, 0.1% NP40, 1mM PMSF, 5 units/mL Bensonase nuclease, 0.2 mM β-mercaptoethanol.

Extraction procedure   Cells were harvested by centrifugation and pellets were stored in -80ºC. Prior to purification, the cell pellet was resuspended in lysis buffer. Cells were disrupted by sonication (10 minutes) and samples were centrifuged for 60 min at 70000 g.

Purification buffers   NiNTA Elution buffer (EB): 20mM Tris-HCl pH 7.5, 500mM NaCl,  250 mM Imidazole, 1mM  DTT

Gel Filtration buffer: 20mM Tris pH 7.5,150mM NaCl, 1mM  DTT.

Purification procedure  Column 1: Affinity purification, open Ni-NTA column Procedure: The supernatant was incubated with 6mL of 50% slurry Ni-NTA beads by rocking. After 1 hour incubation at 4ºC, the beads were washed with 50 mL of lysis buffer. The protein was eluted using ~20mL EB. The Column 2: Gel Filtration (Superdex S75 16/60 Hi-Load, GE Healthcare). Then the fractions containing protein were identified on a SDS-PAGE gel.

Protein stock concentration    10mg/ml.

Crystallization 25% PEG-400, 0.2 M magnesium chloride, 0.1 M hepes, pH 7.5

Data collection