METTL21B

PDB:4QPN

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:SGC cDNA collection AT17-D9
Entry Clone Source:MGC clone collection
SGC Clone Accession:PBC004-B05
Tag:N-terminal His6-tag, removed before crystallization
Host: Sf9 insect cell

Construct

Prelude:METTL21B:M1-A226
Sequence:

gMADPGPDPESESESVFPREVGLFADSYSEKSQFCFCGHVLTITQNFGSRLGVAARVWDAALSLCNYFESQNVDFRGKKVIELGAGT
GIVGILAALQGGDVTITDLPLALEQIQGNVQANVPAGGQAQVRALSWGIDHHVFPANYDLVLGADIVYLEPTFPLLLGTLQHLCRPH
GTIYLASKMRKEHGTESFFQHLLPQHFQLELAQRDEDENVNIYRARHREPRPA

Vector:pFBOH-MHL

Growth

Medium:
Antibiotics:
Procedure: Plasmid transfer vector pFBOH-MHL containing the target gene was transformed into DH10Bac E.coli cells (Invitrogen) to obtain a recombinant viral DNA. SF9 cells were then transfected with Bacmid DNA using Cellfectin reagent (Invitrogen), and recombinant baculovirus was generated and sequentially amplified from P1 to P3 viral stocks. Sf9 cells grown in HyQ® SFX Insect Serum Free Medium to a density of 4 million cells per milliliter of media and with viability not less than 97 % were infected with 10 mL of P3 viral stock for each 0.8L of cell culture. Cells were harvested by centrifugation (4000 RPM, 15 minutes) after 72 hours of incubation on a shaker at 100 RPM and 27 °C. Harvested cells were washed with cold PBS buffer, then flash frozen in liquid nitrogen and stored at -80°C until purified.

Purification

Procedure
The cell lysate was centrifuged to remove insoluble material, and the supernatant was loaded onto a DEAE-cellulose (DE52, Whatman, MA, USA) anion-exchange resin followed by a nickel-NTA agarose column (Qiagen, MD, USA). Bound proteins were eluted with Elution buffer. The eluted

sample was mixed with TEV in a 1:4 molar ratio (TEV to protein) and then dialyzed and subjected to anion-exchange chromatography using a HiTrap Q column (GE Healthcare, NJ, USA) previously equilibrated with the Ion Exchange A buffer. Protein was eluted with a linear gradient of 0-500 mM NaCl using Ion Exchange B buffer, and further purified by size exclusion chromatography on a Superdex 75 16/60 column (GE Healthcare, NJ, USA). The peak fractions containing target protein were pooled, concentrated using amicon centrifugal filter and stored at -80 °C before crystallization. The purity and molecular weight of the final protein sample were confirmed by SDS-PAGE and LC-MS, respectively.

Extraction

Procedure
Frozen cells were thawed and suspended in 150 mL extraction buffer and lysed using a Microfluidizer at 13,000 psi. The lysate was then clarified by centrifugation at ~38000 x g (15,500 rpms) for 1 hour.
Concentration:20 mg/mL
Ligand
S-Adenosyl-L-homocysteine (SAH)**MassSpec:**cut version, expected is 24968 Da and the measured value was 24969.9 Da
Crystallization: Protein sample was incubated with SAH (final concentration of 5mM) for overnight and then set up using SGC and Red Wing (RW) screen conditions at room temperature. Crystals were initially found in RW screen condition C08 (20% PEG3350 and 0.2M Tri-Lithium Citrate) and used for structure determination.

Mineral oil : Paratone oil = 50 : 50
NMR Spectroscopy:
Data Collection:
Data Processing: