

METTL18

PDB:4RFQ

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:SGC cDNA collection AT6-C11

Entry Clone Source:MGC clone collection

SGC Clone Accession:PBC004-E03

Tag:N-terminal His6-tag, removed before crystallization

Host:BL21-V2R-pRARE2

Construct

Prelude:METTL18 :H63-G372

Sequence:

gHKSMENAAPSQDTSPLSAASSSRNLEPHGKQPSLRAAKEHAMPKDLKKMLENKVIETLPGFQHVKLSVVKTIILLKENFPYEGGLK
IWECTFDLLAYFTKAKVKFAGKKVLDLGC GSGLLGITAFKGG SKEIHFQDYNMVIDEVL PNVVANSTLEDEENDVNEPDVKRCRK
PKVTQLYKCRFFSGEWSEFCKLVLSSEKLFVKYDLILTSETIYNPDYYSNLHQTFRLRLSKNGRVLLASKAHYFGVGGGVHLFQKFV
EERDVFKTRILKIIDEGLKRFIIEITFKFPG

Vector:pET28-MHL

Growth

Medium:

Antibiotics:

Procedure:LEX Bubbling. The target protein was expressed in E. coli by inoculating each 50 mL of overnight culture grown in Luria-Bertani medium into a 2 L of Terrific Broth medium in the presence of 50 mg/mL kanamycin and 30 mg/mL chloramphenicol at 37 °C. When OD600 reached ~3.0, the temperature of the medium was lowered to 18 °C and the culture was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before harvested and then flash frozen in liquid nitrogen and stored at -80 degree 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 cation-exchange chromatography using a HiTrap SPcolumn (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 16,000 psi. The lysate was then clarified by centrifugation at ~38000 x g (15,500 rpms) for 1 hour.

Concentration: 37.5 mg/mL

Ligand

S-Adenosyl-L-homocysteine (SAH) **MassSpec:** cut version, expected is 33021.1 Da and the measured value was 33022.5 Da

Crystallization: Protein sample was incubated with SAH (final concentration of 5mM) for overnight and set up using SGC and Red Wing (RW) screen conditions at room temperature. Crystals were initially found in RW screen condition G01 (2.0M (NH₄)₂SO₄, 0.2 K/Na tartrate, 0.1M Na-Citrate pH5.6, 5mM Taurine) and used for structure determination.

Mineral oil : Paratone oil = 50 : 50

NMR Spectroscopy:

Data Collection:

Data Processing: