CAMKMT

PDB:4PWY

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:SGC cDNA collection AT72-H7
Entry Clone Source:MGC clone collection
SGC Clone Accession:PBC002-A04
Tag:N-terminal His6-tag, removed before crystallization
Host:BL21-V2R-pRARE2

Construct

Prelude:CAMKMT:S61-G323
Sequence:

gSVRRFESFNLFSVTEGKERETEEEVGAWVQYTSIFCPEYSISLRHNSGSLNVEDVLTSFDNTGNVCIWPSEEVLAYYCLKHNNIFR
ALAVCELGGGMTCLAGLMVAISADVKEVLLTDGNEKAIRNVQDIITRNQKAGVFKTQKISSCFLRWDNETDVSQLEGHFDIVMCADC
LFLDQYRASLVDAIKRLLQPRGKAMVFAPRRGNTLNQFCNLAEKAGFCIQRHENYDEHISNFHSKLKKENPDIYEENLHYPLLLILT
KHG

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 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 16,000 psi. The lysate was then clarified by centrifugation at ~38000 x g (15,500 rpms) for 1 hour.
Concentration:18 mg/mL
Ligand
S-Adenosyl-L-homocysteine (SAH)**MassSpec:**cut version, expected is 29984 Da and the measured value was 29984.6 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 SGC screen condition D11 (2.4 M Malonate, pH 7.0) and further optimized under the condition with 2.0 M Malonate pH 6.7 using a hanging drop method.

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