MTMR6

PDB:2YF0

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_004676
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:MTMR6A-k040
Tag:C-terminal hexahistidine tag -ahhhhhh
Host:*E.coli* BL21(DE3) R3 pRARE, where R3 denotes a derivative of BL21(DE3) resistant to a strain of T1 bacteriophage (SGC Oxford) and the pRARE plasmid originating from the Rosetta strain (Novagen) supplies tRNAs for rare codons.

Construct

Prelude:
Sequence:

MEHIRTTKVEQVKLLDRFSTSNKSLTGTLYLTATHLLFIDSHQKETWILHHHIASVEKLALTTSGCPLVIQCKNFRTVHFIVPRERD
CHDIYNSLLQLSKQAKYEDLYAFSYNPKQNDSERLQGWQLIDLAEEYKRMGVPNSHWQLSDANRDYKICETYPRELYVPRIASKPII
VGSSKFRSKGRFPVLSYYHQDKEAAICRCSQPLSGFSARCLEDEHLLQAISKANPVNRYMYVMDTRPKLNAMANRAAGKGYENEDNY
SNIRFQFVGIENIHVMRSSLQKLLEVNGTKGLSVNDFYSGLESSGWLRHIKAVMDAAVFLAKAITVENASVLVHCSDGWDRTSQVCS
LGSLLLDSYYRTIKGFMVLIEKDWISFGHKFSERCGQLDGDPKEVSPVFTQFLECVWHLTEQFPQAFEFSEAFLLQIHEHIHSCQFG
NFLGNCQKEREELKLKEKTYSLWPFLLEDQKKYLNPLYSSESHRFTVLEPNTVSFNFKFWRNMYHQFDRTahhhhhh

Vector:pNIC-CH2

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 20 ml TB supplemented with 8 g/l glycerol, 100 µg/ml kanamycin and 34 µg/ml chloramphenicol at 30 °C overnight. The overnight culture (20 ml) was used to inoculate 1.5 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and approximately 200 µl 204 Antifoam A6426 (Sigma). The culture was grown in a LEX bioreactor system (Harbinger Biotechnology) at 37 °C until OD600 reached ~2. The culture was down-tempered to 13 °C over a period of 1 hour before target expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight and cells were harvested the following morning by centrifugation (4,400 x *g*, 10 min, 4 °C). The resulting cell pellet (82.1 g wet cell weight) was resuspended in lysis buffer (1.5 ml/g cell pellet), supplemented with 2000 U Benzonase (Merck) and one tablet of Complete EDTA-free protease inhibitor (Roche Applied Science). The cell suspension was stored at -80 °C.

Purification

Procedure
Columns
IMAC: Ni-charged 5 ml HiTrap Chelating HP (GE Healthcare)
Gel filtration column: HiLoad 16/60 Superdex 200 Prep Grade (GE Healthcare)

Procedure
Purification of the protein was performed as a two step process on an ÄKTAxpress system (GE Healthcare). Prior to purification, columns were equilibrated with IMAC wash1 buffer and gel filtration buffer, respectively. The filtered lysate was loaded onto the Ni-charged HiTrap Chelating column and washed with IMAC wash1 buffer followed by IMAC wash2 buffer. Bound protein was eluted from the IMAC column with IMAC elution buffer and automatically loaded onto the gel filtration column. Fractions containing the target protein were pooled and fresh TCEP was added to a final concentration of 2 mM. The protein was subsequently concentrated using an Amicon Ultra-15 centrifugal filter device, 30,000 NMWL (Millipore) to 36 mg/ml in a volume of 0.95 ml. The detected mass (59606 Da) was very close to the expected mass (59603 Da) and the protein identity therefore confirmed by mass spectrometry.

Extraction

Procedure
The cell suspension was quickly thawed in water. Cells were disrupted by sonication (Vibra-Cell, Sonics) at 80% amplitude for 3 min effective time (pulsed 4s on, 4s off) and cell debris was removed by centrifugation (49,000 x *g*, 20 min, 4 ºC). The supernatant was decanted and filtered through a 0.45 µm flask filter.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained by the sitting drop vapour diffusion method in a 96-well plate. 0.1 µl protein solution (19 mg/ml) was mixed with 0.1 µl of well solution consisting of 0.1 M Bis-Tris pH 5.5 and 2 M ammonium sulfate. The plate was incubated at 4 ºC and crystals appeared within 5 days. The crystals were quickly transferred to a cryo solution consisting of 2.1M ammonium sulfate, 0.1M Bis-Tris pH 5.0, 25% Glycerol, and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Final structure refinement done with 2.65 Å resolution data collected at DIAMOND, beamline I04. Three wavelength MAD data to 2.7Å collected at BESSY BL14-1.
Data Processing: The structure was solved by a combination of low resolution MAD phasing using autoSHARP and subsequent molecular replacement using the MTMR2 (PDB: 1ZSQ) structure and MOLREP. The space group was $P6_4$ 2 2 with cell dimensions a=b=85.73 Å and c=448.4 Å. One monomer were located in the asymmetric unit. Coot was used for model building and at the final stages BUSTER was used for refinement with a single TLS group using data in the 30-2.65 Å resolution interval. The final R values were: R=25.7% and Rfree=28.8%. Coordinates for the crystal structure were deposited in the Protein Data Bank, accession code 2YF0.