NUDT6

PDB:3H95

Revision

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Revised by:created
Revision Date:created
Entry Clone Accession:gi|14602646, BC009842
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:NUDT6A-k041
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*sm
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:

mhhhhhhssgvdlgtenlyfqsmSHQVGVAGAVFDESTRKILVVQDRNKLKNMWKFPGGLSEPEEDIGDTAVREVFEETGIKSEFRS
VLSIRQQHTNPGAFGKSDMYIICRLKPYSFTINFCQEECLRCEWMDLNDLAKTENTTPITSRVARLLLYGYREGFDKIDLTVEELPA
VYTGLFYKLYHKELPENYKTMKGID

Vector:pNIC-Bsa4

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 for 4 hours. The culture was down-

tempered to 18 °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 (34 g wet cell weight) was resuspended in lysis buffer (1.5 ml/g cell pellet), supplemented with 1000 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 1 ml HiTrap Chelating HP (GE Healthcare)
Gel filtration column: HiLoad 16/60 Superdex 75 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 two HiTrap Chelating columns connected in series and washed with IMAC wash1 buffer followed by IMAC wash2 buffer. Bound protein was eluted from the IMAC columns 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, 10,000 NMWL (Millipore) to 36.7 mg/ml in a volume of 250µl. The identity of the protein was 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
None**MassSpec:**
Crystallization: Crystals were obtained by the hanging drop vapour diffusion method in a 24-well plate containing 500 µl of well solution. Protein solution was diluted to 18.3 mg/ml in GF buffer with 2 mM TCEP. Then it was incubated 15min on ice with 5 mM magnesium chloride. 2 µl of this solution was mixed with 1 µl of well solution consisting of 0.4 M tri-ammonium citrate and 10% PEG 3350. The plate was incubated at 20 ºC and crystals appeared within 5 days. The crystals were quickly transferred to cryo solution containing 0.2 M di-ammonium hydrogen citrate, 0.3 M NaCl, 22% PEG 3350 and 20% glycerol and flash frozen in liquid nitrogen.

NMR Spectroscopy:
Data Collection: Diffraction data to 1.7 Å resolution was collected at ESRF beamline ID23-1.
Data Processing: The structure was solved by molecular replacement using the structure of Ndx1 from T. thermophilus as template (PDB: 1VCD). The space group was P41 21 2 with cell dimensions a=b=54.53 Å c=133.71 Å. One monomer was located in the asymmetric unit. ArpWarp was used for completing the model. Iterative cycles of manual improvement and refinement were made respectively in Coot and Refmac. Data in the interval 19.08-1.7 Å resolution was used and at the end of the refinement the R values were: R=19.6% and Rfree=22.1%. Coordinates for the crystal structure were deposited in the Protein Data Bank, accession code 3H95.