NUDT16L1

PDB:3KVH

Revision

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Entry Clone Accession:gi|13623247
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:NUDT16L1A-k017
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:

MVPELKQISRVEAMRLGPGWSHSCHAMLYAANPGQLFGRIPMRFSVLMQMRFDGLLGFPGGFVDRRFWSLEDGLNRVLGLGLGCLRL
TEADYLSSHLTEGPHRVVAHLYARQLTLEQLHAVEISAVHSRDHGLEVLGLVRVPLYTQKDRVGGFPNFLSNAFVSTAKCQLLFALK
VLNMMPEEKLVEALAAATEKQKKALEKLLPASSahhhhhh

Vector:pNIC-CH2

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 70 ml TB supplemented with 8 g/l glycerol, 100 µg/ml kanamycin and 34 µg/ml chloramphenicol at 37 ºC overnight. The overnight culture was used to inoculate three 2 l bottles, each containing 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 ºC until OD600 reached ~2. The culture was down-tempered to 18 ºC 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 (4,430 x *g*, 10 min, 4 ºC). The resulting cell pellet (83 g wet cell weight) was resuspended in lysis buffer (1.5 ml/g cell pellet), supplemented with 6000 U Benzonase (Merck) and three tablets of Complete EDTA-free protease inhibitor (Roche Applied Science). The cell suspension was stored at -80 ºC.

Purification

Procedure
Columns
IMAC: Ni-charged 5 ml HiTrap Chelating HP (GE Healthcare)
Gel filtration column: HiLoad 16/60 Superdex 75 Prep Grade (GE Healthcare)

Procedure
IMAC columns were equilibrated with IMAC wash1 buffer, and gel filtration columns were equilibrated with GF buffer. Purification of the protein was performed on an ÄKTAxpress system (GE Healthcare). 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 identified by SDS-PAGE, pooled, and fresh TCEP was added to a final concentration of 2 mM. The protein was concentrated using an Vivaspin 20 centrifugal filter device (10,000 MWCO; Vivascience) to 10.95 mg/ml in a volume of 3.6 ml. 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). Supernatant was then frozen and stored at -80C. After thawing, it 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. 0.2 µl protein solution (10.95 mg/ml) was mixed with 0.1 µl of well solution consisting of 0.1 M Hepes pH 7.5 and 20% (v/v) Jeffamine M-600. The plate was incubated at 20 ºC and crystals (650 µM long plates) appeared in one night. The crystals were quickly transferred to a cryo solution consisting of 0.1 M Hepes pH 7.6, 22% Jeffamine M-600, 20% Glycerol and 0.3 M NaCl, and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data to 1.7 Å resolution was collected at MAX-LAB, beamline I911-3.

Data Processing: The structure was solved by molecular replacement using NUDT16 as template (PDB: 3COU). The space group was C2 with cell dimensions a=68.81 Å b=60.278 Å c=60.44 Å, β=118.64°. One monomer was located in the asymmetric unit. Model building was initially performed using Arp/wARP, then by iterative cycles of manual improvment of the model in Coot and retrained refinement with Refmac5. Data in the interval 27.0-1.70 Å resolution was used and at the end of the refinement the R values were: R=18.5% and Rfree=21.7%. Coordinates for the crystal structure were deposited in the Protein Data Bank, accession code 3KVH.