NUDT10

PDB:3MCF

Revision

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Entry Clone Accession:BC050700
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:NUDT10A-s001
Tag:C-terminal hexahistidine tag starting with an additional alanine
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:

MKKRAACLCFRSEREDEVLLVSSSRYPDRWIVPGGGMEPEEEPGGAAVREVYEEAGVKGKLGRLLGVFEQNQDPEHRTYVYVLTVTE
LLEDWEDSVSIGRKREWFKVEDAIKVLQCHKPVHAEYLEKLKahhhhhh

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 30 °C overnight. The overnight culture (70 ml) was used to inoculate 3 bottles of 1.5 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and approximately 200 µl 204 Antifoam A6426 (Sigma). Cultures were grown in a LEX bioreactor system (Harbinger Biotechnology) at 37 °C until OD_{600} reached ~2.

Cultures were 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 (117.9 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 °C.

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 Amicon Ultra-15 centrifugal filter device (5,000 NMWL; Millipore) to 13.2 mg/ml in a volume of 0.2 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). 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. 0.2 µl protein solution (13.2 mg/ml) was mixed with 0.1 µl of well solution consisting of 0.01 M Na acetate trihydrate pH 4.6, 0.1 M tri-Na citrate dihydrate pH 5.5, 0.12 M tri-Na citrate dihydrate unbuffered and 19.8% w/v PEG3000 . The plate was incubated at 4 ºC and crystals appeared within 5 days. The crystals were quickly transferred to a cryo solution consisting of 21% w/v PEG3000, 0.22 M tri-Na citrate dihydrate pH 5.5, 0.01 M Na acetate trihydrate pH 4.6, 25% glycerol, and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data to 2.0 Å resolution was collected at BESSY, beamline BL14.2.
Data Processing: The structure was solved by molecular replacement using as template a model

produced by Swiss-Model based upon MS0616 (PDB: 2DUK). The space group was P212121 with cell dimensions a=56.39 Å b=79.55 Å c=88.73 Å. After automatic model building carried on in ArpWarp, two monomers were located in the asymmetric unit. Iterative cycles of manual model building in Coot and refinement in PHENIX led to the final model. Data in the interval 32.5-2.0 Å resolution was used and at the end of the refinement the R values were: R=17.5% and Rfree=21.7%. Coordinates for the crystal structure were deposited in the Protein Data Bank, accession code 3MCF.