NUDT18

PDB:3GG6

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession:BC016902
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*sm
Host: *E.coli* BL21(DE3) (Novagen)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*smSAPAGEPPAPVRLRKNVCYVVLAVFLSEQDEVLLIQEAKRECRGSWYLPAGRMEPGETIVEAL
QREVKEEAGLHCEPETLLSVEERGPSWVRFVFLARPTGGILKTSKEADAESLQAAWYPRTSLPTPLRAHDILHLVELAAQYRQQARH
PLIL

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 10 ml TB supplemented with 8 g/l glycerol, 100 µg/ml kanamycin at 30 °C overnight. The overnight culture (10 ml) was used to inoculate 0.75 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 ~3. The bottle 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 (13.4 g wet cell weight) was resuspended in lysis buffer (2.0 ml/g cell pellet), supplemented with 1000 U Benzonase (Merck) and 0.5 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 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.

Tag removal
The N-terminal histidine tag was proteolytically removed by incubating the target protein with His-tagged TEV protease at a molar ratio of 15:1 at 4 ºC overnight. The proteolytic reaction went to completion, as judged by SDS-PAGE. 20 mM imidazole were added to the reaction mixture. The target protein was then purified from tag and protease by first incubating the protein sample with 1 ml Ni-agarose (Qiagen), pre-equilibrated with GF buffer containing 20 mM imidazole. After 18 hours incubation at 4 ºC the protein:agarose mix was loaded onto a gravity column. The flow through containing TEV-cleaved target protein was collected and concentrated using a Vivaspin 20 centrifugal filter device with 10,000 MWCO (Sartorius) to 35.6 mg/ml in a volume of 0.18 ml and stored at -80 ºC. 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. The protein was diluted with GF buffer, containing 2 mM TCEP, to 15 mg/ml after addition of 5 mM of MgCl2 and 2´deoxyguanosine.The mix was incubated on ice for 15 minutes. 0.1 µl of

the protein solution was mixed with 0.2 µl of well solution consisting of 0.1 M tri-sodium citrate dihydrate, pH 5.5 and 20% (w/v) PEG3000. The plate was incubated at 20 ºC and rod-shaped crystals appeared after 11 days. The crystal was transferred to cryo solution consisting of 0.1 M tri-sodium citrate dihydrate, pH 5.5, 22% PEG3000, 0.3 M NaCl and 25% glycerol, and flash-frozen in liquid nitrogen.

NMR Spectroscopy:

Data Collection:217˚ with an oscillation range of 1˚ were collected on Diamond (I03) on a crystal diffracting to 2.1Å.

Data Processing: Data were processed in P21 21 21 using XDS and XSCALE. Molecular replacement solution was found by PHASER using the PDB entry 2B0V as a probe. Cycles of manual model building in COOT, automated model building in ARP-WARP and refinement in PHENIX led to the deposited model with R and Rfree values of 17% and 23% respectively.