NUMBL

PDB:3F0W

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:NUMBLA-k019
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:

mhhhhhhssgvdlgtenlyfq*smASRPHQWQADEDAVRKGTCSFPVRYLGHVEVEESRGMHVCEDAVKKLKAMGRKSVKSVLWVSA
DGLRVVDDKTKDLLVDQTIEKVSFCAPDRNLDKAFSYICRDGTTRRWICHCFLALKDSGERLSHAVGCAFAACLERKQRREK

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 37 °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 0.2 ml Dow Corning anti-foam RD emulsion 63213 4D (BDH Silicone Products). The culture was grown in a LEX bioreactor system (Harbinger Biotechnology) at 37 °C until OD600 reached ~2. 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 (15.2 g wet cell weight) was resuspended in lysis buffer (2 ml/g cell pellet), supplemented with 2000 U Benzonase (Merck) and 1 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)

Purification:
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 with 10,000 NMWL (Millipore) to 13.4 mg/ml in a volume of 1.8 ml.

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 (49,00 x *g*, 20 min, 4 ºC). The supernatant was decanted and filtered through a 0.45 µm flask filter.
Concentration:
Ligand
MassSpec:
Crystallization: The crystal was obtained by the hanging drop vapour diffusion method using a 24-well plate containing 500 µl well solution. 1 µl of the protein solution (13.4 mg/ml) was mixed with 1 µl of well solution consisting of 0.1 M sodium acetate trihydrate, pH 4.7, 0.1 M lithium sulfate monohydrate and 24% PEG 8000. The plate was incubated at 4 ºC and crystals appeared within 2 days. The crystal was quickly transferred to cryo solution containing 0.1 M sodium acetate trihydrate, pH 4.5, 0.1 M lithium sulfate monohydrate, 30% PEG-8000 and 17 % glycerol and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data to 2.7 Å resolution was collected from a single crystal at ESRF (ID23-1). The crystal belonged to space group P3221 with cell parameters of a=b=55.1 Å and c=99.4 Å. Data was 99.1% complete and has Rmerge of 6.6%.
Data Processing: The structure was solved by molecular replacement using MOLREP with NMR model of mouse Numb protein (1WJ1) as a search model. The asymmetric unit consisted of one polypeptide chain. Structure was refined with Phenix and finally with REFMAC5. Three TLS groups were used for the protein chain according to suggestion of TLSMD server. Final model has a good geometry as analyzed with Molprobity and final R-values were R=24.2% and Rfree=28.1%. The coordinates and structure factors were deposited in PDB with accession code 3F0W.