TNKS1

PDB:2RF5

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC098394
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m)
Host:*E.coli* Rosetta2(DE3) (Novagen)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*sMQGTNPYLTFHCVNQGTILLDLAPEDKEYQSVEEEMQSTIREHRDGGNAGGIFNRYNVIRIQKV
VNKKLRERFCHRQKEVSEENHNHHNERMLFHGSPFINAIIHKGFDERHAYIGGMFGAGIYFAENSSKSNQYVYGIGGGTGCPTHKDR
SCYICHRQMLFCRVTLGKSFLQFSTIKMAHAPPGHHSVIGRPSVNGLAYAEYVIYRGEQAYPEYLITYQIMKPEAPSQTATAAEQ

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were streaked onto LB-agar plates. 5-10 colonies were used to inoculate 20 ml TB supplemented with 8 g/l glycerol, 100 µg/ml kanamycin and 34 µg/ml chloramphenicol. The cells were grown at 30 ºC 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 PPG P2,000 81380 anti-foam solution (Fluka). 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 (5,500 x *g*, 10 min, 4 ºC). The resulting cell pellet (38.2 g wet cell weight) was resuspended in lysis buffer (2 ml/g cell pellet), supplemented with one tablet of Complete EDTA-free protease inhibitor (Roche Applied Science). The cell suspension was stored at -80 ºC.

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 and fresh TCEP was added to a final concentration of 2 mM. The protein was subsequently concentrated using a Amicon Ultra-15 centrifugal filter device, 10,000 NMWL (Millipore) to 22.8 mg/ml in a volume of 0.28 ml. The identity of the protein was confirmed by mass spectrometry.

Extraction

Procedure
The cell suspension was quickly thawed in water and 2500 U Benzonase (Merck) was added. 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 (49,100 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 (22.8 mg/ml) was mixed with 0.1 µl of well solution consisting of 0.18 M Mg-formate pH 5.9, 3% 1,6-hexanediol, 18% PEG-3350. The plate was incubated at 20 ºC and a rod shaped crystals appeared in three days. The crystal was quickly transferred to a cryo solution consisting of well solution complemented with 0.3 M NaCl and 20% glycerol and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data to 2.3 Å resolution was collected at BESSY beamline 14.1. As data contained intense ice rings a lower resolution dataset from identically grown crystal was also collected. After removal of ice rings two datasets were scaled together. Data were processed with XDS in space group I212121 (a=79.95 Å, b=81.24 Å and c=82.72 Å).
Data Processing: The structure was solved by molecular replacement with MOLREP using the PARP12 (PDB code 2PQF) model with truncated side chains as a search model. Model building was done with COOT and refinement with REFMAC5. TLS refinement with 4 TLS groups was used in the refinement process. At the end of the refinement the R values were: R= 19.3% and Rfree= 25.0%.