TNKS2

PDB:3MHK

Revision

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Entry Clone Accession:NM_025235
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SGC Clone Accession:TNKS2A-s001
Tag:C-terminal hexahistidine tag: ahhhhhh
Host:*Escherichia coli* BL21(DE3) R3 pRARE

Construct

Prelude:
Sequence:

MGTILIDLSPDDKEFQSVEEEMQSTVREHRDGGHAGGIFNRYNILKIQKVCNKKLWERYTHRRKEVSEENHNHANERMLFHGSPFVN
AIIHKGFDERHAYIGGMFGAGIYFAENSSKSNQYVYGIGGGTGCPVHKDRSCYICHRQLLFCRVTLGKSFLQFSAMKMAHSPPGHHS
VTGRPSVNGLALAEYVIYRGEQAYPEYLITYQIMRPEGMVDGahhhhhh

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Fresh overnight cultures of *E. coli* strain BL21(DE3) R3 pRARE cells (including 100 µg/ml kanamycin and 34 µg/ml chloramphenicol) transformed with TNKS2 expression construct were used to inoculate 4.5 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and anti-foam 204 in three 2-liter flasks. Cells were grown in a large scale expression system (Harbinger Biotechnology and Engineering) at 37°C until the OD600 reached ~2. The culture was down-tempered to 18°C for 1 h. Expression of TNKS2 was induced by adding 0.5 mM IPTG and growth continued over night at 18°C. Cells were harvested by centrifugation at 4400 x *g* for 10 min. The pellet (71g wet cell weight) was resuspended in 100ml lysis buffer supplemented with Complete EDTA-free Protease Inhibitor (Roche Biosciences) and benzonase Suspended cells were stored at -80°C until further use.

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
Purification of the protein was performed on an ÄKTAxpress system (GE Healthcare). Prior to purification, IMAC column was equilibrated with IMAC wash1 buffer and gel filtration column with gel filtration buffer. The filtered lysate was loaded onto the IMAC column, and therafter 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. Eluting fractions were analyzed by SDS-PAGE and target protein was pooled. Fresh TCEP was added to a final concentration of 2 mM. Purified protein was concentrated to 11.3 mg/ml using an Amicon Ultra-15 centrifugal filter device (Millipore, 10,000 MWCO). The starting methionine was cleaved off according to 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 (49000 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 (7.1 mg/ml) including 2-(2-pyridyl)-7,8-dihydro-5h-thiino[4,3-d]pyrimidin-4-ol (5:1 ligand:protein ratio) was mixed with 0.1 µl of well solution consisting of 1.34M K2H-phosphate and 0.06M NaH2-phosphate. The plate was incubated at 4 ºC. Crystals appeared after two weeks and continued to grow for six more weeks. Crystals were quickly transferred to a cryo solution consisting of 1.2M KH2PO4, 30% Glycerol, 0.2M NaCl and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data to 2.3 Å resolution was collected at ESRF beamline ID-29 using wavelength 0.97908Å.
Data Processing: Data were indexed and integrated in space group $P3_121$ using XDS software. The structure was solved by molecular replacement using the human tankyrase-2 apo structure (pdb: 3kr7) as model template. Molrep was used to solve the structure. The asymmetric unit contained one protein monomer. The cell dimensions are a = b = 92.5Å, c = 53.5Å. Refmac5 was used for refinement and Coot for model building. Data in the interval 46.2-2.3 Å resolution were used and refined to R = 17.95% and R_{free} = 22.73%. Coordinates for the crystal structure were deposited in the Protein Data Bank, with accession code 3mhk.