SNTB2

PDB:2VRF

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:IMAGE:5805707
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal, TEV cleavable hexahistidine tag. Tag sequence: mhhhhhhssgvdlgtenlyfq(*)sm
Host:E. coli BL21(DE3)-R3

Construct

Prelude:
Sequence:

smPVRRVRVVKQEAGGLGISIKGGRENRMPILISKIFPGLAADQSRALRLGDAILSVNGTDLRQATHDQAVQALKRAGKEVLLEVKF
IREVNTVV

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:10 µl of BL21(DE3)-R3 glycerol stock were inoculated into 5ml of TB with 50 µg/ml of kanamycin and 34 µg/ml chloramphenicol and grown overnight at 37°C, 200rpm. 10ml of overnight culture were added to 1L of TB with 50ug/ml kanamycin and incubated at 37°C, 160rpm. After the OD600 reached 1.2, the temperature was dropped to 18°C and 500ul of 1M IPTG was added to the final concentration of ~0.5mM. The culture was then incubated with shaking overnight at 18°C, 160rpm. The following morning the 3L culture was harvested and centrifuged for 10min at 4000rpm. Supernatant was discarded and cell pellets were resuspended in 60ml of a lysis buffer and frozen at -80°C.

Purification

Procedure
Column 1: Ni-NTA, 3mlBuffers: Binding buffer: 50mM HEPES pH 7.5, 500mM NaCl, 20mM Imidazole, 5% glycerol, 1mM PMSF, 0.5mM TCEP; Washing buffer: 50mM HEPES pH 7.5, 500mM NaCl, 40mM Imidazole, 5% glycerol, 1mM PMSF, 0.5mM TCEP; Elution buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 250mM Imidazole, 0.5mM TCEP.Procedure: The column was packed with the Ni-NTA slurry and equilibrated with 15ml of binding buffer. The

supernatant was loaded onto the column and the flow through was collected. The column was washed with 30ml of binding buffer and then 30ml of washing buffer. The protein was eluted with 6ml of elution buffer and applied to the GF columnColumn 2: Superdex 200, HiPrep 16/60 (Amersham)GF buffer: 10mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 0.5mM TCEPProcedure: Gel filtration was carried out on AKTA Purifier. The extinction at 280nm was monitored and fractions were collected and analyzed by SDS-PAGE. Positive fractions were pooled for TEV cleavage. TEV cleavage: The His-tag was cleaved with 1 mg TEV per 40 mg target protein at 4°C overnight. The protein was purified on Ni-NTA using buffers as above. Protein was characterised by the mass spec

Extraction

Procedure
Lysis buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5mM Imidazole, 5% glycerol + EDTA-free Complete (1 tablet/50ml). The thawed cells were broken by sonication (5min, 10s on-pulse, 20s off-pulse) followed by centrifugation for 60 min at 20.000rpm.
Concentration: Concentration and buffer exchange: Using Amicon Ultra-15 concentrators with 10kDa cutoff, the sample was concentrated to 45mg/ml. Concentrations were determined from the absorbance at 280nm using NanoDrop.
Ligand
MassSpec: Calculated mass of the construct was 10329Da. The exact mass was confirmed by the mass spec.
Crystallization: Crystals were grown by vapor diffusion at 20°C in 300nl sitting drops. The drops were prepared by mixing 200nl of protein solution and 100nl of precipitant consisting of 0.1M HEPES pH 7.0 and 30% jeffamine M-600 pH 7.0. Crystals were transferred to a cryo-protectant consisting of 15% ethylene glycol and 85% well solution before flash-cooling in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Resolution: 1.95Å, X-ray source: SLS beam X10SA
Data Processing: