SNX17

PDB:3FOG

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC050590
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:SNX17A-k036
Tag:C-terminal hexahistidine tag: ahhhhhh
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:

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MHFSIPETESRSGDSGGSAYVAYNIHVNGVLHCRVRYSQLLGLHEQLRKEYGANVLPAFPPKKLFSLTPAEVEQRREQLEKYMQAVR
QDPLLGSSETFNSFLRRAQQEahhhhhh
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Vector:pNIC-CH2

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 15 ml TB supplemented with 8 g/l glycerol, 100 µg/ml kanamycin and 34 µg/ml chloramphenicol at 30 °C overnight. The overnight culture (15 ml) was used to inoculate 750 ml 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 ~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 (14.5 g wet cell weight) was resuspended in lysis buffer (2 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 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, 5,000 NMWL (Millipore) to 14.9 mg/ml in a volume of 1.2 ml. The identity of the protein was confirmed by mass spectrometry.

Extraction

Procedure
The cell suspension was quickly thawed in water and diluted with lysis buffer to a final volume of 68 ml. 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. 0.4 µl protein solution (14.9 mg/ml) was mixed with 0.2 µl of well solution consisting of 0.1 M bis-Tris propane, pH 7.5, 0.2 M sodium fluoride and 20% PEG 3350. The plate was incubated at 4 ºC and crystals appeared in two days. The crystals were quickly transferred to a cryo solution consisting of well solution complemented with 20% glycerol and 0.3 M NaCl, and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data to 2.8 Å resolution was collected from a single crystal at BESSY (BL14-2). The crystal belonged to space group P321 with cell parameters of a=89.89 Å, b=89.89 Å, c=39.26 Å, α= 90°, β=90°, γ= 120°.
Data Processing: The structure was solved by molecular replacement using MOLREP with the cytokine-independent survival kinase CISK-PX (1XTE) as a search model. The asymmetric unit consisted of one polypeptide chain. Structure was refined with PHENIX. Final R-values were R=23.5% and Rfree=27.9%. The coordinates and structure factors were deposited to PDB with accession code 3FOG.