STARD13

PDB:2PSO

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|28302262
Entry Clone Source: In-house cloning
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m).
Host:pNIC-Bsa4

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*sMEESGATFHTYLNHLIQGLQKEAKEKFKGWVTCSSTDNTDLAFKKVGDGNPLKLWKASVEVEAP
PSVVLNRVLRERHLWDEDFVQWKVVETLDRQTEIYQYVLNSMAPHPSRDFVVLRTWKTDLPKGMCTLVSLSVEHEEAQLLGGVRAVV
MDSQYLIEPCGSGKSRLTHICRIDLKGHSPEWYSKGFGHLCAAEVARIRNSFQPLIAEGPETKI

Vector:*E.coli* Rosetta 2(DE3) (Novagen)

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 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, 34 µg/ml chloramphenicol and approximately 200 µl BREOX FMT 30 anti-foam solution (Cognis Performance Chemicals UK Ltd)). The culture was grown in a LEX bioreactor system (Harbinger Biotechnology) at 37 ºC until OD600 reached ~2.2. 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 (28.5 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 an Amicon Ultra-15 centrifugal filter device, 10,000 NMWL (Millipore) to 4.3 mg/ml in a volume of 1.4 ml. Some precipitation was noted during concentration. The identity of the protein was confirmed by mass spectrometry.

Extraction

Procedure
The cell suspension was quickly thawed in water and 2000 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. Because some precipitation of salts was observed, the sample was diluted with 50 ml IMAC wash1 buffer, filtered again, and diluted with 25 ml water.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained by the hanging drop vapour diffusion method in a 24-well plate containing 500 µl well solution. 0.8 µl of the protein solution (4.3 mg/ml) was mixed with 0.4 µl of well solution consisting of 0.1 M Tris-HCl pH 8.0, 0.125 M NaCl and 20 % MPD. The plate was incubated at 4 ºC and crystals appeared within three days. The crystal was picked up with a loop and frozen directly in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data was collected at the ESRF beamline ID14-4 and processed with XDS in space group P43 (a=b=78.24 Å, c=212.72 Å).
Data Processing: The structure was solved by molecular replacement using PDB entry 1JSS as an input to MRBUMP. The model that was edited with CHAINSAW produced the best solution with PHASER. At this stage the model consisted of two monomers in the asymmetric unit. After rigorous editing of the models and refinement with PHENIX a third molecule was located with MOLREP using partially refined monomer as an input. Model building was done with COOT and REFMAC5 was used in the final refinement cycles.