FIGNL1

PDB:3D8B

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC051867
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*sm
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:

mhhhhhhssgvdlgtenlyfq*smVPPIPKQDGGEQNGGMQCKPYGAGPTEPAHPVDERLKNLEPKMIELIMNEIMDHGPPVNWEDI
AGVEFAKATIKEIVVWPMLRPDIFTGLRGPPKGILLFGPPGTGKTLIGKCIASQSGATFFSISASSLTSKWVGEGEKMVRALFAVAR
CQQPAVIFIDEIDSLLSQRGDGEHESSRRIKTEFLVQLDGATTSSEDRILVVGATNRPQEIDEAARRRLVKRLYIPLPEASARKQIV
INLMSKEQCCLSEEEIEQIVQQSDAFSGADMTQLCREASLGPIRSLQTADIATITPDQVRPIAYIDFENAFRTVRPSVSPKDLELYE
NWNKTFGCGK

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 10 ml TB supplemented with 8 g/l glycerol, 100 µg/ml kanamycin and 34 µg/ml chloramphenicol at 30 °C overnight. The overnight culture (10 ml) was used to inoculate 0.75 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and approximately 100 µl PPG P2,000 81380 anti-foam solution (Fluka). 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 (17.9 g wet cell weight) was resuspended in lysis buffer (1.5 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 an Amicon Ultra-15 centrifugal filter device with 10,000 NMWL (Millipore) to 35.8 mg/ml in a volume of 1.3 ml. The identity of the protein was confirmed by 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 (49,000 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 of the protein solution (35.8 mg/ml) including 20 mM ADP and 10 mM MgCl2 was mixed with 0.1 µl of well solution containing 0.1 M Bis-Tris, pH 5.5, 0.2 M NaCl and 25% PEG3350. The plate was incubated at 4 °C and crystals appeared within one day. The crystal was briefly transferred to cryo solution containing 0.1 M Bis-Tris, pH 5.5, 0.3 M NaCl, 25% PEG3350 and 20% glycerol, and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data to 2.0 Å resolution was collected at the ESRF beamline ID 23-1.
Data Processing: The structure was solved by molecular replacement using the structure of Spastin from *Drosophila melanogaster* (PDB entry 3B9P) as search model. The space group was P41212 with cell dimensions a=b=85.43 Å c=197.58 Å. Two monomers were located in the asymmetric unit. ARP/wARP was initially used for automatic model building. Refmac5 was thereafter used for refinement and Coot for model building. Refinement using TLS-parameters were done in the later stages. Data in the interval 25.0-2.0 Å resolution was used and at the end of the refinement the values for R=19.81% and Rfree=23.88%. Coordinates for the crystal structure were deposited in the Protein Data Bank, accession code 3D8B.