DDX41

PDB:2P6N

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
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-Gold(DE3)pRARE2, where BL21-Gold(DE3) cells (Stratagene) have been transformed with pRARE2 originating from the Rosetta2 strain (Novagen). The pRARE2 plasmid supplies tRNAs for rare codons.

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*smGAASLDVIQEVEYVKEEAKMVYLLECLQKTPPPVLIFAEKKADVDAIHEYLLLKGVEAVAIHG
GKDQEERTKAIEAFREGKKDVLVATDVASKGLDFPAIQHVINYDMPEEIENYVHRIGRTG**C**SGNTGIATTFINKACDESVLMDLKAL
LLEAKQKVPPVLQVLHCG

Vector:pNIC-Bsa4

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 and approximately 200 µl 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. 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 (4,400 x *g*, 10 min, 4 ºC). The resulting cell pellet (14.4 g wet cell weight) was resuspended in lysis buffer (5 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 19.1 mg/ml in a volume of 0.25 ml. 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 (49,000 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. 0.1 µl protein solution (19.1 mg/ml) was mixed with 0.2 µl of well solution consisting of 0.1 M bis-Tris pH 5.5, 0.2 M lithium sulfide and 25% (w/v) PEG 3350. The plate was incubated at 20 ºC. Crystals appeared within three days and continued to grow for ten more days to reach their maximal size (approx. 120 µm × 50 µm × 50 µm). The crystals were quickly transferred to a cryo solution consisting of well solution with 25% glycerol, and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data was collected at ESRF beamline ID14-4.
Data Processing: An additional peak, off-centred from origin (0,0,0.25) was discovered in Patterson function due to translation pseudo symmetry. The structure was solved by molecular replacement using the helicase domain from the human protein DDX3X as search model (PDB entry: 2I4I) with the program MolRep. The asymmetric unit contained two protein monomers. The space group was P6522 with cell dimensions a=b=68.01 Å c=305.60 Å. Refmac5 was used for refinement and Coot for model building. NCS and TLS restrained refinement was used. The TLS groups were selected using the tlsmd server http://skuld.bmsc.washington.edu/~tlsmd/. Data in the interval 29.7-2.6 Å resolution was used and at the end of the refinement the R values were: R=24.6% and Rfree=29.6%. The coordinates for the crystal structure were deposited in the Protein Data Bank, accession code 2P6N.