DDX5

PDB:3FE2

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC016027
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:

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mhhhhhhssgvdlgtenlyfq*smRTAQEVETYRRSKEITVRGHNCPKPVLNFYEANFPANVMDVIARQNFTEPTAIQAQGWPVALS
GLDMVGVAQTGSGKTLSYLLPAIVHINHQPFLERGDGPICLVLAPTRELAQQVQQVAAEYCRACRLKSTCIYGGAPKGPQIRDLERG
VEICIATPGRLIDFLECGKTNLRRTTYLVLDEADRMLDMGFEPQIRKIVDQIRPDRQTLMWSATWPKEVRQLAEDFLKDYIHINIGA
LEL
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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 °C overnight. 20 ml of the overnight culture were used to inoculate 1.5 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and approximately 500 µl Dow Corning anti-foam RD emulsion 63213 4D (BDH Silicone Products). 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 (29.6 g wet cell weight) was resuspended in lysis buffer (1.5 ml/g cell pellet), supplemented with 2000 U Benzonase (Merck) and one 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 two HiTrap Chelating columns connected in series and washed with IMAC wash1 buffer followed by IMAC wash2 buffer. Bound protein was eluted from the IMAC columns 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 17.6 mg/ml in a volume of 1.2 ml. The purified protein started to precipitate after concentration why precipitate was removed and the protein sample was quickly aliquoted and frozen.

Tag removal
The N-terminal histidine tag was proteolytically removed by incubating DDX5 with His-tagged TEV protease (van den Berg, S., *J. Biotech* **121**, 291-298 (2006)) at a molar ratio of 30:1 at 4 ºC overnight. The proteolytic reaction went near to completion, as judged by SDS-PAGE. Target protein was purified from tag and protease by passing the reaction mixture over a Ni-charged 1 ml HiTrap Chelating HP column (GE Healthcare) pre-equilibrated with IMAC wash1 buffer. The cleaved protein was concentrated and the buffer was changed to GF buffer containing 2 mM TCEP using a Vivaspin 20 centrifugal filter device with 10,000 MWCO (Sartorius). The final protein concentration was determined to 17.5 mg/ml in a volume of 0.7 ml. The identity of the protein was confirmed by mass spectrometry.

Extraction

Procedure
The cell suspension was quickly thawed in water and diluted to 2 x 68 ml with lysis buffer. 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 using a 96-well plate. 0.2 µl of the protein sample (diluted to 15.8 mg/ml) including 20 mM ADP and 10 mM MgCl2 was mixed with 0.1 µl of well solution consisting of 0.1 M Bis-Tris pH 6.4, 0.25 M lithium sulfate monohydrate, 17.5% PEG 3350. The plate was incubated at 4 ºC and crystals appeared within 10 hours. Cryo solution containing 0.1 M Bis-Tris pH 5.5, 0.2 M lithium sulfate, 25% PEG 3350, 0.2 M NaCl and 15% glycerol was added directly to the drop. Crystals were mounted and flash-frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data to 2.6 Å resolution was collected from a single crystal at Bessy (BL14-2). Crystal belonged to C2221 space group with cell parameters of a=84.57 Å, b=106.87 Å, c=117.32 Å. The asymmetric unit contains two monomers.
Data Processing: The structure was solved by molecular replacement using MOLREP with

human DDX3X protein structure (2I4I) residues 3-241 as a search model. ADP was located at the active site. Structure was refined with REFMAC5. Final R-values were R=20.8% and Rfree=27.3%. Coordinates and structure factors are deposited to PDB with accession code 3FE2.