HSPA6

PDB:3FE1

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession:BC035665
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:HSPA6A-k007
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*smELAVGIDLGTTYSCVGVFQQGRVEILANDQGNRTTPSYVAFTDTERLVGDAAKSQAALNPHNT
VFDAKRLIGRKFADTTVQSDMKHWPFRVVSEGGKPKVRVCYRGEDKTFYPEEISSMVLSKMKETAEAYLGQPVKHAVITVPAYFNDS
QRQATKDAGAIAGLNVLRIINEPTAAAIAYGLDRRGAGERNVLIFDLGGGTFDVSVLSIDAGVFEVKATAGDTHLGGEDFDNRLVNH
FMEEFRRKHGKDLSGNKRALRRLRTACERAKRTLSSSTQATLEIDSLFEGVDFYTSITRARFEELCSDLFRSTLEPVEKALRDAKLD
KAQIHDVVLVGGSTRIPKVQKLLQDFFNGKELNKSINPDEAVAYGAAVQAAVLMGD
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Vector:pNIC-Bsa4

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 0.75 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and approximately 0.4 ml 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 (15 g wet cell weight) was resuspended in lysis buffer (3 ml/g cell pellet), supplemented with 1250 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 200 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 25 mg/ml in a volume of 0.41 ml.

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 (49,100 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. 0.2 µl of the protein solution (diluted to 21 mg/ml) including 20 mM ADP and 10 mM MgCl2 was mixed with 0.1 µl of well solution containing 0.1 M citric acid, pH 3.2, 0.15 M di-sodium hydrogen phosphate, 16% (v/v) PEG 300. The plate was incubated at 20 °C and crystals appeared within 5 days. The crystals were briefly transferred to cryo solution containing 0.1 M citric acid, 0.15 M Na2HPO4, 40% PEG 300, 0.2 M NaCl and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data to 2.2 Å resolution was collected from a single crystal at BESSY (BL14-1). The crystal belonged to space group C121 with cell parameters of a=236.45 Å, b=105.4 Å, c=73.59 Å, α= 90°, β=101.04°, γ= 90°.
Data Processing: The structure was solved by molecular replacement using MOLREP with the human Hsp70 ATPase domain (2E88) as a search model. The asymmetric unit consisted of three polypeptide chains. ADP and Mg2+ ions were located in the active site of the enzyme. The structure was refined with REFMAC5. Final R-values were R=20.1% and Rfree=23.8%. Coordinates and structure factors were deposited in the PDB with accession code 3FE1.