PARP15

PDB:3BLJ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC101701
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*smNLPEHWTDMNHQLFCMVQLEPGQSEYNTIKDKFTRTCSSYAIEKIERIQNAFLWQSYQVKKRQ
MDIKNDHKNNERLLFHGTDADSVPYVNQHGFNRSCAGKNAVSYGKGTYFAVDASYSAKDTYSKPDSNGRKHMYVVRVLTGVFTKGRA
GLVTPPPKNPHNPTDLFDSVTNNTRSPKLFVVFFDNQAYPEYLITFTA
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Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 60 ml TB supplemented with 8 g/l glycerol, 100 µg/ml kanamycin and 34 µg/ml chloramphenicol at 30 ºC overnight. The overnight culture was used to inoculate 3 x 1.5 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and approximately 200 µl PPG P2,000 81380 anti-foam solution (Fluka). The culture was grown in a LEX bioreactor system (Harbinger Biotechnology) at 37 ºC until OD600 reached ~2. The bottle 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 (4,000 x *g*, 10 min, 4 ºC). The resulting cell pellet (65.1 g wet cell weight) was resuspended in lysis buffer (3 ml/g cell pellet), supplemented with 6000 U Benzonase (Merck) and three tablets of Complete EDTA-free protease inhibitor (Roche Applied Science). The cell suspension was stored at -80 ºC.

Purification

Procedure

Columns
IMAC 1: Ni-charged 5 ml HisTrap HP (GE Healthcare)
Desalting column: HiPrep 26/10 desalting (GE Healthcare)
IMAC 2: 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 four step process on an ÄKTAxpress system (GE Healthcare). Prior to purification, columns were equilibrated with IMAC wash1 buffer (IMAC columns) and gel filtration buffer (gel filtration and desalting column), respectively. The filtered lysate was loaded onto the Ni-charged HisTrap column and washed with IMAC wash1 buffer followed by IMAC wash3 buffer. Bound protein was eluted from the IMAC column with IMAC elution buffer and automatically loaded onto the desalting column. The next morning fractions containing the target protein were pooled and loaded onto the Ni-charged HiTrap Chelating column. The protein was 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 32.45 mg/ml in a volume of 1.1 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 (49,100 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. 0.1 µl protein solution (32.4 mg/ml) was mixed with 0.2 µl of well solution consisting of 0.2 M ammonium chloride and 20% (w/v) PEG 3350. The plate was incubated at 20 ºC and long plate shaped crystals appeared within 14 days. The crystals were quickly transferred to a cryo solution consisting of well solution complemented with 20% glycerol and 0.3 M NaCl, and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data to 2.2 Å resolution was collected at MAX-lab beamline I911-3.
Data Processing: The structure was solved by molecular replacement using PARP-12 as template (PDB: 2PQF). The space group was P212121 with cell dimensions a=45.21 Å b=68.12 Å c=158.28 Å. Two monomers were located in the asymmetric unit. Automatic model building using Arp/warp was performed; Refmac5 was used for refinement and Coot for model building. Data in the interval 20.0-2.20 Å resolution was used and at the end of the refinement the R values were: R=18.25% and Rfree=24.34%. Coordinates for the crystal structure were deposited in the Protein Data Bank, accession code 3BLJ.