SYNJ2BP

PDB:2JIK

Revision

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Entry Clone Accession:gi|8922964
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SGC Clone Accession:SYNJ2BPA-c007
Tag:N-terminal, TEV cleavable hexahistidine tag
Host:BL-21(DE3)-R3-Rosetta (A homemade phage resistant version of BL21(DE3) containing the pRARE2 plasmid from Rosetta II (DE3) cells).

Construct

Prelude:
Sequence:

smDYLVTEEEINLTRGPSGLGFNIVGGTDQQYVSNDSGIYVSRIKENGAAALDGRLQEGDKILSVNGQDLKNLLHQDAVDLFRNAGY
AVSLRVQHRL*ESSI*

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:Transformation: The construct DNA was transformed into homemade chemically competent cells of the expression strain by a standard heat shock procedure.

Glycerol stock prepataion: A number of colonies from the transformation were used to innoculate 1 ml of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day glycerol stocks were prepared from this overnight culture.

Expression: A glycerol stock was used to innoculate 50 ml of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day this starter culture was used to innoculate 2x 1L of TB media (18 ml starter culture into each) containing 50 µg/ml kanamycin. After 7 hours the temperature was reduced to 25°C (OD 6.4). After a further 1 hour the cells were induced by the addition of 0.5 mM IPTG. The expression was continued overnight.

Cell harvest: Cells were spun down, resuspended in Lysis Buffer and the resuspended cell pellets were stored in a -80°C freezer.

Purification

Procedure
Column 1: Ni-NTA (0.5 ml)
Column 2: Gel filtration. Hiload S200 16/60 - 120 ml volume.

The clarified cell extract was passed through the column twice. The column was then washed with 50 ml of Binding Buffer followed by 30 ml of Wash Buffer. The protein was eluted with 15 ml of Elution Buffer.

The gel filtration column was pre-equilibrated with Gel Filtration Buffer. The eluant from column 1 was concentrated to a volume of 2 ml and loaded on the gel filtration column at a flow rate of 1.0 ml/min. Eluted proteins were collected in 1.8 ml fractions. The fractions containing protein were identified on a coomasie blue stained gel.

TEV protease digestion: The gel filtration fractions containing SYNJ2BPA were pooled and TEV protease solution was added. The digestion was left overnight at 4°C.
Rebinding of impurities to Ni-NTA: The protein was mixed with Ni-NTA resin to bind the impurities. After 90 minutes the resin was spun down and the supernatent collected.

Extraction

Procedure
The resuspended cell pellet was lysed using an Emulsiflex C5 high-pressure homogeniser, collecting a final volume of approximately 50 ml after dilution with Lysis Buffer. PEI was added to a final concentration of 0.15 % and the cell debris and precipitated DNA were spun down.
Concentration: The TEV protease cleaved SYNJ2BPA was concentrated to 8.3 mg/ml (SYNJ2BPA-c007) or 13.2 mg/ml (SYNJ2BPA-c002) (concentrations were measured using a nanodrop machine), distributed into aliquots and frozen at -80°C.
Ligand
MassSpec: Expected: 11023.1
Measured: 11023.3
Crystallization: Crystals grew from a 1:1 ratio of protein to precipitant solution, using the vapour diffusion method. The conditions was 20% PEG 6000, 1 M LiCl, 0.1 M citrate pH 4.0 for construct SYNJ2BPA-c007 that crystallised in the P21 spacegroup. Crystals were grown in 150 nL drops at 20°C
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected by equilibration into precipitant solution containing 20% ethylene glycol, and then flash frozen in liquid nitrogen. Data was collected to a resolution of 1.35 Å (P21) or 1.5 Å (I4) or at the Swiss Light Source, beamline X10.
Data Processing: