IMPA2

PDB:2FVZ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC017176
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m).
Host:E. coli Bl21(DE3)-Codon-Plus-RIPL (Stratagene)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsMKPSGEDQAALAAGPWEECFQAAVQLALRAGQIIRKALTEEKRVSTKTSAADLVTETDHLVEDLI
ISELRERFPSHRFIAEEAAASGAKCVLTHSPTWIIDPIDGTCNFVHRFPTVAVSIGFAVRQELEFGVIYHCTEERLYTGRRGRGAFC
NGQRLRVSGETDLSKALVLTEIGPKRDPATLKLFLSNMERLLHAKAHGVRVIGSSTLALCHLASGAADAYYQFGLHCWDLAAATVII
REAGGIVIDTSGGPLDLMACRVVAASTREMAMLIAQALQTINYGRDDEK

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure:100 µL competent Bl21(DE3)-Codon-Plus RIPL cells (Stratagene) were transformed with 3 µL plasmid. Held 20min on ice, heat-shocked in a waterbath for 45 sec at 42°C. Held on ice for 30 sec. Added 400µL SOC and incubated in shaker for 1 h at 37°C. Cells were spinned down and resuspended in 50µl SOC and plated on LA plates with 50 mg/l Kanamycin (Kan)and 20 mg/l Chloramphenicol (Cm). One colony was used to inoculate 40 ml of TB + Kan 50 mg/l + 20 mg/ml Cm. The inoculation culture was shaken at 37 degrees until early logphase (1 OD600). The inoculation culture was added to three TunAir flasks (Shelton Scientific) with 750 ml of phosphate buffered TB with 50 mg/l Kan and 20 mg/ml Cm. The culture was incubated at 37°C, until OD600 reached of approximately 1.1. The temperature was lowered to 18°C and the culture was induced with 0.5 mM IPTG for 18 hours.

Purification

Procedure

Columns: 1 ml Hi-Trap Chelating (Ni-charged). (GE Healthcare). Superdex 75 HiLoad 16/60 (GE Healthcare).

The sample was purified automatically on an ÄKTA-Xpress (GE Healthcare). Briefly, sample was loaded on the IMAC column, eluted in a storage loop and then loaded on the gel filtration column. Elution fractions were pooled based on SDS-PAGE analysis. Protein was estimated by SDS-PAGE analysis to be more than 95% pure. Fresh TCEP was added to the pooled samples so that the concentration of TCEP was 2 mM.

Concentration was performed by use of Amicon Ultra 15 (Millipore) with 10 000 MW CO. Centrifugation was performed at 15 deg in swing-out buckets for 15 minutes at 3000 g. Yield of purified protein per liter of culture was 5.6 mg.

Extraction

Procedure
Cells were harvested by centrifugation (OD600 7.6; WCW 54.7 g) and pellets were resuspended in 60 ml of lysis buffer (50 mM Hepes, 500 mM NaCl, 10% glycerol, 10 mM imidazole, 0.5 mM TCEP and 2 tablets Complete EDTA-free protease inhibitor (Roche Biosciences)).
After thawing, 4 µl of a 250 U/?l benzonase (Novagen) stock solution was added. Cells were then disrupted by high pressure homogenization with a high-pressure homogenizer (Stansted) (3 passes) prior to centrifugation for 30 min at 49000 g in a Sorvall SA-800 rotor. The soluble fraction was decanted and filtered through 0.45 µm.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained using the hanging drop method at 20°C. Drops were prepared using 1 µl of protein (25 mg/ml concentration) and 1 µl of the well solution (16% Peg 6000, 20 mM MES pH 6.5). Large bi-pyramidial crystals grew within one week.
NMR Spectroscopy:
Data Collection: Data were collected at beamline i911-5 at MAXLAB, (Lund, Sweden) to 2.4 Å resolution. The crystals belonged to spacegroup P21 but were perfectly pseudo-merohedrally twinned emulating primitive orthorombic symmetry.
Data Processing: The structure was solved with molecular replacement (MOLREP, CCP4) and refined against the twinned intensities using SHELX-97.