PRPSAP2

PDB:2JI4

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:TC118400; SGC construct PRPSAP2A-c008
Entry Clone Source: Origene
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m).
Host:BL21(DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmNITKGGLVLFSANSNSSCMELSKKIAERLGVEMGKVQVYQEPNRETRVQIQESVRGKDVFIIQT
VSKDVNTTIMELLIMVYACKTSCAKSIIGVIPYFPYSKQCKMRKRGSIVSKLLASMMCKAGLTHLITMDLHQKEIQGFFNIPVDNLR
ASPFLLQYIQEEIPDYRNAVIVAKSPASAKRAQSFAERLRLGIAVIHGEAQDAESDLVDGRHSPPMVRSVAAIHPSLEIPMLIPKEK
PPITVVGDVGGRIAIIVDDIIDDVDSFLAAAETLKERGAYKIFVMATHGLLSSDAPRRIEESAIDEVVVTNTIPHEVQKLQCPKIKT
VDISMILSEAIRRIHNGESMSYLFRNIGLDD

Vector:pNIC-BSA4

Growth

Medium:TB
Antibiotics:
Procedure:20mL TB media in 100mL shake flask, supplemented with 8 g/L glycerol, 34 µg/ml Chloramphenicol and 100 microG/mL Kanamycin, was inoculated with Rosetta 2 cells and the culture left to grow overnight at 30 degC, 175 RPM. The overnight cultures was used to inoculate fresh 1.5L TB media supplemented with 50 microG/mL kanamycin and approximately 100 microL BREOX (anti-foam solution). The large scale cultivations were grown in tunair flasks 37degC until OD600 reaches approximately 1.8-2. The flasks were then moved to 18degC and induced with 0,5 mM IPTG after 1 hour. Cultures were allowed to grow overnight at 18degC.

Purification

Procedure
Columns:HiTrap Chelating Ni column; GF column: HiLoadÂ□ 16/60 Superdex 200 Prep Grade

Procedure
Purification was performed on an ÄKTAXpress. Prior to purification, columns were equilibrated with IMAC Bind/Wash1 Buffer (HiTrap Chelating) and Gel filtration buffer (Superdex 200). The protein sample was loaded on the HiTrap Chelating column that was washed with IMAC Bind/Wash1 Buffer followed by IMAC Wash2 Buffer. Bound protein was eluted from the IMAC columns with IMAC Elution Buffer and loaded in the Gel filtration column. The protein was finally concentrated to 7.4 g/L and frozen in -80 degC.

Extraction

Procedure
Harvest by centrifugation, rotor F8S-4X 1000y (=SLC-6000) at 5000 rpm for 10 minutes in 4degC and freeze the pellet at -80degC. Prior to purification the cell pellet was briefly thawed in warm water and resuspended in 50 mM NaSO4, 500 mM NaCl, 10% glycerol, 10 mM Imidazole, 0.5 mM TCEP, pH 7.5 supplemented with one tablet Complete EDTA-free protease inhibitor tablet and 4 microL/50ml benzonase. Cells were disrupted by sonication (4s on 4 off 3 min 80% amplitude) and samples were centrifuged for 20 min at 20500 rpm. The soluble fraction was filtered through 0.45 microM and subjected to further purification on the ÄKTAXpress.
Concentration:7.4 mg/mL
Ligand
MassSpec:
Crystallization: Crystals were obtained by the hanging drop vapour diffusion method using 24-well plates from Hampton Research by mixing 1microL of the protein solution (7.4 g/L) with 1uL of the well solution consisting of 4.1M Ammonium Chloride and 0.1M BisTrisPropane pH 7.0 at room temperature. Crystals were cryo protected by a fast dip in 0.1M BisTrisPropane pH 7.0, 3.5M Ammonium chloride and 14% Glycerol.
NMR Spectroscopy:
Data Collection:X-ray data in space group H32 (104.4, 104.4, 192.0, $\alpha = \beta = 90$, $\gamma = 120$) was collected at BESSY (BL14.1) and processed using XDS and XSCALE.
Data Processing: The structure was solved by molecular replacement using MOLREP with the human PAP39 structure (2C4K) as our search model. Final cycles of model building were performed in COOT and REFMAC5 was used for refinement.