PREX1

PDB:3QIK

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC053616
Entry Clone Source: Open Biosystems
(actual DNA used HPC0A0-B07, a chimeric full length DNA of BC053616 and synthesized DNA)
SGC Clone Accession:HPC0AJ-H05
Tag:N-terminal His6-tag, removed before crystallization
Host:BL21-V2R-pRARE2

Construct

Prelude:PREX1:K607-A706
Sequence:

gKNKQLRNDFKLVENILAKRLLILPQEEDYGFDIEEKNKAVVVKSVQRGSLAEVAGLQVGRKIYSINEDLVFLRPFSEVESILNQSF
CSRRPLRLLVATKA

Vector:pET28-MHL

Growth

Medium: Terrific Broth medium in the presence of 50 mg/mL kanamycin and 25 mg/mL chloramphenicol
Antibiotics:
Procedure:LEX Bubbling. The target protein was expressed in *E. coli* by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 2 L of growth medium at 37 °C. When OD_{600} reached ~3.0, the temperature of the medium was lowered to 15 °C and the culture was induced with 1 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 °C.

Purification

Procedure
The lysate was centrifuged at 15,000 rpm for 45 minutes and the supernatant were mixed with 4 mL 50% flurry of Ni-NTA beads and incubated at 4 degree Celsius on rotary shaker for one hour. The mixture was then centrifuged at 2300 rpm for 5 min and the supernatant discarded. The beads were then washed with 50 mL binding buffer containing 5 mM Imidazole twice. Bound proteins were eluted using 15 mL elution buffer. The flow-through was collected and further

purified by a Superdex-75 gel filtration column pre-equilibrated with gel filtration buffer. Fractions containing the target protein were pooled and concentrated using Amicon Ultra-15 centrifugal filter (mwco 10 kDa). The purity of the preparation is tested by SDS-PAGE to be greater than 95%.

Extraction

Procedure
Frozen cells from 4L TB culture were thawed and resuspended in 5 mL extraction buffer per gram of cell pellets with freshly added 0.5% CHAPS, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 uL benzonase (Sigma Catalog # E1014, 250U/uL), and lysed using sonication for 5 min at 100 W, 10 sec on/10 sec off duty cycle.
Concentration:6.9 mg/mL
Ligand
MassSpec: Uncut version native protein expected 13638.7, measured 13639.0
Cut version, expected 11502.4, measured 11494.5, not sure about the cause of -7.9 difference
Crystallization: Crystal was initially obtained from SGC-I screen condition B12.
Crystal used for structure refinement was grown in 1.2 M Li_2SO_4, 0.5 M $(NH_4)_2SO_4$, 0.1 M Sodium Citrate pH 6.2 in sitting drop setup, using 1 uL protein + 1 uL well solution against 1 mL reservoir buffer at room temperature. Crystal used for phasing was Pt derivative of optimized crystal grown in 0.5 M $(NH_4)_2SO_4$, 1.2 M Li_2SO_4, 0.1 M Sodium Citrate pH 5.6. Crystal was harvested from original drop and soaked in 4 uL of the mother liquid containing 1 mM K_2PtI_6 for 6 hours and harvested for diffraction test.

Crystals grow to a mountable size within one week.
Cryo used paratone-N
NMR Spectroscopy:
Data Collection:
Data Processing: