

PLXNB2

PDB:4E71

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:SGC cDNA collection 20-E3

Entry Clone Source:Codon Devices

SGC Clone Accession:HPC09R-A10

Tag:N-terminal His6-tag, removed before crystallization

Host:BL21-V2R-pRARE2

Construct

Prelude:PLXNB2:L1453-G1562

Sequence:

gLLGDDVEYAPLTVSVIVQDEGVDAIPVKVLNCDTISQVKEIIDQVYRGQPCSCWPRPDSVVLEWRPGSTAQILSDLDLTSQREG
WKRVTLMHYNVLDGATLILSKVG

Vector:pET28-MHL

Growth

Medium:

Antibiotics:

Procedure:LEX Bubbling. The target protein was expressed in E. coli by inoculating each 50 mL of overnight culture grown in Luria-Bertani medium into a 2 L of Terrific Broth medium in the presence of 50 mg/mL kanamycin and 30 mg/mL chloramphenicol at 37 degree. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 degree 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 degree.

Purification

Procedure

The clarified lysate was mixed with 5 mL of 50% slurry of Talon beads and incubated at 4 C on rotary shaker for 1 hours. The mixture was then centrifuged at ~1000 x g (2000 rpms) for 5 min and the supernatant discarded. The beads were washed with 50 mL Binding Buffer followed by 50 mL of Washing Buffer. The protein was eluted with 10 mL of Elution Buffer. The eluted protein was mixed with TEV protease in a 1:4 molar ratio (TEV to protein) and dialyzed in Dialysis buffer overnight (Dialysis cassette has a 3 kDa molecular weight cut off). The dialyzed protein was further purified on a home-packed 26/60 Superdex-75 gel filtration column that was

pre-equilibrated with Gel Filtration Buffer. The flow rate is 2 mL/min. Fractions containing the protein were collected and concentrated with Amicon Ultra-15 centrifugal filter (3 kDa molecular weight cut off). The purity of the protein preparation was greater than 95% as judged by SDS-PAGE.

Extraction

Procedure

Frozen cells from 4L TB culture were thawed and re-suspended in 500 mL Extraction Buffer. Cells were lysed by 50 times ten second pulses sonication on ice at 100W with ten second break between each pulse. The lysate was clarified by centrifugation at $\sim 38000 \times g$ (16,000 rpms) for 1 hour.

Concentration: 39 mg/mL

Ligand

MassSpec: cut version, expected is 12337.0 Da and the measured value was 12337.5 Da

Crystallization: Crystal was initially found in RedWing (RW) screen condition F06 (3.5 M NaFormate, 0.1 M Tris pH 8.5)

Crystal used for structure refinement was grown in an optimized RW-F06 condition, i.e., 2.5 M NaFormate, 0.1M Glycine pH 9.6 in sitting drop setup, using 0.5uL protein + 0.5uL well solution against 100 uL reservoir buffer at room temperature.

Crystals grow to a mountable size within two days. Crystals were further dehydrated for two days by adding glycerol to the well solution at a final concentration of 15% before crystals are mounted.

Cryo used mineral oil

NMR Spectroscopy:

Data Collection:

Data Processing: