PTPRO

PDB:2GJT

Revision

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Entry Clone Accession:gi|13677213|ref|NM_030667.1|
Entry Clone Source: Purely Proteins Ltd.
SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.
Host:BL21 (DE3)R3 (Phage resistant strain)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmNPVQLD DFDAYIKDMAKDSDYKFSLQFEELKLIGL DIPHFAADLPLNRCKNRYTNILPYDFS
RV RLVSMNEEEGADYINANYIPGYNSPQEYI ATQGPLPETRNDFWKMVLQQKSQIIVMLT QCNEKRRVKCDHYWPFTEEPIAYG
DITVE MISEEEQDDWACRHFRINYADEMQDVMHF NYTAWPDHGVPTANAAESILQFVHMVRQQ ATKSKGPMIIHCSAGVGRTGT
FIALDRLL QHIRDHEFVDILGLVSEMRSYRMSMVQTE EQYIFIHQCVQLMWMKKKQQFCISDV

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:1ml from a 10 ml overnight culture containing 50µg/ml kanamycin was used to inoculate 1 litre of LB containing 50µg/ml kanamycin. Cultures were grown at 37°C until the OD 600 reached ~0.3 then the temperature was adjusted to 18°C. Expression was induced for 4 hours using 1 mM IPTG at an OD 600 of 0.8. The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen. Binding buffer : 50mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5% glycerol; 0.5 mM TCEP.

Purification

Procedure

Column 1 : Ion exchange - Nucleic acid removal. DEAE cellulose (DE52, Whatman), 10 g of resin in 2.5 x 20 cm column. The resin was hydrated in 2.5M NaCl, then washed with 20 ml binding buffer prior to loading the sample.
Supernatant was applied by gravity flow, followed by a wash with 100 ml binding buffer. The column flow-through was collected.

Column 2: Ni-affinity. Ni-NTA (Qiagen), 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.
The flowthrough from column 1 was loaded by gravity flow on the Ni-NTA column. The column was then washed with 100 ml wash buffer at gravity flow. The protein was eluted by gravity flow by applying 5-ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150 and 250 mM); fractions were collected until essentially all protein was eluted.

Enzymatic treatment: (His tag cleavage using TEV) Samples containing PTPRO were pooled and TEV protease added for overnight incubation at 4°C. Cleaved products and TEV protease were removed by binding to Ni-NTA agarose after buffer exchange to 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP using a 10 kDa cut-off concentrator .

Column 3: Size Exclusion Chromatography. Superdex S200 16/60 HiLoad
TEV-cleaved PTPRO, after re-binding to NiNTA, was directly applied to a S200 16/60 HiLoad gel filtration column equilibrated in 50 mM HEPES pH 7.5, 300 mM NaCl, 10 mM DTT using either an ÄKTAprime or ÄKTAxpress system

Extraction

Procedure
Frozen pellets were thawed and cells lysed using a high pressure cell disrupter. The lysate was centrifuged at 17,000 rpm for 30 minutes and the supernatant collected for purification.
Concentration: Protein was concentrated to 10 mg/ml using an Amicon 10 kDa cut-off concentrator
Ligand
MassSpec:LC- ESI -MS TOF confirmed the correct mass expected for this construct. Predicted mass of TEV-cleaved protein = 34596; Measured = 34596
Crystallization: Crystals were grown at 4°C in 600 nl sitting drops mixing 450 nl of protein with 150 nl of a solution containing 0.1M HEPES pH 8.0; 10% isopropanol; 20% PEG 4K
NMR Spectroscopy:
Data Collection: Resolution: 2.19 Å; Crystals were cryo-protected using the well solution and 20% ethylene glycol and flash frozen in liquid nitrogen. X-ray source: Diffraction data were collected at the SLS beamline X10 at a single wavelength (0.979 Å).
Data Processing: