PTPN11

PDB:3B7O

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|33356177
Entry Clone Source: Purely Proteins
SGC Clone Accession:n/a
Tag: Tag sequence: TEV-cleavable N-terminal His6 tag mhhhhhhssgvdlgtenlyfq
Host:BL21 (DE3) Rosetta

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*SMAETTDKVKQGFWEEFETLQQQECKLLYSRKEGQRQENKNKNRYKNILPFDHTRVVLHDGDPNE
PVSDYINANIIMPEFETKCNNSKPKKSYIATQGCLQNTVNDFWRMVFQENSRVIVMTTKEVERGKSKCVKYWPDEYALKEYGVMRVR
NVKESAAHDYTLRELKLSKVGQGNTERTVWQYHFRTWPDHGVPSDPGGVLDFLEEVHHKQESIMDAGPVVVHCSAGIGRTGTFIVID
ILIDIIREKGVDCDIDVPKTIQMVRSQRSGMVQTEAQYRFIYMAVQHYIETLQRRI

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:ml from a 10 ml overnight culture containing 50 µg/ml kanamycin was used to inoculate 1 litre of LB containing 50 µg/ml kanamycin. Cultures were grown at 37°C until the OD600 reached ~0.3 then the temperature was adjusted to 18°C. Expression was induced overnight using 1 mM IPTG at an OD600 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, 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 mM and 250 mM); fractions were collected until essentially all protein was eluted.Column 3: Size Exclusion Chromatography. Superdex S200 16/60 HiLoadEluted protein , was directly applied to a S200 16/60 HiLoad gel filtration column equilibrated in 10 mM HEPES, pH 7.5 250 mM NaCl, 10 mM DTT using an ÄKTAxpress system. PTPN11 eluted at a retention time corresponding to the monomeric protein. Eluted fractions were 95% pure as judged by SDS-PAGE

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:13.5 mg/ml in SEC buffer using a centricon with a 10kDa cut off
Ligand
MassSpec:ESI-MS revealed that the protein had the expected mass of 36824 Da.
Crystallization: The PTPN11 was crystallized at 20°C using the sitting-drop vapor diffusion method. 100nl of concentrated protein was mixed with 100nl of well solution and equilibrated with 100 µl of the well solution. Diffraction quality crystals were obtained in a solution containing 20% PEG 3350 and 0.15M Na (malate).
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected using 25% ethylene glycol and flash frozen in liquid nitrogen. Diffraction data were collected to 1.6Å at the Swiss light source beam-line X10SA at a single wavelength of 1.00721 Å.
Data Processing: