SPSB4

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Revision

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Entry Clone Accession:gi|18254460
Entry Clone Source:MGC
SGC Clone Accession:SPSB4A-c009
Tag:mhhhhhhssgvdlgtenlyfq*sm
Host:BL21(DE3)-R3

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*smGAEPGRPARLDQLLDMPAAGLAVQLRHAWNPEDRSLNVFVKDDDRLTFHRHPVAQSTDGIRGK
VGHARGLHAWQINWPARQRGTHAVVGVATARAPLHSVGYTALVGSDAESWGWDLGRSRLYHDGKNQPGVAYPAFLGPDEAFALPDSL
LVVLDMDEGTLSFIVDGQYLGVAFRGLKGKKLYPVVSAVWGHCEVTMRYINGLDPE

Vector:pNIC28-Bsa4.

Growth

Medium:LB
Antibiotics:
Procedure:1ml from a 50 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 ~0.5 then the temperature was adjusted to 18°C. Expression was induced for 4 hours using 0.5 mM IPTG at an OD600 of 0.8. The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen.

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.
Column 2: Ni-affinity. Ni-NTA (Qiagen), 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.
Column 3: Size Exclusion Chromatography. Superdex S75 16/60 HiLoad

Supernatant was applied by gravity flow, followed by a wash with 50 ml binding buffer. The column flow-through was collected.

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. 10 mM DTT was added for overnight storage together with TEV protease for cleavage of the N-terminal hexahistidine tag.
Protein was applied to a S75 16/60 HiLoad gel filtration column equilibrated in 50 mM HEPES pH 7.5, 150 mM NaCl, 5 mM DTT using an ÄKTAprime system

Extraction

Procedure
Frozen pellets were thawed and fresh 1 mM PMSF and 0.5 mM TCEP were added. Cells were lysed by sonication. The lysate was centrifuged at 17,000 rpm for 30 minutes and the supernatant collected for purification.
Concentration: Protein was concentrated to 9.0 mg/ml using an Amicon 10 kDa cut-off concentrator.
Ligand
MassSpec:LC- ESI -MS TOF. Expected mass (before TEV cleavage): 25192; and Observed mass (before TEV cleavage): 25192
Crystallization: Crystals were grown at 4°C in 150 nl sitting drops mixing 100 nl of protein with 50 nl of a solution containing 14.4% PEG 10K; 0.16M Ca(ac)2; 20% glycerol; 0.08M cacodylate pH 6.5. The crystals were cryo-protected using 25% ethylene glycol which was added to the drop 30 seconds prior to mounting and flash freezing in liquid nitrogen.
NMR Spectroscopy:
Data Collection:2.2 Å. X-ray source: Diffraction data were collected at the SLS beamline X10 at a single wavelength (0.9537 Å).
Data Processing: