FDPS + NE58025

PDB:3CP6

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:IMAGE:4132071
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal tag: mgsshhhhhhssgrenlyfqghm
Host:BL21(DE3)

Construct

Prelude:
Sequence:

ghmNGDQNSDVYAQEKQDFVQHFSQIVRVLTEDEMGHPEIGDAIARLKEVLEYNAIGGKYNRGLTVVVAFRELVEPRKQDADSLQRA
WTVGWCVELLQAFFLVADDIMDSSLTRRGQICWYQKPGVGLDAINDANLLEACIYRLLKLYCREQPYYLNLIELFLQSSYQTEIGQT
LDLLTAPQGNVDLVRFTEKRYKSIVKYKAAFYSFYLPIAAAMYMAGIDGEKEHANAKKILLEMGEFFQIQDDYLDLFGDPSVTGKIG
TDIQDNKCSWLVVQCLQRATPEQYQILKENYGQKEAEKVARVKALYEELDLPAVFLQYEEDSYSHIMALIEQYAAPLPPAVFLGLAR
KIYKRRKafter tag removal

Vector:p11-Toronto

Growth

Medium:
Antibiotics:
Procedure: Overnight cultures in LB (10 ml with100 µg/ml ampicillin) were used to inoculate 1 litre of LB medium containing 100 µg/ml ampicillin. Cultures were grown at 37°C until they reached an OD600 of 0.6-0.8 and then induced with 1 mM IPTG. The temperature was adjusted to 18°C and expression was allowed to continue overnight. The cells were collected by centrifugation.

Purification

Procedure
Column 1: 2ml Ni-NTA agaroseBuffers: Binding: 50 mM HEPES pH 7.5, 5 mM imidazole, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP; Wash: 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM imidazole, 5% glycerol, 0.5 mM TCEP; Elution: 50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM imidazole, 5% glycerol, 0.5 mM TCEP.Procedure: Approximately 75 ml of bacterial lysate was loaded by gravity onto a 2 ml Ni-NTA agarose columns pre-equilebrated with binding buffer. The columns were then washed twice with 30ml binding buffer, then twice with 12.5ml of wash

buffer. Protein was then eluted with 12.5 ml of elution buffer and collected as 1.5ml fractions. Fractions containing purified protein were pooled and concentrated to a volume of less than 5mls using a Vivaspin concentrator with 10 kD MW cutoff.Column 2: Hiload 16/60 Superdex 200 prep grade 120 mlBuffers: 10 mM Hepes pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEPProcedure: The concentrated protein was loaded onto the column at 1 ml/min using an AKTA purifier system. Eluted protein was collected in 1 ml fractions.

Extraction

Procedure
The cell pellet was resuspended in 50 mM HEPES pH 7.5, 5 mM imidazole, 500 mM NaCl, 5% glycerol.and lysed using a high pressure cell disruptor. The lysate was centrifuged at 17,000 RPM for 30 minutes at 4°C and the supernatant was collected.
Concentration: All fractions containing pure protein were pooled and concentrated to 85 mg/ml using a Vivaspin concentrator with 10 kD MW cutoff.
Ligand
NE58025**MassSpec:**Characterisation of the protein by mass spectrometry revealed MW of 40.697kDa after TEV cleavage, coinciding with the predicted mass of the T201A mutant.
Crystallization:NE58025 1R6S was prepared as a 100 mM stock solution in 100mM Tris HCl pH 7.7. MgCl2 was prepared as a 100 mM aquaeus stock solution. NE58025 1R6S and MgCl2 were added to the protein to a final concentration of 2 mM each and a final protein concentration of 15 mg/ml. Crystals were grown at 20°C in 300 nl sitting drops by mixing 100 nl of protein solution and 200 nl of precipitant consisting of 0.2M NH4Cl pH6.3, 20% PEG 6000, 10% Ethylene glycol. Crystals were mounted using 20% ethylene glycol as a cryoprotectant before flash freezing.
NMR Spectroscopy:
Data Collection: Resolution: 2.0Å; X-ray source: Rotating anode, FR-E superbright.
Data Processing: