MGC35130

PDB:1YRV

Revision

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Entry Clone Accession:gi 20988674
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SGC Clone Accession:ubc53.001.150; plate SDC006:D3
Tag:
Host:E.coli BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsMHGRAYLLLHRDFCDLKENNYKGITAKPVSEDMMEWEVEIEGLQNSVWQGLVFQLTIHFTSEYNYAPP
VVKFITIPFHPNVDPHTGQPCIDFLDNPEKWNTNYTLSSILLALQVMLSNPVLENPVNLEAARILVKDESLYRTILRLFNRP

Vector:

Growth

Medium:
Antibiotics:
Procedure:MGC35130 was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin at 37ºC to an OD600 of 7.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15ºC. The culture was centrifuged and the cell pellets were collected and stored at -80ºC.

Purification

Procedure
The cleared lysate was loaded onto a TALON metal-affinity resin column from BD Biosciences at 4ºC. The column was washed with wash buffer A (10mM Tris-HCl pH 8.0, 0.5 M NaCl, 5% glycerol, 10 mM imidazole, 1 mM ß-mercaptoethanol), wash buffer B (same as wash buffer A but containing 0.05% Tween with protease 20) and again wash buffer A, and the protein was eluted with 10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 200 mM imidazole, 1 mM ß-mercaptoethanol. The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with 20 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 2mM dithiothreitol (DTT) and concentrated by ultrafiltration.

Extraction

Procedure
The cell pellet was resuspended in lysis buffer (10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 2 mM imidazole, 1 mM ß-mercaptoethanol) inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF) and lysed using Microfluidizer. The lysate was cleared by centrifugation.
Concentration:48 mg/mL
Ligand
MassSpec:
Crystallization: Purified MGC35130 was crystallized using the hanging drop vapor diffusion method. Crystals grew when the protein (12 mg/mL) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 11% PEG MME 5000, 5% Tacsimate, 15mM DTT, 0.1M HEPES, pH 7.4.
NMR Spectroscopy:
Data Collection:
Data Processing: