CCDC101

PDB:3MEV

Revision

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Entry Clone Accession:Q96ES7
Entry Clone Source:MGC AT16-F2 (Q96ES7)
SGC Clone Accession:SGF29_6; plate JMC01N:E01
Tag:N-terminal tag: MGSSHHHHHHSSRENLYFQG
Host:BL21 (DE3) Codon plus RIL (Stratagene)

Construct

Prelude:
Sequence:

MHHHHHHSSRENLYFQGRRGVLMTLLQQSAMTLPLWIGKPGDKPPPLCGAIPASGDYVARPGDKVAARVKAVDGDEQWILAEVVSYS
HATNKYEVDDIDEEGKERHTLSRRRVIPLPQWKANPETDPEALFQKEQLVLALYPQTTCFYRALIHAPPQRPQDDYSVLFEDTSYAD
GYSPPLNVAQRYVVACKEPKKK

Vector:pET28a-MHC

Growth

Medium:
Antibiotics:
Procedure:A 250 mL flask containing LB (Sigma L7658) supplemented with 50 µg/mL kanamycin (BioShop Canada KAN 201) was inoculated from a glycerol stock of the bacteria. The flask was shaken overnight (16 hours) at 250 rpm at 37 °C. Using the Lex system, a 2L bottle (VWR 89000-242) containing 1800 mL of TB (Sigma T0918) supplemented with 1.5% glycerol, 50 ug/ mL kanamycin and 600 µl antifoam 204 (Sigma A-8311) was inoculated with 50 mL overnight LB culture, and incubated at 37 °C. The temperature of the media was reduced to 15 °C one hour prior to induction and induced at OD600 = 6 with 100 µM isopropyl-thio-β-D-galactopyranoside (BioShop Canada IPT 001). Cultures were aerated overnight (16 hours) at 15 °C, and cell pellets collected by centrifugation and frozen at -80 °C.

Purification

Procedure
IMAC: Unclarified lysate was mixed with 2-3 mL of Ni-NTA superflow Resin (Qiagen) per 40 mL lysate. The mixture was incubated with mixing for at least 45 minutes at 4oC. The mixture was than loaded onto an empty comLum (BioRad) and washed with 100 mL wash buffer.

Samples were eluted from the resin by exposure to 2-3 column volumes (approx. 10-15 mL) of elution buffer. Concentration of eluted protein was estimated by OD280. Gel filtration chromatography: An XK 26x65 column (GE Healthcare) packed with HighLoad Superdex 75 resin (GE Healthcare) was pre-equilibrated with gel filtration buffer for 1.5 column volumes using an AKTA explorer (GE Healthcare) at a flow rate of 1.0 mL/min. The dialyzed sample from the IMAC step (approx. 15 mL) was loaded onto the column at 1.5 mL/min, and 2mL fractions were collected into 96-well plates (VWR 40002-012) using peak fractionation protocols). Fractions observed by a UV absorption chromatogram to contain the protein were pooled.

Extraction

Procedure
Frozen cell pellet contained in bags (Beckman 369256) obtained from 2L of culture were thawed by soaking in warm water. Each cell pellet was resuspended in 25-40 mL lysis buffer and homogenized using an Ultra-Turrax T8 homogenizer (IKA Works) at maximal setting for 30-60 seconds per pellet. Cell lysis was accomplished by sonication (Virtis408912, Virsonic) on ice: the sonication protocol was 10 sec pulse at half-maximal frequency (5.0), 10 second rest, for 10 minutes total sonication time per pellet.
Concentration: Purified proteins were concentrated using 15 mL concentrators with a 5,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore) at 3750 rpm, typically resulting in a final concentration around 20 mg/mL.
Ligand
MassSpec:
Crystallization: Crystals of SGF29 tandem tudor domain were grown at 291 K using the sitting drop method by mixing equal volumes of 20-28% PEG3350, 0.1M Bis-Tris pH 5.5, 5mM H3[R2A]K4me3 peptide was present in the protein stock solution as binding partner. The crystals were cryoprotected by cryoprotectant consisting of 100% reservoir solution and 20% glycerol.
NMR Spectroscopy:
Data Collection:
Data Processing: