Molecular Biology

Entry Clone Accession: BC008716
Entry Clone Source: MGC
SGC Construct ID: DDR1A-c002
Protein Region: P601-V913
Vector: pFB-LIC-Bse
Tag: N-6HIS;N-TEV
Host: DH10Bac

Sequence (with tag(s)):
MGHHHHHHSSGVDLGTENLYFQSMPRVDFPRSRLRFKEKLGEGQFGEVHLCEVDSPQDL
VSLDFPLNVRKGHPLLVAVKILRPDATKNARNDFLKEVKIMSRLKDPNIIRLLGVCVQDDP
LCMITDYMENGDLNQFLSAHQLEDKAAEGAPGDGQAAQGPTISYPMLLHVAAQIASGM
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CILMGKFTTASDVWAFGVTLWEVLMLCRAQPFGQLTDEQVIENAGEFFRDQGRQVYLSR
PPACPQGLYELMLRCWSRESEQRPPFSQLHRFLAEDALNTV
Sequence after tag cleavage:
SMPRVDFPRSRLRFKEKLGEGQFGEVHLCEVDSPQDLVSLDFPLNVRKGHPLLVAVKILRP
DATKNARNDFLKEVKIMSRLKDPNIIRLLGVCVQDDPLCMITDYMENGDLNQFLSAHQL
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DNA Sequence:
GGCTTAGGAAGTATTAACTGATCTCTGCCCTAGTTCTCATGTGTTAAATATGGATAGTA
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ACTAATATATGGACCTAGCTTGAGGCAATTTTAATCCCCTGCACTAGGCAGGTAATAAT
AAAGGTTGAGTTTTCCACAAAAAAAAAAAAAA

Protein Expression

Medium: Insect Xpress;
Antibiotics: Ampicillin

Procedure: Sf9 cells at a density of $2x10^6$/ml were infected with recombinant DDR1 baculovirus (virus stock P2; 3ml of virus stock per 1000ml cell culture). Cells were shaken at 110rpm at 27°C in an Infors shaker with a radii of 25mm. After 72 hours since infection the cultures were harvested by centrifugation at 900g for 20 mins. Cell pellets were resuspended in 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM Imidazole, 5 % glycerol plus Merck Set III protease inhibitor and stored at -20°C.

Protein Purification

Procedure: Cells were lysed by sonication and lysates clarified by centrifugation at 21,000rpm following the addition of 0.125% polyethyleneimine. The resulting supernatant was incubated with Ni-sepharose resin (equilibrated in 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM Imidazole, 5 % glycerol), mixing by inversion, at 4ºC for 1 hour. This was centrifuged at 700g for 5mins, the supernatant removed and resin resuspended in 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM Imidazole, 5 % glycerol. This was applied to a gravity column and washed and eluted with 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, and 30-250mM imidazole. Fractions containing protein (as seen by SDS-PAGE) were treated with TEV protease and 10mM DTT overnight at 4ºC prior to gel filtration on an S200 gel filtration column using 50 mM HEPES pH 7.5, 300 mM NaCl and 1mM TCEP as the running buffer. Additional purification was carried out by loading fractions from gel filtration onto a Ni-IMAC gravity column equilibrated in 50 mM HEPES pH 7.5, 300 mM NaCl and 1mM TCEP and collecting the flow through. The flow through was concentrated to 13.6 mg/ml using a centrifugal concentrator.
Columns: Column 1: Ni-IMAC; Column 2: Superdex 16/60 S200 gel filtration; Column 3: Ni Rebind
Concentration: 13.6 mg/ml
Mass-spec Verification: Correct cleaved intact mass observed.

Structure Determination

Crystallization: Protein was incubated with the compound PK012130a (3-(2-imidazo[1,2-a]pyrazin-3-ylethynyl)-~{N}-[3-[(4-methylpiperazin-1-yl)methyl]-5-(trifluoromethyl)phenyl]-4-propan-2-yl-benzamide) dissolved in DMSO at a final concentration of 1mM for 4 hours on ice prior to 0.22um spin filtration and the setting up of crystal plates. Crystals were grown using the sitting drop vapor diffusion method at 20ºC. Crystals were grown in 150 nl drops consisting of 2:1 mother liquor (10% ethylene glycol, 0.2M sodium sulfate, 24% PEG3350, 0.1M bis-tris-propane pH 7.1) to protein (13.6 mg/ml) with compound at 1mM. 20% ethylene glycol was added to the crystal as a cryoprotectant and the crystal was mounted and flash frozen in liquid nitrogen.
Data Collection: Data was collected at beamline I03 at 100K.
Data Processing: Data was processed to a resolution of approximately 2.1 Å using XDS and either xia2 or autoPROC, or DIALS.