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
RYLATLNFVHRDLATRNCLVGENFTIKIADFGMSRNLYAGDYYRVQGRAVLPIRWMAWE
CILMGKFTTASDVWAFGVTLWEVLMLCRAQPFGQLTDEQVIENAGEFFRDQGRQVYLSR
PPACPQGLYELMLRCWSRESEQRPPFSQLHRFLAEDALNTV
Sequence after tag cleavage:
SMPRVDFPRSRLRFKEKLGEGQFGEVHLCEVDSPQDLVSLDFPLNVRKGHPLLVAVKILRP
DATKNARNDFLKEVKIMSRLKDPNIIRLLGVCVQDDPLCMITDYMENGDLNQFLSAHQL
EDKAAEGAPGDGQAAQGPTISYPMLLHVAAQIASGMRYLATLNFVHRDLATRNCLVGEN
FTIKIADFGMSRNLYAGDYYRVQGRAVLPIRWMAWECILMGKFTTASDVWAFGVTLWEV
LMLCRAQPFGQLTDEQVIENAGEFFRDQGRQVYLSRPPACPQGLYELMLRCWSRESEQR
PPFSQLHRFLAEDALNTV
DNA Sequence:
CCATGGGCCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTG
TACTTCCAATCCATGCCCAGAGTGGATTTCCCTCGATCTCGACTCCGCTTCAAGGAGA
AGCTTGGCGAGGGCCAGTTTGGGGAGGTGCACCTGTGTGAGGTCGACAGCCCTCAA
GATCTGGTTAGTCTTGATTTCCCCCTTAATGTGCGTAAGGGACACCCTTTGCTGGTAGC
TGTCAAGATCTTACGGCCAGATGCCACCAAGAATGCCAGGAATGATTTCCTGAAAGA
GGTGAAGATCATGTCGAGGCTCAAGGACCCAAACATCATTCGGCTGCTGGGCGTGTG
TGTGCAGGACGACCCCCTCTGCATGATTACTGACTACATGGAGAACGGCGACCTCAAC
CAGTTCCTCAGTGCCCACCAGCTGGAGGACAAGGCAGCCGAGGGGGCCCCTGGGGA
CGGGCAGGCTGCGCAGGGGCCCACCATCAGCTACCCAATGCTGCTGCATGTGGCAGC
CCAGATCGCCTCCGGCATGCGCTATCTGGCCACACTCAACTTTGTACATCGGGACCTG
GCCACGCGGAACTGCCTAGTTGGGGAAAATTTCACCATCAAAATCGCAGACTTTGGC
ATGAGCCGGAACCTCTATGCTGGGGACTATTACCGTGTGCAGGGCCGGGCAGTGCTGC
CCATCCGCTGGATGGCCTGGGAGTGCATCCTCATGGGGAAGTTCACGACTGCGAGTG
ACGTGTGGGCCTTTGGTGTGACCCTGTGGGAGGTGCTGATGCTCTGTAGGGCCCAGC
CCTTTGGGCAGCTCACCGACGAGCAGGTCATCGAGAACGCGGGGGAGTTCTTCCGGG
ACCAGGGCCGGCAGGTGTACCTGTCCCGGCCGCCTGCCTGCCCGCAGGGCCTATATG
AGCTGATGCTTCGGTGCTGGAGCCGGGAGTCTGAGCAGCGACCACCCTTTTCCCAGC
TGCATCGGTTCCTGGCAGAGGATGCACTCAACACGGTGTGACAGTAAAGGTGGATAC
GGATCCGAATTCGAGCTCCGTCGACAAGCTT

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 1000 ml cell culture). Cells were shaken at 110rpm at

27°C in an Infors shaker with a radius of 25 mm. 72 hours after infection the cultures were harvested by centrifugation at 900 g 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: Stored cell pellets were thawed and sonicated on ice for 5 mins (5 min cycle of 5 seconds on/10 seconds off). 1ml of 5% PEI (0.125 %) was added to the lysate. Lysate was centrifuged for 60 min at 4 degrees in a JA 25.50 rotor at 60,000 g (22,000 rpm). The supernatant was loaded onto Ni-IMAC resin (equilibrated in 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM Imidazole, 5 % glycerol) and rotated gently at 4ºC for 1 hour. 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. The final fraction contained 1M imidazole. Fractions containing protein (as seen by SDS-PAGE) were treated with TEV protease overnight at 4ºC prior to gel filtration on an S200 gel filtration column using 50 mM HEPES pH 7.5, 300 mM NaCl and 0.5 mM 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 0.5 mM TCEP and collecting the flow through, wash and first elution (30 mM imidazole). The flow through was concentrated to 8.4 mg/ml using a centrifugal concentrator.
Columns: Column 1: Ni-IMAC, then TEV; Column 2: S200; Column 3: ni rebind;
Concentration: 8.41 mg/ml
Mass-spec Verification: Intact mass confirmed by LC-MS as 38147.1 Da

Structure Determination

Crystallization: *Crystallization Condition:* 20% Polyethylene glycol 3350, 10% ethylene glycol, 0.1M Bis-tris propane pH 6.5, 0.2M Sodium Acetate (20 deg C)*; Protein Concentration:* 8.72 mg/ml*; Crystallization Ligands: Compound 1:* D2099 inhibitor;
In order to enhance the compound solubility in aqueous solution, 100 µl of protein stock at 8.4 mg/ml was diluted in 2 ml of gel filtration buffer. 2 µl of compound stock (to reach 1 mM proportion with the protein) was added to the dilution and mixed with a P1000 pipette to allow as much compound as possible to dissolve. The mixture was concentrated again to 100 µl using a 10kDa cut-off Amicon centrifugal device, transferred to a 1.5 ml tube and spun down for 10 mins at 4 degrees in a microfuge at maximum speed to precipitate the remaining insoluble compound.
Data Collection: Data was collected at beamline I03 at 100K, wavelength 0.97949.
Data Processing: Data was processed to a resolution of ~2.3 Å using Phaser, Aimless, XDS and buster.