RFXANK

PDB:3UXG

Revision

Revision Type:created
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Entry Clone Accession:NP_003712.1.
Entry Clone Source:MGC CM32-D8 (NP_003712.1)
SGC Clone Accession:RFXANK_6; plate JMC029A03
Tag:N-terminal tag: MGSSHHHHHHSSGRENLYFQG
Host:BL21 (DE3) Codon plus RIL (Stratagene)

Construct

Prelude:
Sequence:

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MGSSHHHHHHSSGRENLYFQGSALPATLDSLSIHQLAAQGELDQLKEHLRKGDNLVNKPDERGFTPLIWASAFGEIETVRFLLEWGA
DPHILAKERESALSLASTGGYTDIVGLLLERDVDINIYDWNGGTPLLYAVRGNHVKCVEALLARGADLTTEADSGYTPMDLAVALGY
RKVQQVIENHILKLFQSNLVPADPE
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Vector:pET28-MHL

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 aglycerol 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 µg/ 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 hourprior to induction and induced at OD600 = 6 with 100 µM isopropyl-thio-ß-D-galactopyranoside (BioShop Canada IPT 001). Cultures wereaerated 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 withmixing for at least 45 minutes at 4oC. The mixture was then 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 proteinwas estimated by OD280. pTEV was added to eluted protein at 1:20 for eluted protein and dialyze against gel filtration buffer overnight toremove His-tag.

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. Thedialyzed 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-wellplates (VWR 40002-012) using peak fractionation protocols). Fractions observed by a UV absorption chromatogram to contain the protein werepooled.

Extraction

Procedure
Frozen cell pellet contained in bags (Beckman 369256) obtained from 2L of culture were thawed by soaking in warm water. Each cell pelletwas resuspended in 25-40 mL lysis buffer and homogenized using an Ultra-Turrax T8 homogenizer (IKA Works) at maximal setting for 30-60seconds 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 30-50 mg/mL.
Ligand
MassSpec:
Crystallization:RFXANK (residues 90 to 260) and the RFX5 peptide (residues 167 to 183) were mixed in a ratio of 1:5 and crystallized in a buffer containing0.1 M bis-tris HCl (pH 6.5), 0.2 M ammonium acetate, and 25% PEG 3350. RFXANK-R199H (residues 90 to 260) and the HDAC4 peptide(residues 343 to 359) were mixed in a ratio of 1:3 and crystallized in a buffer containing: 0.1 M hepes (pH 7.5), 0.2 M NaCl, and 25% PEG3350.14-3-3γ was purified as described previously (1) and concentrated to 30 mg/ml in a buffer containing 20 mM tris-HCl (pH 7.5), 150 mM NaCl,1 mM dithiothreitol (DTT). 14-3-3γ was then mixed with the pSer350 HDAC4 peptide (residues 343 to 359) in a ratio of 3:1 and crystallized ina buffer containing 0.3 M magnesium acetate and 20% PEG3350. Before flash-freezing in liquid nitrogen, all of the crystals were soaked in acryoprotectant consisting of 85 to 90% reservoir solution and 10 to 15% glycerol (v/v).
NMR Spectroscopy:
Data Collection:
Data Processing: