CXXC1

PDB:3QMC

Revision

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SGC Clone Accession:CXXC1_13; plate JMC022H12
Tag:N-terminal tag: MGSSHHHHHHSSGRENLYFQG
Host:BL21 (DE3) Codon plus RIL (Stratagene)

Construct

Prelude:
Sequence:

MHHHHHHSSRENLYFQGQIKRSARMCG ECEACRRTED CGHCDFCRDM KKFGGPNKIR QKCRLRQCQL RARESYKYFP SS

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 froma 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 (VWR89000-242) 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 °Cone hour prior to induction and induced at OD600 = 6 with 100 µM isopropyl-thio-β-D-galactopyranoside (BioShop Canada IPT 001). Cultureswere 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 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

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 into96-well plates (VWR 40002-012) using peak fractionation protocols). Fractions observed by a UV absorption chromatogram to contain theprotein 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 pelletwas 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 athalf-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 human CFP1 CXXC domain was mixed with differentCpG DNAs at a molar ratio of 1:1.2 and then crystallized using thehanging dropvapour diffusion method at 18 °C. CFP1 and CpG DNA was crystallized in a buffer containing 0.1-M Hepes sodium, pH 7.5,0.2-M $CaCl_2$, 28% PEG 400 (GCGG, CCGG1 and ACGG DNAs) or 0.1-M Hepes sodium, pH 7.5, 0.1-M $MgCl_2$, 30%550 MME (TCGT,ACGT and TCGA DNAs). Before flash-freezing crystals in liquid nitrogen, crystals were soaked in a cryoprotectant consisting of 100%reservoir solution and 12% glycerol.
NMR Spectroscopy:
Data Collection:
Data Processing: