NFATC2IP

PDB:2L76
Entry Clone Accession:AT129-A8
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:HS00387_244_338
Tag:MGSSHHHHHHSSGRENLYF
Vector:p15Tvlic

Sequence:

QGQEDEVVLVEGPTLPETPRLFPLKIRCRADLVRLPLRMSEPLQSVVDHMATHLGVSPSRILLLFGETELSPTATPRTLKLGVADII
DCVVLTSS

Growth

Medium: 50 ml of M9 minimal media (100 µM ZnSO4, 8.55 mM NaCl, 47.6 mM Na2HPO4, 22 mM KH2PO4, 100 mM MgSO4, 2 mM biotin, 1.5 mM thiamine.HCl, 10 mM ZnSO4, and 0.1 M CaCl2) supplemented with 15NH4Cl, 13C6-D-glucose, and 50 µg/ml kanamycin

Procedure: Competent BL21 (DE3) cells (Invitrogen, C6000-03) were transformed and incubated overnight (18 hours) at 220 rpm at 37 °C in a 125 ml flask containing 50 ml of M9 minimal media (100 uM ZnSO4, 8.55 mM NaCl, 47.6 mM Na2HPO4, 22 mM KH2PO4, 100 mM MgSO4, 2 mM biotin, 1.5 mM thiamine.HCl, 10 mM ZnSO4, and 0.1 M CaCl2) supplemented with 15NH4Cl, 13C6-D-glucose, and 50 µg/ml kanamycin. The overnight starter culture was transferred to a 2 L flask containing 1 L of M9 minimal media supplemented with supplemented with 15NH4Cl, 13C6-D-glucose, and 50 µg/ml kanamycin, and incubated at 37 °C. When the OD_{600} reached a value of 1.0, protein expression was induced with 100 µM isopropyl-thio-ß-D-galactopyranoside and the cells were incubated overnight (15.5 hours) at 220rpm at 15 °C.

Purification

Procedure: The frozen cell pellet stored in a 50 ml Falcon tube obtained from 1L of culture was thawed by soaking in warm water, and resuspended in 40 mL lysis buffer. The cell pellet was lysed by sonication (Branson Sonicator, probe catalog #) on ice for 10 minutes total sonication time (10 sec pulses at half-maximal frequency with 10 second rest)]. The lysate was clarified by centrifugation for 20 min at 4 °C. The supernatant was mixed with 2 mL of Ni2+ affinity beads per 40 mL lysate. The mixture was incubated with mixing for 20 minutes at 4 °C. The lysate was spun at 2000 rpm for 6 minutes, and the supernatant was decanted. The remaining resin was resuspended and washed twice with lysis buffer, followed by two washes with with 5 mL of cold wash buffer. The washed resin was transferred to a gravity filter column and further washed with 2 mL of wash buffer. Samples were eluted from the resin by exposure to 5mL of elution buffer.

Buffer exchange & protein concentration: The purified protein was exchanged from elution buffer into MOPS-based NMR buffer by ultracentrifugation using 5 mL concentrators with a 5,000 molecular weight cut-off (VivaSpin 2 MES) at 3000 rpm, resulting in a final volume of 300ul (final protein concentration of 0.9 mM). The concentrated protein was transferred to a 5mm Shigemi NMR tube.

Extraction

Procedure: Cell pellets were collected by centrifugation (7000 rpm, 20 mins) and frozen in 50 mL Falcon tubes at -80 °C for storage.

Structure Determination

MassSpec: MW=12624.52 g/mol
NMR Spectroscopy: A series of spectra (3D 1H-13C NOESY, 3D 1H-15N NOESY, 2D 1H-13C Constant Time HSQC, 3D HNCO, 3D HNCA, 3D CBCA(CO)NH, 3D HBHA(CO)NH, 3D 1H-13C Aromatic NOESY, 3D (H)CCH-TOCSY, and 3D H(C)CH-TOCSY) were generated using a 500MHz Bruker AVANCE spectrometer, a 600MHz Bruker AVANCE spectrometer and a 800MHz Bruker AVANCE spectrometer. NMR data was processed and analyzed using Topspin, NMRPipe, NMRDraw, Sparky, Abacus, FMC-GUI, CNS, TALOS, PALES, PSVS, and WhatIF.