

LMNB2

PDB:2LLL

Revision

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Entry Clone Accession:AU46-B6

Entry Clone Source:MGC

SGC Clone Accession:jmc29e12

Tag:MHHHHHHSSGRENLYFQG

Host:BL21 (DE3) cells (Invitrogen, C6000-03)

Construct

Prelude:

Sequence:

MHHHHHHSSGRENLYFQGSASGSVSIEEIDLEGKFVQLKNNSDKDQSLGNWRIKRQVLEGEETIAYKFTPKYILRAGQMVTVWAAGAG
VAHSPPSTLVWKGQSSWGTGESFRTVLVNADGEEVAMRTVKKSSVMRENENG

Vector:pET28-MHL

Growth

Medium:

Antibiotics:

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, 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 0.5 L of 2X M9 minimal media 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 1mM isopropyl-thio-β-D-galactopyranoside and the cells were incubated overnight (15.5 hours) at 220rpm at 15°C . Cell pellets were collected by centrifugation (7000 rpm, 20 mins) and frozen in 50 mL Falcon tubes at -80°C for storage.

Purification

Procedure

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 (15.4mM tris.HCl, 100 uM ZnSO₄ 100uL, 0.5 mM NaCl, and 500 mM imidazole; pH 8.5).

Extraction

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 25 mL lysis buffer (15.4mM tris.HCl, 100 uM ZnSO₄ 100uL, 0.5 mM NaCl, and 15 mM imidazole; pH 8.5). The cell pellet was lysed by sonication (Branson Sonicator) on ice for 5 minutes and 30 secs total sonication time (30 sec pulses at amplitude of 72% frequency with 30 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 25 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 (15.4mM tris.HCl, 100 uM ZnSO₄ 100uL, 0.5 mM NaCl, and 30 mM imidazole; pH 8.5).

Concentration:

Ligand

MassSpec:

Crystallization:

NMR Spectroscopy: Buffer exchange & protein concentration. The purified protein was exchange from elution buffer into phosphate-based NMR buffer (for H₂O experiments: 10 mM sodium phosphate, 300 mM NaCl, 1 mM Benzamidine, 0.01% NaN₃, 0.01 mM ZnSO₄, 10% D₂O, and 90% H₂O, pH 7.0; for 10 mM Tris-HCl, 400 mM NaCl, 1 mM Benzamidine, 0.01% NaN₃, 0.01 mM ZnSO₄, and 100% D₂O, pH 7.0) 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.

Data Collection: 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 600MHz Bruker AVANCE spectrometer and a 800MHz Bruker AVANCE spectrometer. NMR data was processed and analyzed using Topspin, NMRPipe, NMRDraw, Sparky, FMC-GUI, CYANA, CNS, TALOS, and PSVS.

Data Processing: The assigned chemical shifts and spectral peak lists were deposited on 2011. 11.11 into the BMRB database with the ID code 18053 with the following authors: Lemak, A., Yee, A., Houliston, S., Garcia, M., Xu C., Min, J., and Arrowsmith, C.H.