LAMMER

PDB:3LLT

Revision

Revision Type:created
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Entry Clone Accession:AAK38173.1
Entry Clone Source:
SGC Clone Accession:PP-LAMMER:D534-E875
Tag:mgsshhhhhhssgrenlyfq
Host:BL21(DE3)-V2R-pACYC LamP

Construct

Prelude:
Sequence:

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DDEIVHFSWKKGMLLNNAFLVIRKMGDGTFGRVLLCQHIDNKKYYAVKVVRNIKKYTRSAKIEADILKKIQNDDINNNNIVKYHGKF
MYYDHMCLIFEPLGPSLYEIITRNNYNGFHIEDIKLYCIEILKALNYLRKMSLTHTDLKPENILLDDPYFEKSLITVRRVTDGKKIQ
IYRTKSTGIKLIDFGCATFKSDYHGSIINTRQYRAPEVILNLGWDVSSDMWSFGCVLAELYTGSLLFRTHEHMEHLAMMESIIQPIP
KNMLYEATKTNGSKYVNKDELKLAWPENASSINSIKHVKKCLPLYKIIKHELFCDFLYSILQIDPTLRPSPAELLKHKFLE
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Vector:pET15-MHL

Growth

Medium:TB
Antibiotics:
Procedure:PF14_0431 was expressed in E. coli BL21(DE3)-V2R-pACYC LamP cells in Terrific Broth (TB) in the presence of carbenicillin /chloramphenicol (100 microg/mL and 34 microg/mL respectively). A single colony was inoculated into 25 mL of LB with of in a 25 mL(100 microg/mL and 34 microg/mL respectively) Falcon tube and incubated with shaking at 220 rpm overnight at 37 degC. Then the culture was transfered into 1 L of TB with 100 microg/mL carbenicillin, 34 microg/mL chloramphenicol and 0.5 mL of antifoam (Sigma) in a 1 L bottle and cultured using the LEX system to an OD 600 of ~5, cooled to 15 degC, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 degC.

Purification

Procedure
The cleared lysate was loaded onto a column prepacked with 10 g DE52 (Whatman) anion exchange resin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer); and subsequently onto a 1.0 - 2.5 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer

at approximately 1 - 1.5 mL/min. After the lysate was loaded, the DE52 was further washed with 20 mL of Binding Buffer. The Ni-NTA column was then washed with 200 mL of Wash Buffer at 2 - 2.5 mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer. TCEP was then added to 5 mM after approximately 15 minutes after elution.

The sample was then loaded onto a Superdex S200 26/60 column equilibrated with Gel Filtration Buffer. The fractions from the peak eluting at 232 mL corresponding to monomeric protein were pooled and concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The protein sample identity and purity were evalulated by mass spectroscopy and SDS-PAGE gel. The concentrated protein was stored at 4 degC.

Extraction

Procedure
The culture was harvested by centrifugation. Pellets from 4 L of culture were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with the addition of 400uL/ L protease inhibitors (1 tablet dissolved in 25ml of ddH2O, Roche complete EDTA-free). Resuspended pellets stored at -80 degC were thawed overnight at 4 degC on the day before purification. Prior to mechanical lysis, each pellet from 1 L of culture was pretreated with 0.5 % CHAPS, 100 ul of protease inhibitors(1 tablet dissolved in 25ml of ddH2O, Roche complete EDTA-free) and 500 units of benzonase for 40 minutes at room temperature. Cells were mechanically lysed with a microfluidizer (Microfluidizer Processor, M-110EH) at approximately 18000 psi; and the cell lysate was centrifuged using a Beckman JA-25.50 rotor at ~75000 x g (24000 rpms) for 20 minutes at 10 degC.
Concentration:
Ligand
MassSpec:
Crystallization: The protein was crystallized at 18 degC in 0.2M Na Acetate, 1.85 M NH4SO4, 0.1M Na Cacodylate pH5.6 with ligands 5 mM AMPPNP using the Sitting drop vapor diffusion method.
NMR Spectroscopy:
Data Collection:
Data Processing: