NEDD4L

PDB:2ONI
Entry Clone Accession:gi:21619659
Entry Clone Source:nedd4l.BC032597.MGC.AT55-F3.pCMV-SPORT6
SGC Clone Accession:
Tag:mhhhhhhssgrenlyfqG
Host:BL21 (DE3)
Vector:p28a-LIC-TEV

Sequence:

mhhhhhhssgrenlyfqGSREFKQKYDYFRKKLKKPADIPNRFEMKLHRNNIFEESYRRIMSVKRPDVLKARLWIEFESEKGLDYGG
VAREWFFLLSKEMFNPYYGLFEYSATDNYTLQINPNSGLCNEDHLSYFTFIGRVAGLAVFHGKLLDGFFIRPFYKMMLGKQITLNDM
ESVDSEYYNSLKWILENDPTELDLMFCIDEENFGQTYQVDLKPNGSEIMVTNENKREYIDLVIQWRFVNRVQKQMNAFLEGFTELLP
IDLIKIFDENELELLMCGLGDVDVNDWRQHSIYKNGYCPNHPVIQWFWKAVLLMDAEKRIRLLQFVTGTSRVPMNGFAELYGSNGPQ
LFTIEQWGSPEKLPRAHTCFNRLDLPPYETFEDLREKLLMAVEN

Growth

Medium: Terrific Broth (TB)

Procedure: NEDD4L was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin at 37 degC to an OD_{600} of 7.5. Protein expression was then induced with isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15 degC. The culture was centrifuged and the cell pellets were collected and stored at -80 degC. To produce SeMet derivative of NEDD4L, the same bacterial cells were grown using M9 SeMet High-Yield growth media kit package (Medicilon) according to manufacturer's instructions.

Purification

Procedure: The cleared lysate was loaded onto a TALON metal-affinity resin column (BD Biosciences) at 4 degC (1.5 ml settled gel volume per liter original cell culture). The column was washed with 10 ml wash buffer A, 10 mlwash buffer B and then with 30 ml wash buffer A, and the protein was eluted with 6 ml elution buffer. The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with GF buffer and concentrated by ultrafiltration to a final protein concentration of 10 mg/ml using Amicon Ultra centrifugal filter with 10kD cutoff.

Extraction

Procedure: The cell pellet was resuspended in lysis buffer containing protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF) and lysed using Microfluidizer. The lysate was cleared by centrifugation.

Structure Determination

Crystallization: Crystals were grown in hanging drops by mixing 2 microL protein solution with 2 microL well solution (1.7 M sodium/potassium phosphate, pH 6.0, 1 mM DTT) at 21 degC. For cryoprotection, the crystals were soaked in 2 M sodium-potassium phosphate, pH 7.0, 1 mM DTT, 25% ethylene glycol and 2 mg/ml Nedd4l.574.947.