

UBQLN3

PDB:1YQB

Revision

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Tag:N-terminal His-tag with integrated thrombin-cleavage site MGSSHHHHHHSSGLVPRGS.

Host:E.coli BL21 (DE3)

Construct

Prelude:

Sequence:

mgsshhhhhssglvprgsPHLIKVTVKTPKDKEDFSVTDCTIQQLKEEISQRFKAHPDQLVLIFAGKILKDPDSLAQCGVRDGLT
VHLVIKRQHRAMG

Vector:p28a-LIC

Growth

Medium:

Antibiotics:

Procedure:Ubiquilin 3 was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin at 37°C to an OD600 of 5.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15°C. The culture was centrifuged and the cell pellets were collected and stored at -80°C.

Purification

Procedure

The cleared lysate was loaded onto a TALON metal-affinity resin column from BD Biosciences at 4°C. The column was washed with wash buffer A (10mM Tris-HCl pH 8.0, 0.5 M NaCl, 5% glycerol, 10 mM imidazole, 1 mM β -mercaptoethanol), wash buffer B (same as wash buffer A but containing 0.05% Tween 20) and again wash buffer A, and the protein was eluted with 10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 200 mM imidazole, 1 mM β -mercaptoethanol. The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare) equilibrated with 20 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 2mM dithiothreitol and concentrated by ultrafiltration.

Extraction

Procedure

The cell pellet was resuspended in lysis buffer (10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 2 mM imidazole, 1 mM β -mercaptoethanol) with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF) and lysed using Microfluidizer. The lysate was cleared by centrifugation.

Concentration: 36 mg/mL

Ligand

MassSpec:

Crystallization: Purified ubiquilin 3 was crystallized using the sitting drop vapor diffusion method. Crystals grew when the protein (18 mg/mL) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 2M ammonium sulfate, 0.1 M bis-Tris, pH 6.5.

NMR Spectroscopy:

Data Collection:

Data Processing: