UEV3

PDB:3DL2

Revision

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Tag:MGSSHHHHHHSSGLVPR*GS
Host:*E. coli* BL21 (DE3)

Construct

Prelude:
Sequence:

MGSSHHHHHHSSGLVPR*GSSKSWANHENKTVNKITVVGGGELGIACTLAISAKGIADRLVLLDLSEGTKGATMDLEIFNLPNVEIS
KDLSASAHSKVVIFTVNSLGSSQSYLDVVQSNVDMFRALVPALGHYSQHSVLLVASQPVEIMTYVTWKLSTFPANRVIGIGCNLDSQ
RLQYIITNVLKAQTSGKEVWVIGEQGEDKVLTWSGQEEVVSHTSQVQLSNRAMELLRVKGQRSWSVGLSVADMVDSIVNNKKKVHSV
SALAKGYYDINSEVFLSLPCILGTNGVSEVIKTTLKEDTVTEKLQSSASSIHSLQQQLKL

Vector:PET28a-LIC

Growth

Medium: Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin
Antibiotics:
Procedure: The protein was expressed in *E. coli* BL21 (DE3) grown in growth medium at 37®C to an OD_{600} of 5.5. Protein expression was induced 0.05 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15®C. The culture was centrifuged (12,000 x g, 15 minutes) and cell pellets collected and stored at -80®C.

Purification

Procedure
The cleared lysate was loaded onto a 3 mL TALON metal-affinity resin column (BD Biosciences) at 4ºC. The column was washed with 10 mL Wash buffer A, 10 mL Wash buffer B, and 10 mL Wash buffer A. The protein was eluted with 6 mL Elution buffer. His-tag was removed by incubation of the protein with thrombin (1 U per mg protein, overnight at 4 °C). The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with Gel Filtration buffer. Fractions containing protein (analyzed by SDS-PAGE) were pooled and concentrated by ultrafiltration using an Amicon Ultra centrifugal filter with 10 kD cutoff.

Extraction

Procedure
The cell pellet from a 2 L culture was resuspended in 50 ml Lysis buffer, lysed using a Microfluidizer at 18,000 PSI, and cleared by centrifugation at 40,000 x g for 30 min.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were grown in hanging drops by mixing 2 ul UBE2V3 solution (6 mg/ml) with 2 ul well solution (1.6 M Na/KPO4, pH 6.5, 0.2 M NaCl, 2 mM DTT) at 18°C. The crystals were cryoprotected by immersion in the well solution mixed in 1:1 ratio with a water solution containing 20% (w/v) sucrose, 4% (w/v) glucose, 18% (v/v) glycerol and 18% (v/v) ethylene glycol.
NMR Spectroscopy:
Data Collection:
Data Processing: