Apg4a

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Revision

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Entry Clone Accession:BC061696
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Tag:
Host:E.coliBL21 (DE3)

Construct

Prelude:
Sequence:

MHHHHHHSSGRENLYFQGPDTDELVWILGKQHLLKTEKSKLLSDISARLWFTYRRKFSPI
GGTGPSSDAGWGCMLRCGQMMLAQALICRHLGRDWSWEKQKEQPKEYQRILQCFLDRKDC
CYSIHQMAQMGVGEGKSIGEWFGPNTVAQVLKKLALFDEWNSLAVYVSMDNTVVIEDIKK
MCRVLPLSADTAGDRPPDSLTASNQSKGTSAYCSAWKPLLLIVPLRLGINQINPVYVDAF
KECFKMPQSLGALGGKPNNAYYFIGFLGDELIFLDPHTTQTFVDTEENGTVNDQTFHCLQ
SPQRMNILNLDPSVALGFFCKEEKDFDNWCSLVQKEILKENLRMFELVQKHPSHW

Vector: pET28-MHL

Growth

Medium:
Antibiotics:
Procedure: Apg4a was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) with 50 µg/ml of kanamycin at 37ºC to an OD600 of 7.5. Cells were induced by isopropyl-1-thio-D-galactopyranoside (IPTG) at 0.1 mM overnight at 15ºC. Cell pellets were harvested by centrifugation and stored at -80ºC.

Purification

Procedure
The lysate was mixed with 2-3 mL of HisLink Protein Purification Resin (Promega V8821) per construct. The mixture is incubated with mixing for at least 20 minutes at 4degC. The lysate is spun at 500xg for 3 minutes to pellet the HisLink resin. The lysate is carefully decanted off the resin, and then 50 mL of lysis buffer is added to wash the resin. The resin is allowed to settle for 5 minutes, then poured off and washed 3 more times with fresh lysis buffer. The washed resin is then loaded onto a gravity column, and then washed with a column volume of low imidazole buffer. Samples are eluted from the HisLink resin by exposure to 10-14 mL elution buffer at a 1mL/min flow rate. To cut the (His)6 tag off of the protein, 5 units of TEV protease per milligram of protein is added to the sample and is stored without shaking, overnight, at 4degC. An XK 16x65 column (part numbers 18-1031-47 and 18-6488-01, GE Healthcare) packed with HighLoad Superdex 200 resin (10-1043-04, GE Healthcare) is pre-equilibrated with gel filtration buffer for 1.5 column volumes using an AKTAxpress at a flow rate of 3 mL/min. 5 mL of sample is loaded onto the column at 1.5 mL/min, and 2mL fractions are collected into 96-well plates using peak fractionation protocols. Peak fractions are analyzed for purity using SDS-PAGE and/or mass spectrometry and pooled.

Extraction

Procedure
The cell pellet was resuspended in lysis buffer (10 mM Tris-HCl, pH 8.0, 0.1 M NaCl, 10 mM imidazole and protease inhibitors (0.1µM phenylmethyl sulfonyl fluoride and Sigma general protease inhibitor (Sigma P2714, resuspended and diluted according to manufacturerÂ□s instructions) and lysed using sonication (10 sec pulse at maximal frequency, 10 second rest, for 6 minutes total sonication time, Virsonic).
Concentration:
Ligand
MassSpec:
Crystallization: Purified proteins are concentrated using 15 mL concentrators with an appropriate molecular weight cut-off (Amicon Ultra-15 10,000 MWCO, UFC901024) to a final concentration of 10 mg/mL for crystallography. The experimental conditions were sitting drop, 18oC, 23.5% PEG3350, 0.2M NaCl, 0.1M SPG buffer pH5.4, 5% ethylene glycol. Cryoprotection was achieved using 20% ethylene glycol.

NMR Spectroscopy:
Data Collection:
Data Processing: