ARHGAP11A

PDB:3EAP

Revision

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Entry Clone Accession:gi|7661858
Entry Clone Source:MGC AT68-D2
SGC Clone Accession:HPC077-H08
Tag:N-terminal tag: mhhhhhhssgrenlyfq*g
Host:BL21-CodonPlus(DE3)-RIL

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgMWDQRLVRLALLQHLRAFYGIKVKGVRGQCDRRRHETAATEIGGKIFGVPFNALPHSAVPEYGHIPSFL
VDACTSLEDHIHTEGLFRKSGSVIRLKALKNKVDHGEGCLSSAPPCDIAGLLKQFFRELPEPILPADLHEALLKAQQLGTEEKNKAT
LLLSCLLADHTVHVLRYFFNFLRNVSLRSSENKMDSSNLAVIFAPNLLQTSEGHEKMSSNTEKKLRLQAAVVQTLIDYASDIGRVPD
FILEKIPAML

Vector:pET28-mhl (GI:134105571)

Growth

Medium: Terrific Broth
Antibiotics:
Procedure:LEX Bubbling. The target protein was expressed in E. coli by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 1.8 L of Terrific Broth medium in the presence of 50 µg/mL kanamycin and 50 µg/mL chloramphenicol at 37 degC. When OD600 reached ~3.0, the temperature of the medium was lowered to 18 µC and the culture was induced with 1 mM IPTG. The cells were allowed to grow overnight before they were harvested and flash frozen in liquid nitrogen and stored at -80 degC.

Purification

Procedure
The lysate was centrigued at 15,000 rpm for 45 minutes and the supernatants were mixed with 6 mL 50% Ni-NTA beads, and incubated at 4 degC for 1.5 hours. The supernatant was then passed through a gravity column (Poly-Prep, Bio-Rad, Catalog #731-1550) and the beads were washed using 50 mL washing buffer twice. The protein bound to beads were eluted using 20 mL elution buffer twice. The flow-through was collected and loaded onto Supderdex-75 gel filtration

column. Eluted fractions were pooled and concentrated using amicon centrifugal filter (m.w. cut-off 10,000). The purity of the proteins was higher than 90% judged by SDS-PAGE

Extraction

Procedure
Frozen cells from 9L TB culture were thawed and resuspended in 700 mL extraction buffer with freshly added 1mM PMSF/Benzomidine, 5U/ml of Benzonase (Sigma Catalog # E1014, 250U/microL), 0.5% CHAPS, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and lysed using microfludizer(17000 psi).
Concentration:27.6 mg/mL (22.4 mg/mL for SeMet labeled protein).
Ligand
MassSpec: Native: 30423.35, expected 30423.05
SeMet: 30659.06, expected 30657.53
Crystallization: Crystallization was setup using sitting drops with Red Wings and SGC-I screens initially. Only condition SGC-B10 with 1:100 Endoproteinase Glu-C gives rod like crystals. Optimization was done using hanging drop vaporization, crystals usually appear in 2-3 days.Crystal used for data collection was grown at 0.1M Tris pH 8.0, 6% PEG 8000, 0.2 M NaCl. The protein stock solution was supplemented with 5% Ethylene Glycol, and 1:100 (m:m) Endoproteinase Glu-C. SeMet labeled protein grown under the same condition was also used to confirm the location of the Methionine.
NMR Spectroscopy:
Data Collection:
Data Processing: