ARHGAP27

PDB:3PP2

Revision

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Tag:C-terminal His-tag
Host:BL21-V2R-pRARE2

Construct

Prelude:ARHGAP27:A150-H548:CH
The purified protein contains two domains but the crystallized fragement contains only the PH domain.
Sequence:

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MAVRTKTLDKAGVLHRTKTADKGKRLRKKHWSASWTVLEGGVLTFFKDSKTSAAGGLRQPSKFSTPEYTVELRGATLSWAPKDKSSR
KNVLELRSRDGSEYLIQHDSEAIISTWHKAIAQGIQELSAELPPEESESSRVDFGSSERLGSWQEKEEDARPNAAAPALGPVGLESD
LSKVRHKLRKFLQRRPTLQSLREKGYIKDQVFGCALAALCERERSRVPRFVQQCIRAVEARGLDIDGLYRISGNLATIQKLRYKVDH
DERLDLDDGRWEDVHVITGALKLFFRELPEPLFPFSHFRQFIAAIKLQDQARRSRCVRDLVRSLPAPNHDTLRMLFQHLCRVIEHGE
QNRMSVQSVAIVFGPTLLRPEVEETSMPMTMVFQNQVVELILQQCADIFPPHHHHHHH
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Vector:pNIC-CH

Growth

Medium: Terrific Broth medium in the presence of 50 mg/mL kanamycin and 25 mg/mL chloramphenicol
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 2 L of Terrific Broth medium in the presence of 50 mg/mL kanamycin and 25 mg/mL chloramphenicol at 37 °C. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 °C and the culutre was induced with 1 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 °C.

Purification

Procedure
The lysate was centrifuged at 15,000 rpm for 45 minutes and the supernatants were mixed with 4 mL 50% flurry of Ni-NTA beads and incubated at 4 degree on rotary shaker for one hour. The

mixture was then centrifuged at 2300 rpm for 5 min and the supernant discarded. The beads were then washed with 50 mL binding buffer containing 5 mM Imidazole twice. Bound proteins were eluted using 15 mL elution buffer. The flow-through was collected and further purifed by a Superdex-200 gel filtraton column pre-equilibrated with gel filtration buffer. Fractions containing the protein were collected and loaded on to a Source 30S ion exchange column pre-equilibrated with low salt ion exchange buffer and eluted gradiently using high salt ion exchange buffer. Target protein elutes at 500 mM NaCl concentration. Fractions containing the target protein were pooled and concentrated using Amicon Ultra-15 centrifugal filter (mwco 10 kDa). The purity of the preparation is tested by SDS-PAGE to be greater than 95%.

Extraction

Procedure
Frozen cells from 4L TB culture were thawed and resuspended in 5mL extraction buffer per gram of cell pellets with freshly added 0.5% CHAPS, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 uL benzonase (Sigma Catalog # E1014, 250U/uL), and lysed using sonication for 5 min at 100 W, 10 sec on/10 sec off duty cycle.
Concentration:35.6 mg/mL (in 20 mM MES pH 6.5, 500 mM NaCl)
Ligand
MassSpec:native protein expected 46335.96
measured 46211.1 (-124.9). DNA sequencing verified.
The difference could be caused by a missing Met combined with some modification.
Crystallization: Crystal was initially obtained from Red Wings screen H05.
Crystal used for structure determination was grown in 1.4M Sodium Citrate, 0.1 M HEPES pH 7.5 in the presense of 1:100 (w/w) endoproteinase Glu-C V8 in hanging drop setup, using 2uL protein 2uL well solution again 300 uL reservoir buffer at room temperature.

Crystals grow to a mountable size within one week.
Cryo used well solution + 20% glycerol
NMR Spectroscopy:
Data Collection:
Data Processing: