GNE

PDB:3EO3

Revision

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Entry Clone Accession:NP_005467
Entry Clone Source:MGC: 20-E6
SGC Clone Accession:HPC068-E12
Tag:N-terminal tag: mhhhhhhssgrenlyfq*g
Host:BL21-CodonPlus(DE3)-RIL

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgTLSALAVDLGGTNLRVAIVSMKGEIVKKYTQFNPKTYEERINLILQMCVEAAAEAVKLNCRILGVGIST
GGRVNPREGIVLHSTKLIQEWNSVDLRTPLSDTLHLPVWVDNDGNCAALAERKFGQGKGLENFVTLITGTGIGGGIIHQHELIHGSS
FCAAELGHLVVSLDGPDCSCGSHGCIEAYASGMALQREAKKLHDEDLLLVEGMSVPKDEAVGALHLIQAAKLGNAKAQSILRTAGTA
LGLGVVNILHTMNPSLVILSGVLASHYIHIVKDVIRQQALSSVQDVDVVVSDLVDPALLGAASMVLDYTTRR

Vector:pET28-mhl (GI:134105571)

Growth

Medium:
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 25 µg/mL chloramphenicol at 37 degC. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 degC and the culutre was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 degC. For selenomethionine (SeMet) labeling, prepackaged M9 SeMET growth media kit (Medicilon) was used following manufacturer instructions.

Purification

Procedure
The lysate was centrigued at 15,000 rpm for 45 minutes and the supernatants were mixed with 5 mL 50% flurry of Ni-NTA beads and incubated at 4 degC 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 washing buffer containing 30 mM and 75 mM Imidazole, and finally the elution buffer. The flow-trough was collected and further purifed by a Superdex-75 gel filtraton column pre-equilibrated with gel filtration buffer. Fractions were collected and digested with TEV protease. TEV protease was removed from the treated protein sample by adding 100 uL 50% flurry of Ni-NTA beads and the sample was purified with supderdex-75 gel filtration again. Fractions containing the protein were collected and concentrated with Amicon Ultra-15 centrifugal filter. The purify of the preparation is tested by SDS-PAGE to be around 99%.

Extraction

Procedure
Frozen cells from 1.8L TB culture were thawed and resuspended in 150 mL extraction buffer with freshly added 0.5% CHAPS, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 microL benzonase (Sigma Catalog # E1014, 250U/microL), and lysed using microfluidizer at 15,000 PSI.
Concentration:40 mg/mL
Ligand
MassSpec: Expected mass for native protein 35640.00. The mass results of native and SeMet labeled proteins match with the expected.
Crystallization: Crystallization was setup using sitting drops with Red Wings and SGC-I screens initially. Initial hits were found for conditions RW-D11, RW-G11 and RW-A09 with 1:100 Chymotrypsin (w/w) and 5mM ADP as additives.

Native crystal used for structure determination was grown in 15% PEG4000, 0.2M NH4Ac, 0.1 M NaCitrate, pH 5.6 with 1:100 Chymotrypsin (w/w) and 5mM ADP in sitting drop setup.SeMet crystal used for structure determination was grown in 14.55% PEG4000, 0.2M NH4Ac, 0.1M NaCitrate, pH 6.0 with 1:100 Chymotrypsin (w/w) and 5mM ADP in sitting drop setup.Crystals grow to a mountable size with 24 hours.
NMR Spectroscopy:
Data Collection:
Data Processing: