GMIP

PDB:3QWE

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC126436.1
Entry Clone Source:MGC CM19-D6
SGC Clone Accession:HPC09D-E11
Tag:N-terminal His6-tag, removed before crystallization
Host:BL21-V2R-pRARE2

Construct

Prelude:GMIP:G80-P357
Sequence:

gGEELDLRLIRTKGGVDAALEYAKTWSRYAKELLAWTEKRASYELEFAKSTMKIAEAGKVSIQQQSHMPLQYIYTLFLEHDLSLGTL
AMETVAQQKRDYYQPLAAKRTEIEKWRKEFKEQWMKEQKRMNEAVQALRRAQLQYVQRSEDLRARSQGSPEDSAPQASPGPSKQQER
RRRSREEAQAKAQEAEALYQACVREANARQQDLEIAKQRIVSHVRKLVFQGDEVLRRVTLSLFGLRGAQAERGPRAFAALAECCAPF
EPGQRYQEFVRALRPEAP

Vector:pET28-MHL

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 each 100 mL of overnight culture grown in Luria-Bertani medium into a 2 L of growth medium at 37 °C. When OD_{600} reached ~3.0, the temperature of the medium was lowered to 15 °C and the culture 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 °.

Selenomethionine labeling of the protein used the M9 SeMet growth media kit from Medicilon following the manufacturer\'s instructions.

Purification

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

half hour. The mixture was then centrifuged at 2300 rpm for 5 min and the supernatant discarded. The beads were then washed with 50 mL binding buffer containing 25 mM and 50 mM Imidazole. Bound proteins were eluted using 10 mL elution buffer. The His6 was removed by digesting with 1:10 (w/w) TEV:protein overnight in dialysis against gel filtration buffer. The protein is further purified by a Superdex-75 gel filtration column pre-equilibrated with gel filtration buffer. 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 90%.

Extraction

Procedure
Frozen cells from 4L TB culture were thawed and resuspended in 5mL extraction buffer per gram of cell pellets with freshly added 0.2% NP40, and supplemented with protease inhibitor 1 mM PMSF, and 10 U/mL benzonase (Sigma Catalog # E1014, 250U/uL), and lysed using sonication for 8 min at 110 W, 10 sec on/10 sec off duty cycle.
Concentration:15 mg/mL
Ligand
MassSpec: Uncut version native protein expected 34268.8, measured 34270.8
SeMet cut version, expected 32372.2 (for 5 SeMet for +47.94 each), measured 32372.05
Crystallization: Crystal was initially obtained from SGC-I screen condition A08.
Crystal used for structure refinement was grown in a dehydrated drop of Hampton additive screen in condition SA08 (i.e. in 20% PEG 1500, 0.2 M NaCl, 0.1 M HEPES pH 7.5, 5% Ethyl Glycol, 3% Glucose monohydrate) in sitting drop setup, using 0.5 uL protein + 0.5 uL well solution against 100 uL reservoir buffer at room temperature. The crystals were dehydrated using 5% glycerol added into well solution and incubated further overnight.

Crystal used for phasing was SeMet labelled, grown in SA08-96 well optimization plate (10-30% PEG1500, 0.2 M NaCl, 0.1 M HEPES pH 6.0-9.5, 5% Ethyl Glycol) well H2 (i.e. 12% PEG1500, 0.2M NaCl, 0.1M HEPES pH 9.5, 5% Ethylene glycol).

Crystals grow to a mountable size within two days.
Cryo used paratone-N
NMR Spectroscopy:
Data Collection:
Data Processing: