# GIMAP4

**PDB:**3LXX

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**AU86-H10:BC020657.1
**Entry Clone Source:**MGC
**SGC Clone Accession:**HPC09G-G04
**Tag:**mhhhhhhssgrenlyfq*g
**Host:**BL21-V2R-pRARE2

## Construct

**Prelude:**GIMAP4:G20-M240
Tag not removed
**Sequence:**

mhhhhhhssgrenlyfqgGPGRQEPRNSQLRIVLVGKTGAGKSATGNSILGRKVFHSGTAAKSITKKCEKRSSSWKETELVVVDTPG
IFDTEVPNAETSKEIIRCILLTSPGPHALLLVVPLGRYTEEEHKATEKILKMFGERARSFMILIFTRKDDLGDTNLHDYLREAPEDI
QDLMDIFGDRYCALNNKATGAEQEAQRAQLLGLIQRVVRENKEGCYTNRMYQRAEEEIQKQTQAM

**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 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 °C.

For selenomethionine (SeMet) labeling, prepackaged M9 SeMET growth media kit (Medicilon) was used following manufacturer instructions.

## Purification

**Procedure**
The lysate was centrifuged at 16,000 rpm for 60 minutes and the supernatant was mixed with 6 mL 50% Ni-NTA beads, and incubated at 4 degrees Celsius on roller drum for 1 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 binding buffer followed by 50 mL washing buffer. The protein bound to beads were then eluted using 15 mL elution buffer. The flow-through was collected and loaded onto Supderdex-75 26/60 gel filtration column. Eluted fractions were pooled and added 5mM MgCl2, and concentrated using Amicon centrifugal filter (m.w. cut-off 10,000 ). GDP was then added to the concentrated protein solution to a final concentration of 5 mM. The purity of the proteins was higher than 95% judged by SDS-PAGE.

## Extraction

**Procedure**
Frozen cells from 6L culture were thawed and resuspended in 400 mL extraction buffer with freshly added final concentration of 1 mM PMSF/Benzamidine, 0.5% CHAPS and 5 U/mL Benzonase (Sigma Catalog # E1014, 250U/uL), and supplemented with 1 mL protease inhibitor cocktail (SIGMA Catalog # P8849), and lysed using sonication at 10 seconds 50% duty cycle for 9 minutes at 120 W.
**Concentration:**17 mg/mL
**Ligand**
GDP, $Mg^{2+}$**MassSpec:**Native protein expected:

- 27001.6 (Native)
- 27283.2 (SeMet Average)

Measured:

- Native: 27002.0
- SeMet: 27287.4

**Crystallization:**Crystals were obtain from initial screen conditions RW A03 and E07.
Crystal used for structure determination was grown in RW E07 optimization plate, containing 25% PEG-3350, 0.25 M Sodium/Potassium Tartrate, 0.1 M HEPES pH 7.8, with 1:100 (w/w) dispase added. 2 uL protein solution + 2 uL well solution in hanging drop setup.
Crystals grow to a mountable size within 3 days.
Cryo used N-Paratone.
**NMR Spectroscopy:**

**Data Collection:**
**Data Processing:**