GRLF1

PDB:3C5H

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:CDV GRLF1 (SGC template 25-E5; camR)
Entry Clone Source: Codon Devices Synthesized
SGC Clone Accession:HPC074-D01
Tag:mhhhhhhssgrenlyfq*g
Host:BL21-CodonPlus(DE3)-RIL

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgTYNISVVGLSGTEKEKGQCGIGKSCLCNRFVRPSADEFHLDHTSVLSTSDFGGRVVNNDHFLYWGEVSR
SLEDCVECKMHIVEQTEFIDDQTFQPHRSTALQPYIKRAAATKLASAEKLMYFCTDQLGLEQDFEQKQMPDGKLLVDGFLLGIDVSR
GMNRNFDDQLKFVSNLYNQLAKTKKPIVVVLTKCDEGVERYIRDAHTFALSKKNLQVVETSARSNVNVDLAFSTLVQLIDK

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 25 µg/mL chloramphenicol at 37 °reeC. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 µC 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 °C.

Purification

Procedure
The lysate was centrigued at 15,000 rpm for 45 minutes and the supernatants were mixed with 3 mL 50% Ni-NTA beads, and incubated at 4 °reeC for 1 hours. The supernant was then passed through a gravity column (Poly-Prep, Bio-Rad, Catalog #731-1550) and the beads were washed using 10 mL washing buffer once. The protein bound to beads were eluted using 8 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). Five times protein concentration of GppNHp was added to the protein and incubated for 1 hour at 4 degree before concentrating. The purity of the proteins was higher than 95% judged by SDS-PAGE. Selenomethionine labeling of the protein used the M9 SeMet growth media kit from Medicilon following the manufacturer\\\'s instructions.

Extraction

Procedure
Frozen cells were thawed and resuspended in 150 mL extraction buffer with freshly added 0.5% CHAPS and 2mM BME, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 µL benzonase (Sigma Catalog # E1014, 250U/µL), and lysed using microfluidizer at 15,000 PSI.
Concentration:12~20 mg/mL
Ligand
GppNHp (?)**MassSpec:**Native: 28907.11, expected 28905.70SeMet: 29141.84
Crystallization: Only one condition from SGC, Red Wings screen kits gives crystal in the initial screen: RD09, and the conditions were further optimized.
Crystal used for phasing was SeMet labeled, and was grown in sitting drop of 33.18% PEG 4000, 0.1 M Tris pH 9.0, 0.2 M MgCl2, 0.1 mM DTT, using protein stock of 18.0 mg/mL, no cryo used.
Crystal used for refinement was grown in sitting drop of 25.91% PEG 4000, 0.1 M Tris pH 8.0, 0.2 M MgCl2, 0.1 mM DTT, using protein stock of 12.6 mg/mL, 1:1 mixed paratone/mineral oil was used as cryo.
Crystals appears in the drop in 1 to 2 days.

(lasted updated Y.TONG 20080220)
NMR Spectroscopy:
Data Collection:
Data Processing: