FGD5

PDB:3MPX

Revision

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Entry Clone Accession:BC132814
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SGC Clone Accession:HPC09X-H08
Tag:MHHHHHHSSGRENLYFQG
Host:BL21-V2R-pRARE2

Construct

Prelude:FGD5:G648-G1063Tag not removed
Sequence:

mhhhhhhssgrenlyfqgGQSRALVIAQELLSSEKAYVEMLQHLNLDFHGAVMRALDDMDHEGRDTLAREELRQGLSELPAIHDLHQ
GILEELEERLSNWESQQKVADVFLAREQGFDHHATHILQFDRYLGLLSENCLHSPRLAAAVREFEQSVQGGSQTAKHRLLRVVQRLF
QYQVLLTDYLNNLCPDSAEYDNTQGALSLISKVTDRANDSMEQGENLQKLVHIEHSVRGQGDLLQPGREFLKEGTLMKVTGKNRRPR
HLFLMNDVLLYTYPQKDGKYRLKNTLAVANMKVSRPVMEKVPYALKIETSESCLMLSASSCAERDEWYGCLSRALPEDYKAQALAAF
HHSVEIRERLGVSLGERPPTLVPVTHVMMCMNCGCDFSLTLRRHHCHACGKIVCRNCSRNKYPLKYLKDRMAKVCDGCFGELKKRG

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:LEX Bubbling. The Se-Met target protein was expressed in E. coli by inoculating 50 mL of overnight culture grown in Luria-Bertani medium into a 2 L of M9 salt medium in the presence of NIAAC, Thiamine and Vitamine B12 mix, fifteen mineral supplements, 0.5% glycerol, 50 mg/mL kanamycin and 35 mg/mL chloramphenicol at 37 degree. When OD600 reached ~1.2, the temperature of the medium was lowered to 18 degree. Then, the inhibitory amino acid cocktail (IAAC) and Se-Met mix were added to the culture and the culture was induced with IPTG at a 1 mM final concentration. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 degree.

Purification

Procedure
The lysate was centrifuged at 15,000 rpm for 45 minutes and the supernatants were mixed with 8 mL 50% flurry of Ni-NTA beads and incubated at 4 degree on rotary shaker for one hour. The mixture was then centrifuged at 2500 rpm for 5 min and the supernant discarded. The beads were

then washed with washing buffer, and finally the elution buffer. The flow-through was collected and further purifed by a Superdex-75 gel filtraton column pre-equilibrated with gel filtration buffer. Fractions containing the protein were collected and concentrated with Amicon Ultra-15 centrifugal filter. 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 150 mL extraction buffer 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 microfluidizer at 15,000 PSI.
Concentration:14.3 mg/mL
Ligand
MassSpec:protein expected 50177, measured 50229
Crystallization: Crystallization was setup using in situ proteolysis method in sitting drops with Red Wings and SGC-I screens initially. Diffracting crystals were found from initial screen plate for Red Wings A05.
Crystal used for structure determination was grown in 25% PEG 3350, 0.2M
Li2SO4, 0.1M HEPES buffer at pH 7.5, with 1% w/w trypsin.
Crystals grow to a mountable size within 1 week
NMR Spectroscopy:
Data Collection:
Data Processing: