TJP3

PDB:3KFV

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:SGC cDNA library: 20-E5:TJP3
Entry Clone Source: Codon Devices Synthesized
SGC Clone Accession:HPC092-C03
Tag:C-terminal: HHHHHH
Host:BL21-V2R-pRARE2

Construct

Prelude:TJP3:G508-L808:cH6
Tag not removed
Sequence:

```
mGDSFYIRTHFELEPSPPSGLGFTRGDVFHVLDTLHPGPGQSHARGGHWLAVRMGRDLREQERGIIPNQSRAEQLASLEAAQRAVGV
GPGSSAGSNARAEFWRLRGLRRGAKKTTQRSREDLSALTRQGRYPPYERVVLREASFKRPVVILGPVADIAMQKLTAEMPDQFEIAE
TVSRTDSPSKIIKLDTVRVIAEKDKHALLDVTPSAIERLNYVQYYPIVVFFIPESRPALKALRQWLAPASRRSTRRLYAQAQKLRKH
SSHLFTATIPLNGTSDTWYQELKAIIREQQTRPIWTAEDQLhhhhhh
```

Vector:pET28a-LIC-CHis (NCBI GI:145307000) Digested with NcoI and BseRI

Growth

Medium:
Antibiotics:
Procedure:LEX Bubbling. Selenomethionine labeling of the target protein was carried out using the M9 SeMet growth media kit from Medicilon (Cat. MD045004) following manufacturer's instructions. Briefly, 50 mL overnight *E. coli* Luria-Bertani culture was inoculated into a 2 L of M9 medium in the presence of NIAAC, thiamine and Vitamin B12 mix, fifteen mineral supplements, in the presence of 50 µg/mL kanamycin and 35 µg/mL chloramphenicol at 37 °C. When OD_{600} reached ~1.2, 40 mL stock solution containing inhibitory amino acid cocktail (IAAC) and Se-Met were added to the M9 culture and the temperature of the medium was lowered to 18 °C. After fifteen minutes, the culture was induced with IPTG at a 1 mM final concentration. The cells were allowed to grow overnight before being harvested and flash frozen in liquid nitrogen and stored at -80 °C.

Purification

Procedure

The lysate was centrifuged at 15,000 rpm for 45 minutes and the supernatants were mixed with 5 mL 50% flurry of TalonÂ Cobalt beads and incubated at 4 C on rotary shaker for one hour. The mixture was then centrifuged at 2300 rpm for 5 min and the supernant was discarded. The beads were then washed with washing buffers containing 30 mM and 75 mM imidazole, and finally the bound protein was eluted using the elution buffer. The elutant was collected and further purified on a Superdex-200 gel filtration 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 2L M9 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:15.2 mg/mL
Ligand
MassSpec: Se-Met protein expected 34945, measured 36596
Crystallization: Crystallization was setup in sitting drops with Red Wings and SGC-I screens initially. Diffracting crystals were found from condition SGC-G4. Optimization was carried out in hanging drop setup. Crystal used for structure determination was grown in 5% MPD, 1.4 M $(NH_4)_2SO_4$, 0.1 M HEPES buffer at pH 7.5, with 10 mM ATP in the protein stock solution, in hanging drop setup. Paratone was used as cryoprotectant. Crystals grow to a mountable size within 1 week.
NMR Spectroscopy:
Data Collection:
Data Processing: