MUT

PDB:3BIC

Revision

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Entry Clone Accession:gi|4557767
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Tag:C-terminal hexahistidine tag and Flag tag, TEV cleavable: aenlyfq*shhhhhhdykddddk
Host:BL21(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

MSPHYLRQVKESSGSRLIQQRLLHQQQPLHPEWAALAKKQLKGKNPEDLIWHTPEGISIKPLYSKRDTMDLPEELPGVKPFTRGPYP
TMYTFRPWTIRQYAGFSTVEESNKFYKDNIKAGQQGLSVAFDLATHRGYDSDNPRVRGDVGMAGVAIDTVEDTKILFDGIPLEKMSV
SMTMNGAVIPVLANFIVTGEEQGVPKEKLTGTIQNDILKEFMVRNTYIFPPEPSMKIIADIFEYTAKHMPKFNSISISGYHMQEAGA
DAILELAYTLADGLEYSRTGLQAGLTIDEFAPRLSFFWGIGMNFYMEIAKMRAGRRLWAHLIEKMFQPKNSKSLLLRAHCQTSGWSL
TEQDPYNNIVRTAIEAMAAVFGGTQSLHTNSFDEALGLPTVKSARIARNTQIIIQEESGIPKVADPWGGSYMMECLTNDVYDAALKL
INEIEEMGGMAKAVAEGIPKLRIEECAARRQARIDSGSEVIVGVNKYQLEKEDTVEVLAIDNTSVRNRQIEKLKKIKSSRDQALAER
CLAALTECAASGDGNILALAVDASRARCTVGEITDALKKVFGEHKANDRMVSGAYRQEFGESKEITSAIKRVHKFMEREGRRPRLLV
AKMGQDGHDRGAKVIATGFADLGFDVDIGPLFQTPREVAQQAVDADVHAVGVSTLAAGHKTLVPELIKELNSLGRPDILVMCGGVIP
PQDYEFLFEVGVSNVFGPGTRIPKAAVQVLDDIEKCLEKKQQSVaenlyfq*shhhhhhdykddddk

Vector:pNIC-CTHF

Growth

Medium:
Antibiotics:
Procedure:10µl of BL21(DE3)-R3 glycerol stock were inoculated into 5ml of Terrific broth medium supplemented with kanamycin (50 µg/ml) and chloramphenicol (34µg/ml) and grown overnight at 37°C, 200rpm. In the morning 1L of TB supplemented with the same antibiotics was inoculated with 10ml of the overnight culture and incubated at 37°Cwith intensive shaking (160rpm). After the OD600 reached 1.5, the temperature was changed to 18°C and IPTG was added to the final concentration of ~0.1mM. The culture was incubated at 18°C with shaking (160rpm) for additional 18h. The following morning the 3 l culture was harvested and centrifuged for 10min at 4000rpm. Supernatant was discarded and the cell pellets were resuspended in 75ml of a lysis buffer and frozen at -80°C.

Purification

Procedure
Step 1: Ni-affinity, Ni-Sepharose - (GE Healthcare) Acta-Express; Step 2: Superdex 200 Column, HiPrep 16/60 (Amersham); Step 3: Ion exchange -5ml HiTrap Q SepharoseThe cell extract was loaded on the AKTA Express system The extinction at 280nm was monitored and fractions were collected and analyzed by SDS-PAGE. MUTA containing fractions were diluted with IEX buffer A to a concentration of NaCl of 50 mM. The protein was loaded onto a HiTrap Q Sepharose column, and eluted with a NaCl gradient (50-300mM). Fractions containing protein were analysed by SDS-PAGE.

Extraction

Procedure
The thawed cells were broken by 5 passes at 16.000 psi through a high pressure homogeniser, followed by centrifugation for 45 min at 20.000rpm at 4°C.
Concentration: Using Amicon Ultra-15 concentrators with 30kDa cutoff, the sample was concentrated to 11mg/ml. Concentrations were determined from the absorbance at 280 nm using a NanoDrop spectrophotometer.
Ligand
MassSpec: The calculated mass of the construct was 84724Da, and the observed mass (ESI-MS) was 84510Da, compatible with an N-terminal methionine and serine deletion.
Crystallization: Crystals were grown by vapor diffusion at 20°C in 150nl sitting drops. Vitamin B12 was added to a final concentration of 5mM prior to crystallisation. The drops were prepared by mixing 50nl of protein solution and 100nl of precipitant consisting of 1.60M Na/KPO4; 0.1M HEPES pH 7.5. Crystals were flash-cooled in liquid nitrogen with 25% glycerol as cryoprotectant.
NMR Spectroscopy:
Data Collection: Resolution: 2.65Å; X-ray source: Swiss Light source (SLS), beamline X-10.
Data Processing: