NT5C3A

PDB:2CN1

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC015856
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated thrombin protease cleavage site: mgsshhhhhhssglvprg*s(m).
Host:

Construct

Prelude:
Sequence:

MGSSHHHHHHSSGLVPRGSNPTRVEEIICGLIKGGAAKLQIITDFDMTLSRFSYKGKRCPTCHNII DNCKLVTDECRKKLLQLKEK
YYAIEVDPVLTVEEKYPYMVEWYTKSHGLLVQQALPKAKL KEIVAESDVMLKEGYENFFDKLQQHSIPVFIFSAGIGDVLEEVIRQ
AGVYHPNVKVVSNF MDFDETGVLKGFKGELIHVFNKHDGALRNTEYFNQLKDNSNIILLGDSQGDLRMADGVAN VEHILKIGYLN
DRVDELLEKYMDSYDIVLVQDESLEVANSILQKIL

Vector:p28A-LIC

Growth

Medium:
Antibiotics:
Procedure:BL21 (DE3) pGro7 cells from glycerol stocks were grown in 20 ml Terrific Broth media supplemented with 8 g/l glycerol, 100 µg/ml kanamycin and 34 µg/ml chloramphenicol at 30 ºC over night. The following morning, 20 ml of the over night culture inoculated 1500 ml of Terrific Broth media supplemented with 50 µg/ml kanamycin, 8 g/l glycerol and approximately 50 µl BREOX (antifoam) in glass flasks in the Large Scale Expression System (LEX). Cells were grown at 37 ºC until OD600nm of 2.2 and were down-tempered to 18 ºC for 45 minutes in water bath. Expression of chaperones (GroEL and GroES) was induced by addition of L-arabinose to a final concentration of 0.5 mg/ml. After 30 minutes, expression of target protein was induced by addition of IPTG to a concentration of 0.5 mM. Protein expression was allowed to continue over night at 18 ºC.

Purification

Procedure

Purification was performed on an AKTA Xpress system. Prior to purification, columns were equilibrated with Binding and wash1 Buffer (HiTrap Chelating) and Gel filtration buffer (Superdex 75). The lysate was loaded on the HiTrap Chelating column and washed with Binding and wash1 Buffer followed by Wash2 Buffer. Bound protein was eluted from the IMAC columns with Elution Buffer and loaded on the Gel filtration column. The purified protein was concentrated using an Amicon Ultra 15 (Millipore) with 10 000 MW, the final concentration was 14 mg/ml. Fresh TCEP was added to the sample to a final concentration of 2 mM.

Extraction

Procedure
Cells were harvested by centrifugation and pellets were resuspended in 50mM Sodium-Fosfate pH 7.5, 500mM NaCl, 10% glycerol, 10 mM Imidazole, 0.5 mM TCEP supplemented with one tablet Complete EDTA-free protease inhibitor tablet per cell pellet and freezed at -80 ºC. The frozen cells were briefly thawed in warm water and 2000 U of Benzonase was added. Cells were disrupted by High Pressure Homogenization run twice at 10 000 PSI and samples were centrifuged for 20 minutes at 40000×g. The soluble fraction was filtered through 0.45 µm and subjected to further purification on the ÄKTAxpress.
Concentration:
Ligand
MassSpec:
Crystallization: Sitting drops containing 0.1 µl of protein solution (14 mg/mL) + 0.1µl well solution containing 24% PEG1500 and 20% glycerol was left to equilibrate against well solution. Crystals appeared after 5 days. Seeding into pre-equilibrated drops was necessary to reproduce the crystals.
NMR Spectroscopy:
Data Collection:
Data Processing: Native data to 2.7 Å was collected at ESRF, beam-line ID14.4. The space group is C2221 and the cell parameters are 86 101 77 90 90 90. The structure was solved with one polypeptide per asymmetric unit, by Molecular Replacement using mouse NT5C3 (pdb entry: 2bdu) as template structure. Refmac was used for refinement and Coot for model building. TLS refinement with 10 TLS groups was used in Refmac. The final model starts at asparagine 14 and ends at the C-terminus of NT5C3, which is leucine 286.