CHN1

PDB:2OSA

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_001813
Entry Clone Source:MGC clone AT8-G1
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with thrombin cleavage site: mhhhhhhssglvprgs
Host:E.coli BL21 (DE3) codon plus RIL

Construct

Prelude:
Sequence:

gsKHVKKVYSCDLTTLVKAHTTKRPMVVDMCIREIESRGLNSEGLYRVSGFSDLIEDVKMAFDRDGEKADISVNMYEDINIITGALK
LYFRDLPIPLITYDAYPKFIESAKIMDPDEQLETLHEALKLLPPAHCETLRYLMAHLKRVTLHEKENLMNAENLGIVFGPTLMRSPE
LDAMAALNDIRYQRLVVELLIKNEDILF

Vector:p28a-LIC

Growth

Medium:TB
Antibiotics:
Procedure: The target was expressed in E. coli by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into 1.8 L of Terrific Broth medium in the presence of 50 microG/mL kanamycin and chloramphenicol at 37degC. When OD600 was ~3.0, the culture was induced with 1mM IPTG and the temperature was reduced to 15degC, and the cells were allowed to grow overnight.

Purification

Procedure
The supernatant was loaded onto 5 mL HiTrap Ni-NTA column (Pharmacia Amersham) equilibrated with binding buffer at 4 ºC using peristaltic pump. The HiTrap Ni-NTA column was washed stepwise with 25 mL of binding buffer, 25 mL of binding buffer with 30 mM imidazole, and 25 mL of binding buffer with 50 mM imidazole. The His-tagged protein was eluted with a linear gradient of imidazole from 50 mM to 500 mM in 50 mL. The fractions corresponding to the correct eluted protein peaks were combined. The protein was further purified on a Superdex 75 (GE Healthcare) gel filtration column, equilibrated with gel filtration buffer. Protein peak

fractions were combined, and DTT was added to a final concentration of 10 mM. The protein was concentrated using an Amicon Ultra (m.w cutoff 10 kDa) centrifugal filter and the concentration for protein stock solution was estimated by Bradford to be 60 -110 mg/mL (in different batches).

Extraction

Procedure
Cultures were harvested by centrifugation and the cell pellets were stored at -80 ºC before use. Cells from 1.8L culture were thawed and resuspended in 150 mL of binding buffer with 0.5% CHAPS (Sigma) and 1 mM phenylmethyl sulfonyl fluoride (PMSF) and lysed with microfluidizer. The lysate was centrifuged at 38,400 xg for 45 min and collected the supernatant.
Concentration:79.7 mg/mL
Ligand
MassSpec: Measured 23114.29 D, expected 23113.90 D.
Crystallization: Crystallization trials were set up using the sitting drop vapor diffusion method at room temperature. The protein drop was equilibrated against a reservoir solution (1:1 volume ratio) containing 30.0% Jeffamine ED-2001, 0.1M HEPES pH 7.0. Crystals reached a size of about 50 microns within two to three days.
NMR Spectroscopy:
Data Collection:
Data Processing: