CHN1

PDB:3CXL

Revision

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Entry Clone Accession:BC011393
Entry Clone Source:MGC AT8-G1
SGC Clone Accession:HPC074-A05
Tag:mhhhhhhssgrenlyfq*g
Host:BL21-CodonPlus(DE3)-RIL

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgVWKSYLYQLQQEAPHPRRITCTCEVENRPKYYGREFHGMISREAADQLLIVAEGSYLIRESQRQPGTYT
LALRFGSQTRNFRLYYDGKHFVGEKRFESIHDLVTDGLITLYIETKAAEYIAKMTINPIYEHVGYTTLNREPAYKKHMPVLKETHDE
RDSTGQDGVSEKRLTSLVRRATLKENEQIPKYEKIHNFKVHTFRGPHWCEYCANFMWGLIAQGVKCADCGLNVHKQCSKMVPNDCKP
DLKHVKKVYSCDLTTLVKAHTTKRPMVVDMCIREIESRGLNSEGLYRVSGFSDLIEDVKMAFDRDGEKADISVNMYEDINIITGALK
LYFRDLPIPLITYDAYPKFIESAKIMDPDEQLETLHEALKLLPPAHCETLRYLMAHLKRVTLHEKENLMNAENLGIVFGPTLMRSPE
LDAMAALNDIRYQRLVVELLIKNEDILF

Vector:pET28-mhl (GI:134105571)

Growth

Medium: Terrific Broth
Antibiotics:
Procedure:LEX Bubbling. The target protein was expressed in E. coli by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 1.8 L of Terrific Broth medium in the presence of 50 µg/mL kanamycin and 25 µg/mL chloramphenicol at 37 °reeC. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 µC and the culture was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before they were harvested and flash frozen in liquid nitrogen and stored at -80 °C.

Purification

Procedure
The lysate was centrigued at 15,000 rpm for 45 minutes and the supernatants were mixed with 3 mL 50% Ni-NTA beads, and incubated at 4 °reeC for 1 hours. The supernatant was then passed through a gravity column (Poly-Prep, Bio-Rad, Catalog #731-1550) and the beads were washed using 10 mL washing buffer twice. The protein bound to beads were eluted using 8 mL elution

buffer twice. The flow-through was collected and loaded onto Supderdex-200 gel filtration column. Eluted fractions were pooled and concentrated using amicon centrifugal filter (m.w. cut-off 10,000). The purity of the proteins was higher than 97% judged by SDS-PAGE.

Extraction

Procedure
Frozen cells from 1.8L TB culture were thawed and resuspended in 150 mL extraction buffer with freshly added 0.5% CHAPS and 2mM BME, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 µL benzonase (Sigma Catalog # E1014, 250U/µL), and lysed using sonication at 20 seconds 50% duty cycle for 5 minutes at 100W.
Concentration:24.2 mg/mL
Ligand
Zn**MassSpec:**Native: 53721.10, expected 53714.77
Crystallization: Crystallization was setup using sitting drops with Red Wings and SGC-I screens initially. Bipyramid crystals were seen at condition SC04 around one month after setup. Optimization was done using hanging drop vaporization, it usually takes 10 days or more for crystals to grown.
Crystal used for data collection was grown at 1.4M (NH4)2SO4, 100mM Glycine pH 9.5, 10 mM MgCl2, 5% MPD.

Last updated by ytong 20080428
NMR Spectroscopy:
Data Collection:
Data Processing: