CENTA1

PDB:3FEH

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC033747
Entry Clone Source:MGC AT53-B3
SGC Clone Accession:HPC077-H02
Tag:N-terminal tag: mhhhhhhssgrenlyfq*g
Host:BL21-CodonPlus(DE3)-RIL

Construct

Prelude: Tag not removed
CENTA1:K3-F370
Sequence:

mhhhhhhssgrenlyfqgKERRRAVLELLQRPGNARCADCGAPDPDWASYTLGVFICLSCSGIHRNIPQVSKVKSVRLDAWEEAQVE
FMASHGNDAARARFESKVPSFYYRPTPSDCQLLREQWIRAKYERQEFIYPEKQEPYSAGYREGFLWKRGRDNGQFLSRKFVLTEREG
ALKYFNRNDAKEPKAVMKIEHLNATFQPAKIGHPHGLQVTYLKDNSTRNIFIYHEDGKEIVDWFNALRAARFHYLQVAFPGASDADL
VPKLSRNYLKEGYMEKTGPKQTEGFRKRWFTMDDRRLMYFKDPLDAFARGEVFIGSKESGYTVLHGFPPSTQGHHWPHGITIVTPDR
KFLFACETESDQREWVAAFQKAVDRPMLPQEYAVEAHF

Vector:pET28-mhl (GI:134105571)

Growth

Medium: Terrific Broth
Antibiotics: Kanamycin 50 µg/mL Chloramphenicol 25 µg/mL
Procedure:LEX Bubbling. The target protein was expressed in *E. coli* by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into 2L Terrific Broth medium in the presence of 50 µg/mL kanamycin and 50 µg/mL chloramphenicol at 37 degC. When OD600 reached ~3.0, the temperature of the medium was lowered to 18 degC and the culture was induced with 1 mM IPTG. The cells were allowed to grow overnight before they were harvested and flash frozen in liquid nitrogen and stored at -80 degC.

Purification

Procedure
The lysate was centrigued at 15,000 rpm for 45 minutes and the supernatants were mixed with 1.5 mL 50% Ni-NTA beads, and incubated at 4 degC for 1.5 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 10 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 95% judged by SDS-PAGE.

Extraction

Procedure
Frozen cells were thawed and resuspended in 80 mL extraction buffer with freshly added 1mM PMSF/Benzomidine, 5U/ml of Benzonase (Sigma Catalog # E1014, 250U/µL), 0.5% CHAPS, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and lysed using microfludizer(17000 psi).
Concentration:43.3 mg/mL (14.0 mg/mL for SeMet labeled protein)
Ligand
Zn**MassSpec:**Native: 44928.82, expected 44925.78
SeMet: 45259.89, expected 45254.01
Crystallization: Buffer for protein is 20mM HEPES pH7.3, 300mM NaCl, 1mM TCEP, 5% Glycerol.
Crystallization was setup using sitting drops with Red Wings and SGC-I screens initially. Plate crystals were seen in conditions RW-G08, G09 and H02.Native crystal for structure determination was grown in 25% P3350, 0.2M NH4Ac, and 0.1M Bis-Tris pH 6.5 in sitting drop setting (0.5uL protein + 0.5uL well solution), crystals usually appear in 4-7 day, cryoprotectant used 20% P3350 + 20% EG.
SeMet crystal used for structure determination was grown in 30% P3350, 0.2M NH4Ac, and 0.1M Bis-Tris pH 6.0 in hanging drop setting (1uL + 1uL). Same cryo used.
NMR Spectroscopy:
Data Collection:
Data Processing: