CENTA1+KIF13B

PDB:3FM8

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:CENTA1: BC033747
KIF13B: BC005977
Entry Clone Source:CENTA1: MGC AT53-B3
KIF13B: OpenBiosystem CloneID:4103715
SGC Clone Accession:CENTA1: HPC060-A04
KIF13B: HPC079-B11
Tag: Both have N-terminal tag: mhhhhhhssgrenlyfq*g
Host:BL21-CodonPlus(DE3)-RIL

Construct

Prelude: Tag not removed
CENTA1:M1-P374
KIF13B:G440-P545
Sequence:

CENTA1:

mhhhhhhssgrenlyfqgMAKERRRAVLELLQRPGNARCADCGAPDPDWASYTLGVFICLSCSG
IHRNIPQVSKVKSVRLDAWEEAQVEFMASHGNDAARARFESKVPSFYYRPTPSDCQLLR
EQWIRAKYERQEFIYPEKQEPYSAGYREGFLWKRGRDNGQFLSRKFVLTEREGALKYFN
RNDAKEPKAVMKIEHLNATFQPAKIGHPHGLQVTYLKDNSTRNIFIYHEDGKEIVDWFNA
LRAARFHYLQVAFPGASDADLVPKLSRNYLKEGYMEKTGPKQTEGFRKRWFTMDDRRL
MYFKDPLDAFARGEVFIGSKESGYTVLHGFPPSTQGHHWPHGITIVTPDRKFLFACETESD
QREWVAAFQKAVDRPMLPQEYAVEAHFKHKP
KIF13B:
mhhhhhhssgrenlyfqgGIKVGDDKCFLVNLNADPALNELLVYYLKEHTLIGSANSQDIQLCGM
GILPEHCIIDITSEGQVMLTPQKNTRTFVNGSSVSSPIQLHHGDRILWGNNHFFRLNLP
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 for CENTA1, cut-off 5,000 for KIF13B-FHA). The purity of the proteins was higher than 95% judged by SDS-PAGE. The complex was formed by mixing purified CENTA1 and KIF13B at 1:1 ratio and run through a Superdex-75 column. The fractions were collected and concentrated using Amicon centrifugal filter and then used to setup crystallization.

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:28.2 mg/mL (complex concentration using Bradford measurement)
Ligand
Zn**MassSpec:**CENTA1: Native: 45619.63, expected 45618.66
KIF13b: Native: 13951.10, expected 13950.80
Crystallization: Buffer for the protein is gel filtration buffer.
Crystal used for data collection was grown in Optimized SGC-B12 condition:Well solution: 1.20 M Li2SO4, 0.1M Sodium Citrate pH 6.0 0.5M (NH4)2SO4Protein complex was added 5% MPD and then mixed with well solution at 1uL:1uL ratio, and setup as hanging drop in a 24-well optimization plate. Crystals grown to a size of 60micron in about 3-4 weeks. Cryo used 1.6M Li2SO4 and 0.5M (NH4)2SO4.
NMR Spectroscopy:
Data Collection:
Data Processing: