Materials and Method

Note: To our best knowledge, this should represent an accurate description of the materials and methods required to reproduce our work. If any of the content on this page is difficult to interpret or should you have trouble repeating our work, do not hesitate to contact us as soon as possible in order for us to provide additional information and advice.

Entry Clone Source: MGC

Entry Clone Accession: BC031878

SGC Construct ID: HDHD3A-c108

GenBank GI number: gi|13654294

Vector: pNIC-CTHF. Details [PDF]; Sequence [FASTA] or [GenBank]

Amplified construct sequence:

```
CTTAAGAAGGAGATATACTATGCGACTGCT
GACGTGGGATGTGAAGGACACGCTGCTCAG
GCTCCGCCACCCCTTAGGGGAGGCCTATGC
CACCAAGGCCCGGGCCCATGGGCTGGAGGT
GGAGCCCTCAGCCCTGGAACAAGGCTTCAG
GCAGGCATACAGGGCTCAGAGCCACAGCTT
CCCCAACTACGGCCTGAGCCACGGCCTAAC
CTCCCGCCAGTGGTGGCTGGATGTGGTCCT
GCAGACCTTCCACCTGGCGGGTGTCCAGGA
TGCTCAGGCTGTAGCCCCCATCGCTGAACA
GCTTTATAAAGACTTCAGCCACCCCTGCAC
CTGGCAGGTGTTGGATGGGGCTGAGGACAC
CCTGAGGGAGTGCCGCACACGGGGTCTGAG
ACTGGCAGTGATCTCCAACTTTGACCGACG
GCTAGAGGGCATCCTGGGGGGCCTTGGCCT
GCGTGAACACTTCGACTTTGTGCTGACCTC
CGAGGCTGCTGGCTGGCCCAAGCCGGACCC
CCGCATTTTCCAGGAGGCCTTGCGGCTTGC
TCATATGGAACCAGTAGTGGCAGCCCATGT
TGGGGATAATTACCTCTGCGATTACCAGGG
GCCTCGGGCTGTGGGCATGCACAGCTTCCT
GGTGGTTGGCCCACAGGCACTGGACCCCGT
GGTCAGGGATTCTGTACCTAAAGAACACAT
CCTCCCCTCTCTGGCCCATCTCCTGCCTGC
CCTTGACTGCCTAGAGGGCTCAGCAGAGAA
CCTCTACTTCCAATCGCACCATCATCACCA
CCATGATTACAAGGATGACGACGATAAGTG
AGGATCC
```

Final protein sequence (tag sequence in lowercase):

```
MRLLTWDVKDTLLRLRHPLGEAYATKARAH
GLEVEPSALEQGFRQAYRAQSHSFPNYGLS
HGLTSRQWWLDVVLQTFHLAGVQDAQAVAP
IAEQLYKDFSHPCTWQVLDGAEDTLRECRT
RGLRLAVISNFDRRLEGILGGLGLREHFDF
VLTSEAAGWPKPDPRIFQEALRLAHMEPVV
AAHVGDNYLCDYQGPRAVGMHSFLVVGPQA
LDPVVRDSVPKEHILPSLAHLLPALDCLEG
Saenlyfq*shhhhhhdykddddk
```

Tags and additions: C-terminal TEV-cleavable (at *) his-tag with the following sequence aenlyfq*shhhhhhdykddddk

Host: BL21(DE3)-R3-pRARE2

Growth medium, induction protocol: Cells from the glyerol stock were cultured in 600 ml of LB media with 50 µgml of Kanamycin at 37°C overnight. The cells then were washed and cultured in 12L MD

media with 40mg of Selenomethionine/L at 37°C. When the OD reached 0.754, 0.1 mM (final concentration) of IPTG was added and the temperature was decreased to 18°C for overnight culture.
Extraction buffer, extraction method: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 30 mM Imidazole. Complete Protease Inhibitor Cocktail Tablets (Roche) were added (one tablet/50ml buffer). The cells were harvested by centrifugation at 4,000 g for 10 min. The pellet from 1 L culture was resuspended in 25 ml of extraction buffer. The sample was homogenized by using the EmulsiFlex-05 homogenizer (Glen Creston) and then centrifuged at 37505 g. The supernatant was kept for further purification.
Column 1: Ni-NTA
Column 1 Buffers: **Binding buffer:**500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 30 mM Imidazole; **Washing Buffer**: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 30 mM Imidazole; **Elution Buffer I**: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 60 mM Imidazole; **Elution Buffer II:** 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 125 mM Imidazole; **Elution Buffer III**: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 250 mM Imidazole.
Column 1 Procedure: The column was packed by 6 ml of Ni-NTA slurry and equilibrated with 15 ml of binding buffer. The supernatant was loaded onto the column and the flow through was collected. The column was washed with 2x20 ml of washing buffer. The protein was eluted with 10 ml of elution buffer I, II & III respectively.
Column 2: Superdex 200 Hiload 16 60
Column 2 Buffers: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 0.5 mM TCEP.
Column 2 Procedure: Sample from previous elute I and II was concentrated to 5 ml before loaded onto the AKTA Purifier. AKTA Purifier was run at 4°C. Fractions were analyzed by SDS - PAGE and the most purified fractions were collected.
Mass spectrometry characterization: 29824 (Selenomethionine labelled, as expected)
Protein concentration: 8.3 mg/ml
Crystallization: Crystals were grown by vapor diffusion at 20°C in 150nl sitting drops. The drops were prepared by mixing 50nl of protein solution and 100nl of precipitant consisting of 0.49M NaH_2PO_4 and 0.91M K_2HPO_4. Crystals were flash-cooled in liquid nitrogen
Data Collection: Resolution: 1.55 Å; X-ray source: Diamond beamline IO2

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