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| <b>Entry Clone Source:</b> MGC  |
| <b>Entry Clone Accession:</b> IMAGE:3686411   |
| <b>SGC Construct ID:</b> pNIC-CTHF  |
| <b>Vector:</b> pNIC_CTHF. Details [ <a href="#">PDF</a> ]; Sequence [ <a href="#">FASTA</a> ] or [ <a href="#">GenBank</a> ]  |
| <b>Final protein sequence (Tag sequence in lowercase):</b><br>MKEDLNRLKKLTHAAPCMLFMKGTP<br>QEPRCGFSKQMVEILHKHNIQFSSFD<br>IFSDEEVHQGLKAYSSWPTYPQLYVS<br>GELIGGLDIIKELEASEELDTICPKA<br>aenlyfq^shhhhhdykdddk<br><br>^ TEV cleavage site   |
| <b>Tags and additions:</b> TEV protease cleavable C-terminal histidine tag  |
| <b>Host:</b> BL21 (DE3)R3-pRARE2 (Phage resistant strain).  |
| <b>Expression:</b> TB supplemented with 50 µg/ml Kanamycin and 34 µg/ml chloramphenicol. 4 litre TB in two 4L flasks were innoculated with 40 ml overnight culture and grown at 37°C until OD <sub>600</sub> reached 2.5. The temperature was then decreased to 18°C and the protein expression induced with 0.1 mM IPTG over night   |
| <b>Cell harvest:</b> The cells were collected by centrifugation (4000 RPM, 30 minutes) and frozen at -80°C  |
| <b>Cell Lysis:</b> Cell pellets were dissolved in approximately 150ml lysis buffer and broken by homogenization by 5 passes at 12,000 psi. The cell debris was pelleted at 35,000 x g and the supernatant used for further purification<br><b>Lysis buffer:</b> 50 mM HEPES pH 7.5, 500 mM NaCl, 10 mM Imidazole, 5% glycerol, 0.5 mM TCEP, 1 mM PMSF and 3 U/ml of Benzonase<br><b>Extraction buffer, extraction method:</b> The cell pellet was resuspended in a total volume of 200 ml lysis buffer and the cells disrupted by sonication. Nucleic acids and cell debris were removed by adding 0.15% PEI, followed by centrifugation for 60 minutes at 40,000xg. The supernatant was further clarified by filtration (0.20 mm). |
| <b>Column 1:</b> Ni-sepharose, HisTrap FF, 5 ml (GE healthcare)   |
| <b>Column 1 Buffers:</b><br><b>Binding buffer:</b> 50 mM HEPES pH 7.5, 500 mM NaCl, 10 mM Imidazole, 0.5 mM TCEP<br><b>Wash and elution buffer:</b> 50 mM HEPES pH 7.5, 500 mM NaCl, 10-300 mM imidazole  |
| <b>Column 1 Procedure:</b> The cell lysate was applied onto a 5 ml Ni-Sepharose FF gravity column equilibrated with binding buffer. The column was subsequently washed with 10 column volumes of binding buffer and the protein eluted using a stepwise gradient of imidazole. All fractions were collected and analysed by 4-12% SDS-PAGE.   |
| <b>Column 2:</b> Hiload 16/60 Superdex 200 prep grade 120 ml (GE/Amersham Biosciences).   |
| <b>Column 2 Buffer:</b> 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP  |
| <b>Column 2 Procedure:</b> The fractions containing TXNL2 from the Ni-affinity chromatography were concentrated in amicon (3K) to 4ml. The protein was filtered through a 0.20µm PVDF   |

filter and applied onto a Superdex S200 column at 1.2 ml/min. The eluted proteins were collected in 1.8 ml fractions and analysed by SDS-PAGE.

**Enzymatic treatment:** TEV cleavage

**Column 3:** Ni-Sepharose FF (TEV clean up)

**Column 3 Wash Buffer:** 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol

**Column 3 Procedure:** The N-terminal histidine-tag was cleaved with 150 µg of TEV protease per 10 mg protein at 4°C for 48 hours. The protein was then applied to a 0.5 ml Ni-sepharose column and the flow through collected. The column washed with 2.5 ml buffer and the flow through and the wash fraction analysed by SDS-PAGE.

**Concentration:** The protein was concentrated in Amicon 3K to 36.8 mg/ml. The protein concentration was determined spectrophotometrically using the predicted molar extinction coefficient 11460(M<sup>-1</sup> cm<sup>-1</sup>) and the predicted mass of 12727.7 Da

**Mass spectrometry characterization:**

Observed mass: 12728.1 Da

Expected mass: 12727.7 Da

**Crystallisation:** TXNL2A was crystallised by vapor diffusion at 4°C from a sitting drop consisting of 50 nl protein (36.8 mg/ml) and 100 nl well solution containing 0.1 M Bis-Tris pH 5.5, 25% PEG3350. The crystal was transferred to a cryo protectant composed of 25% ethylene glycol before flash-cooling in liquid nitrogen

**Data collection:**

**Resolution:** 1.8 Å

**X-ray source:** Diamond Light Source beamline I03.