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: IMAGE:3686411
SGC Construct ID: TXNL2A-c002
GenBank GI number: gi|95113651
Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]
Tag and additions: TEV-cleavable (*), N-terminal histidine tag. Tag sequence: `mhhhhhhssgvdlgtenlyfq*sm`. **Note:** Due to an cloning artefact, a C-terminal histidine tag was added with the sequence: `kvkvdtdpnsssvdklaaalehhhhhh`.

Protein sequence:
`sMAAGAAEAAVAAVEEVGSAGQFEELLRL`
`KAKSLLVVHFWAPWAPQCAQMNEVMAELA`
`KELPQVSFVKLEAEGVPEVSEKYEISSVP`
`TFLFFKNSQKIDRLDGAHAPELTKKVQRH`
`ASSGSFLPSA`

Host: BL21(DE3)-R3-pRARE2
Growth medium, induction protocol: Medium: TB supplemented with 50 µg/ml Kanamycin and 34 ug/ml chloramp. 4 liter TB in two 4-L flask were inoculated with 40 ml overnight culture and grown at 37°C until OD_{600} reached 2.0 . The temperature was then decreased to 18°C and the protein expression induced with 0.1 mM IPTG over night. The cells were collected by centrifugation (4000 RPM, 30 minutes) and frozen at -80°C.
Extraction buffer, extraction method: Lysis buffer: 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. The cell pellet was resuspended in a total volume of 250 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 µm).
Column 1: Ni-Sepharose FF, 4 ml (GE/Amersham Biosciences)
Buffers: **Binding buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 10 mM Imidazole, 5% glycerol, 0.5 mM TCEP. **Wash and elution buffers:** 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol 10-250 mM imidazole.
Procedure: The cell lysate was applied onto a 4 ml Ni-Sepharose FF 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.
Column 2: Hiload 16/60 Superdex 200 prep grade 120 ml (GE/Amersham Biosciences)
Buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP
Procedure: The fractions containing TXNL2A from the Ni-affinity chromatography were incubated with 0.5 mM TCEP and concentrated in amicon (3K) to 4 ml. The protein was filtered through a 0.20 µm 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)
Buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol

Procedure: The N-terminal histidine-tag was cleaved with 150 µg of TEV protease per 10 mg protein at 4°C for 16 hours. The TEV cleaved protein was applied to a 0.5 ml Ni-sepharose column and the flow through collected. The column was washed with 5 ml buffer and the flow through and the wash fraction were analysed by SDS-PAGE.
Concentration: The protein was concentrated in Amicon (5 K) to 53.8 mg/ml. The protein concentration was determined spectrophotometrically using the predicted molar extinction coefficient 12490 (M^{-1} cm^{-1}) predicted mass of 16605.8 Da .
Mass spectrometry characterisation: The mass determined for TXNL2Ap009 was determined with ESI-MS to 16605.8 Da, in agreement with the predicted mass of the protein with an C-terminal histidine tag.
Crystallisation: Crystals of the TXN domain in TXNL2A were grown by vapor diffusion at 20°C, combined with microseeding procedures. Sitting drops consisting of 100 nl protein (28 mg/ml) and 50 nl well solution are equilibrated against well solution containing 1.4 M ammonium citrate pH 7 for 1hr. At this stage, drops were seeded with 20 nl of 10^{-5} (v/v) dilution of a microseed suspension from initial needle clusters grown in 1.7 M ammonium citrate pH 7. Seeds were crushed using the Hampton Seed Bead Kit, and diluted into 50µl of precipitant solution. The crystal was transferred to a cryoprotectant composed of 50 % Malonate pH 7.0 before flash-cooling in liquid nitrogen.
Data Collection: Resolution: 1.55 Å; **X-ray source:** Diamond light source I02.