Click on the iSee icon to launch an enhanced annotation with interactive 3D representations and animated transitions. Please note that a web plugin (activeICM) is required to access this enhanced functionality. Instructions for the installation and use of the web plugin are available.

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

Amplified construct sequence:

CTTAAGAAGGAGATATACTATGAGCAGCTC
AGAAGATAAAATAACAGTCCACTTTATAAA
CCGTGATGGTGAAACATTAACAACCAAAGG
AAAAGTTGGTGATTCTCTGCTAGATGTTGT
GGTTGAAAATAATCTAGATATTGATGGCTT
TGGTGCATGTGAGGGAACCCTGGCTTGTTC
AACCTGTCACCTCATCTTTGAAGATCACAT
ATATGAGAAGTTAGATGCAATCACTGATGA
GGAGAATGACATGCTCGATCTGGCATATGG
ACTAACAGACAGATCACGGTTGGGCTGCCA
AATCTGTTTGACAAAATCTATGGACAATAT
GACTGTTCGAGTGCCTGAAACAGTGGCTGA
TGCCAGACAATCCATTGATGTGGGCAAGAC
CTCCGCAGAGAACCTCTACTTCCAATCGCA
CCATCATCACCACCATGATTACAAGGATGA
CGACGATAAGTGAGGATCC

Final protein sequence (tag sequence in lowercase)

MSSSEDKITVHFINRDGETLTTKGKVGDSL
LDVVVENNLDIDGFGACEGTLACSTCHLIF
EDHIYEKLDAITDEENDMLDLAYGLTDRSR
LGCQICLTKSMDNMTVRVPETVADARQSID
VGKTSaenlyfq

Host: BL21(DE3)-R3-pRARE2

Tags and additions: TEV-cleavable (*), C-terminal hexa-histidine tag. Tag sequence: aenlyfq*shhhhhhdykddddk

Growth medium, induction protocol: Medium: TB supplemented with 50 µg/ml Kanamycin and 34 µg/ml chloramphenicol. 4 litre TB in two 4-L flask were inoculated with 40 ml overnight culture and grown at 37°C until OD600 reached 2.5. The temperature was then decreased to 18°C and the protein expressionÂ induced with 0.1 mM IPTG overnight . The cells were collected by centrifugation (4000 RPM, 30 minutes) and frozen at -80°C.

Extraction buffer: 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.

Extraction method: 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 mm).

Column 1: Ni-sepharose, HisTrap FF, 5 ml (GE healthcare) Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 10 mM Imidazole, 5% glycerol, 0.5 mM TCEP. Wash buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 20 mM Imidazole, 5% glycerol and 0.5 mM TCEP. Elution buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 300 mM Imidazole, 5% glycerol 0.5 mM TCEP. Buffer exchange:10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP.
Purification procedure: The cell extract was applied onto the column at 4 ml/minute on an AKTA-express system (GE healthcare). The column was then washed with 20 column volumes of binding buffer, 10 column volumes of wash buffer, and then eluted with 5 column volumes elution buffer at 5 ml/min. The eluted peak of A280 was automatically collected into capillary loops. Protein eluted from the Ni-sepharose was diluted in 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP and concentrated in Amicon 3K to 0.8 ml 22 mg/ml.
Enzymatic treatment: TEV cleavage.
Column 3: Ni-Sepharose FF (TEV clean up)
Buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP
Concentration: The tev-cleaved protein was concentrated in Amicon 3K to 14.7 mg/ml. The protein concentration was determined spectrophotometrically using the predicted molar extinction coefficient 4470 (M-1 cm-1) and predicted mass of 14558.3 Da .
Mass spec characterization: The mass determined for FDX1A-p010 was 14427 Da, in agreement with the predicted mass of the protein lacking the N-terminal Methionine. Masses were determined by LC-MS, using an Agilent LC/MSD TOF system with reversed-phase HPLC coupled to electrospray ionisation and an orthogonal time-of-flight mass analyser. Proteins were desalted prior to mass spectrometry by rapid elution off a C3 column with a gradient of 5-95% acetonitrile in water with 0.1% formic acid.
Crystallisation: FDX1A was crystallised by vapor diffusion at 20°C from a sitting drop consisting of 75 nl protein (12 mg/ml) and 75 nl 1.75 M K3 Citrate. The crystal was transferred to a cryo protectant composed of 1.8 M Malonate pH 7.0 before flash-cooling in liquid nitrogen.
Data Collection: Resolution**:** 2.54 Å. **X-ray source:** Diamond light source I02.