FigureS1. Purity of recombinant AtTRXo1 and AtTRXo2. Sample proteins (10 μg) were separated by 15% SDS-PAGE and stained with Coomassie Brilliant Blue.

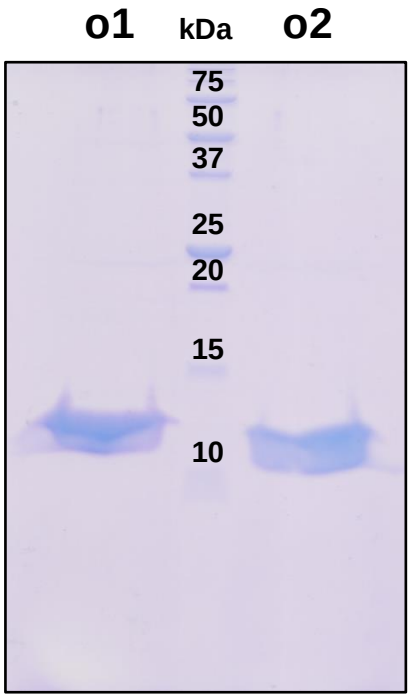

Figure S2. Analytical gel filtration of *E. coli* IscS. This analysis was performed by loading 100 μg of protein onto a Superdex S200 10/300 column. The presence of the polypeptide and of the PLP cofactor have been detected by the absorbance at 280 nm (blue line) and 420 nm (red line), respectively.

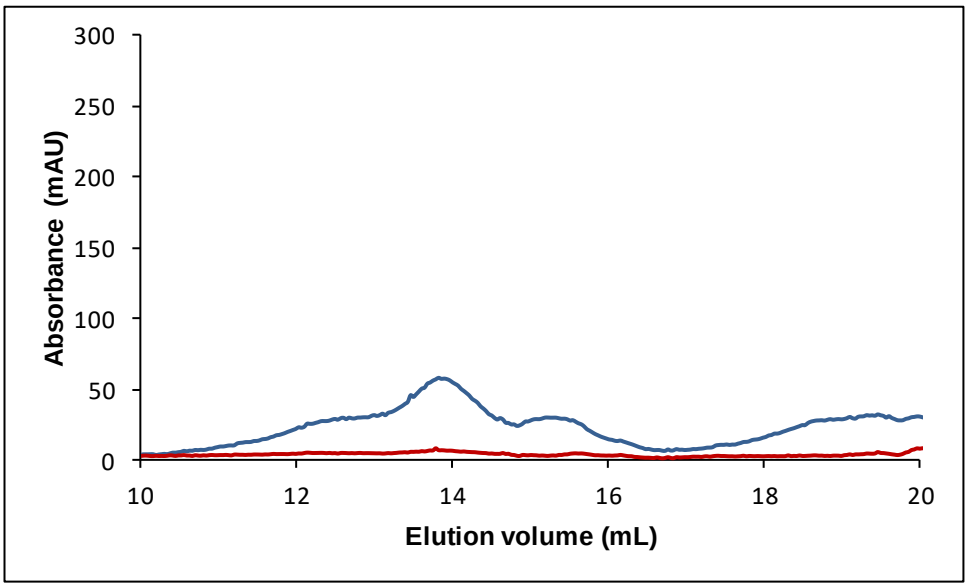

Figure S3. Electrospray ionization mass spectrometry analysis of NFU4. Mass spectrum of NFU4 protein determined for an untreated (A) or a reduced protein (B) as described in the "Materials and Methods" section. Species with a mass of 22,0351.1 and 22037,4 Da represent oxidized and reduced forms, respectively. Other species with sodium or Tris adduct are visible (species with a mass of 22,056.8 and 22,057.6 Da).

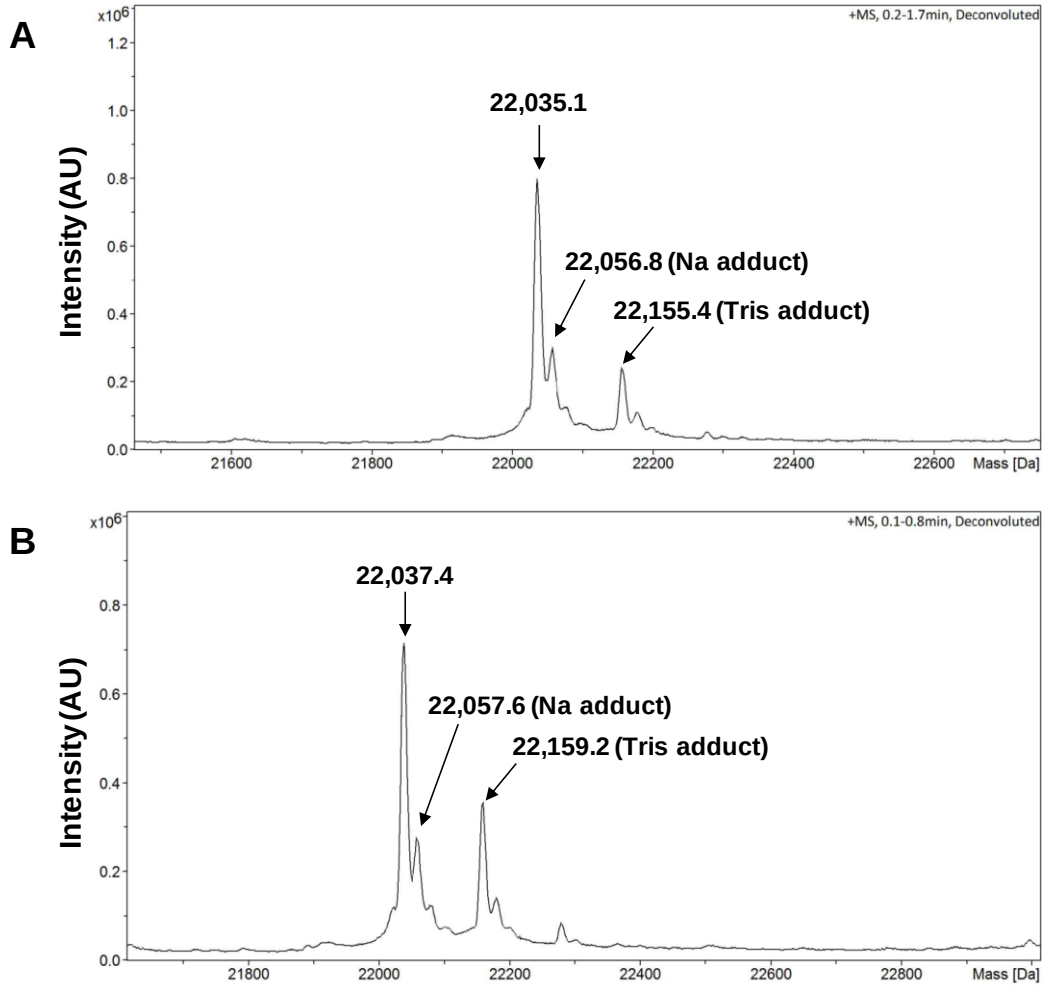

Figure S4. Electrospray ionization mass spectrometry analysis of NFU5. Mass spectrum of NFU5 protein determined for an untreated (A) or reduced protein (B) as described in the "Materials and Methods" section. Species with a mass of 21,690.9 and 21693.0 Da represent oxidized and reduced forms, respectively. Other species with sodium or Tris adduct are visible (species with a mass of 21,714.7 Da, 21,812.5 and 21,813.3 Da).

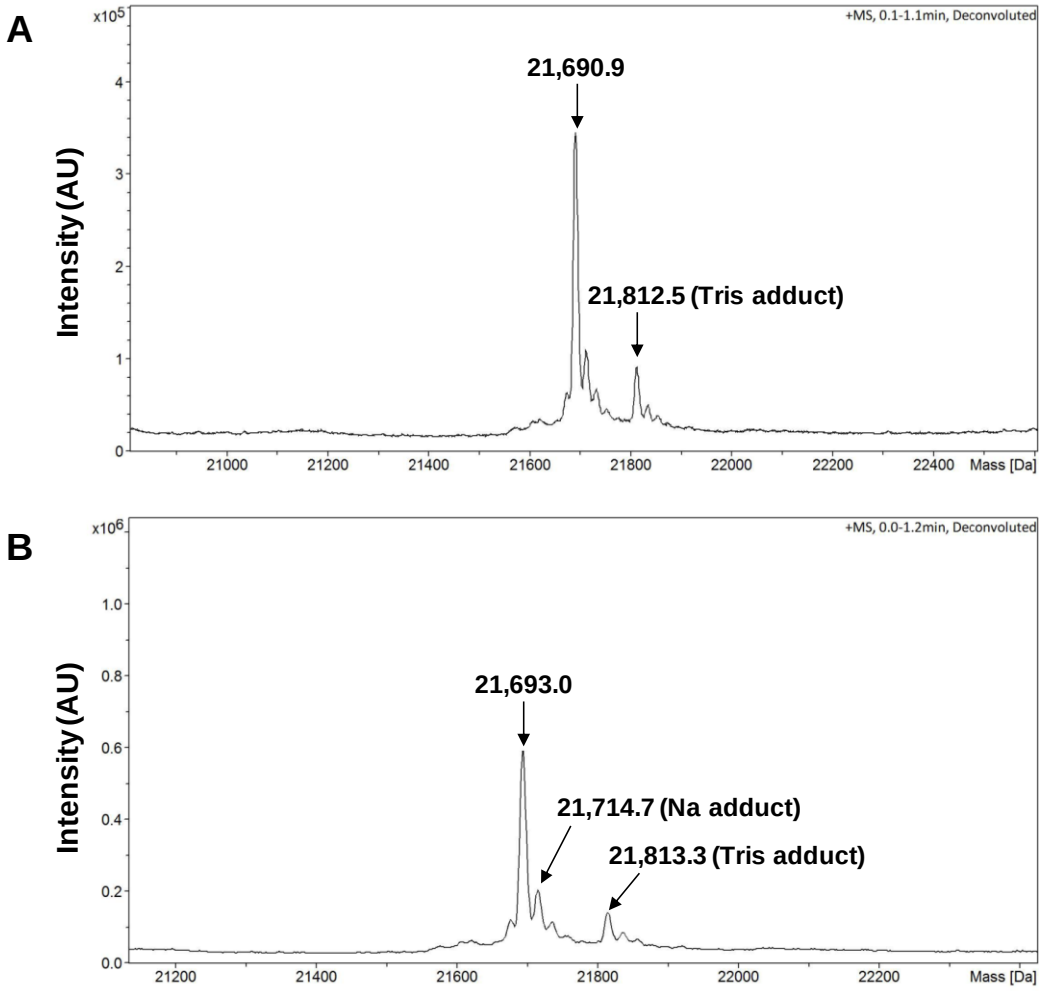

Figure S5. The disulfide bridge of mitochondrial NFUs is reduced by NTR/TRXo system but not by NTR/GRXS15 system. The reduction of as-purified, oxidized forms of NFU4 (A) or NFU5 (B) was assessed after a 15 min incubation in the presence of the following reducing systems: NTR: NADPH + NTR ; TRXo1: NADPH + NTR + TRXo1 ; TRXo2: NADPH + NTR + TRXo2 ; GRXS15: NADPH + NTR + GRXS15. The ability of these three oxidoreductases to reduce NFU proteins was evaluated using either 1 µM or 10 µM. After alkylation with 2 kDa mPEG maleimide, proteins were separated on non-reducing SDS-PAGE. Reduced (Red) and oxidized (Ox) proteins served as controls. The stars indicate the alkylated (*) forms of the oxidoreductases in the respective regeneration systems when visible.

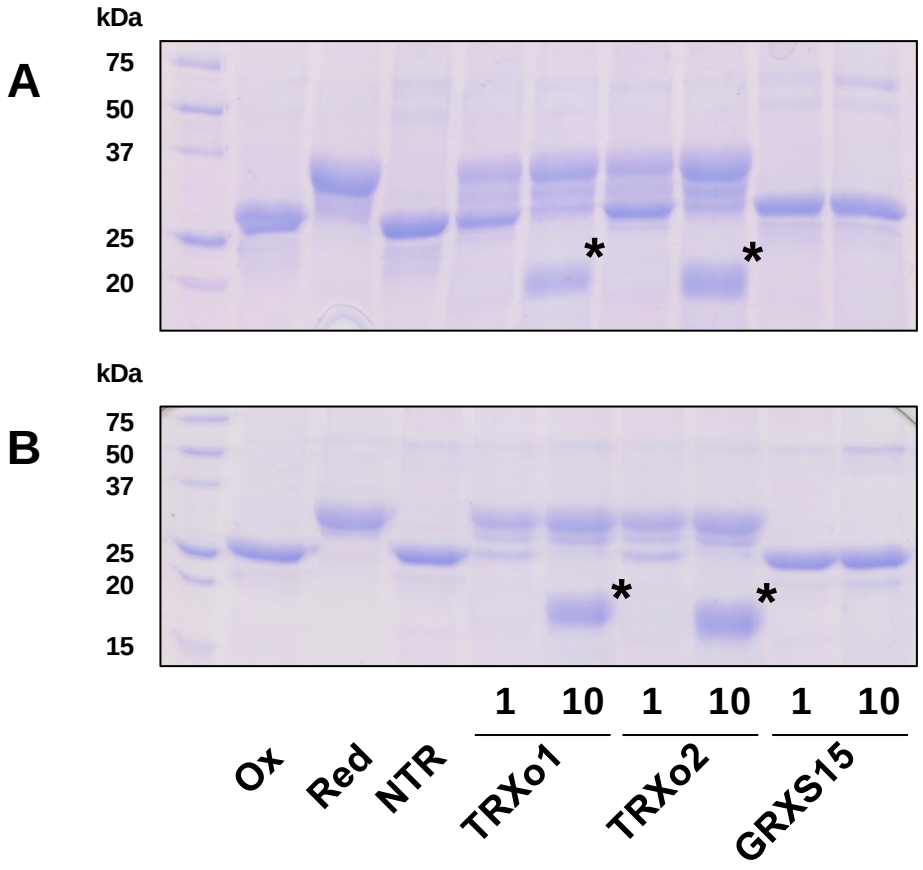

Table S1. Primers used for cloning and site-directed mutagenesis experiments. The *Nco*I, *Nde*I and *Bam*HI restriction sites used for cloning are underlined in the primers. The mutagenic codons are in bold.

Name	**Sequence**
TRXo1 for	5′ CCCCCCCCATATGGAAAATGGTGTTGTTCTA 3′
TRXo1 rev	5′ CCCCGGATCCTCACTTGTAGAGCTGTTC 3′
TRXo2 for	5′ CCCCCCCCATATGCGATCCAGCTTTGTAGTG 3′
TRXo2 rev	5′ CCCCGGATCCTCACTTGTAGAGTTGTTC 3′
TRXo2 C37S for	5′ TTCACTGCCGCATGG**TCT**GGACCTTGCAGGCTT 3′
TRXo2 C37S rev	5′ AAGCCTGCAAGGTCC**AGA**CCATGCGGCAGTGAA3′
TRXo2 C40S for	5′ GCATGGTGTGGACCT**AGC**AGGCTTATCTCTCCT3′
TRXo2 C40S rev	5′ AGGAGAGATAAGCCT**GCT**AGGTCCACACCATGC3′
NFU4 for	5′ CCCCCATGGCTTTTATCCAAACCCAATCA 3′
NFU4 rev	5′ CCCCGGATCCCTACTCTACTCTCATCTC 3′
NFU5 for	5′ CCCCCATGGCTTTTATCCAAACC 3′
NFU5 rev	5′ CCCCGGATCCTCACTCCATTGGACCAGA 3′
IscS for	5′ CCCCCCCCATATGTACGGAGTTTATAGAGCA 3′
IscS rev	5′ CCCCGGATCCTTAATGATGAGCCCATTC 3′