Structural and mechanistic insights into an unusual thiol disulfide oxidoreductase

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Table S1: NMR and refinement statistics for Dtrx structures. Structural statistics and restraint violations of the 20 selected structures representative of Dtrx in solution at pH 5.7 and 290K.

	Reduced	Oxidized
NMR distance and dihedral constraints Distance constraints		
Effective distance restraints	1846	2108
Short range ($\|i-j\| \leq 1$)	1152	1261
Medium range ($1 < \|i-j\| < 5$)	276	415
Long range ($\|i-j\| \geq 5$)	418	432
Average number of restraints per residue	17	19
H-bond restraints	48	70
Dihedral angle restraints from TALOS	156	156
Structures statistics		
Restraint violations		
Distance >0,5Å	0	1
Dihedral >5°	0	5
Energies (kcal/mol)		
E_{total}	-2951	-2853
E_{bond}	58	57
E_{angle}	338	349
$E_{dihedral}$	975	992
E_{VdW}	-850	-866
$E_{electric}$	-7713	-7090
Average ensemble RMSD (Å)		
Backbone	0.94±0.15	0.59±0.11
Heavy atoms	1.31±0.14	0.94±0.11
Deviations from idealized geometry		
bonds (Å)	0.0097	0.0100
angles (°)	2.465	2.441
Ramachandran plot (%)*		
Most favorable region	86.5	83.4
Additionally allowed region	13.2	16.3
Generously allowed region	0.2	0.3
Disallowed region	0.0	0.0

* calculated using PROCHECK v. 3.5.4

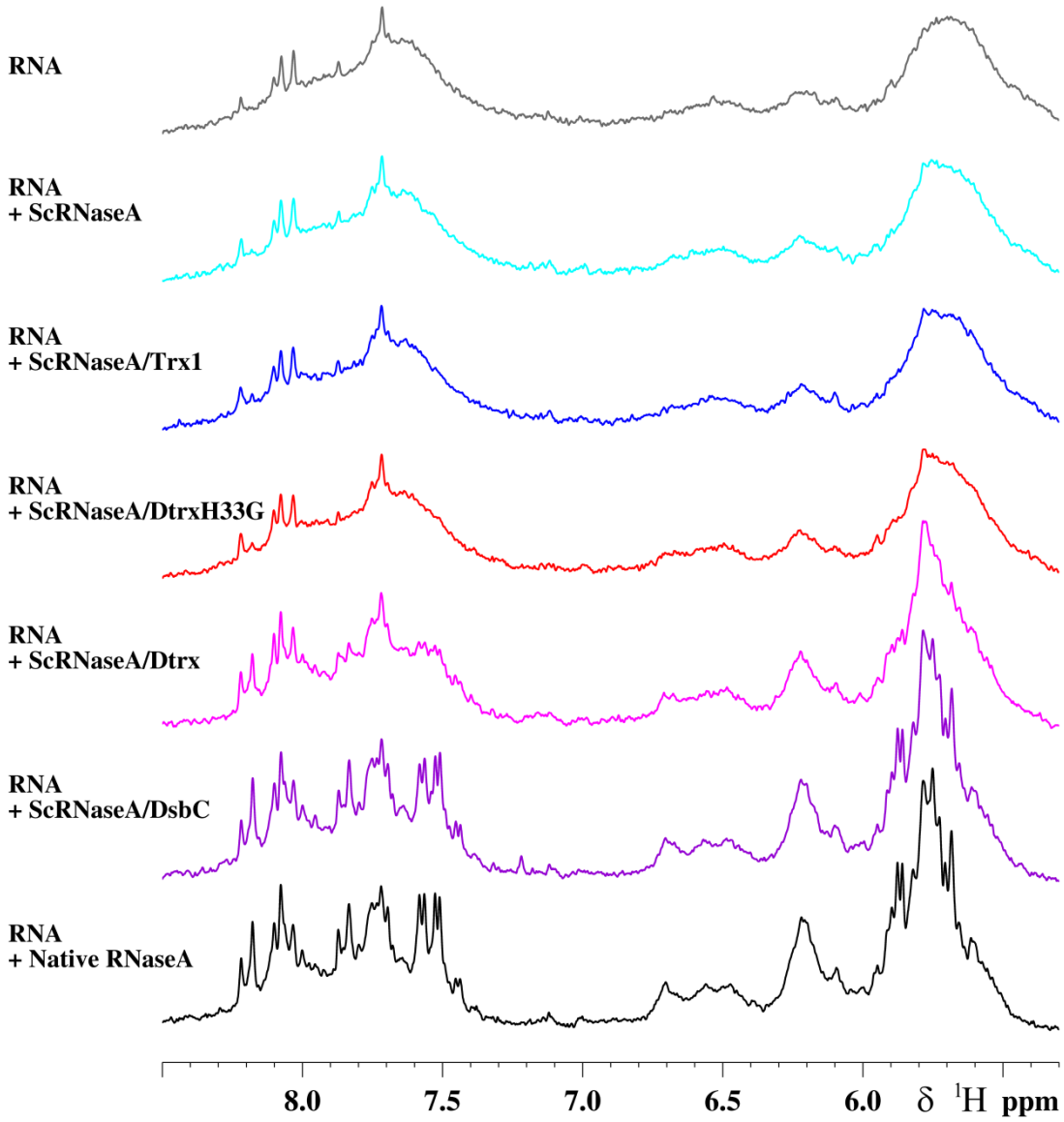

Figure S1: Scrambled RNaseA (ScRNase) refolding assay: the cleavage of RNA by RNaseA was followed by 1D ^{1}H-NMR.

1D 1H-NMR spectra of RNA with different mixture proteins: ScRNaseA (cyan), Trx1 and ScRNaseA (blue), DtrxH33G and ScRNaseA (red), Dtrx and ScRNaseA (pink), DsbC and ScRNaseA (purple), Native RNaseA (black). The assay was performed at 298K on a Bruker Avance III DRX 500 MHz spectrometer after 30 min of reaction. The NMR sample of RNA was 2 mg/ml in phosphate buffer 100 mM at pH 7 and 10 % D_2O. For the determination of the percentage of the RNaseA activity of native RNaseA and reshuffling of ScRNaseA after incubation with proteins, the mean intensity of several isolated peaks in RNA spectrum was used relative to RNA spectrum in the presence of native RNaseA. RNA spectrum (grey) in presence of scrambled RNaseA is used as blank.

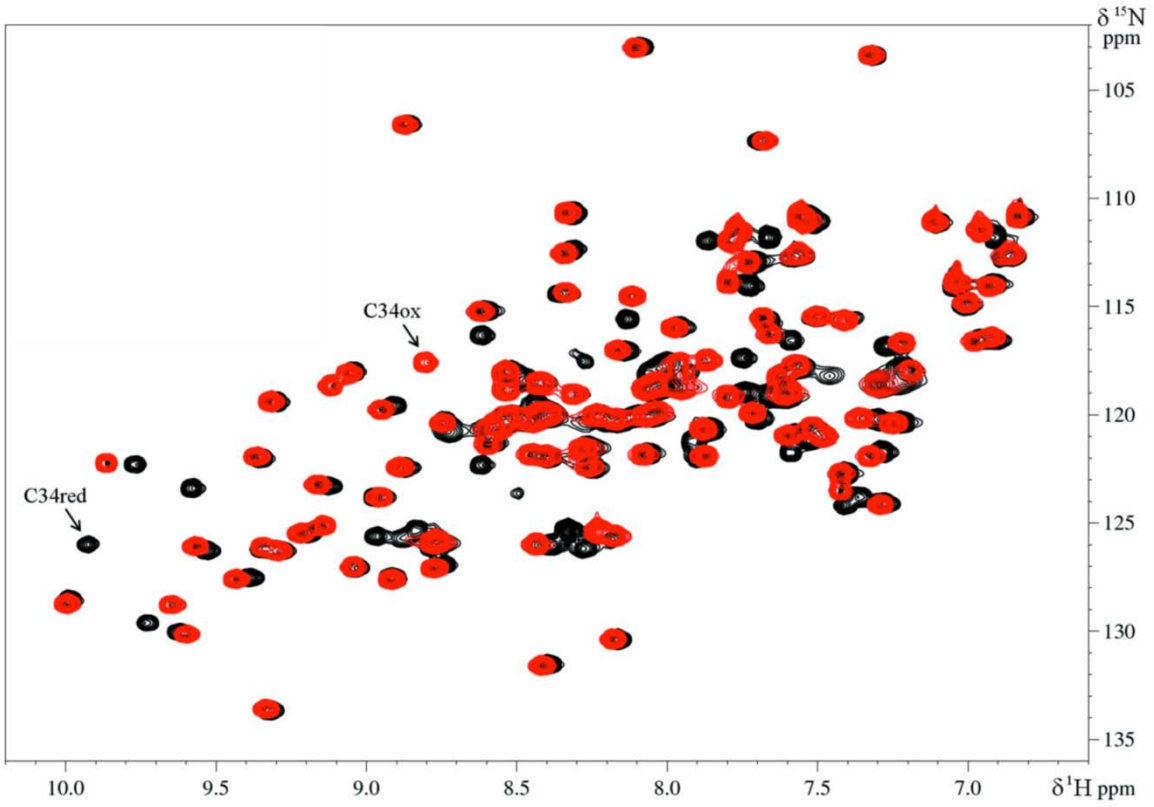

Figure S2: Titration of the redox potential of the disulfide bond of Dtrx. Superimposition of the ^{1}H-^{15}N HSQC spectra of Dtrx in the reduced (black) and oxidized (red) states at 298 K and pH 7. Arrows show the NH resonances of C34 under oxidizing (1.1 mM GSSG/1.8 mM GSH) and reducing (5.2 mM GSSG/23.6 mM GSH) conditions.

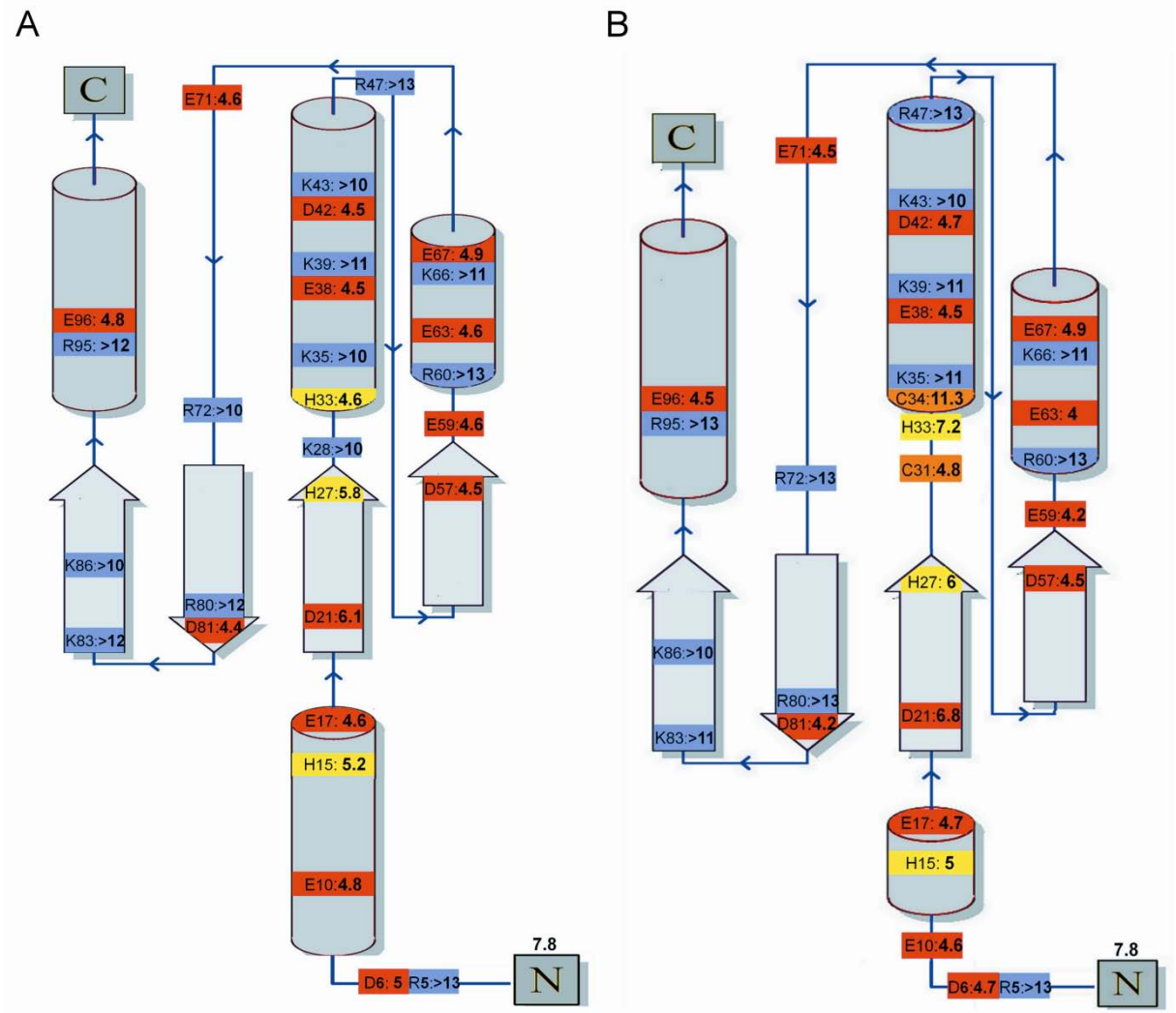

Figure S3: pKa of ionisable residues of reduced and oxidized Dtrx. Topology of the oxidized (A) and reduced (B) Dtrx forms. β-strands are shown as arrows and α-helices as cylinders. The pKas of ionisable residues determined by NMR are indicated. Acid residues Asp, Glu, are boxed in red, basic residues, Arg, Lys, are in blue, histidines are in yellow and cysteines are in orange.

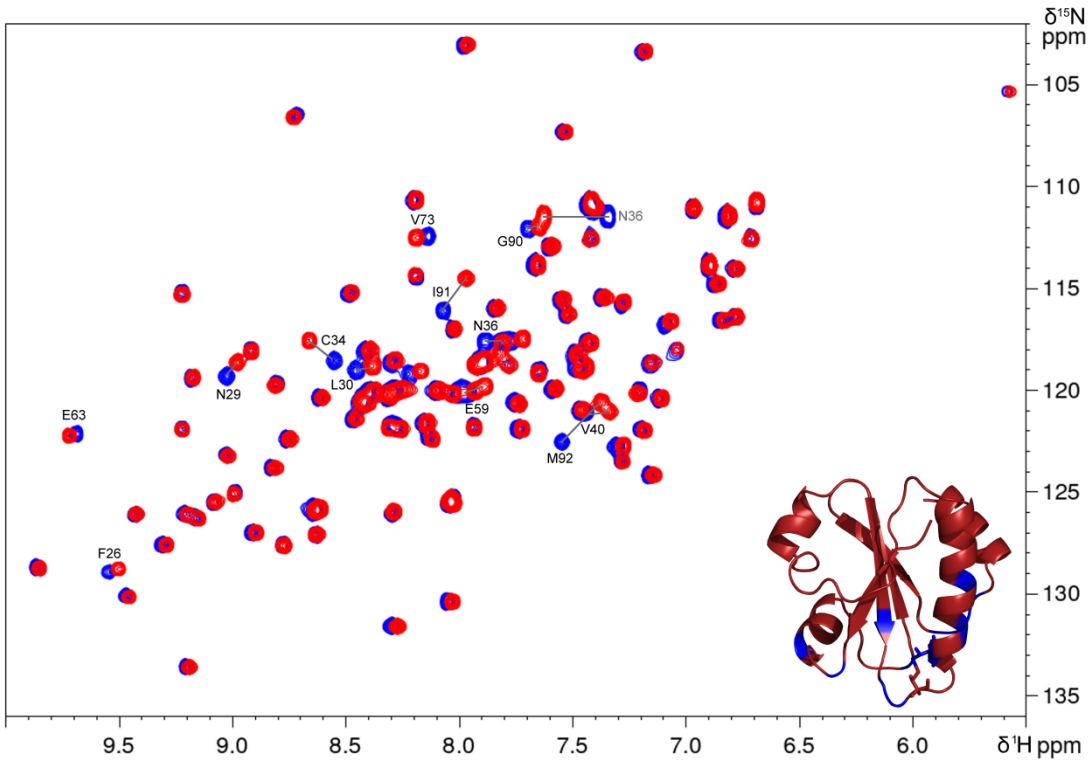

Figure S4: Superimposition of $^{1}H,^{15}N$-HSQC spectra of Dtrx and DtrxH33G. The samples were prepared in 100 mM potassium phosphate buffer pH 7, 50 mM NaCl. The spectra were recorded at 298 K on a 600 MHz NMR spectrometer. Residues undergoing chemical shift changes are labeled in the spectra and reported in blue on the Dtrx structure.

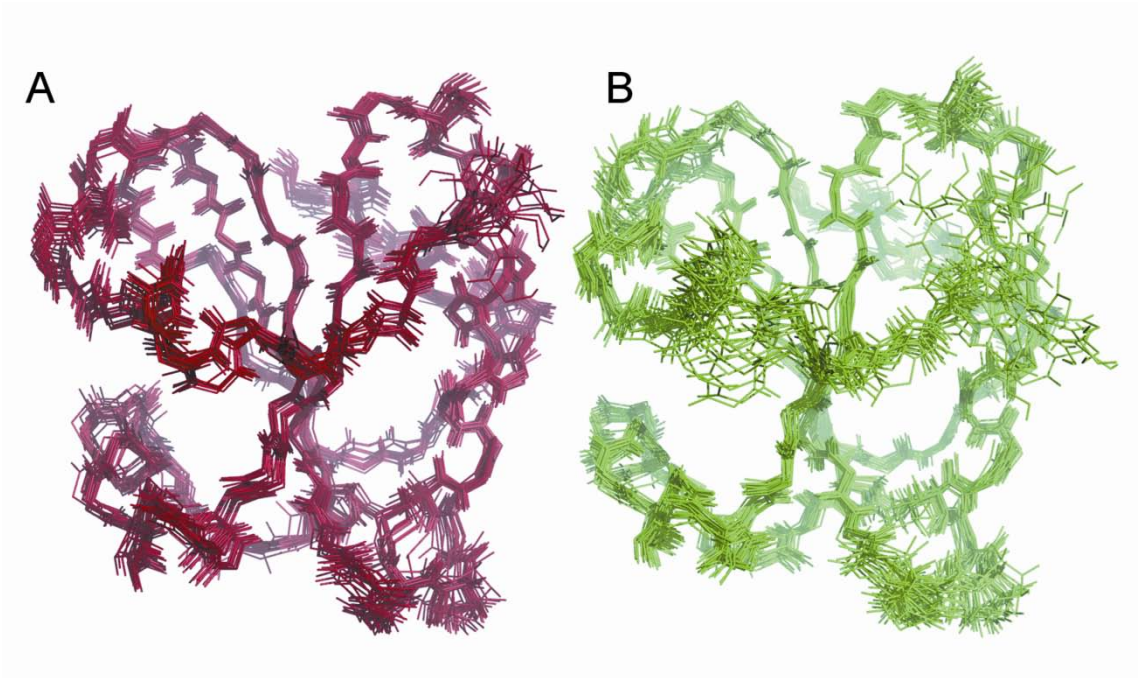

Figure S5: Solution structures of the reduced and oxidized forms of Dtrx. Superimposition of 20 representative structures of Dtrx in the oxidized (A) and reduced (B) states. The structures superimposed for minimal rmsd of all protein backbone atoms N, Cα and CO.

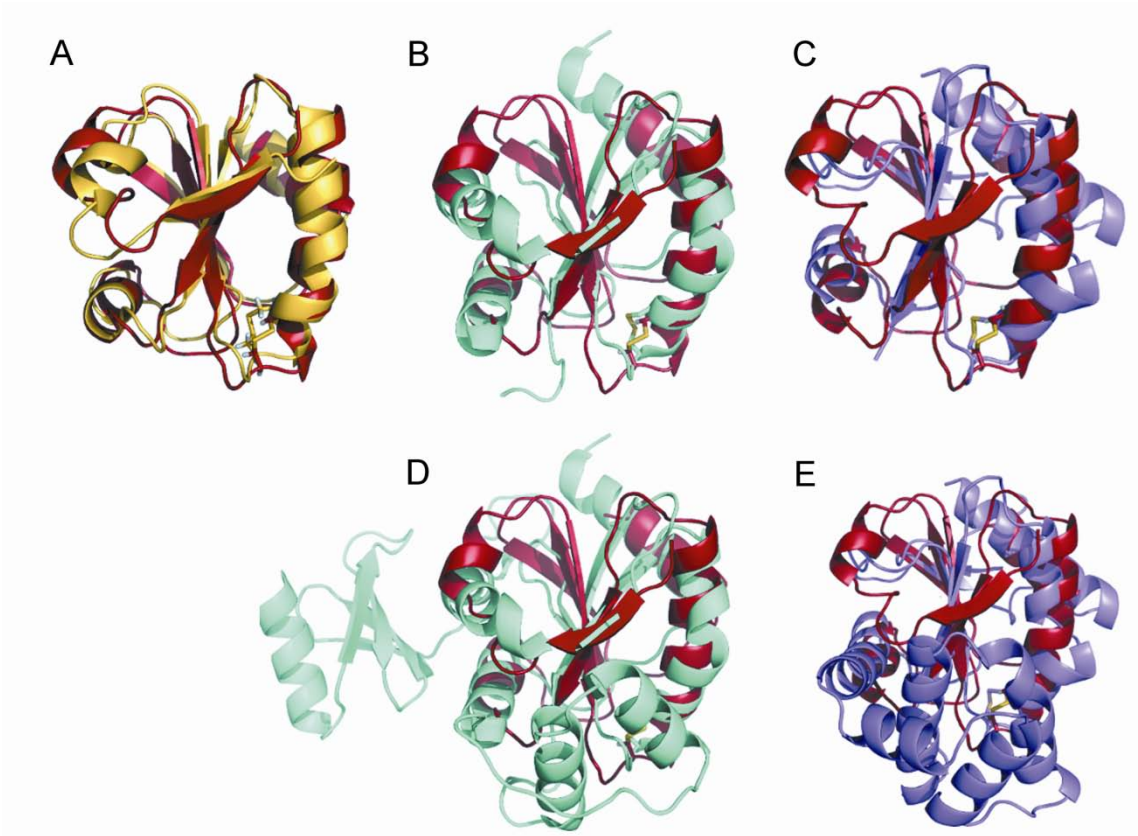

Figure S6: Structural comparisons. Overlay of the oxidized structures of Dtrx (red) with (A) *E. coli* Trx (yellow) (PDB entry 1XOA), (B) *E. coli* DsbA thioredoxin domain without the inserted helical domain F62-Q136 (blue) (PDB entry 1A2J), (C) *E. coli* DsbC thioredoxin domain without the inserted helical domain L126-V163 (purple) (PDB entry 1EEJ), (D) *E. coli* DsbA (blue), (E) *E. coli* DsbC (purple). The catalytic disulfide bond is shown as yellow sticks.