Supplementary information to the paper "Structural insights into a fusion protein between a glutaredoxin-like and a ferredoxin-disulfide reductase domain from an extremophile bacterium" by Zannini et al

Primer names	Sequences (5′ to 3′)	Cloned domain
DpGRX-FDR1 for	CCCCCCCCATATGACTGATAAAAAGATA	Full-length
DpGRX-FDR1 rev	CCCCCCCGGATCCTTAGTCTAACTCATAGTG	Full-length
DpGRX-FDR1 for2	CCCCCCCCATATGCGTACTGAGGTAGATGAA	FDR domain (AA 83-196)
DpGRX-FDR1 rev2	CCCCCCCGGATCCTTACCCTATTTTTTCTTTTATTTCCTG	GRX domain (AA 1-83)

Table S1: Primers used in this study for PCR cloning.
Restriction sites are underlined.

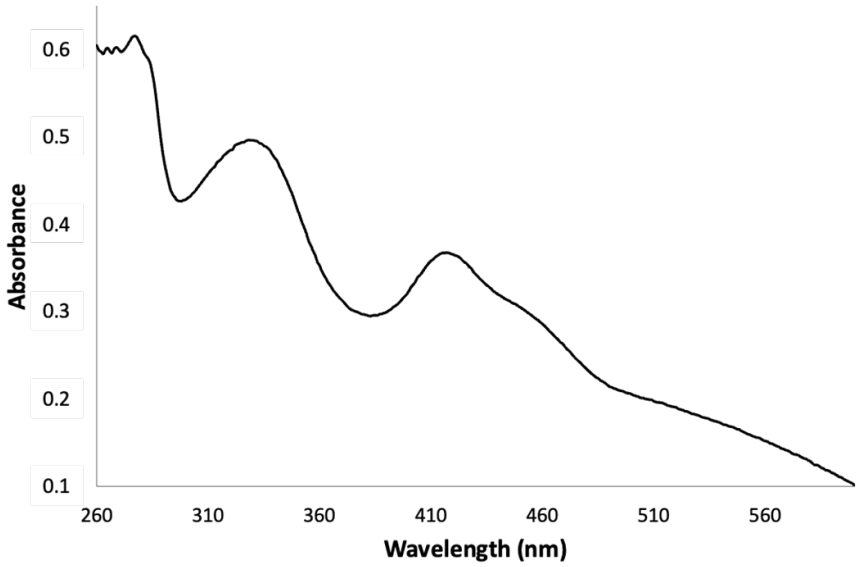

Figure S1: UV-Visible absorption spectrum of the anaerobically-purified GRX domain of DpGRX-FDR.

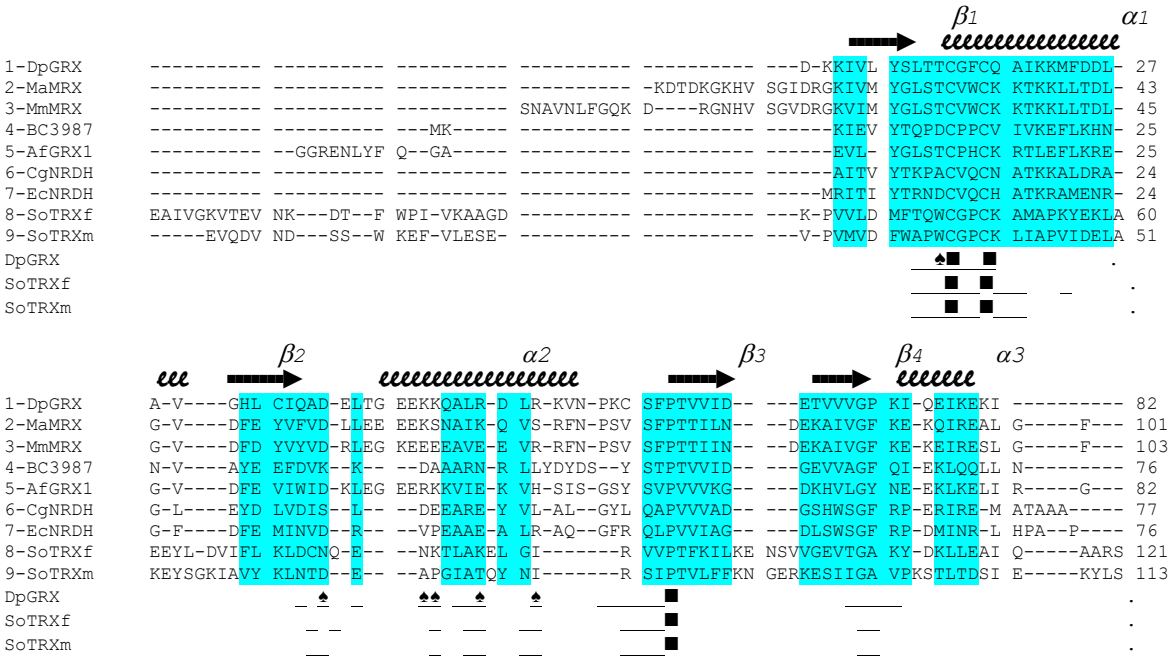

Figure S2: Structure-based sequence alignment of the GRX-like domain of DpGRX-FDR1 with its structural homologs and spinach TRX-*f* and TRX-*m*.

The sequence alignment was generated with mTM-align. Sequences included in the alignment were retrieved from the RCSB PDB: DpGRX, N-terminal domain of DpGRX-FDR1 (this study); MaMRX, methanoredoxin from *Methanosarcina acetivorans* (PDB ID 5cax), methanoredoxin from *Methanosarcina mazei* (PDB ID 3nzn); BC3987 thioredoxin-like from *Bacillus cereus* (PDB ID 3zij); AfGRX1 glutaredoxin 1 from *Archaeoglobus fulgidus* (PDB ID 3ic4); CgNRDH Nrdh-redoxin from *Corynebacterium glutamicum* (PDB ID 4fiw); NrdH-redoxin from *Escherichia coli* (PDB ID 1h75); SoTRXf TRX f from spinach (PDB ID 2pu9); SoTRXm TRX m from spinach (PDB ID 2puk). Secondary structures are labelled and shown using arrows (β-strands) and squiggles (α helices). Common regions *i.e.* regions with no gaps and with pairwise residue distances less than 4Å are highlighted blue. Invariant residues are marked with ■. Residues that are hydrogen bonded to DpFDR domain in GRX/FDR complex are marked with ♠. The underlined positions correspond to residues at the interface with the target enzyme (FTR in the case of TRX f and m and DpFDR in the case of DpGRX).

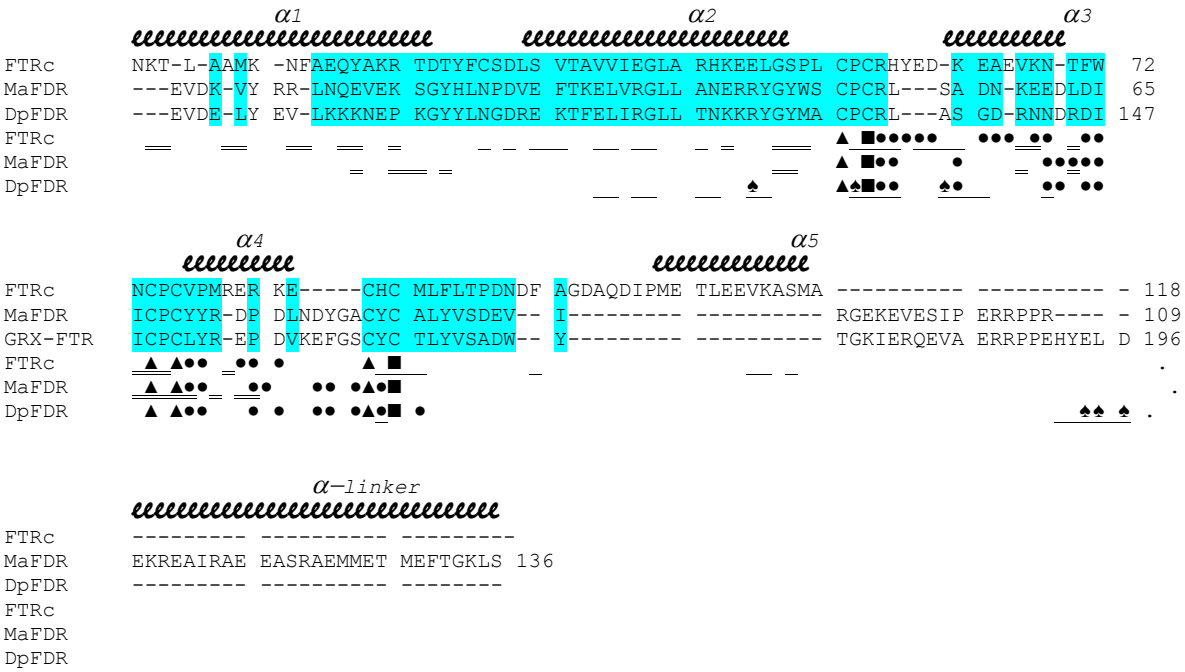

Figure S3: Structure-based sequence alignment of SynFTRc and FDR domains of MaFDR-RBX and DpGRX-FDR1 highlighting their common regions.

The sequence alignment was generated with mTM-align. Sequences were retrieved from the RCSB PDB : SynFTRc (PDB ID 1dj7), MaFDR (PDB ID 4tpu), DpFDR (this study). Secondary structures are labelled and shown using arrows (β-strands) and squiggles (α helices). Common regions *i.e.* regions with no gaps and with pairwise residue distances less than 4Å are highlighted blue. Cysteine residues that coordinate the iron-sulfur center in the resting state are marked with ▲. Cysteine residues that form disulfide bridge in the resting state are marked with ■. Residues near the FTRc iron-sulfur center and covered by FTRv subunit are marked with ●. Residues near the FDR iron-sulfur center and covered by FDR C-terminal tail are marked with ●. Residues that are hydrogen bonded to DpGRX domain in DpFDR/DpGRX complex are marked with ♠. The underlined positions correspond to residues at the interface with the target enzyme (TRX in the case of FTR and GRX domain in the case of DpGRX-FDR1). The double underlined positions correspond to residues at the interface with the electron donor (FDX in the case of FTR and the rubredoxin-like domain in the case of MaFDR-RBX).