Supporting Information

The amino acids motif –^{32}GSSYN^{36}- in the catalytic domain of *E. coli* flavorubredoxin NO reductase is essential for its activity

Maria C. Martins, Susana F. Fernandes, Bruno A. Salgueiro, Jéssica C. Soares, Célia V. Romão, Cláudio M. Soares, Diana Lousa, Filipe Folgosa * and Miguel Teixeira *

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal

* Correspondence: miguel@itqb.unl.pt, Tel: 351 214469309, f.folgosa@itqb.unl.pt, 351 214469321

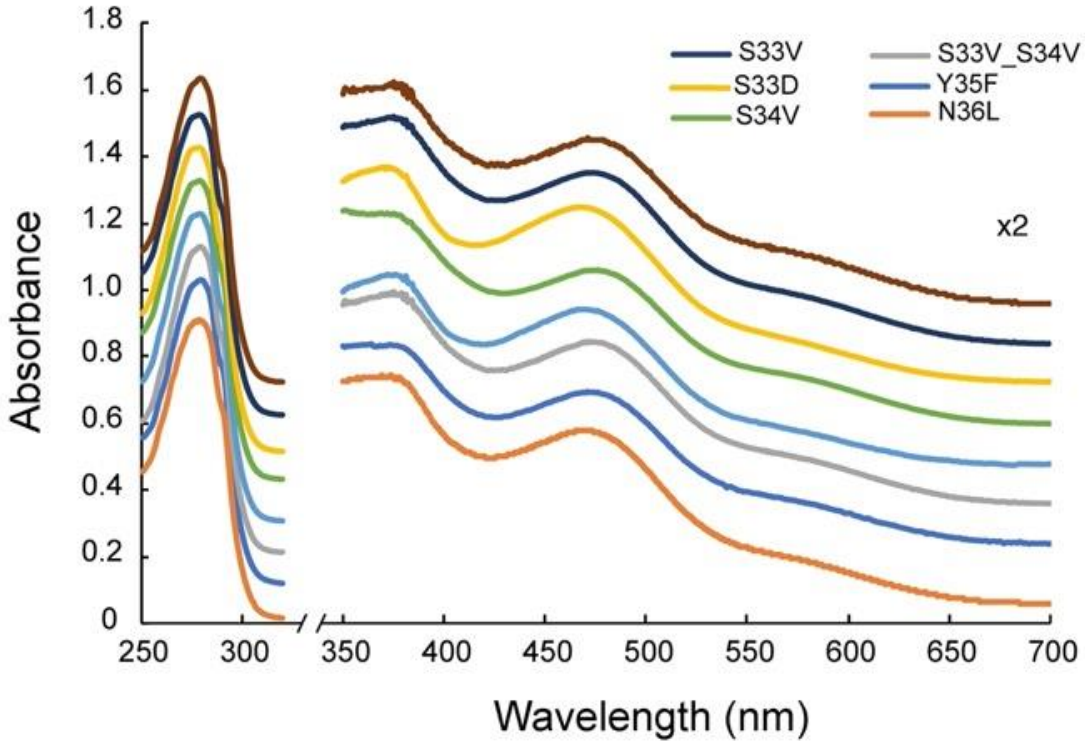

Figure S1 – UV-Visible spectra of FDP wt and site-directed mutants in the as-purified state. The protein concentration was 30 μM in 50 mM Tris-HCl pH 7.5, 18% glycerol.

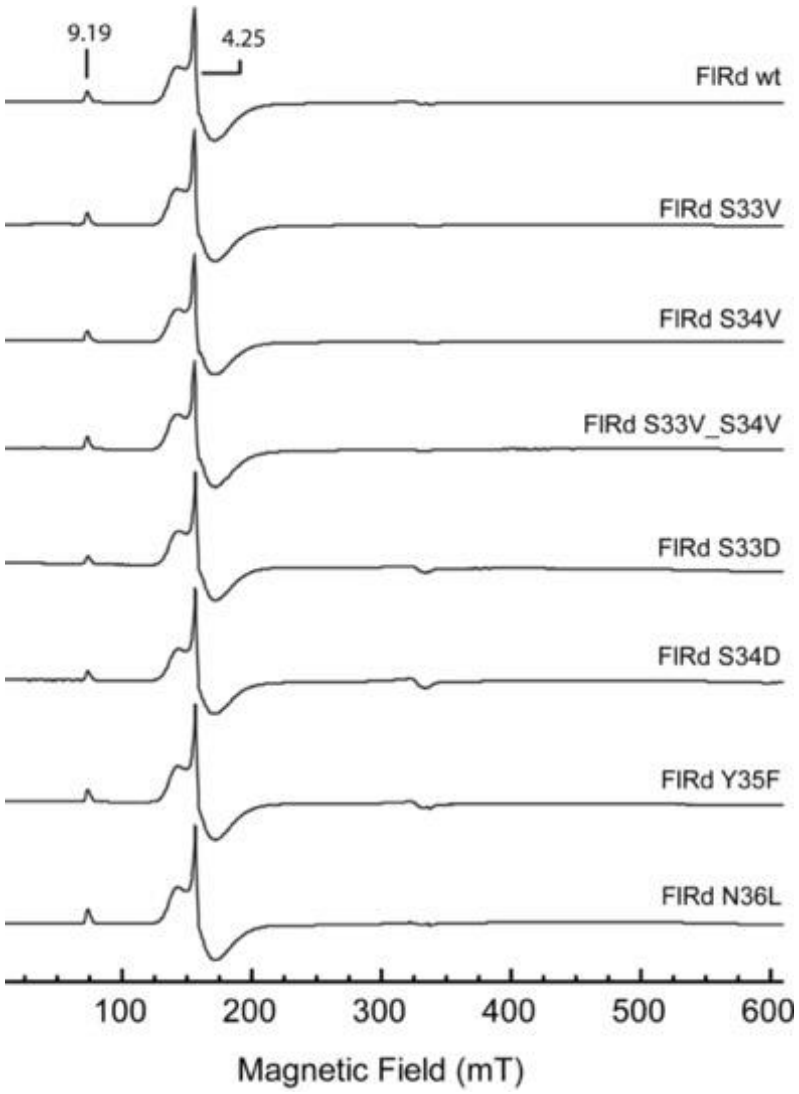

Figure S2 – EPR spectra of ferric rubredoxin sites of FDP wt and site-directed mutants. EPR spectra of the *E. coli* wt FDP and its mutants in the as-purified state, recorded at 7 K, with a microwave frequency of 9.39 GHz, a modulation amplitude of 1.0 mT and a microwave power of 2 mW. The protein concentration was 200μM in 50 mM Tris-HCl pH 7.5, 18% glycerol.

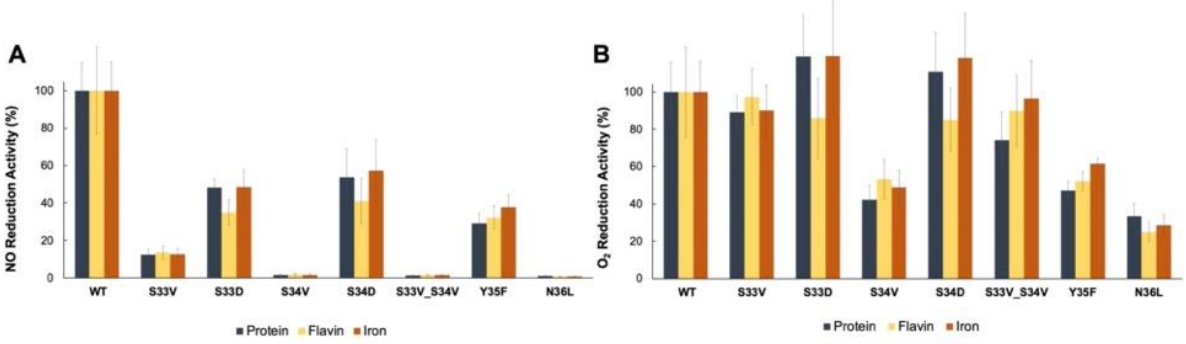

Figure S3 – NO and O_2 reduction activities of FDP wt and site-directed mutants taking into consideration the cofactor incorporation. A. NO reductase activities in percentages in relation to the wt. **B.** O_2 reductase activities in percentages in relation to the wt. The blue bars represent the activities calculated based on the protein concentration (same as Figure 4), while yellow and orange bars represent the corresponding activities corrected to the FMN and iron incorporation for each protein, respectively. The activities represented are averages calculated based on at least three assays for each protein. The error bars represent the standard deviations. The FMN and iron incorporation for each protein used for the calculations are presented in Table 1.

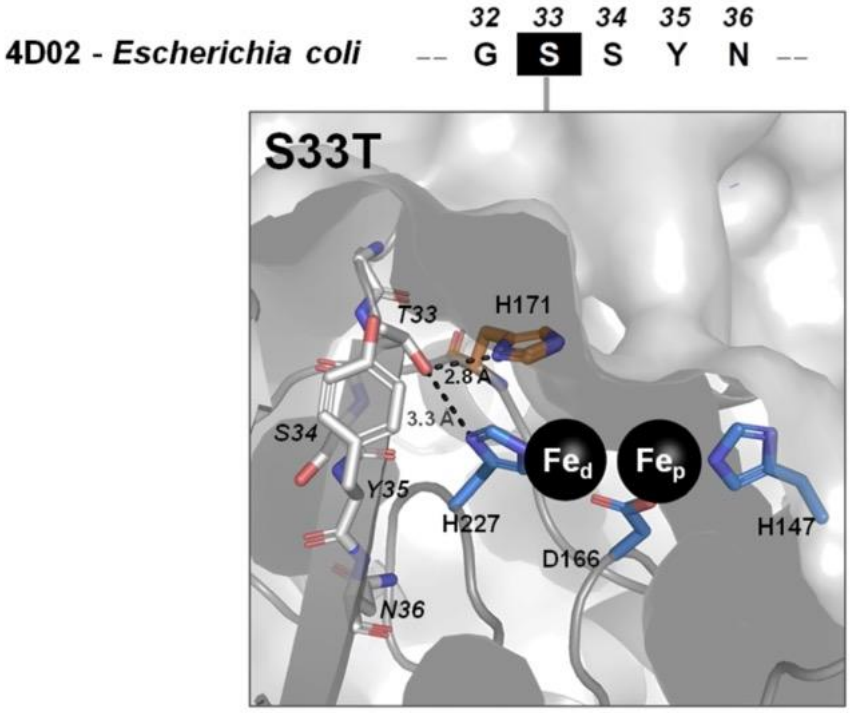

Figure S4 - Homology based models of the S33T variant. The interaction of residue 33 with the surrounding residues is shown for the homology-based model of the variant S33T. The figure was built with PyMOL using the X-ray structure for the WT (PDB code: 4D02) and the structure corresponding to the best model generated for the variant. The protein secondary structure elements are shown using a grey cartoon representation. The residues coordinating the metal center are displayed using sticks with the carbons colored in blue, the iron atoms as black spheres. The distance between residue 33 and histidines 171 and 227 is represented by a dashed line and labelled with the corresponding value.