Supplementary material

A two-component NOX-like system in bacteria is involved in the electron transfer chain to the methionine sulfoxide reductase MsrP

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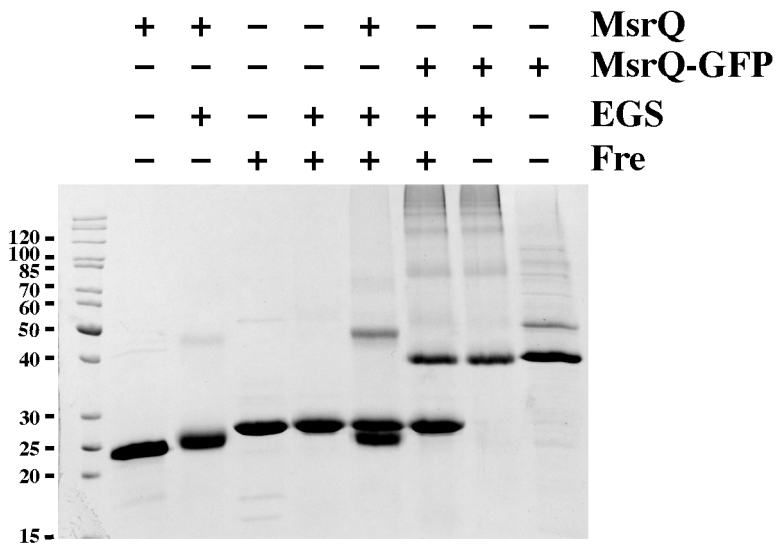

Figure S1: Interaction analysis between Fre and MsrQ or between Fre and MsrQ-GFP fusion. Purified proteins ((MsrQ, 10 μL at 1 mg/mL), MsrQ-GFP (10 μL at 1 mg/mL) and Fre (10 μL at 1 mg/mL) were first pre-incubated during 1 hour, alone or together, without the cross-linking reagent. As indicated above the gels, the cross-linking reactions were then started, by adding (+) or not (-) 0.5 mM EGS. After 20 min incubation, the reactions were immediately subjected to a SDS-PAGE analysis.

From this SDS-PAGE analysis, a clear crosslink between MsrQ and Fre is observed (see the band at 50 kDa, lane 4 from the right,). No crosslink is observed between Fre and the MsrG-GFP fusion, where GFP is present on the cytosolic side of MsrQ (see lane 3 from the right, showing no additional crosslink band when comparing to control lane 2). These data demonstrate that the crosslink interface between Fre and MsrQ is located on the cytosolic side of MsrQ.

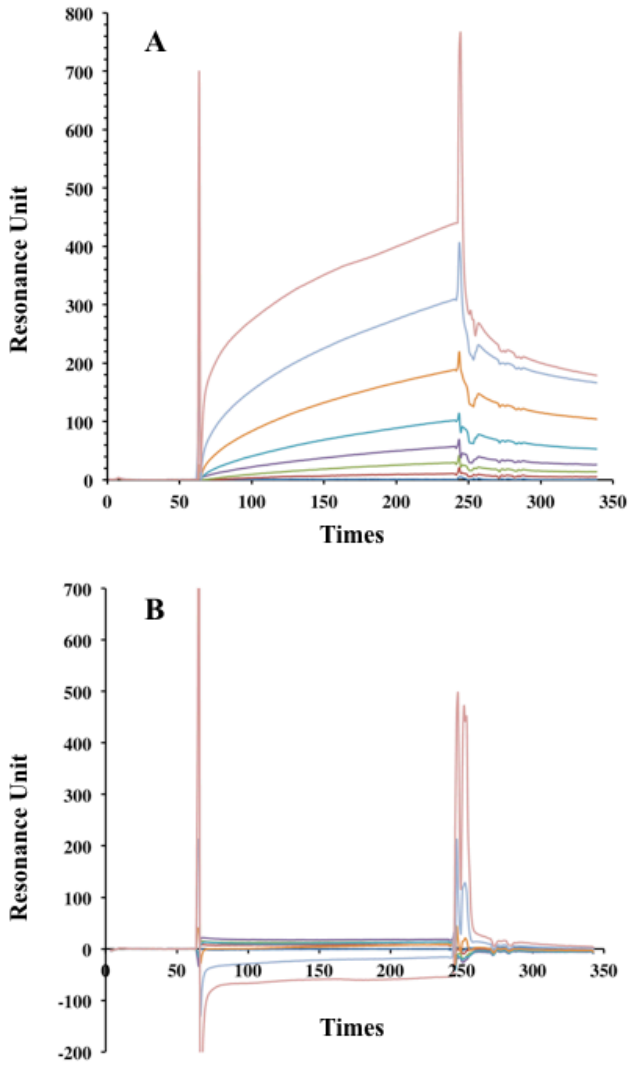

Figure S2: Surface plasmon resonance analysis of MsrQ binding to immobilized *E.coli* flavin reductase Fre (A) or to Bovine Serum Albumine (B). The purified MsrQ protein (60 μL) was injected over a Fre and BSA functionalized surface at a flow rate of 20 μL/min with 50 mM Tris-HCl pH 8.0; 0.016% DDM and 50 mM NaCl as running Buffer. The sensorgram was obtained for a range of MsrQ concentration at 120, 60, 30, 10, 3, 1 and 0.3 μM (from top to bottom).

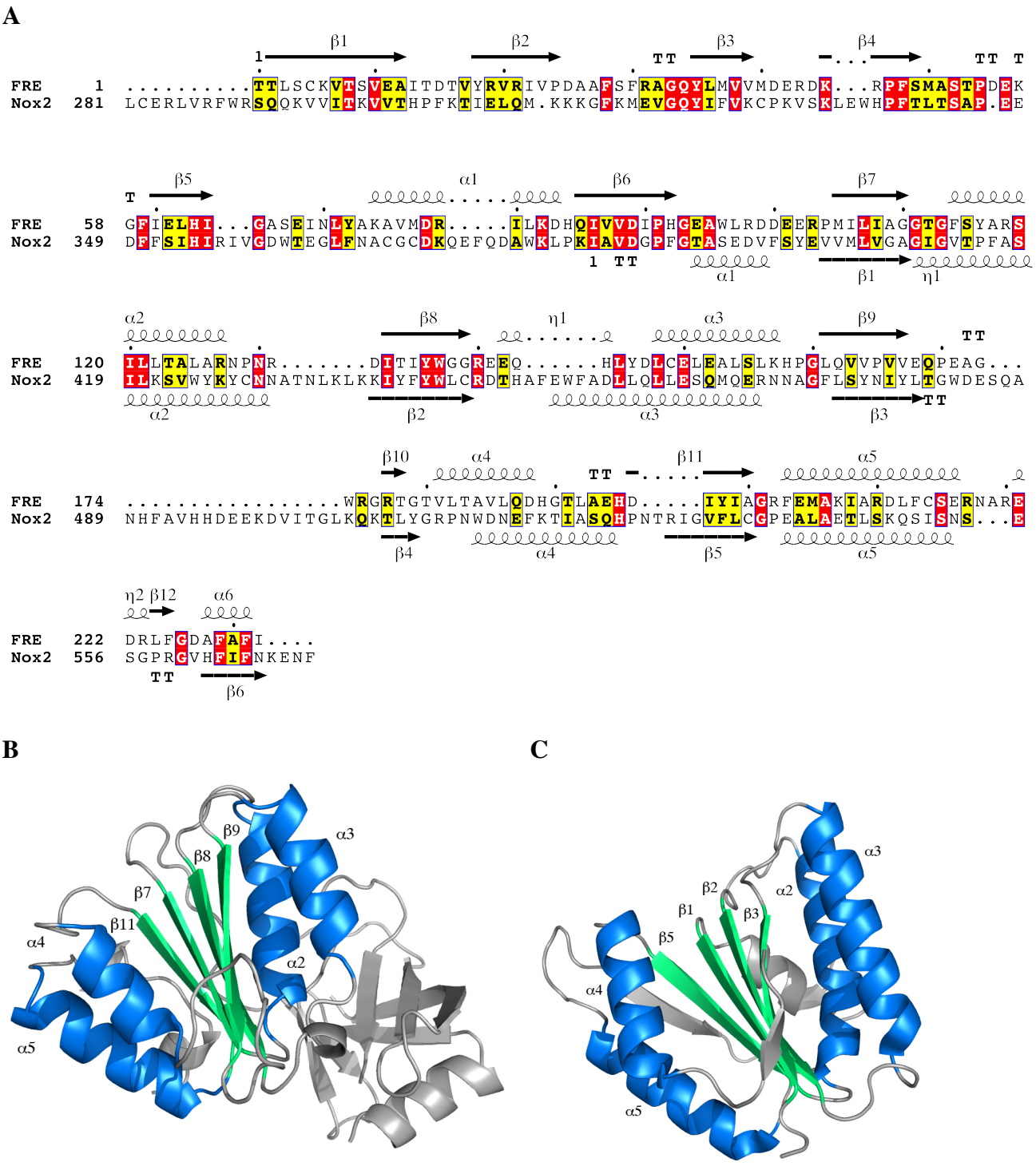

Figure S3: Structural relationship between the *E. coli* flavin reductase (Fre) and the cytosolic dehydrogenase domain of human NOX2. A) Sequence alignment between Fre (uniprot code P0AEN3) and the C-terminal dehydrogenase domain of Nox2 (AA 281 to 570 – uniprot code P04839) performed by COBALT (1) and shown with ESPript 3.06 (2). The secondary structure elements of Fre (PDB : 1QFJ) and truncated C-terminal domain of Nox2 (385-570) (PDB : 3A1F) are superimposed on the alignment. B) and C) Conserved secondary structures drawn with Pymol (3). The structures have the same orientation (green for sheet and blue for helix). B) Fre (PDB : 1QFJ) containing both NADPH and flavin binding domain, C) C-terminal truncated dehydrogenase domain of human Nox2, residues 385-570, corresponding only to the NADPH binding domain (PDB : 3A1F).

1- Papadopoulos JS, Agarwala R. COBALT: constraint-based alignment tool for multiple protein sequences. Bioinformatics. 2007 May 1;23(9):1073-9. Epub 2007 Mar 1. PubMed PMID: 17332019.

2- Gouet P, Courcelle E, Stuart DI, Métoz F. ESPript: analysis of multiple sequence alignments in PostScript. Bioinformatics. 1999 Apr;15(4):305-8. PubMed PMID: 10320398.

3- The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC.