## **Supporting Information**

## Maher et al. 10.1073/pnas.1800195115



**Fig. S1.** Conserved residues in SdhE including the RGxXE motif form part of the interaction surface with SdhA. (A). Structure of *Escherichia coli* SdhAE complex highlighting regions of SdhE, colored blue, orange, and red, that contribute to the binding interface with SdhA. Noncontact regions of SdhE are colored green. (*B*). Entire primary sequence of *E. coli* SdhE aligned with the complete or core domain of homologous proteins from other bacteria, fungi, animals, and plants. Amino acid residue numbering is indicated for *E. coli* SdhE only, directly above the sequence. Secondary structure elements of SdhE are also shown above the alignment and are colored coded as defined in *A: E. coli* (P64559), *Serratia* spp. (G4V4G2), *Shigella flexneri* (Q83JU2), *Salmonella typhimurium* (Q7CPV1), Yersinia pestis (Q8ZHJ5), Vibrio cholera (Q9KPA2), Haemophilus influenza (P44025), Pasteurella multocida (Q9CK44), Mannheimia succiniciproducens (Q65QC6), Shewanella oneidensis (Q8EH90), Ralstonia solanacearum (Q8XXX1), Wigglesworthia glossinidia brevipalpis (Q8D2B6), Idiomarina loihiensis (Q5R0Z7), *Pseudomonas aeruginosa* (Q9I5G9), Xanthomonas oryzae (Q5H0G1), Methylococcus capsulatus (Q3EBC2), Mus musculus (Q82C61), Danio rerio (A3KP74), Drosophila melanogaster (Q4V519, A12897), Aedes aegypti (Q178L7), Saccharomyces cerevisiae (Q08230), Schizosaccharomyces pombe (Q10440), Caenorhabditis elegans (Q9NA72), Dictyostelium discoideum (Q54B20), and Arabidopsis thaliana (Q9FI44). The codes in parenthesis indicate primary accession numbers.

Parameter	Value	
Data collection		
Space group	P212121	
Cell dimensions		
a, b, c, Å	53.0, 100.0, 232.1	
α, β, γ, °	90, 90, 90	
X-ray source	AUS MX2	
λ, Å	0.954	
Detector	ADSC Quantum 315r	
Resolution range, Å	50–2.15 (2.23–2.15)*	
Observed reflections	284,545	
Unique reflections	63,285	
Completeness, %	93.0 (94.8)	
Multiplicity	4.5 (4.4)	
<i σ(i)=""></i>	8.6 (1.6)	
R <sub>merge</sub> , %	14.3 (80.0)	
R <sub>pim</sub> , %	7.2 (41.1)	
Refinement		
Reflections in working set <sup>†</sup>	47,878	
Reflections in test set	2,561	
Protomers (SdhAE complexes) per ASU 2		
R <sub>work</sub> , %	19.9 (26.0)	
R <sub>free</sub> , %	25.8 (28.0)	
rmsd bond lengths, Å	0.009	
rmsd bond angles, °	1.39	
B factors, Å <sup>2</sup>		
SdhA	15	
SdhE	18	
Ramachandran <sup>‡</sup>		
Favored, %	97.64	
Allowed, %	2.20	
Outliers, %	0.16	
PDB ID code	6C12	

## Table S1. Data collection and refinement statistics

\*Values in parenthesis are for highest-resolution shell.

<sup>+</sup>Following anisotropic correction of data (1).

<sup>‡</sup>Calculated using MolProbity (2).

1. Strong M, et al. (2006) Toward the structural genomics of complexes: Crystal structure of a PE/PPE protein complex from Mycobacterium tuberculosis. Proc Natl Acad Sci USA 103:8060-8065.

2. Chen VB, et al. (2010) MolProbity: All-atom structure validation for macromolecular crystallography. Acta Crystallogr D Biol Crystallogr 66:12-21.

## Table S2. Distances between the covalent FAD cofactor and structural elements putatively involved in covalent flavinylation

Base, Å (proton abstraction, C8 methyl group)	N1-C <sub>2</sub> =O <sub>2</sub> stabilization	Amide near N5 (Å)
His365 Νδ1-C8α (4.4)	Helix dipole (residues 415–429)	Gln62 NH (3.4)
His354 Nδ1-C8α (4.0)	Helix dipole (residues 405–419)	Gln50 NH (3.1)
His354 Nδ1-C8α (4.2)	Helix dipole (residues 405–419)	Gln50 NH (4.4)
	Base, Å (proton abstraction, C8 methyl group) His365 Νδ1-C8α (4.4) His354 Νδ1-C8α (4.0) His354 Νδ1-C8α (4.2)	Base, Å (proton abstraction, C8 methyl group)N1-C2=O2 stabilizationHis365 N $\delta$ 1-C8 $\alpha$ (4.4)Helix dipole (residues 415-429)His354 N $\delta$ 1-C8 $\alpha$ (4.0)Helix dipole (residues 405-419)His354 N $\delta$ 1-C8 $\alpha$ (4.2)Helix dipole (residues 405-419)

\*PDB ID code 1ZOY (1). <sup>†</sup>PDB ID code 2WDQ (2).

<sup>‡</sup>This work.

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1. Sun F, et al. (2005) Crystal structure of mitochondrial respiratory membrane protein complex II. Cell 121:1043–1057. 2. Ruprecht J, Yankovskaya V, Maklashina E, Iwata S, Cecchini G (2009) Structure of Escherichia coli succinate:quinone oxidoreductase with an occupied and empty quinone-binding site. J Biol Chem 284:29836-29846.