The Solvent-Exposed Fe-S D-cluster Contributes to Oxygen-Resistance in *D. vulgaris* Ni-Fe Carbon Monoxide Dehydrogenase

Supporting Information

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Table S1. Crystallographic data collection and refinement statistics for C45G/T50C Dv CODH.

	as-isolated	O_2-exposed < 20 min	O_2-exposed 2 hours	O_2-exposed 2 days
Data collection				
Space group	$P2_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions				
a, *b*, *c* (Å)	65.6, 147.4, 66.1	66.1, 111.4, 193.9	65.5, 111.8, 194.5	65.6, 112.1, 194.4
α, β, γ (°)	90.0, 110.8, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Wavelength (Å)	0.9791	0.9791	0.9791	0.9791
Resolution (Å) ‡	100–1.83	100–2.50	100–2.21	100–2.78
	(1.87–1.83)	(2.55–2.50)	(2.26–2.21)	(2.84–2.78)
Unique reflections ‡	102654 (7601)	50165 (3647)	72260 (5304)	36890 (2707)
R_{sym} (%) ‡	8.6 (77.7)	19.0 (68.7)	13.2 (78.2)	15.1 (95.2)
$CC_{1/2}$ ‡	99.9 (82.9)	98.3 (66.6)	99.5 (68.3)	99.4 (67.7)
$< I / \sigma(I) >$ ‡	13.8 (2.4)	6.8 (2.0)	10.1 (2.1)	11.6 (2.0)
Completeness (%) ‡	99.7 (99.8)	99.5 (99.5)	99.8 (99.9)	99.8 (100)
Multiplicity ‡	6.8 (6.7)	4.7 (4.6)	5.3 (5.5)	6.2 (6.2)
Refinement				
Resolution (Å)	73.68–1.83	73.63–2.50	97.71–2.21	73.45–2.78
R_{work}/R_{free} §	0.147/0.178	0.181/0.211	0.174/0.203	0.179/0.214
No. atoms				
protein	9400	9201	9238	9133
B-cluster	16	16	16	16
C-cluster	36¶	18	18	18
D-cluster	8	8	0	0
water	803	351	684	118
Average *B*-factors (Å^2)				
protein	29.0	26.9	31.4	41.4
B-cluster	20.5	20.9	22.7	41.2
C-cluster	28.4	52.4	55.3	75.7

D-cluster	23.0	34.0	–	–
water	39.8	29.7	37.0	34.6
R.m.s. deviations				
bond lengths (Å)	0.009	0.005	0.004	0.004
bond angles (°)	1.15	1.15	0.71	0.82
Rotamer outliers (%)	0.62	0.32	0.63	0.65
Ramachandran (%)				
favored	96.6	96.5	96.3	96.8
allowed	3.1	3.2	3.3	3.0
disallowed	0.3	0.3	0.4	0.2
Residues (of 629)				
chain A	4–629	5–629	4–629	5–40; 43–629
chain B	4–628	4–629	4–627	4–40; 43–629

‡Values in parenthesis indicate highest resolution bin.
§A total of 4–5% of reflections were set aside during refinement for cross-validation.
¶For this structure, the C-clusters were refined as a mixture of reduced (80%) and oxidized (20%) conformations, resulting in the presence of 36 C-cluster atoms rather than 18.

Table S2. Crystallographic data collection statistics for iron anomalous data.

	as-isolated Fe peak*	O_2-exposed < 20 min Fe peak*	O_2-exposed 2 hours Fe peak*
Data collection			
Space group	$P2_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions			
a, *b*, *c* (Å)	65.8, 147.5, 66.1	66.1, 111.6, 194.2	65.6, 112.1, 195.2
α, β, γ (°)	90.0, 110.8, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Wavelength (Å)	1.7389	1.7389	1.7389
Resolution (Å) ‡	100–2.27 (2.32–2.27)	100–2.80 (2.86–2.80)	100–2.60 (2.66–2.60)
Unique reflections ‡	105228 (7670)	67913 (5089)	85290 (2666)
R_{sym} (%) ‡	13.4 (78.7)	19.2 (89.3)	12.2 (84.0)
$CC_{1/2}$ ‡	99.2 (68.7)	98.6 (68.7)	99.5 (64.1)
$< I / \sigma(I) >$ ‡	8.1 (2.2)	8.4 (2.0)	11.1 (1.8)
Completeness (%) ‡	98.0 (97.9)	99.4 (99.4)	99.7 (99.3)
Multiplicity ‡	4.2 (4.1)	5.2 (5.2)	4.6 (4.5)

*Bijvoet pairs were not merged during data processing.
‡Values in parenthesis indicate highest resolution bin.

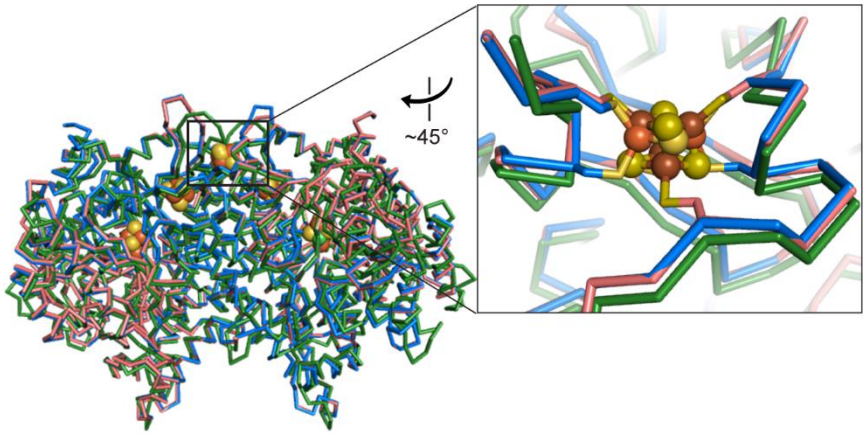

Figure S1.
The structure of C45G/T50C Dv CODH aligns well with those of other CODHs. Shown is the C45G/T50C Dv CODH structure (pink) aligned with that of WT Dv CODH (blue, Cα r.m.s.d of 0.27 Å for 1250 Cα atoms) and *C. hydrogenoformans* CODH II (green, Cα r.m.s.d of 1.27 Å for 1250 Cα atoms). Proteins are shown as the Cα trace of each structure with metalloclusters shown as spheres with Fe in orange, S in yellow, and Ni in green. The inset shows structural similarity in the vicinity of the D-cluster between CODHs regardless of the cluster type at this position. In the inset, the [4Fe-4S] D-cluster of C45G/T50C Dv CODH is displayed along with the [2Fe-2S] D-cluster of WT Dv CODH, with the [4Fe-4S] cluster colored in darker shades.

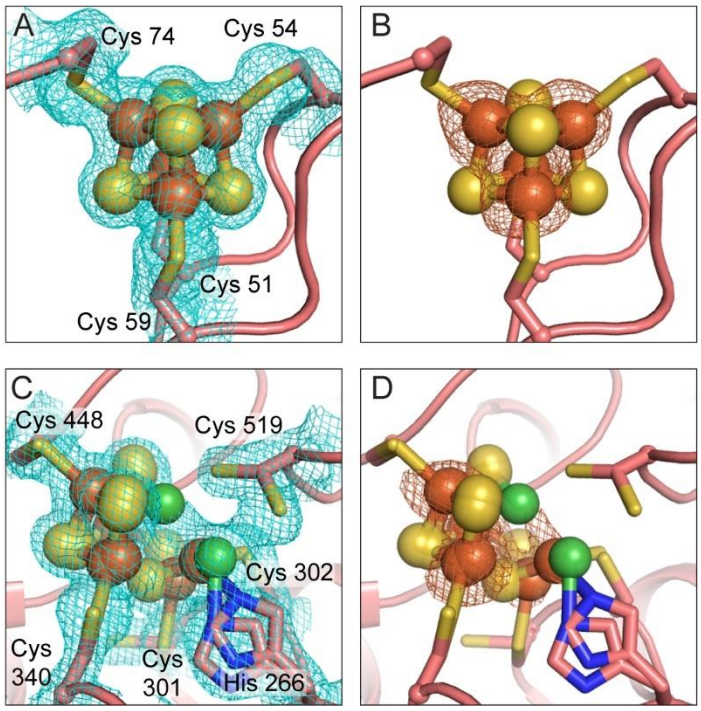

Figure S2.
The B- and C-clusters of as-isolated C45G/T50C Dv CODH. A) 2*Fo*−*Fc* electron density (blue mesh; contoured to 1σ) for the B-cluster. B) Iron anomalous difference map (orange mesh; contoured to 5σ) for the B-cluster. C) 2*Fo*−*Fc* electron density (blue mesh; contoured to 1σ) for the C-cluster. Note that the C-cluster is observed in both the reduced (80% occupancy) and oxidized (20% occupancy) conformations, with the Ni ion refined with an occupancy of 20% in each conformation. D) Iron anomalous difference map (orange mesh; contoured to 5σ) for the C-cluster. Protein is shown as in Figures 2 and 4.

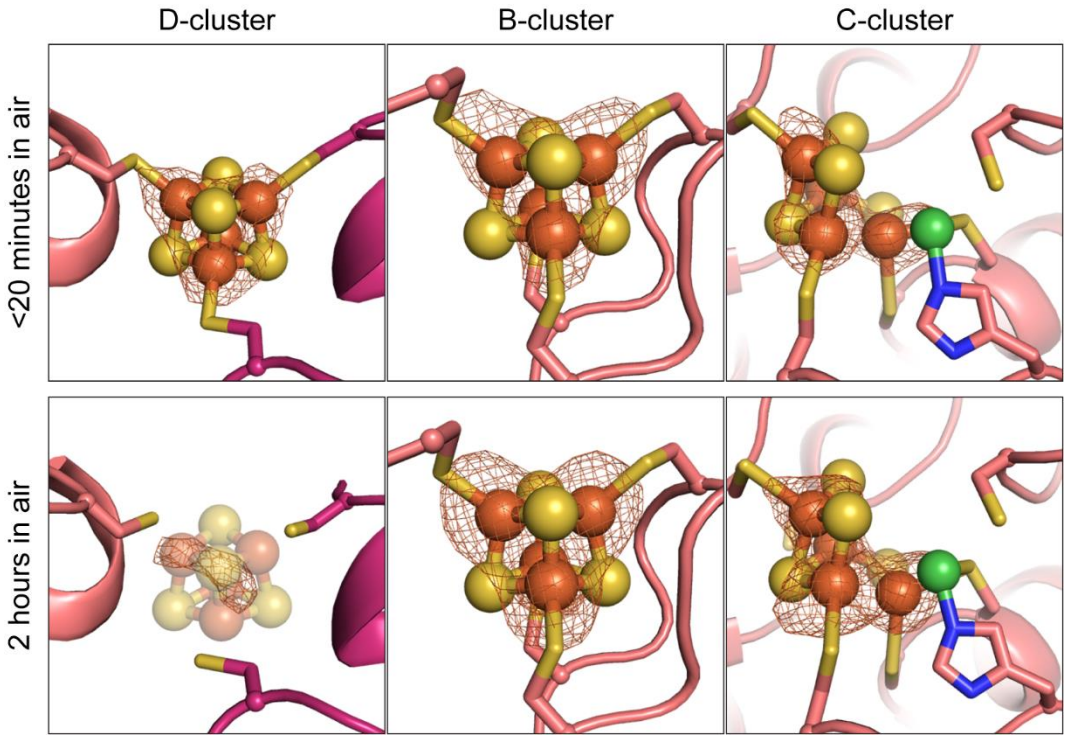

Figure S3.
Iron anomalous diffraction data for air-exposed C45G/T50C Dv CODH. Iron anomalous difference maps (orange mesh, contoured to 4σ) calculated from data collected at the iron peak wavelength (7130 eV, 1.7389 Å). Protein is shown as in Figures 2 and 4.

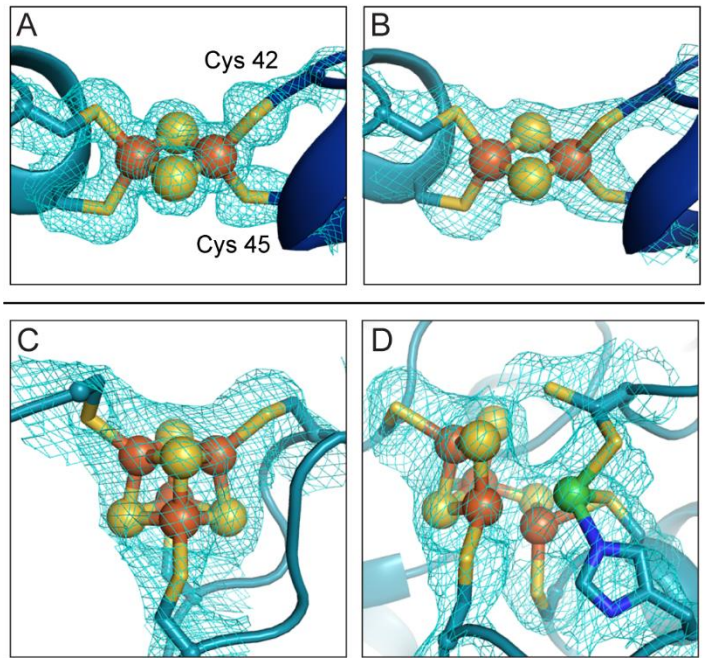

Figure S4.
Stability of the metalloclusters in WT Dv CODH over time in air. A) [2Fe-2S] D-cluster of WT Dv CODH in crystals maintained under anaerobic conditions (PDB ID 6B6W). B) D-cluster of WT Dv CODH after the crystal was exposed for 2 days to ambient atmospheric conditions (PDB ID 6B6Y). C) The B-cluster of WT Dv CODH after 2 days in air (PBD ID 6B6Y). D) The oxidized C-cluster of WT Dv CODH after 2 days in air (PBD ID 6B6Y). 2*Fo*−*Fc* electron density (blue mesh) contoured to 1σ. Protein shown as in Figures 2 and 4 with protein ribbon representation in blue. Figure panels have been reproduced with permission from Reference 1.

References

(1) Wittenborn, E. C.; Merrouch, M.; Ueda, C.; Fradale, L.; Léger, C.; Fourmond, V.; Pandelia, M.-E.; Dementin, S.; Drennan, C. L. Redox-Dependent Rearrangements of the NiFeS Cluster of Carbon Monoxide Dehydrogenase. *eLife* **2018**, **7**, e39451.