Supporting Information

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Fig. 51. Predicted quinone-binding cavities in published complex I structures. (A) The active state of bovine complex I (PDB ID: 5LC5) (6). (B) The bovine structure with Q10 modeled in using MD simulations (Q10 was removed for the channel prediction). (C) Complex I from the porcine respirasome (PDB ID: 5GUP) (7). (D) *T. thermophilus* complex I (PDB ID: 4HEA) (10). (E) *Y. lipolytica* complex I (PDB ID: 4WZ7) (9). (F) Ovine complex I (PDB ID: 5LNK) (8) in which the cavity is blocked by the $\beta 1-\beta 2^{49}$ ^{kDa} loop; the cavity from *A* is overlaid as a mesh for comparison. (G) The active state of bovine complex I (PDB ID: 5LC5) with the modeled Q10 structure overlaid. (H) The bovine structure with Q10 modeled in using MD simulations. Cluster N2 is shown as spheres and Y108^{49kDa} is shown because its hydroxyl oxygen was used as the starting point for cavity searches. Channels were predicted using the Caver 3.0 plugin in PyMOL (33) with a probe size of 1.4 Å or 1.1 Å for *E*.



Fig. 52. Conformational changes in the modeled ubiquinone-bound states. (*A*) The sidechain of F224^{ND1} responds to the isoprenoid chain in modeled structures with different species bound. For Q1–Q6 and the ubiquinone-free state, it obstructs the channel at isoprenoid-7, whereas for Q8 and Q10 it has moved away. Also shown are variations in the positions of the sidechains of Y108 and H59 in the presence and absence of the ubiquinone headgroup. (*B*) The charged residues within 5 Å of isoprenoids 4–7 form two distinct groups of interacting residues. Predicted hydrogen bonds are shown with dashed yellow lines. (C) The sidechains in the righthand set (including residues on a nearby helix) show less variation in their positions between the different states than those in the lefthand set that are predominantly present on loops.



Fig. S3. Comparison of the protein environments of quinone molecules with different isoprenoid chain lengths in modeled structures. (*A*) The percentage of residues within 5 Å of each isoprenoid unit that are classified as hydrophobic (A, F, I, L, M, P, V, W, and Y). (*B*) The percentage of residues within 5 Å of each isoprenoid unit that are classified as hydrophobic (C, D, E, G, H, K, N, Q, R, S, and T). (*C*) The percentage of residues within 5 Å of each isoprenoid unit that are classified as hydrophilic (C, D, E, G, H, K, N, Q, R, S, and T). (*C*) The percentage of residues within 5 Å of each isoprenoid unit that are classified of residues within 5 Å of each isoprenoid unit that are classified as hydrophilic (C, D, E, G, H, K, N, Q, R, S, and T). (*C*) The percentage of residues within 5 Å of each isoprenoid unit that are classified of the percentage of residues within 5 Å of each isoprenoid unit that are classified as hydrophilic (C, D, E, G, H, K, N, Q, R, S) and T). (*C*) The percentage of residues within 5 Å of each isoprenoid unit that are classified as hydrophilic (C, D, E, G, H, K, N, Q, R, S) and T). (*C*) The percentage of residues within 5 Å of each isoprenoid unit that are classified as hydrophilic (C, D, E, G, H, K, N, Q, R, S) and T). (*C*) The percentage of residues within 5 Å of each isoprenoid unit that are classified as hydrophilic (C, D, E, G, H, K, N, Q, R, S) and T). (*C*) The percentage of residues within 5 Å of each isoprenoid unit that are classified as hydrophilic (C, D, E, G, H, K, N, Q, R, S) and T). (*C*) The percentage of residues within 5 Å of each isoprenoid unit that are classified as hydrophilic (C, D, E, G, H, K, N, Q, R, S) and T). (*C*) The percentage of residues within 5 Å of each isoprenoid unit that are classified as hydrophilic (C, D, E, G, H, K, N, Q, R) and T). (*C*) The percentage of residues within 5 Å of each isoprenoid unit that are classified as hydrophilic (C, D, E, G, H, K, N, Q) and T). (*C*) The percentage of residues within 5 Å of each isoprenoid unit tha



Fig. S4. Estimated relative free energies for solvation of quinone in the complex I binding site for each quinone variant. The free energies of solvation were estimated using continuum electrostatics calculations on 50 snapshots extracted from the MD simulations, by the MM-GBSA method (36). The calculation provides a free-energy change relative to quinone in a reference medium, here the low dielectric membrane slab. All values are given relative to the value for Q1. Although this simplified MM-GBSA model accounts for electrostatic solvation free energies, it does not account for entropic contributions. A complete treatment would require computationally demanding free-energy calculations that are not currently justified by the resolution of available structural data.

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Quinone	<i>К</i> _М (mM)	V _{max} (µmol⋅min ⁻¹ ⋅mg ⁻¹)	$k_{\rm cat}$ (s ⁻¹)	$k_{\rm cat}/K_{\rm M}$ (m ${ m M}^{-1}\cdot{ m s}^{-1}$)	R ²
Q1	4.1 ± 0.95	10.2 ± 0.69	168 ± 11	41 ± 9.8	0.9575
Q2	8 ± 3.1	8.4 ± 0.41	138 ± 6.9	17 ± 6.2	0.8500
Q4	10 ± 2.8	27 ± 2.7	439 ± 44	42 ± 12	0.9615
Q6	5.4 ± 0.94	22 ± 1.2	363 ± 20	66 ± 12	0.9787
Q8	1.0 ± 0.23	20.1 ± 0.93	332 ± 15	319 ± 71	0.9591
Q10	0.49 ± 0.081	22.5 ± 0.58	371 ± 9.5	764 ± 130	0.9771