Supplementary Data for:

Water exit pathways and proton pumping mechanism in B-type Cytochrome *c* oxidase from molecular dynamics simulations

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1. H++ parameters, protonation states

H++ cannot recognize non-standard complex ligands such as heme automatically. We therefore manually supplied PQR files (PDB+charge+radius) with appropriate charges. For standard residues, the charges from the Amber ff12SB force field were used. The charges of cofactors were obtained through QM calculations as described in the methods section of the manuscript and in section 3 of this supporting information. The pH was set to 6.5. General atomic van der Waals radii were used (C: 1.70 Å; N: 1.55 Å; Fe: 1.00 Å; O: 1.50 Å; Cu: 1.40 Å; H: 1.20 Å).

All Arg, Tyr and Lys residues are predicted to be protonated with high pKa values (Arg > 11.7, Tyr > 8.9, Lys > 8.7). All but two Glu residues are predicted to have pKa values < 5.4, while Glu203 and $Glu131^{II}$ have pKa values of 7.0 and 6.7, respectively. We have assumed that all Glu residues in their standard, deprotonated state. All Asp residues apart from Asp372 have pKa values < 4.1 and are thus deprotonated. Most His residues have rather low pKa values while His462, His552, and $His8^{II}$ have pKa values around 6.7 and His376 has a pKa > 12. We have assumed that all His residues apart from His376 are in their default protonation state (singly protonated on the ε-nitrogen atom).

Table S1. Non-standard protonation states of titratable residues and their pKa value as determined by H++.

Residue	Protonation state	pKa
Asp372	protonated	10.1
His376	doubly protonated	> 12

The equilibrium protonation state thus has all residues in their default protonation state with the exception of Asp372, which is protonated and His376, which is doubly protonated. We refer to this as state D in the manuscript.

2. Hydrogen bond analysis of states F to A

These protonation states are equivalent to protonation states A to F in the mechanism presented in the manuscript (see Figure 1). The difference is that His376 is protonated at the other imidazole nitrogen atom.

Table S2. Molecular dynamics simulations of protonation states omitted in the manuscript (from state A' to state E'). These correspond to the states between state F and state A.

Simulation No.	Protonation states/ Equivalent state				
	$PRAa_3$ protonated	Asp372 protonated	His376 protonated[a]	$Glu126^{II}$ protonated	Equivalent state[b]
B'	yes	no	δ	no	B
C'	yes	yes	δ	no	C
E'	no	yes	ε	yes	E

a: ε=epsilon-protonated histidine; δ=delta-protonated histidine; ε,δ=doubly protonated histidine.

b: the corresponding states in the mechanism from state A to state F presented in Figure 1.

Table S3. Hydrogen bond occupancies for simulations of different protonation states omitted in the manuscript (state F to state A).[a]

Simulation	Acceptor[b]	Donor[b]	Occupancy[c]
B'	OD2(Asp372)	O2A($PRAa_3$)	99.4%
	OD1(Asp372)	ND1(δ-His376)	80.9%
	O(Asp372)	N(δ-His376)	44.9%
C'	O1A($PRAa_3$)	OD2(Asp372H)	33.0%
	OD1($PRAa_3$)	OD2(Asp372H)	3.4%
	NE2(δ-His376)	O2A($PRAa_3$)	10.6%
	O1A($PRAa_3$)	ND1(δ-His376)	4.0%
	OD1(Asp372H)	ND1(δ-His376)	67.0%
	O(Asp372H)	N(δ-His376)	44.0%
	OD1(Asp372H)	O2A($PRAa_3$)	3.4%
E'	O1A($PRAa_3$)	OD2(Asp372H)	28.2%
	O2A($PRAa_3$)	OD2(Asp372H)	18.9%
	O(Asp372H)	N(ε-His376)	69.5%

a: Only occupancies larger than 3% are listed.

b: Name of the heavy atom involved in the hydrogen bond, with the residue name in parenthesis.

c: Occupancy is the fraction of simulation frames during which the bond is present. Hydrogen bonds are defined through an angle cutoff of 135° and a distance cutoff of 3.0 Å between the hydrogen bonded atoms.

3. Coordinates and charges for the DNC, Cu_A and heme b cofactors

The tables contain two sets of charges, ESP charges and final charges. The ESP charges were directly obtained from an ESP charge fit as outlined in the methods section of the manuscript. The final charges were obtained by a) averaging the charges on methyl group hydrogen atoms and b) scaling the charges of all atoms in the cluster model and the remaining backbone atoms of the coordinating residues such that the total charge is maintained after removing the hydrogen link atom.

The DNC cluster model includes heme a_3, Cu_B, and the side chains of residues His384, His283, His282, His233 and Tyr237. Charges for the DNC cluster models are shown together with their coordinates in Tables S4 and S5, while corresponding backbone charges are shown in Tables S6 and S7.

The Cu_A pair cluster model contains the Cu_A pair and the side chains of coordinating histidine and cysteine residues $His114^{II}$, $His157^{II}$, $Cys149^{II}$, and $Cys153^{II}$. Charges and coordinates for the Cu_A pair are in table S8, and modified backbone charges in table S9.

The heme b cluster model used for the charge derivation contains the heme b group, His72, and His386. The charges for the cluster model and the backbone atoms are in tables S10 and S11, respectively.

Table S4. DNC cluster model for charge derivation with deprotonated $PRAa_3$. The hydrogen link atoms have a zero final charge (atom number 145, 146, 147, 180, 192).

		Coordinates			ESP charge	Final charge
1	H	15.0919	-13.8809	20.1325	0.0352	0.0362
2	H	16.6276	-13.0180	20.0467	0.0451	0.0462
3	H	17.0009	-14.5366	17.9313	0.0389	0.0400
4	H	15.7954	-15.5568	18.7164	0.0842	0.0853
5	H	18.4473	-14.8542	20.2099	0.1421	0.1432
6	H	17.2901	-18.3890	18.7306	0.0520	0.0531
7	H	15.8539	-17.4826	19.2064	0.0607	0.0618
8	H	16.6360	-18.5198	20.2743	0.0695	0.0706
9	C	1.7052	5.0366	20.0171	0.0327	0.0338
10	C	2.5005	3.9189	19.4383	-0.2017	-0.2006
11	N	2.1330	2.5878	19.5847	-0.1958	-0.1947
12	C	3.7791	3.9715	18.9697	-0.1289	-0.1278
13	C	3.1731	1.8615	19.2102	-0.0565	-0.0554
14	N	4.2091	2.6648	18.8349	0.0185	0.0196
15	H	19.7420	-17.4231	20.5996	0.0633	0.0644
16	H	18.4790	-17.9990	21.6875	0.0595	0.0606

17	H	0.6379	4.7971	20.0193	-0.0093	0.0462
18	H	1.8414	5.9519	19.4298	0.0844	0.0462
19	H	4.4177	4.8066	18.7288	0.2145	0.2156
20	H	3.2385	0.7877	19.2139	0.1901	0.1912
21	H	19.0363	-16.4097	21.7462	0.0438	0.0449
22	C	8.9253	3.5915	19.8306	0.1397	0.1408
23	C	7.9032	2.7638	19.1149	-0.0565	-0.0555
24	C	8.2584	1.6533	18.3216	-0.2832	-0.2822
25	C	6.5426	3.0546	19.2539	-0.2839	-0.2828
26	C	7.3024	0.8660	17.7217	-0.2492	-0.2481
27	C	5.5632	2.2892	18.6453	0.0752	0.0762
28	C	5.8940	1.1222	17.8509	0.2669	0.2680
29	O	5.0095	0.3853	17.3131	-0.5142	-0.5131
30	H	8.7263	4.6613	19.6959	0.0159	0.0170
31	H	9.9356	3.3733	19.4741	0.0114	0.0170
32	H	9.3105	1.4106	18.1889	0.2442	0.2453
33	H	6.2465	3.8911	19.8783	0.2195	0.2205
34	H	7.5943	0.0112	17.1170	0.1864	0.1875
35	H	7.6219	-1.6739	21.4141	0.0342	0.0353
36	C	-3.9015	1.7831	16.2314	0.1298	0.1308
37	C	-2.5712	1.8074	16.8908	-0.1190	-0.1179
38	N	-1.3792	1.9118	16.2100	-0.1152	-0.1141
39	C	-2.2079	1.7375	18.2074	-0.2345	-0.2335
40	C	-0.3650	1.9224	17.0959	-0.2200	-0.2189
41	N	-0.8383	1.8181	18.3290	-0.0926	-0.0915
42	H	-4.1527	0.7769	15.8744	0.0266	0.0257
43	H	-3.9218	2.4631	15.3730	0.0122	0.0257
44	H	-1.2717	1.9899	15.2023	0.3655	0.3665
45	H	-2.8452	1.6487	19.0705	0.2440	0.2451
46	H	0.6708	2.0184	16.8138	0.2306	0.2317
47	C	-4.8473	4.0392	21.4640	0.0533	0.0544
48	C	-3.4886	3.4587	21.6105	-0.1030	-0.1019
49	N	-3.1769	2.5046	22.5537	-0.0825	-0.0814
50	C	-2.3513	3.5927	20.8591	-0.2078	-0.2067
51	C	-1.9140	2.0896	22.3445	-0.1487	-0.1476
52	N	-1.3768	2.7226	21.3093	-0.2300	-0.2289
53	H	-4.8133	4.8994	20.7903	0.0625	0.0477
54	H	-5.2545	4.3669	22.4261	0.0228	0.0477
55	H	-3.7988	2.1687	23.2995	0.3363	0.3373
56	H	-2.1921	4.2314	20.0038	0.2140	0.2151
57	H	-1.4311	1.3352	22.9393	0.2075	0.2086

58	H	3.8308	-1.2935	25.7518	0.2184	0.2195
59	O	1.9694	-0.4511	21.5257	-0.3621	-0.3610
60	O	0.8883	0.5650	21.8126	-0.2053	-0.2042
61	H	5.8797	-1.9695	25.8732	0.2219	0.2230
62	H	8.0960	-1.0879	25.8925	0.1986	0.1997
63	H	8.1053	-0.8938	24.0480	0.2087	0.2098
64	H	5.5243	-2.9624	19.8722	0.1934	0.1945
65	H	4.2108	-3.5199	16.2748	0.1501	0.1512
66	C	18.7850	-17.1890	21.0420	-0.0417	-0.0407
67	C	17.6370	-15.4070	19.7580	-0.1812	-0.1801
68	H	2.2678	-3.3433	15.6797	0.0394	0.0404
69	H	0.8434	-4.0948	16.4115	0.0312	0.0323
70	H	0.9006	-2.3415	16.1811	0.0420	0.0431
71	H	-0.5438	-2.5978	18.0708	0.2077	0.2088
72	H	-4.1202	-1.5898	19.1771	0.1274	0.1284
73	H	-4.3550	-0.4799	21.1809	0.0361	0.0372
74	H	-3.8374	-0.7215	22.8268	0.0389	0.0399
75	C	-0.0504	-7.2186	24.2237	0.0790	0.0801
76	C	0.6855	-6.1386	23.5033	-0.1353	-0.1342
77	N	1.6740	-6.3813	22.5761	-0.0062	-0.0051
78	C	0.5171	-4.7753	23.4703	-0.3402	-0.3391
79	C	2.0667	-5.2166	22.0289	-0.2785	-0.2774
80	N	1.3804	-4.2092	22.5483	-0.1384	-0.1374
81	H	-0.5938	-7.8401	23.5005	0.0346	0.0444
82	H	-0.7849	-6.7658	24.8951	0.0698	0.0444
83	H	2.0554	-7.2912	22.3316	0.3357	0.3368
84	H	-0.1680	-4.1808	24.0514	0.2395	0.2406
85	H	2.8329	-5.1513	21.2770	0.2045	0.2055
86	H	-5.1647	-2.8448	21.0769	-0.0764	-0.0754
87	C	6.8030	-7.9630	15.0190	-0.0407	-0.0396
88	H	1.4274	1.1326	22.4077	0.2502	0.2513
89	H	7.9427	-2.8246	22.7205	0.0457	0.0468
90	H	-1.9426	1.1189	25.6722	-0.0863	-0.0852
91	H	-0.7758	1.2841	26.9937	-0.0604	-0.0593
92	H	-1.1161	-1.1334	27.5814	0.0573	0.0584
93	H	-2.2045	-1.3205	26.2201	0.0227	0.0237
94	H	2.6225	-1.3856	27.6150	0.0147	0.0158
95	H	2.1009	0.2953	27.4412	0.0378	0.0389
96	H	1.0250	-0.8921	28.1904	0.0220	0.0230
97	H	-2.2682	-1.2859	24.0422	0.1871	0.1882
98	Fe	1.6141	-2.1268	21.9575	0.4395	0.4405

99	C	-1.3346	-1.4745	23.5349	-0.0272	-0.0261
100	C	-0.0124	-2.5249	19.0104	-0.1332	-0.1321
101	C	4.5890	-2.8159	20.3851	-0.0531	-0.0520
102	C	3.3003	-1.5397	24.8408	-0.1930	-0.1919
103	N	1.0789	-1.6410	23.8254	0.1781	0.1792
104	C	-0.1873	-1.4132	24.2971	-0.1301	-0.1290
105	C	-0.1482	-1.0686	25.7005	-0.2147	-0.2136
106	C	1.1727	-1.0627	26.0609	-0.0519	-0.0508
107	C	1.9198	-1.4256	24.8777	-0.1759	-0.1748
108	C	1.7602	-0.7480	27.3938	0.1017	0.1028
109	O	-2.8389	-2.3055	17.7965	-0.4954	-0.4944
110	C	-1.3252	-0.7663	26.5701	0.0598	0.0609
111	C	-1.6614	0.7362	26.6566	0.2609	0.2620
112	C	-2.7853	0.9669	27.6592	0.4787	0.4798
113	O	-2.4948	0.9059	28.8815	-0.7587	-0.7576
114	O	-3.9714	1.1442	27.2059	-0.7640	-0.7629
115	N	2.1724	-2.6752	20.0848	0.0876	0.0887
116	C	1.3481	-2.7453	18.9983	-0.1616	-0.1605
117	C	2.1030	-3.0591	17.8035	-0.0964	-0.0953
118	C	3.4106	-3.1704	18.1919	-0.0752	-0.0741
119	C	3.4394	-2.8982	19.6173	0.0380	0.0390
120	C	1.5026	-3.2181	16.4479	0.0408	0.0419
121	N	3.5648	-2.2357	22.5255	0.0598	0.0609
122	C	4.6471	-2.4888	21.7285	-0.2038	-0.2027
123	C	5.8788	-2.3386	22.4712	-0.0753	-0.0742
124	C	5.5164	-1.9723	23.7450	-0.0284	-0.0274
125	C	4.0628	-1.9142	23.7524	-0.0777	-0.0766
126	C	7.2416	-2.5720	21.9207	0.0711	0.0722
127	C	6.3393	-1.7205	24.9167	-0.2565	-0.2555
128	C	7.5808	-1.2139	24.9432	-0.3863	-0.3853
129	N	-0.3164	-2.0543	21.3867	0.0920	0.0931
130	C	-0.7816	-2.2003	20.1159	-0.1840	-0.1829
131	C	-2.2022	-1.9401	20.0820	0.0182	0.0193
132	C	-2.5856	-1.6360	21.3779	-0.1942	-0.1932
133	C	-1.3946	-1.7272	22.1719	-0.1071	-0.1060
134	C	-3.1014	-1.9481	18.9466	0.2491	0.2501
135	C	-3.9369	-1.2284	21.8667	0.0210	0.0221
136	C	-4.9505	-2.3656	22.0367	0.2532	0.2543
137	C	-6.2418	-1.8845	22.6968	0.5372	0.5383
138	O	-7.3503	-2.2440	22.1689	-0.7923	-0.7913
139	O	-6.1501	-1.1874	23.7390	-0.7474	-0.7464

140	C	12.4300	-8.1250	17.4050	-0.0016	-0.0005
141	C	16.5530	-14.8040	18.8770	-0.0331	-0.0320
142	C	16.8450	-17.7870	19.5090	-0.0677	-0.0666
143	C	17.7330	-16.6840	20.0880	-0.2765	-0.2754
144	Cu	0.2004	2.0169	20.0962	0.5441	0.5452
145	H	8.8911	3.3924	20.9134	0.0204	0.0000
146	H	-4.6733	2.1049	16.9383	0.0350	0.0000
147	H	-5.5315	3.2947	21.0321	0.0545	0.0000
148	H	7.2455	-3.3914	21.1959	0.0172	0.0182
149	H	-4.5255	-3.1394	22.6939	-0.0567	-0.0557
150	H	0.5853	-7.8951	24.8206	0.0257	0.0000
151	C	4.5067	-3.4170	17.2279	0.0169	0.0180
152	O	5.3603	-2.3076	17.2075	-0.4783	-0.4772
153	C	5.1040	-4.8050	17.5250	0.1115	0.1125
154	C	6.1600	-5.2870	16.4770	-0.0843	-0.0832
155	C	7.1680	-6.3090	16.8880	-0.3044	-0.3033
156	C	7.3270	-7.5380	16.3810	-0.1688	-0.1677
157	C	8.1390	-8.6170	17.0680	0.0037	0.0048
158	C	9.5840	-8.6600	16.4480	-0.0771	-0.0760
159	C	10.4260	-9.6870	17.2000	-0.2850	-0.2839
160	C	11.6400	-9.4220	17.7130	-0.2017	-0.2007
161	C	12.2440	-10.5320	18.5670	-0.0420	-0.0409
162	C	13.2020	-11.3920	17.7400	-0.0767	-0.0756
163	C	13.9360	-12.4900	18.4300	-0.2284	-0.2273
164	C	15.2740	-12.5690	18.4610	-0.2221	-0.2211
165	C	15.8830	-13.5520	19.4750	-0.0339	-0.0329
166	C	16.2360	-11.6990	17.6670	-0.0435	-0.0424
167	H	5.5554	-2.0706	16.2979	0.3859	0.3869
168	H	5.5759	-4.7629	18.4956	-0.0259	-0.0249
169	H	4.2928	-5.5175	17.5497	-0.0134	-0.0123
170	H	6.7264	-4.4282	16.1483	0.0384	0.0395
171	H	5.6321	-5.7132	15.6367	0.0880	0.0891
172	H	7.8914	-6.1326	17.6702	0.1941	0.1952
173	H	7.4692	-7.7919	14.1864	0.0418	0.0429
174	H	5.8137	-7.6108	14.7668	0.0547	0.0558
175	H	6.6789	-9.0357	14.9997	0.0547	0.0558
176	H	7.6436	-9.5662	16.9267	0.0687	0.0697
177	H	8.1919	-8.3920	18.1230	0.0102	0.0112
178	H	10.0542	-7.6916	16.5344	0.1051	0.1061
179	H	9.5333	-8.9440	15.4072	0.0755	0.0766
180	H	10.1318	-10.7088	17.3890	0.1909	0.1920

181	H	13.4301	-8.0722	17.8092	0.0692	0.0702
182	H	11.9129	-7.1974	17.6015	0.0403	0.0414
183	H	12.6135	-8.0589	16.3428	0.0310	0.0321
184	H	11.4492	-11.1542	18.9510	0.0697	0.0708
185	H	12.7845	-10.0894	19.3906	0.0288	0.0299
186	H	13.9508	-10.7400	17.3150	0.1172	0.1183
187	H	12.6332	-11.8527	16.9459	0.0377	0.0387
188	H	13.4548	-13.3019	18.9550	0.1808	0.1819
189	H	17.2155	-11.5657	18.1020	0.0878	0.0888
190	H	15.8495	-10.7416	17.3500	0.0395	0.0406
191	H	16.4637	-12.1795	16.7269	0.0564	0.0575
192	H	1.8223	5.4178	21.0208	0.0604	0.0000

Table S5. DNC cluster model for charge derivation with protonated $PRAa_3$. The hydrogen link atoms have a zero final charge (atom number 145, 146, 147, 180, 192)

		Coordinates			ESP charge	Charge-derived
1	H	15.0919	-13.8809	20.1325	0.03464	0.0357
2	H	16.6276	-13.0180	20.0467	0.04487	0.0460
3	H	17.0009	-14.5366	17.9313	0.03890	0.0400
4	H	15.7954	-15.5568	18.7164	0.08353	0.0846
5	H	18.4473	-14.8542	20.2099	0.14199	0.1431
6	H	17.2901	-18.3890	18.7306	0.05167	0.0528
7	H	15.8539	-17.4826	19.2064	0.06116	0.0623
8	H	16.6360	-18.5198	20.2743	0.07002	0.0711
9	C	1.7052	5.0366	20.0171	0.02779	0.0289
10	C	2.5005	3.9189	19.4383	-0.20565	-0.2045
11	N	2.1330	2.5878	19.5847	-0.18695	-0.1858
12	C	3.7791	3.9715	18.9697	-0.12277	-0.1217
13	C	3.1731	1.8615	19.2102	-0.06442	-0.0633
14	N	4.2091	2.6648	18.8349	0.01935	0.0205
15	H	19.7420	-17.4231	20.5996	0.06167	0.0628
16	H	18.4790	-17.9990	21.6875	0.05934	0.0604
17	H	0.6379	4.7971	20.0193	-0.00805	0.0477
18	H	1.8414	5.9519	19.4298	0.08630	0.0477
19	H	4.4177	4.8066	18.7288	0.21341	0.2145
20	H	3.2385	0.7877	19.2139	0.19226	0.1934
21	H	19.0363	-16.4097	21.7462	0.04302	0.0441
22	C	8.9253	3.5915	19.8306	0.14331	0.1444
23	C	7.9032	2.7638	19.1149	-0.05221	-0.0511
24	C	8.2584	1.6533	18.3216	-0.28197	-0.2809
25	C	6.5426	3.0546	19.2539	-0.28260	-0.2815

26	C	7.3024	0.8660	17.7217	-0.24689	-0.2458
27	C	5.5632	2.2892	18.6453	0.07542	0.0765
28	C	5.8940	1.1222	17.8509	0.26980	0.2709
29	O	5.0095	0.3853	17.3131	-0.50944	-0.5083
30	H	8.7263	4.6613	19.6959	0.01528	0.0164
31	H	9.9356	3.3733	19.4741	0.01016	0.0164
32	H	9.3105	1.4106	18.1889	0.24483	0.2459
33	H	6.2465	3.8911	19.8783	0.21940	0.2205
34	H	7.5943	0.0112	17.1170	0.18729	0.1884
35	H	7.6219	-1.6739	21.4141	0.03636	0.0375
36	C	-3.9015	1.7831	16.2314	0.11867	0.1198
37	C	-2.5712	1.8074	16.8908	-0.12455	-0.1234
38	N	-1.3792	1.9118	16.2100	-0.11517	-0.1141
39	C	-2.2079	1.7375	18.2074	-0.21990	-0.2188
40	C	-0.3650	1.9224	17.0959	-0.21124	-0.2101
41	N	-0.8383	1.8181	18.3290	-0.10582	-0.1047
42	H	-4.1527	0.7769	15.8744	0.02983	0.0296
43	H	-3.9218	2.4631	15.3730	0.01724	0.0296
44	H	-1.2717	1.9899	15.2023	0.36576	0.3669
45	H	-2.8452	1.6487	19.0705	0.23510	0.2362
46	H	0.6708	2.0184	16.8138	0.22927	0.2304
47	C	-4.8473	4.0392	21.4640	0.05825	0.0594
48	C	-3.4886	3.4587	21.6105	-0.10907	-0.1080
49	N	-3.1769	2.5046	22.5537	-0.07763	-0.0765
50	C	-2.3513	3.5927	20.8591	-0.20215	-0.2010
51	C	-1.9140	2.0896	22.3445	-0.15477	-0.1537
52	N	-1.3768	2.7226	21.3093	-0.22662	-0.2255
53	H	-4.8133	4.8994	20.7903	0.06117	0.0482
54	H	-5.2545	4.3669	22.4261	0.02591	0.0482
55	H	-3.7988	2.1687	23.2995	0.32753	0.3286
56	H	-2.1921	4.2314	20.0038	0.21467	0.2158
57	H	-1.4311	1.3352	22.9393	0.20892	0.2100
58	H	3.8308	-1.2935	25.7518	0.21975	0.2209
59	O	1.9694	-0.4511	21.5257	-0.36285	-0.3617
60	O	0.8883	0.5650	21.8126	-0.20913	-0.2080
61	H	5.8797	-1.9695	25.8732	0.22303	0.2241
62	H	8.0960	-1.0879	25.8925	0.19806	0.1992
63	H	8.1053	-0.8938	24.0480	0.20782	0.2089
64	H	5.5243	-2.9624	19.8722	0.19340	0.1945
65	H	4.2108	-3.5199	16.2748	0.14869	0.1498
66	C	18.7850	-17.1890	21.0420	-0.03964	-0.0385

67	C	17.6370	-15.4070	19.7580	-0.18148	-0.1804
68	H	2.2678	-3.3433	15.6797	0.04014	0.0412
69	H	0.8434	-4.0948	16.4115	0.03248	0.0336
70	H	0.9006	-2.3415	16.1811	0.04165	0.0428
71	H	-0.5438	-2.5978	18.0708	0.20736	0.2085
72	H	-4.1202	-1.5898	19.1771	0.12690	0.1280
73	H	-4.3550	-0.4799	21.1809	0.04307	0.0442
74	H	-3.8374	-0.7215	22.8268	0.03348	0.0346
75	C	-0.0504	-7.2186	24.2237	0.07265	0.0738
76	C	0.6855	-6.1386	23.5033	-0.13931	-0.1382
77	N	1.6740	-6.3813	22.5761	-0.00299	-0.0019
78	C	0.5171	-4.7753	23.4703	-0.33406	-0.3330
79	C	2.0667	-5.2166	22.0289	-0.27973	-0.2786
80	N	1.3804	-4.2092	22.5483	-0.14100	-0.1399
81	H	-0.5938	-7.8401	23.5005	0.03766	0.0471
82	H	-0.7849	-6.7658	24.8951	0.07105	0.0471
83	H	2.0554	-7.2912	22.3316	0.33550	0.3366
84	H	-0.1680	-4.1808	24.0514	0.23665	0.2378
85	H	2.8329	-5.1513	21.2770	0.20605	0.2072
86	H	-5.1647	-2.8448	21.0769	-0.00939	-0.0083
87	C	6.8030	-7.9630	15.0190	-0.04034	-0.0392
88	H	1.4274	1.1326	22.4077	0.25145	0.2526
89	H	7.9427	-2.8246	22.7205	0.04831	0.0494
90	H	-1.9426	1.1189	25.6722	-0.07844	-0.0773
91	H	-0.7758	1.2841	26.9937	-0.05003	-0.0489
92	H	-1.1161	-1.1334	27.5814	0.06431	0.0654
93	H	-2.2045	-1.3205	26.2201	0.02458	0.0257
94	H	2.6225	-1.3856	27.6150	0.01720	0.0183
95	H	2.1009	0.2953	27.4412	0.03969	0.0408
96	H	1.0250	-0.8921	28.1904	0.02342	0.0245
97	H	-2.2682	-1.2859	24.0422	0.19664	0.1977
98	Fe	1.6141	-2.1268	21.9575	0.44011	0.4412
99	C	-1.3346	-1.4745	23.5349	-0.03721	-0.0361
100	C	-0.0124	-2.5249	19.0104	-0.13091	-0.1298
101	C	4.5890	-2.8159	20.3851	-0.05041	-0.0493
102	C	3.3003	-1.5397	24.8408	-0.19502	-0.1939
103	N	1.0789	-1.6410	23.8254	0.18076	0.1819
104	C	-0.1873	-1.4132	24.2971	-0.12683	-0.1257
105	C	-0.1482	-1.0686	25.7005	-0.21019	-0.2091
106	C	1.1727	-1.0627	26.0609	-0.05144	-0.0503
107	C	1.9198	-1.4256	24.8777	-0.17431	-0.1732

108	C	1.7602	-0.7480	27.3938	0.09725	0.0984
109	O	-2.8389	-2.3055	17.7965	-0.48943	-0.4883
110	C	-1.3252	-0.7663	26.5701	0.04694	0.0480
111	C	-1.6614	0.7362	26.6566	0.23613	0.2372
112	C	-2.7853	0.9669	27.6592	0.47523	0.4763
113	O	-2.4948	0.9059	28.8815	-0.75280	-0.7517
114	O	-3.9714	1.1442	27.2059	-0.75699	-0.7559
115	N	2.1724	-2.6752	20.0848	0.08756	0.0887
116	C	1.3481	-2.7453	18.9983	-0.16167	-0.1606
117	C	2.1030	-3.0591	17.8035	-0.09793	-0.0968
118	C	3.4106	-3.1704	18.1919	-0.07031	-0.0692
119	C	3.4394	-2.8982	19.6173	0.03500	0.0361
120	C	1.5026	-3.2181	16.4479	0.04137	0.0425
121	N	3.5648	-2.2357	22.5255	0.05137	0.0525
122	C	4.6471	-2.4888	21.7285	-0.19556	-0.1945
123	C	5.8788	-2.3386	22.4712	-0.07700	-0.0759
124	C	5.5164	-1.9723	23.7450	-0.02498	-0.0239
125	C	4.0628	-1.9142	23.7524	-0.06980	-0.0687
126	C	7.2416	-2.5720	21.9207	0.06528	0.0664
127	C	6.3393	-1.7205	24.9167	-0.26082	-0.2597
128	C	7.5808	-1.2139	24.9432	-0.38052	-0.3794
129	N	-0.3164	-2.0543	21.3867	0.09934	0.1004
130	C	-0.7816	-2.2003	20.1159	-0.18319	-0.1821
131	C	-2.2022	-1.9401	20.0820	0.00919	0.0103
132	C	-2.5856	-1.6360	21.3779	-0.16610	-0.1650
133	C	-1.3946	-1.7272	22.1719	-0.13020	-0.1291
134	C	-3.1014	-1.9481	18.9466	0.25222	0.2533
135	C	-3.9369	-1.2284	21.8667	0.03232	0.0334
136	C	-4.9505	-2.3656	22.0367	0.17618	0.1773
137	C	-6.2418	-1.8845	22.6968	0.46496	0.4661
138	O	-7.3503	-2.2440	22.1689	-0.62648	-0.6254
139	O	-6.1501	-1.1874	23.7390	-0.42985	-0.4287
140	C	12.4300	-8.1250	17.4050	-0.00022	0.0009
141	C	16.5530	-14.8040	18.8770	-0.03241	-0.0313
142	C	16.8450	-17.7870	19.5090	-0.06797	-0.0669
143	C	17.7330	-16.6840	20.0880	-0.27605	-0.2749
144	Cu	0.2004	2.0169	20.0962	0.54198	0.5431
145	H	8.8911	3.3924	20.9134	0.02044	0.0000
146	H	-4.6733	2.1049	16.9383	0.03827	0.0000
147	H	-5.5315	3.2947	21.0321	0.05410	0.0000
148	H	7.2455	-3.3914	21.1959	0.01941	0.0205

149	H	-4.5255	-3.1394	22.6939	0.00640	0.0075
150	H	0.5853	-7.8951	24.8206	0.02921	0.0000
151	C	4.5067	-3.4170	17.2279	0.01985	0.0210
152	O	5.3603	-2.3076	17.2075	-0.48081	-0.4797
153	C	5.1040	-4.8050	17.5250	0.11221	0.1133
154	C	6.1600	-5.2870	16.4770	-0.08346	-0.0824
155	C	7.1680	-6.3090	16.8880	-0.30430	-0.3032
156	C	7.3270	-7.5380	16.3810	-0.16953	-0.1684
157	C	8.1390	-8.6170	17.0680	0.00315	0.0043
158	C	9.5840	-8.6600	16.4480	-0.07652	-0.0754
159	C	10.4260	-9.6870	17.2000	-0.28616	-0.2851
160	C	11.6400	-9.4220	17.7130	-0.20108	-0.2000
161	C	12.2440	-10.5320	18.5670	-0.04289	-0.0418
162	C	13.2020	-11.3920	17.7400	-0.07755	-0.0764
163	C	13.9360	-12.4900	18.4300	-0.22965	-0.2285
164	C	15.2740	-12.5690	18.4610	-0.22084	-0.2197
165	C	15.8830	-13.5520	19.4750	-0.03313	-0.0320
166	C	16.2360	-11.6990	17.6670	-0.04201	-0.0409
167	H	5.5554	-2.0706	16.2979	0.38714	0.3882
168	H	5.5759	-4.7629	18.4956	-0.02624	-0.0251
169	H	4.2928	-5.5175	17.5497	-0.01427	-0.0132
170	H	6.7264	-4.4282	16.1483	0.03867	0.0398
171	H	5.6321	-5.7132	15.6367	0.08775	0.0889
172	H	7.8914	-6.1326	17.6702	0.19408	0.1952
173	H	7.4692	-7.7919	14.1864	0.04150	0.0426
174	H	5.8137	-7.6108	14.7668	0.05479	0.0559
175	H	6.6789	-9.0357	14.9997	0.05525	0.0564
176	H	7.6436	-9.5662	16.9267	0.06910	0.0702
177	H	8.1919	-8.3920	18.1230	0.01011	0.0112
178	H	10.0542	-7.6916	16.5344	0.10552	0.1066
179	H	9.5333	-8.9440	15.4072	0.07550	0.0766
180	H	10.1318	-10.7088	17.3890	0.19135	0.1925
181	H	13.4301	-8.0722	17.8092	0.06867	0.0698
182	H	11.9129	-7.1974	17.6015	0.03978	0.0409
183	H	12.6135	-8.0589	16.3428	0.03024	0.0313
184	H	11.4492	-11.1542	18.9510	0.07013	0.0712
185	H	12.7845	-10.0894	19.3906	0.02901	0.0301
186	H	13.9508	-10.7400	17.3150	0.11841	0.1195
187	H	12.6332	-11.8527	16.9459	0.03795	0.0391
188	H	13.4548	-13.3019	18.9550	0.18142	0.1825
189	H	17.2155	-11.5657	18.1020	0.08668	0.0878

190	H	15.8495	-10.7416	17.3500	0.03878	0.0399
191	H	16.4637	-12.1795	16.7269	0.05547	0.0566
192	H	1.8223	5.4178	21.0208	0.06160	0.0000
193	H	-7.1048	-0.9224	24.0518	0.46111	0.4622

Table S6. Charges for backbone atoms of His and Tyr residues (His384, His283, His282, His233, Tyr237) coordinating to the DNC metal atoms for deprotonated $PRAa_3$.

His	Final charge	Tyr	Final charge
N	-0.4146	N	-0.4146
H	0.2730	H	0.2730
C	0.5984	C	0.5984
O	-0.5668	O	-0.5668
CA	0.0199	CA	-0.0003
HA	0.0892	HA	0.0887

Table S7. Charges for backbone atoms of His and Tyr residues (His384, His283, His282, His233, Tyr237) coordinating to the DNC metal atoms for protonated $PRAa_3$.

His	Final charge	Tyr	Final charge
N	-0.4146	N	-0.4146
H	0.2730	H	0.2730
C	0.5984	C	0.5984
O	-0.5668	O	-0.5668
CA	0.0199	CA	-0.0003
HA	0.0892	HA	0.0887

Table S8. Charges and coordinates for the Cu_A cluster model. Hydrogen link atoms are number 13, 17, 26, 32.

		Coordinates			ESP charge	final charge
1	C	-18.1522	3.4218	30.9910	-0.5867	-0.5705
2	C	-18.4372	4.0988	32.2910	0.2846	0.3009
3	N	-18.0862	3.5418	33.4700	-0.3921	-0.3758
4	C	-19.1292	5.2208	32.6120	-0.2926	-0.2764
5	C	-18.5032	4.2728	34.4970	0.1060	0.1223
6	N	-19.0922	5.3598	33.9740	-0.2500	-0.2337
7	C	-17.3262	-1.2892	32.7420	-0.1664	-0.1502
8	S	-16.2912	0.2128	32.4560	-0.2338	-0.2176
9	H	-19.6192	5.8816	31.9122	0.2687	0.2850
10	H	-18.3871	4.0325	35.5435	0.1858	0.2020
11	H	-16.8843	-2.2592	32.5680	0.1287	0.1331

12	H	-18.3448	-1.2880	32.3830	0.1621	0.1331
13	H	-14.2758	4.2522	35.3758	0.1973	0.0000
14	H	-15.9522	4.7541	35.1563	0.1658	0.1615
15	H	-15.1644	3.8174	34.0830	0.0726	0.1615
16	H	-13.0305	-0.4114	38.3905	0.2054	0.1983
17	H	-14.6802	-0.6694	37.8227	0.1719	0.0000
18	C	-15.2762	3.9118	35.1530	-0.3300	-0.3138
19	S	-15.8732	2.2378	35.8850	-0.1755	-0.1593
20	C	-13.6912	-0.3412	37.5390	-0.5037	-0.4875
21	C	-13.1622	-0.9202	36.2650	0.2216	0.2378
22	N	-13.7682	-0.7712	34.9990	-0.2755	-0.2593
23	C	-12.0592	-1.6832	36.0970	-0.3347	-0.3185
24	C	-12.9812	-1.3902	34.1110	-0.0770	-0.0608
25	N	-11.9722	-1.9772	34.7520	-0.1515	-0.1353
26	H	-17.5280	-1.3916	33.7980	0.0597	0.0000
27	H	-13.8185	0.7251	37.4243	0.1688	0.1983
28	H	-11.3836	-1.9951	36.8797	0.2965	0.3127
29	H	-13.1364	-1.4126	33.0424	0.2594	0.2756
30	Cu	-15.0792	0.5298	34.4350	0.6429	0.6591
31	Cu	-17.3092	1.6468	34.0310	0.7492	0.7654
32	H	-17.1981	3.6589	30.5440	0.2001	0.0000
33	H	-18.9293	3.5034	30.2454	0.2147	0.2103
34	H	-18.0835	2.3555	31.1479	0.1674	0.2103
35	H	-19.5469	6.0861	34.5087	0.4225	0.4388
36	H	-11.2365	-2.5180	34.3202	0.4178	0.4340

Table S9. Charges for backbone atoms of His and Cys residues (His114^{II}, His157^{II}, Cys149^{II}, and Cys153^{II}) coordinating to the Cu_A cluster model.

His	Final charge	Cys	Final charge
N	-0.3990	N	-0.3990
H	0.2886	H	0.2886
C	0.6140	C	0.6140
O	-0.5512	O	-0.5512
CA	-0.0414	CA	-0.0184
HA	0.1527	HA	0.0675

Table S10. Charges and coordinates for the heme b cluster model. Hydrogen link atoms are number 96,97.

		Coordinate			ESP Charge	Final Charge
1	C	0.9340	-1.0870	39.2210	-0.0383	-0.0373

2	H	0.8140	-1.4670	40.2360	0.0587	0.0585
3	H	-0.0480	-0.9390	38.7710	0.0626	0.0585
4	C	1.6030	-2.1620	38.4400	-0.0487	-0.0477
5	N	2.5720	-2.9730	39.0090	-0.0535	-0.0525
6	H	2.9370	-2.8770	39.9460	0.3700	0.3710
7	C	2.9390	-3.8990	38.1110	-0.3042	-0.3032
8	H	3.7030	-4.6410	38.3440	0.2730	0.2740
9	N	2.2270	-3.7030	37.0160	-0.1621	-0.1611
10	C	1.3550	-2.6620	37.1780	-0.2284	-0.2274
11	H	0.6630	-2.4010	36.3770	0.2287	0.2297
12	C	3.2440	-8.0580	30.7870	0.0416	0.0426
13	H	2.4380	-8.6640	30.3730	0.0364	0.0349
14	H	3.9520	-8.7030	31.3080	0.0329	0.0349
15	C	2.6240	-7.1720	31.8280	0.0398	0.0408
16	N	1.3040	-6.7990	31.8030	-0.1891	-0.1881
17	H	0.6680	-7.0710	31.0670	0.3910	0.3920
18	C	1.0040	-6.0550	32.8530	-0.1911	-0.1901
19	H	0.0010	-5.6600	33.0120	0.2181	0.2191
20	N	2.1010	-5.9340	33.5650	-0.0857	-0.0847
21	C	3.1400	-6.6420	32.9560	-0.3022	-0.3012
22	H	4.1250	-6.6710	33.4210	0.2001	0.2011
23	Fe	2.1890	-4.8630	35.2990	0.4212	0.4212
24	N	0.4910	-5.6790	35.6350	0.1963	0.1973
25	C	-0.8450	-5.1970	35.3160	-0.3499	-0.3489
26	C	-1.8010	-6.2940	35.3980	0.2177	0.2187
27	C	-3.2170	-6.2310	35.0040	0.1095	0.1105
28	H	-3.5850	-7.2440	34.8400	0.0259	0.0269
29	H	-3.3040	-5.6590	34.0800	-0.0213	-0.0203
30	C	-4.0620	-5.5770	36.0540	-0.0743	-0.0733
31	H	-3.4960	-4.7980	36.5650	-0.0004	0.0006
32	H	-4.1970	-6.4270	36.7230	0.0575	0.0585
33	C	-5.3850	-5.0460	35.6580	0.5908	0.5918
34	O	-6.3060	-4.7340	36.4620	-0.6547	-0.6537
35	O	-5.7620	-5.0000	34.2800	-0.6863	-0.6853
36	C	-1.2010	-7.3010	36.0470	-0.0887	-0.0877
37	C	-1.7740	-8.6880	36.4110	-0.0608	-0.0598
38	H	-2.0620	-9.2130	35.5000	0.0693	0.0703
39	H	-1.0170	-9.2670	36.9400	0.0271	0.0281
40	H	-2.6480	-8.5640	37.0500	0.0862	0.0872
41	C	0.1470	-6.9350	36.2410	-0.2117	-0.2107
42	C	1.0730	-7.7580	36.8300	-0.1624	-0.1614

43	H	0.8840	-8.7230	37.2780	0.3431	0.3441
44	C	2.4860	-7.3740	36.8720	-0.1868	-0.1858
45	N	3.1100	-6.2990	36.3710	0.1403	0.1413
46	C	3.4200	-8.2000	37.4900	-0.0894	-0.0884
47	C	3.1340	-9.4510	38.3370	0.0456	0.0466
48	H	2.6000	-10.1850	37.7330	-0.0156	-0.0146
49	H	4.0750	-9.8800	38.6820	0.0437	0.0447
50	H	2.5240	-9.1760	39.1970	0.0342	0.0352
51	C	4.6550	-7.5910	37.4240	0.0338	0.0348
52	C	5.9280	-7.8980	37.8100	-0.2082	-0.2072
53	H	6.7450	-7.7400	37.1220	0.1888	0.1898
54	C	6.1960	-8.4500	39.1760	-0.4311	-0.4301
55	H	5.3790	-8.6080	39.8640	0.2155	0.2165
56	H	7.2060	-8.6900	39.4730	0.1961	0.1971
57	C	4.4050	-6.3990	36.5980	-0.0152	-0.0142
58	C	5.4660	-5.7080	36.1880	-0.2586	-0.2576
59	H	6.3010	-5.4300	36.8140	0.2511	0.2520
60	C	5.2520	-4.6650	35.1230	0.0518	0.0528
61	N	3.9500	-4.2580	34.8740	0.1297	0.1307
62	C	6.1400	-3.7540	34.8090	0.0106	0.0116
63	C	7.6230	-3.9210	34.9700	-0.1511	-0.1501
64	H	7.9610	-4.7680	34.3730	0.0981	0.0991
65	H	8.1300	-3.0160	34.6340	0.1028	0.1038
66	H	7.8570	-4.1000	36.0190	0.0479	0.0489
67	C	5.5750	-2.9280	33.7950	0.0036	0.0046
68	C	6.3250	-1.7880	33.1430	-0.0166	-0.0156
69	H	5.6060	-1.0490	32.8220	0.1283	0.1293
70	C	7.6350	-1.5870	32.9290	-0.5394	-0.5384
71	H	8.3540	-2.3260	33.2500	0.2601	0.2611
72	H	7.9700	-0.6860	32.4360	0.2572	0.2581
73	C	4.3060	-3.1120	34.0770	-0.2437	-0.2427
74	C	3.2700	-2.2920	33.4430	-0.2148	-0.2138
75	H	3.4260	-1.4390	32.8000	0.3112	0.3122
76	C	1.9600	-2.4710	33.6580	-0.0985	-0.0975
77	N	1.2270	-3.3930	34.2810	0.1245	0.1255
78	C	0.9420	-1.5050	33.2340	-0.0871	-0.0861
79	C	1.2530	-0.3830	32.2350	-0.0005	0.0005
80	H	1.5840	-0.8170	31.2920	0.0088	0.0098
81	H	0.3560	0.2120	32.0650	0.0795	0.0805
82	H	2.0400	0.2540	32.6370	0.0068	0.0078
83	C	-0.2980	-1.9000	33.6120	-0.1975	-0.1965

84	C	-0.0930	-3.1810	34.2820	0.0226	0.0236
85	C	-1.1870	-3.8610	34.7050	-0.1226	-0.1216
86	H	-2.1730	-3.4460	34.5540	0.3053	0.3063
87	C	-1.6580	-1.4520	33.1780	-0.0301	-0.0291
88	H	-2.3980	-2.2470	33.2670	0.0068	0.0078
89	H	-1.6560	-1.0700	32.1570	0.0648	0.0658
90	C	-2.0490	-0.3000	34.1390	0.2817	0.2827
91	H	-1.3490	0.5310	34.0530	-0.0303	-0.0293
92	H	-2.0450	-0.6730	35.1630	-0.0446	-0.0436
93	C	-3.4230	0.1660	33.7830	0.4773	0.4783
94	O	-3.9190	1.2320	34.1700	-0.6035	-0.6025
95	O	-4.2460	-0.5330	33.0330	-0.6108	-0.6098
96	H	1.5490	-0.2190	39.3350	0.0511	0.0000
97	H	3.6720	-7.4750	29.9990	0.0325	0.0000

Table S11. Charges for backbone atoms of His residues (His72, His386) coordinating to the heme b cluster model.

His	Final charge
N	-0.4147
H	0.2729
C	0.5983
O	-0.5669
CA	0.0198
HA	0.0891

4. Stability of MD trajectories

In order to check the stability of our trajectories after equilibration, we have investigated the RMSD deviation of C_α backbone atoms and the area per lipid. We can clearly see that the simulations are stable with an RMSD of 1 Å to 1.5 Å throughout the simulations (Figure S1) and a stable area per lipid of approximately 60 $Å^2$ (Figure S2).

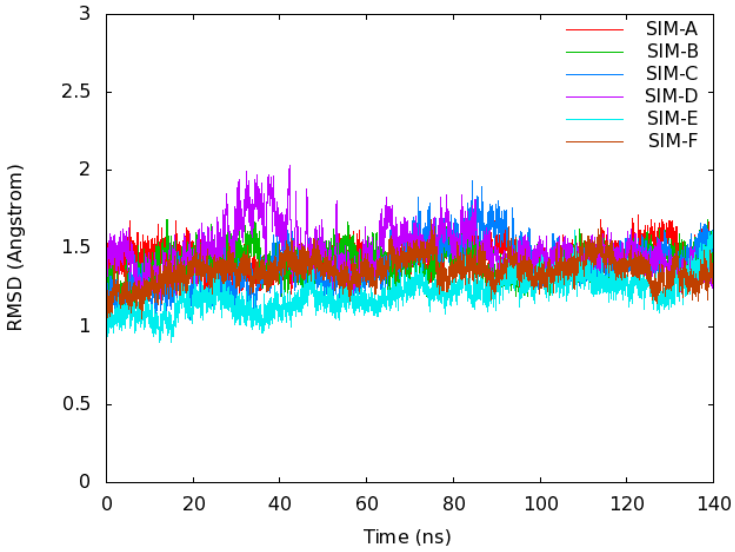

Figure S1. The RMSD of CcO protein Cα-backbone atoms for all simulations with protonation states considered in this work. The crystal structure serves as the reference structure.

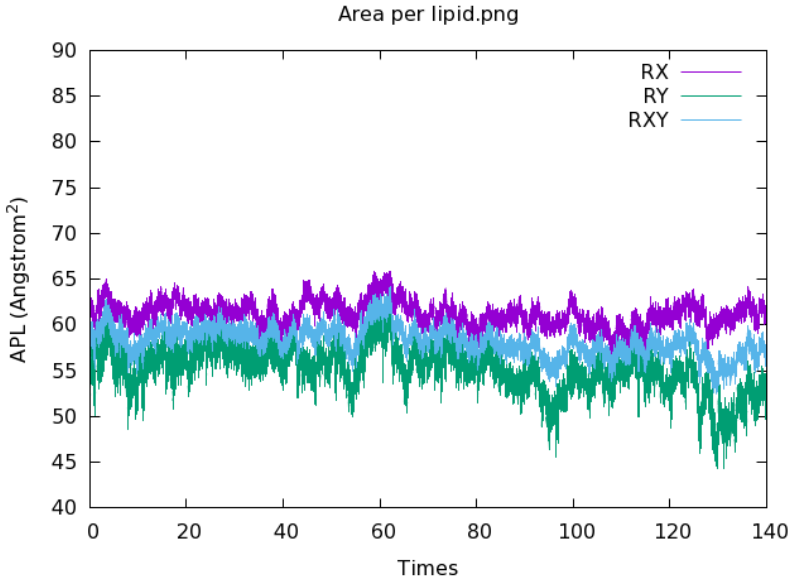

Figure S2. The area per lipid for simulation A. Results for simulations of all the other protonation states are equivalent. For details how the area per lipid was calculated, see below.

The area per lipid (A_{lipid}) was obtained from the lateral area A_{system} of the simulation box, the approximate lateral area occupied by the protein, $A_{protein}$ and the number of lipids in each leaflet (n_{lipid}):

$$A_{lipid} = \frac{A_{system} - A_{protein}}{n_{lipid}}$$

The lateral area of the protein was approximately circle shaped and $A_{protein}$ was therefore calculated as the area of a circle:

$$A_{protein} = \pi R^2$$

R is the radius of the protein. Because the protein at the lipid:water interface was not perfectly circle shaped, slightly different diameters were measured in the X and Y dimensions. Three different $A_{protein}$ values, and thereby three different area per lipid values (Figure S1), were calculated using the radius of the protein in the X dimension (RX), its radius in the Y dimension (RY) and the average of the two values (RXY).

5. Distance analysis for the salt bridges Arg225–Asp287 and Arg225–Glu126^{II}

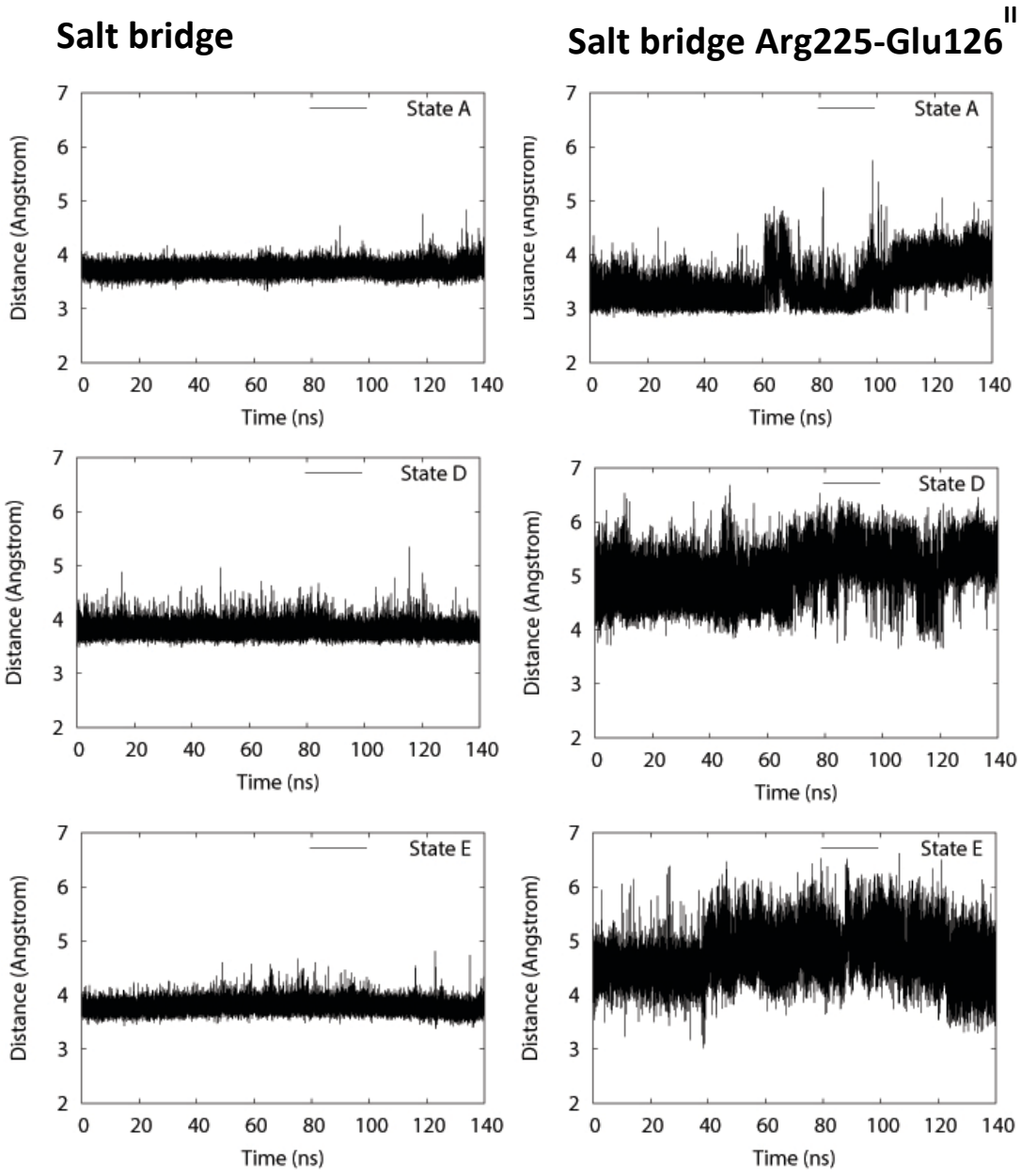

Figure S3. The distances between the center of mass of the side chain groups for the salt bridges Arg225-Asp287 (left) and Arg225-Glu126^{II} (right) for protonation states A (top), D (middle) and E (bottom).

6. Distance analysis for the residues at each side of the entrance of water pathway 2

The distances between the residues at each side of the entrance of water exit pathway 2 for two representative simulations that are shown in the manuscript to describe the water pathways are shown in Figure S4. The distance is the center-of-mass distance between the side chains of the residues.

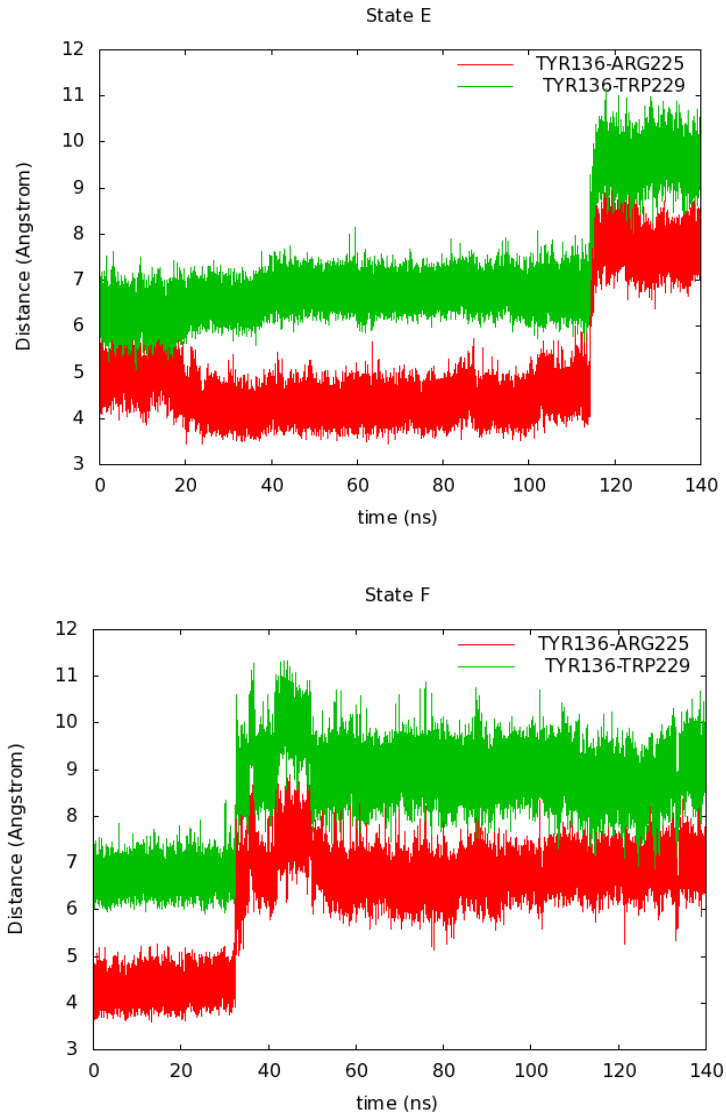

Figure S4. The distances between the centers of mass (COM) of the side chain groups of residues Tyr136 and Arg225 and of residues Tyr136 and Trp229 in state E and F.

7. Comparison of exit pathway P1 to pathway exit-R in aa_3-type CcOs

Here we compare the water exit pathway P1 that we observed in our MD simulations to the alternative exit-R pathway that has been proposed by Sugitani and Stuchebrukhov [*Biochim. Biophys. Acta, Bioenerg.*, 1787 (2009) 1140-1150] based on structural comparisons to water transport pathways in aa_3-type CcOs. Figure S5 shows that the observed pathway (top panels) is consistent with the pathway proposed by Sugitani *et al.* (bottom right panel) based on comparison to the exit-R pathway in aa3-type CcOs (bottom left panel).

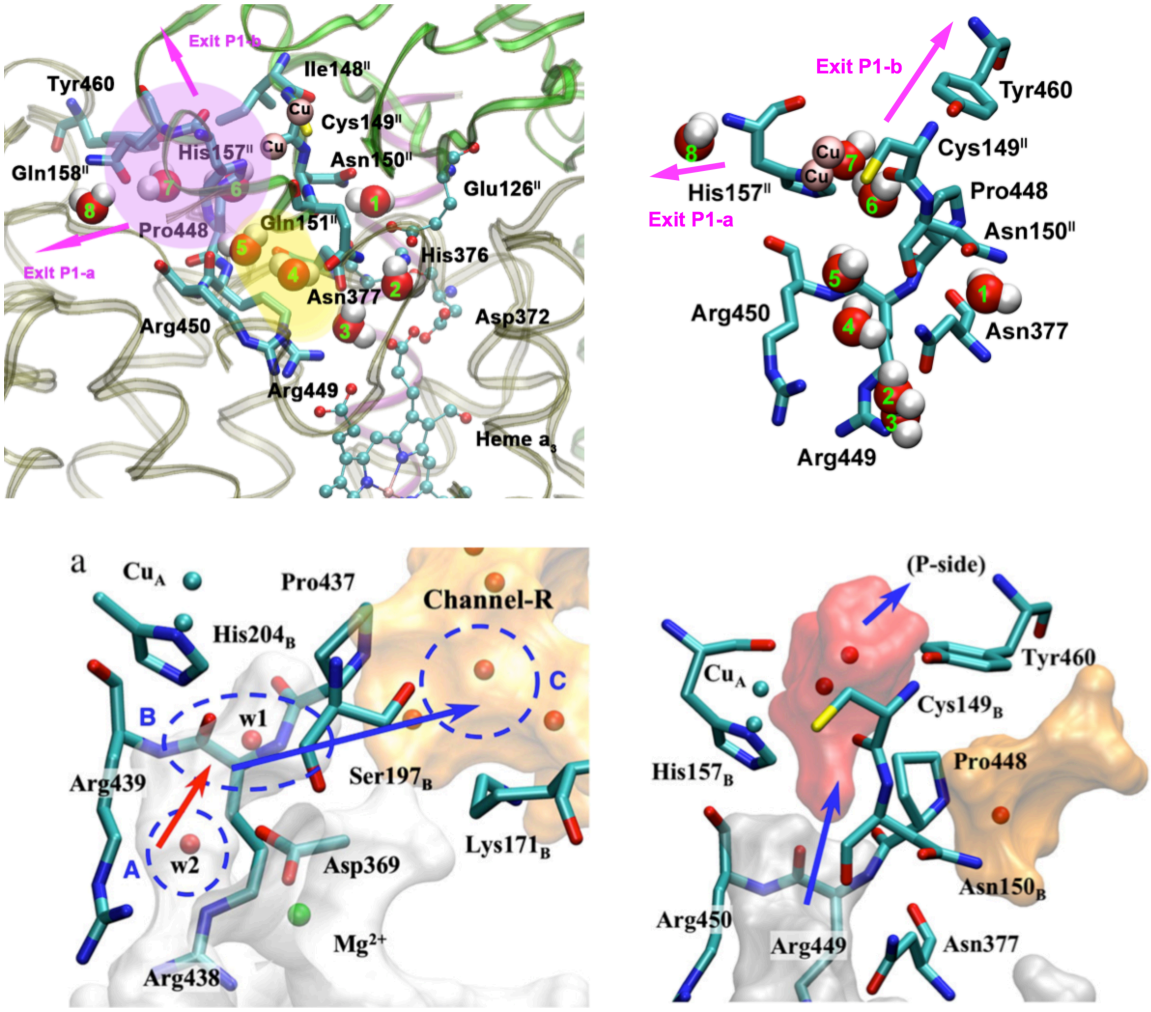

Figure S5. Top: Water exit pathway P1 observed in our MD simulations of ba_3-type CcO from *Thermus thermophilus* represented in two different orientations. Bottom left: Water exit-R pathway in aa3-type CcOs identified by Sugitani and Stuchebrukhov. Bottom right: Alternative exit-R pathway proposed for ba_3-type CcOs by Sugitani and Stuchebrukhov based on structural comparisons. The figures in the bottom panel were reproduced from R. Sugitani, A.A. Stuchebrukhov, *Biochim. Biophys. Acta*, 1787 (2009) 1140-1150.

8. Water occupancy of water exit pathways P1 and P2 for all simulations

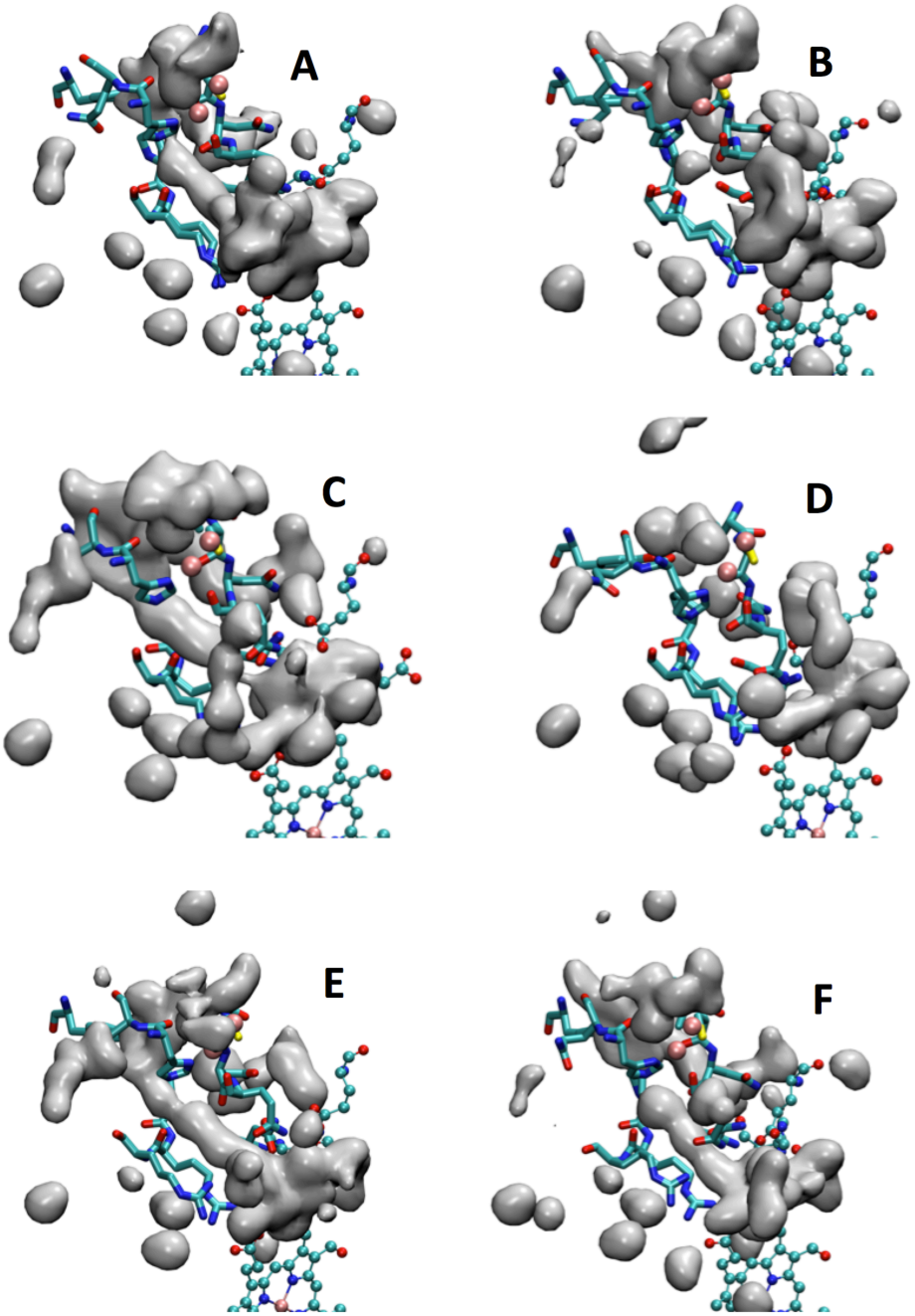

Figure S6. Water exit pathway P1. Isosurface plots at 25% water occupancy averaged over the entire trajectory for all protonation states A to D. The absence of water along pathway P1 indicates that this pathway is disrupted in state D.

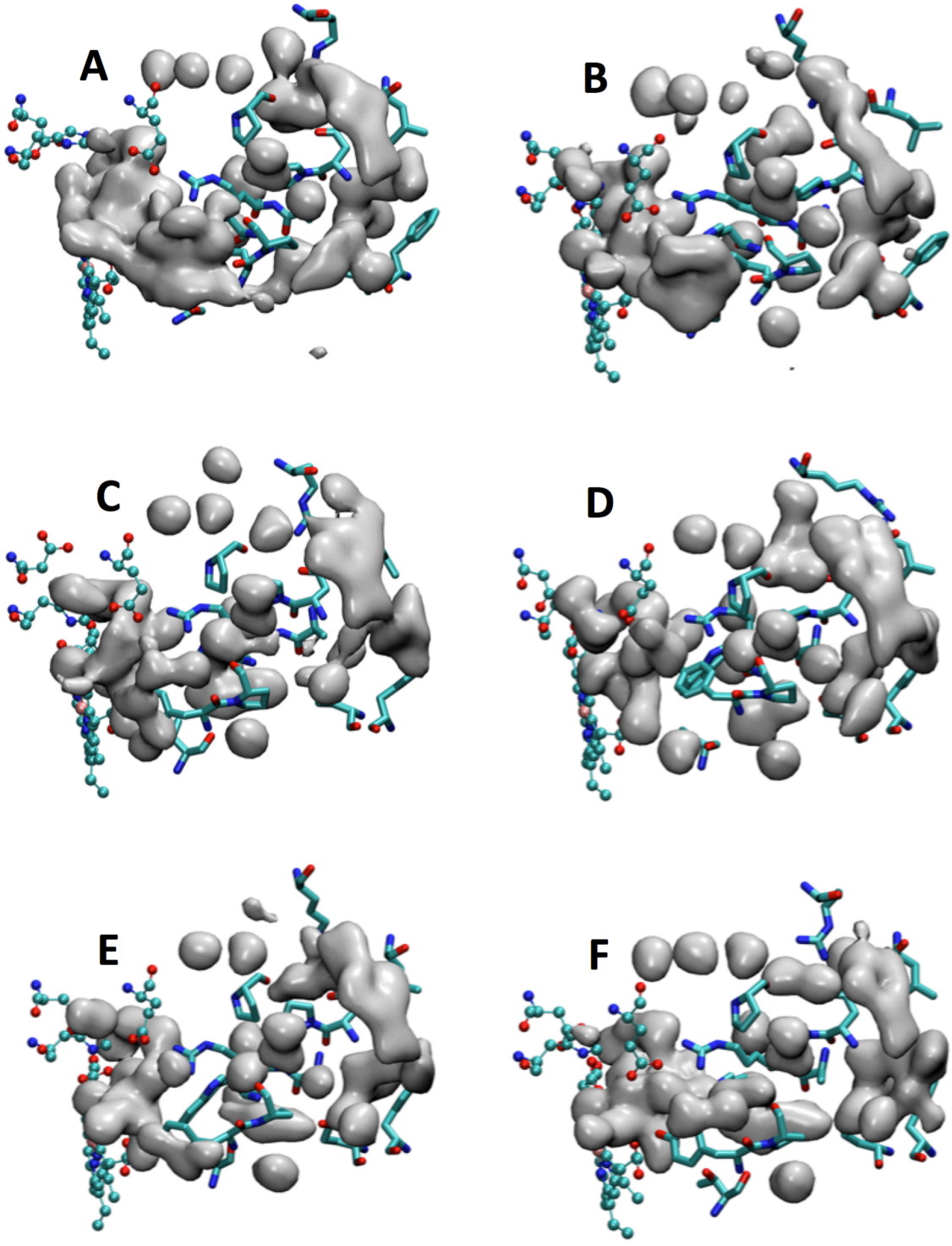

Figure S7. Water exit pathway P2. Isosurface plots at 25% water occupancy averaged over the entire trajectory for all protonation states A to D. The conformation of residue Tyr136 that blocks the entry to pathway P2 can clearly be seen for simulation D.