Supporting Information

Proton Transfer in the K-Channel Analogue of B-Type Cytochrome *c* Oxidase from *Thermus thermophilus*

Anna Lena Woelke, Anke Wagner, Gegham Galstyan, Tim Meyer, Ernst-Walter Knapp*

Institute of Chemistry and Biochemistry, Freie Universität Berlin, D-14195 Berlin, Germany

* corresponding author: E W Knapp, knapp@chemie.fu-berlin.de

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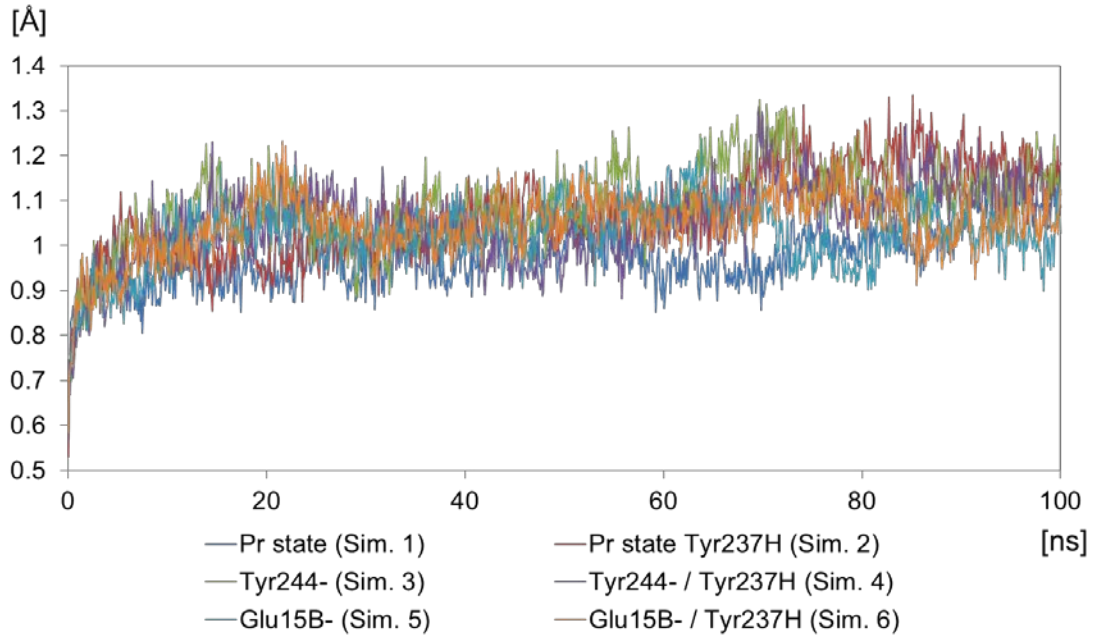


Figure S1: Root mean square deviation (RMSD) of C*c*O backbone atoms relative to the crystal structure PDB-id 3S8F [2] obtained by MD simulations for all simulations performed (for simulation details see Table I in main manuscript).

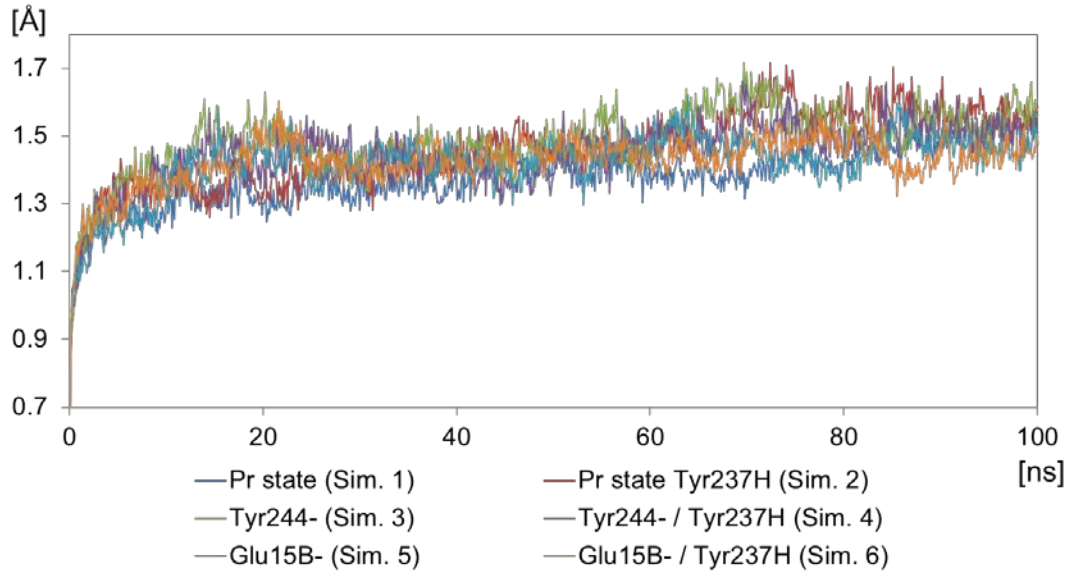


Figure S2: Root mean square deviation (RMSD) of C*c*O of all protein atoms relative to the crystal structure PDB-id 3S8F [2] obtained by MD simulations for all simulations performed (for simulation details see Table I in main manuscript).

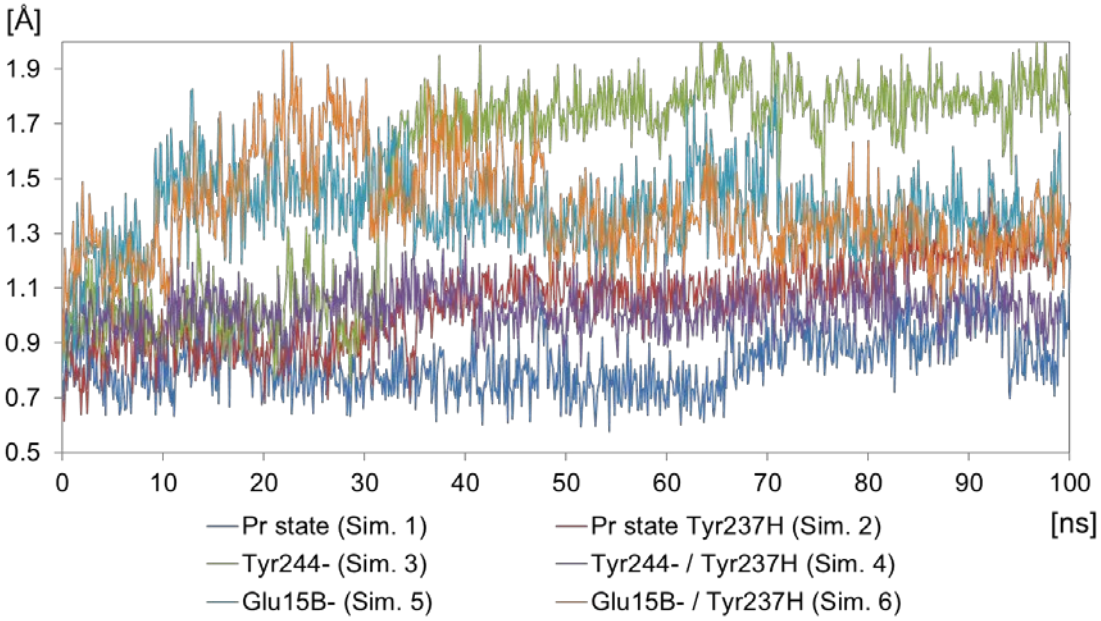


Figure S3: Root mean square deviation (RMSD) of C*c*O of K-channel analogue residues. MD simulation frames were aligned on the backbone atoms of the crystal structure (PDB-id 3S8F [2]). Then, RMSDs were measured for all atoms of the K-channel analogue residues (Tyr237, Ser309, Tyr244, Thr312, Tyr248, Thr315, Glu15B).

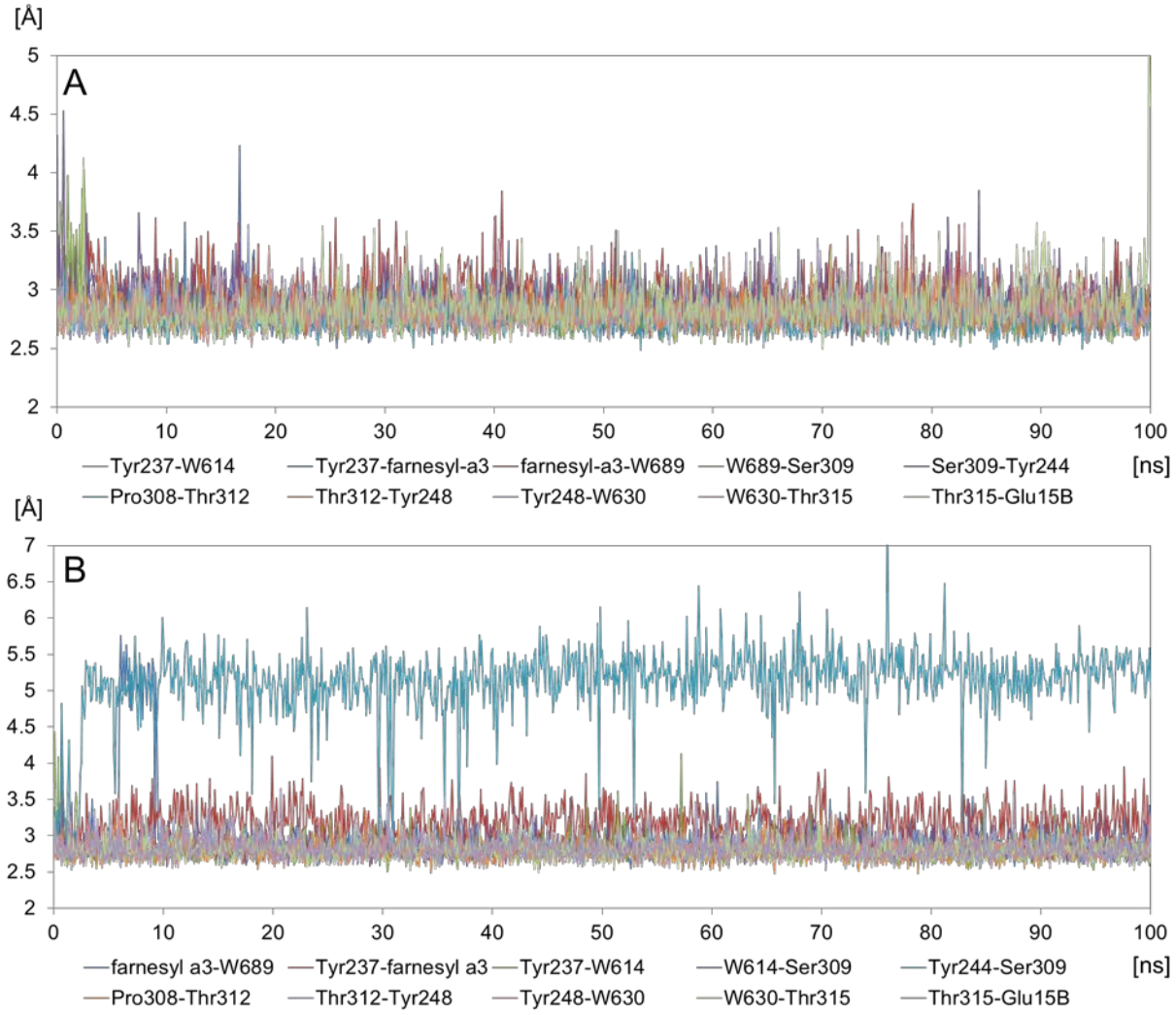


Figure S4: Hydrogen-bonding distances between polar oxygen atoms in the K-channel analogue. Distances between oxygen atoms were recorded for the 100 ns MD trajectory in (A) P_R state and (B) with Tyr237H (for simulation details see Table I, no. 1+2 in main manuscript).

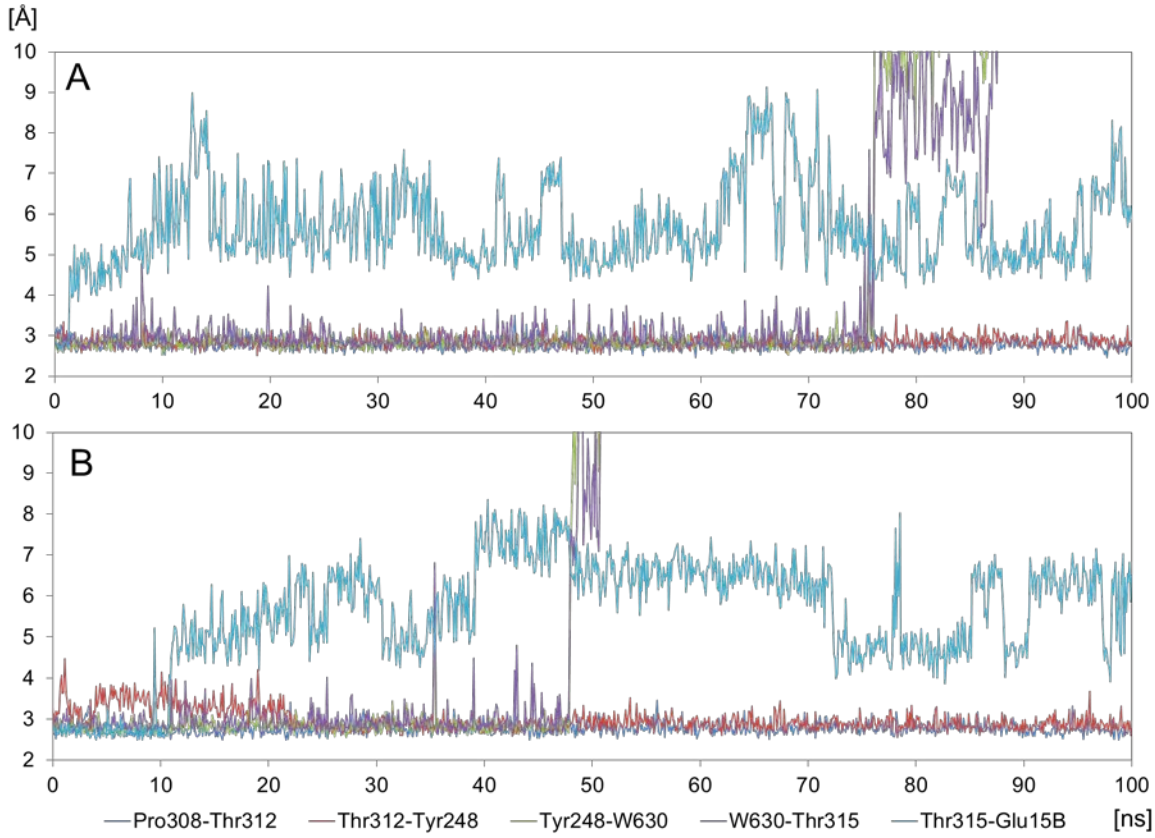


Figure S5: Hydrogen-bonding distances between polar oxygen atoms at the entrance of the K-channel analogue. Distances between oxygen atoms were recorded for the 100 ns MD trajectory with deprotonated Glu15B in (A) P_R state and (B) with Tyr237H (for simulation details see Table I, no. 5+6 in main manuscript).

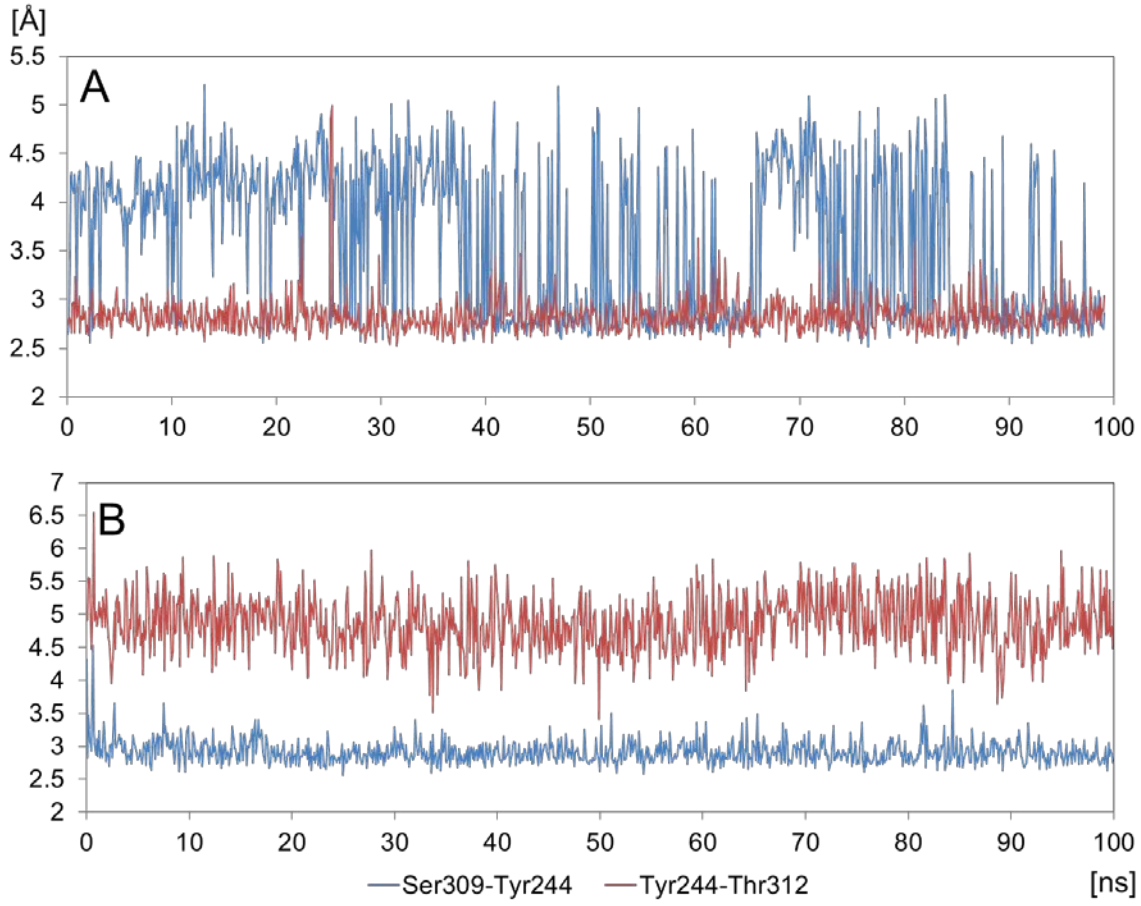
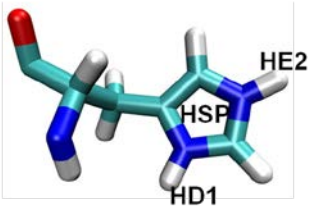
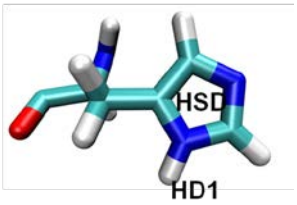
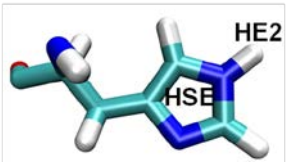


Figure S6: Hydrogen-bonding distances between polar oxygen atoms in the middle of the K-channel analogue. Distances between oxygen atoms were recorded for the 100 ns MD trajectory with (A) deprotonated Tyr244 and protonated Tyr237 and (B) in P_R state (for simulation details see Table I, no. 3+1 in main manuscript).

Table S1: Protonation states of the histidines at pH=7 in C*c*O (PDB-id 2GSM).







The different protonation states of histidine are called HSP, HSD and HSE in the CHARMM[1] program.

AA	72 (A)	142 (A)	233 (A)	282 (A)	283 (A)	298 (A)	376 (A)	384 (A)	386 (A)
HisType	HSD	HSE	HSE	HSD	HSD	HSE	HSP	HSD	HSD

440 (A)	462 (A)	552 (A)	5 (B)	8 (B)	40 (B)	114 (B)	117 (B)	157 (B)
HSD	HSE	HSD	HSE	HSD	HSE	HSE	HSE	HSE

Table S2: Geometry-optimized coordinates and atomic charges for the BNC cofactors.

a) CuA in oxidized state (used for all calculations)

Cu1	-30.3270214596	148.9184511061	13.7615419482	0.311997
Cu2	-28.4194902574	147.0332691630	14.5574729498	0.311997
H3	-25.2084464886	148.7142486770	14.3672519247	0.132877
N4	-26.4180447344	147.0350061074	13.7613730923	-0.213947
C5	-25.2792104960	147.8039855783	13.7948458658	0.024317
C6	-26.3104004498	145.9874156863	13.0149433953	0.097385
H7	-27.0472576587	145.2276439342	12.8076710978	0.104494
N8	-25.0273624388	146.0202440434	12.5116533800	-0.193361
C9	-24.3662249887	147.1531184579	12.9921076707	-0.208905
H10	-23.3478442625	147.3702223383	12.7093699160	0.196572
C11	-30.6264341984	149.2368093769	16.9497028022	-0.005121
H12	-30.4054167205	148.7254743565	17.8889034834	0.051160
H13	-31.5650897477	148.8611676073	16.5406165089	0.051160
S14	-29.2290393107	148.9763774079	15.7792855141	-0.310848
N15	-29.0538688961	152.3462818304	14.3272468683	-0.905063
H16	-29.1249085687	151.3658659433	14.5726316043	0.331005
C17	-28.6592991126	152.5455030526	12.9512767613	0.210796
H18	-28.7145301876	153.6090198907	12.6975313624	0.052321
H19	-27.6134786516	152.2410036342	12.8189304878	0.028775
C20	-29.5141552252	151.6904314385	11.9991776517	0.415545
O21	-29.5435779802	150.4575644807	12.1093223297	-0.425493
N22	-30.4223645331	152.3424376739	11.2262762183	-0.713060
H23	-30.3115998988	153.3212532065	11.0056001449	0.340823
H24	-30.9970633615	151.7859976103	10.6064085380	0.378953
C25	-28.7727473311	147.3833845238	11.2819231234	0.101646
H26	-28.3851671725	146.5076054906	10.7559089054	0.022120
H27	-27.9553689119	148.0159583505	11.6245714914	0.022120
S28	-29.8141884519	146.8154649626	12.6947431327	-0.352773
H29	-33.2808170799	147.8994134365	12.8801623517	0.131131
N30	-32.2897306953	149.6188265009	13.7066361207	-0.147998
C31	-33.3333857278	148.9529431160	13.1069528429	-0.031274
C32	-32.6434209137	150.9186094890	13.8600279503	-0.018683
H33	-31.9863883607	151.7192103060	14.1609385453	0.133179
N34	-33.8670035199	151.0708698156	13.3622134927	-0.158875
C35	-34.3319449733	149.8509296044	12.8869753737	-0.154431
H36	-35.3179097335	149.7535293719	12.4594951925	0.177053
C37	-28.8008645918	144.5433665465	17.5359531210	-0.094367
H38	-29.8338392323	144.8572342928	17.3734838364	0.092250
H39	-28.5264599668	144.7327389645	18.5758743440	0.092250
S40	-27.7683885608	145.5455451624	16.4155875678	-0.220580
C41	-26.0783119079	145.0085812476	16.8369832484	-0.222799
H42	-25.4015144887	145.6254723219	16.2425678160	0.123502
H43	-25.8812513278	145.1873340764	17.8960677150	0.123502
H44	-25.9205965198	143.9567008975	16.5879251985	0.123502
H45	-24.6123510469	145.3410336601	11.8886099439	0.319269
H46	-34.4022407918	151.9283429563	13.3556921216	0.320702
H47	-29.7656802426	152.9419634016	14.7208235833	0.389642
H48	-30.6985977966	150.3070776053	17.1522811330	0.051160
H49	-29.3996092214	147.9516399790	10.5936179505	0.022120
H50	-28.7102417113	143.4791274433	17.3070069628	0.092250

b) heme a in oxidized state (used for all calculations)

H1	1.0239320577	-1.3183000967	38.9398941612	0.181210
C2	1.4909700298	-2.1324224093	38.4082267686	-0.126252
N3	2.4668918501	-2.9237006327	39.0000844501	-0.204047
C4	1.2861378809	-2.6540293935	37.1656361891	-0.136193
C5	2.8746383679	-3.8623337411	38.1188188228	-0.004414
N6	2.1887508740	-3.7038229060	37.0079671504	0.023538
H7	3.3010617514	-7.6716097547	31.0262020711	0.183593
C8	2.8189386081	-7.0847620378	31.7920567270	-0.118088
N9	1.4768473618	-6.7487184046	31.7139378525	-0.212660
C10	3.2757388779	-6.5531276053	32.9675072483	-0.203911
C11	1.1211226057	-6.0245334916	32.7849481888	-0.007644
N12	2.1757196738	-5.8833510427	33.5439998740	0.091744
C13	-1.2033153115	-3.8952969679	34.7482843924	-0.333782
C14	1.0683498935	-7.7360338829	36.8568703076	-0.215239
C15	5.5237873650	-5.7221106855	36.2039619395	-0.212895

C16	3.2880141988	-2.3070323447	33.4493402981	-0.167627
C17	-0.8764851168	-5.1961099437	35.3457753388	0.199004
C18	-1.8233223516	-6.2856272242	35.4378607023	-0.327948
C19	-1.2429350809	-7.2886238244	36.0795785368	0.222301
C20	0.1202440891	-6.9210944822	36.2681782075	0.065534
C21	-1.8264598055	-8.6265806947	36.4280023243	-0.360348
H22	-2.8629855560	-6.2001035017	35.1433657427	0.178094
C23	2.4403890906	-7.4095341963	36.9145825438	0.122940
C24	3.4545716847	-8.2428732404	37.5502690519	0.057027
C25	4.6855252720	-7.6285874638	37.4710702589	-0.005936
C26	4.4177819827	-6.4252747661	36.6297624112	0.096625
C27	3.1206281311	-9.4520075595	38.3799785215	-0.295983
C28	5.9908924779	-8.0940867938	37.9343020613	-0.101349
C29	6.2508615605	-8.6390349075	39.1317923800	-0.319434
C30	5.2927391525	-4.6809894736	35.1270879572	0.028820
C31	6.1902587286	-3.7680275644	34.8045987395	0.102032
C32	5.6135142001	-2.9276240257	33.7719798447	0.054588
C33	4.2854766480	-3.1099013493	34.0684863600	0.039909
C34	7.6814172627	-3.9478418006	34.9632157130	-0.302515
C35	6.1880366110	-1.8085038431	33.0423748568	-0.137651
C36	7.4852979481	-1.5995773340	32.7586685527	-0.301693
C37	1.9841093651	-2.4706336464	33.6606378982	0.134036
C38	0.9696371805	-1.4641848169	33.2148692074	0.086746
C39	-0.2914101054	-1.9201908563	33.6367409301	-0.324167
C40	-0.0831411170	-3.1818975699	34.2985367627	0.238467
C41	1.2140368575	-0.3478005172	32.2433300212	-0.292696
H42	-1.2466043426	-1.4523509067	33.4352140027	0.176273
N43	0.4488573285	-5.6781516584	35.6588858845	-0.276989
N44	3.0986426869	-6.3168742427	36.4000216410	-0.276989
N45	3.9187898095	-4.2543203730	34.8720651383	-0.276989
N46	1.2361344097	-3.3832150463	34.2856998002	-0.276989
Fe47	2.1751087947	-4.8471192658	35.3013198502	0.425000
H48	3.6397265093	-4.5970138349	38.3053640219	0.165195
H49	0.6146164615	-2.3374821273	36.3912458139	0.159761
H50	2.8430697625	-2.7919136913	39.9280966895	0.328887
H51	4.2467451855	-6.5573114858	33.4300010250	0.208630
H52	0.1355122674	-5.6284978918	32.9649365663	0.154493
H53	0.8633838827	-7.0028468966	30.9525158105	0.331262
H54	0.7600638035	-8.6704523913	37.3064450892	0.149311
H55	3.5870562216	-1.4559726303	32.8553294127	0.123769
H56	6.5144645763	-5.9699224250	36.5474862973	0.159482
H57	-2.2148318142	-3.5576469344	34.5826922848	0.198560
H58	7.7878610479	-0.7104617433	32.2147749139	0.147502
H59	8.2731175650	-2.2929914264	33.0225151671	0.159937
H60	5.4857200706	-1.0615661919	32.6816602697	0.140246
H61	7.2570469372	-8.9496295613	39.3957844083	0.151779
H62	5.4862963802	-8.7680336755	39.8914043546	0.161680
H63	6.8266151829	-7.9505769088	37.2503030047	0.123733
H64	4.0100335071	-10.0638624727	38.5495863659	0.098044
H65	2.7327612910	-9.1636237884	39.3650748215	0.098044
H66	2.3689667545	-10.0915887293	37.9047498233	0.098044
H67	8.1660145356	-2.9956206190	35.2032700204	0.100690
H68	7.9384912620	-4.6471427722	35.7621634222	0.100690
H69	8.1319283170	-4.3239521776	34.0382515860	0.100690
H70	-1.7381775272	-8.8385647174	37.4993707519	0.116140
H71	-2.8878358844	-8.6613100376	36.1705969314	0.116140
H72	-1.3216482278	-9.4381362872	35.8919764633	0.116140
H73	0.3003409076	0.2268552205	32.0703620493	0.101366
H74	1.9848331649	0.3546700968	32.5842350113	0.101366
H75	1.5444352287	-0.7431634898	31.2747706025	0.101366

c) heme a3 in state Fe(IV)=O (used for all calculations)

H38	-5.6190862081	-37.1675029949	20.5613171826	0.157824
N39	-6.8442915675	-36.1095730724	21.9895348831	-0.207126
H40	-7.6268375641	-36.7394697787	22.0790002153	0.316613
C41	-5.7335756117	-36.2909980218	21.1794846745	-0.138747
C42	-6.7010807601	-34.9238803102	22.6259582968	0.126215
H43	-7.4049957889	-34.5116211504	23.3320808668	0.105198
N44	-5.5503090507	-34.3588445223	22.2633277446	-0.335061
C45	-4.9452440472	-35.1883148349	21.3642905634	0.009826
H46	-3.9944289009	-34.9449101126	20.9190309940	0.086811
H47	0.2104785816	-34.9125771942	23.4328657543	0.160168

Fe48	-4.5184204499	-32.6416521663	22.8989580956	0.562061
N49	-2.8648006690	-33.7281769651	23.5761712994	-0.176399
N50	-5.3538540863	-32.7407635446	24.8568666373	-0.150605
N51	-5.9981861510	-31.3041549976	22.3726331576	-0.128398
N52	-3.5098871971	-32.2565793661	21.1056674638	-0.143865
C53	-1.7169820310	-33.9358358875	22.8478186742	0.206583
C54	-0.7880789194	-34.5975744229	23.7155774277	-0.290598
C55	-1.3746727485	-34.7646064123	24.9451906915	-0.063552
C56	-2.6944957702	-34.2251146125	24.8564708510	0.141559
C57	-4.8681833255	-33.5332831366	25.8795556988	0.067069
C58	-5.8658039558	-33.5129892003	26.9273318970	0.103347
C59	-6.9007876164	-32.7165331703	26.5310038895	-0.244257
C60	-6.5700017633	-32.2272437564	25.1999903716	0.123513
C61	-7.0682504703	-30.9200853988	23.1543873432	-0.017331
C62	-7.8484796438	-29.9605157689	22.4099332908	0.121088
C63	-7.2568045052	-29.7793826608	21.2031439710	-0.017751
C64	-6.0791782061	-30.6262827956	21.1599354178	0.044192
C65	-3.9463843793	-31.4023295552	20.1319859056	0.040356
C66	-2.9222565032	-31.3503961606	19.1140617013	0.167079
C67	-1.8971720724	-32.1559626221	19.5033762582	-0.385701
C68	-2.2764200639	-32.7262818211	20.7697046249	0.232631
C69	-1.5084520632	-33.5986734494	21.5199937226	-0.272957
H70	-0.5642673621	-33.9143238441	21.0845843150	0.140189
C71	-3.6557684319	-34.2007007233	25.8690995714	-0.212156
H72	-3.3668655714	-34.7215607305	26.7754817657	0.154845
C73	-7.3508851258	-31.3797296227	24.4232413008	-0.170412
H74	-8.2513585223	-31.0123450454	24.8996544773	0.158541
C75	-5.1727804438	-30.7339681631	20.1287022193	-0.173241
H76	-5.3804094412	-30.1468408745	19.2396427284	0.123366
C77	-0.7203503779	-35.3821699896	26.0968366526	0.401299
H78	0.2265947671	-35.9117239622	25.8461089048	-0.020246
O79	-1.1043938401	-35.3280839796	27.2558809620	-0.434365
C80	-5.7514258601	-34.2579373433	28.2295129919	-0.317330
H81	-4.7245613363	-34.5749254799	28.4271077037	0.104881
H82	-6.3742225561	-35.1614413140	28.2337424518	0.078587
H83	-6.0742325566	-33.6439578606	29.0778891479	0.111725
C84	-9.0845923040	-29.2873417000	22.9300720215	-0.278931
H85	-9.1282162297	-28.2434647254	22.6031701617	0.098724
H86	-9.1166819348	-29.2956137384	24.0230636382	0.077371
H87	-9.9993755422	-29.7844369728	22.5824207187	0.082733
C88	-7.6499638239	-28.9027824467	20.1007983288	-0.083917
H89	-6.8378605767	-28.5861745273	19.4469330884	0.121726
C90	-8.8730359307	-28.4174290989	19.8345425230	-0.378785
H91	-9.0308815006	-27.7465306164	18.9954392673	0.146703
H92	-9.7565007008	-28.7127535234	20.3888194855	0.158767
C93	-2.9973778799	-30.5271309401	17.8598757756	-0.300927
H94	-3.0532303148	-29.4538695223	18.0792403279	0.095647
H95	-3.8803334318	-30.7804087529	17.2598835186	0.078603
H96	-2.1151961718	-30.6908857339	17.2343759044	0.097210
C97	-8.0998014255	-32.3290711967	27.3565826724	0.319502
H98	-7.9081735958	-32.5670307831	28.4131962024	-0.016557
O99	-8.4723804561	-30.9546555075	27.2234886252	-0.613663
H100	-7.6842528621	-30.4249545479	27.4062866800	0.391412
H101	-8.9966290949	-32.8820456318	27.0496103428	0.034867
H102	-0.9698969828	-32.3452916349	18.9777700864	0.176710
O104	-3.7173545313	-31.3470686937	23.4935894406	-0.352657

d) CuB in PR state with Cu(II)-OH Tyr237-O- (used for Tyr237 deprotonated)

H1	-0.8264348977	-26.9346109600	26.1064242774	0.114395
N2	-2.2615098291	-28.5572664326	25.9604539222	-0.272336
C3	-1.8463080878	-27.2596556179	26.2418658027	-0.150851
C4	-3.5420971306	-28.6758556889	26.2606412562	0.239017
H5	-4.2047558321	-29.5248796211	26.1757183499	0.073658
N6	-4.0075359556	-27.4746763060	26.6779303239	0.018397
C7	-2.9594099957	-26.5594534038	26.6421526776	-0.139733
H8	-3.0851688944	-25.5565598424	27.0152909734	0.104747
H9	-7.4669767381	-24.5607221949	25.8510568872	0.096415
C10	-7.1688404465	-25.5584168702	26.1647318218	-0.355662
C11	-8.1455072571	-26.5516344439	26.3458615786	-0.025632
H12	-9.1974766433	-26.3140259532	26.2026918731	0.065093
C13	-7.7482593517	-27.8206578078	26.7208140444	-0.352689

H14 -8.4567905795 -28.6277836644 26.8823833952 0.101035
C15 -6.3642335389 -28.1607556020 26.8967965953 0.468574
O16 -5.9999644106 -29.3344808020 27.2440125686 -0.663003
C17 -5.8139427879 -25.8303667570 26.3081465185 -0.047149
H18 -5.0892850961 -25.0611900854 26.0485474013 0.091091
C19 -5.3883832049 -27.1014775020 26.7276621459 -0.156292
H20 0.7852770016 -33.3204222733 28.4027251972 0.151168
N21 -0.7357574042 -31.8060817970 28.5174237514 -0.138017
H22 -0.9905625006 -31.8829941978 29.4918222852 0.276540
C23 0.2228504796 -32.5635976410 27.8780136765 -0.274163
C24 -1.3050648697 -30.9706270807 27.6300361874 0.122950
H25 -2.0769605107 -30.2493471192 27.8480220333 0.070911
N26 -0.7548904222 -31.1951466430 26.4312355829 -0.347171
C27 0.2014882313 -32.1910163383 26.5710586696 0.066167
H28 0.7862817376 -32.5415473094 25.7365729296 0.093837
H29 3.7200734096 -31.2644666827 23.6833759693 0.099653
N30 1.9002902498 -31.5525148359 22.5596236380 -0.247127
H31 2.2419632600 -32.1739304486 21.8404850570 0.274181
C32 2.6731337940 -31.0195297081 23.5882368589 -0.084616
C33 0.6629101046 -31.0570231261 22.6444618907 0.048863
H34 -0.1651386774 -31.2846729282 21.9914555394 0.153108
N35 0.6137614190 -30.2121193382 23.6871710819 -0.100284
C36 1.8581144913 -30.1821177181 24.2830129771 -0.176187
H37 2.0642151958 -29.5799968425 25.1542071162 0.134585
Cu103 -1.0845506669 -29.8341269628 24.8938509096 0.345165
O106 -1.7536237560 -29.0975303164 23.2951111386 -1.015467
H107 -2.0035987608 -28.1759530489 23.4461681401 0.336828

e) CuB in PR state with Cu(II)-OH Tyr237-OH (used for Tyr237 protonated)

H1 -0.8903030011 -26.9122738263 26.1209729916 0.163635
N2 -2.2944940036 -28.5611061666 25.9699135968 -0.278232
C3 -1.9042401272 -27.2567284765 26.2489171452 -0.090344
C4 -3.5629998273 -28.7017951339 26.2571793843 0.114349
H5 -4.1555995250 -29.5928522102 26.1474667632 0.102382
N6 -4.0697007471 -27.5087776324 26.6699145053 0.115890
C7 -3.0311687802 -26.5748483892 26.6373482137 -0.178669
H8 -3.1643250919 -25.5708792226 27.0043431557 0.178374
H9 -7.4289088137 -24.4878232579 25.8404578774 0.143010
C10 -7.1677891328 -25.4920844851 26.1555426415 -0.156157
C11 -8.1532012886 -26.4580572992 26.3452393144 -0.046268
H12 -9.2010846602 -26.2104477308 26.2097203661 0.132988
C13 -7.7974924142 -27.7498716023 26.7265000525 -0.251665
H14 -8.5623231913 -28.5071009605 26.8828991695 0.148767
C15 -6.4586858996 -28.1008086512 26.8894203036 0.255685
O16 -6.0817660469 -29.3642035986 27.2643312960 -0.514649
C17 -5.8258681756 -25.8433330865 26.3050153643 -0.130014
H18 -5.0605022373 -25.1208508519 26.0473666949 0.142600
C19 -5.4570884075 -27.1219376779 26.7247512580 0.007477
H20 0.8816957634 -33.3136059777 28.3882102465 0.182608
N21 -0.6722779252 -31.8329388865 28.5058845328 -0.159156
H22 -0.9231420263 -31.9243597913 29.4806466206 0.319914
C23 0.3037379213 -32.5667058229 27.8661568171 -0.148849
C24 -1.2572878384 -31.0061882653 27.6199517629 0.065660
H25 -2.0434563230 -30.3068276519 27.8476507982 0.128051
N26 -0.6989880281 -31.2141920885 26.4215024389 -0.275759
C27 0.2781578347 -32.1892493218 26.5585397689 -0.003117
H28 0.8732574990 -32.5246893992 25.7253295240 0.111948
H29 3.7403608534 -31.2684578974 23.6634306582 0.154390
N30 1.9002898232 -31.5594265318 22.5739080537 -0.191458
H31 2.2255561008 -32.1901799057 21.8541887948 0.316181
C32 2.6929735597 -31.0204729792 23.5826282592 -0.074571
C33 0.6673097257 -31.0564169619 22.6763088870 0.081909
H34 -0.1710067264 -31.2888561002 22.0381824965 0.176658
N35 0.6401165951 -30.2000763563 23.7120840579 -0.220788
C36 1.8964372889 -30.1715901007 24.2847790246 -0.096136
H37 2.1260542437 -29.5638892556 25.1459890337 0.152475
Cu103 -1.0521976077 -29.8551141939 24.8881634956 0.593722
O106 -1.7456079573 -29.1113523064 23.3121095522 -0.760961
H107 -2.0223354844 -28.1942349722 23.4410356975 0.367948
H41 -6.8166525527 -29.9729106423 27.1083927072 0.420178

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