

# Laccase Redox Potentials: pH Dependence and Mutants - A QM/MM Study

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## Supporting Information

### *Method calibration - Details*

The following tables contain the free energies, SCF energies, tetrahydrofuran (THF) solvent corrections for the 6-31G\* basis calculations, as well as the corresponding 6-311++G\*\* single point energies, all for the corresponding density functionals tested. Definitions: see main article.

**Table S1: BP86 D<sup>-</sup>/D vs. Fc/Fc<sup>+</sup> standard results.**

BP86	D <sup>-</sup>	D	Fc	Fc <sup>+</sup>
E <sub>small</sub> / Hartree	-2254.07904442	-2254.07904442	-511.218711492	-510.971919144
E <sub>large, CPCM</sub> / Hartree	-2254.13884291	-2254.00322708	-511.222404852	-511.033294087
ΔG / Hartree	0.584536	0.588109	0.130034	0.12803
E <sub>ARP</sub> / eV		3.6902944266		5.1459656449
G <sub>ARP</sub> / eV		3.7875207016		5.0914340314
E <sub>RRP</sub> / eV				-1.4556712183
G <sub>RRP</sub> / eV				-1.3039133298

**Table S2: PBE D<sup>-</sup>/D vs. Fc/Fc<sup>+</sup> standard results.**

PBE	D <sup>-</sup>	D	Fc	Fc <sup>+</sup>
E <sub>small</sub> / Hartree	-2251.62706433	-2251.5445891	-510.633509462	-510.389157494
E <sub>large, CPCM</sub> / Hartree	-2251.68636941	-2251.5551081	-510.637177339	-510.450576515
E <sub>ARP</sub> / eV		3.5718019108		5.0776666766
G <sub>ARP</sub> / eV		3.6690281858		5.023135063
E <sub>RRP</sub> / eV				-1.5058647658
G <sub>RRP</sub> / eV				-1.3541068773

**Table S3: PBE0 D<sup>-</sup>/D vs. Fc/Fc<sup>+</sup> standard results.**

PBE0	D <sup>-</sup>	D	Fc	Fc <sup>+</sup>
E <sub>small</sub> / Hartree	-2251.73365043	-2251.65165944	-510.583056964	-510.320595789
E <sub>large, CPCM</sub> / Hartree	-2251.79680892	-2251.6642571	-510.587376177	-510.381877925
E <sub>ARP</sub> / eV		3.6069184739		5.1504094544
G <sub>ARP</sub> / eV		3.7041447489		5.0587887245
E <sub>RRP</sub> / eV				-1.9849733726
G <sub>RRP</sub> / eV				-1.7961263676

**Table S4: M06 D<sup>-</sup>/D vs. Fc/Fc<sup>+</sup> standard results.**

M06	D <sup>-</sup>	D	Fc	Fc <sup>+</sup>
E <sub>small</sub> / Hartree	-2252.68286828	-2252.59043132	-510.581503938	-510.547496519
E <sub>large, CPCM</sub> / Hartree	-2252.74517215	-2252.60139975	-510.585830723	-510.60852135
E <sub>ARP</sub> / eV		3.912245985		5.5498379043
G <sub>ARP</sub> / eV		4.00947226		5.4953062907
E <sub>RRP</sub> / eV				-1.9429194303
G <sub>RRP</sub> / eV				-1.7911615418

Table S5: M06L D<sup>-</sup>/D vs. Fc/Fc<sup>+</sup> standard results.

M06L	D <sup>-</sup>	D	Fc	Fc <sup>+</sup>
E <sub>small</sub> / Hartree	-2253.76696466	-2253.6901104	-511.115148803	-510.873280848
E <sub>large, CPCM</sub> / Hartree	-2253.82674696	-2253.70005328	-511.118369509	-510.934492311
E <sub>ARP</sub> / eV		3.4475103769		5.0035530436
G <sub>ARP</sub> / eV		3.5447366519		4.9490214301
E <sub>RRP</sub> / eV				-1.5560426668
G <sub>RRP</sub> / eV				-1.4042847782

Table S6: ωB97XD D<sup>-</sup>/D vs. Fc/Fc<sup>+</sup> standard results.

ωB97XD	D <sup>-</sup>	D	Fc	Fc <sup>+</sup>
E <sub>small</sub> / Hartree	-2253.40201897	-2253.31745569	-510.95804028	-510.691373958
E <sub>large, CPCM</sub> / Hartree	-2253.46621644	-2253.33017401	-510.962376547	-510.752640794
E <sub>ARP</sub> / eV		3.701902803		5.7072001134
G <sub>ARP</sub> / eV		3.7991290781		5.6526684999
E <sub>RRP</sub> / eV				-2.0052973104
G <sub>RRP</sub> / eV				-1.8535394218

Table S7: B3LYP D<sup>-</sup>/D vs. Fc/Fc<sup>+</sup> standard results.

B3LYP	D <sup>-</sup>	D	Fc	Fc <sup>+</sup>
E <sub>small</sub> / Hartree	-2253.97006754	-2253.88539546	-511.102550506	-510.840817802
E <sub>large, CPCM</sub> / Hartree	-2254.02970873	-2253.89610602	-511.106340047	-510.902350865
E <sub>ARP</sub> / eV		3.6355146453		5.5508279632
G <sub>ARP</sub> / eV		3.7327409203		5.4962963497
E <sub>RRP</sub> / eV				-1.915313318
G <sub>RRP</sub> / eV				-1.7635554294

Table S8: CAM-B3LYP D<sup>-</sup>/D vs. Fc/Fc<sup>+</sup> standard results.

CAM-B3LYP	D <sup>-</sup>	D	Fc	Fc <sup>+</sup>
E <sub>small</sub> / Hartree	-2252.91972074	-2252.83332628	-510.803977942	-510.535975252
E <sub>large, CPCM</sub> / Hartree	-2252.98164841	-2252.8451429	-510.808041573	-510.597518022
E <sub>ARP</sub> / eV		3.7145038507		5.7286371873
G <sub>ARP</sub> / eV		3.8117301258		5.6741055738
E <sub>RRP</sub> / eV				-2.0141333366
G <sub>RRP</sub> / eV				-1.862375448

Table S9: B3PW91 D<sup>-</sup>/D vs. Fc/Fc<sup>+</sup> standard results.

B3PW91	D <sup>-</sup>	D	Fc	Fc <sup>+</sup>
E <sub>small</sub> / Hartree	-2253.29874767	-2253.21445684	-510.994970705	-510.732080485
E <sub>large, CPCM</sub> / Hartree	-2253.3611971	-2253.22640614	-510.999144218	-510.793309724
E <sub>ARP</sub> / eV		3.6678485723		5.6010414567
G <sub>ARP</sub> / eV		3.7650748473		5.5465098431
E <sub>RRP</sub> / eV				-1.9331928844
G <sub>RRP</sub> / eV				-1.7814349958

The following table contains the electronic and free energies of a Cu(Im)<sub>2</sub>(SMe) (Im = imidazole, Me = methyl) Cu(I)/Cu(II) redox pair M/M<sup>+</sup>. Starting geometry of the Cu(I) optimisation taken from the QM/MM optimised laccase geometry, by removing surplus atoms and replacing the omitted link carbon atoms by hydrogen. Cu(II) optimisation from Cu(I) optimised structure.

**Table S10: BP86 M/M<sup>+</sup> vs. Fc/Fc<sup>+</sup> differences in thermal contributions to Gibbs free energies. Fc/Fc<sup>+</sup> as in Table S 1.**

BP86	M	M <sup>+</sup>	Fc	Fc <sup>+</sup>
ΔG / Hartree	0.129229	0.131341	0.130034	0.12803
ΔΔG <sub>ARP</sub> / eV		0.057470443		-0.0545316135

The following tables contain information on the change in energies upon computing the M06 energies on the basis of PBE0 and BP86 optimised geometries (and vice versa) of the D<sup>-</sup>/D pair. The energies shown are computed from a gas phase optimised geometry, but including CPCM for THF. The basis was 6-31G\* for both optimisation and THF single point. To relate the results to Tables S1 to S4, use the following relation:

$$E_{\text{CPCM}} = E_{\text{small}} + \Delta E_{\text{CPCM}} \quad (\text{S1})$$

**Table S11:** E<sub>CPCM</sub> values for different D<sup>-</sup>/D geometries computed with different functionals, M06 and BP86

Geometry	BP86 energies			M06 energies		
	D <sup>-</sup> / Hartree	D / Hartree	Δ / eV	D <sup>-</sup> / Hartree	D / Hartree	Δ / eV
BP86	-2253.7174213	-2253.589677	3.4760	-2252.3406767	-2252.204353	3.7095
M06	-2253.7012823	-2253.576341	3.3998	-2252.3524242	-2252.217729	3.6652

**Table S12:** E<sub>CPCM</sub> values for different D<sup>-</sup>/D geometries computed with different functionals, M06 and PBE0

Geometry	PBE0 energies			M06 energies		
	D <sup>-</sup> / Hartree	D / Hartree	Δ / eV	D <sup>-</sup> / Hartree	D / Hartree	Δ / eV
PBE0	-2251.4403851	-2251.314869	3.4154	-2252.3495372	-2252.214652	3.6704
M06	-2251.4363746	-2251.312015	3.3839	-2252.3524242	-2252.217729	3.6652

The following tables contain information of the effect of optimising the redox pairs either in gas phase or in THF before computing the redox potentials.

**Table S13:** G<sub>large</sub> + ΔE<sub>CPCM</sub> values and derived redox potentials for different geometries from PBE0 optimisation with or without CPCM correction for THF.

Optimisation in	D <sup>-</sup> / Hartree	D / Hartree	Fc / Hartree	Fc <sup>+</sup> / Hartree
<b>Gas phase</b>	-2251.06150914	-2251.19933594	-510.253444348	-510.452697945
<b>THF</b>	-2251.06282567	-2251.2002968	-510.253550802	-510.45307166
<b>Gas phase</b>	G <sub>ARP</sub> / eV	3.7504579803	G <sub>ARP</sub> / eV	5.4219661413
			G <sub>RRP</sub> / eV	-1.6715081611
<b>THF</b>	G <sub>ARP</sub> / eV	3.7407797073	G <sub>ARP</sub> / eV	5.429238683
			G <sub>RRP</sub> / eV	-1.6884589757

**Table S14:** G<sub>large</sub> + ΔE<sub>CPCM</sub> values and derived redox potentials for different geometries from M06 optimisation with or without CPCM correction for THF.

Optimisation in	D <sup>-</sup> / Hartree	D / Hartree	Fc / Hartree	Fc <sup>+</sup> / Hartree
<b>Gas phase</b>	-2252.006552	-2252.15084907	-510.482616447	-510.665915961
<b>THF</b>	-2252.00748578	-2252.15093806	-510.482730561	-510.666292378
<b>Gas phase</b>	G <sub>ARP</sub> / eV	3.9265229818	G <sub>ARP</sub> / eV	4.9878334625
			G <sub>RRP</sub> / eV	-1.0613104806
<b>THF</b>	G <sub>ARP</sub> / eV	3.9035350768	G <sub>ARP</sub> / eV	4.9949710901
			G <sub>RRP</sub> / eV	-1.0914360134

*Tests including an Onsager model for the region beyond 35 Å radius*

To check if the presence of the point charges outside the 35 Å water sphere are relevant for the actual RP, we have devised a series of calculations for three different snapshots from our set. First, we removed all point charges beyond a radius of 37 Å and computed the RP, 2 Å more than the 35 Å sphere as individual water molecules or hydrogen atoms could still stick out of the sphere further than 35 Å. Then, we reintroduced the removed system charge by summing it up into a single charge, which we placed at the average of all removed point charge coordinates; this position was modified such that the distance to the centre of the sphere was 37 Å. For this system, we computed the RP as well.

Finally, we repeated the above calculations, but including an Onsager model at 37 Å. The results are displayed in the following table. Note that we do not include the MM part for the reported values, as they do not change the differences upon introducing the Onsager model or upon point charge relocation.

Table S15: Changes upon deleting/relocating charges beyond 37 Å radius (amounting to +4.80 charge), and the effect of a Onsager model for the bulk water. 800 ps snapshot is most relocated from the centre of the sphere, 100 ps snapshots chosen arbitrarily.

	Orginal	Cut charges	Neutralised	Cut/Onsager	Cut, neutr./O
100 ps / NI <sub>neutral</sub>	4.03245	2.35891	4.22613	2.35932	4.22656
100 ps / NI <sub>low-pH</sub>	5.11265	4.33670	5.29414	4.33742	5.29488
800 ps / NI <sub>low-pH</sub>	4.71502	3.93878	4.89905	3.93957	4.89986

It can be seen that as long as the total system charge is conserved, the changes in RP upon changes in the point charge field are within the range of the standard deviation. Furthermore, the introduction of an Onsager model has no effect beyond 0.001 eV.

*Additional force field terms for NI, Red and NI<sub>low-pH/mut</sub>*

The following tables contain the additional “bonds” and “angles” that were constructed to keep the Cu coordination spheres intact. A pictorial representation of the T1 site constraints (valid for all models except NI<sub>low-pH/mut</sub>) is given in **Error! Reference source not found.**.. The values in the tables correspond to a QM/MM optimised NI<sub>neutral</sub> model reference structure, see main article for details. The force constant for all given constraints was 500 kcal mol<sup>-1</sup> Å<sup>-2</sup>. Charges were simple integers for all ions, test calculations showed no differences in RP when employing NBO derived charges (not

**Table S16: T1 site restraints, for all models except the F463M mutant**

Bonds (A-B)			Angles (A-B-C)			
A	B	Value / Å	A	B	C	Value / °
N <sub>δ</sub> (His395)	Cu(T1)	1.9773 <sup>a</sup>	C <sub>ε</sub> (His395)	N <sub>δ</sub> (His395)	S <sub>γ</sub> (Cys453)	150.70
N <sub>δ</sub> (His458)	Cu(T1)	1.9773 <sup>a</sup>	C <sub>ε</sub> (His395)	N <sub>δ</sub> (His395)	N <sub>δ</sub> (His458)	91.23 <sup>a</sup>
S <sub>γ</sub> (Cys453)	Cu(T1)	2.1724	N <sub>δ</sub> (His395)	N <sub>δ</sub> (His458)	C <sub>ε</sub> (His458)	91.23 <sup>a</sup>
H <sub>δ</sub> (Ile455)	Cu(T1)	3.0041	N <sub>δ</sub> (His395)	H <sub>δ</sub> (Ile455)	N <sub>δ</sub> (His458)	51.63
H <sub>δ2</sub> (Phe463)	Cu(T1)	2.8807	C <sub>ε2</sub> (Phe463)	C <sub>δ2</sub> (Phe463)	Cu(T1)	82.22
N <sub>δ</sub> (His395)	S <sub>γ</sub> (Cys453)	3.7310 <sup>a</sup>	H <sub>ε2</sub> (Phe463)	C <sub>ε2</sub> (Phe463)	Cu(T1)	86.44
N <sub>δ</sub> (His458)	S <sub>γ</sub> (Cys453)	3.7310 <sup>a</sup>				
C <sub>ε</sub> (His395)	C <sub>ε</sub> (His458)	3.3426				
H <sub>ε2</sub> (Phe463)	N <sub>δ</sub> (His395)	2.4138				

<sup>a</sup> Averaged from two corresponding reference structure values

**Table S17: T1 site restraints for the F463M mutant model. Differences to Table S16 highlighted (italics).**

Bonds (A-B)			Angles (A-B-C)			
A	B	Value / Å	A	B	C	Value / °
N <sub>δ</sub> (His395)	Cu(T1)	1.9773 <sup>a</sup>	C <sub>ε</sub> (His395)	N <sub>δ</sub> (His395)	S <sub>γ</sub> (Cys453)	150.70
N <sub>δ</sub> (His458)	Cu(T1)	1.9773 <sup>a</sup>	C <sub>ε</sub> (His395)	N <sub>δ</sub> (His395)	N <sub>δ</sub> (His458)	91.23 <sup>a</sup>
S <sub>γ</sub> (Cys453)	Cu(T1)	2.1724	N <sub>δ</sub> (His395)	N <sub>δ</sub> (His458)	C <sub>ε</sub> (His458)	91.23 <sup>a</sup>
H <sub>δ</sub> (Ile455)	Cu(T1)	3.0041	N <sub>δ</sub> (His395)	H <sub>δ</sub> (Ile455)	N <sub>δ</sub> (His458)	51.63
S <sub>δ</sub> (Met463)	Cu(T1)	3.6545	C <sub>β</sub> (Met463)	C <sub>γ</sub> (Met463)	Cu(T1)	92.32 <sup>b</sup>
N <sub>δ</sub> (His395)	S <sub>γ</sub> (Cys453)	3.7310 <sup>a</sup>	C <sub>ε</sub> (Met463)	S <sub>δ</sub> (Met463)	Cu(T1)	95.68 <sup>b</sup>
N <sub>δ</sub> (His458)	S <sub>γ</sub> (Cys453)	3.7310 <sup>a</sup>				
C <sub>ε</sub> (His395)	C <sub>ε</sub> (His458)	3.3426				
C <sub>ε</sub> (Met463)	N <sub>δ</sub> (His395)	3.8114 <sup>b</sup>				

<sup>a</sup> Averaged from two corresponding reference structure values <sup>b</sup> Value of initial mutant structure

shown).

**Table S18: TNC site restraints for the NI models.**

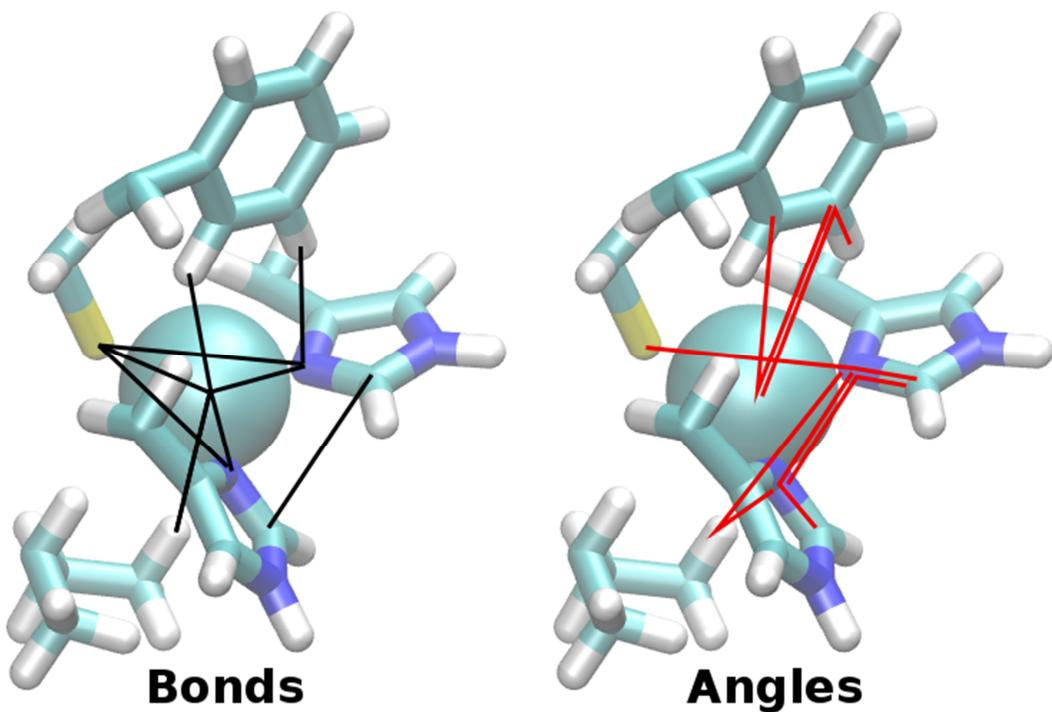
Bonds (A-B)			Angles (A-B-C)			
A	B	Value / Å	A	B	C	Value / °
Cu(T2)	O(OH/T2)	1.9118	O(O <sup>2-</sup> )	Cu(T2)	O(OH/T2)	175.35
Cu(T2)	O(O <sup>2-</sup> )	1.9316	Cu(T3B)	O(O <sup>2-</sup> )	Cu(T3A)	99.32
Cu(T3B)	Cu(T3A)	3.0119	Cu(T3B)	Cu(T2)	Cu(T3A)	50.94
O(O <sup>2-</sup> )	O(OH/T3)	2.4940	Cu(T3B)	O(OH/T3)	Cu(T3A)	100.87
N <sub>ε</sub> (His64)	N <sub>ε</sub> (His398)	4.0663	N <sub>ε</sub> (His398)	Cu(T2)	O(OH/T2)	95.67
			Cu(T3B)	Cu(T3A)	N <sub>ε</sub> (His109)	107.21 <sup>a</sup>
			Cu(T3A)	Cu(T3B)	N <sub>ε</sub> (His452)	107.21 <sup>a</sup>
			N <sub>ε</sub> (His454)	Cu(T3A)	N <sub>ε</sub> (His109)	97.51 <sup>b</sup>
			N <sub>ε</sub> (His454)	Cu(T3A)	N <sub>δ</sub> (His66)	97.51 <sup>b</sup>
			N <sub>ε</sub> (His109)	Cu(T3A)	N <sub>δ</sub> (His66)	97.51 <sup>b</sup>
			N <sub>ε</sub> (His452)	Cu(T3B)	N <sub>ε</sub> (His111)	97.51 <sup>b</sup>
			N <sub>ε</sub> (His452)	Cu(T3B)	N <sub>ε</sub> (His400)	97.51 <sup>b</sup>
			N <sub>ε</sub> (His111)	Cu(T3B)	N <sub>ε</sub> (His400)	97.51 <sup>b</sup>
			N <sub>ε</sub> (His64)	Cu(T2)	O(O <sup>2-</sup> )	90.36
			H(OH/T2)	O(OH/T2)	Cu(T2)	125.24

<sup>a</sup> Averaged from two corresponding reference structure values <sup>b</sup> Average of six reference values

**Table S19: TNC site restraints for the Red models. Differences to Table S 18 highlighted (italics).**

Bonds (A-B)			Angles (A-B-C)			
A	B	Value / Å	A	B	C	Value / °
Cu(T2)	O(OH/T2)	1.9118	<i>O(H<sub>2</sub>O/T3)</i>	<i>Cu(T2)</i>	<i>O(OH/T2)</i>	<i>175.53<sup>c</sup></i>
<i>Cu(T2)</i>	<i>O(H<sub>2</sub>O/T3)</i>	<i>4.1285<sup>c</sup></i>	<i>Cu(T3B)</i>	<i>O(H<sub>2</sub>O/T3)</i>	<i>Cu(T3A)</i>	<i>132.84<sup>c</sup></i>
Cu(T3B)	Cu(T3A)	3.0119	Cu(T3B)	Cu(T2)	Cu(T3A)	50.94
N <sub>ε</sub> (His64)	N <sub>ε</sub> (His398)	4.0663	N <sub>ε</sub> (His398)	Cu(T2)	O(OH/T2)	95.67
			Cu(T3B)	Cu(T3A)	N <sub>ε</sub> (His109)	107.21 <sup>a</sup>
			Cu(T3B)	Cu(T3A)	N <sub>ε</sub> (His452)	107.21 <sup>a</sup>
			N <sub>ε</sub> (His454)	Cu(T3A)	N <sub>ε</sub> (His109)	97.51 <sup>b</sup>
			N <sub>ε</sub> (His454)	Cu(T3A)	N <sub>δ</sub> (His66)	97.51 <sup>b</sup>
			N <sub>ε</sub> (His109)	Cu(T3A)	N <sub>δ</sub> (His66)	97.51 <sup>b</sup>
			N <sub>ε</sub> (His452)	Cu(T3B)	N <sub>ε</sub> (His111)	97.51 <sup>b</sup>
			N <sub>ε</sub> (His452)	Cu(T3B)	N <sub>ε</sub> (His400)	97.51 <sup>b</sup>
			N <sub>ε</sub> (His111)	Cu(T3B)	N <sub>ε</sub> (His400)	97.51 <sup>b</sup>
			N <sub>ε</sub> (His64)	Cu(T2)	<i>O(H<sub>2</sub>O/T3)</i>	<i>90.13<sup>c</sup></i>
			H(OH/T2)	O(OH/T2)	Cu(T2)	125.24

<sup>a</sup> Averaged from two corresponding reference structure values <sup>b</sup> Average of six reference values <sup>c</sup> From crystal

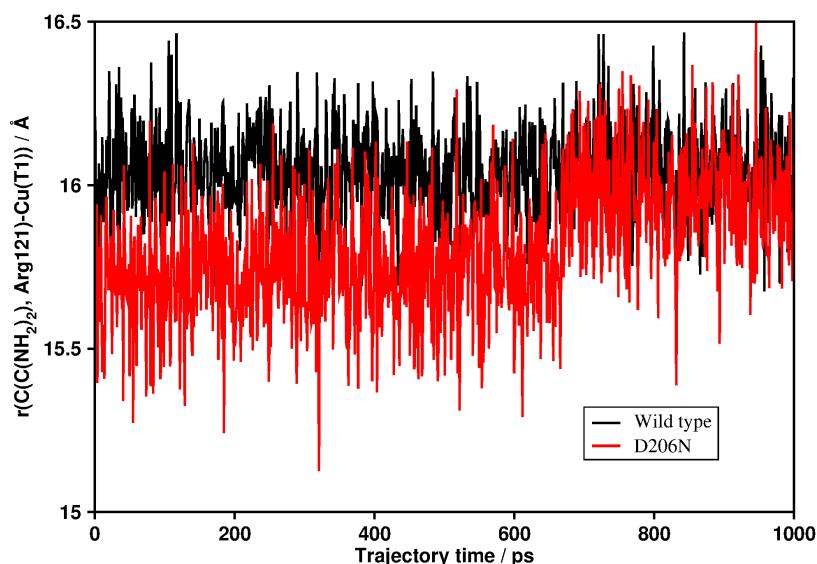


**Scheme S1:** Restraining distances (“bonds”) and angles defined to keep the T1 site intact during the MD simulation. A visual representation of the effect during the MD simulation is given in the main article.

#### *Surface charge location*

We provide a video file (mpg format) that shows the location of the charges changed for the gradual de-protonation scheme. The video is available from the publisher’s web page. The video displays a 360 degree rotation about an arbitrary axis crossing through the T1 Cu centre; the residues of interest are coloured red. It can be seen that most of them are located at or near the surface of the enzyme.

#### *Arg121 dynamics*



**Scheme S2:** Distance graph for Arg121, analogous to the inset of Figure 5 in the main paper. Being 5 Å more distant to T1 Cu than Asp456, and showing no obvious correlation to the RPs in Tables 2 and 4 of the main paper, the Arg121 switch does not appear to explain the change in RP upon D206N exchange.

*Effect of Cu(I) QM/MM optimisation starting geometry*

The following tables contain the energies and resulting redox potentials of all computed models, including those Cu(I) T1 laccase models that are optimised starting directly from the molecular dynamics snapshot.

**Table S20: Energies of the Ni<sub>low-pH</sub> model, depending on the optimisation protocol, in Hartree. Differences in eV.**

BP86/MM	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-3128.519023	-3128.679299	-3128.699709	4.36	4.92
200 ps	-3128.435934	-3128.6146	-3128.605991	4.86	4.63
300 ps	-3128.467643	-3128.643474	-3128.640599	4.78	4.71
400 ps	-3128.444642	-3128.60542	-3128.609288	4.37	4.48
500 ps	-3128.564453	-3128.736272	-3128.736303	4.68	4.68
600 ps	-3128.597082	-3128.763957	-3128.766942	4.54	4.62
700 ps	-3128.538991	-3128.711112	-3128.7261	4.68	5.09
800 ps	-3128.437276	-3128.624396	-3128.620537	5.09	4.99
900 ps	-3128.626453	-3128.827211	-3128.832013	5.46	5.59
1000 ps	-3128.571081	-3128.732622	-3128.741826	4.40	4.65
<b>Average</b>				<b>4.72</b>	<b>4.83</b>
<b>Std. Dev.</b>				<b>0.33</b>	<b>0.31</b>
M06/MM <sup>a</sup>	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-1685.322075	-1685.501772	-1685.522446	4.89	5.45
200 ps	-1685.239141	-1685.437753	-1685.429044	5.40	5.17
300 ps	-1685.269612	-1685.466972	-1685.461924	5.37	5.23
400 ps	-1685.248753	-1685.428615	-1685.433597	4.89	5.03
500 ps	-1685.368710	-1685.560401	-1685.560464	5.22	5.22
600 ps	-1685.399706	-1685.587408	-1685.590430	5.11	5.19
700 ps	-1685.339410	-1685.533817	-1685.546430	5.29	5.63
800 ps	-1685.237736	-1685.446985	-1685.439130	5.69	5.48
900 ps	-1685.429237	-1685.649397	-1685.653930	5.99	6.11
1000 ps	-1685.374625	-1685.555122	-1685.565174	4.91	5.19
<b>Average</b>				<b>5.28</b>	<b>5.37</b>
<b>Std. Dev.</b>				<b>0.34</b>	<b>0.30</b>

<sup>a</sup> Using the BP86 QM/MM geometry

**Table S21:** Energies of the Ni<sub>neutral</sub> model, depending on the optimisation protocol, in Hartree. Differences in eV.

BP86/MM	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-3125.835266	-3125.970664	-3125.972698	3.68	3.74
200 ps	-3125.854843	-3125.973195	-3125.97143	3.22	3.17
300 ps	-3125.812557	-3125.943717	-3125.944401	3.57	3.59
400 ps	-3122.45571	-3122.587286	-3122.58701	3.58	3.57
500 ps	-3125.659198	-3125.788916	-3125.788927	3.53	3.53
600 ps	-3125.723147	-3125.860365	-3125.858172	3.73	3.67
700 ps	-3125.826676	-3125.955012	-3125.95175	3.49	3.40
800 ps	-3125.809016	-3125.941234	-3125.941243	3.60	3.60
900 ps	-3126.003653	-3126.137492	-3126.132413	3.64	3.50
1000 ps	-3125.911491	-3126.050656	-3126.049665	3.79	3.76
<b>Average</b>				<b>3.57</b>	<b>3.53</b>
<b>Std. Dev.</b>				<b>0.15</b>	<b>0.16</b>
M06/MM <sup>a</sup>	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-1682.638870	-1682.791016	-1682.793103	4.14	4.20
200 ps	-1682.658063	-1682.792088	-1682.792486	3.65	3.66
300 ps	-1682.615952	-1682.765407	-1682.766047	4.07	4.08
400 ps	-1679.257657	-1679.406028	-1679.405733	4.04	4.03
500 ps	-1682.462352	-1682.610401	-1682.610371	4.03	4.03
600 ps	-1682.525591	-1682.679715	-1682.678589	4.19	4.16
700 ps	-1682.629696	-1682.775007	-1682.772832	3.95	3.89
800 ps	-1682.611893	-1682.763229	-1682.762072	4.12	4.09
900 ps	-1682.805628	-1682.957226	-1682.952876	4.13	4.01
1000 ps	-1682.710680	-1682.871371	-1682.868273	4.37	4.29
<b>Average</b>				<b>4.07</b>	<b>4.04</b>
<b>Std. Dev.</b>				<b>0.18</b>	<b>0.17</b>

<sup>a</sup> Using the BP86 QM/MM geometry

**Table S22: Energies of the Red<sub>low-pH</sub> model, depending on the optimisation protocol, in Hartree. Differences in eV.**

BP86/MM	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-3116.640103	-3116.811611	-3116.812387	4.67	4.69
200 ps	-3116.650926	-3116.813527	-3116.813513	4.42	4.42
300 ps	-3116.456006	-3116.621569	-3116.624171	4.51	4.58
400 ps	-3116.738239	-3116.926984	-3116.926991	5.14	5.14
500 ps	-3116.884428	-3117.049023	-3117.050636	4.48	4.52
600 ps	-3116.795151	-3116.962592	-3116.96738	4.56	4.69
700 ps	-3116.765323	-3116.925046	-3116.925263	4.35	4.35
800 ps	-3116.863628	-3117.031335	-3117.030979	4.56	4.55
900 ps	-3116.754825	-3116.920982	-3116.922411	4.52	4.56
1000 ps	-3116.651835	-3116.815278	-3116.817255	4.45	4.50
<b>Average</b>				<b>4.56</b>	<b>4.60</b>
<b>Std. Dev.</b>				<b>0.21</b>	<b>0.20</b>
M06/MM <sup>a</sup>	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-1673.442559	-1673.634466	-1673.635565	5.22	5.25
200 ps	-1673.450294	-1673.633668	-1673.633662	4.99	4.99
300 ps	-1673.259241	-1673.445303	-1673.447852	5.06	5.13
400 ps	-1673.539766	-1673.748508	-1673.748628	5.68	5.68
500 ps	-1673.684050	-1673.870969	-1673.872619	5.09	5.13
600 ps	-1673.598007	-1673.785303	-1673.790708	5.10	5.24
700 ps	-1673.567575	-1673.747617	-1673.747868	4.90	4.91
800 ps	-1673.666300	-1673.854020	-1673.853989	5.11	5.11
900 ps	-1673.557504	-1673.744180	-1673.745734	5.08	5.12
1000 ps	-1673.455010	-1673.638367	-1673.640509	4.99	5.05
<b>Average</b>				<b>5.12</b>	<b>5.16</b>
<b>Std. Dev.</b>				<b>0.20</b>	<b>0.20</b>

<sup>a</sup> Using the BP86 QM/MM geometry

**Table S23: Energies of the Red<sub>neutral</sub> model, depending on the optimisation protocol, in Hartree. Differences in eV.**

BP86/MM	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-3125.197495	-3125.308232	-3125.308218	3.01	3.01
200 ps	-3125.346866	-3125.444326	-3125.457596	2.65	3.01
300 ps	-3125.235669	-3125.349079	-3125.34921	3.09	3.09
400 ps	-3125.313982	-3125.425621	-3125.432406	3.04	3.22
500 ps	-3125.189628	-3125.311593	-3125.313339	3.32	3.37
600 ps	-3125.360959	-3125.476235	-3125.48009	3.14	3.24
700 ps	-3125.316579	-3125.430667	-3125.43843	3.10	3.32
800 ps	-3125.393477	-3125.510904	-3125.510342	3.20	3.18
900 ps	-3125.266078	-3125.403022	-3125.391656	3.73	3.42
1000 ps	-3125.426838	-3125.55455	-3125.552737	3.48	3.43
<b>Average</b>				<b>3.17</b>	<b>3.23</b>
<b>Std. Dev.</b>				<b>0.27</b>	<b>0.15</b>
M06/MM <sup>a</sup>	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-1682.000128	-1682.129292	-1682.129337	3.51	3.52
200 ps	-1682.149786	-1682.266050	-1682.279479	3.16	3.53
300 ps	-1682.038433	-1682.170505	-1682.170571	3.59	3.60
400 ps	-1682.115956	-1682.246343	-1682.253361	3.55	3.74
500 ps	-1681.991875	-1682.132431	-1682.146076	3.82	4.20
600 ps	-1682.163316	-1682.297926	-1682.301302	3.66	3.75
700 ps	-1682.119017	-1682.251921	-1682.259487	3.62	3.82
800 ps	-1682.194967	-1682.331000	-1682.329932	3.70	3.67
900 ps	-1682.068981	-1682.224704	-1682.213561	4.24	3.93
1000 ps	-1682.229460	-1682.374169	-1682.374304	3.94	3.94
<b>Average</b>				<b>3.68</b>	<b>3.77</b>
<b>Std. Dev.</b>				<b>0.27</b>	<b>0.20</b>

<sup>a</sup> Using the BP86 QM/MM geometry

**Table S24:** Energies of the Ni<sub>low-pH</sub>/F463M model, depending on the optimisation protocol, in Hartree. Differences in eV.

BP86/MM	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-3368.653649	-3368.818065	-3368.819625	4.47	4.52
200 ps	-3368.685454	-3368.854029	-3368.849478	4.59	4.46
300 ps	-3368.66805	-3368.836689	-3368.837574	4.59	4.61
400 ps	-3368.805128	-3368.968493	-3368.968571	4.45	4.45
500 ps	-3368.780037	-3368.926591	-3368.929475	3.99	4.07
600 ps	-3368.79445	-3368.964894	-3368.961091	4.64	4.53
700 ps	-3368.398493	-3368.56112	-3368.562876	4.43	4.47
800 ps	-3368.68569	-3368.866248	-3368.867121	4.91	4.94
900 ps	-3368.571505	-3368.723972	-3368.724436	4.15	4.16
1000 ps	-3368.789273	-3368.953173	-3368.949144	4.46	4.35
<b>Average</b>				<b>4.47</b>	<b>4.46</b>
<b>Std. Dev.</b>				<b>0.24</b>	<b>0.12</b>
M06/MM <sup>a</sup>	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-1925.509130	-1925.696304	-1925.697819	5.09	5.13
200 ps	-1925.539110	-1925.729392	-1925.725288	5.18	5.07
300 ps	-1925.522636	-1925.713940	-1925.714785	5.21	5.23
400 ps	-1925.659260	-1925.846024	-1925.845650	5.08	5.07
500 ps	-1925.634192	-1925.803233	-1925.806232	4.60	4.68
600 ps	-1925.649111	-1925.842148	-1925.838477	5.25	5.15
700 ps	-1925.252506	-1925.437694	-1925.439532	5.04	5.09
800 ps	-1925.540102	-1925.744022	-1925.744828	5.55	5.57
900 ps	-1925.425686	-1925.601873	-1925.602279	4.79	4.81
1000 ps	-1925.641194	-1925.829275	-1925.825911	5.12	5.03
<b>Average</b>				<b>5.09</b>	<b>5.08</b>
<b>Std. Dev.</b>				<b>0.24</b>	<b>0.23</b>

<sup>a</sup> Using the BP86 QM/MM geometry

**Table S25: Energies of the Ni<sub>low-pH/D206N</sub> model, depending on the optimisation protocol, in Hartree. Differences in eV.**

BP86/MM	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-3125.579176	-3125.748519	-3125.747263	4.61	4.57
200 ps	-3125.454301	-3125.623424	-3125.626782	4.60	4.69
300 ps	-3125.625381	-3125.788478	-3125.789637	4.44	4.47
400 ps	-3125.602814	-3125.744914	-3125.773112	3.87	4.63
500 ps	-3125.653971	-3125.845489	-3125.848403	5.21	5.29
600 ps	-3125.806105	-3125.965503	-3125.958185	4.34	4.14
700 ps	-3125.499807	-3125.667079	-3125.664174	4.55	4.47
800 ps	-3125.660612	-3125.806965	-3125.808698	3.98	4.03
900 ps	-3125.65608	-3125.81676	-3125.822816	4.37	4.54
1000 ps	-3125.67143	-3125.828965	-3125.828962	4.29	4.29
<b>Average</b>				<b>4.43</b>	<b>4.51</b>
<b>Std. Dev.</b>				<b>0.35</b>	<b>0.33</b>
M06/MM <sup>a</sup>	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-1682.382627	-1682.571100	-1682.570023	5.13	5.10
200 ps	-1682.256119	-1682.443629	-1682.447385	5.10	5.20
300 ps	-1682.427857	-1682.610024	-1682.611375	4.96	4.99
400 ps	-1682.402135	-1682.563618	-1682.590729	4.39	5.13
500 ps	-1682.456653	-1682.665519	-1682.668417	5.68	5.76
600 ps	-1682.608085	-1682.787501	-1682.779570	4.88	4.67
700 ps	-1682.302405	-1682.489008	-1682.485093	5.08	4.97
800 ps	-1682.464543	-1682.628952	-1682.631902	4.47	4.55
900 ps	-1682.458291	-1682.638478	-1682.644753	4.90	5.07
1000 ps	-1682.474401	-1682.651318	-1682.651404	4.81	4.82
<b>Average</b>				<b>4.94</b>	<b>5.03</b>
<b>Std. Dev.</b>				<b>0.34</b>	<b>0.32</b>

<sup>a</sup> Using the BP86 QM/MM geometry

*Effect of functional choice for the laccase system (PBE results)*

The following tables contain the energies and resulting redox potentials of all computed models when employing the PBE functional instead of M06, including Cu(I) energies optimised directly from the MD snapshot.

**Table S26:** Energies of the Ni<sub>low-pH</sub> model, depending on the optimisation protocol when computed with the PBE functional, in Hartree. Differences in eV.

PBE/MM <sup>a</sup>	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-1684.761215	-1684.781714	-1684.597090	4.47	5.02
200 ps	-1684.696996	-1684.688553	-1684.514432	4.97	4.74
300 ps	-1684.725676	-1684.722824	-1684.546262	4.88	4.80
400 ps	-1684.687702	-1684.692442	-1684.523471	4.47	4.60
500 ps	-1684.818399	-1684.818443	-1684.642544	4.79	4.79
600 ps	-1684.846373	-1684.849372	-1684.675185	4.66	4.74
700 ps	-1684.793712	-1684.808793	-1684.617504	4.79	5.21
800 ps	-1684.706450	-1684.703360	-1684.515997	5.18	5.10
900 ps	-1684.909255	-1684.913973	-1684.704485	5.57	5.70
1000 ps	-1684.814856	-1684.824349	-1684.649542	4.50	4.76
<b>Average</b>				<b>4.87</b>	<b>4.94</b>
<b>Std. Dev.</b>				<b>0.33</b>	<b>0.32</b>

<sup>a</sup> Using the BP86 QM/MM geometry

**Table S27:** Energies of the Ni<sub>neutral</sub> model, depending on the optimisation protocol when computed with the PBE functional, in Hartree. Differences in eV.

PBE/MM <sup>a</sup>	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-1682.051465	-1682.053703	-1681.912568	3.78	3.84
200 ps	-1682.054786	-1682.052873	-1681.932697	3.32	3.27
300 ps	-1682.024756	-1682.025409	-1681.890114	3.66	3.68
400 ps	-1678.668420	-1678.668136	-1678.533146	3.68	3.67
500 ps	-1681.869932	-1681.869935	-1681.736758	3.62	3.62
600 ps	-1681.941470	-1681.939014	-1681.800725	3.83	3.76
700 ps	-1682.036028	-1682.033729	-1681.905155	3.56	3.50
800 ps	-1682.022617	-1682.023432	-1681.887504	3.68	3.70
900 ps	-1682.218850	-1682.214385	-1682.082016	3.72	3.60
1000 ps	-1682.131974	-1682.131585	-1681.989810	3.87	3.86
<b>Average</b>				<b>3.66</b>	<b>3.63</b>
<b>Std. Dev.</b>				<b>0.15</b>	<b>0.16</b>

<sup>a</sup> Using the BP86 QM/MM geometry

**Table S28: Energies of the Red<sub>low-pH</sub> model, depending on the optimisation protocol when computed with the PBE functional, in Hartree. Differences in eV.**

PBE/MM <sup>a</sup>	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-1672.894324	-1672.895155	-1672.718792	4.78	4.80
200 ps	-1672.895843	-1672.895834	-1672.729057	4.54	4.54
300 ps	-1672.704391	-1672.706998	-1672.534165	4.63	4.70
400 ps	-1673.009047	-1673.009096	-1672.816182	5.25	5.25
500 ps	-1673.131162	-1673.132871	-1672.962977	4.58	4.62
600 ps	-1673.045241	-1673.050713	-1672.874086	4.66	4.81
700 ps	-1673.007533	-1673.007757	-1672.843532	4.46	4.47
800 ps	-1673.113527	-1673.113789	-1672.942070	4.67	4.67
900 ps	-1673.003650	-1673.005149	-1672.833146	4.64	4.68
1000 ps	-1672.897491	-1672.899587	-1672.730156	4.55	4.61
<b>Average</b>				<b>4.66</b>	<b>4.71</b>
<b>Std. Dev.</b>				<b>0.22</b>	<b>0.21</b>

<sup>a</sup> Using the BP86 QM/MM geometry

**Table S29: Energies of the Red<sub>neutral</sub> model, depending on the optimisation protocol when computed with the PBE functional, in Hartree. Differences in eV.**

PBE/MM <sup>a</sup>	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-1681.389704	-1681.389691	-1681.275232	3.11	3.11
200 ps	-1681.525715	-1681.538864	-1681.424336	2.76	3.12
300 ps	-1681.430475	-1681.430630	-1681.313373	3.19	3.19
400 ps	-1681.506985	-1681.513840	-1681.391573	3.14	3.33
500 ps	-1681.393187	-1681.406736	-1681.267392	3.42	3.79
600 ps	-1681.557612	-1681.561489	-1681.438543	3.24	3.35
700 ps	-1681.511635	-1681.519374	-1681.393887	3.20	3.41
800 ps	-1681.592007	-1681.591412	-1681.470853	3.30	3.28
900 ps	-1681.484093	-1681.473177	-1681.343872	3.82	3.52
1000 ps	-1681.635532	-1681.633649	-1681.504103	3.58	3.53
<b>Average</b>				<b>3.29</b>	<b>3.39</b>
<b>Std. Dev.</b>				<b>0.28</b>	<b>0.19</b>

<sup>a</sup> Using the BP86 QM/MM geometry

**Table S30: Energies of the Ni<sub>low-pH/F463M</sub> model, depending on the optimisation protocol when computed with the PBE functional, in Hartree. Differences in eV.**

PBE/MM <sup>a</sup>	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-1924.870858	-1924.872438	-1924.701472	4.61	4.65
200 ps	-1924.905903	-1924.901501	-1924.732995	4.71	4.59
300 ps	-1924.888903	-1924.889750	-1924.715709	4.71	4.74
400 ps	-1925.020431	-1925.020662	-1924.852673	4.56	4.57
500 ps	-1924.978799	-1924.981578	-1924.827474	4.12	4.19
600 ps	-1925.016812	-1925.013433	-1924.842091	4.75	4.66
700 ps	-1924.613387	-1924.615045	-1924.446116	4.55	4.60
800 ps	-1924.918995	-1924.919902	-1924.733569	5.05	5.07
900 ps	-1924.776208	-1924.776644	-1924.619196	4.27	4.28
1000 ps	-1925.005997	-1925.002086	-1924.836828	4.60	4.50
<b>Average</b>				<b>4.59</b>	<b>4.58</b>
<b>Std. Dev.</b>				<b>0.26</b>	<b>0.24</b>

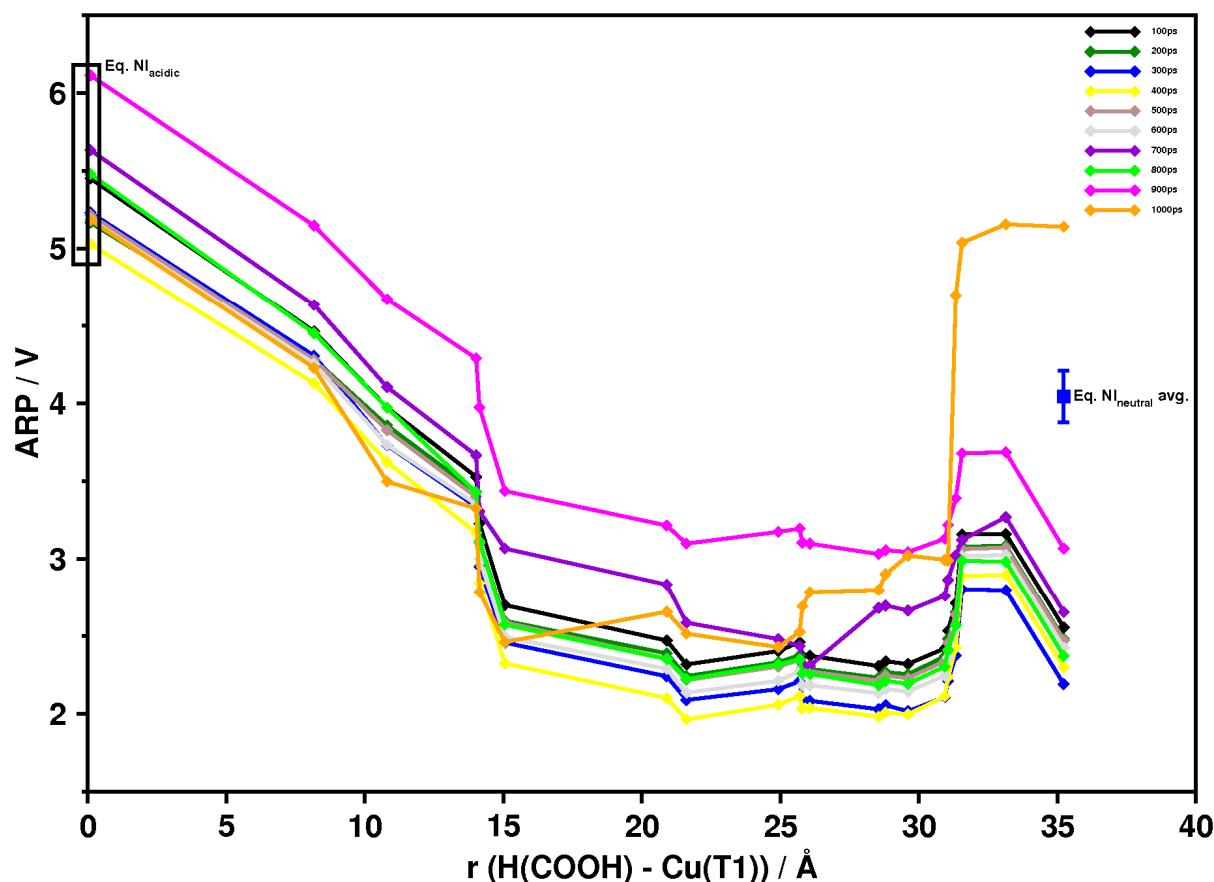
<sup>a</sup> Using the BP86 QM/MM geometry

**Table S31: Energies of the Ni<sub>low-pH/D206N</sub> model, depending on the optimisation protocol when computed with the PBE functional, in Hartree. Differences in eV.**

PBE/MM <sup>a</sup>	E(Cu(II))	E(Cu(I))		ΔE (in eV)	
Time	Direct	Direct	From Cu(II)	Direct	From Cu(II)
100 ps	-1681.831666	-1681.830473	-1681.658382	4.72	4.68
200 ps	-1681.706320	-1681.709820	-1681.533613	4.70	4.79
300 ps	-1681.871844	-1681.872957	-1681.704638	4.55	4.58
400 ps	-1681.827528	-1681.855206	-1681.681538	3.97	4.73
500 ps	-1681.928465	-1681.931522	-1681.733428	5.31	5.39
600 ps	-1682.048743	-1682.041157	-1681.885131	4.45	4.25
700 ps	-1681.749758	-1681.746724	-1681.578395	4.66	4.58
800 ps	-1681.889564	-1681.891953	-1681.739899	4.07	4.14
900 ps	-1681.899537	-1681.905472	-1681.735084	4.47	4.64
1000 ps	-1681.912102	-1681.912092	-1681.750464	4.40	4.40
<b>Average</b>				<b>4.51</b>	<b>4.61</b>
<b>Std. Dev.</b>				<b>0.36</b>	<b>0.34</b>

<sup>a</sup> Using the BP86 QM/MM geometry

*Individual evolution of each snapshot in the gradual de-protonation scheme*

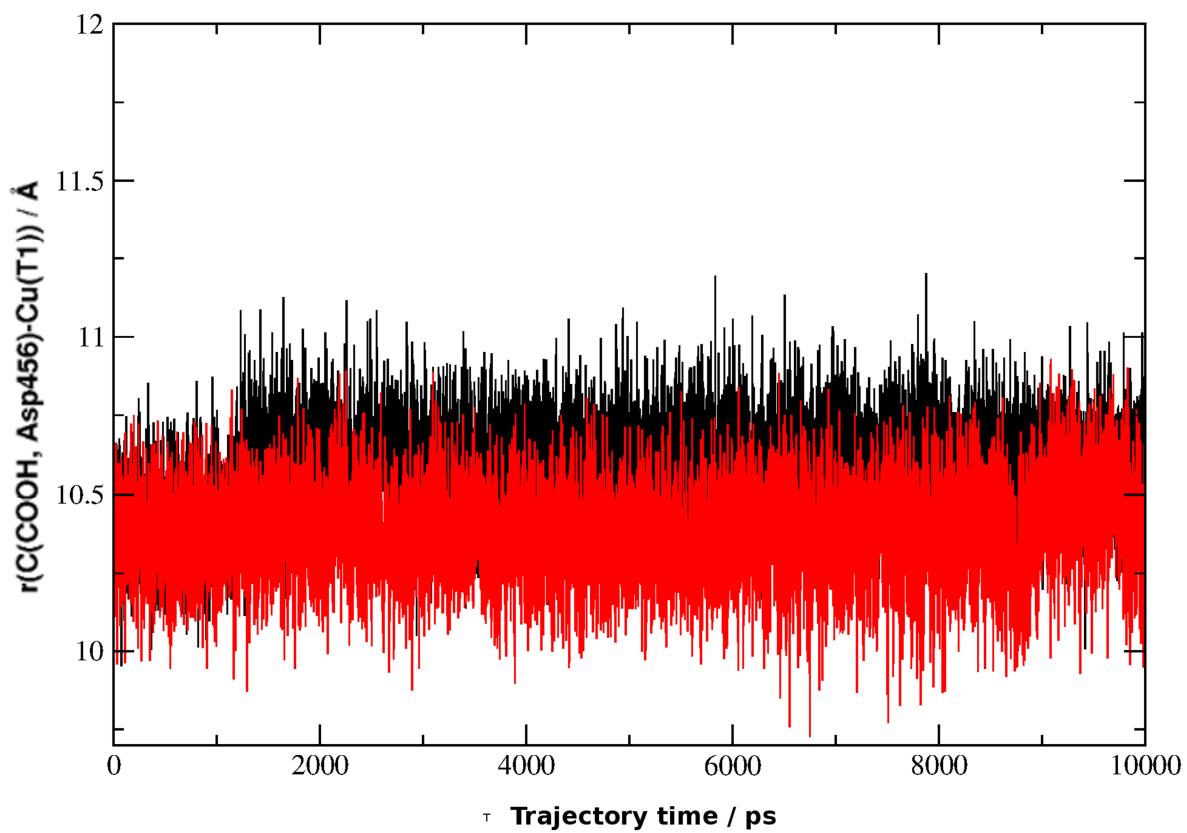


Scheme S1: ARP at different point charge field compositions, due to gradual replacement of uncharged Glu or Asp residues by their charged counterparts. Distance measured to the carboxylic proton in each Glu or Asp residue, based on the 600 ps snapshot (arbitrary choice, standard deviation of  $r$  is less than 0.1 Å over all snapshots, omitted). Corresponding ARPs from the equilibrated QM/MM trajectories indicated for comparison (NI<sub>neutral</sub> shows standard deviation of RP as error bars).

Table S32: Reduced sampling tests (showing M06 only, wild types). Showing average absolute redox potentials (ARPs) for the given systems, full sets (first column), and four subsets of 5 snapshots each.

	Avg. ARP	ARP, for odds	ARP, for even	ARP, 100-500	ARP, 600-1000
NI <sub>low-pH</sub>	5.370	5.530	5.211	5.220	5.521
Red <sub>low-pH</sub>	5.162	5.109	5.214	5.238	5.085
NI <sub>neutral</sub>	4.044	4.042	4.045	4.000	4.088
Red <sub>neutral</sub>	3.770	3.813	3.727	3.715	3.825

*Extended trajectories of the D206N mutant*



**Scheme S4:** Wild type (black) and D206N (red) distances between Asn456 and Cu(T1) along a 10 ns trajectory. Also along the extended trajectory, the wild type prefers a different conformation compared to the mutant.