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Supplementary Information

Figure S1. A blue crystal of *Mv* BOx showing the frequently observed 'sheet-like' morphology.

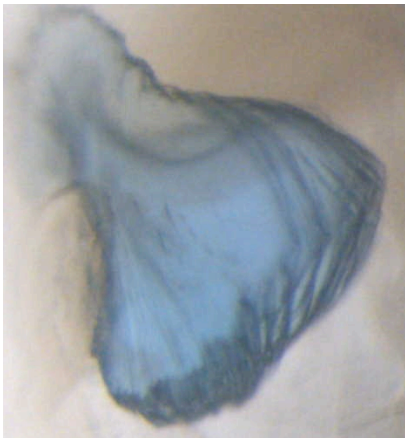

Figure S2. Cartoon representations of laccases from four organisms, highlighting the segments of the structure that differ significantly from each other. Regions where the structure is conserved in all four enzymes are coloured grey. The structures that do not overlap are coloured to match the colours used in Table S2. The orientation of the coppers in all four structures is the same.

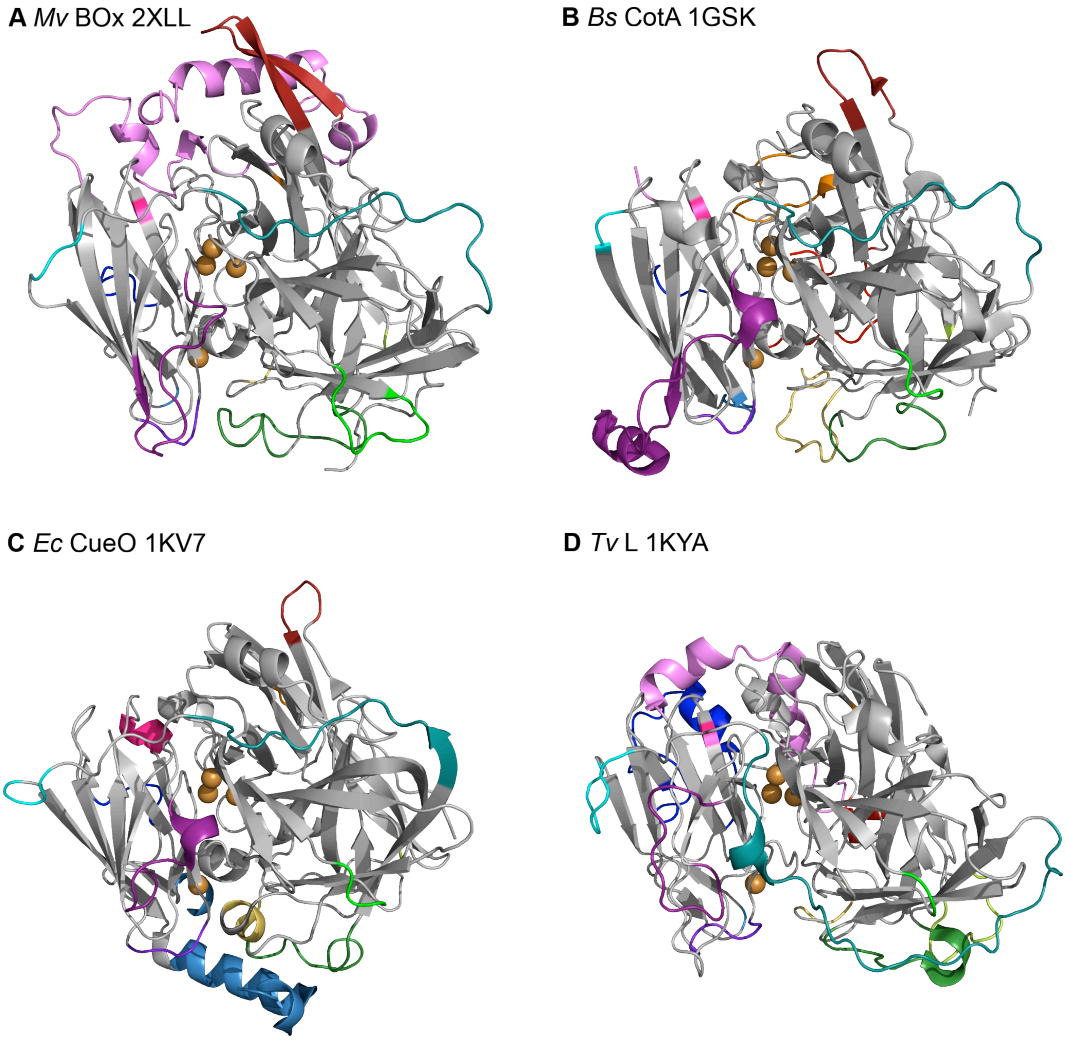

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Figure S3. Distances from the T1 Cu atom (Panel A) and the TNC (Panel B) to atoms on the surface of *Mv* BOx. Colour coding: 8–10 Å = red, 10–12 Å = magenta, 12–14 Å = orange, 14–16 Å = green, 16–18 Å = blue, >18 Å = grey. Cu atoms are depicted as cyan spheres within the semi-transparent surface.

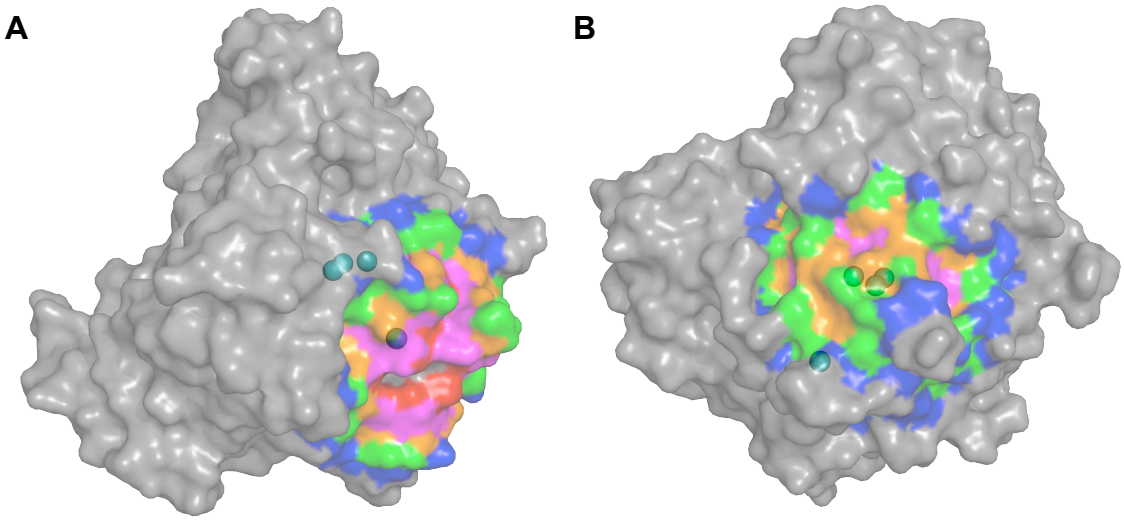

Figure S4. Cyclic voltammograms recorded during the modification of graphite electrodes with bilirubin. Experimental conditions were: 0.1 M phosphate pH 8.5, T = 25 °C, ω = 4000 rpm, ν = 0.1 V/s, projected electrode surface area ~0.03 cm^2.

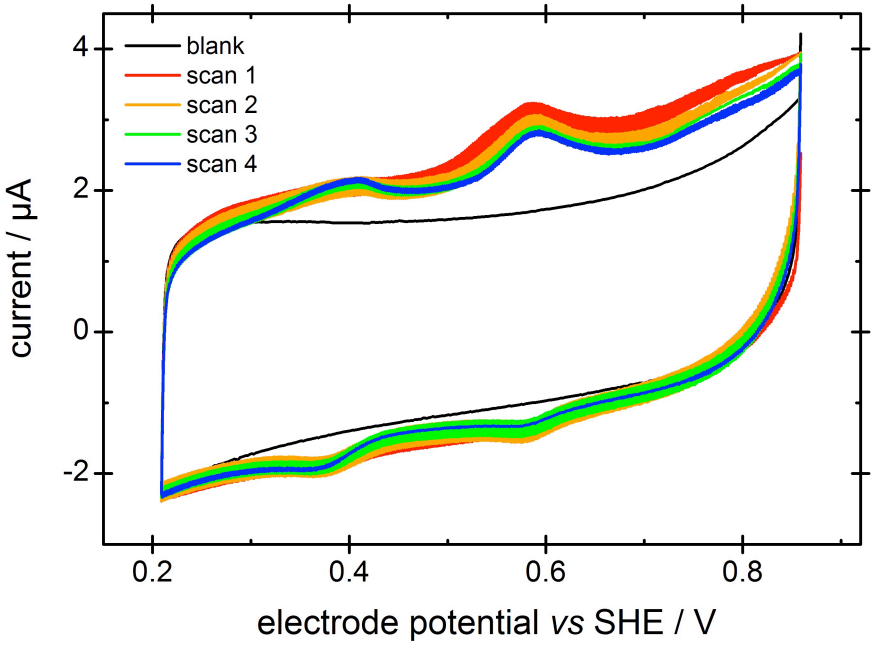

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Table S1. List of selected residues mentioned in the main text. In the columns detailing channels through the protein, residues sufficiently close to the TNC that they are common to multiple pathways are shown in blue.

Residues for which insufficient side-chain density was recorded	The amino-acid residues that form the putative substrate binding-site in *Mv* BOx	Residues forming the channel from T2 Cu to the surface (green in Fig. 1, main text)	Residues forming the first channel from T3 Cu atoms to the surface (yellow in Fig. 1, main text)	Residues forming the second channel from T3 Cu atoms to the surface (beige in Fig. 1, main text)
Asp-53	Asn-197	His-94	Phe-46	Pro-18
Asn-186	Ser-198	His-96	His-48	Pro-67
Asp-323	Trp-200	Leu-95	Leu-59	Gly-68
Asp-338	Ser-231	Gly-97	Met-65	Pro-69
Gln-372	Ser-233	Ser-98	Ser-66	Thr-70
Glu-500	Met-273	Phe-99	Pro-67	Gln-72
Leu-501	Gly-304	Asp-105	His-94	His-94
Gln-514	Thr-305	Gln-126	His-96	His-96
Glu-518	Asp-306	Arg-129	Trp-132	Trp-132
Ile-520	Trp-361	His-401	His-134	His-134
	Gly-395	Ile-402	Asp-135	Asp-135
	Trp-396	His-403	His-136	His-136
	Thr-397	Les-404	Thr-141	Thr-141
	His-398	Val-405	Ala-145	Ala-145
	Pro-399	Asp-406	Tyr-146	Ala-150
	Trp-433	Asp-430	Gly-148	Gly-151
	Asn-499	His-445	Gln-149	Leu-152
	His-462	Ala-447	Ala-150	Met-154
			Thr-179	Gly-168
			Lys-181	Tyr-169
			Gly-208	Asp-173
			Ala-228	Ile-174
			His-401	Pro-175
			His-403	Ile-177
			His-456	Thr-179
			His-458	Asn-207
			Leu-460	Arg-224
			Glu-463	Leu-226
				Ala-228
				Ala-229
				His-401
				His-403
				His-456
				His-458
				Leu-460
				Glu-463

Table S2. Manual structural alignment of *Mv* BOx (PDB 2XLL), *Bs* CotA (PDB 1GSK), *Ec* CueO (PDB 1KV7), and laccase III from *Trametes versicolor* (PDB 1KYA). X-ray–determined crystal structures were aligned to atoms comprising the T1 Cu, the two His and one Cys coordinating it, and the conserved Pro adjacent to one coordinating His. Color coding refers to significantly different loops between the structures and matches the colors in Figure S2. Yellow-highlighted residues were not resolved in the structure but appear in the amino acid sequence. Blue-highlighted residues for the first coordination sphere around the T1 Cu. Residue numbers for *Ec* CueO (1KV7) omit the protein's Tat-type signal peptide and are therefore shifted from the index number in the PDB by 28 residues.

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2XLL     VAQISPQYPMFTVPLPIPPVKQPRLTVTNPVNGQEI-WYYEVEIKPFTHQVYPDLGSADLVGYDGMSPGP  69
1GSK     -----MTLEKFVDALPIPDTLKPVQQSK------EK-TYYEVTMEECTHQLHRDLPPTRLWGYNGLFPGP  58
1KV7     ---------AERPTLPIPDLLTTDA-----------RNRIQLTIGAGQSTFGGK--TATTWGYNGNLLGP  48
1KYA     --------------------GIGP----------------VADLTITNAAVSPDGFSRQAVVV-NGGTPGP  34

2XLL     TFQVPRGVETVVRFINNAE------------------------APNSVHLHGSFS--RAAFDGWAE--DI 111
1GSK     TIEVKRNENVYVKWMNNLPSTHF--LPIDHTIHHSDSQHEEPEVKTVVHLHGGVT--PDDSDGYPE--AW 121
1KV7     AVKLQRGKAVTVDIYNQLT------------------------EETTLHWHGLEV--PGEVDGGPQ--GI  90
1KYA     LITGNMGDRFQLNVIDNLT-NHTML------------------KSTSIHWHGFFQKGTNWADGPAFINQC  85

2XLL     TEP-GS-----------FKDYYYPNRQSARTLWYHDHAMHITAENAYRGQAGLYMLTDPAEDALN---LP 166
1GSK     FSK--D-FEQTGPYFKR-EVYHYPNQQRGAILWYHDHAMALTRLNVYAGLVGAYIIHDPKEKRLK---LP 185
1KV7     IPP--GG----------KRSVTLNVDQPAATCWFHPHQHGKTGRQVAMGLAGLVVIEDDEILKLM---LP 145
1KYA     PISSGH-----------SFLYDFQVPDQAGTFWYHSHL--ST--QYCDGLRGPFVVYDPNDPA--ADLYD 138

2XLL     SGYGEFD--IPMILTSKQYTANGNLVTTNGE----------------------LNSFWGDVIHVNGQP- 210
1GSK     SD--EYD--VPLLITDRTINEDGSLFYPS--APENPSPSLPNPSI---------VPAFCGETILVNGKV- 239
1KV7     KQWGIDD--VPVIVQDKKFSADGQIDYQL---------------DVMT-----AAVGWFGDTLLTNGA-- 191
1KYA     V----DNDDTVITLVDWYH------VAAK---------------------LGPAFPLGADATLINGKGR- 176

2XLL     ---------WPFKNVEP-RKY-RFRFLDAAVSRSFGLYFADTDAIDT-RLPFKVIASDSGLLEHPADTSL 268
1GSK     ---------WPYLEVEP-RKY-RFRVINASNTRTYNLSLD-------NGGDFIQIGSDGGLLPRSVKLNS 291
1KV7     ---------IYPQHAAP-RGWLRLRLLNGCNARSLNFATS-------DNRPLYVIASDGGLLPEPVKVSE 244
1KYA     SPSTTTADL-SVISVTPGKRY-RFRLVSLSCDPNYTFSID-------GH-NMTIIETDSINT-APLVVDS 235

2XLL     LYISMAERYEVVFDFSDYAGKTIELRNLG----GSIGGIGTDTDYDN----------------------- 286
1GSK     FSLAPAERYDIIIDFTAYEGESIILANSA-----------------GCGGDVNPE-------------- 309
1KV7     LPVLMGERFEVLVEVNDNK-PFDLVTLPSQMGM------AIAP-------------FDKP---------- 285
1KYA     IQIFAAQRYSFVLEANQAV-DNYWIRANP-----------------------------NFGNVGFTGG 252

2XLL     -TDKVMRFVVADDTTQPDTSVVPANLR-------------------------DVPF-PSPTT------- 346
1GSK     TDANIMQFRVTKPLAQKDESRKPKYLA-------------------------SYPSVQHERI------- 366
1KV7     ---HPVMRIQP--IAISASGALPDTLS-------------------------SLPA-LPSLEG------ 317
1KYA     I--NSAILRYD----------------GAAAVEPTTTQTTSTAPLNEVNLHPLVATA-VP----GSPVAG 320

2XLL     --NTPRQFRFGRTG-------------------------------------------------------PT 360
1GSK     --QNIRTLKLAGTQDEYGR-------------------------------------------------PV 385
1KV7     --LTVRKLQLSMDP-----MLDMMGMQMLMEKYGDQAMAGMDHSQMMGHMGHGNMNHMNHGGKFDFHH-A 379
1KYA     GVDLAINMAFNFNG-------------------------------------------------------TN 336

2XLL     WTINGVAFADV-------------------------QNR-LLANVPVGTVERWELINAGN--GW----- 496
1GSK     LLLNNKRW---HD-------------------------PVTETPKVGTTEIWSIINPT---RG----- 417
1KV7     NKINGQAF---D-MN-----------------------KPMFAAAKGQYERWVISGV--GDMM----- 413
1KYA     FFINGASFT------PPTVPVLLQIISGAQNAQDLLPSGS-V-YSLPSNADIEISFPAT------AAAPG 392

2XLL     -THPIHIHLVDFKVISRTSGNNAR-----------------------------TVMPYESGLKDVVWL 434
1GSK     -THPIHLHLVSFRVLDRRP-----------------FDIARYQESGELSYTGPAVPPPPSEKGWKDTIQA 469
1KV7     -LHPFHIHGTQFRILS--------ENGK-------------------------PPAAHRAGWKDTVKV 447
1KYA     APHPFHLHGHAFAVVR--------SAGSTVYNYDNP-------------------------IFRDVVST 428

2XLL     GRR---ETVVVEAHYAPFPGV-----YMFHCHNLIHEDHDMMAAFNATV---LPDYGYNATVFVDPMEEL 493
1GSK     HAG---EVLRIAATFGPYSGR-----YVWHCHILEHEDYDMMRPMDITDPHK---------------- 513
1KV7     EGN----VSEVLVKF-NHDA-PKEHAYMAHCHLLEHEDTGMMLGFTV----------------------- 488
1KYA     GTPAAGDNVTIRFRT-DNPGP-----WFLHCHIDFHLEAGFAVVFAED----------------------- 470

2XLL     WQARPYELGEFQAQSGQFSVQAVTERIQTMAEYRPYAAADE---------------------------- 534
1GSK     ------------------------------------------------------------------- 513
1KV7     ------------------------------------------------------------------- 488
1KYA     ----------------------------------------IPDVASANPVPQAWSDLCPTYDARDPSDQ 499
```

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Table S3. Bond lengths and angles between Cu atoms within the trinuclear cluster, determined from the crystal structure of *Mv* BOx using PyMOL. Values are averages across the four protein molecules (A–D) in the asymmetric unit, with standard deviations (if non-zero) given in brackets.

Bond Lengths / Å (σ)	
Bonds to $T3_a$	
His_{96}-N – Cu	2.2
His_{134}-N – Cu	2.2
His_{458}-N – Cu	2.3
Bonds to $T3_b$	
His_{136}-N – Cu	2.13 (0.05)
His_{403}-N – Cu	2.05 (0.06)
His_{456}-N – Cu	2.13 (0.05)
Bonds to T2	
His_{94}-N – Cu	2.05 (0.06)
His_{401}-N – Cu	1.93 (0.05)
Cu-Cu bond lengths	
$T3_a$ – $T3_b$	4.9
$T3_a$ – T2	4.1 (0.08)
$T3_b$ – T2	4.1 (0.08)
Angles / ° (σ)	
∠ T2 – $T3_a$ – $T3_b$	53.3 (1.03)
∠ T2 – $T3_b$ – $T3_a$	53.4 (0.78)
∠ $T3_a$ – T2 – $T3_b$	73.3 (0.34)