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Supporting Information

Quantum Refinement Does Not Support Dinuclear Copper Sites in Crystal Structures of Particulate Methane Monooxygenase

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Methods

Quantum refinement

Quantum refinement is standard crystallographic refinement supplemented by QM calculations for a small, but interesting part of the protein.[1,2] Crystallographic refinement software change the protein model (coordinates, occupancies, B factors, etc.) to improve the fit of the observed and calculated structure-factor amplitudes (usually estimated as the residual disagreement, the *R* factor). Owing to the limited resolution normally obtained for biomolecules, the experimental data are supplemented by some chemical information, usually in the form of a MM force field.[3] Then, the refinement takes the form of a minimization or simulated annealing calculation by molecular dynamics using an energy function of the form

$$E_{\mathrm{cryst}} = w_{\mathrm{A}}\, E_{\mathrm{Xray}} + E_{\mathrm{MM}} \qquad (1)$$

where E_{Xray} is a penalty function that describes how well the model agrees with the experimental data (we have used a maximum-likelihood refinement target using amplitudes, MLF[4,5]). E_{MM} is an empirical (molecular mechanics, MM) energy function with bond, angle, dihedral and non-bonded terms, and w_{A} is a weight factor, which is necessary because E_{MM} is in energy units whereas E_{Xray} is unit-less. It determines the relative importance of the crystallographic raw data and the MM force field for the final structure.

Quantum chemistry can be introduced in this function by replacing the MM potential for a small region of the protein (system 1) by QM calculations, yielding a QM energy for system 1, E_{QM1}. To avoid double counting, we must then subtract the MM energy of system 1, E_{MM1},

$$E_{\mathrm{cqx}} = w_{\mathrm{A}}\, E_{\mathrm{Xray}} + E_{\mathrm{MM12}} + w_{\mathrm{QM}}\, E_{\mathrm{QM1}} - E_{\mathrm{MM1}} \qquad (2)$$

Thereby, we introduce an accurate energy function for the system of interest. Such an energy function is implemented in the software ComQum-X,[1] which is a combination of the QM software Turbomole[6] and the crystallography and NMR system (CNS), [7,8] version 1.3. The factor w_{QM} in Eq. 5 is another weight, which is needed because the CNS MM force field is based on a statistical analysis of crystal structures.[9] Therefore, the force constants are not energy-derived, as is the QM term, but they are in arbitrary statistical units. Experience has shown that the CNS force constants are typically three times larger than energy-based force constants,[9] and $w_{\mathrm{QM}} = 3$ has therefore been used throughout this work.[1] Crystallographic refinement is traditionally performed without any electrostatic interactions, because hydrogen atoms are not discerned in the structure. We have followed this custom and excluded electrostatics and hydrogen atoms from all crystallography and MM calculations (but they are of course included in the QM calculations).

Two crystal structures were studied in this investigation, viz. the revised pMMO structure from *M. capsulatus* (Bath) (3RGB) at 2.8 Å resolution[10] and the 2.68-Å structure of pMMO from *Methylocystis sp.* strain M (3RFR).[10] Coordinates, occupancies, B factors and structure factors were obtained from the protein databank files. From these files, we also obtained the space group, unit-cell parameters, resolution limits, *R* factors and the test set used for the evaluation of the R_{free} factor.

The full protein was used in all calculations, including all crystal water molecules. In each cycle of the geometry optimization, the surrounding protein was allowed to relax by one cycle of crystallographic minimization and one cycle of individual B-factor refinement. However, the new coordinates and B factors were accepted only if the *R* factor was reduced. The w_{A} factor was determined by CNS in the first iteration of the refinement and then kept fixed. It was 4.91 for 3RGB and 4.93 for 3RFR. Electron-density maps were generated using *phenix.maps*.[11]

We used the standard CNS MM force field for all atoms (protein_rep.param, water_rep.param, and ion.param). For the junctions, where a bond is broken between the QM and MM regions, we employed the hydrogen link-atom approach i.e. the QM systems were truncated by H atoms (called HL below), replacing a C atom in the protein (called CL). The force field for the QM system was constructed automatically from that of the protein. For the HL atoms, we employed the same angle and dihedral parameters as for the CL atoms. For the XL–HL bond (where XL is the atom in the QM system bound to HL), we used as the equilibrium bond length ($r^0_{\text{XL-HL}}$) that obtained by geometry optimisation with the TPSS-D3/def2-SV(P) approach for a model of the isolated ligand (e.g. imidazole). The force constant ($k_{\text{XL-HL}}$) was obtained from the corresponding force constant and equilibrium bond length of the XL–CL bond according to:

$$k_{\text{XL-HL}} = k_{\text{XL-CL}} \left(\frac{r^0_{\text{XL-HL}}}{r^0_{\text{XL-CL}}}\right)^2 \tag{3}$$

Van der Waals parameters of 0.0188 kJ/mol and 2.2272 Å were used for all junction atoms (taken from the HA atom type).

The quality of the models was compared using the real-space difference density Z-score[12] (RSZD), calculated by EDSTATS in the CCP4 suite,[13] which measures the local accuracy of the model. The maximum of the absolute values of the positive and negative RSZD (combined RSZD) for the Cu ion and all the first-sphere ligands was taken as the quality metric (individual values for each of the groups are given in the supplementary material). It is called RSZD in the main text and RSZD_{max} in this supplementary material (to differ it from RSZD for the individual residues and atoms, which also will be discussed). RSZD is typically < 3.0 for a good model. In addition, the strain energy of the active site (ΔE_{str}) was calculated, i.e. the difference in the QM energy of the QM region when optimised in the crystal or freely in vacuum.[14]

QM calculations

All QM calculations were performed with the Turbomole software, version 7.1.[6] We employed the density-functional theory (DFT) method TPSS[15] and the def2-SV(P)[16] basis set. The calculations were sped up by expanding the Coulomb interactions in an auxiliary basis set, the resolution-of-identity (RI) approximation.[17,18] Empirical dispersion corrections were included with the DFT-D3 approach[19] and Becke–Johnson damping,[20] as implemented in Turbomole.

The QM system consisted of one or two Cu ions, two imidazole groups modelling the sidechains of His-137 and 139, and $H_2N–(CH_2)_2$–imidazole, as a model of His-33 (sidechain and backbone). Sometimes, one or two water molecules were added or models of Glu-35, Asp-79C (modelled by an acetate groups), Tyr-374 (modelled by phenol) or His-137 was extended to imidazole–$(CH_2)_2$–CO–NH–CH_3, including the backbone towards the next residue. The Cu ions were always modelled in both the Cu^+ and Cu^{2+} states, in the latter case with unrestricted formalism. For dimeric Cu sites, we employed three oxidation states: fully reduced, fully oxidised and the mixed-valence Cu^+–Cu^{2+} state. After some test calculations, we decided to use the ferromagnetically coupled triplet state for the fully oxidised state, which was lower in energy than the antiferromagnetically coupled open-shell singlet state (obtained with the broken-symmetry approach), owing to the lack of any strong bridging ligand.

For the study of the reaction mechanism (Figure 6 in the main article), structures with the same QM model (plus a methane molecule) were optimised in gas phase with the TPSS-D3/def2-SV(P) approach. Then, more accurate single-point energies were calculated with the def2-TZVPD[21,22] basis set and both the TPSS-D3 and B3LYP-D3[23–25] methods. These calculations were run in a COSMO continuum solvent.[26,27] The default optimised COSMO radii (and a water solvent radius of 1.3 Å),[28] whereas a radius of 2 Å was used for the metals.[29] The dielectric constant was 4, whereas we used default values for all other parameters. Frequency calculations were performed at the TPSS-D3/def2-SV(P) level of theory and from these, zero-point energies were calculations and it was confirmed that transition states had one and minima had no imaginary frequencies.

Details of quantum refined structures of protomer 1 of the 3RGB structure

We started by performing quantum refinement of site A in the first protomer of the 3RGB structure using a dinuclear Cu site (called C2W0 in the tables to indicate that it contains two Cu ions and no extra water molecules). This gave structures quite similar to the starting crystal geometry as can be seen in Figures S1a and S1b: The two Cu ions are on top of each other, but in variance to the starting structure (Figure 1a in the main article), all three imidazole groups are directed towards the Cu ions with the ND1 (His-33 and His-137; called N_A and N_B in the following and in the Tables) or NE2 (His-139; N_C) atoms binding. One of the Cu ions (Cu_A) coordinates to all four N atoms, whereas the other (Cu_B) coordinates to only one or two of them (N_A and possibly also N_C). The distance to the His-33 backbone amide group (N_D) is typically longer than that to the imidazole groups. The detailed distances depend on the oxidation states of the two Cu ions and are listed in Table S1. In the (fully) reduced state, the two Cu_B–N distances (to N_A and N_C) are short (2.02–2.03 Å), whereas the four Cu_A–N distances are longer and much more varying (2.07–2.63 Å). This reflects the preference of Cu^+ for low coordination numbers. However, in the mixed-valence (MV) and (fully) oxidised states, the Cu_A–N distances to the imidazole groups are short, 2.05–2.15 Å, that to N_D is somewhat longer, 2.24–2.30 Å, whereas only the Cu_B–N_A distance is short (2.08–2.13 Å) and the Cu_B–N_C bond is essentially broken, 2.89–2.93 Å. The Cu–Cu distance is 2.21–2.25 Å. The spin densities show that Cu_B is actually in the reduced state also in the fully oxidised structure. Instead, the spin is spread over Cu_A (0.34 *e*) and the ligands. Also in the MV state, there is no spin on Cu_B and only 0.41 *e* spin on Cu_A.

If we set $w_A = 0$ in Eqn. 2, i.e. turn off the restraint towards the experimental data, thereby obtaining a QM/MM calculation with the CNS MM force field, the MV and especially the oxidised structures reorganise significantly, as can be seen in Table S1 (method Q). However, the reduced structure remains more intact, with both Cu ions two-coordinate with Cu–N distances of 1.89–2.08 Å and a Cu–Cu distance of 2.46 Å. This reflects the intrinsic preferences of the metals and the ligands in the protein.

Further information about the preferences of the metal can be obtained by freely optimising the metals and the four ligands in vacuum, i.e. ignoring the covalent and steric influence of the protein (besides the covalent link between N_A and N_D). For the reduced site, this gave little changes from the QM/MM structure (cf. Table S1, method V). For the MV and oxidised states, the differences were larger, but mainly related to Cu_B, which bound with side-on interactions with the imidazole rings, because all N atoms were occupied in a square-planar coordination to Cu_A.

To obtain structures that better fitted the experimental data, we attempted to model the site in other ways. First, we tried to use the model suggested by Yoshizawa and coworkers in their QM/MM studies of the reaction mechanism,[30–32] i.e. a dimeric model with the two Cu ions and the four N ligands in the same plane (Figure S2a). We could successfully obtain such a structure by model building and then refining the structure with $w_A = 0$. The structures are described in Table S1 (called Y2W0a). Cu_A binds to His-33 (both sidechain and backbone amide) and His-137 with Cu_A–N = 1.97–2.09 Å. The latter group bridges to Cu_B, which also binds to His-139 with distances of 1.86–1.99 Å. The Cu–Cu distance is 2.35–2.45 Å, actually increasing with the oxidation state of the Cu ions. However, if we turned on the restraint towards the experimental data, the site went back to something similar to the original QM-refined structures, i.e. with the four N atoms mainly coordinating to Cu_A.

A prime problem with site A, especially in the higher oxidation states, is that it contains too few ligands for two metal ions. Therefore, we tried to enhance the site with two water molecules. After many attempts, we were able to obtain some reasonable structures and the best (in terms of $RSZD_{max}$) are shown in Tables S1 and S2 (called Y2W2), and in Figure 2b. In the MV and oxidised structures, Cu_A is still coordinated by all four N atoms in a square plane (Cu–N distances of 1.98–2.13 Å) and also by one of the water molecules in an axial position (2.52–2.53 Å). N_B and N_C bridges to Cu_B but with a quite poor geometry (2.07–2.19 Å). Cu_B also binds to the other water molecule (2.02–2.04 Å). The $RSZD_{max}$ scores are higher for the reduced structure, but appreciably better for the MV and oxidised states (1.5) than the structures started from the original crystal structure. The strain energies are also appreciably better, 149–159 kJ/mol. However, the $2F_o - F_c$ electron-density maps in Figure 3a in the main article show that there is no electron density around the water molecules.

Consequently, we also optimised the corresponding structures without the water molecules (Y2W0 in Tables S1 and S2). They are more similar for all three oxidation states (except that N_C does not bind to Cu_A in the reduced state) with Cu_A–N distances of 1.91–2.19 Å and Cu_B–N distances of 1.90–2.28 Å to N_B and N_C. They gave slightly better $RSZD_{max}$ scores (1.3 for the MV and oxidised states), but somewhat higher strain energies (170–220 kJ/mol).

Yoshizawa and coworkers have also sometimes suggested structures in which nearby residues coordinate to site A.[30–32] Therefore, we tried to enhance the QM system with models of the sidechains Glu-35, Asp-79C or Tyr-374 and then repeated the QM refinement. However, it turned out that these residues were too far away in the crystal structure to coordinate to any of the Cu ions and therefore the structures were closely similar to those obtained without any added residues (Table S1). However, if the QM region was extended with the carbonyl group of His-137, this group was found to coordinate to Cu_B with Cu–O distances of 2.30–2.52 Å in all three oxidation states as can be seen in Figure S2c. This led to a shortening of the Cu_A–N_D bond (2.16–2.19 Å) and a slight elongation of the Cu_A–N_B bond (2.11–2.23 Å), but otherwise the structures were similar to the original QM-refined structures. Yet, these structures reproduced the crystallographic data quite poorly, with $RSZD_{max}$ scores of 2.6–2.7 (Table S2). The strain energies were also nearly as high as for the original C2W0 structures, 178–226 kJ/mol.

Finally, we instead tried to model site A with a single Cu ion (C1W0 in Tables S1 and S2). This gave structures that were similar to that of Cu_A in the dinuclear sites. The geometry was distorted square planar with a rather small N_B–Cu–N_D angle of 117° (reduced state) to 133° (oxidised state), as can be seen in Figure S3a and S3b. Otherwise, the geometry was quite regular. In the oxidised state, the Cu–N distances were 1.85–2.22 Å, with the longest bond to N_D. In the reduced state, the three bonds to the sidechains were similar (1.84–2.23 Å), but the bond to N_D increased to 2.62 Å. If the same structures were optimised with $w_A = 0$, the structures became more square-planar with N_B–Cu–N_D angles of 139° and 165°. The Cu–N distances become more similar, 1.97–2.10 Å for the oxidised state and 1.96–2.12 (N_A–N_C) and 2.33 Å (N_D) for the reduced state. If the structures were instead optimised in vacuum, the reduced state became tetrahedral with all four Cu–N distances of 1.99–2.05 (N_A–N_C) and 2.14 Å (N_D), whereas the oxidised state became almost perfectly square planar (N_B–Cu–N_D angle of 169°) with Cu–N distances of 1.99–2.02 (N_A–N_C) and 2.07 Å (N_D). In all three oxidised structures, the spin of the Cu ion was ~0.5 *e*.

There remains some positive difference density on top of the Cu ion in this structure, which was interpreted as an additional Cu ion in the original crystal structure. Yet, it is too close to the Cu ion and with a too low density to be another metal. Therefore, we tried to model it as a water molecule instead (C1W1 in Tables S1 and S2). In the reduced state, the water molecule did not coordinate to Cu (as could be expected from the preference of Cu^+ for low coordination numbers). Therefore, the structure did not change significantly from that obtained without the water molecule. However, for the oxidised state, the water molecule coordinated with a Cu–O distance of 2.20 Å as can be seen in Figure S4. As a consequence of the increased coordination number, the distance to the other ligands increased by 0.03–0.07 Å. If optimised with QM/MM or in vacuum, the Cu–O distance increased somewhat (2.25–2.31 Å) and the Cu–N distances became more similar.

The other protomers of the 3RGB structure

Next, we studied site A in the other two protomers of the 3RGB structure. As mentioned in the main text, the structure of these two sites is different from that in the first protomer. This was also reflected in the quantum-refined structures of this site, started from the crystal structure with two Cu ions (C2W0): For all three oxidation states, strongly distorted structures were obtained with Cu_A coordinating to His-137 and bidentately to His-33 as can be seen in Figure S5a. His-137 bridges to Cu_B, which also interacts with the sidechain of His-139, but with a far-from-ideal, side-on geometry. The corresponding distances are listed in Table S1. The $RSZD_{max}$ scores were worse than in protomer 1, 2.8–2.9, whereas the strain energies were similar, 178–247 kJ/mol (Table S2). Protomer 3 gave similar structures (also described in Tables S1 and S2). However, the $RSZD_{max}$ scores were somewhat smaller, 2.0–2.8, as was the strain energies (160–224 kJ/mol).

To check whether this striking difference in the metal-site geometry is really dictated by the crystallographic data, we used the trifold non-crystallographic symmetry to copy the structure of protomer 1 to site A in protomer 2 and 3 also. However, the structures converged back to the original structures again.

Next, we also optimised Yoshizawa-type dinuclear structures in the other two protomers (Y2W2). They are also presented in Tables S1, S2 and Figure S5b, and it can be seen that the structures are rather similar to those in protomer 1, but with some variation. For protomer 2, these structures fitted the crystallographic data better than the other dinuclear structures with $RSZD_{max}$ scores of 1.9–2.2 and strain energies of 110–144 kJ/mol. However, for protomer 3, they were much worse in terms of $RSZD_{max}$ (2.9–3.2), but the strain energies were still lower (104–160 kJ/mol).

We also optimised the corresponding structures without water molecules (Y2W0). From the results in Tables S1 and S2, it can be seen that they were similar to the corresponding structures with water, except that for Cu_B in protomer 3, N_D was replaced by N_A. In protomer 3, these structures gave lower $RSZD_{max}$ scores than the structures with water, but higher strain energies, whereas both measures were worse for protomer 2.

Next, we tried to model a single Cu ion in both protomers. This gave structures that were similar to the corresponding structures in protomer 1 (Figure S5c). The Cu–N distances (shown in Table S1) were also rather similar. The electron-density maps were also better than those of the dinuclear Cu model, with $RSZD_{max}$ of 1.1–1.7 for protomer 2 and 1.5–2.0 for protomer 3. The strain energies were also much lower than for the dinuclear structures, 37–76 kJ/mol.

Finally, we also tried a single Cu ion with an additional water molecule. It gave results similar to that of protomer 1, with the difference that the water molecule did not coordinate to

Cu in protomer 3. However, in both protomers, the structures without the axial water molecule gave lower RSZD$_{max}$ scores and strain energies (Table S2).

The 3RFR crystal structure

Finally, we considered the 3RFR crystal structure. As mentioned in the introduction, it contains a dinuclear site A only in protomer 1, whereas this site is mononuclear in the other two protomers. Therefore, we performed quantum-refinement also of that site, using the 3RFR crystallographic data. With two Cu ions in the oxidised state (C2W0), the dinuclear site gave a structure similar to that of the first protomer in the 3RGB crystal (Table S1). However, for the reduced state, the structure was distorted and less similar. The side chains of His-137 and 139 coordinate primarily to Cu$_A$, whereas both N$_A$ and N$_D$ coordinate primarily to Cu$_B$, although all atoms except N$_B$ are within 3 Å also of the other Cu ion. The Cu–Cu distance is 2.24 Å. These structures fitted the crystallographic raw data quite poorly with RSZD$_{max}$ scores of 1.6–1.9 and very high strain energies, 292–357 kJ/mol.

However, much more reasonable structures were obtained with a single Cu ion in this site. Both the reduced and oxidised states gave almost perfectly square-planar structures with N$_B$–Cu–N$_D$ angles of 171–177°. The Cu–N distances were similar to those of the 3RGB structure, 1.94–2.40 Å for the reduced state and 1.96–2.15 Å for the oxidised state (Table S1). These structures also fitted the experimental data better, with RSZD$_{max}$ = 1.3–1.4 and strain energies of 32 (oxidised state) or 88 kJ/mol (reduced state). Structures with a coordinated water molecule could also be obtained for both oxidation states (Cu–O distances of 2.43–2.50 Å). However, they gave larger RSZD$_{max}$ scores of 2.3 and 2.6.

For the other two protomers, the original crystal structure contained mononuclear sites. This was confirmed by the quantum-refinement, which gave structures similar to the other mononuclear sites in the 3RGB or 3RFR structures (Tables S1 and S2). However, no structures were obtained with a bound water molecule, not even for the oxidised state. The structures fitted the experimental data appreciably better than for protomer 1, with RSZD$_{max}$ = 0.4–0.9 without the water molecule. On the other hand, the strain energies were similar, 37–47 kJ/mol for the oxidised state and 89–101 kJ/mol for the reduced state.

Discussion of different RSZD scores

In this study, we have used quantum refinement to re-refine the putative dinuclear Cu site A in two crystal structures of pMMO. We have tried several different dinuclear structures, as well as a mononuclear structure in all three protomers in both enzymes and also tried to add one or two extra water molecules. We have observed some variation among the two crystal structures and the three protomers.

In terms of the strain energies, our results are quite conclusive (Table 1 in the main article): The mononuclear models always give lower strain energies than the dinuclear models (37–85 kJ/mol, compared to 104–300 kJ/mol). For the same crystal and protomer, the best mononuclear structure always give a 63–85 kJ/mol lower strain energy than the best dinuclear structure. This confirms the impression that the dinuclear structures are not reasonable, even after the quantum refinement.

The RSZD$_{max}$ results are slightly less conclusive (Tables 1 and S2). For the 3RFR structure and protomers 2 and 3 of the 3RGB structure, the mononuclear sites still give the lowest scores (1.3 compared to 1.6 in 3RFR, 1.1 compared to 1.9 in protomer 2 and 1.5 compared to 1.8 for protomer 3 of 3RGB). However, for protomer 1 in 3RGB, the lowest scores are actually obtained for the Y2W0 structure (1.3, compared to 1.5 for the best mononuclear site). On the other hand, it is not entirely clear how to compare RSZD scores between sites of different composition. The RSZD scores are calculated for individual residues (viz. for His-33, 137 and 139, as well as for the Cu ions and water molecules; the individual RSZD result are shown in Table S2). These are not the same for all systems and they do not cover the same space in the various structures. Moreover, it is unclear how to combine 4–7 RSZD results to a single score in and unambiguous manner: We have used the maximum value (RSZD$_{\text{max}}$, called simply RSZD in the main article) , but other measures are also conceivable. Table S2 show two additional scores, obtained from the sum or the average. In fact, the mononuclear site gives the lowest RSZD score if the sum is considered (3.1, compared to 3.4), whereas the Y2W2 structure gives the best results if the average is considered (0.5 compared to 0.7).

A third argument against a dinuclear site is the large variation of the dinuclear structures in the various protomers and crystals: The geometries vary extensively (cf. Table S1) and the best one also varies between the crystals and protomers (the Y2W0 structure is best in protomer 1, the Y2W2 in protomer 2, whereas C2W0 is best in protomer 3 of the 3RGB structures.

Taken all results together, we find no conclusive evidence that the crystal structures of pMMO show a dinuclear Cu site. Instead, a mononuclear site fits the experimental data at least as well and it also gives chemically much more reasonable structures that are similar in both crystals and all protomers. Such a site is more similar to the mononuclear Cu site in the lytic polysaccharide monooxygenases, although it contains an additional histidine residue and lacks the nearby tyrosine residue.[33] The main evidence for a dinuclear site comes from EXAFS investigations, showing a striking reproducibility of the Cu–Cu interaction in pMMOs from four different methanotrophs.[10,34–36] One possibility is that the dicopper site detected by EXAFS is disrupted upon crystallization. On the other hand, EXAFS fits are known to give multiple local minima, so that different structures can give equally good fits.[37,38] This will be examined in future studies by a similar combination of computational and EXAFS methods.[39]

Reaction mechanism

To provide a further check of the suggested mononuclear Cu site, we studied the reaction of the suggested mononuclear active site with a molecule of methane. Yoshizawa and Shiota have already studied with QM method putative reaction mechanisms of mono- and dinuclear Cu sites in pMMO.[30] However, their mononuclear model came from site B in the protein and therefore contained two His side chains and an acetate group and no His brace or amino group. Therefore, we repeated their study using our preferred mononuclear site in Figure 4 in the main article, i.e. with three His side chains, one of which is connected to a terminal amino group, as is shown in Figure 6 in the main article. Geometries were optimised at the TPSS-D3/def2-SV(P) level of theory in gas phase. Then, more accurate energies were calculated with the def2-TZVDP basis set with both the TPSS-D3 and B3LYP-D3 methods in a COSMO continuum solvent with a dielectric constant of 4. All energies are given in Table S3. Reported energies in Figure 6 and in the text are B3LYP-D3/def2-TZVPD energies, including the zero-point energy, obtained from frequencies at the TPSS-D3/def2-SV(P) level of theory.

Following the previous study, we started with a Cu(III) ion with an oxo ligand (or equivalently Cu(II)–O$^{\bullet-}$; net charge +1; called RS in Figure 6). All structures were studied in both the singlet and triplet states. In both cases, there was 0.5 spin on the Cu ion, 0.05–0.06 spins on each His ligand and the remainder on O (0.7 for the singlet and 1.3 for the triplet). In the singlet, the O spin was opposite to that of the other atoms and groups. Unlike the study by Yoshizawa and Shiota,[30] the two states were not degenerate; instead the triplet was 85 kJ/mol more stable than the singlet. Next, a methane molecule was added to the complex, forming a weak hydrogen bond to the oxo group (2.58 Å CH–O distance; R). It had nearly the same energy as the RS structure (plus a free methane molecule).

A transition state (TS1) for the transfer of one of the hydrogen atoms of the methane molecule could be located. It had a CH–O distance of 1.17 Å and was 22 kJ/mol higher in energy than RS for the triplet. It is characterised by a single imaginary frequency of 1475*i* cm^{-1}, similar to that found by Yoshizawa and Shiota (1445*i* cm^{-1}).[30] This transition state leads to an intermediate with Cu–OH interacting with a methyl radical (Im1). It had a O–H distance of 0.99 Å and a OH–C distance of 2.18 Å and it was 16 kJ/mol more stable than RS. The Cu ion still had 0.5 spin, whereas 0.3 spin resided on OH and there was 0.95 spin on the methyl radical.

This intermediate could rearrange essentially barrierless to a second intermediate (Im2) in which the methyl radical also binds to the Cu ion. In this intermediate, the spins on Cu and OH have increased slightly, whereas that on the methyl radical has decreased strongly to 0.6. For the triplet state, Im2 is 10 kJ/mol less stable than Im1. However, for the singlet-state structures (which up to Im1 has been 75–150 kJ/mol less stable than the triplet states), Im2 is strongly stabilised and actually becomes 46 kJ/mol more stable than the triplet state (and 52 kJ/mol more stable than ^{3}RS). Therefore, we suggest, in accordance with the study of Yoshizawa and Shiota,[30] that the spin state changes from triplet to singlet at this point. ^{1}Im2 is a closed-shell singlet, indicating a Cu(III)–OH^-–CH_3^- complex.

Finally, the methanol product (P) can form by a recombination of OH and the methyl group. The corresponding transition state (TS2) has a rather complicated structure because both groups are still bound to Cu. The C–O distance is 1.91 Å and the imaginary frequency is 396*i* cm^{-1}, slightly smaller than that obtained by Yoshizawa and Shiota (473*i* cm^{-1}).[30] It is still a closed-shell species and it is 53 kJ/mol higher in energy than ^{1}Im2. In the product, methanol has dissociated from the Cu(I) ion and is 217 kJ/mol more stable than the starting point (^{3}RS).

From the energy components in Table S3, it can be seen that the effect of an increase in the basis set from def2-SV(P) to def2-TZVPD (for the TPSS-D3 method) is quite small for the triplet state (up to 17 kJ/mol, in general reducing the relative energies), whereas it is larger for the R, TS1, and Im1 states (up to 67 kJ/mol), increasing the energies. Likewise, the effect of changing the DFT functional from TPSS to B3LYP has a relatively small effect on the triplet energies (up to 24 kJ/mol, increasing the energies), but a much larger effect on the singlet energies (again up to 67 kJ/mol), although it is mainly an effect of decreasing the relative energy of all open-shell singlet states by 50–67 kJ/mol. Thus, the singlet and triplet states are much closer in energy at the TPSS level of theory. The zero-point energy has a rather small effect on all energies, up to 17 kJ/mol with a varying sign (but nearly the same energy for the singlet and triplet states).

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Table S1. Cu–ligand distances (Å) in the various complexes. The results for different crystal structures and protomers are presented in separated sections of the table. The structures are described with a four-letter code, giving the number of Cu ions (C2 and C1; Y2 represent Yoshizawa-like structures with two Cu ions, such as those in Figure S2) and the number of water molecules, W2, W1 or W0 (the Glu35, Asp79, Tyr374 and His137 structures of 3RGB, subunit A all represent C2W0 structures, but with enhanced QM regions). The methods are quantum refinement (R), QM/MM (Q; i.e. quantum refinement with $w_A = 0$) and vacuum optimisations (V). State is the oxidation state of the Cu ion(s), reduced (Red; one or two Cu^I), oxidised (Ox; one or two Cu^{II}) or mixed valence (MV; Cu^I+Cu^{II}). Cu–ligand distances are reported only if they were shorter than 3.0 Å. N_A, N_B and N_C are the coordinating sidechain atoms of His-33, His-137 and His-139, respectively. N_D is the backbone (amino-terminal) N atom or His-33. O_1 and O_2 are the O atoms of the water molecules that are present in some structures. In the structure with the QM region enhanced with the backbone of His137, O_1 represents the carbonyl oxygen.

Structure	Method	State	Distance to Cu_A							Distance to Cu_B					
			Cu_B	N_A	N_B	N_C	N_D	O_1	O_2	N_A	N_B	N_C	N_D	O_1	O_2
3RGB, protomer 1															
C2W0	R	Red	2.21	2.34	2.07	2.63	2.25			2.02		2.03			
		MV	2.25	2.07	2.10	2.06	2.24			2.08		2.89			
		Ox	2.25	2.05	2.15	2.06	2.30			2.13		2.93			
	Q	Red	2.46	2.76	2.01		2.08			1.89		1.89			
		MV	2.69	2.15	2.03	2.00	2.09			1.94					
		Ox	5.49	2.05	2.13	2.05	2.09								
	V	Red	2.54	2.72	1.91		1.96			1.90		1.89			
		MV	3.63	2.05	2.06	2.00	2.07			2.67	2.64				
		Ox	5.67	2.09	2.06	2.09	2.07								
Y2W0a	Q	Red	2.35	1.97	2.07		2.09				1.97	1.87			
		MV	2.43	1.97	2.08		2.07				1.99	1.86			
		Ox	2.45	2.01	2.07		2.08				1.99	1.87			
	R	Red	2.27	2.01	2.01	1.93				2.09			1.98		
		MV	2.59	1.91	2.17	2.09	2.02				2.15	2.11			
		Ox	3.54	1.98	2.03	2.03	2.02								
	V	Red	2.41	2.00	2.02		2.01				1.97	1.90			
Y2W2	R	Red	2.51	2.01	2.06	1.99	2.99	2.23		2.17			2.02	2.08	2.07
		MV	2.51	1.94	2.07	2.12	1.98	2.53			2.19	2.09			2.04
		Ox	2.51		2.19	2.09		2.04		1.94	2.07	2.12	1.98		2.53
		Ox,alt	2.60	1.98	2.06	2.13	1.98	2.52			2.19	2.07			2.02
	V	Red	2.56	1.98	2.08	1.96				2.50			1.95		1.94
		MV	2.46		2.15	1.96		2.06	2.18	1.98	2.01		2.00		2.89
		Ox	3.66			2.08		2.00	2.09	2.06	2.00	2.39	2.02		
Y2W0	R	Red	2.18	1.94	2.19		1.95				1.90	1.83			
		MV	2.42	1.91	2.08	2.13	2.00				2.21	2.11			
		Ox	2.47	1.98	2.05	2.13	2.00				2.28	2.11			
	V	Red	2.41	2.00	2.02		2.01				1.97	1.90			
		MV	3.93	1.98	2.10	2.10	2.06				2.85	2.83			
		Ox	3.89	2.12	2.07	2.08	2.06				2.89	2.83			
Glu35	R	Red	2.21	2.45	2.09	2.61	2.15			2.01		2.01	2.94		
		MV	2.21	2.34	2.07	2.62	2.17			2.03		2.03	2.94		
		Ox	2.20	2.26	2.07	2.57	2.16			2.05		2.08	2.95		
Asp79	R	Red	2.21	2.34	2.07	2.64	2.25			2.01		2.02			
		MV	2.21	2.34	2.07	2.64	2.25			2.01		2.02			
		Ox	2.25	2.09	2.10	2.09	2.25			2.06		2.79			

Tyr374	R	Red	2.21	2.35	2.06	2.64	2.24			2.01		2.02			
		MV	2.21	2.35	2.06	2.64	2.24			2.01		2.02			
		Ox	2.21	2.27	2.06	2.62	2.24			2.03		2.04			
His137	R	Red	2.25	2.23	2.11	2.49	2.19			2.09		2.19		2.52	
		MV	2.30	2.01	2.21	2.03	2.16			2.14		2.97		2.30	
		Ox	2.28	2.01	2.23	2.05	2.18			2.15		2.94		2.39	
	V	Red	2.49	2.90	1.92		1.98			1.93		1.93		2.13	
		MV	2.42		1.97	2.02	2.03	2.52		1.88			2.91	1.89	
		Ox	2.55		1.99	2.07	2.05	2.31		1.91			2.90	1.90	
C1W0	R	Red		1.84	2.23	1.90	2.62								
		Ox		1.85	2.13	1.94	2.22								
	Q	Red		1.96	2.12	2.02	2.33								
		Ox		1.97	2.06	2.01	2.10								
	V	Red		2.01	1.99	2.05	2.14								
		Ox		1.99	2.02	2.02	2.07								
C1W1	R	Red		1.87	2.25	1.93	2.55	3.09							
		Ox		1.89	2.16	1.99	2.29	2.20							
	Q	Ox		2.00	2.08	2.03	2.09	2.25							
	V	Red		2.28	2.01	2.02	2.06								
		Ox		2.02	2.03	2.04	2.08	2.31							
3RGB, protomer 2															
C2W0	R	Red	2.29	1.85		2.12	2.18				1.97	2.27			
		MV	2.36	1.84		2.12	2.13				1.95	2.23			
		Ox	2.40	1.86		2.13	2.13				1.96	2.25			
	V	Red	2.42	1.93		2.01	2.09				1.90	1.97			
		MV	2.55	1.94		2.04	2.07				1.94	1.96			
		Ox	3.62	2.00		2.10	2.07				1.94	2.02			
Y2W2	R	Red	2.51	1.92	2.06	2.03	2.34		2.83		2.88	2.47		1.95	1.94
		MV	2.96	1.89	2.07	2.05	2.18		2.28		2.31	2.14		1.93	
		Ox	3.00	1.92	2.07	2.07	2.19		2.30		2.28	2.14		1.93	
	V	Red	2.59	2.34	1.93		1.99		2.96			1.91		2.16	1.95
		MV	2.47	1.93		1.97	2.08		2.83		1.93	2.49		2.12	2.03
		Ox	2.96	2.07	2.10	2.24	2.07				2.21	2.07		2.04	2.05
Y2W0	R	Red	2.45	1.87	2.26	1.96	2.30				2.39	2.09			
		MV	2.92	1.87	2.07	2.01	2.15				2.45	2.24			
		Ox	3.12	1.93	2.05	2.01	2.13				2.59	2.36			
	V	Red	2.41	1.92		2.01	2.10				2.75	2.02			
		MV	3.89	1.98	2.10	2.10	2.07				2.84	2.82			
		Ox	3.90	2.12	2.07	2.09	2.06				2.90	2.83			
C1W0	R	Red		1.88	2.13	1.95	2.49								
		Ox		1.89	2.04	2.01	2.20								
	V	Red		2.01	1.98	2.05	2.14								
		Ox		1.99	2.02	2.02	2.07								
C1W1	R	Ox		1.91	2.02	2.02	2.21	2.37							
	V	Ox		2.02	2.03	2.04	2.08	2.34							
3RGB, protomer 3															
C2W0	R	Red	2.29	1.98		2.24	2.03				1.95	2.04			
		MV	2.30	1.95		2.17	2.00				1.93	2.06			
		Ox	2.33	1.98		2.15	2.00				1.93	2.08			
	V	Red	2.41	1.93		2.01	2.09				1.90	1.97			
		MV	2.56	1.94		2.03	2.07				1.89	1.95			
		Ox	3.62	2.00		2.10	2.07				1.94	2.02			
Y2W2	R	Red	2.47	2.21	2.04	2.01	2.21	2.89			2.93	2.65		1.94	1.92
		MV	3.94	2.00	2.02	2.01	2.11					2.72		1.89	
		Ox	3.93	2.07	2.01	2.01	2.11					2.74		1.92	

	V	Red	2.59	2.32	1.93		1.99	2.95			1.91		2.17	1.95
		MV	4.76	1.99	2.02	2.10	2.04						1.93	
		Ox	4.93	2.08	2.05	2.10	2.04						1.92	
Y2W0	R	Red	2.45	2.04	2.35	1.95	2.19			2.38	2.06			
		MV	2.77	2.01	2.08	2.00	2.11			2.36	2.23			
		Ox	2.86	2.09	2.06	1.99	2.11			2.47	2.29			
	V	Red	2.42	1.93		2.01	2.09			1.90	1.97			
		MV	3.90	1.98	2.11	2.10	2.07			2.84	2.82			
		Ox	3.90	2.12	2.07	2.08	2.06			2.90	2.83			
C1W0	R	Red		2.03	2.19	1.92	2.27							
		Ox		2.00	2.02	1.97	2.12							
	V	Red		2.01	1.99	2.05	2.14							
		Ox		1.99	2.02	2.02	2.07							
C1W1	R	Ox		2.02	2.01	1.98	2.12	3.05						
	V	Ox		2.02	2.03	2.04	2.08	2.34						
3RFR, protomer 1														
C2W0	R	Red	2.24	2.73	2.06	1.92	2.93		2.09		2.79	2.34		
		MV	2.34	2.23	2.07	1.92	2.54		2.36		3.00	2.46		
		Ox	2.32	2.34	2.11	1.93	2.70		2.32		2.94	2.17		
C1W0	R	Red		2.12	2.26	1.94	2.39							
		Ox		2.05	2.09	1.96	2.15							
C1W1	R	Red		2.06	2.19	1.92	2.76	2.43						
		Ox		2.04	2.08	1.96	2.14	2.50						
3RFR, protomer 2														
C1W0	R	Red		2.12	2.08	2.06	2.56							
		Ox		2.01	2.01	2.03	2.30							
C1W1	R	Ox		2.02	2.01	2.03	2.28							
3RFR, protomer 3														
C1W0	R	Red		2.00	2.31	1.96	2.34							
		Ox		2.00	2.10	1.96	2.16							
C1W1	R	Ox		2.01	2.08	1.96	2.15							

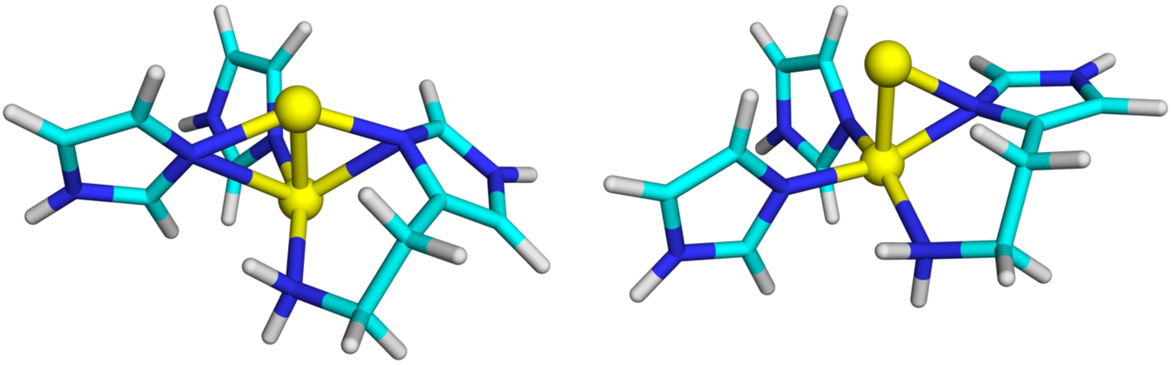
Table S2. RSZD scores for each ligand in the active site, together with the maximum, sum and average. The last two columns give R_{free} and R of the structures. PM, Struct and State are the protomer, the structure and the oxidation state of site A.

PM	Struct	State	RSZD										R_{free}	R
			H33	H137	H139	Cu_A	Cu_B	Wat_1	Wat_2	Max	Sum	Av		
3RGB crystal structure														
1	start		1.0	2.0	0.8	4.7				4.7	8.5	2.1		
	C2W2	Red	0.2	2.0	0.8	2.1	1.5			2.1	6.6	1.3	0.2831	0.2627
		MV	0.4	1.8	0.8	0.9	1.9			1.9	5.8	1.2	0.2832	0.2627
		Ox	0.5	1.9	0.9	0.8	2.1			2.1	6.2	1.2	0.2832	0.2627
	Y2W2	Red	1.3	1.6	0.4	0.0	0.8	0.4	3.2	3.2	7.7	1.1	0.2879	0.2437
		MV	1.0	1.5	0.5	0.1	0.6	0.2	0.3	1.5	4.2	0.6	0.2878	0.2437
		Ox	0.6	1.5	0.4	0.1	0.4	0.1	0.3	1.5	3.4	0.5	0.2878	0.2437
		Ox,alt	3.0	2.5	4.3	1.4	4.2	0.7	2.0	4.3	18.1	2.6	0.2879	0.2439
	Y2W0	Red	1.8	1.5	1.8	2.7	0.9			2.7	8.7	1.7	0.2881	0.2439
		MV	0.7	1.3	0.4	1.2	0.9			1.3	4.5	0.9	0.2879	0.2438
		Ox	0.8	1.3	0.6	1.1	0.6			1.3	4.4	0.9	0.2879	0.2438
	His137	Red	0.3	2.7	1.4	2.5	1.4			2.7	8.3	1.7	0.2831	0.2627
		MV	1.5	2.7	0.8	1.9	2.6			2.7	9.5	1.9	0.2831	0.2627
		Ox	0.4	2.3	0.7	1.5	2.6			2.6	7.5	1.5	0.2832	0.2627
	C1W0	Red	0.2	1.7	0.3	0.9				1.7	3.1	0.8	0.2824	0.2627
		Ox	0.3	1.6	0.4	1.2				1.6	3.5	0.9	0.2823	0.2626
	C1W1	Red	0.3	1.7	0.2	0.7		0.6		1.7	3.5	0.7	0.2827	0.2626
		Ox	0.3	1.5	0.3	0.7		0.5		1.5	3.3	0.7	0.2822	0.2626
2	C2W2	Red	1.3	1.5	1.4	2.0	2.8			2.8	9.0	1.8	0.2832	0.2626
		MV	1.3	1.5	1.6	2.1	2.9			2.9	9.4	1.9	0.2832	0.2626
		Ox	1.3	1.5	1.6	2.1	2.9			2.9	9.4	1.9	0.2832	0.2626
	Y2W2	Red	1.7	0.3	1.6	0.7	0.5	0.8	2.2	2.2	7.8	1.1	0.2832	0.2626
		MV	1.2	0.7	1.3	1.6	0.4	0.5	1.9	1.9	7.6	1.1	0.2822	0.2626
		Ox	1.2	0.6	1.5	1.5	0.3	0.5	2.1	2.1	7.7	1.1	0.2823	0.2626
	Y2W0	Red	1.7	0.4	0.8	2.5	0.4			2.5	5.8	1.2	0.2823	0.2625
		MV	1.2	0.6	0.6	2.7	0.8			2.7	5.9	1.2	0.2823	0.2625
		Ox	1.4	0.7	0.7	2.8	1.0			2.8	6.6	1.3	0.2823	0.2625
	C1W0	Red	1.1	0.5	0.4	0.7				1.1	2.7	0.7	0.2823	0.2625
		Ox	1.7	0.5	0.4	0.3				1.7	2.9	0.7	0.2823	0.2625
	C1W1	Ox	1.1	0.4	1.1	0.4		2.4		2.4	5.4	1.1	0.2822	0.2625
3	C2W2	Red	0.3	2.0	1.4	0.1	1.9			2.0	5.7	1.1	0.2830	0.2627
		MV	0.5	1.9	1.5	0.5	2.6			2.6	7.0	1.4	0.2830	0.2627
		Ox	0.5	1.8	1.6	0.5	2.8			2.8	7.2	1.4	0.2830	0.2627
	Y2W2	Red	0.3	1.5	0.0	0.1	1.4	0.2	3.0	3.0	6.5	0.9	0.2828	0.2627
		MV	0.3	1.5	0.2	0.9	1.6	0.0	3.2	3.2	7.7	1.1	0.2821	0.2627
		Ox	0.5	1.5	0.2	0.9	1.6	0.0	2.9	2.9	7.6	1.1	0.2821	0.2627
	Y2W0	Red	0.3	1.5	0.3	1.0	1.8			1.8	4.9	1.0	0.2827	0.2627
		MV	0.2	1.5	0.4	0.9	2.1			2.1	5.1	1.0	0.2819	0.2627
		Ox	0.2	1.6	0.5	0.9	2.2			2.2	5.4	1.1	0.2819	0.2627
	C1W0	Red	0.1	1.5	0.3	1.0				1.5	2.9	0.7	0.2822	0.2626
		Ox	0.4	1.6	0.0	1.3				1.6	3.3	0.8	0.2829	0.2627
	C1W1	Ox	0.4	1.6	0.0	2.0		1.6		2.0	5.6	1.1	0.2829	0.2627
3RFR crystal structure														
1	C2W2	Red	0.0	0.1	0.1	0.6	2.7			2.7	3.5	0.7	0.2763	0.2483
		MV	0.0	0.0	0.3	0.5	1.6			1.6	2.4	0.5	0.2763	0.2483
		Ox	0.0	0.4	0.4	0.6	1.9			1.9	3.3	0.7	0.2763	0.2483
	C1W0	Red	0.4	0.0	0.1	1.4				1.4	1.9	0.5	0.2765	0.2483
		Ox	0.1	0.0	0.1	1.3				1.3	1.5	0.4	0.2765	0.2483
	C1W1	Red	0.6	0.0	0.1	1.3		2.6		2.6	4.6	0.9	0.2767	0.2484
		Ox	0.0	0.0	0.1	0.9		2.3		2.3	3.3	0.7	0.2764	0.2483
2	C1W0	Red	0.2	0.0	0.4	0.1				0.4	0.7	0.2	0.2765	0.2485
		Ox	0.4	0.1	0.4	0.2				0.4	1.1	0.3	0.2765	0.2485
	C1W1	Ox	0.3	0.1	0.4	0.5		1.3		1.3	2.6	0.5	0.2764	0.2484
3	C1W0	Red	0.0	0.3	0.2	0.9				0.9	1.4	0.4	0.2762	0.2484
		Ox	0.0	0.3	0.2	0.9				0.9	1.4	0.4	0.2762	0.2484
	C1W1	Ox	0.0	0.2	0.2	1.0		0.5		1.0	1.9	0.4	0.2760	0.2483

Table S3. Energy components of the various reactant states in the reaction with methanol. Five energies are given for each reactant in both the triplet ($S = 1$) and singlet state ($S = 0$): TPSS-D3/def2-SV(P) optimised geometries, TPSS-D3/def2-TZVPD and B3LYP-D3/def2-TZVPD single point energies, obtained in a COSMO continuum solvent with a dielectric constant of 4, zero-point energies (ZPE) from TPSS-D3/def2-SV(P) frequencies and the total energies, which are the sum of the ZPE and the B3LYP-D3/def2-TZVPD energies. The reference energy is always the triplet RS state.

Energy	QM		QM+COSMO		QM+COSMO		ZPE		Total	
Method	TPSS-D3		TPSS-D3		B3LYP-D3		TPSS-D3		B3LYP-D3	
Basis	def2-SV(P)		def2-TZVPD		def2-TZVPD		def2-SV(P)		def2-TZVPD	
S	1	0	1	0	1	0	1	0	1	0
RS	0.0	19.4	0.0	33.0	0.0	85.2	0.0	-0.3	0.0	84.8
R	-21.0	-2.5	-11.7	24.6	-1.2	74.2	2.9	2.9	1.7	77.1
TS1	24.0	28.1	20.7	61.1	35.1	124.4	-13.2	-12.6	21.9	111.8
Im1	6.9	6.8	-10.0	74.2	-8.8	141.2	-6.8	-7.0	-15.6	134.2
Im2	-20.1	-87.0	-32.2	-100.0	-8.1	-60.8	1.9	9.0	-6.2	-51.8
TS2	125.6	-30.9	113.2	-34.7	132.4	-5.5	-8.3	6.2	124.2	0.7
P	-32.9	-237.1	-16.5	-226.9	9.0	-233.6	13.7	16.9	22.6	-216.7

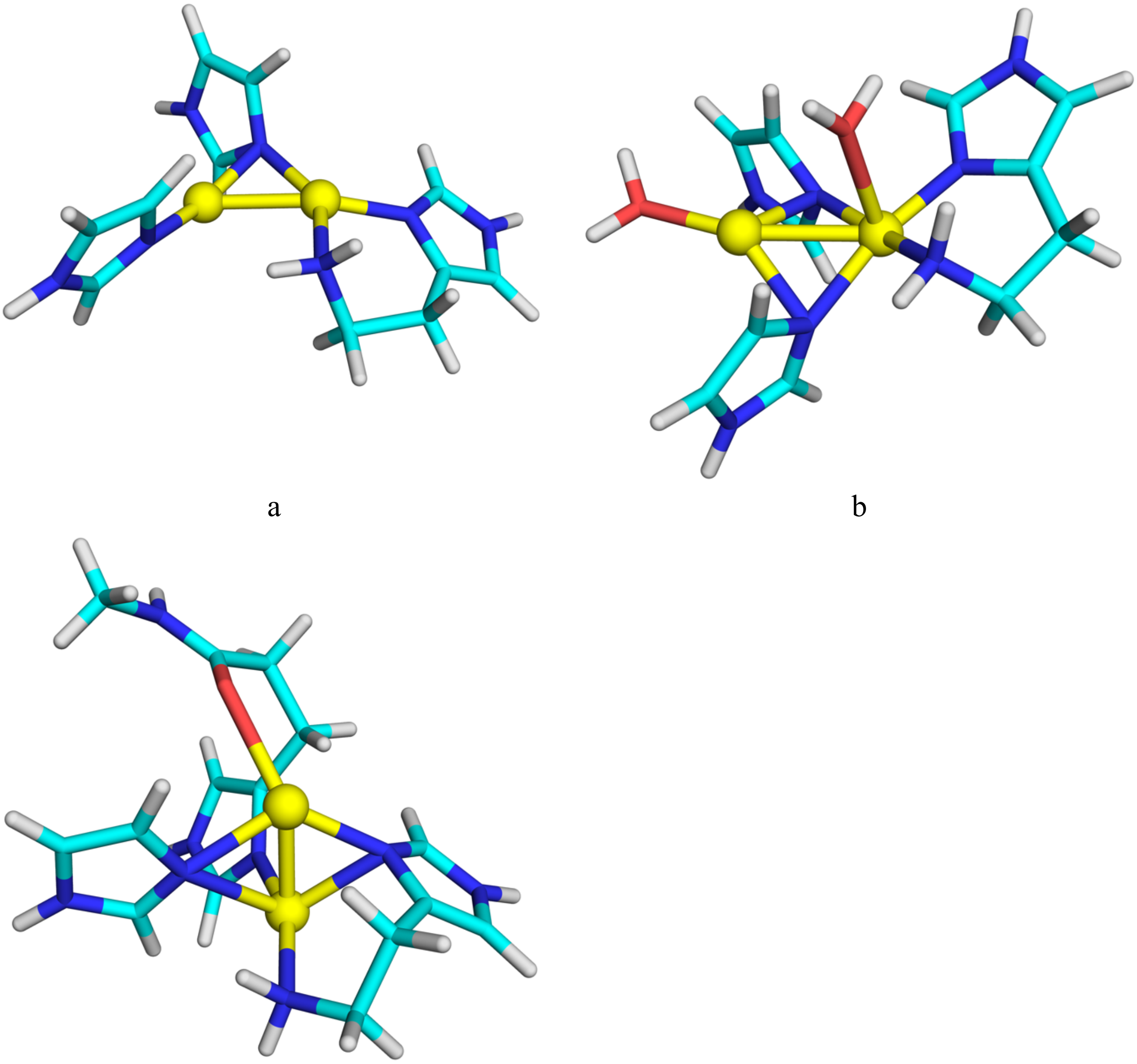
Figure S1. QM-refined structures of site A in the first protomer of the 3RGB structure, modelled by two Cu ions in the (a) fully reduced and (b) fully oxidised states.



a

b

Figure S2. (a) The Yoshizawa-type structure of pMMO (first protomer of the 3RGB structure, Y2W0a) obtained by QM/MM (i.e. QM-refinement with $w_A = 0$) for the reduced state. (b) A quantum-refined structure with two extra water molecules for the MV state (Y2W2). (c) The quantum-refined structure of site A with the backbone carbonyl group coordinating to Cu_B (reduced state).

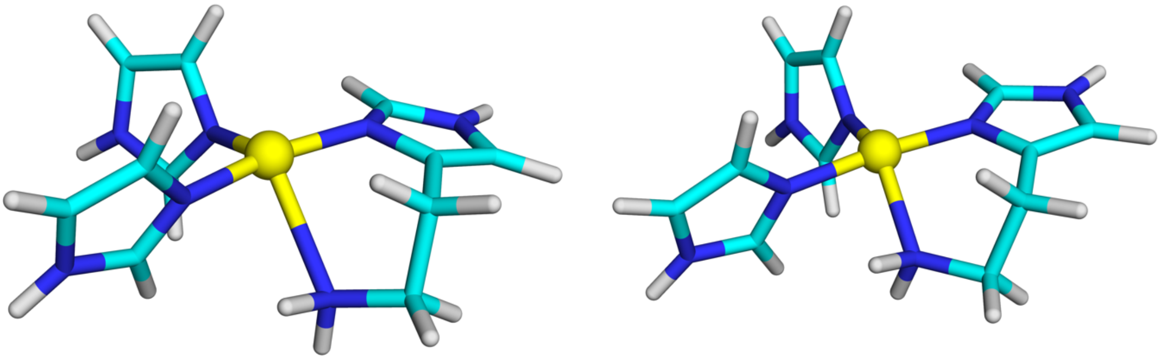


a

b

c

Figure S3. QM-refined structures of site A, modelled with a single Cu ion (C1W0) in the (a) reduced and (b) oxidised states.



a b

Figure S4. QM-refined structures of site A, modelled with a single Cu ion and the water molecule in the (a) oxidised state (C1W1). Part (b) shows the electron-density maps of the oxidised state. The $2mF_o–DF_c$ maps are contoured at 1.0 σ and the $mF_o – DF_c$ maps are contoured at +3.0 σ (green) and –3.0 σ (red).

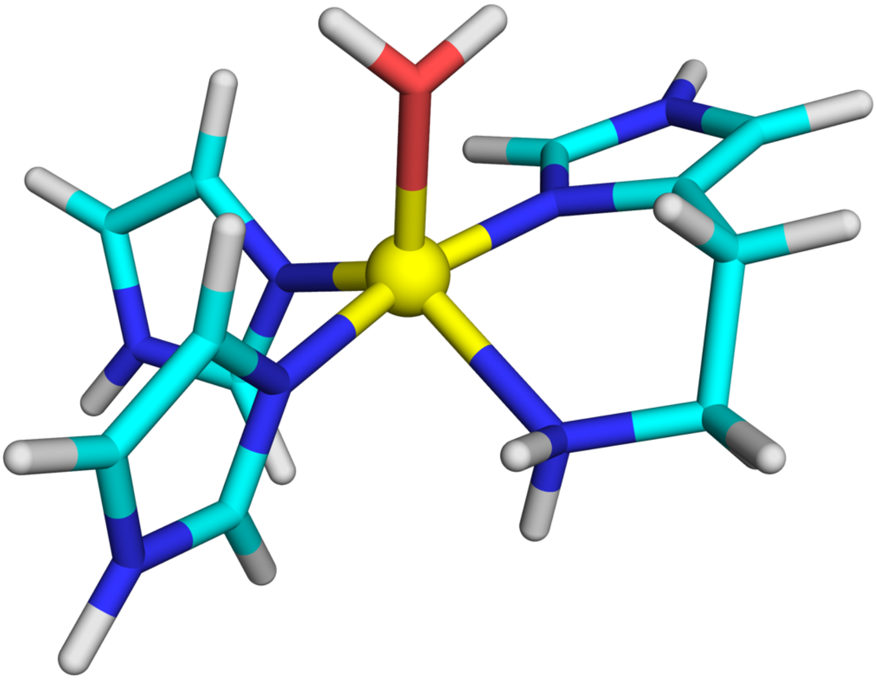
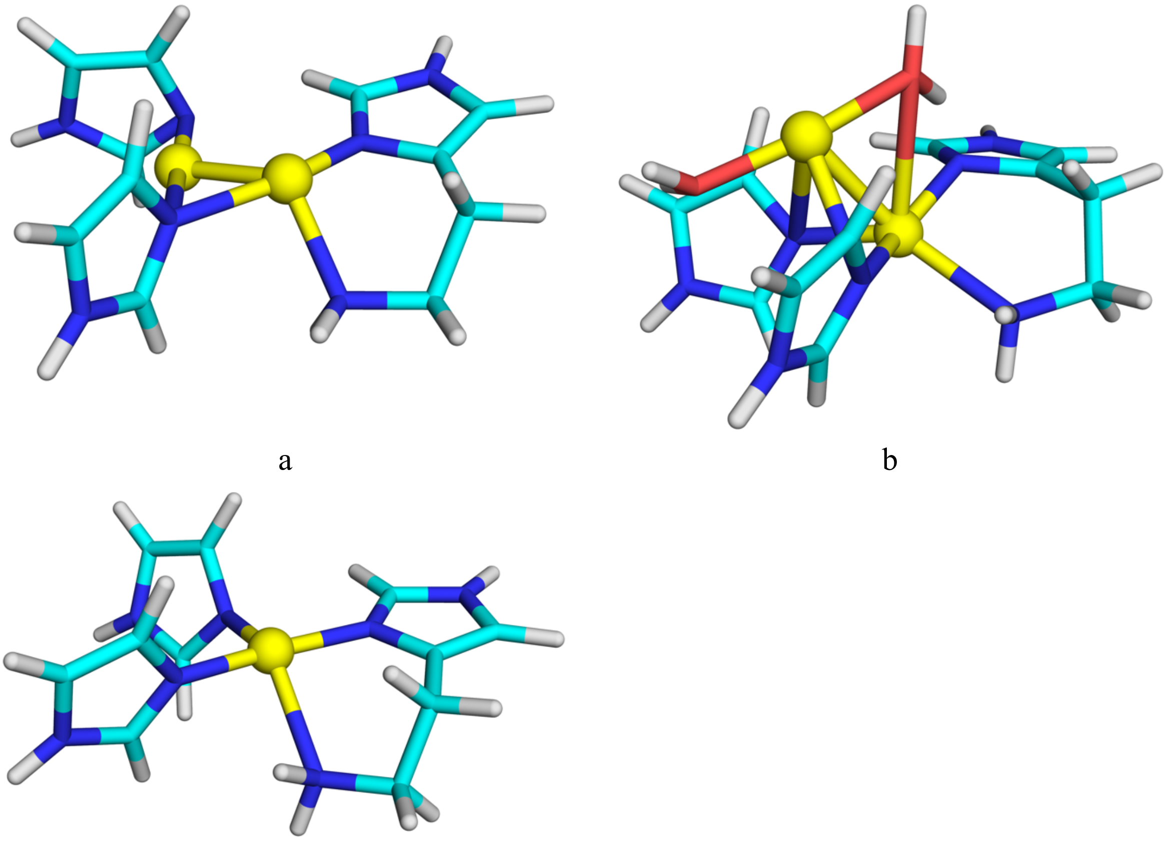


Figure S5. QM-refined structures of site A in the second protomer of the 3RGB structure, modelled (a) with a two Cu ions (C2W0) in the reduced state, (b) the Yoshizawa-type structure (Y2W2) or (c) with a single Cu ion (still in the reduced state).



a

b

c

Table S4. Coordinates of the QM system for the mononuclear site in all optimised structures.

```
REMARK PDB files of the mononuclear copper site in pMMO obtained by quantum refinement
REMARK L. Cao, O. Caldararu, A. C. Rosenzweig, U. Ryde
REMARK Quantum refinement does not support dinuclear copper sites in crystal structures
REMARK of particulate methane monooxygenase
REMARK Angew. Chemie 2017
REMARK Supplementary material
REMARK 18 structures are given:
REMARK 2 Crystal structures: 3RGB or 3RFR
REMARK 3 subunits
REMARK Reduced, Cu(I), oxidised, Cu(II), or oxidised state with an extra water molecule
REMARK Only heavy atoms in the QM system are included; other atoms are not moved
REMARK
REMARK 3RGB, Subunit 1, Reduced state
REMARK DATE:04-Nov-2016  15:59:39       created by user: lili
REMARK CNS VERSION:1.3
ATOM      1  CB  HIS    33      39.558 130.222  81.449  1.00 73.31      A    C 0
ATOM      2  CG  HIS    33      39.536 130.282  82.943  1.00 72.87      A    C 0
ATOM      3  CD2 HIS    33      40.433 130.882  83.811  1.00 72.68      A    C 0
ATOM      4  ND1 HIS    33      38.383 129.977  83.651  1.00 72.38      A    N 0
ATOM      5  CE1 HIS    33      38.558 130.382  84.908  1.00 72.38      A    C 0
ATOM      6  NE2 HIS    33      39.798 130.929  85.043  1.00 72.37      A    N 0
ATOM      7  C   HIS    33      39.582 131.594  79.449  1.00 73.42      A    C 0
ATOM      8  N   HIS    33      37.660 131.761  81.212  1.00 73.34      A    N 0
ATOM      9  CA  HIS    33      39.101 131.627  80.915  1.00 73.37      A    C 0
ATOM     10  CB  HIS   137      35.988 128.077  85.420  1.00 43.65      A    C 0
ATOM     11  CG  HIS   137      35.055 129.223  85.194  1.00 43.97      A    C 0
ATOM     12  CD2 HIS   137      33.793 129.437  85.714  1.00 43.67      A    C 0
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ATOM     14  CE1 HIS   137      34.440 131.182  84.497  1.00 44.62      A    C 0
ATOM     15  NE2 HIS   137      33.425 130.694  85.267  1.00 43.95      A    N 0
ATOM     16  CB  HIS   139      33.507 127.696  78.902  1.00 49.60      A    C 0
ATOM     17  CG  HIS   139      34.226 128.599  79.774  1.00 50.45      A    C 0
ATOM     18  CD2 HIS   139      35.368 128.398  80.520  1.00 51.09      A    C 0
ATOM     19  ND1 HIS   139      33.792 129.883  80.036  1.00 50.61      A    N 0
ATOM     20  CE1 HIS   139      34.668 130.428  80.938  1.00 51.73      A    C 0
ATOM     21  NE2 HIS   139      35.625 129.541  81.267  1.00 51.75      A    N 0
ATOM     22 CU+1 CU1   416      36.806 129.802  82.728  1.00 64.05      A   CU 1
END
REMARK 3RGB, Subunit 1, Oxidised state
REMARK DATE:04-Nov-2016  15:59:39       created by user: lili
REMARK CNS VERSION:1.3
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ATOM      3  CD2 HIS    33      40.522 130.877  84.072  1.00 74.26      A    C 0
ATOM      4  ND1 HIS    33      38.438 130.187  83.638  1.00 73.84      A    N 0
ATOM      5  CE1 HIS    33      38.532 130.410  84.955  1.00 73.92      A    C 0
ATOM      6  NE2 HIS    33      39.789 130.817  85.246  1.00 74.06      A    N 0
ATOM      7  C   HIS    33      39.578 131.593  79.450  1.00 75.04      A    C 0
ATOM      8  N   HIS    33      37.650 131.186  81.051  1.00 75.22      A    N 0
ATOM      9  CA  HIS    33      39.071 131.617  80.906  1.00 75.00      A    C 0
ATOM     10  CB  HIS   137      36.012 128.058  85.421  1.00 43.37      A    C 0
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ATOM     13  ND1 HIS   137      35.514 130.203  84.285  1.00 44.35      A    N 0
ATOM     14  CE1 HIS   137      34.554 131.143  84.346  1.00 44.08      A    C 0
ATOM     15  NE2 HIS   137      33.575 130.760  85.205  1.00 43.00      A    N 0
ATOM     16  CB  HIS   139      33.513 127.691  78.906  1.00 47.68      A    C 0
ATOM     17  CG  HIS   139      34.225 128.591  79.782  1.00 48.49      A    C 0
ATOM     18  CD2 HIS   139      35.316 128.377  80.597  1.00 49.04      A    C 0
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ATOM     20  CE1 HIS   139      34.602 130.431  80.945  1.00 49.68      A    C 0
ATOM     21  NE2 HIS   139      35.525 129.530  81.346  1.00 49.68      A    N 0
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END
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REMARK DATE:08-Nov-2016  15:55:59       created by user: lili
REMARK CNS VERSION:1.3
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ATOM      3  CD2 HIS A  33      40.598 130.819  84.076  1.00 71.43      A    C 0
ATOM      4  ND1 HIS A  33      38.442 130.380  83.686  1.00 70.70      A    N 0
ATOM      5  CE1 HIS A  33      38.584 130.569  84.998  1.00 70.88      A    C 0
ATOM      6  NE2 HIS A  33      39.886 130.832  85.265  1.00 71.25      A    N 0
ATOM      7  C   HIS A  33      39.579 131.595  79.450  1.00 72.08      A    C 0
ATOM      8  N   HIS A  33      37.642 131.281  81.056  1.00 72.17      A    N 0
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ATOM     10  CB  HIS A 137      36.006 128.065  85.426  1.00 42.08      A    C 0
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ATOM     14  CE1 HIS A 137      34.532 131.157  84.383  1.00 43.48      A    C 0
ATOM     15  NE2 HIS A 137      33.541 130.746  85.214  1.00 42.16      A    N 0
ATOM     16  CB  HIS A 139      33.514 127.692  78.904  1.00 46.16      A    C 0
ATOM     17  CG  HIS A 139      34.206 128.617  79.773  1.00 47.19      A    C 0
ATOM     18  CD2 HIS A 139      35.312 128.435  80.569  1.00 47.74      A    C 0
ATOM     19  ND1 HIS A 139      33.747 129.896  80.001  1.00 47.61      A    N 0
ATOM     20  CE1 HIS A 139      34.555 130.464  80.937  1.00 48.85      A    C 0
ATOM     21  NE2 HIS A 139      35.502 129.588  81.320  1.00 48.45      A    N 0
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ATOM     23 CU+1 CU1 A 417      36.893 129.893  82.710  1.00 66.97      A   CU 1
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REMARK DATE:11-Apr-2017  12:41:20       created by user: lili
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ATOM      1  CB  HIS E  33      35.739 157.866  32.437  1.00 62.03      E    C 0
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ATOM      3  CD2 HIS E  33      36.188 160.006  30.997  1.00 62.91      E    C 0
ATOM      4  ND1 HIS E  33      34.150 159.748  31.878  1.00 61.28      E    N 0
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ATOM      6  NE2 HIS E  33      35.362 161.058  30.613  1.00 63.11      E    N 0
ATOM      7  C   HIS E  33      35.696 155.460  32.461  1.00 61.00      E    C 0
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ATOM     10  CB  HIS E 137      31.830 162.236  33.284  1.00 41.72      E    C 0
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ATOM     15  NE2 HIS E 137      29.020 160.518  31.646  1.00 41.95      E    N 0
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ATOM     20  CE1 HIS E 139      30.674 156.975  33.565  1.00 45.65      E    C 0
ATOM     21  NE2 HIS E 139      31.617 157.843  33.947  1.00 45.55      E    N 0
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END
REMARK 3RGB, Subunit 2, Oxidised state
REMARK DATE:11-Apr-2017  12:41:20       created by user: lili
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ATOM     18  CD2 HIS E 139      31.508 157.937  35.293  1.00 42.78      E    C 0
ATOM     19  ND1 HIS E 139      30.095 156.358  34.689  1.00 42.57      E    N 0
ATOM     20  CE1 HIS E 139      30.635 156.896  33.567  1.00 43.27      E    C 0
ATOM     21  NE2 HIS E 139      31.505 157.859  33.911  1.00 43.06      E    N 0
ATOM     22 CU+1 CU1 E 416      32.717 158.882  32.685  1.00 60.13      E   CU 1
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REMARK DATE:28-Apr-2017  22:41:54       created by user: lili
REMARK CNS VERSION:1.3
ATOM      1  CB  HIS E  33      35.886 157.873  32.189  1.00 59.80      E    C 0
ATOM      2  CG  HIS E  33      35.398 159.200  31.646  1.00 60.12      E    C 0
ATOM      3  CD2 HIS E  33      36.154 160.227  31.120  1.00 61.04      E    C 0
ATOM      4  ND1 HIS E  33      34.064 159.621  31.606  1.00 59.31      E    N 0
ATOM      5  CE1 HIS E  33      34.010 160.833  31.058  1.00 59.75      E    C 0
ATOM      6  NE2 HIS E  33      35.265 161.230  30.758  1.00 61.48      E    N 0
ATOM      7  C   HIS E  33      35.679 155.459  32.468  1.00 58.72      E    C 0
ATOM      8  N   HIS E  33      33.688 156.900  32.464  1.00 59.81      E    N 0
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ATOM     12  CD2 HIS E 137      29.577 161.680  32.189  1.00 39.80      E    C 0
ATOM     13  ND1 HIS E 137      31.171 160.110  32.113  1.00 40.08      E    N 0
ATOM     14  CE1 HIS E 137      30.074 159.621  31.530  1.00 39.60      E    C 0
ATOM     15  NE2 HIS E 137      29.098 160.551  31.562  1.00 40.03      E    N 0
ATOM     16  CB  HIS E 139      30.186 156.696  37.125  1.00 41.73      E    C 0
ATOM     17  CG  HIS E 139      30.582 157.002  35.765  1.00 40.91      E    C 0
ATOM     18  CD2 HIS E 139      31.427 157.943  35.234  1.00 41.13      E    C 0
ATOM     19  ND1 HIS E 139      30.090 156.276  34.711  1.00 41.47      E    N 0
ATOM     20  CE1 HIS E 139      30.600 156.792  33.563  1.00 42.20      E    C 0
ATOM     21  NE2 HIS E 139      31.427 157.802  33.856  1.00 41.45      E    N 0
ATOM     22  O   HOH E 416      33.826 159.548  34.573  1.00 48.02      E    O 0
ATOM     23 CU+1 CU1 E 417      32.656 158.842  32.632  1.00 62.64      E   CU 1
END
REMARK 3RGB, Subunit 3, Reduced state
REMARK DATE:26-Jun-2017  15:10:27       created by user: lili
REMARK CNS VERSION:1.3
ATOM      1  CB  HIS I  33      34.208 101.593  33.639  1.00 65.19      I    C 0
ATOM      2  CG  HIS I  33      33.709 100.358  32.950  1.00 63.32      I    C 0
ATOM      3  CD2 HIS I  33      34.393  99.184  32.704  1.00 62.66      I    C 0
ATOM      4  ND1 HIS I  33      32.362 100.165  32.640  1.00 62.85      I    N 0
ATOM      5  CE1 HIS I  33      32.236  98.899  32.241  1.00 61.69      I    C 0
ATOM      6  NE2 HIS I  33      33.443  98.278  32.260  1.00 61.82      I    N 0
ATOM      7  C   HIS I  33      34.334 102.858  35.741  1.00 67.59      I    C 0
ATOM      8  N   HIS I  33      32.195 102.113  34.878  1.00 66.92      I    N 0
ATOM      9  CA  HIS I  33      33.615 101.692  35.082  1.00 66.67      I    C 0
ATOM     10  CB  HIS I 137      29.516  99.980  30.220  1.00 57.16      I    C 0
ATOM     11  CG  HIS I 137      28.741  99.823  31.519  1.00 56.37      I    C 0
ATOM     12  CD2 HIS I 137      27.384  99.728  31.741  1.00 56.56      I    C 0
ATOM     13  ND1 HIS I 137      29.413  99.669  32.729  1.00 55.16      I    N 0
ATOM     14  CE1 HIS I 137      28.481  99.502  33.657  1.00 55.62      I    C 0
ATOM     15  NE2 HIS I 137      27.235  99.536  33.102  1.00 55.52      I    N 0
ATOM     16  CB  HIS I 139      28.174 106.072  33.340  1.00 48.56      I    C 0
ATOM     17  CG  HIS I 139      28.627 104.733  33.712  1.00 48.95      I    C 0
ATOM     18  CD2 HIS I 139      29.466 103.864  33.053  1.00 49.86      I    C 0
ATOM     19  ND1 HIS I 139      28.257 104.090  34.875  1.00 49.19      I    N 0
ATOM     20  CE1 HIS I 139      28.859 102.855  34.888  1.00 49.72      I    C 0
ATOM     21  NE2 HIS I 139      29.588 102.693  33.784  1.00 49.20      I    N 0
ATOM     22 CU+1 CU1 I 417      30.789 101.270  33.302  1.00 71.43      I   CU 1
END
REMARK 3RGB, Subunit 3, Oxidised state
REMARK DATE:26-Jun-2017  15:10:27       created by user: lili
REMARK CNS VERSION:1.3
ATOM      1  CB  HIS I  33      34.380 101.309  33.784  1.00 63.58      I    C 0
ATOM      2  CG  HIS I  33      33.727 100.174  33.039  1.00 61.90      I    C 0
ATOM      3  CD2 HIS I  33      34.320  99.009  32.604  1.00 61.51      I    C 0
ATOM      4  ND1 HIS I  33      32.350 100.069  32.782  1.00 61.73      I    N 0
ATOM      5  CE1 HIS I  33      32.130  98.862  32.244  1.00 60.88      I    C 0
ATOM      6  NE2 HIS I  33      33.303  98.208  32.119  1.00 61.20      I    N 0
ATOM      7  C   HIS I  33      34.319 102.861  35.740  1.00 65.57      I    C 0
ATOM      8  N   HIS I  33      32.308 102.351  34.484  1.00 64.60      I    N 0
ATOM      9  CA  HIS I  33      33.570 101.702  35.050  1.00 64.69      I    C 0
ATOM     10  CB  HIS I 137      29.528  99.989  30.215  1.00 54.82      I    C 0
ATOM     11  CG  HIS I 137      28.775  99.851  31.514  1.00 53.92      I    C 0
ATOM     12  CD2 HIS I 137      27.490  99.400  31.715  1.00 54.16      I    C 0
ATOM     13  ND1 HIS I 137      29.356 100.079  32.760  1.00 52.36      I    N 0
ATOM     14  CE1 HIS I 137      28.437  99.793  33.682  1.00 52.88      I    C 0
ATOM     15  NE2 HIS I 137      27.299  99.382  33.081  1.00 53.20      I    N 0
ATOM     16  CB  HIS I 139      28.178 106.071  33.333  1.00 47.84      I    C 0
ATOM     17  CG  HIS I 139      28.630 104.730  33.706  1.00 48.14      I    C 0
ATOM     18  CD2 HIS I 139      29.488 103.855  33.079  1.00 48.88      I    C 0
ATOM     19  ND1 HIS I 139      28.249 104.112  34.877  1.00 48.41      I    N 0
ATOM     20  CE1 HIS I 139      28.858 102.896  34.950  1.00 48.99      I    C 0
ATOM     21  NE2 HIS I 139      29.614 102.716  33.861  1.00 48.33      I    N 0
ATOM     22 CU+1 CU1 I 417      30.869 101.290  33.339  1.00 73.67      I   CU 1
END
REMARK 3RFR, Subunit 1, Reduced state
REMARK DATE:11-Apr-2017  17:59:04       created by user: lili
REMARK CNS VERSION:1.3
ATOM      1  CB  HIS A  29      49.904  34.452  46.113  1.00 78.48      A    C 0
ATOM      2  CG  HIS A  29      50.430  35.676  46.800  1.00 78.54      A    C 0
ATOM      3  CD2 HIS A  29      49.880  36.941  46.776  1.00 78.53      A    C 0
ATOM      4  ND1 HIS A  29      51.518  35.666  47.672  1.00 78.55      A    N 0
ATOM      5  CE1 HIS A  29      51.621  36.906  48.148  1.00 78.05      A    C 0
ATOM      6  NE2 HIS A  29      50.649  37.701  47.635  1.00 79.24      A    N 0
ATOM      7  C   HIS A  29      49.125  32.190  46.334  1.00 78.25      A    C 0
ATOM      8  N   HIS A  29      51.510  32.812  46.840  1.00 79.15      A    N 0
ATOM      9  CA  HIS A  29      50.085  33.184  46.967  1.00 78.41      A    C 0
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ATOM     10  CB  HIS A 133      55.147  36.089  48.320  1.00 49.64      A    C 0
ATOM     11  CG  HIS A 133      54.624  36.076  49.695  1.00 53.16      A    C 0
ATOM     12  CD2 HIS A 133      55.244  36.654  50.785  1.00 52.66      A    C 0
ATOM     13  ND1 HIS A 133      53.443  35.493  50.085  1.00 55.37      A    N 0
ATOM     14  CE1 HIS A 133      53.328  35.721  51.388  1.00 55.22      A    C 0
ATOM     15  NE2 HIS A 133      54.400  36.430  51.852  1.00 54.43      A    N 0
ATOM     16  CB  HIS A 135      55.203  29.470  50.334  1.00 53.71      A    C 0
ATOM     17  CG  HIS A 135      54.332  30.604  50.095  1.00 53.64      A    C 0
ATOM     18  CD2 HIS A 135      54.265  31.598  49.142  1.00 53.89      A    C 0
ATOM     19  ND1 HIS A 135      53.299  30.837  50.978  1.00 53.37      A    N 0
ATOM     20  CE1 HIS A 135      52.639  31.952  50.561  1.00 55.61      A    C 0
ATOM     21  NE2 HIS A 135      53.217  32.453  49.466  1.00 54.70      A    N 0
ATOM     22 CU+2 CU2 A 419      52.513  34.050  48.621  1.00 79.73      A   CU 2
END
REMARK 3RFR, Subunit 1, Oxidised state
REMARK DATE:11-Apr-2017  17:59:04       created by user: lili
REMARK CNS VERSION:1.3
ATOM      1  CB  HIS A  29      50.208  34.382  45.978  1.00 79.02      A    C 0
ATOM      2  CG  HIS A  29      50.621  35.625  46.705  1.00 78.80      A    C 0
ATOM      3  CD2 HIS A  29      50.048  36.876  46.632  1.00 78.59      A    C 0
ATOM      4  ND1 HIS A  29      51.581  35.637  47.721  1.00 78.91      A    N 0
ATOM      5  CE1 HIS A  29      51.585  36.876  48.235  1.00 78.27      A    C 0
ATOM      6  NE2 HIS A  29      50.669  37.636  47.602  1.00 79.12      A    N 0
ATOM      7  C   HIS A  29      49.122  32.198  46.333  1.00 79.01      A    C 0
ATOM      8  N   HIS A  29      51.420  32.758  47.297  1.00 80.48      A    N 0
ATOM      9  CA  HIS A  29      50.050  33.231  46.983  1.00 79.19      A    C 0
ATOM     10  CB  HIS A 133      55.148  36.089  48.284  1.00 49.30      A    C 0
ATOM     11  CG  HIS A 133      54.589  36.034  49.630  1.00 52.69      A    C 0
ATOM     12  CD2 HIS A 133      55.082  36.685  50.738  1.00 51.36      A    C 0
ATOM     13  ND1 HIS A 133      53.475  35.302  49.989  1.00 55.17      A    N 0
ATOM     14  CE1 HIS A 133      53.275  35.525  51.296  1.00 54.77      A    C 0
ATOM     15  NE2 HIS A 133      54.231  36.362  51.770  1.00 53.16      A    N 0
ATOM     16  CB  HIS A 135      55.205  29.470  50.334  1.00 53.53      A    C 0
ATOM     17  CG  HIS A 135      54.334  30.603  50.099  1.00 53.54      A    C 0
ATOM     18  CD2 HIS A 135      54.293  31.613  49.163  1.00 53.64      A    C 0
ATOM     19  ND1 HIS A 135      53.285  30.818  50.965  1.00 53.36      A    N 0
ATOM     20  CE1 HIS A 135      52.627  31.931  50.572  1.00 55.24      A    C 0
ATOM     21  NE2 HIS A 135      53.234  32.452  49.495  1.00 54.11      A    N 0
ATOM     22 CU+2 CU2 A 419      52.498  34.050  48.630  1.00 81.48      A   CU 2
END
REMARK 3RFR, Subunit 1, Oxidised state with an extra water molecule
REMARK DATE:11-Apr-2017  17:45:49       created by user: lili
REMARK CNS VERSION:1.3
ATOM      1  CB  HIS A  29      50.553  34.233  45.910  1.00 75.20      A    C 0
ATOM      2  CG  HIS A  29      50.819  35.534  46.606  1.00 75.45      A    C 0
ATOM      3  CD2 HIS A  29      50.157  36.737  46.466  1.00 75.65      A    C 0
ATOM      4  ND1 HIS A  29      51.642  35.620  47.728  1.00 75.53      A    N 0
ATOM      5  CE1 HIS A  29      51.484  36.847  48.242  1.00 75.15      A    C 0
ATOM      6  NE2 HIS A  29      50.588  37.537  47.505  1.00 75.75      A    N 0
ATOM      7  C   HIS A  29      49.116  32.206  46.325  1.00 75.32      A    C 0
ATOM      8  N   HIS A  29      51.127  32.728  47.746  1.00 76.22      A    N 0
ATOM      9  CA  HIS A  29      50.006  33.265  46.969  1.00 75.23      A    C 0
ATOM     10  CB  HIS A 133      55.148  36.084  48.289  1.00 50.55      A    C 0
ATOM     11  CG  HIS A 133      54.598  36.053  49.642  1.00 53.60      A    C 0
ATOM     12  CD2 HIS A 133      55.109  36.707  50.741  1.00 52.24      A    C 0
ATOM     13  ND1 HIS A 133      53.488  35.330  50.018  1.00 56.05      A    N 0
ATOM     14  CE1 HIS A 133      53.304  35.559  51.325  1.00 55.02      A    C 0
ATOM     15  NE2 HIS A 133      54.270  36.392  51.785  1.00 53.81      A    N 0
ATOM     16  CB  HIS A 135      55.210  29.462  50.331  1.00 52.38      A    C 0
ATOM     17  CG  HIS A 135      54.333  30.586  50.113  1.00 52.71      A    C 0
ATOM     18  CD2 HIS A 135      54.285  31.610  49.191  1.00 52.23      A    C 0
ATOM     19  ND1 HIS A 135      53.292  30.784  50.991  1.00 52.42      A    N 0
ATOM     20  CE1 HIS A 135      52.632  31.904  50.616  1.00 54.19      A    C 0
ATOM     21  NE2 HIS A 135      53.233  32.440  49.542  1.00 52.77      A    N 0
ATOM     22 CU+2 CU2 A 419      52.508  34.052  48.698  1.00 77.21      A   CU 2
ATOM     23  O   HOH A 420      53.618  33.361  46.562  1.00 36.62      A    O 0
END
REMARK 3RFR, Subunit 2, Reduced state
REMARK DATE:28-Nov-2016  12:00:25       created by user: lili
REMARK CNS VERSION:1.3
ATOM      1  CB  HIS E  29      16.569 -12.883  54.958  1.00 78.25      E    C 0
ATOM      2  CG  HIS E  29      15.439 -12.937  55.949  1.00 78.11      E    C 0
ATOM      3  CD2 HIS E  29      14.085 -13.056  55.712  1.00 78.62      E    C 0
ATOM      4  ND1 HIS E  29      15.669 -13.011  57.321  1.00 76.42      E    N 0
ATOM      5  CE1 HIS E  29      14.484 -13.218  57.890  1.00 77.56      E    C 0
ATOM      6  NE2 HIS E  29      13.500 -13.248  56.952  1.00 79.31      E    N 0
ATOM      7  C   HIS E  29      18.769 -12.489  54.377  1.00 79.15      E    C 0
ATOM      8  N   HIS E  29      17.642 -11.035  56.072  1.00 79.37      E    N 0
ATOM      9  CA  HIS E  29      17.897 -12.410  55.592  1.00 78.91      E    C 0
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ATOM     10  CB  HIS E 133      14.307 -10.420  60.056  1.00 64.38      E    C 0
ATOM     11  CG  HIS E 133      15.150 -11.339  60.881  1.00 66.46      E    C 0
ATOM     12  CD2 HIS E 133      15.146 -11.545  62.247  1.00 67.43      E    C 0
ATOM     13  ND1 HIS E 133      16.102 -12.150  60.303  1.00 66.98      E    N 0
ATOM     14  CE1 HIS E 133      16.676 -12.835  61.284  1.00 65.96      E    C 0
ATOM     15  NE2 HIS E 133      16.124 -12.498  62.481  1.00 66.39      E    N 0
ATOM     16  CB  HIS E 135      20.623  -7.700  60.115  1.00 47.31      E    C 0
ATOM     17  CG  HIS E 135      19.864  -8.938  59.747  1.00 48.28      E    C 0
ATOM     18  CD2 HIS E 135      18.658  -9.176  59.124  1.00 48.99      E    C 0
ATOM     19  ND1 HIS E 135      20.391 -10.189  59.998  1.00 48.89      E    N 0
ATOM     20  CE1 HIS E 135      19.512 -11.126  59.553  1.00 49.05      E    C 0
ATOM     21  NE2 HIS E 135      18.449 -10.538  59.021  1.00 48.87      E    N 0
ATOM     22 CU+2 CU2 E 420      16.767 -11.565  58.421  1.00 82.94      E   CU 2
END
REMARK 3RFR, Subunit 2, Oxidised state
REMARK DATE:28-Nov-2016  12:00:25       created by user: lili
REMARK CNS VERSION:1.3
ATOM      1  CB  HIS E  29      16.481 -12.623  54.981  1.00 80.85      E    C 0
ATOM      2  CG  HIS E  29      15.406 -12.855  56.012  1.00 80.62      E    C 0
ATOM      3  CD2 HIS E  29      14.133 -13.330  55.797  1.00 81.09      E    C 0
ATOM      4  ND1 HIS E  29      15.595 -12.757  57.396  1.00 79.64      E    N 0
ATOM      5  CE1 HIS E  29      14.478 -13.195  57.985  1.00 80.54      E    C 0
ATOM      6  NE2 HIS E  29      13.577 -13.540  57.043  1.00 81.64      E    N 0
ATOM      7  C   HIS E  29      18.765 -12.495  54.379  1.00 81.43      E    C 0
ATOM      8  N   HIS E  29      17.839 -11.183  56.363  1.00 82.06      E    N 0
ATOM      9  CA  HIS E  29      17.879 -12.461  55.607  1.00 81.34      E    C 0
ATOM     10  CB  HIS E 133      14.298 -10.410  60.035  1.00 64.90      E    C 0
ATOM     11  CG  HIS E 133      15.154 -11.333  60.826  1.00 66.47      E    C 0
ATOM     12  CD2 HIS E 133      15.085 -11.667  62.161  1.00 67.06      E    C 0
ATOM     13  ND1 HIS E 133      16.213 -12.014  60.257  1.00 66.88      E    N 0
ATOM     14  CE1 HIS E 133      16.789 -12.742  61.218  1.00 66.30      E    C 0
ATOM     15  NE2 HIS E 133      16.125 -12.555  62.381  1.00 66.13      E    N 0
ATOM     16  CB  HIS E 135      20.627  -7.695  60.116  1.00 47.65      E    C 0
ATOM     17  CG  HIS E 135      19.882  -8.934  59.755  1.00 48.47      E    C 0
ATOM     18  CD2 HIS E 135      18.636  -9.171  59.224  1.00 48.90      E    C 0
ATOM     19  ND1 HIS E 135      20.453 -10.177  59.929  1.00 48.82      E    N 0
ATOM     20  CE1 HIS E 135      19.573 -11.126  59.543  1.00 49.03      E    C 0
ATOM     21  NE2 HIS E 135      18.456 -10.538  59.117  1.00 48.70      E    N 0
ATOM     22 CU+2 CU2 E 420      16.855 -11.559  58.403  1.00 86.08      E   CU 2
END
REMARK 3RFR, Subunit 2, Oxidised state with an extra water molecule
REMARK DATE:12-Apr-2017  14:20:10       created by user: lili
REMARK CNS VERSION:1.3
ATOM      1  CB  HIS E  29      16.503 -12.684  54.985  1.00 81.59      E    C 0
ATOM      2  CG  HIS E  29      15.410 -12.873  56.005  1.00 81.34      E    C 0
ATOM      3  CD2 HIS E  29      14.109 -13.263  55.779  1.00 81.73      E    C 0
ATOM      4  ND1 HIS E  29      15.605 -12.809  57.390  1.00 80.25      E    N 0
ATOM      5  CE1 HIS E  29      14.461 -13.186  57.970  1.00 81.19      E    C 0
ATOM      6  NE2 HIS E  29      13.540 -13.459  57.022  1.00 82.26      E    N 0
ATOM      7  C   HIS E  29      18.771 -12.496  54.383  1.00 82.28      E    C 0
ATOM      8  N   HIS E  29      17.794 -11.169  56.352  1.00 82.90      E    N 0
ATOM      9  CA  HIS E  29      17.887 -12.464  55.627  1.00 82.16      E    C 0
ATOM     10  CB  HIS E 133      14.300 -10.410  60.034  1.00 64.98      E    C 0
ATOM     11  CG  HIS E 133      15.159 -11.324  60.816  1.00 66.53      E    C 0
ATOM     12  CD2 HIS E 133      15.113 -11.656  62.154  1.00 67.04      E    C 0
ATOM     13  ND1 HIS E 133      16.202 -12.017  60.231  1.00 67.00      E    N 0
ATOM     14  CE1 HIS E 133      16.792 -12.747  61.181  1.00 66.38      E    C 0
ATOM     15  NE2 HIS E 133      16.150 -12.551  62.356  1.00 66.09      E    N 0
ATOM     16  CB  HIS E 135      20.624  -7.703  60.117  1.00 47.70      E    C 0
ATOM     17  CG  HIS E 135      19.873  -8.929  59.759  1.00 48.53      E    C 0
ATOM     18  CD2 HIS E 135      18.628  -9.156  59.220  1.00 49.10      E    C 0
ATOM     19  ND1 HIS E 135      20.431 -10.180  59.932  1.00 48.90      E    N 0
ATOM     20  CE1 HIS E 135      19.547 -11.123  59.537  1.00 48.90      E    C 0
ATOM     21  NE2 HIS E 135      18.438 -10.522  59.106  1.00 48.78      E    N 0
ATOM     22  O   HOH E 419      18.763 -14.119  58.597  1.00 76.39      E    O 0
ATOM     23 CU+2 CU2 E 420      16.863 -11.579  58.389  1.00 84.92      E   CU 2
END
REMARK 3RFR, Subunit 3, Reduced state
REMARK DATE:28-Nov-2016  12:00:25       created by user: lili
REMARK CNS VERSION:1.3
ATOM      1  CB  HIS I  29      71.009 -19.302  34.919  1.00 76.64      I    C 0
ATOM      2  CG  HIS I  29      72.127 -20.295  35.141  1.00 76.90      I    C 0
ATOM      3  CD2 HIS I  29      72.990 -20.827  34.204  1.00 76.91      I    C 0
ATOM      4  ND1 HIS I  29      72.545 -20.746  36.406  1.00 77.70      I    N 0
ATOM      5  CE1 HIS I  29      73.631 -21.503  36.216  1.00 76.48      I    C 0
ATOM      6  NE2 HIS I  29      73.923 -21.588  34.891  1.00 76.21      I    N 0
ATOM      7  C   HIS I  29      70.053 -17.100  35.655  1.00 78.29      I    C 0
ATOM      8  N   HIS I  29      70.122 -19.079  37.154  1.00 78.47      I    N 0
ATOM      9  CA  HIS I  29      70.843 -18.324  36.121  1.00 77.51      I    C 0
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ATOM     10  CB  HIS I 133      72.019 -23.668  38.446  1.00 68.19      I    C 0
ATOM     11  CG  HIS I 133      73.118 -23.008  39.295  1.00 69.03      I    C 0
ATOM     12  CD2 HIS I 133      74.179 -23.625  39.951  1.00 69.37      I    C 0
ATOM     13  ND1 HIS I 133      73.222 -21.629  39.434  1.00 68.64      I    N 0
ATOM     14  CE1 HIS I 133      74.313 -21.403  40.158  1.00 68.41      I    C 0
ATOM     15  NE2 HIS I 133      74.932 -22.579  40.489  1.00 69.35      I    N 0
ATOM     16  CB  HIS I 135      68.328 -19.987  42.685  1.00 43.79      I    C 0
ATOM     17  CG  HIS I 135      69.341 -19.969  41.642  1.00 43.91      I    C 0
ATOM     18  CD2 HIS I 135      69.639 -20.796  40.577  1.00 43.76      I    C 0
ATOM     19  ND1 HIS I 135      70.149 -18.862  41.521  1.00 43.89      I    N 0
ATOM     20  CE1 HIS I 135      70.893 -19.016  40.385  1.00 44.83      I    C 0
ATOM     21  NE2 HIS I 135      70.614 -20.184  39.803  1.00 43.38      I    N 0
ATOM     22 CU+2 CU2 I 420      71.598 -20.589  38.156  1.00 82.47      I   CU 2
END
REMARK 3RFR, Subunit 3, Oxidised state
REMARK DATE:28-Nov-2016  12:00:25       created by user: lili
REMARK CNS VERSION:1.3
ATOM      1  CB  HIS I  29      71.003 -19.318  34.918  1.00 75.89      I    C 0
ATOM      2  CG  HIS I  29      72.119 -20.308  35.143  1.00 76.17      I    C 0
ATOM      3  CD2 HIS I  29      73.003 -20.823  34.220  1.00 76.17      I    C 0
ATOM      4  ND1 HIS I  29      72.531 -20.771  36.411  1.00 77.04      I    N 0
ATOM      5  CE1 HIS I  29      73.635 -21.520  36.230  1.00 75.75      I    C 0
ATOM      6  NE2 HIS I  29      73.932 -21.579  34.915  1.00 75.55      I    N 0
ATOM      7  C   HIS I  29      70.058 -17.102  35.657  1.00 77.52      I    C 0
ATOM      8  N   HIS I  29      70.266 -19.125  37.215  1.00 77.77      I    N 0
ATOM      9  CA  HIS I  29      70.880 -18.337  36.116  1.00 76.79      I    C 0
ATOM     10  CB  HIS I 133      71.993 -23.684  38.411  1.00 69.80      I    C 0
ATOM     11  CG  HIS I 133      73.028 -22.960  39.244  1.00 70.43      I    C 0
ATOM     12  CD2 HIS I 133      74.103 -23.447  39.972  1.00 70.29      I    C 0
ATOM     13  ND1 HIS I 133      73.043 -21.567  39.284  1.00 70.58      I    N 0
ATOM     14  CE1 HIS I 133      74.097 -21.207  40.035  1.00 70.14      I    C 0
ATOM     15  NE2 HIS I 133      74.761 -22.317  40.452  1.00 69.87      I    N 0
ATOM     16  CB  HIS I 135      68.323 -19.992  42.681  1.00 43.95      I    C 0
ATOM     17  CG  HIS I 135      69.341 -19.971  41.639  1.00 44.15      I    C 0
ATOM     18  CD2 HIS I 135      69.649 -20.779  40.562  1.00 43.82      I    C 0
ATOM     19  ND1 HIS I 135      70.143 -18.857  41.551  1.00 44.25      I    N 0
ATOM     20  CE1 HIS I 135      70.897 -18.964  40.431  1.00 45.19      I    C 0
ATOM     21  NE2 HIS I 135      70.622 -20.123  39.816  1.00 43.42      I    N 0
ATOM     22 CU+2 CU2 I 420      71.602 -20.533  38.166  1.00 83.83      I   CU 2
END
REMARK 3RFR, Subunit 3, Oxidised state with an extra water molecule
REMARK DATE:12-Apr-2017  14:54:54       created by user: lili
REMARK CNS VERSION:1.3
ATOM      1  CB  HIS I  29      70.997 -19.319  34.928  1.00 77.06      I    C 0
ATOM      2  CG  HIS I  29      72.114 -20.311  35.138  1.00 77.31      I    C 0
ATOM      3  CD2 HIS I  29      72.969 -20.855  34.203  1.00 77.29      I    C 0
ATOM      4  ND1 HIS I  29      72.552 -20.752  36.405  1.00 78.09      I    N 0
ATOM      5  CE1 HIS I  29      73.641 -21.518  36.213  1.00 76.86      I    C 0
ATOM      6  NE2 HIS I  29      73.908 -21.606  34.891  1.00 76.67      I    N 0
ATOM      7  C   HIS I  29      70.060 -17.099  35.661  1.00 78.66      I    C 0
ATOM      8  N   HIS I  29      70.286 -19.111  37.236  1.00 78.87      I    N 0
ATOM      9  CA  HIS I  29      70.893 -18.334  36.127  1.00 77.97      I    C 0
ATOM     10  CB  HIS I 133      71.995 -23.681  38.415  1.00 70.54      I    C 0
ATOM     11  CG  HIS I 133      73.017 -22.958  39.248  1.00 71.12      I    C 0
ATOM     12  CD2 HIS I 133      74.087 -23.432  39.993  1.00 70.93      I    C 0
ATOM     13  ND1 HIS I 133      73.038 -21.564  39.263  1.00 71.41      I    N 0
ATOM     14  CE1 HIS I 133      74.088 -21.189  40.012  1.00 70.91      I    C 0
ATOM     15  NE2 HIS I 133      74.745 -22.295  40.457  1.00 70.55      I    N 0
ATOM     16  CB  HIS I 135      68.328 -19.991  42.678  1.00 43.89      I    C 0
ATOM     17  CG  HIS I 135      69.339 -19.974  41.642  1.00 44.04      I    C 0
ATOM     18  CD2 HIS I 135      69.644 -20.780  40.561  1.00 43.68      I    C 0
ATOM     19  ND1 HIS I 135      70.146 -18.862  41.551  1.00 44.25      I    N 0
ATOM     20  CE1 HIS I 135      70.902 -18.969  40.429  1.00 44.79      I    C 0
ATOM     21  NE2 HIS I 135      70.619 -20.126  39.816  1.00 43.21      I    N 0
ATOM     22  O   HOH I 419      73.631 -17.917  38.640  1.00 60.97      I    O 0
ATOM     23 CU+2 CU2 I 420      71.621 -20.507  38.169  1.00 83.12      I   CU 2
END
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