**Supplementary Information**

**Direct detection of coupled proton and electron transfers in human manganese superoxide dismutase**

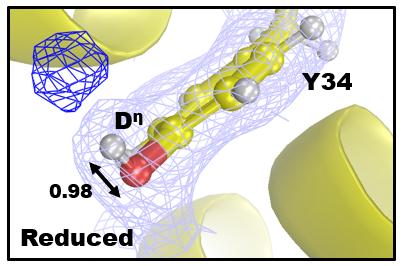
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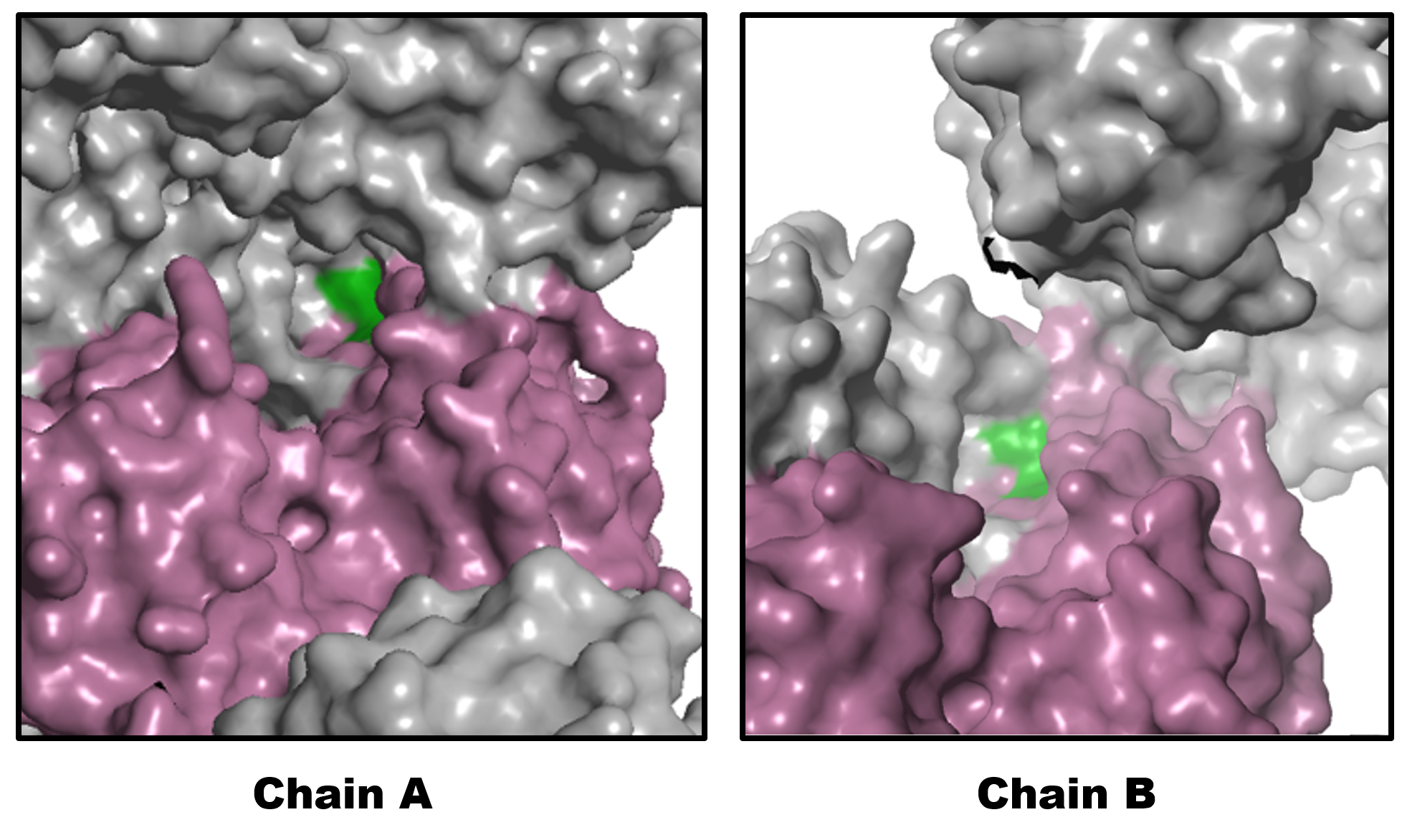
2Eppley Institute for Cancer and Allied Diseases, 986805 Nebraska Medical Center, Omaha, NE 68198-6805, USA

3Second Target Station, Oak Ridge National Laboratory, 1 Bethel Valley Road, Oak Ridge, TN 37831, USA

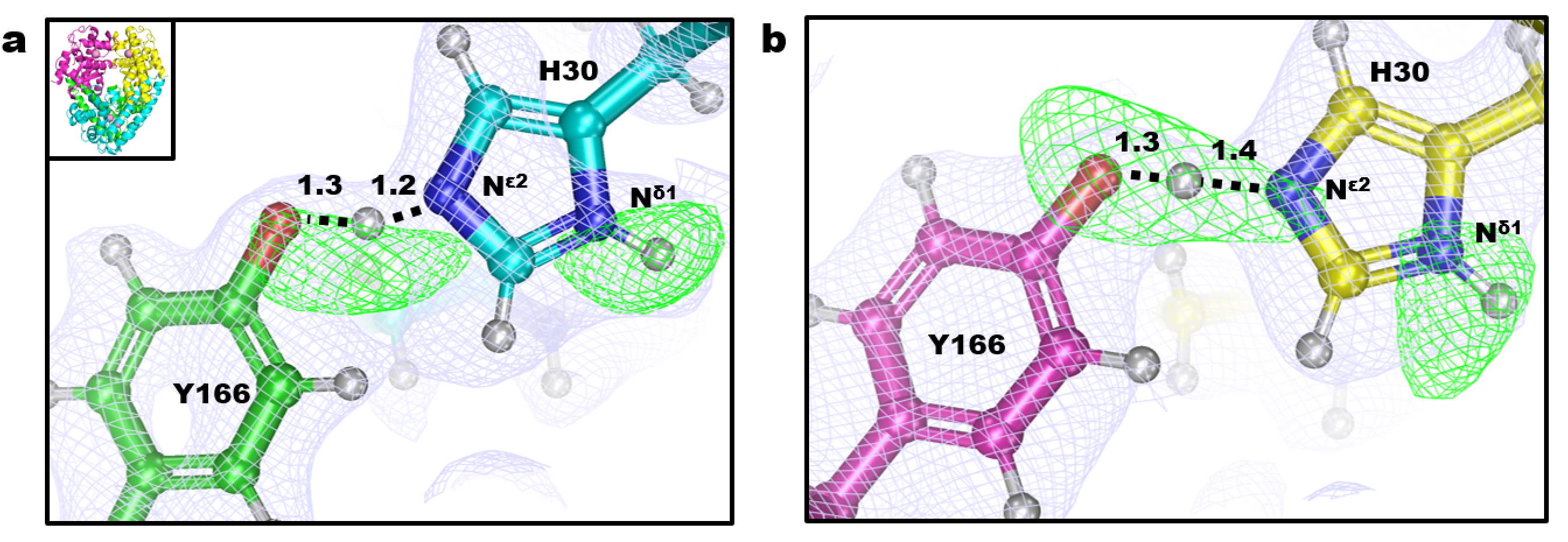
4Neutron Scattering Division, Oak Ridge National Laboratory, 1 Bethel Valley Road, Oak Ridge, TN 37831, USA

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**Figure S1. Residual density for the hydroxyl group of Tyr34 in Mn2+SOD of chain B.** Light blue 2|Fo|-|Fc| nuclear density displayed at 1.0 σ. Dark blue omit |Fo|-|Fc| difference density is displayed at 2.0 σ. Bond length in Å is given.



**Figure S2. Solvent accessibility differences between chains of the asymmetric AB dimer for *P*6122MnSOD.** Magenta depicts the surfaces of the chain for the asymmetric unit and green depicts the surfaces leading to the active site of the chain. Grey depicts the surfaces of symmetry generated asymmetric AB dimers.



**Figure S3.** **Protonation states of His30 and Tyr166 for Mn2+SOD.** Light blue 2|Fo|-|Fc| nuclear density displayed at 1.0 σ. Green omit |Fo|-|Fc| difference density is displayed at 2.5 σ. The inset of (a) shows the tetrameric assembly. Numbers are distances in Å.

**Table S1. Gln143 bonding character from CLPO analysis.** Numbers are calculated bond order.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Five-Coordinate Mn3+  Y166(-)H30(ε)Y34(-) | Five-Coordinate Mn3+  Y166(H)H30(δ)Y34(-) | Six-Coordinate Mn2+  Y166(H)H30(δ)Y34(-) | Five-Coordinate Mn2+  Y166(H)H30(δ)Y34(H) |
| Nε2-Cε1 | 1.36 | 1.37 | 1.56 | 1.52 |
| Oε1-Cε1 | 1.45 | 1.45 | 1.30 | 1.33 |

**Table S2. Charge and energy interactions of donor-acceptor CLPO analysis.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Trp123 Nε1 Lone Pair → Trp123 Cε2-Cδ2 π\*-bond** | | | | |
| State | Donor Occupancy  (e-) | Charge Transfer (e-) | Acceptor Occupancy (e-) | Energy Stabilization (kcal/mol) |
| Five-Coordinate Mn2+  Y166(H)H30(δ)Y34(H) | 1.58 | 0.15 | 0.52 | ↓ 13.52 |
| **Gln143 Nε2 Lone pair → WAT1 O-H σ\*-bond** | | | | |
| State | Donor Occupancy  (e-) | Charge Transfer (e-) | Acceptor Occupancy (e-) | Energy Stabilization (kcal/mol) |
| Six-Coordinate Mn2+  Y166(H)H30(δ)Y34(-) | 1.81 | 0.14 | 0.15 | ↓ 0.46 |
| Five-Coordinate Mn2+  Y166(H)H30(δ)Y34(H) | 1.78 | 0.17 | 0.18 | ↓ 1.39 |

**Table S3. Percent covalence of shared hydrogen atoms in SSHBs bonds from CLPO analysis.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Five-Coordinate Mn3+  Y166(-)H30(ε)Y34(-) | | Five-Coordinate Mn3+  Y166(H)H30(δ)Y34(-) | | Six-Coordinate Mn2+  Y166(H)H30(δ)Y34(-) | | Five-Coordinate Mn2+  Y166(H)H30(δ)Y34(H) | |
| (Gln143)Nε2-H-O(WAT1) | Nε2 | O | Nε2 | O | Nε2 | O | Nε2 | O |
| 0.92 | 0.08 | 0.92 | 0.08 | 0.29 | 0.71 | 0.36 | 0.64 |
| (Tyr166)Oη-H-Nε2(His30) | Oη | Nε2 | Oη | Nε2 | Oη | Nε2 | Oη | Nε2 |
| 0.20 | 0.80 | 0.77 | 0.23 | 0.76 | 0.24 | 0.79 | 0.21 |
| (Tyr34)Oη-H-O(WAT2) | Oη | O | Oη | O | Oη | O | Oη | O |
| 0.15 | 0.85 | 0.23 | 0.77 | 0.20 | 0.80 | 0.90 | 0.10 |
| (His30)Nδ1-H-O(WAT2) | Nδ1 | O | Nδ1 | O | Nδ1 | O | Nδ1 | O |
| 0.14 | 0.86 | 0.89 | 0.11 | 0.88 | 0.12 | 0.92 | 0.08 |

**Table S4. Active Site Bond Lengths of MnSOD Neutron Structures**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Mn3+SOD** | | **Mn2+SOD** | |
| **Mn Covalent Bonds (Å)** | **A** | **B** | **A** | **B** |
| Mn-Nε2(H26) | 2.07 | 2.07 | 2.26 | 2.10 |
| Mn-Nε2(H74) | 2.13 | 2.12 | 2.19 | 2.25 |
| Mn-Oε2(D159) | 1.95 | 1.94 | 2.44 | 2.15 |
| Mn-Nε2(H163) | 2.06 | 2.14 | 2.23 | 2.21 |
| Mn-O(WAT1) | 1.78 | 1.76 | 2.12 | 2.22 |
| Mn-O(OL) | **-** | **-** | 1.82 | - |

**Table S5. Data collection and refinement statistics**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Data Collection Statistics | | | | |
|  | Neutron | | X-ray | |
|  | **Oxidized** | **Reduced** | **Oxidized** | **Reduced** |
| PDB Code | **7KKS** | **7KKW** | **7KKU** | **7KLB** |
| Diffraction Source | MaNDi | | Rigaku FR-E SuperBright | |
| Temperature (K) | 296 | | | |
| Space group | *P*6122 | | | |
| *a*, *b*, *c* (Å) | 81.30, 81.30, 241.840 | 81.33, 81.33, 242.880 | 81.14, 81.14, 241.63 | 81.13, 81.13, 242.12 |
| *α*, *β*, *γ* (°) | 90, 90, 120 | | | |
| Wavelengths (Å) | 2-4 | | 1.5418 | |
| No. of images | 8 | 8 | 180 | 280 |
| Exposure time | 48 h | 48 h | 60 s | 60 s |
| No. of unique reflections | 24556 | 21719 | 31815 | 25718 |
| Total No. of reflections | 196496 | 155817 | 248673 | 194364 |
| Resolution range (Å) | 14.64-2.20 (2.28-2.20) | 14.65-2.30 (2.38-2.30) | 50.00-2.02 (2.07-2.02) | 50.00-2.16 (2.20-2.16) |
| Multiplicity | 8.0 (6.1) | 7.2 (5.7) | 7.8 (3.5) | 7.6 (4.1) |
| I/σ(I) | 7.0 (3.40) | 6.2 (3.3) | 8.3 (2.0) | 4.8 (2.0) |
| Rmerge | 0.284 (0.314) | 0.277 (0.294) | - | - |
| Rmeas | - | - | 0.291 (0.610) | .459 (.683) |
| CC 1/2 | 0.935 (0.275) | 0.943 (0.319) | 0.950 (0.730) | 0.804 (0.638) |
| Rpim | 0.101 (0.129) | 0.102 (0.124) | 0.082 (0.320) | 0.140 (0.326) |
| Data completeness (%) | 98.83 (98.83) | 98.94 (99.16) | 100.0 (100.0) | 97.5 (95.5) |
| Refinement Statistics | | | | |
| Rwork | 0.2565 | 0.2493 | 0.2166 | 0.2075 |
| Rfree | 0.2817 | 0.3021 | 0.2517 | 0.2544 |
| No. of atoms  Protein including D  Solvent  Mn | 6606  6228  376  2 | 6556  6222  332  2 | 3291  3162  127  2 | 3284  3168  98  2 |
| R.m.s. deviations  Bond lengths (Å)  Bond angles (°) | 0.093  0.78 | 0.092  0.64 | 0.002  0.47 | 0.006  0.98 |
| Average *B*-factor  Protein  Water  Mn | 33.62  30.51  25.3 | 30.57  29.19  23.53 | 28.23  30.64  17.00 | 33.82  31.91  24.30 |

**Supplementary Methods**

**Data processing and refinement.** X-ray refinement was performed by removing all non-protein entities in the starting model of 5VF91, simple molecular replacement through rigid-body refinement, and subsequent restrained-positional refinement. With *COOT*2, the protein model was manually fit into |*F*o|-|*F*c| peaks as needed and refined first. New solvent structure and Mn atoms were manually modeled into |*F*o|-|*F*c| density. As D atoms were manually added during iterations of neutron refinement, the stereochemistry weight scale was manually adjusted due to the increased number of atoms at sensitive stereochemical positions. Loose Mn-coordination restraints were derived from the Mn2+SOD X-ray structure and applied to the Mn2+SOD neutron model, whereas the Mn3+SOD neutron model used restraints derived from our own DFT calculations. In both cases, the R-free value was improved with the application of these restraints.

**Computational Details.** Computations from the NWChem 6.8 software package utilized an extra-fine integration grid quadrature known to provide high precision with restricted open-shell John-Sham (ROKS) treatment3-5. The geometry optimizations implemented the B3LYP exchange-correlation functional dispersion corrected according to Becke and Johnson damping (DFT-D3-BJ)6,7. Optimizations were first performed in the gas phase until electron density converged to an energy difference of < 0.0627 kcal/mol between macro iterations. A less strict threshold was used for the solution phase due to its significant increase in computational load. There was no notable difference when optimizations began directly in the solution phase other than longer computational times. The nearest three water molecules found in the neutron structure counterparts, representative of the ordered solvent found at the active site, were included in the QM models in addition to the Mn-ligated solvent.

**Solvation model.** The COSMO solvation model treats solvent as an implicit dielectric continuum (i.e. many solvent molecules need not be explicitly modeled in the QM system). The charge distribution of the continuum is derived using a scaled-conductor boundary condition between the cavity surface and solvent8. The inclusion of several explicit solvent molecules in combination with an implicit solvent model to model explicitly known hydrogen bonds increases the accuracy of energy calculations9. In the case of the present QM system, the explicit water molecules utilized are representative of the ordered solvent found experimentally in the neutron structures and other published X-ray structures1.

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