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Supporting information for article:

Crystal structures of a dodecameric multicopper oxidase from *Marinithermus hydrothermalis*

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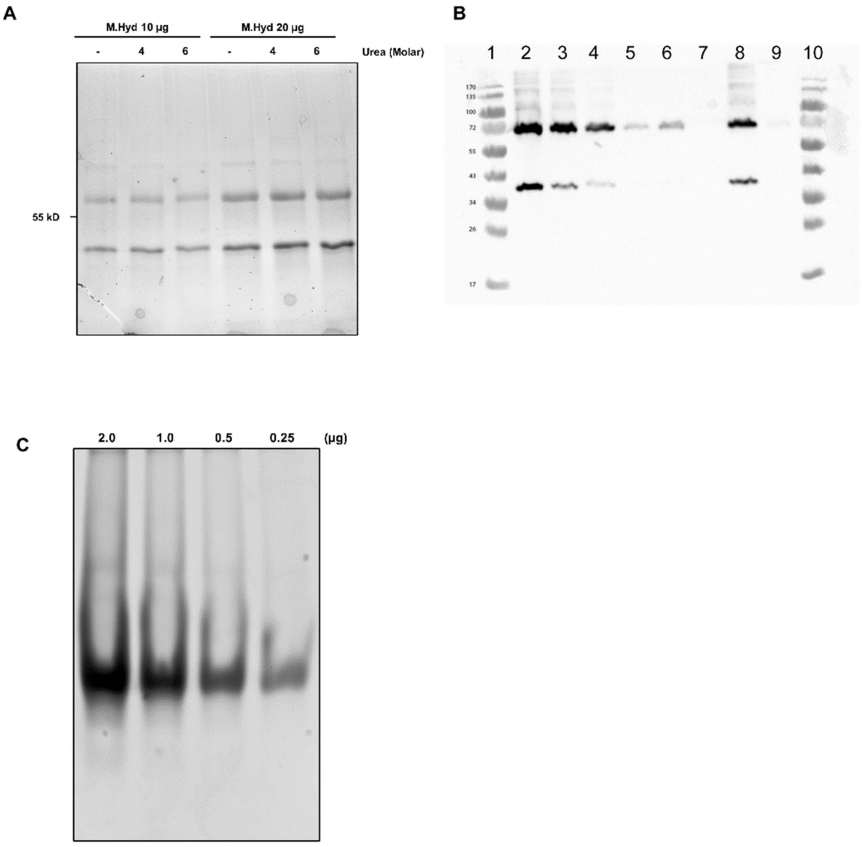

Figure S1 Analysis of MhMCO purity. (A) A stain-free (BioRad) 10% SDS-PAGE image is shown for 10 and 20 µg of MhMCO following purification by ion-exchange chromatography. Final concentrations of urea of 4 and 6 M were added to the samples in an effort to denature the protein. Two major bands are observed, one at ~ 40 kDa and one at ~70 kDa. The ~40 kDa band corresponds to the expected MW for a single polypeptide chain of MhMCO. The gel was imaged on a BioRad ChemiDoc instrument. (B) A western blot probed with anti-6-His antibody for approximately 20, 4, 2, 0.4, and 0.2 µg of ion-exchange purified MhMCO run on a 12.5 % SDS gel is shown in lanes 2-6, markers are shown in lanes 1 and 10, and induced and uninduced samples of crude extracts from the bacterial cell culture are shown in lanes 8 and 9. Two major bands were observed for the ion-exchange purified MhMCO as was true for the SDS-PAGE analysis suggesting that both of the bands are hexa-His-tagged MhMCO. Faint higher molecular weight bands appear in both the SDS-PAGE and western blot images suggesting that these bands are higher MW multimers of MhMCO. (C) Image of a 6% native gel with 0.25-2 µg of sample loaded per lane as indicated. Samples were diluted in 50 mM Tris pH 8.0 and the gel was run in TBE buffer. A single major band is observed in the native gel separation for MhMCO.

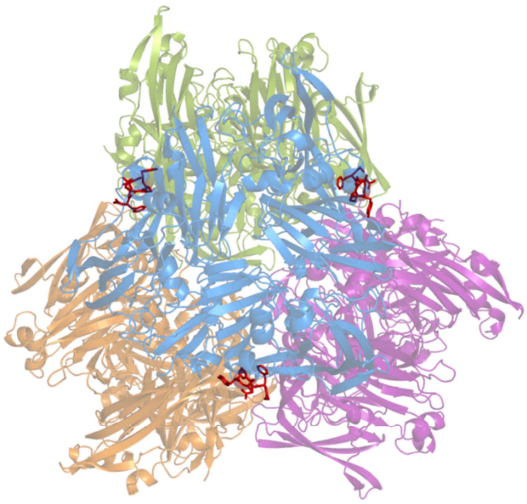

Figure S2 Shown is a cartoon rendering of the orthorhombic dodecameric structure (trimers in orange, blue, purple, and green). The N-termini associated with the blue trimer are shown in red stick renderings. The N-termini are on the outside face of each subunit within the trimeric units and thus do not interfere with formation of the dodecamer.