Supplemental Information

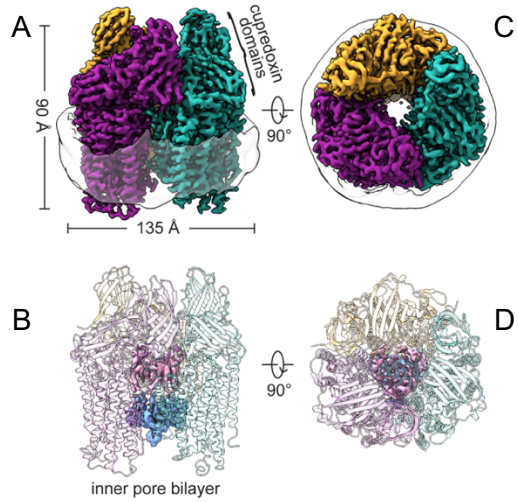

Figure 1: (**A**) CryoEM (2.14 Å resolution) map of *M. capsulatus* (Bath) pMMO showing the nanodisc and the pMMO trimer colored by protomer. (**B**) Model highlighting stabilized lipid bilayer in the inner pore, with periplasmic lipids colored blue and cytoplasmic lipids colored pink. (**C**) Representative micrograph and (**D**) 2D class averages. Adapted from Koo *et al., Science* **2022.**

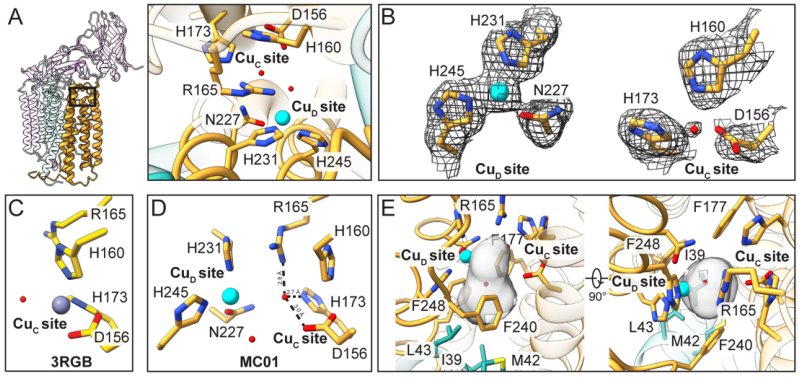

Figure 2: Previously-unobserved metal-binding site and substrate cavity. (**A**) Overall view of three subunits in protomer. (**B**) New Cu$_D$ metal-binding site and empty Cu$_C$ site. Comparison of disrupted crystal structure PmoC site (**C**) and fully-ordered site in the cryo-EM structure (**D**). (**E**) Possible substrate cavity formed in the cryo structure. Adapted from Koo *et al., Science* **2022.**

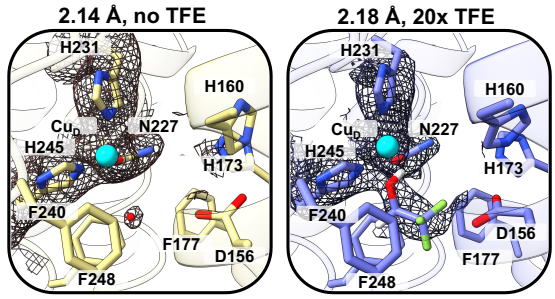

Figure 3: CryoEM structures of pMMO in nanodiscs without (yellow, left) and with (purple, right) trifluoroethanol (TFE). Notably, the map with TFE added (right) shows a large density that connects to the Cu$_D$ site (modeled as TFE with fluorine atoms as green sticks) while the map without TFE (left) has density attributable to a water molecule in this location.

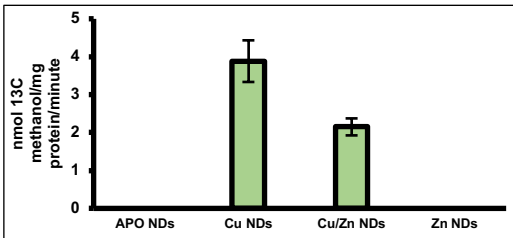

Figure 4: Methane oxidation activity of metal-depleted pMMO reloaded with (from left to right) no metal, 10 eq. Cu(II), 5 eq. Cu(II) + 5 eq. Zn(II), and 10 eq. Zn(II) and reconstituted into nanodiscs (NDs).

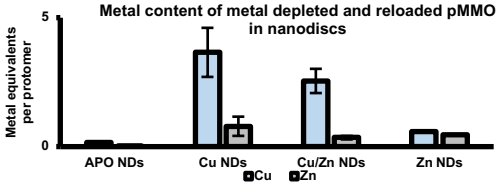

Figure 5: Copper and zinc content per protomer for pMMO in nanodiscs depleted and reloaded with (from left to right) no metal, 10 eq. Cu(II), 5 eq. Cu(II) + 5 eq. Zn(II), and 10 eq. Zn(II).

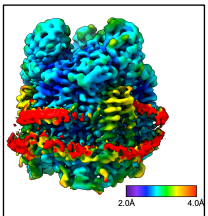

Figure 6: 3.12 Å cryoEM local resolution map for metal depleted pMMO reloaded with 10 eq. Cu(II). Notably, the PmoC subunit shows lower local resolution, likely as a result of prior copper depletion and subunit destabilization.

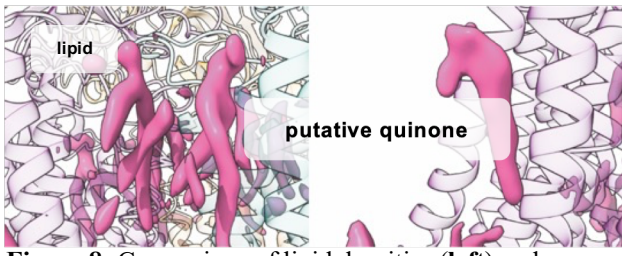

Figure 8: Comparison of lipid densities (**left**) and putative quinone density bound to PmoC (**right**) in the cryoEM map of *M. capsulatus* (Bath) pMMO.

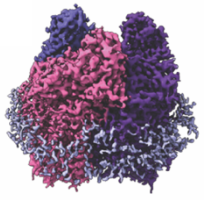

Figure 7: CryoEM structure of of *Methylacidiphilum* sp. str. Yellowstone pMMO in CHAPS at 3.35 Å showing each protomer and the detergent micelle.