Structure *16*

Supplemental Data

Structure of a Copper Pump Suggests a Regulatory Role for Its Metal-Binding Domain

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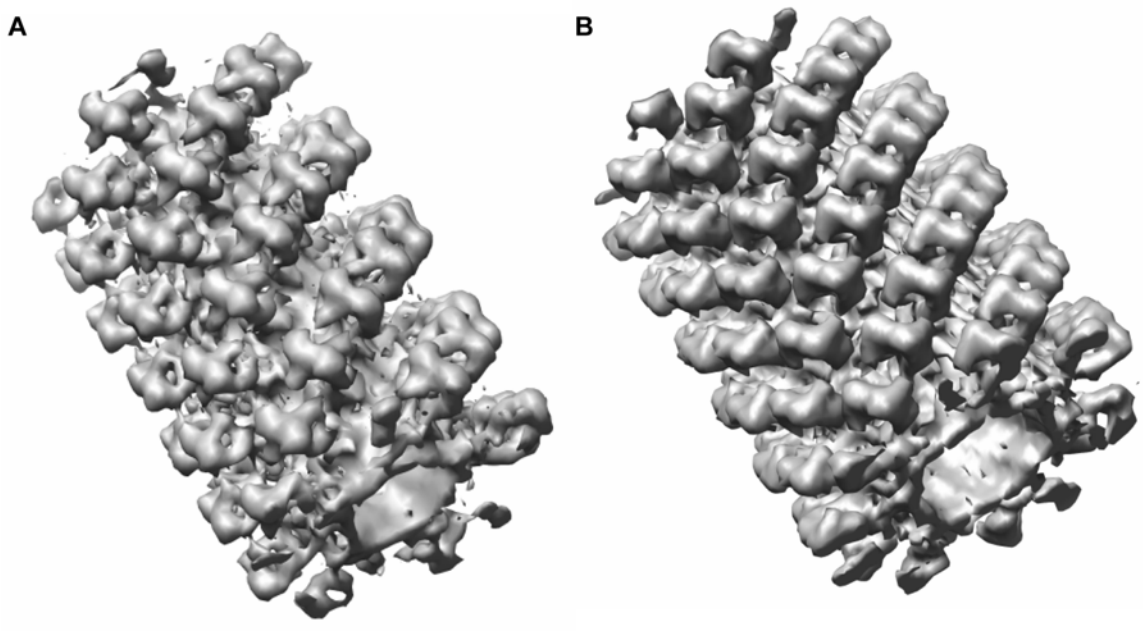

Figure S1. Overview of the Helical Tubes

(A) Tubular crystal of ΔC-CopA. (b) Tubular crystal of ΔNΔC-CopA. Dimeric elements can be seen arranged in a helical assembly. Note that the ΔC-CopA are narrower than ΔNΔC-CopA tubes, which is reflected in the helical symmetries in Table 1.

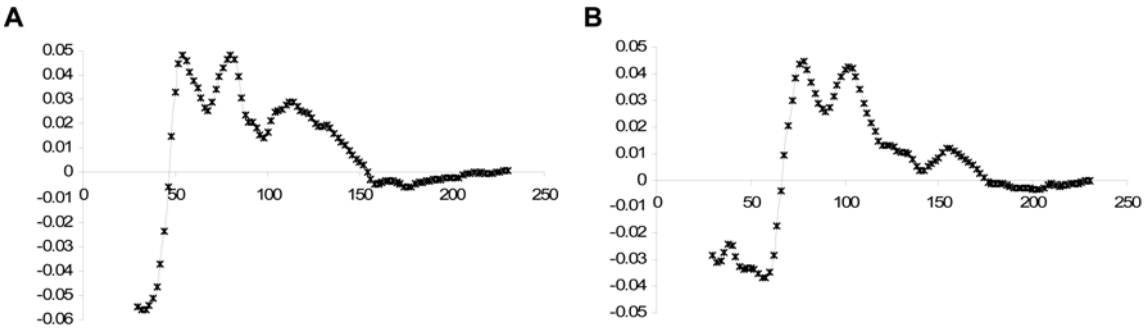

Figure S2. Mean Radial Density Distributions

(A) ΔC-CopA. (B) ΔNΔC-CopA. The twin maxima in both plots reflect the higher scattering at the surfaces of the bilayer, presumably due to the concentration of phosphate. The average density within the membrane is generally higher than on the cytoplasmic side of the membrane (higher radius), explaining the lower contrast within the membrane portion of the map.

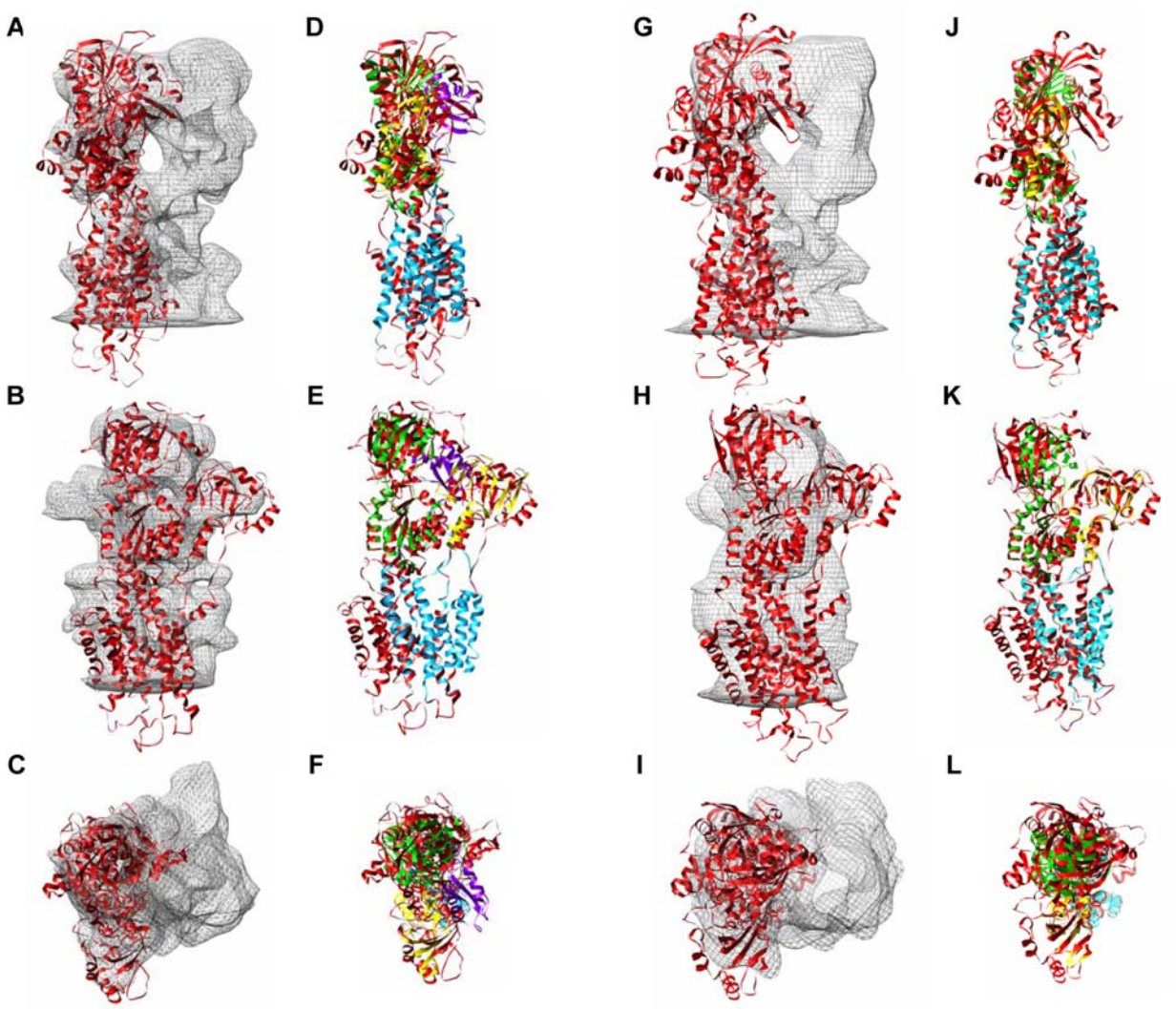

Figure S3. Model Construction for CopA

Prior to docking, atomic coordinates from Ca^{2+}-ATPase were used as a template for positioning isolated domains of CopA. In particular, the N-/P-domain pair from *A. fulgidus* (PDB accession code 2B8E) was aligned with the corresponding Ca^{2+}-ATPase domains as was the A-domain from *A. fulgidus* (2HC8). Transmembrane helices 1-6 were taken from Ca^{2+}-ATPase and the N-terminal metal binding domain from *B. subtilis* (1JWW) was positioned in unoccupied density. For ΔC-CopA, the structure of Ca^{2+}-ATPase in the E2 state (2EAR) was used as a template. For ΔNΔC-CopA, the Ca^{2+}-ATPase structure in the E2-P state with MgF_4 (1WPG) was used. (A-C) Juxtaposition of the Ca^{2+}-ATPase templates within the ΔC-CopA density map. Ca^{2+}-ATPase is a considerably larger molecule due to the presence of many inserted elements of secondary structure, including four additional helices on the C-terminal end of the molecule.

(D-F) Superposition of CopA domains on the Ca^{2+}-ATPase template in the E2 state. (G-I) Superposition of Ca^{2+}-ATPase within the ΔNΔC-CopA map. (J-L) Superposition of CopA domains on the Ca^{2+}-ATPase template in the E2-P state. Colors are as follows. red: Ca^{2+}-ATPase template. cyan: transmembrane helices. green: N-/P-domain pair from *A. fulgidus* CopA. yellow: A-domain from *A. fulgidus* CopA. purple: N-terminal MBD from *B. subtilis* CopA.

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Menkes6   561 ------EG-DGV-----LELVVRGMTCASCVHKIESSLTKHRGILYCSVALATNKAHIKY
Wilson6   557 ---EDYAGSDGN-----IELTITGMTCASCVHNIESKLTRTNGITYASVALATSKALVKF
HAH1        1 ---------MPK-----HEFSVD-MTCGGCAEAVSRVLNKLGG---VKYDIDLPNKKVCI
ATX1        1 ------MAEIKH-----YQFNVV-MTCSGCSGAVNKVLTKLEPDV-SKIDISLEKQLVDV
CopA2      72 --------VTEK-----AEFDIEGMTCAACANRIEKRLNKIEGVANAPVNFALETVTVEY
CopA        1 MVKDTYISSASKTPPMERTVRVTGMTCAMCVKSIETAVGSLEGVEEVRVNLATETAFIRF
                            - β  -  -    α     -    -   β  -  - β  -
Serca1a     1                                                MEAAHSKSTEECLA
                                                               –  α

Menkes6       DPEIIGPRDIIHTIESLGFEASLVKKDRSASH--------------------------        640
Wilson6       DPEIIGPRDIIKIIEEIGFHASLA-----------------------------------        632
HAH1          ESEHS-MDTLLATLKKTGKTVSYLGLE--------------------------------         68
ATX1          YTTLP-YDFILEKIKKTGKEVR-SGKQL-------------------------------         73
CopA2         NPKEASVSDLKEAVDKLGYKLKLKGEQDS------------------------------        147
CopA          DEKRIDFETIKRVIEDLGYGVVDEQAAVSAEVEHLSRMKRKLYVAAFAGVLLLFLAHFISLPYED  125
                    -   α    -     –β–              TM1'         TM1
              YFGVSETTGLTPDQVKRHLEKYGHNELPAEEGKSLWELVIEQFEDLLVRILLLAACISFVLAW     77
               –       –    α     -              -  TM1'-  -     TM1        -
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Figure S4. Sequence Alignments

Six MBDs have been aligned with secondary structure shown below. These sequences correspond to the sixth MBD from MNK (Menkes6), the sixth MBD from WNDP (Wilson6), Atx1 metallochaperone from yeast, Hah1 metallochaperone from humans, the second MBD from CopA from *B. subtilis* (CopA2) and the MBD from copA from *A. fulgidis* (CopA). In the latter case, the sequence is extended through the first transmembrane helix (TM1). At the bottom, the sequence from Ca^{2+}-ATPase (SERCA1a) is shown with an aligned TM1. TM1' corresponds to the bent extension of this helix running within the lipid head groups. There follows an unstructured loop of 11-13 residues connecting the respective N-terminal domains, which in the case of Ca^{2+}-ATPase is a pair of α-helices and in CopA is the MBD.