ARTICLE

Supporting Information

Transmembrane Cu(I) P-type ATPase pumps are electrogenic uniporters

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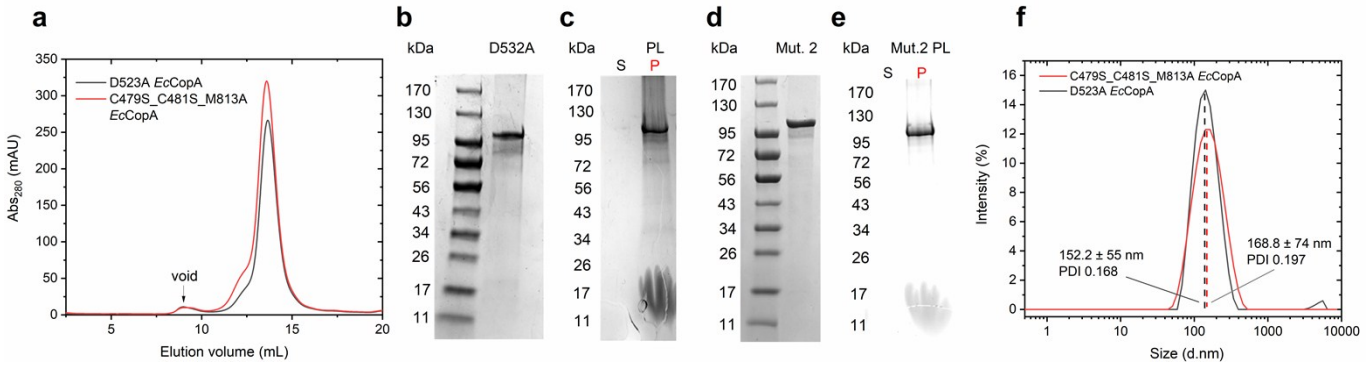

Figure S1: *Ec*CopA mutants' purification and reconstitution in proteoliposomes. (a) Size Exclusion Chromatogram (SEC) elution profile of D523A *Ec*CopA and C479S_C481S_M813A *Ec*CopA purified in DDM micelles. (b) SDS-PAGE of purified D523A *Ec*CopA and (c) corresponding SDS-PAGE analysis of *Ec*CopA incorporation in isolated proteoliposomes (P, pellet) in comparison to non-incorporated protein (S, supernatant). (d) SDS-PAGE of purified C479S_C481S_M813A *Ec*CopA and (e) corresponding SDS-PAGE analysis of C479S_C481S_M813A *Ec*CopA incorporation in isolated proteoliposomes (P, pellet) in comparison to non-incorporated protein (S, supernatant). (f) Dynamic light scattering (DLS) analysis of D523A *Ec*CopA and C479S_C481S_M813A *Ec*CopA proteoliposomes revealing monodisperse vesicle size distribution.

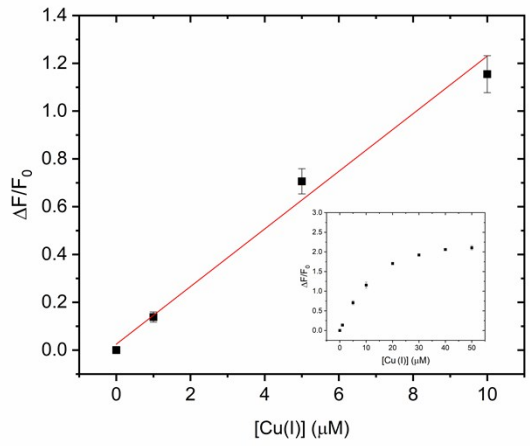

Figure S2: Calibration of CTAP-3 (20 μM) fluorescence in proteoliposome lumen. Relative changes of fluorescence emission (λ_{ex}= 365 nm; λ_{em}= 450 nm) were recorded upon encapsulation of known Cu(I) amounts (0-50 μM) in proteoliposomes prepared under the same conditions utilized for Cu(I) transport assays. A linear dependency (R^2= 0.99) was observed for Cu(I) concentration up to 10 μM and a fluorescence change saturation at approx. 20 μM (inset).