**Supplementary Materials:** The following supporting information can be downloaded at: www.mdpi.com/xxx/ and supplementary materials contain: Figure S1, Figure S2 and Table S1.

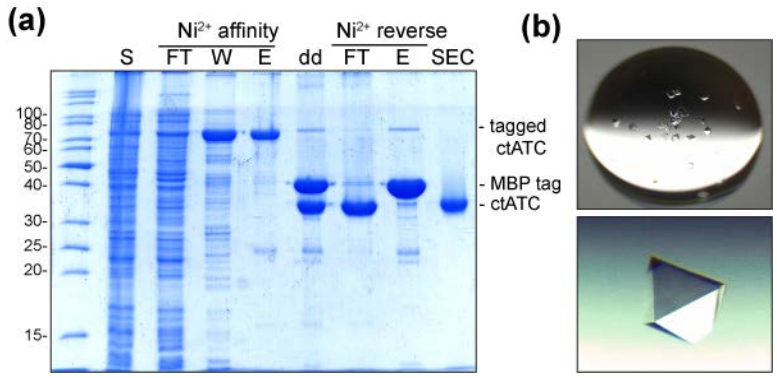


**Figure S1**. **ctATC2Cys purification and crystallization**. **(a)** SDS-PAGE of different steps along the purification of ctATC2Cys: S, soluble fraction of the lysate; $Ni^{2+}$ affinity FT, W, and E, are the flowthrough, wash with 35 mM imidazole and elution of the first affinity column; dd, sample after dialysis and digestion with PreScission; $Ni^{2+}$ reverse FT and E are the flowthrough and elution of the second affinity column; SEC, sample after size exclusion chromatography. **(b)** Bipyramidal-shaped crystals of ctATC2Cys.

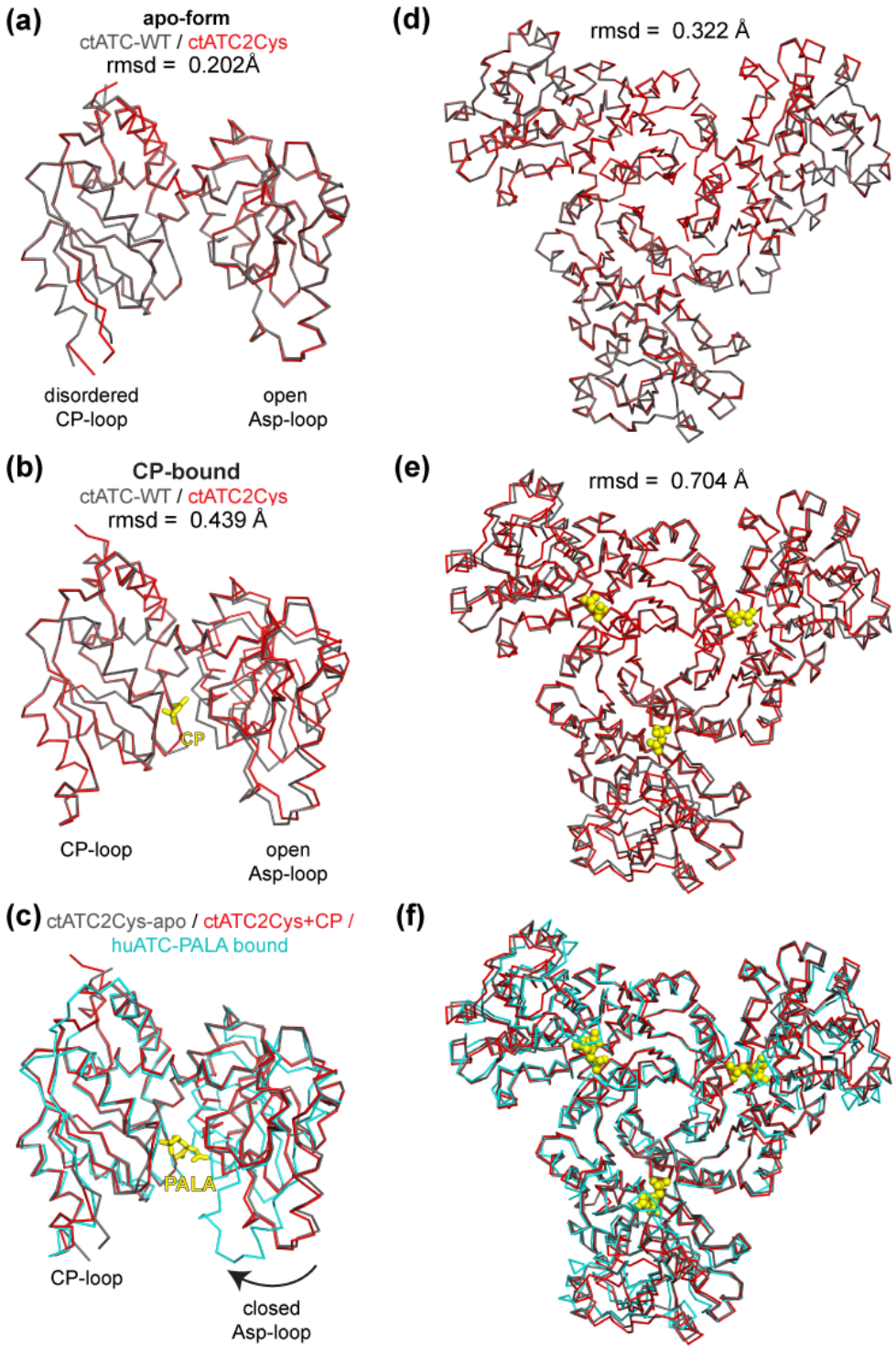


**Figure S2**. Structural similarity of ctATC2Cys and ctATC-WT. Ribbon superposition ctATC-WT (in grey) and ctATC2Cys (in red) subunits in apo form **(a)** or bound to CP **(b)**. **(c)** Superposition of ctATC2Cys apo and CP-bound structures with human ATC bound to PALA (shown in yellow sticks). The arrow indicates the expected movement of the Asp-loop in ctATC2Cys upon binding to PALA. **(d–f)** Superpositions for the entire protein trimer.

**Table S1. Oligonucleotides used for PCR amplification and mutagenesis of ctATC.** Codons for Cys mutations are in red and flanking regions for In-fusion cloning are underscored.

| Oligonucleotide | Sequence 5′- 3′ |
|---|---|
| ctATC-N2045C_Fw | CTG CGA ACC CTG GCC TGC TAC TCT GAT GCT ATC |
| ctATC-N2045C_Rv | GAT AGC ATC AGA GTA GCA GGC CAG GGT TCG CAG |
| ctATC-R2238C_Fw | TAT TTT CGG CAG ATG TGC TAT GGG CTG TAT TGT |
| ctATC-R2238C_Rv | ACA ATA CAG CCC ATA GCA CAT CTG CCG AAA ATA |
| ctATC-Fw | AAGTTCTGTTTCAGGGCCCTCATTCAAGAAATCTCAC |
| ctATC-Rv | ATGGTCTAGAAAGCTTTAGGACATGACCAGTGC |