Supplementary Figures

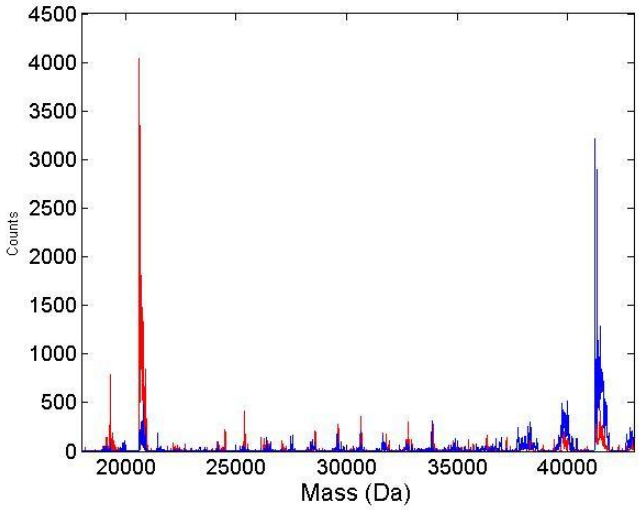

Figure S1. WT_{DTT} crystals are reduced by the soak with DTT. Analysis of dissolved WT_{DTT} crystals were analyzed by mass spectrometry to assess the redox state of the enzyme after being soaked for ~2 minutes with 0.1 M DTT. The spectra of oxidized (blue) and reduced (red) wild type crystals are shown. The theoretical MW of the dimer is 41230.76 Da (C_{1856} H_{2852} N_{482} O566 S_8) and 20616.39 Da for the monomer, with actual values measured at 41231 Da and 20616 Da, respectively. Though the spectra are noisy due to the presence of ionizable reagents present in the crystallization buffer, the primary peak for the WT_{DTT} crystals is the C_P-thiol/thiolate form of the enzyme and is clearly monomeric, indicating that the disulfides of the $LU_{S\text{-}S}$ crystals were indeed reduced by the DTT soak.

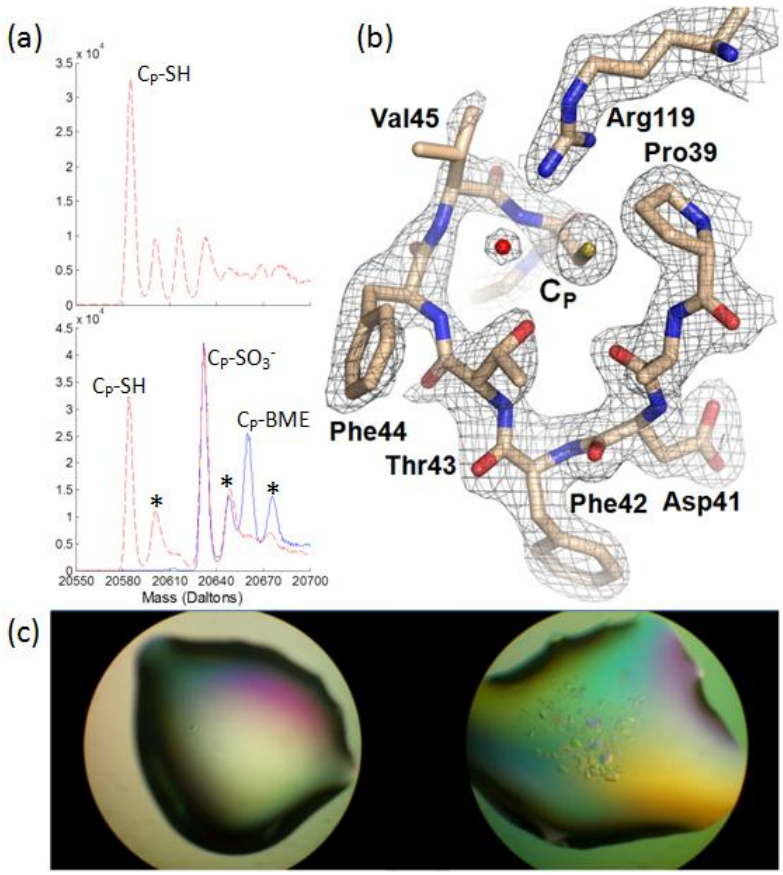

Figure S2. Characterization of the *St*AhpC C165A variant. (a) Mass spectrum of dissolved $C165A_{DTT}$ crystals is shown in the upper panel. The theoretical and actual peaks for the dominant thiol state were 20584.32 Da and 20585 Da, respectively. Smaller peaks may be higher oxidized states (see further comment in panel b). The mass spectrum of C165A crystals pretreated with 100 mM peroxide is shown in the lower panel. For the reduced sample the peaks of interest were (in Da with actual in parenthesis): C_P-SH 20584.32(20584); C_P-SOH 20600.32(20601); C_P-SO_3^- 20632.32(20632). For the oxidized sample the peaks of interest were: C_P-SO_3^- 20632.32(20632); C_P-BME 20660.44(20660), C_P-BME+OH 20676.44(20676). Crystals were found to be C_P-SO_3^- and C_P-BME (BME was in the protein stock) when oxidized (blue) and C_P-SH and C_P-SO_3^- when reduced with 0.1 M DTT (red dashed). We suspect that smaller peaks could be due to Met-oxidation as could be expected at this concentration of peroxide with mass shift of ~16 Da. However, due to noise a more accurate mass of these peaks was not obtained, and further work was not done to confirm their identity. (b) The FF active site of $C165A_{DTT}$. $C165A_{DTT}$ $2F_O$-F_C electron density (contoured at 1.0 ρ_{rms}) for the CD′ active site shows the refined FF model for the C_P-loop, oriented as in Fig. 3a. Because mass spectra of $C165A_{DTT}$ crystals exhibited minor peaks for the approximate molecular weights of higher oxidized states of C_P, we cannot precisely quantify the effect of the mutation on active site stability. (c) Crystallization drop at 24 h time point resulting from a trial using C165A as purified (left panel) compared with using C165A protein treated with by the addition of 10 μM BME for 30 min,

followed by 20 μM peroxide (right panel). These crystals (on the right side of panel c) provided the structure shown in (b).

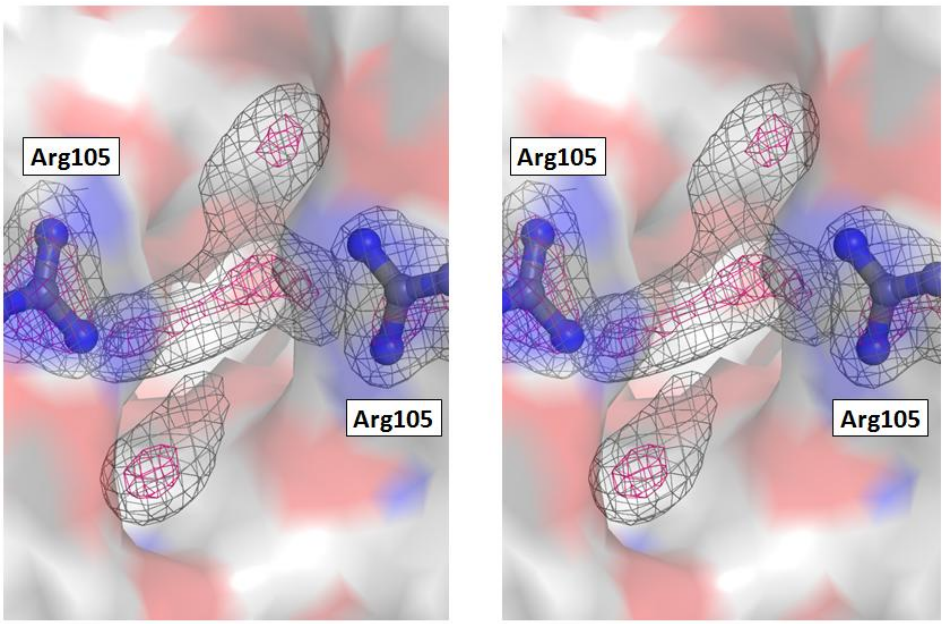

Figure S3. Uninterpreted electron density at the decamer-building interfaces. Shown is $2F_O$-F_C density at the interface between chains B and C of the WT_{DTT} structure (transparent surface with carbons grey, oxygens red, nitrogens blue). Contour levels are 1.2 ρ_{rms} (grey) and 3.0 ρ_{rms} (hot pink). This is representative of density present at all decamer-building interfaces. The site is located near Arg105 (sticks) in a generally electropositive environment having local NCS two-fold symmetry. Strong density of this shape was observed in both the WT_{DTT} and $C165A_{DTT}$ structures, and could not be easily fit with any of the constituents in the crystallization conditions or reagents used during the protein purification process. Also mass spectrometry of the samples did not show clearly-interpretable peaks for a bound small molecule. The strong density and long interaction distances (all ≥3.5 Å) would be consistent with a sulfur atom at some positions. Such density is also seen in electron density maps of C46S (PDB code 1n8j) and $LU_{S\text{-}S}$ (PDB code 1yep) but the density is not as strong and the main peaks were modeled as waters in those structures.