Supplemental Materials

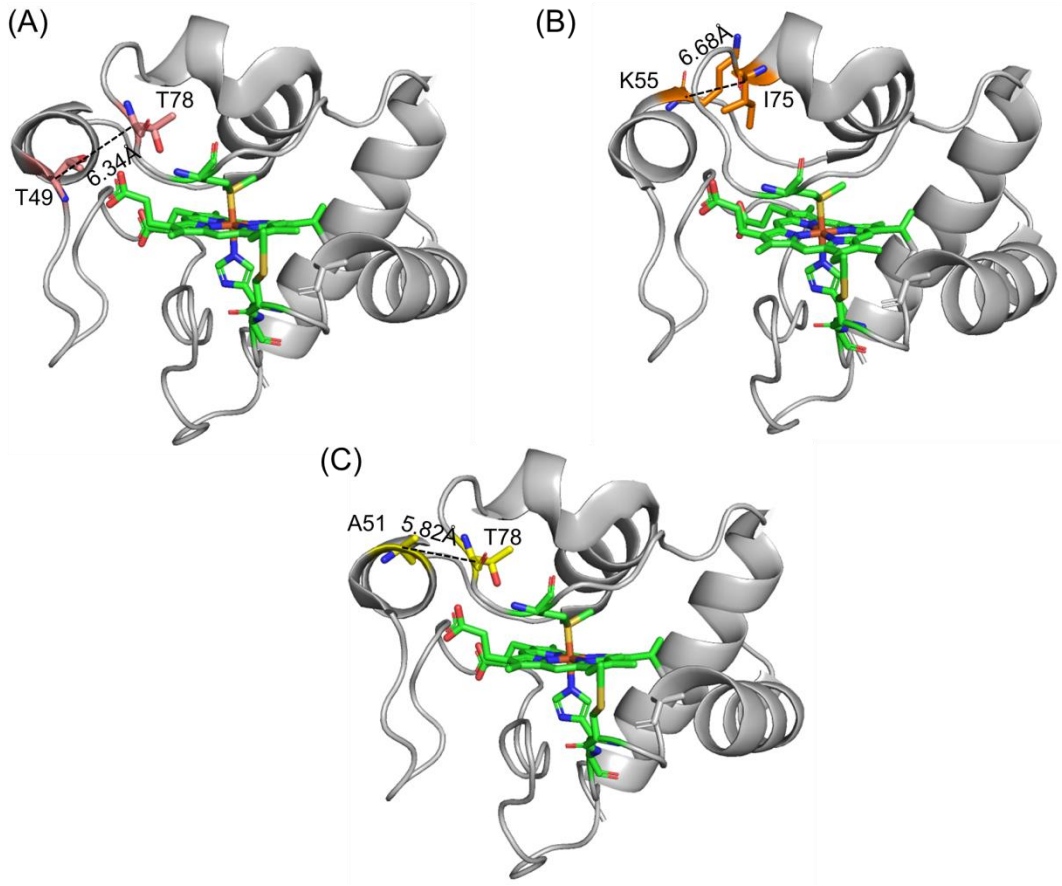

Fig. S1 Possible targets for disulfide engineering as predicted by the web-server Yosshi. The distances between the amino acids in loops C and D are indicated by dashed lines. (A) T49-T78, (B) K55-I75, and (C) A51-T78.

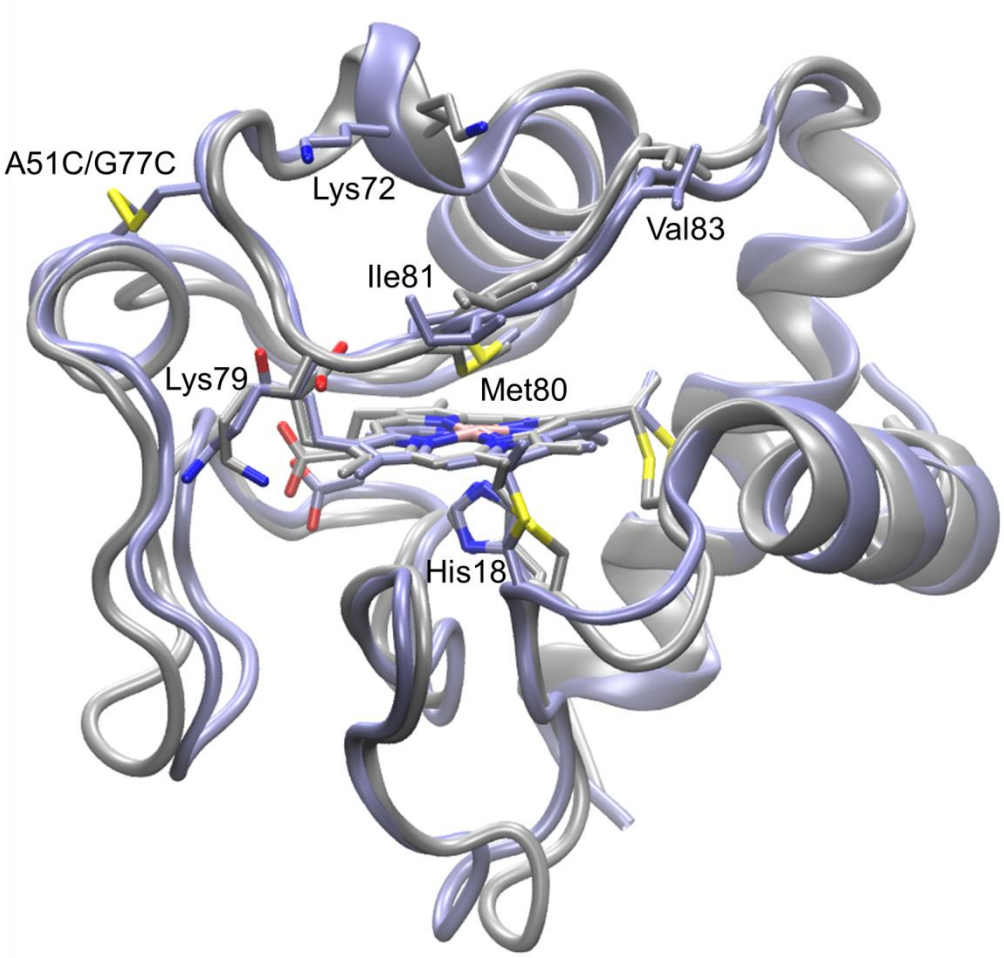

Fig. S2 Overlay of the simulated structure of A51C/G77C *h*Cyt *c* (blue) with the solution structure of WT *h*Cyt *c* (PDB code 2N9J, gray) showing the conformational changes upon formation of the disulfide bond Cys51-Cys77.

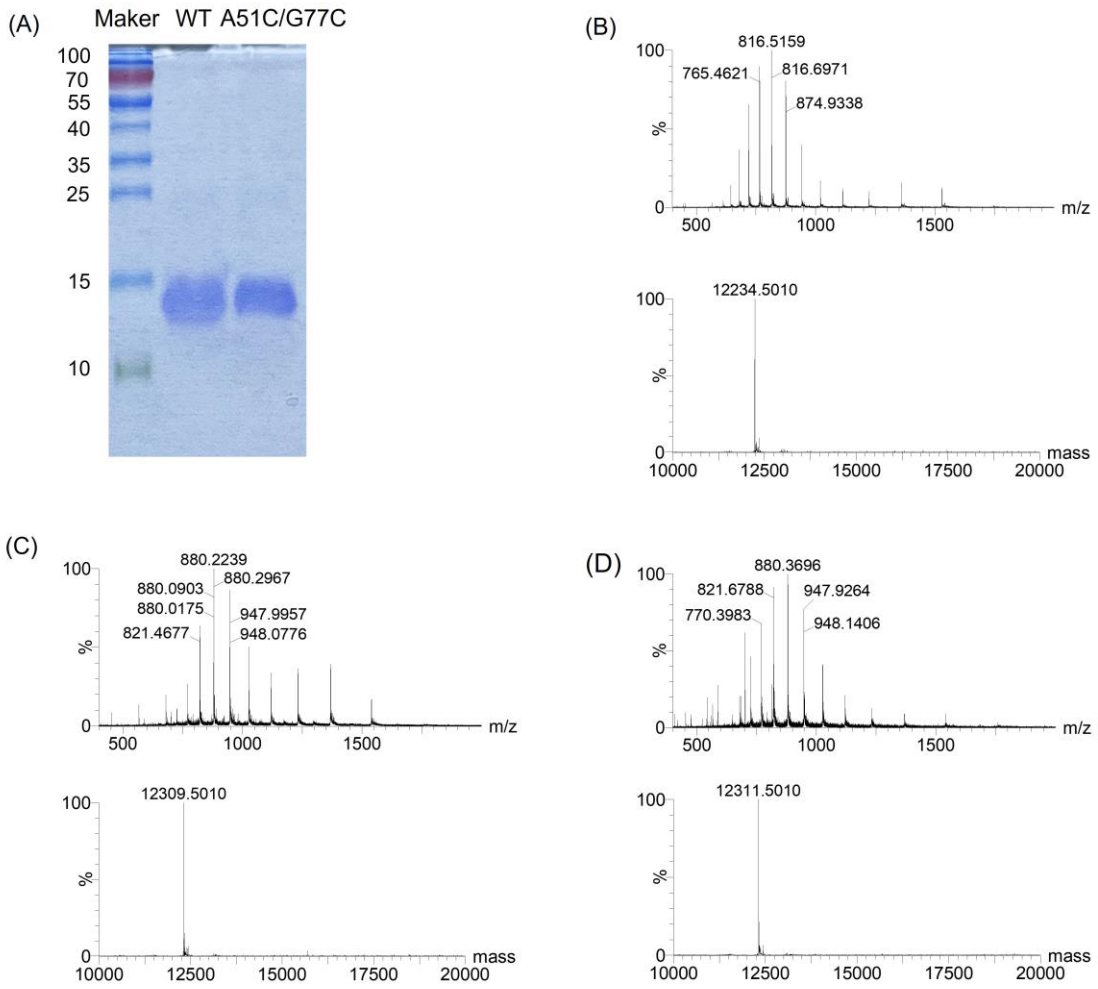

Fig. S3 (A) SDS-PAGE of the purified WT and A51C/G77C *h*Cyt *c*. The protein marker was shown for comparison. ESI-MS spectra of the purified (B) WT *h*Cyt *c*, (C) A51C/G77C *h*Cyt *c* and (D) treated with reducing agent TCEP.

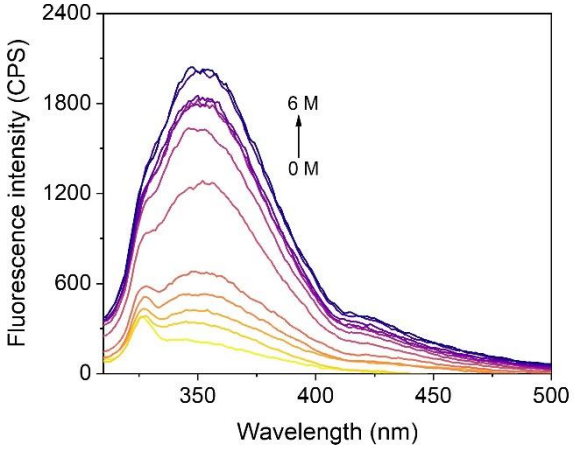

Fig. S4 Fluorescence spectra of WT *h*Cyt *c* varied with the concentration of guanidine hydrochloride (0-6 M).

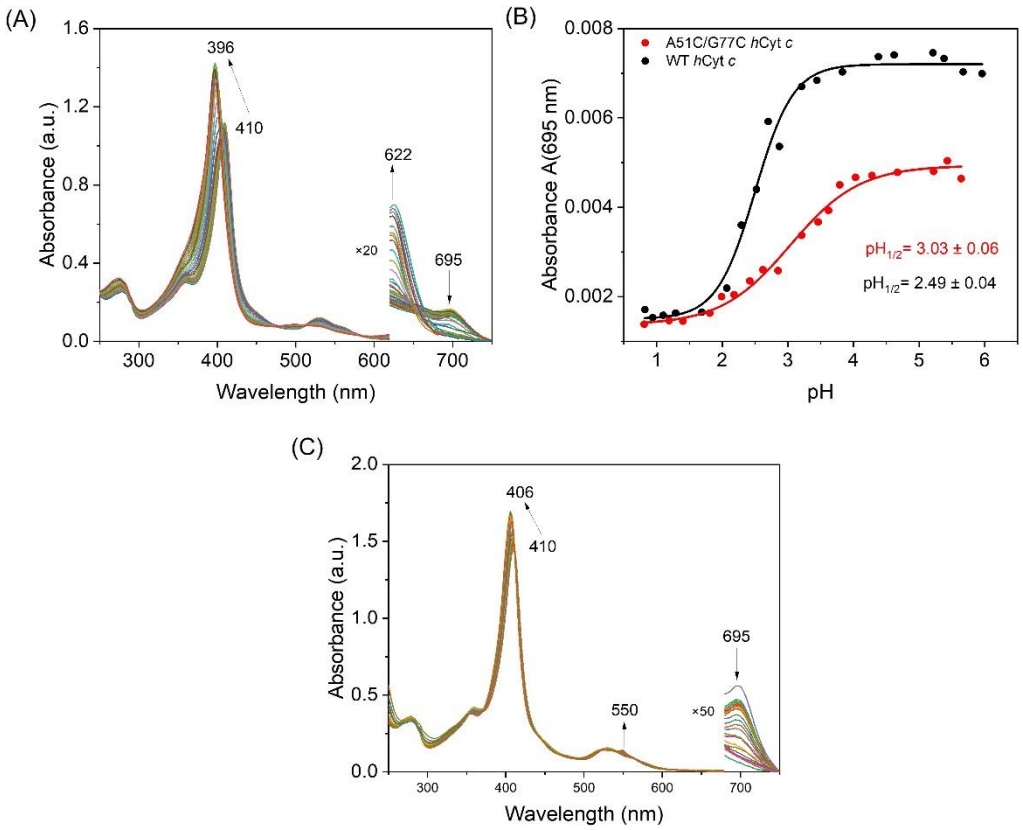

Fig. S5 (A) pH-dependent UV−vis spectra changes of the WT *h*Cyt *c* in acidic unfolding studies; (B) Plot of 695 nm versus pH for acid unfolding of WT and A51C/G77C *h*Cyt *c*; (C) pH-dependent UV−vis spectra changes of the WT *h*Cyt *c* in alkaline transition studies.

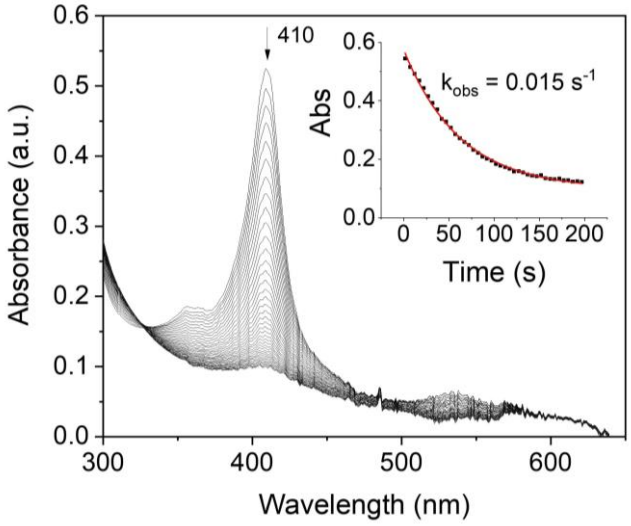

Fig. S6 Time-dependent UV−vis spectra of WT *h*Cyt *c* in a reaction with 100 mM H_2O_2. The spectral change of the Soret band was shown as an inset.

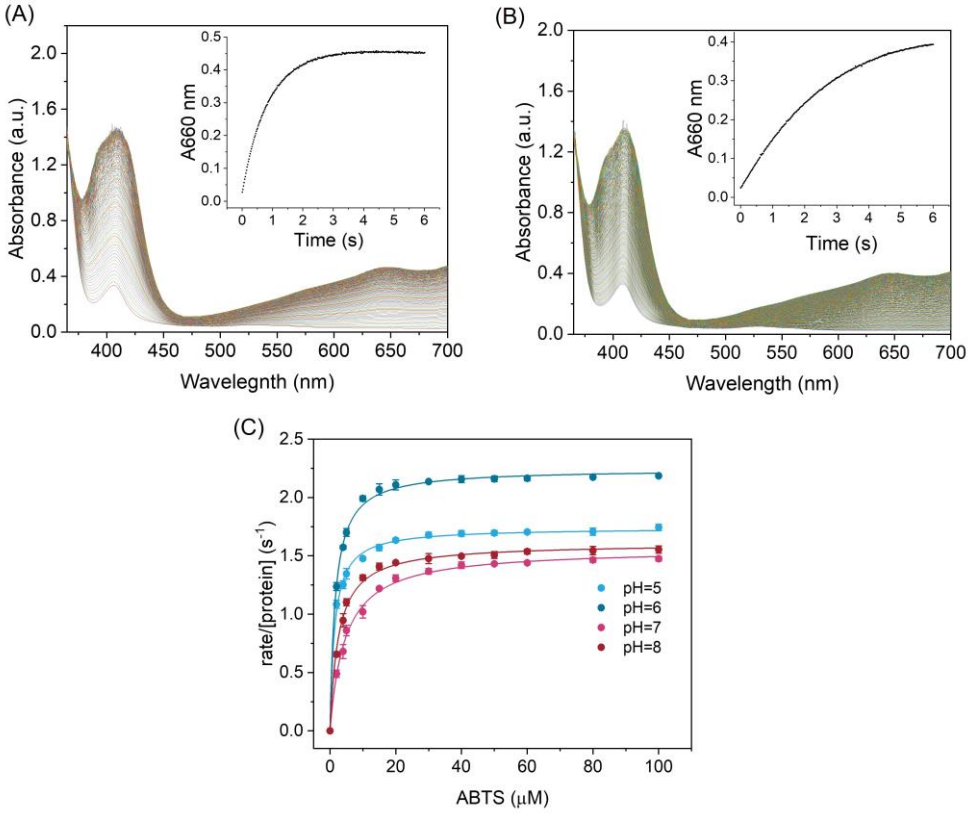

Fig. S7 Peroxidase activity assay using ABTS as a substrate. Stopped-flow kinetic of ABTS (0.1 mM) catalyzed by (A) A51C/G77C and (B) WT *h*Cyt *c* in the presence of H_2O_2. (C) Michaelis−Menten plots vs the concentrations of ABTS for WT *h*Cyt *c* at pH 5−8.