Supplementary Information for

Disruption of a potential disulfide bond of Cys65-Cys141 on the structure and stability of globin X from zebrafish

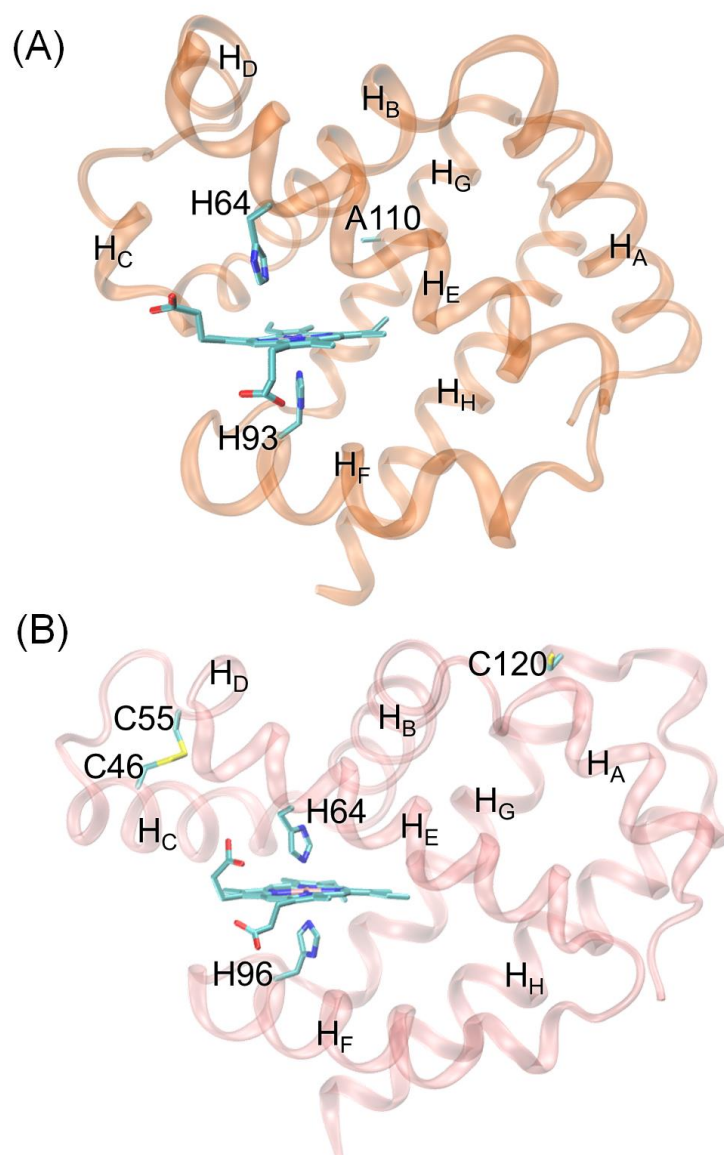Aiqun Pan [a], Xichun Liu [a]*, Hui Han [a], Shu-Qin Gao [b], Ying-Wu Lin [a,b]*

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Figure S1. (A) X-ray crystal structure of human K45R/C110A Mb mutant (PDB code 3RGK), showing the heme site and the location of Ala110. (B) X-ray crystal structure of human Ngb (PDB code 4MPM), showing the bis-His heme coordination, the disulfide bond of Cys46-Cys55 and the location of Cys120. The polypeptide chain with eight α-helices is labeled as A-H.
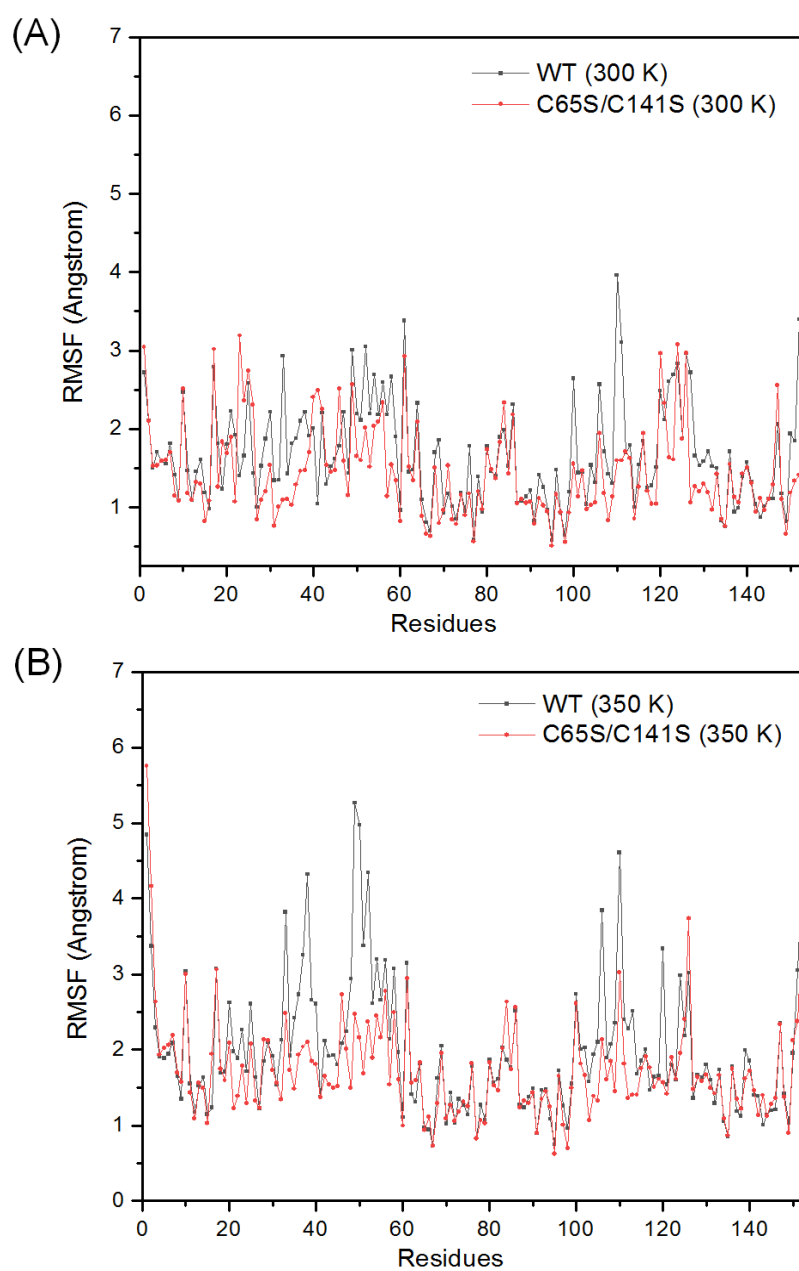
Figure S2. The average RMSF over time of each residue in the trajectories between 100-200 ns for WT and C65S/C141S globin X simulated at 300 K (A) and 350 K (B), respectively.
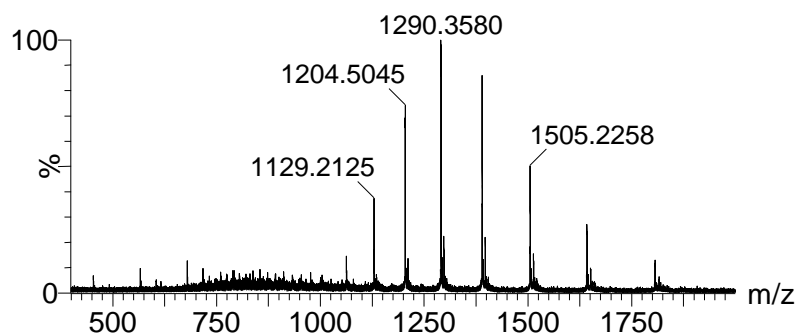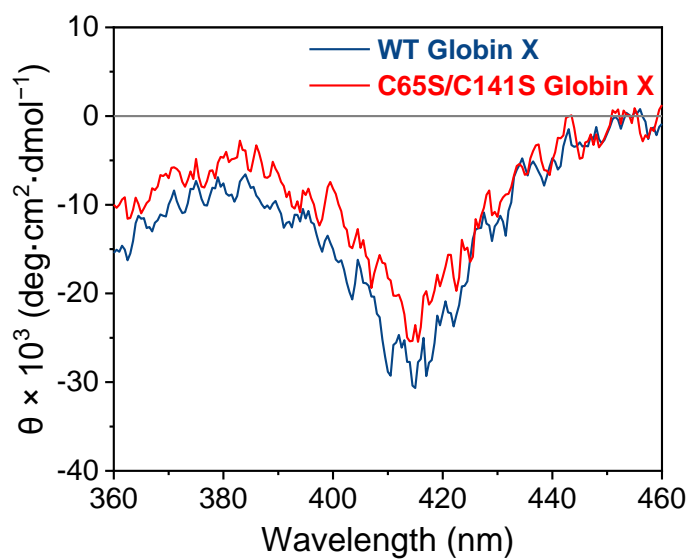
Figure S3. ESI-MS spectra of WT globin X showing the original multiply-charged series.
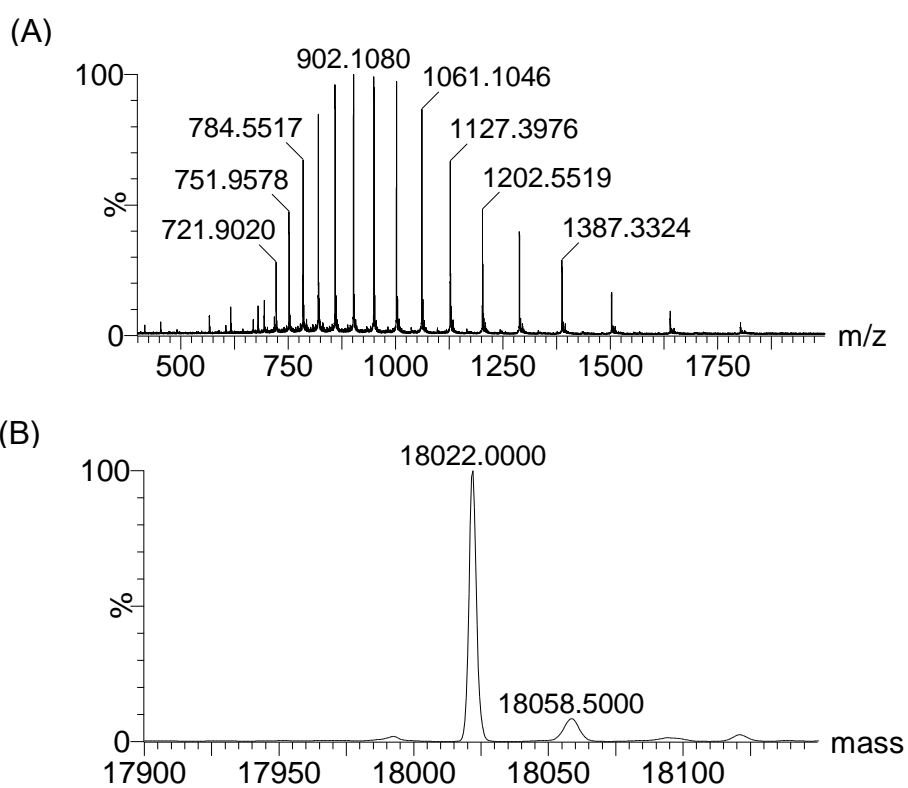
Figure S4. (A) ESI-MS spectra of the purified C65S/C141S globin X showing the corresponding multi-charged peaks. (B) The molecular weight of C65S/C141S globin X was calculated to 18021.80 Da, observed at 18022.00 Da.
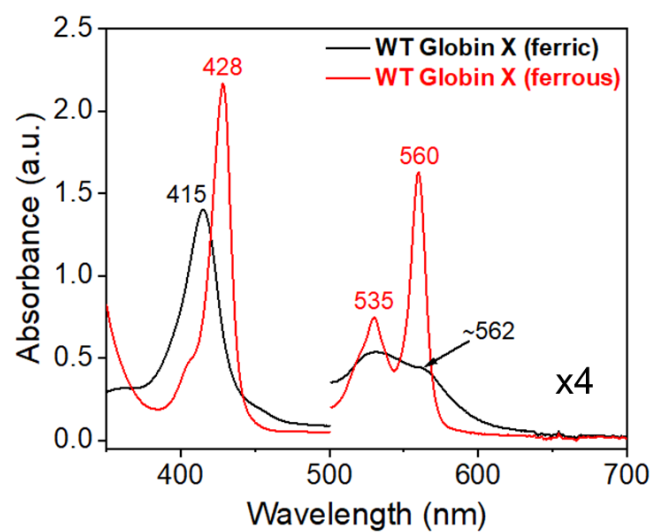
Figure S5. UV-Vis spectra of WT globin X in both ferric and ferrous states.

Figure S6. CD spectra of WT globin X (blue) and C65S/C117S globin X (red) (10 μM protein in 10 mM potassium phosphate buffer, pH 7.4).