Supporting Information

O'Neill et al. 10.1073/pnas.1121549109

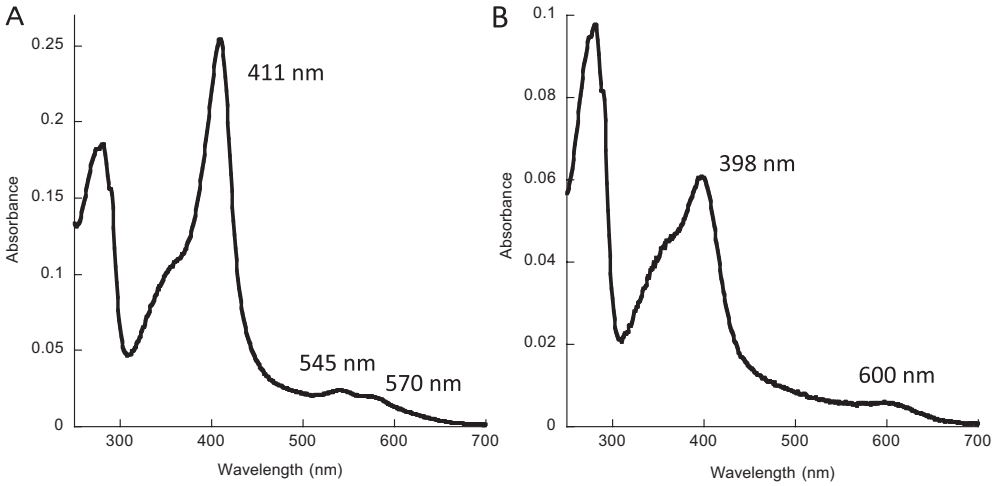

Fig. S1. Absorption spectra of holo-PhuS wild type (*A*) and H209/210/212A (*B*) proteins. Cuvettetes contained 10 μM holo-PhuS in 20 mM Tris•HCl (pH 7.5).

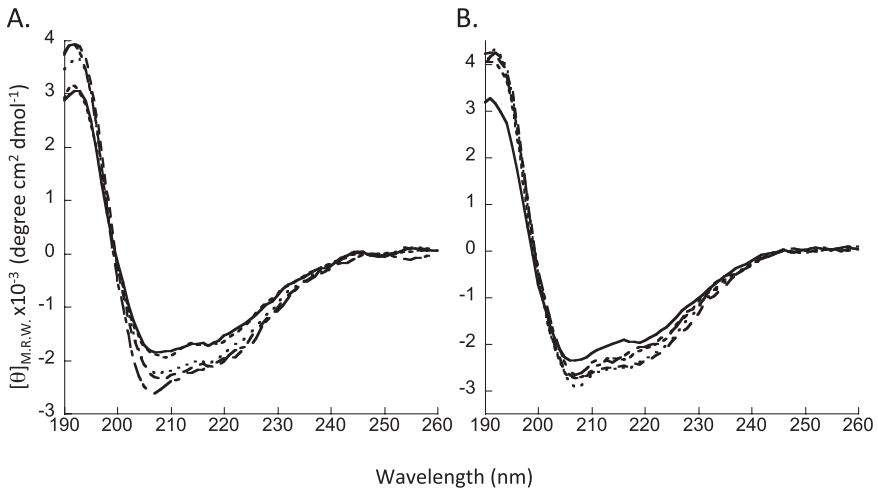

Fig. S2. Circular dichroism spectra of the wild-type and His-mutant apo-PhuS (*A*) and holo-PhuS (*B*) proteins. PhuS wild type (——), H209A (—— - ——), H210A (— — —), H212A (- - - -), H209/210/212A (· · · · · ·); spectra of PhuS (2.5 μM) were recorded in 1 mM sodium phosphate (pH 7.4) at 25 °C.

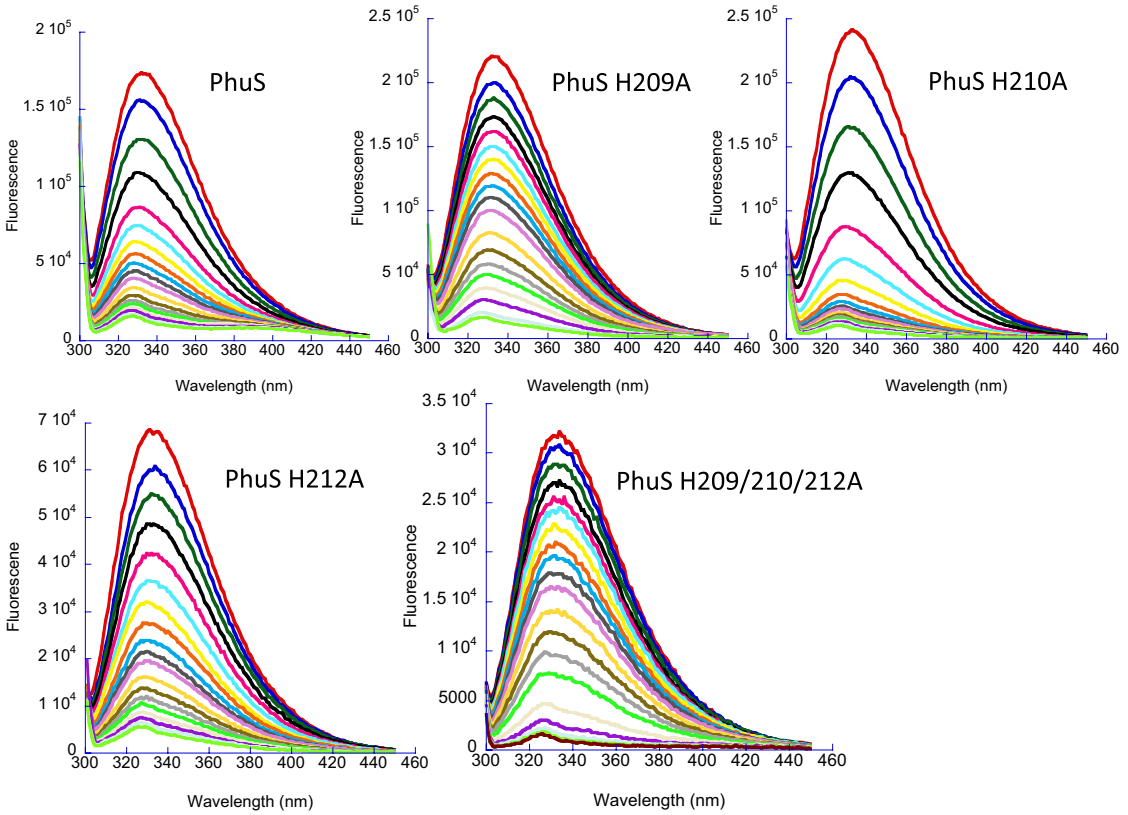

Fig. S3. Fluorescence emission spectra of wild-type and His-mutant PhuS proteins on incremental addition of heme. To 1 μM apo-PhuS proteins in 20 mM Tris•HCl (pH 7.8), heme was added in increments from 0.1 to 50 μM. The binding constant (K_d) was fit to a one-site binding model according to the decrease in Trp fluorescence intensity at 337 nm as a function of increasing heme concentration.

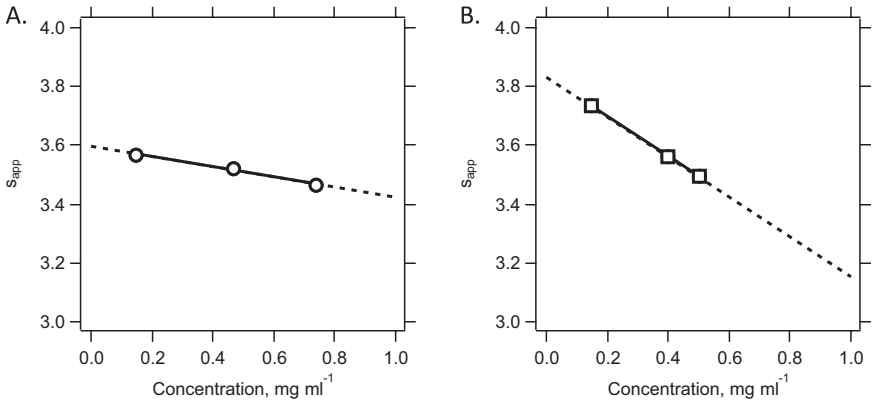

Fig. S4. Concentration dependences of the weight average sedimentation coefficients of holo-PhuS (*A*) and apo-PhuS (*B*). In both cases, points show the experimental weight average sedimentation coefficients as a function of total concentration. Overlaid are linear regressions of the data. The observed values extrapolated to infinite dilution equal 3.60 S and 3.84 S for holo- and apo-Phus, respectively, which correspond to 3.23 S and 3.45 S when corrected to the standard condition of 20 °C in water.

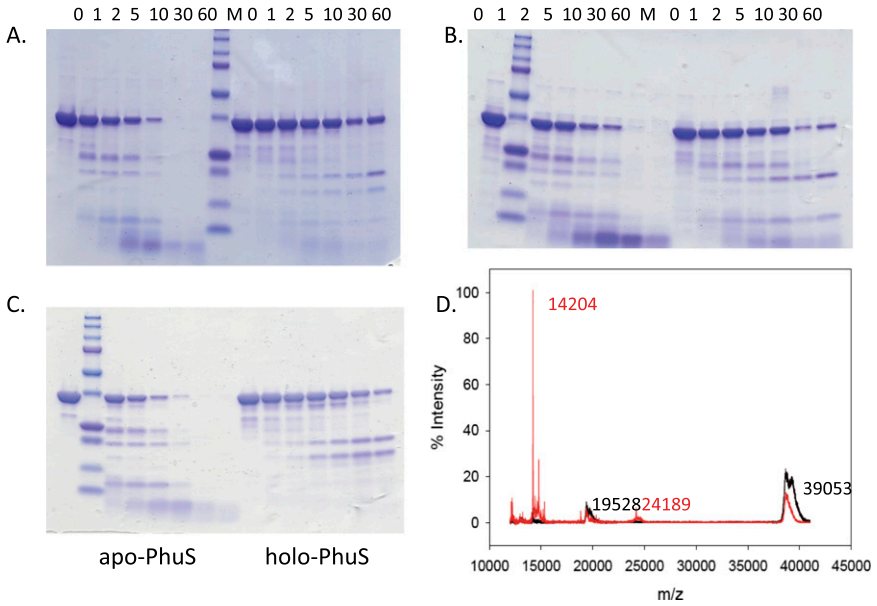

Fig. S5. Limited proteolysis and MALDI-MS spectrum of apo- and holo-PhuS mutants. SDS/PAGE of apo- and holo-PhuS H210A (*A*), apo- and holo-PhuS H209A (*B*), and apo- and holo-PhuS H212A (*C*), after proteolysis at intervals up to 60 min. (*D*) MALDI-MS spectrum of apo- and holo-PhuS H209/210/212A proteolysis after 2 min. The major peptide fragments are highlighted and color coded (red for apo-PhuS and black for holo-PhuS). The peak at ≈39 kDa corresponds to intact PhuS (M+1) and 19528 (M+2).

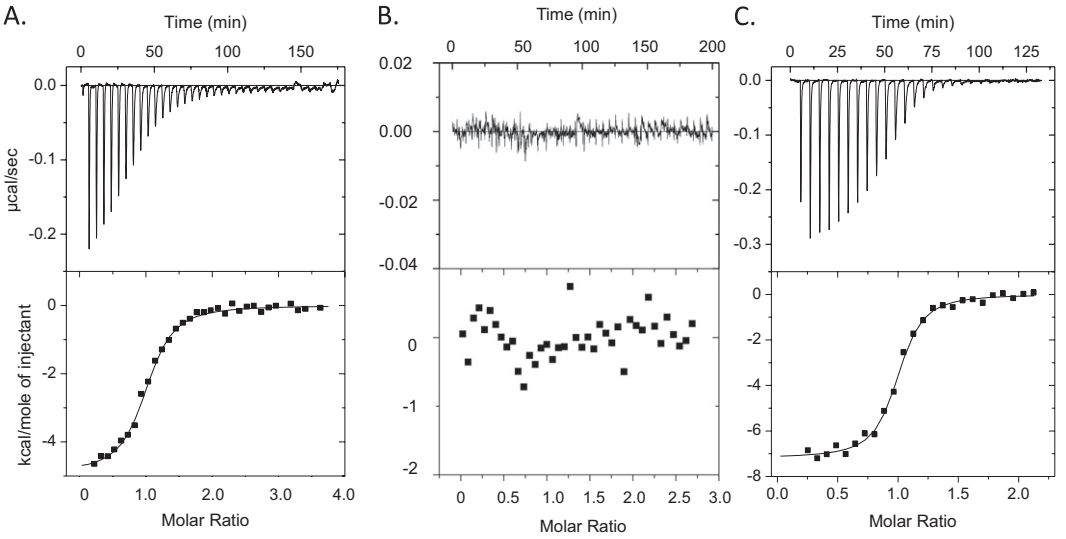

Fig. S6. Isothermal titration calorimetry (ITC) analysis of the interaction of holo-PhuS (*A*), apo-PhuS (*B*), and holo-PhuS H209A (*C*) with apo-HemO. Titrations were performed in 20 mM sodium phosphate (pH 7.5) at 298 K. *Upper*: Time-dependent release of heat during the titration. *Lower*: Peak integrals as a function of the molar ratio of heme to protein. The data were fit to a single binding model with Origin software, supplied by Microcal.