Supplemental Information

Cysteine pK_a values for the bacterial peroxiredoxin AhpC

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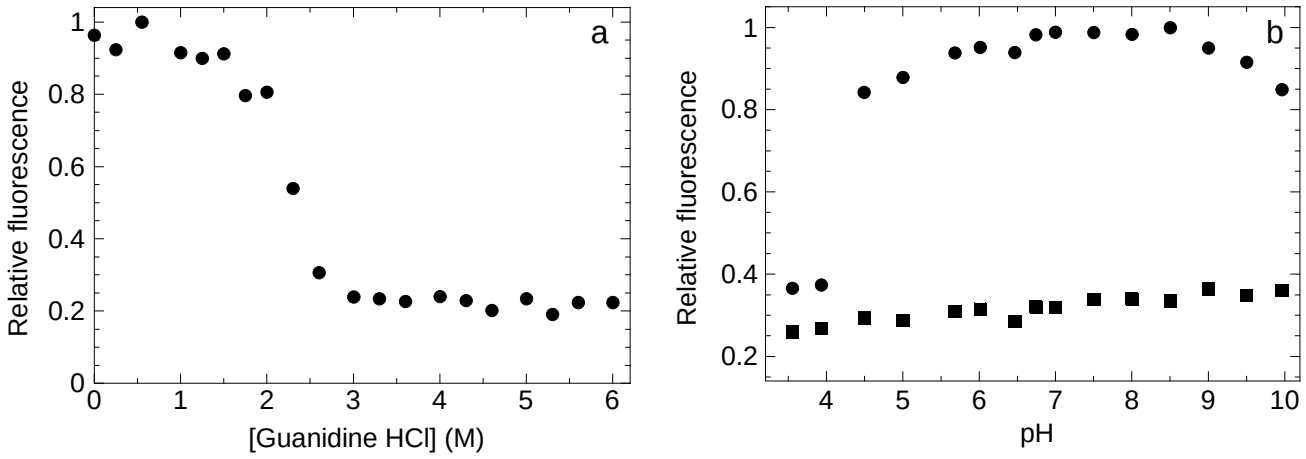

Figure S1. The intrinsic tryptophan fluorescence of *S. typhimurium* AhpC decreases as the protein becomes destabilized and can be used to confirm that AhpC is stable from pH 5-9. (a) AhpC (20 µg) was added to 1.5 mL of buffer containing varying concentrations of GuHCl (0-6 M). After 2 hours at 25 °C, the intrinsic tryptophan fluorescence was measured (λ_{ex} = 280 nm, λ_{em} = 350 nm). (b) AhpC (20 µg) was added to a final volume of 1.5 mL at various pH from 3-10 and incubated at 23 °C for 24 h. The intrinsic tryptophan fluorescence for each sample was measured at λ_{ex}= 280 nm and λ_{em} = 355 nm (●). The fluorescence for each sample was then remeasured under denaturing conditions following the addition of 10 µL of concentrated HCl (■).

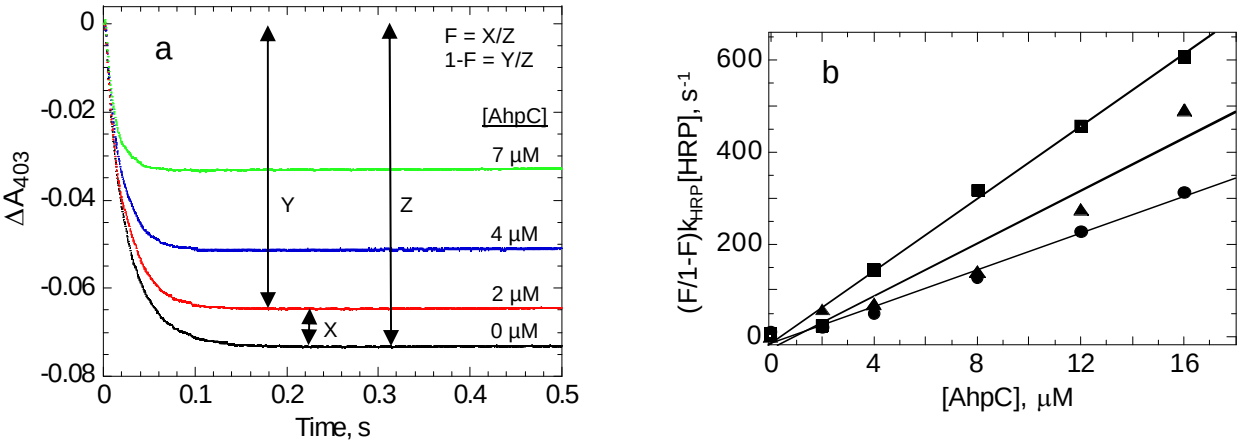

Figure S2: AhpC inhibits the HRP reaction in a concentration-dependent manner and the plot of (F/1-F)k_{HRP}[HRP] *versus* [AhpC] is linear. (a) The change in HRP absorbance at 403 nm was monitored using stopped-flow spectroscopy by mixing one solution containing 7.5 µM HRP and various concentrations of AhpC in 5 mM phosphate 1 mM EDTA, pH 7.0 with another solution containing 3 µM hydrogen peroxide in 2X BPACE buffer at pH 9.0. The final change in absorbance was used to calculate the fraction of inhibition caused by AhpC (F), as shown in the plot. (b) The value for (F/1-F)k_{HRP}[HRP] was plotted *versus* [AhpC] at pH 5.0 (●), pH 6.0 (▲), pH 7.0 (■). The slope of each line provides the k_{AhpC} for H_2O_2 at that pH.