METHODS

Details for the calculations are given elsewhere [1] and summarized here. The inner sphere contribution, ΔG_{in}, and partial charges for the redox site were calculated using broken-symmetry [2] density functional methods [3] (BS-DFT) with the B3LYP/6-31G(++)$_S$**// B3LYP/6-31G** functional/basis set using the program NWChem [4] as previously described. [5] Here, ΔG_{in} = -0.232 eV and ΔG_{SHE} = 0.43 eV. The outer sphere contribution, ΔG_{out}, was calculated using APBS [6], a program for solving the Poisson-Boltzmann equation, as described fully elsewhere. [1] A 51.2 Å cubic grid with a grid spacing of 0.2 Å was used for all calculations. The atomic radii and protein partial charges were from CHARMM22 parameters [7], and the probe radius for all Connolly [8] surfaces was r = 1.4 Å. The dielectric regions were defined as the redox site with $\varepsilon_c = 1$, the protein with $\varepsilon_p = 4$, and the solvent with $\varepsilon_s = 78$, and the ionic concentrations were zero. Building coordinates for hydrogens was carried out in CHARMM [9] version 35b1. Residue 11 of H42Q was labeled as an asparagine in the crystal structure in disagreement with the wt sequence so it was replaced by an aspartic acid as in the wt; however, the results at do not change if the sequence in the crystal structure is used.

The $E°$ as a function of pH were calculated according to

$$A_{\text{His}} \equiv 10^{\text{pK}_a(\text{His})-\text{pH}}$$

$$A_{\text{Neg}} \equiv 10^{\text{pK}_a(\text{Neg})-\text{pH}}$$

$$a = \frac{1}{1 + A_{\text{Neg}}}$$

$$b = \frac{A_{\text{Neg}} - A_{\text{His}}}{\left(1 + A_{\text{Neg}}\right)\left(1 + A_{\text{His}}\right)}$$

$$c = \frac{A_{\text{His}}}{1 + A_{\text{His}}}$$

$$E° = aE_a° + bE_b° + cE_c°$$

where pK_a(His)=6.3, pK_a(Neg)=3.5. $E_a°$, $E_b°$ and $E_c°$ are the calculated reduction potentials for the protein with all ionizable residues protonated, all ionizable residues with pK_a above 5 protonated, and all ionizable residues with pK_a above 8 protonated, respectively.

The error in the calculated $E°$ has been estimated as about 30 mV if high-resolution crystals are used. [10] The deviation appears to be mainly associated with small differences in the redox site geometry. Larger error may be associated with protein dynamics, changes in the redox site, or poorer resolution (larger that 1.5 Å resolution) structures. The experimental data shown here are performed using cyclic voltammetry with $E°$ reproducible to within 2 mV in 50 mM potassium phosphate buffer, 150 mM KCl [11] and are in good agreement with previous studies following the absorption spectra in 25 mM TAGP (Tris-sodium acetate-glycine-potassium phosphate buffer) with NaCl to total ionic strength of 140 mM. [12] The larger difference in the low pH results compared to experiment may be due to the lack of salt in the calculations or one or more glutamates or aspartates may have pK_a below 3.5 in the experiment. The lack of salt would raise the medium and high pH $E°$ since dielectric shielding of the surface charges, which are mostly negative, would increase $E°$. We choose to overestimate rather than underestimate the effects of charged residues by omitting salt in the calculations.

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