Supporting information:

Electrochemical and structural properties of a protein system designed to generate tyrosine Pourbaix diagrams

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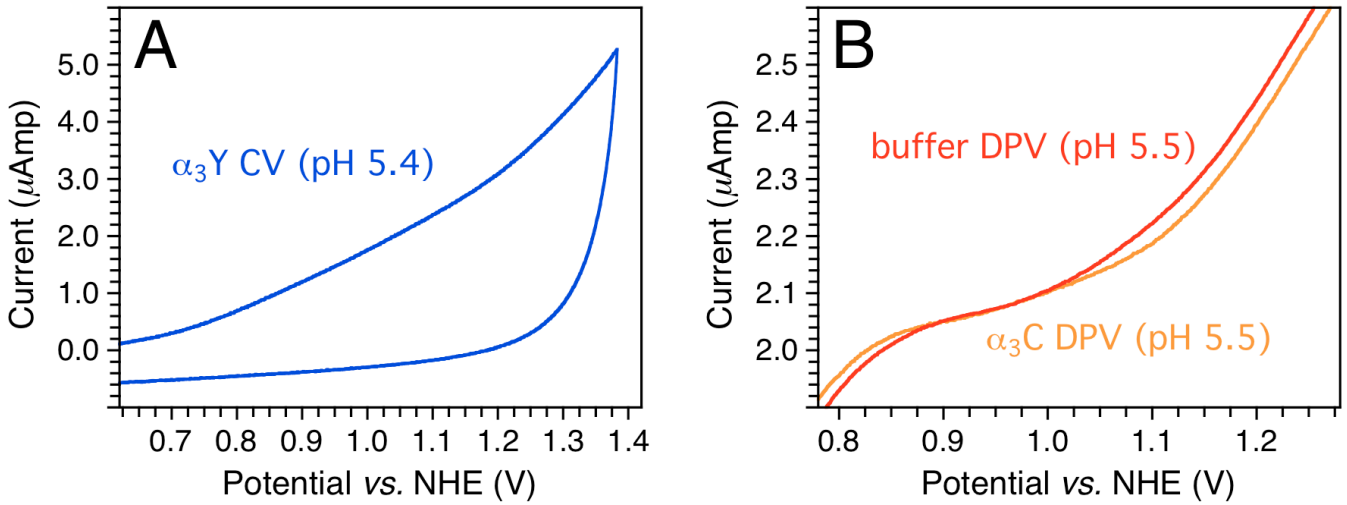

Fig. S1. *Electrochemical properties of α_3Y and control samples*. (A) Cyclic voltammogram of 210 μM α_3Y in 20 mM sodium acetate, 20 mM potassium phosphate, 40 mM KCl, pH 5.43; scan rate 200 mV/s, iR-compensation 103 ohm, temperature 23° C. (B) Differential pulse voltammograms of buffer containing 20 mM sodium acetate, 20 mM potassium phosphate, 40 mM KCl, pH 5.52 (red) and 200 μM α_3C in 20 mM sodium acetate, 20 mM potassium phosphate, 40 mM KCl, pH 5.46 (orange); interval time 0.1 s, step potential 1.05 mV, scan rate 10.5 mV s^{-1}, modulation time 5 ms, modulation amplitude 50 mV, temperature 23° C.

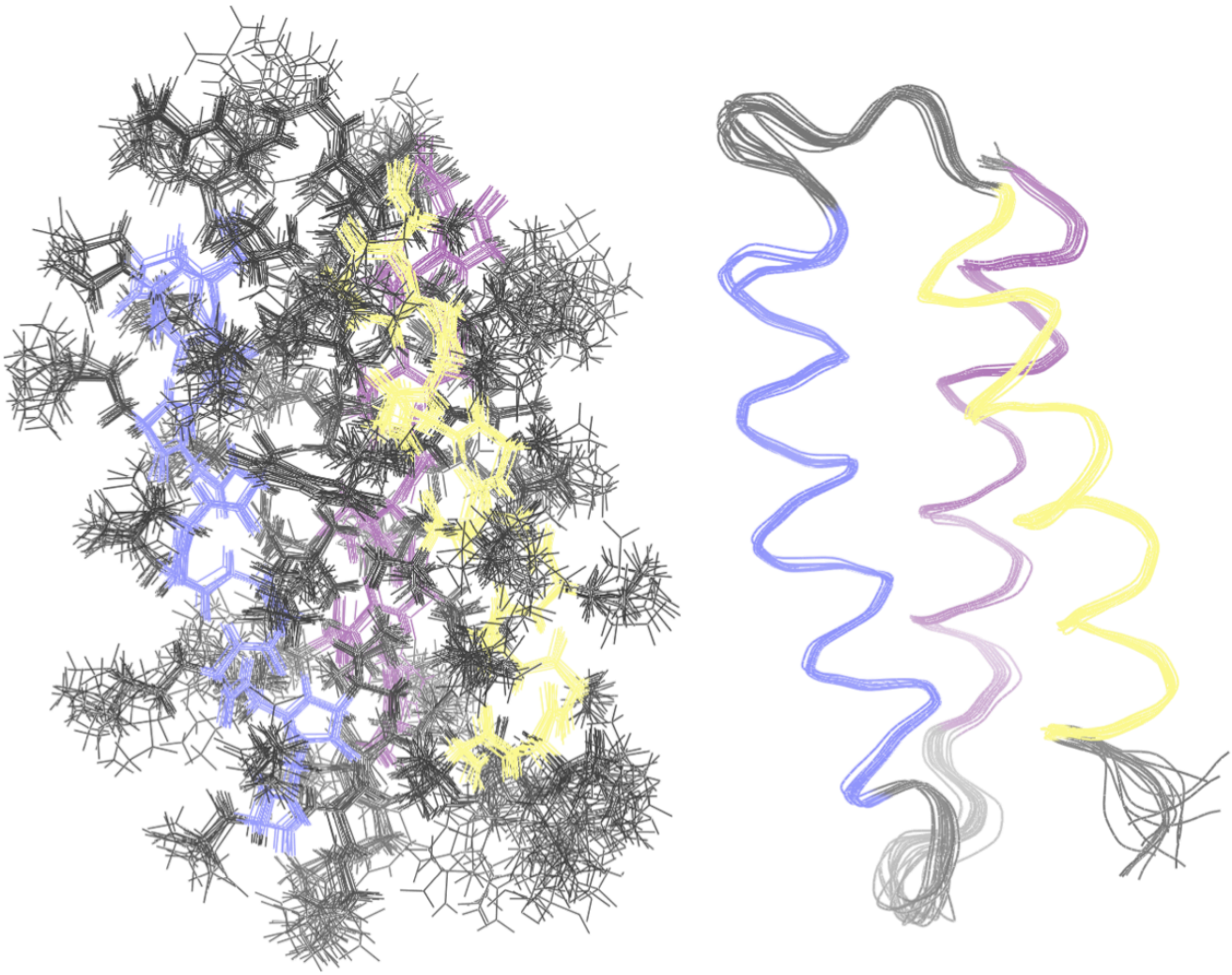

Fig. S2. *Stick and backbone representations of the α_3W solution NMR structure* (1LQ7.pdb; Dai *et al., J. Am. Chem. Soc. 124,* 10952–10953, 2002). Helices 1, 2 and 3 are colored yellow, blue and purple, respectively. The high-quality structure of α_3W (average RMSD from mean coordinates: 0.22 Å (backbone atoms helical residues), 0.33 Å (backbone atoms all residues), 0.76 Å (heavy atoms helical residues) and 0.83 Å (heavy atoms all residues)) was used as a guide to engineer Y32/His interactions in the α_3Y protein. α_3Y is a single-site W32Y variant of α_3W.

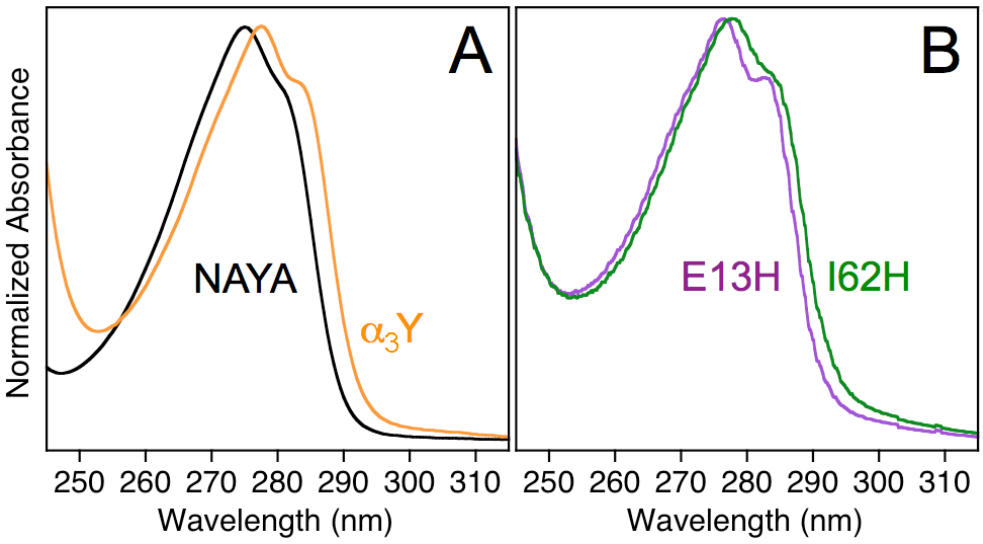

Fig. S3. *Probing for Y32/His interactions in single-site α_3Y-His variants by absorption spectroscopy*. (A) Absorption spectra of α_3Y (orange) and N-acetyl-tyrosinamide (NAYA; black) dissolved in 10 mM potassium phosphate, 30 mM KCl, pH 7.0. (B) Absorption spectra of α_3Y-E13H (purple) and α_3Y-I62H (green) in 10 mM Tris, 15 mM KCl, pH 9.0. The data were collected at room temperature.

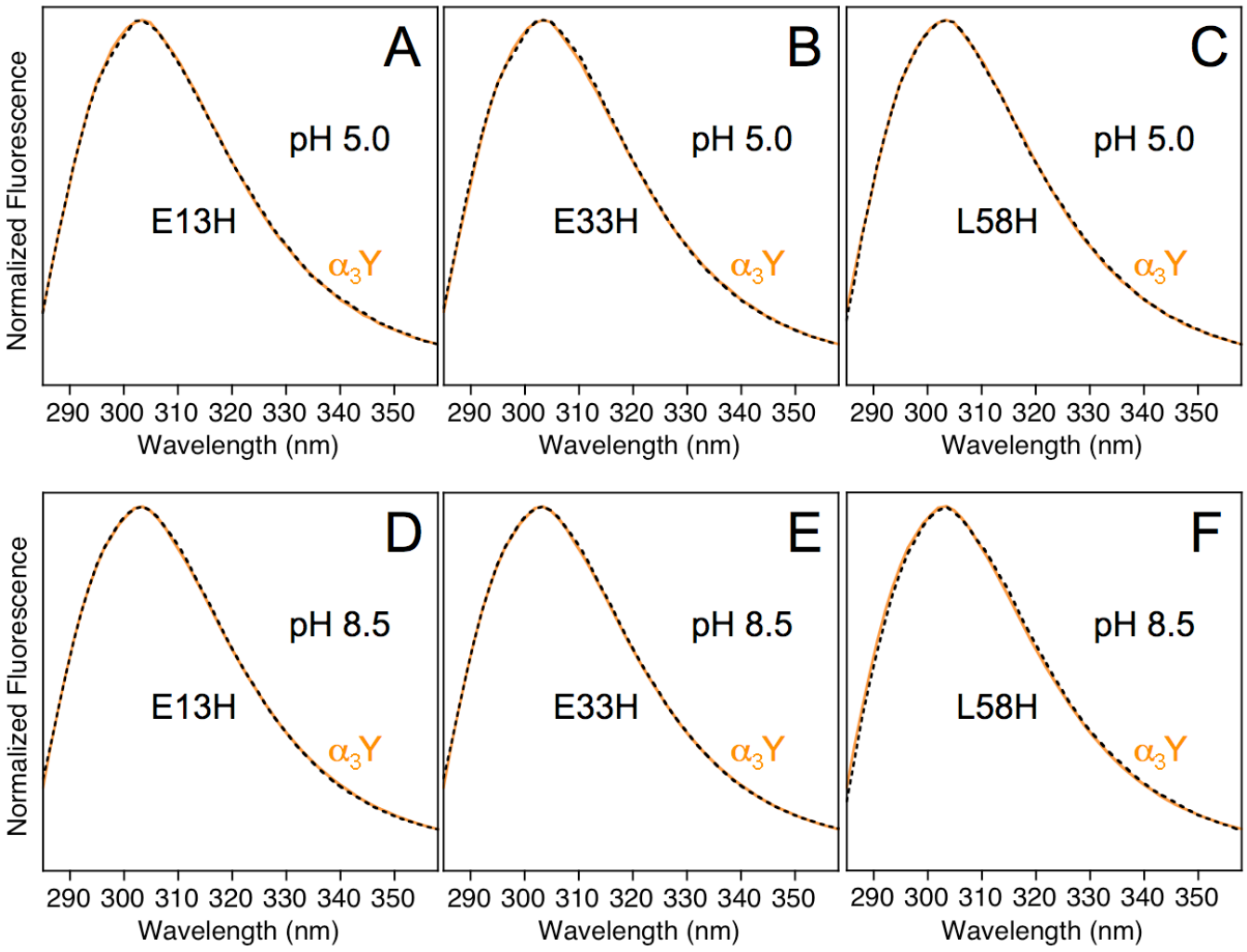

Fig. S4. *Probing for Y32/His interactions in single-site α_3Y-His variants by fluorescence spectroscopy*. Introducing a histidine at position 13, 33 or 58 did not give rise to any significant shift in the Y32 fluorescence emission spectrum. The orange spectra represent α_3Y while the dashed black spectra represent three different α_3Y-His variants at pH 5.0 (panels A, B and C) and at pH 8.5 (panels D, E and F. The pH 5.0 samples were prepared in 15 mM sodium acetate and 30 mM KCl. The pH 8.5 samples were prepared in 15 mM Tris and 30 mM KCl. The spectra were obtained at room temperature and have been smoothened for clarity.

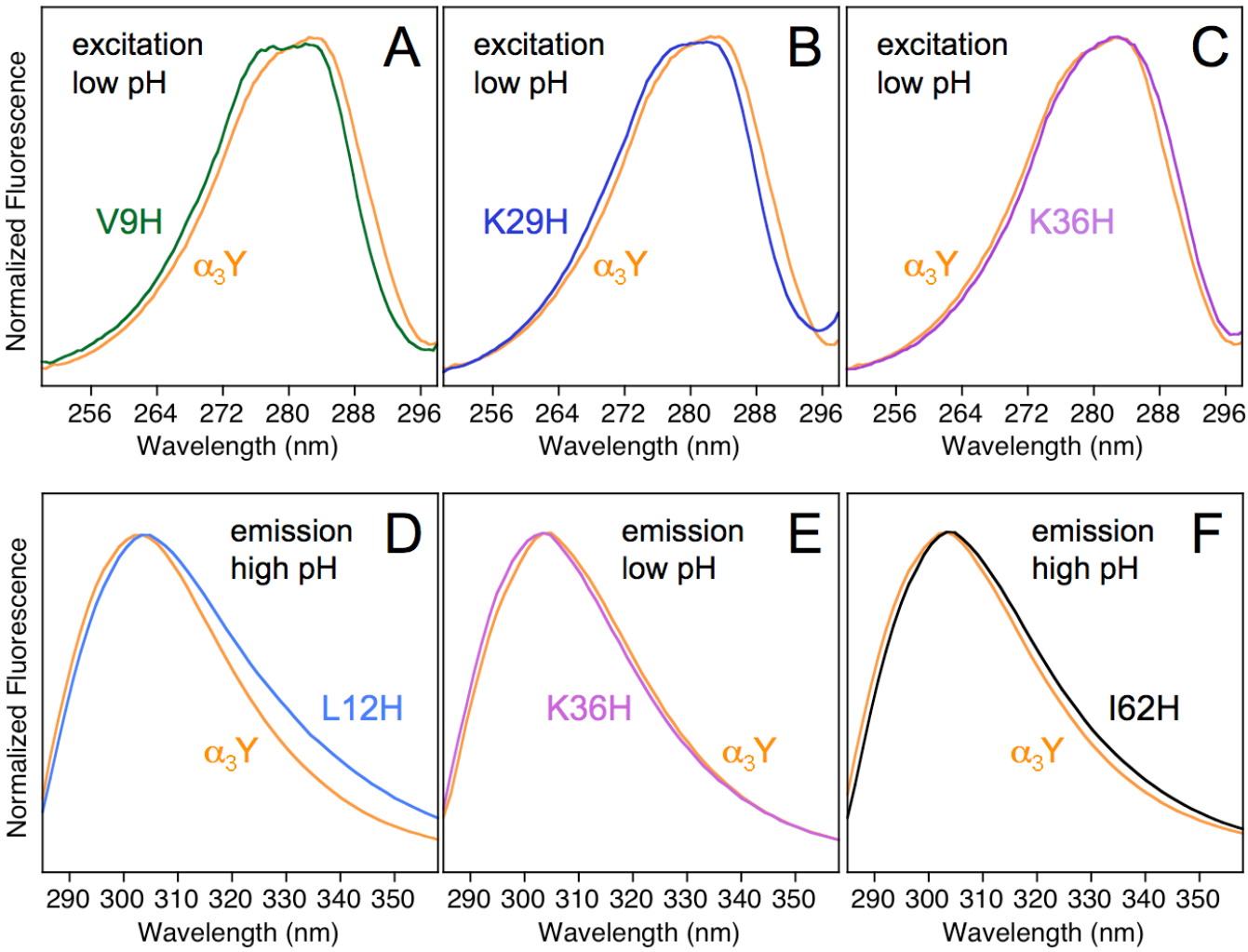

Fig. S5. *Probing for Y32/His interactions in single-site α_3Y-His variants by fluorescence spectroscopy*. Introducing a histidine at position 9, 12, 29, 36 or 62 gave rise to a perturbation of the Y32 fluorescence excitation (panels A, B and C) and/or emission (panels D, E and F) spectra. Samples conditions were as follows: (A) α_3Y (orange) and α_3Y-V9H (green) in 15 mM sodium acetate, 30 mM KCl, pH 5.0; (B) α_3Y (orange) and α_3Y-K29H (blue) in 10 mM sodium acetate, 30 mM KCl, pH 5.5; (C) α_3Y (orange) in 15 mM sodium acetate, 30 mM KCl, pH 5.0 and α_3Y-K36H (magenta) in 10 mM sodium acetate, 30 mM KCl, pH 5.5; (D) α_3Y (orange) and α_3Y-L12H (cyan) in 10 mM Tris, 15 mM KCl, pH 8.2; (E) α_3Y (orange) in 15 mM sodium acetate, 30 mM KCl, pH 5.0 and α_3Y-K36H (magenta) in 10 mM sodium acetate, 30 mM KCl, pH 5.5; (F) α_3Y (orange) and α_3Y-L58H (black) in 15 mM Tris, 30 mM KCl, pH 8.5. The spectra were obtained at room temperature and have been smoothened for clarity.

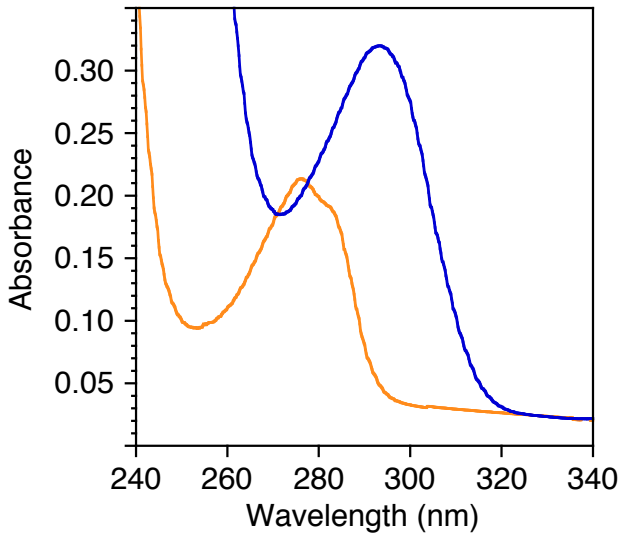

Fig. S6. *Absorption spectrum of α_3Y as a function of pH*. Spectra recorded on α_3Y at pH 7.0 (orange; λ_{max} 277.8 nm) and 12.3 (blue; λ_{max} 293.2 nm). α_3Y was dissolved in 10 mM potassium phosphate, 10 mM HEPES, 10 mM sodium borate, 10 mM CAPS and the spectra collected at room temperature.

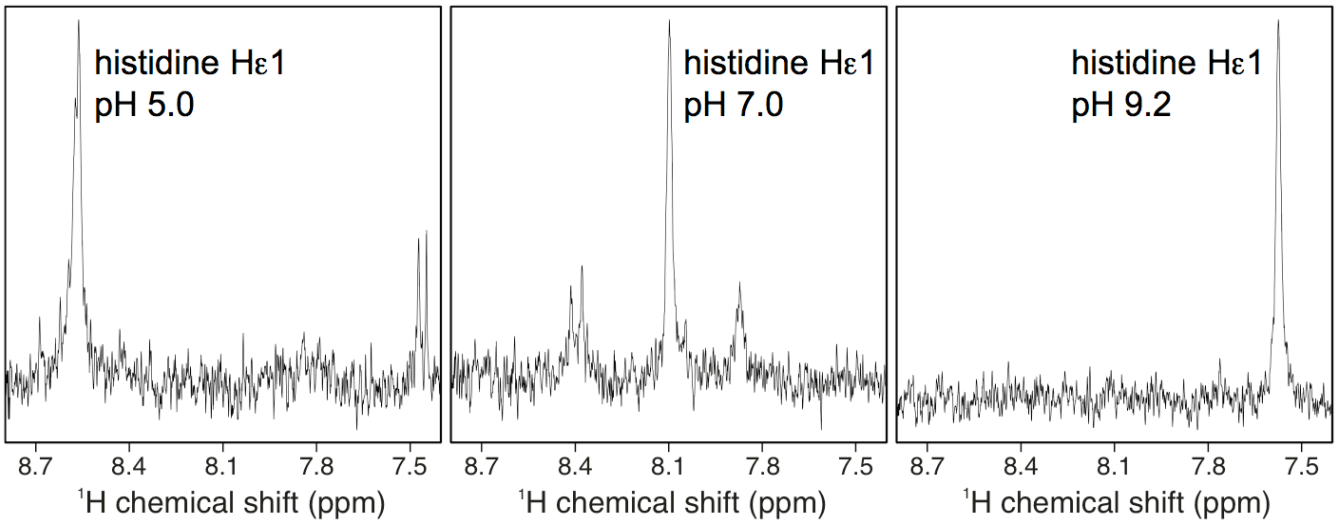

Fig. S7. *One-dimensional NMR spectrum of α_3Y-K36H as a function of pH*. NMR spectra recorded on α_3Y-H36H at pH 5.0 (left panel), 7.0 (middle) and 9.2 (right). The pH-sensitive resonance of the histidine ε1 ring proton shifts more than 1 ppm across the titrated pH range. α_3Y-H36H was dissolved in D_2O containing a buffer cocktail of deuterated sodium acetate, sodium phosphate, sodium borate and 30 mM KCl. The spectra were collected at 25° C.

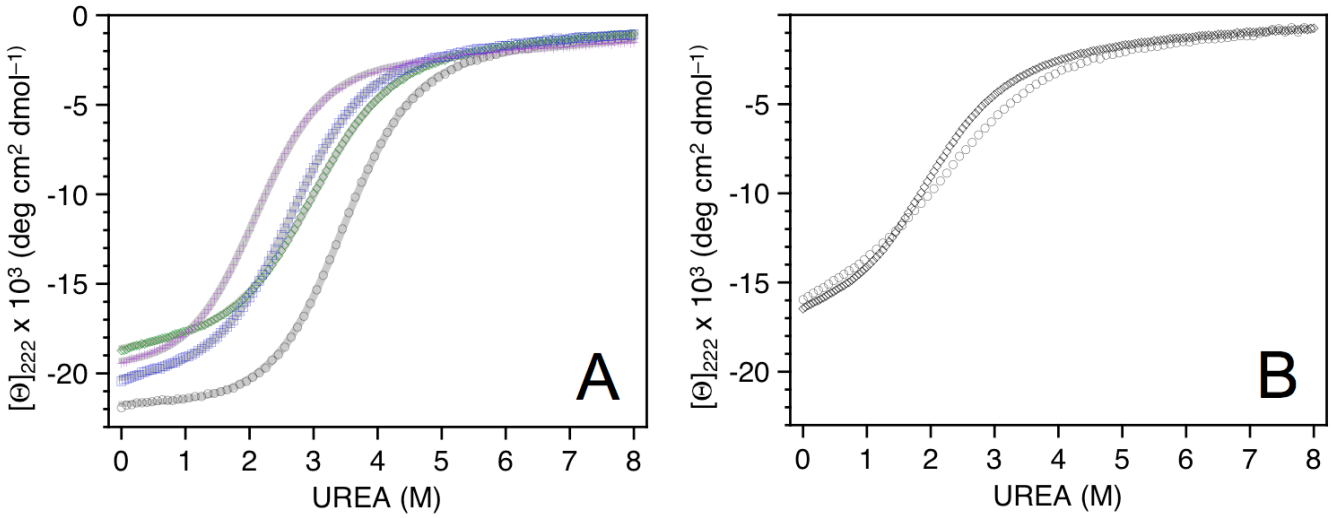

Fig. S8. *Chemical denaturation of α_3Y and histidine variants*. Panel (A) displays urea-induced unfolding/folding transitions of α_3Y (black circles), α_3Y-V9H (green diamonds), α_3Y-K29H (blue squares) and α_3Y-K36H (magenta crosses) while panel (B) displays data obtained on the α_3Y-L12H (circles) and α_3Y-I62H (diamonds) proteins. The grey lines in panel (A) represent nonlinear curve fits to determine the stability of the protein in the absence of denaturant (see Table 1 in the main text; Santoro & Bolen, *Biochemistry 27,* 8063–8068, 1988). The proteins were dissolved in 10 mM sodium acetate, 10 mM potassium phosphate, 10 mM sodium borate, pH 8.2 and the data collected at 25° C.

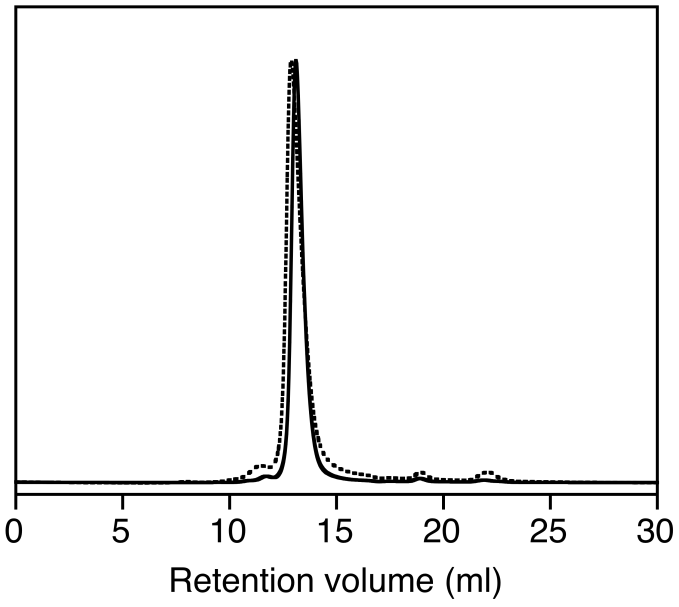

Fig. S9. *Gel filtration of α_3Y and α_3Y-K29H*. Elution profiles of α_3Y (solid line) and α_3Y-K29H (dotted line) on an analytical Superdex™ 75 gel filtration column equilibrated with 10 mM sodium acetate, 10 mM potassium phosphate, 10 mM sodium borate, 100 mM KCl, pH 7.0. The data were collected at room temperature.

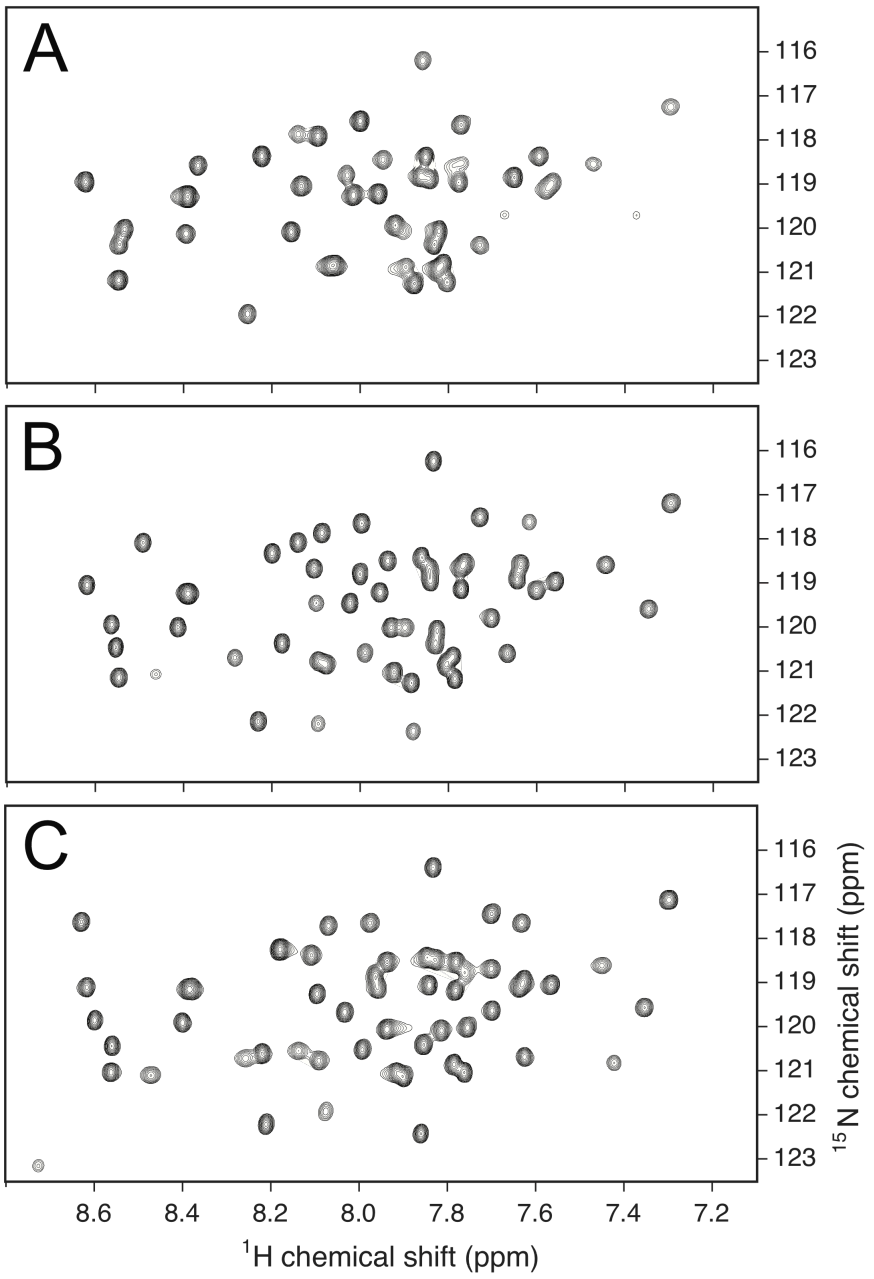

Fig. S10. *Two-dimensional ^{15}N-HSQC NMR spectrum of α_3Y-K36H as a function of pH.* ^{15}N-HSQC spectra of α_3Y-K36H obtained at 35º C and (A) pH 8.4, (B) pH 7.0 and (C) pH 5.5. The protein was dissolved in 20 mM deuterated Tris, 20 mM NaCl, 8% D_2O (pH 8.4) or 20 mM deuterated sodium acetate, 20 mM sodium phosphate, 20 mM NaCl, 8% D_2O (pH 7.0 and 5.5).