Electrostatic Energetics of *Bacillus subtilis* Ribonuclease P Protein Determined by Nuclear Magnetic Resonance-Based Histidine pK_a Measurements

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Supporting Information

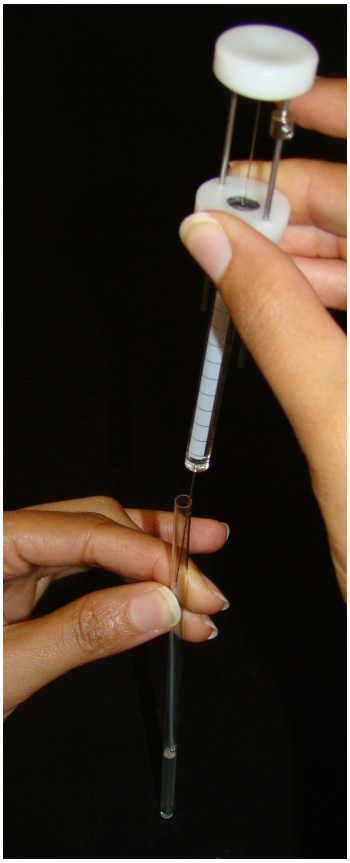
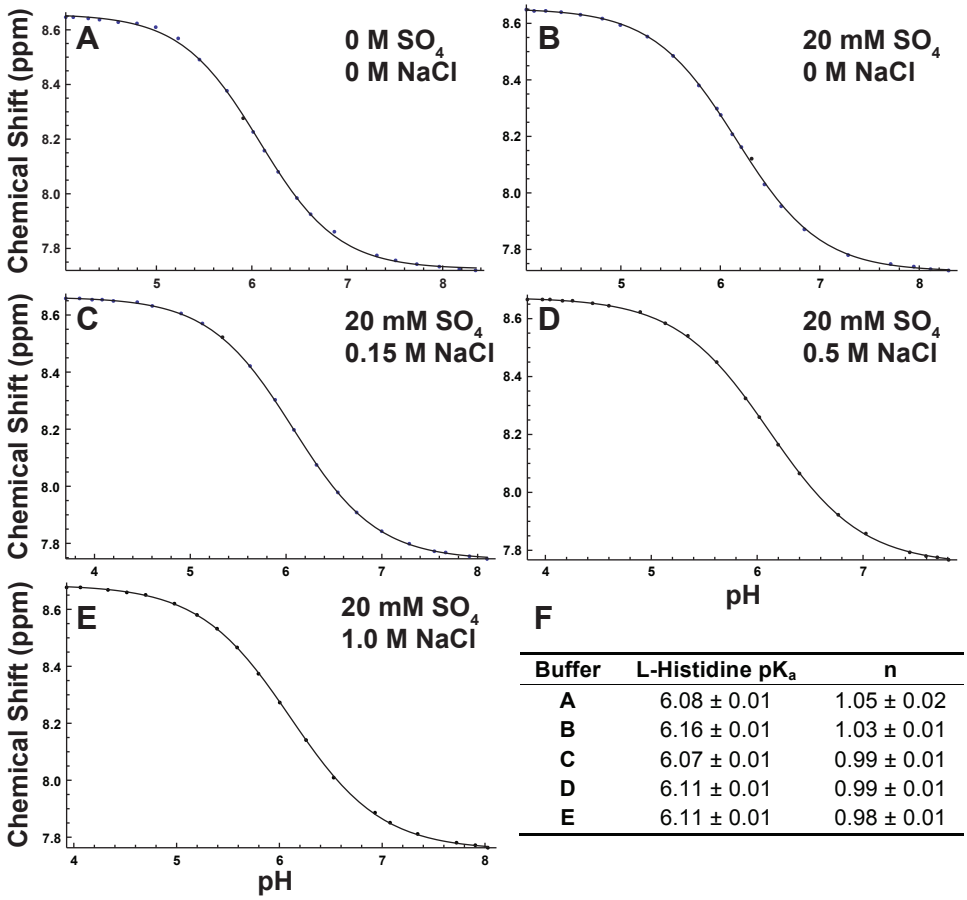


Figure S1: Titration of NMR sample with a 0.5–0.4 µl aliquot of NaOH using a 5.0 µl positive displacement syringe extended with polyethylene tubing.



F

Buffer	L-Histidine pK_a	n
A	6.08 ± 0.01	1.05 ± 0.02
B	6.16 ± 0.01	1.03 ± 0.01
C	6.07 ± 0.01	0.99 ± 0.01
D	6.11 ± 0.01	0.99 ± 0.01
E	6.11 ± 0.01	0.98 ± 0.01

Figure S2: L-Histidine (internal standard) titration curves under different buffer conditions. In addition to the components indicated, each sample had 10 mM pyridine, 90% H_2O, and 10% D_2O. The $^{13}C^{\varepsilon 1}$ protons were used to determine the pK_a values and Hill coefficients for L-Histidine, listed in panel **F**. Uncertainties are based on the standard errors of the fitted parameters.

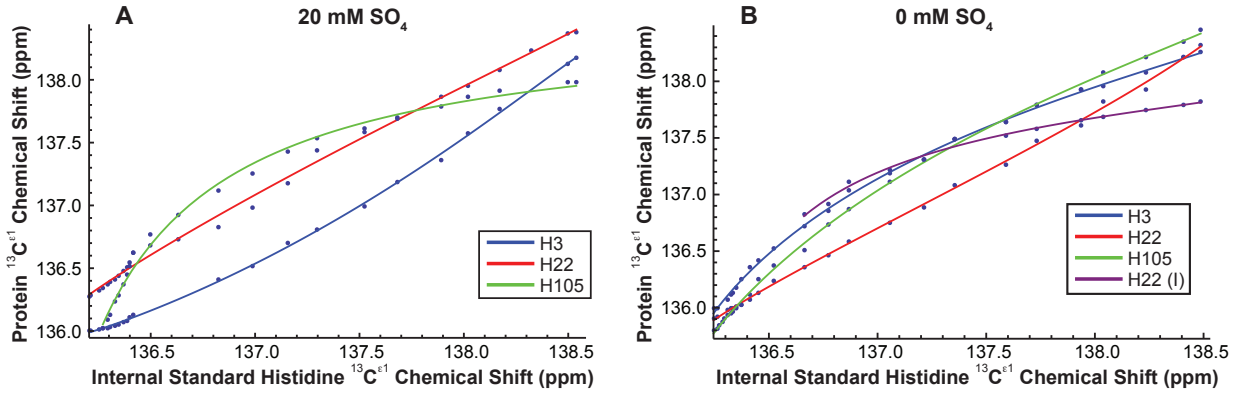


Figure S3: $^{13}C^{\varepsilon 1}$ P protein histidine chemical shifts versus $^{13}C^{\varepsilon 1}$ L-Histidine chemical shifts titration curves for (**A**) sulfate-bound folded and (**B**) unfolded P protein. H3, H22, and H105 are represented as the blue, red, and green curves, respectively. The purple curve in (**B**) represents the H22 intermediate peak.

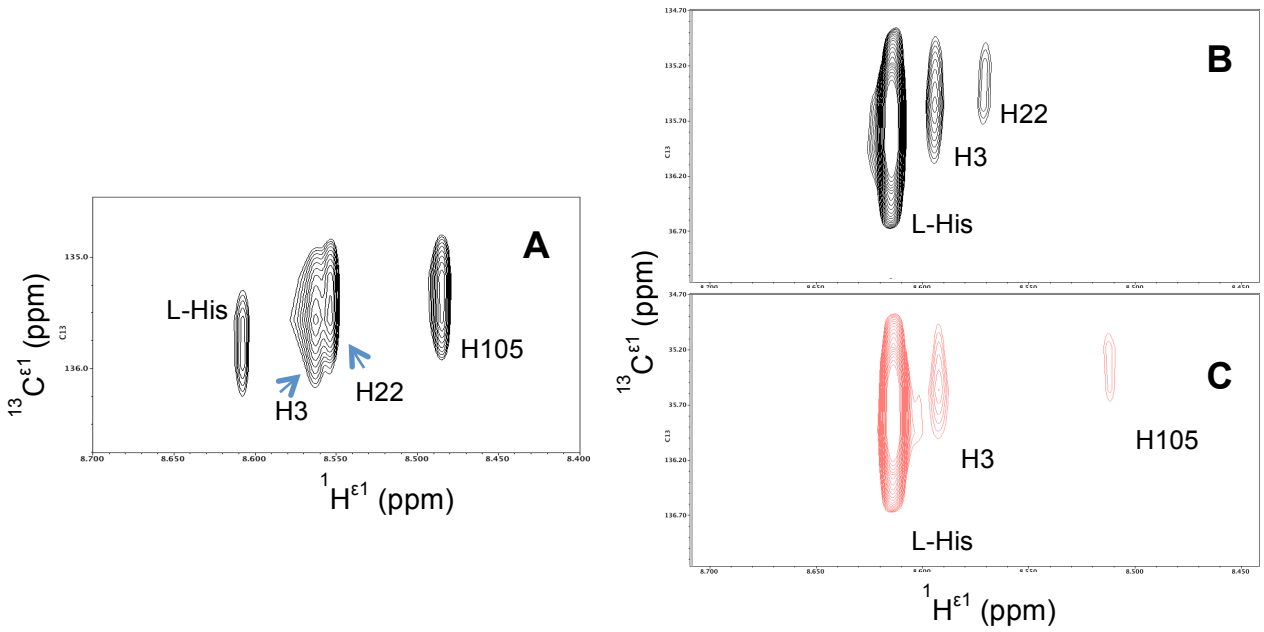


Figure S4: NMR resonances from all three histidine residues in the unfolded state of P protein were assigned by comparing spectra of (**A**) F107W P protein to spectra of two variants, (**B**) H105A and (**C**) H22A, that each lack a different histidine residues. All spectra were collected at a sample pH of 4. Assignment spectra for the sulfate-bound folded state are not shown.

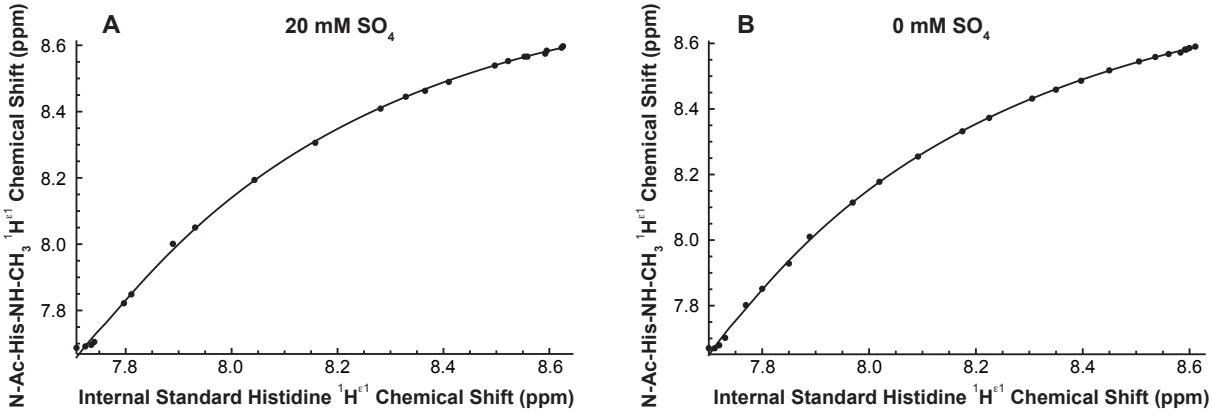


Figure S5: $^{1}H^{\varepsilon1}$ N-acetyl-L-Histidine methylamide chemical shifts versus $^{1}H^{\varepsilon1}$ L-Histidine chemical shifts titration curves in the (**A**) presence and (**B**) absence of 20 mM sodium sulfate. The estimated pK_a of N-acetyl-L-Histidine methylamide was (**A**) 6.52 ± 0.03 and (**B**) 6.44 ± 0.02.

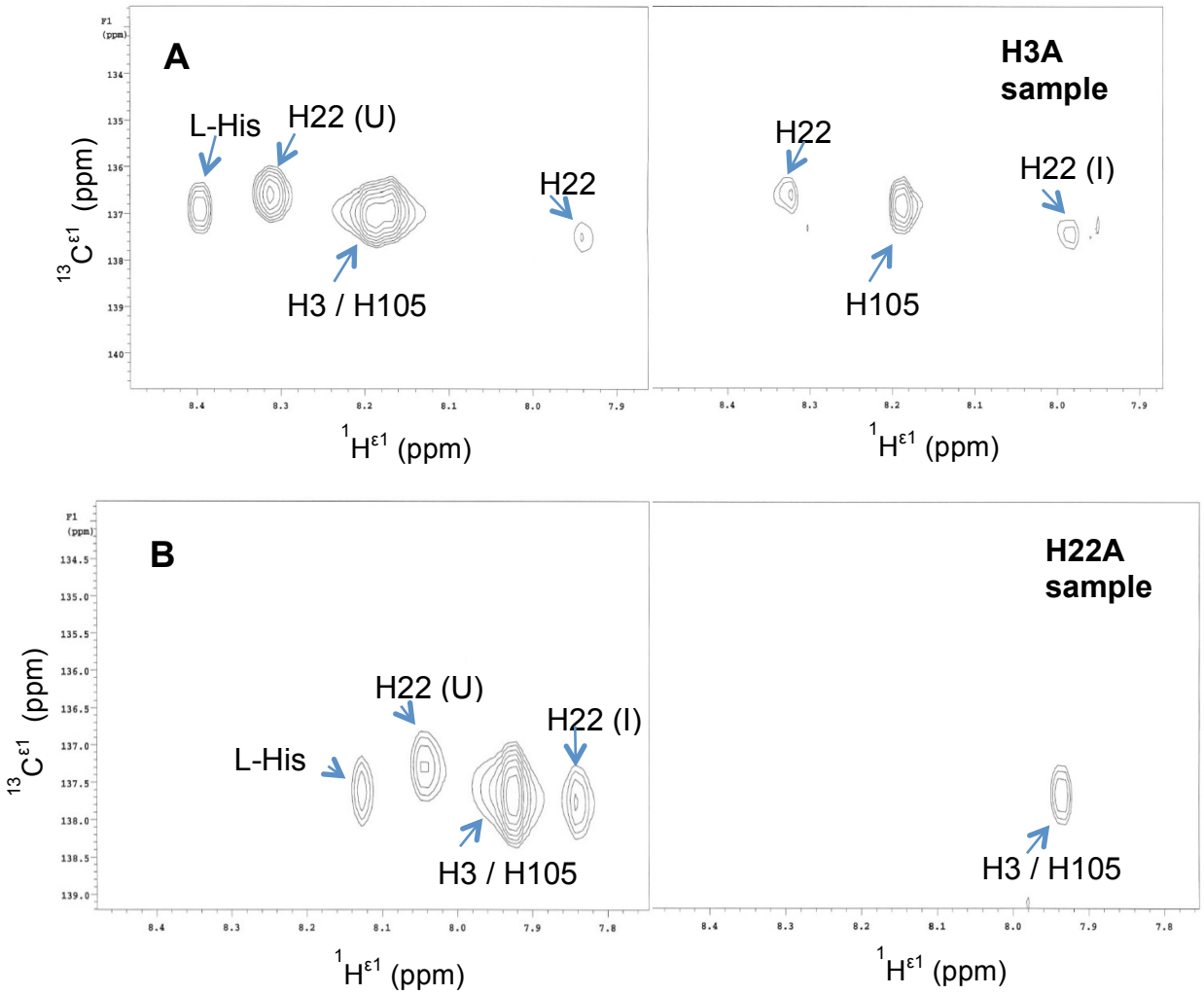


Figure S6: NMR resonance from intermediate peak in the unfolded state of P protein was assigned by comparing spectra of F107W P protein to spectra of two variants, (**A**) H3A and (**B**) H22A, that each lack a different histidine residue. The left sides of panels (**A**) and (**B**) correspond to spectra that were collected at a sample pH of 5.85 and 6.10, respectively.

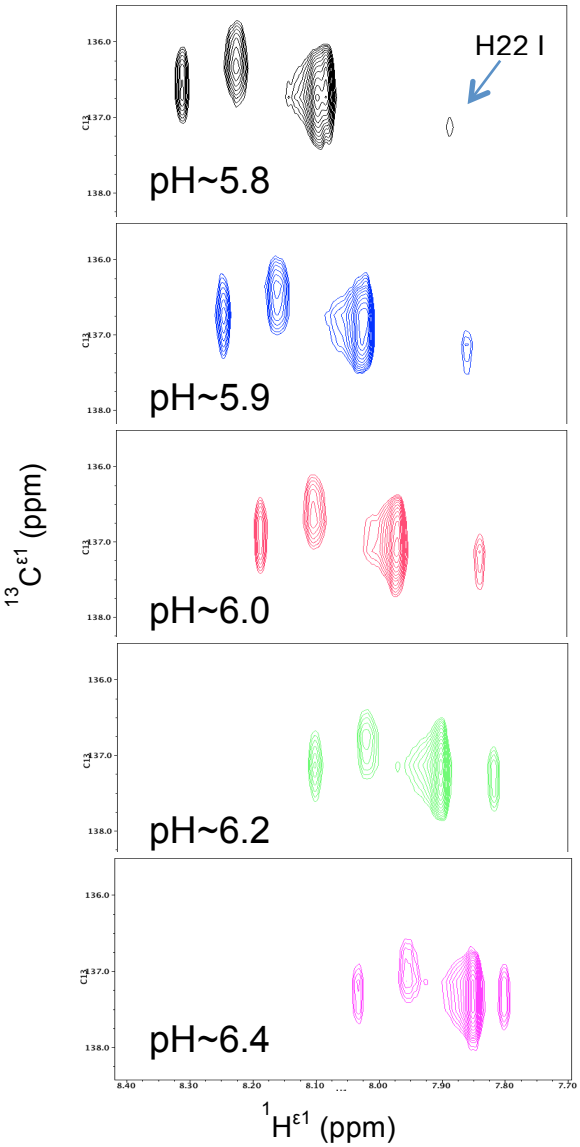


Figure S7: ^{1}H-^{13}C HSQC unfolded P protein spectra in various pH buffers. The intensity of the H22 intermediate peak (H22 I) increases as the pH is increased.

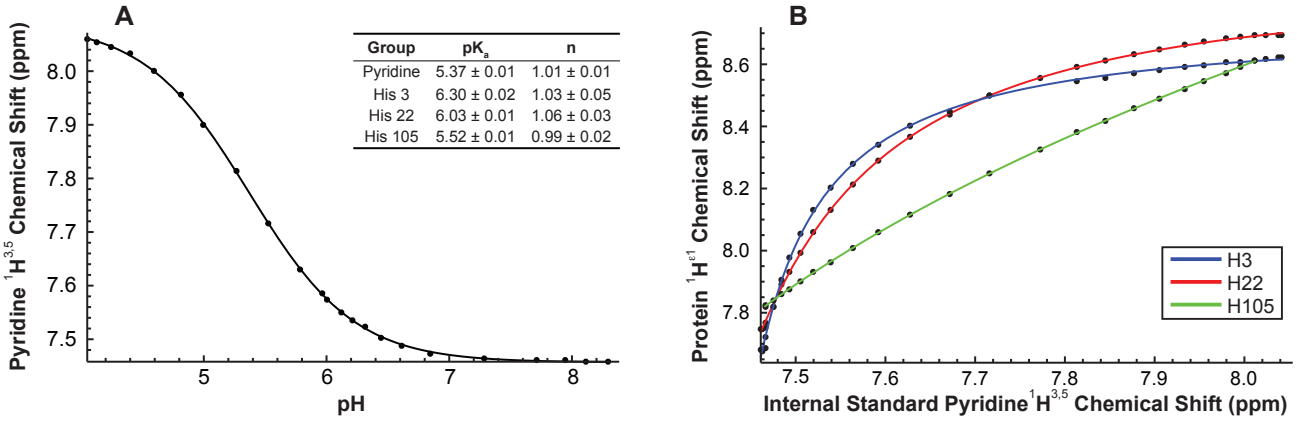


Figure S8: (**A**) Pyridine (internal standard) and (**B**) P protein histidine residue titration curves in the presence of 20 mM sulfate using pyridine as the internal standard. Inset table lists the best-fit pK$_a$ values, using pyridine as an internal standard instead of L-histidine.

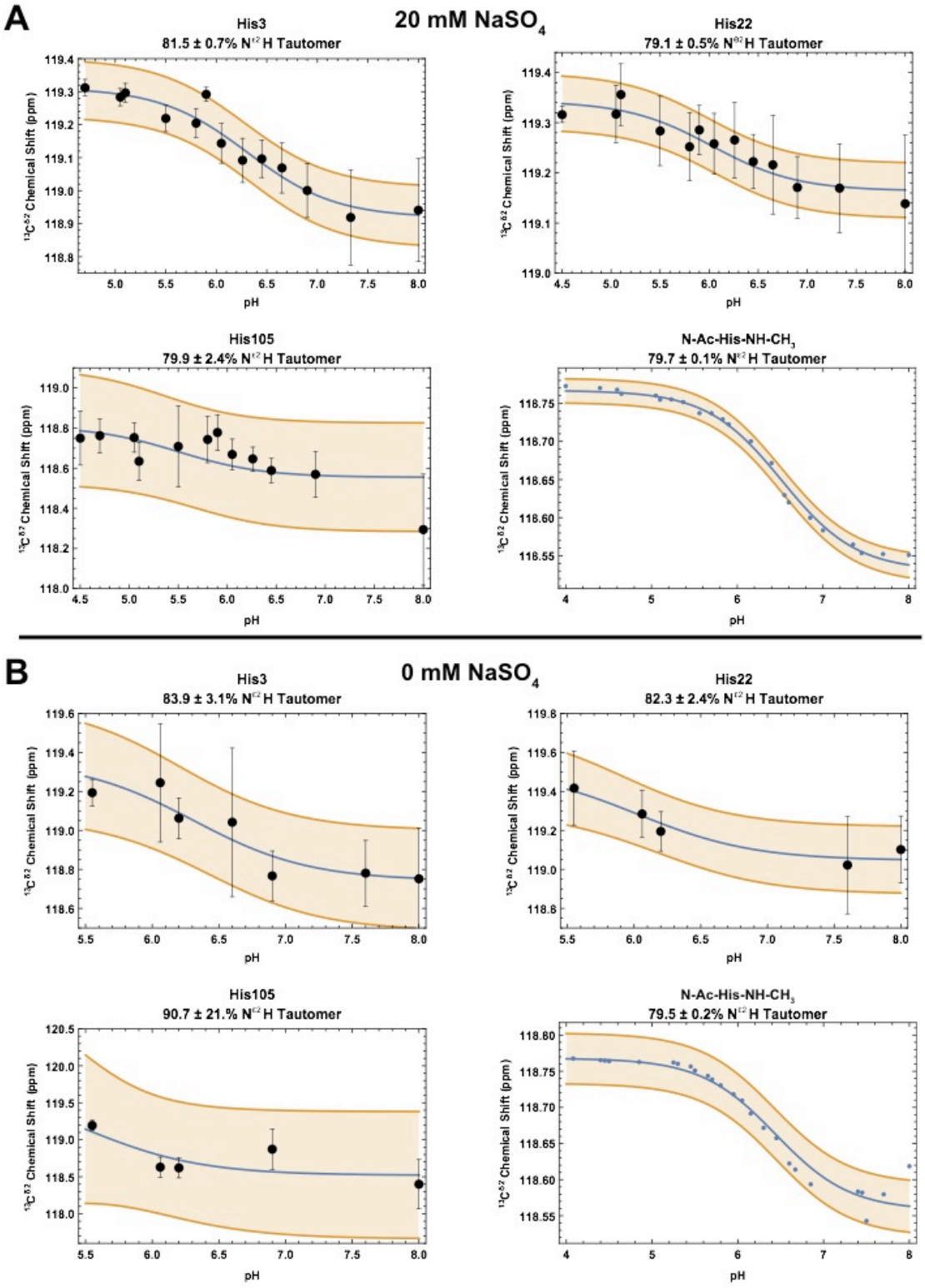


Figure S9: Determination of the tautomeric state of the neutral form of the protein and model compound histidines in (**A**) 20 mM $NaSO_4$ (folded P protein) and (**B**) 0 mM $NaSO_4$ (unfolded P protein). All P protein histidines in both folding and unfolding conditions show very similar tautomeric state populations to the model compound N-acetyl-L-Histidine-methylamide, indicating that the protein histidines are highly solvated and form water hydrogen bonds under all conditions. The error bars represent 95% confidence levels obtained from peak fitting. The beige bands represent 95% confidence levels in the best-fit chemical shift. The fitting function is described in Materials and Methods.

Table S1: Histidine pK_a values and Hill coefficients in unfolded, intermediate, and sulfate-bound folded P protein based on fitting $^{13}C^{\varepsilon 1}$ data. [a]

Residue	pK_a^F	n^F	pK_a^I	n^I	pK_a^U	n^U
His 3	6.32 ± 0.02	1.10 ± 0.06	5.73 ± 0.04	0.94 ± 0.09	5.73 ± 0.04	0.94 ± 0.09
His 22	6.10 ± 0.05	0.99 ± 0.12	5.40 ± 0.55	0.91 ± 0.41	6.11 ± 0.04	0.95 ± 0.09
His 105	5.55 ± 0.05	0.90 ± 0.03	5.80 ± 0.03	0.96 ± 0.08	5.80 ± 0.03	0.96 ± 0.08

[a] Solutions contained 10 mM L-Histidine, 10 mM pyridine in 90% H_2O, 10% D_2O at 25°C with either 0 mM or 20 mM sodium sulfate. The pK_a values were obtained by fitting the data to Eq 5. The $^{13}C^{\varepsilon 1}$ shifts were used to determine the pK_a values. Uncertainties represent the 95% confidence intervals and were obtained as described in Materials and Methods.

Table S2: Solvent exposure of the histidine sidechains in P protein, relative to Gly-His-Gly, calculated as described in Materials and Methods.

Residue	Relative accessible surface area (%)
His 3	70
His 22	61
His 105	57

Table S3: ^1H and ^{13}C chemical shifts of internal standard L-Histidine, Pyridine, and P protein histidine residues when the imidazole ring is fully protonated (pH~4) or deprotonated (pH~8). Uncertainties represent the 95% confidence intervals and were obtained as described in Materials and Methods.

	0 mM Na_2SO_4		20 mM Na_2SO_4	
δ (ppm) / Residue	^1H	^{13}C	^1H	^{13}C
L-Histidine (pH~4)	8.66 ± 0.01	136.25 ± 0.03	8.65 ± 0.01	136.26 ± 0.02
L-Histidine (pH~8)	7.72 ± 0.01	138.59 ± 0.03	7.72 ± 0.01	138.59 ± 0.02
Pyridine (pH~4)	8.08 ± 0.01	129.47 ± 0.05	8.09 ± 0.01	129.49 ± 0.03
Pyridine (pH~8)	7.45 ± 0.01	126.58 ± 0.02	7.46 ± 0.01	126.57 ± 0.01
His 3 (pH~4)	8.61 ± 0.01	135.81 ± 0.06	8.62 ± 0.01	135.99 ± 0.02
His 3 (pH~8)	7.69 ± 0.01	138.33 ± 0.07	7.67 ± 0.01	137.98 ± 0.04
His 22 (pH~4)	8.60 ± 0.01	135.82 ± 0.05	8.70 ± 0.01	136.28 ± 0.05
His 22 (pH~8)	7.67 ± 0.01	138.48 ± 0.08	7.74 ± 0.01	138.46 ± 0.09
His 105 (pH~4)	8.54 ± 0.01	135.65 ± 0.06	8.65 ± 0.01	136.14 ± 0.07
His 105 (pH~8)	7.69 ± 0.01	138.52 ± 0.06	7.81 ± 0.01	137.99 ± 0.05