Supporting Information

Helical Hairpin Structure of Influenza Hemagglutinin Fusion Peptide Stabilized by Charge-Dipole Interactions between the N-terminal Amino Group and the Second Helix.

Justin L. Lorieau, John M. Louis, and Ad Bax

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892.

Hemagglutinin fusion peptide expression and purification. Uniformly $^{13}C/^{15}N$- enriched HAfp23, containing a 7-residue C-terminal extension to facilitate sample handling,[1] was prepared as described previously.[2] The full sequence of the 30-residue peptide is GLFGAIAGFI EGGWTGMIDG WYGSGKKKKD. The G8A mutant was constructed using the QuikChange mutagenesis protocol (Stratagene, Carlsbad, CA) and verified by ESI-MS prior to making samples for NMR experiments.

Sample Preparation for NMR. Samples of $^{13}C,^{15}N$-labeled HAfp23 were lyophilized and dissolved in 100% D_2O (Cambridge Isotope Laboratories) in a N_2 glove box. The final solution consisted of 0.4 mM peptide, 10 mM ^{2}H-Tris pH 7.3 and 130-140 mM ^{2}H-DPC. Measurement of pH was conducted with a pH electrode that was soaked for 1 hour in 1 M KCl in D_2O. A pD correction was not applied to the data. Although the pH meter reading is offset due to the D+ isotope effect on the glass electrode, this offset is largely compensated by a change in measured pK value in D_2O, and therefore an offset correction was not applied to the data. This observation is consistent with results reported by Bundi and Wüthrich.[3] Titrations were conducted by adding small aliquots of either 50 mM ^{2}H-citric acid (Cambridge Isotopes), 50 mM Na_2CO_3 (Mallinckrodt), or 50 mM NaOD (Cambridge Isotopes) in D_2O.

NMR Experiments. Experiments were carried out on a Bruker Avance-III 600 MHz spectrometer, equipped with a four-channel TXI probe, at a sample temperature of 305K. Chemical shifts were referenced indirectly to TSP, using the HDO resonance as an internal reference (4.71 ppm). Chemical shift titration data were collected with 3D versions of either the HACAN CH2-TROSY experiment (Supporting Information Figure S2), or the 3D HCCO or HCACO experiments, adapted with gradient coherence selection.[4] Data were fit by least-squares minimization and errors estimated by leave-one-out jack-knifing of the data.

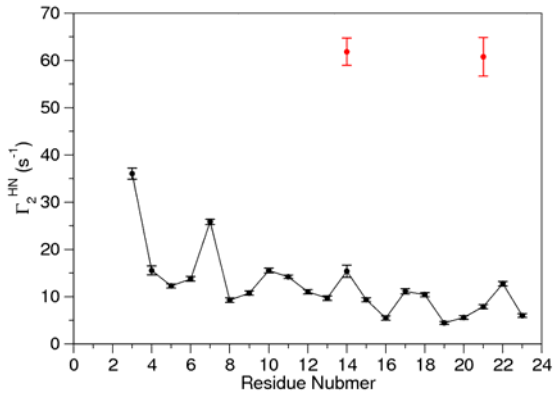

Figure S1. The transverse paramagnetic relaxation enhancement (PRE) of the backbone $^{1}H^{N}$ nuclei in a sample of 0.6 mM ^{13}C,^{15}N-HAfp23 in 130 mM ^{2}H-DPC micelles, containing 1.9 mM 5-doxyl-stearic acid (5-DSA). The relaxation enhancement profile places the amide protons of the hydrophobic residues closer to the micelle core, where the paramagnetic tag is located. Note that the amide protons are shielded from direct contact to the lipid alkyl chains by their relatively bulky side chains. The PRE effects for the Trp14 and Trp21 $H^{\varepsilon 1}$ sidechain protons, not shielded by such effects, are marked in red.

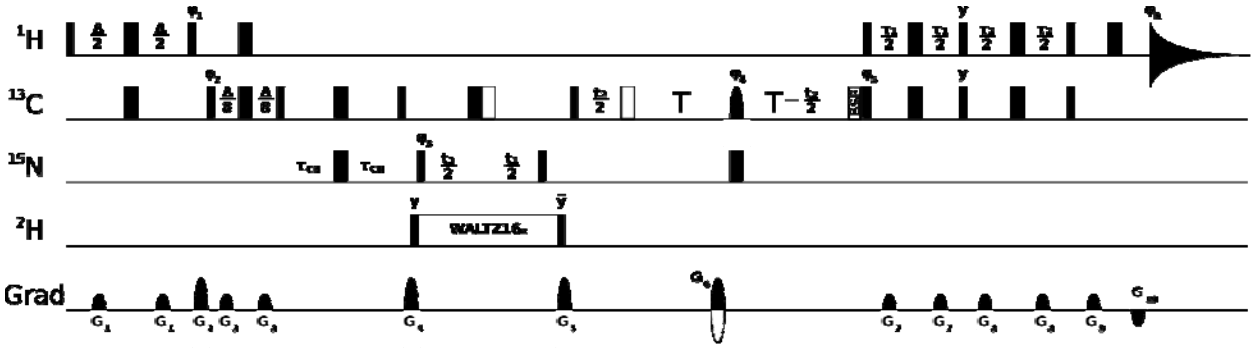

Figure S2. Pulse sequence for the HACAN CH_2-TROSY experiment. Narrow and wide bars represent 90º and 180º pulses, respectively. All pulses on the ^{1}H channel are non-selective. Solid pulses on the ^{13}C channel are centered at the Gly methylene frequency (46 ppm), and the open pulses are centered on the carbonyl frequency (177 ppm). The shaped pulse is a 3.5-ms ReBURP pulse,[5] permitting narrowing of the $^{13}C^{\alpha}$ spectral width to 7 ppm, and the pulse labeled 'BSP' is a carbonyl Bloch-Siegert compensation pulse.[6] The phase of all pulses is 'x', unless otherwise specified. The pulse phases are: φ_1 = -y; φ_2 = 225º, 45º; φ_3 = x,x,x,x,-x,-x,-x,-x; φ_4 = x,x,y,y; φ_5 = x; φ_R = x,-x,-x,x,-x,x,x,-x. Quadrature detection is achieved by States-TPPI acquisition in the t_1 dimension, incrementing φ_3, and by the echo/anti-echo method in the t_2 dimension by inverting the amplitude of the gradient pulse G_6 and the phase of φ_5.[4] Delay durations: Δ = 1.7 ms; τ_{CN} = 12.5 ms; T = 12.5 ms; τ_1 = 1.17 ms, τ_2 = 790 μs. All gradient pulses are applied in the z direction and have sine-bell profiles, with durations $G_{1,\ldots,10}$ of 1.55, 1.0, 0.4, 0.8, 0.6, 1.4, 1.0, 0.64, 0.246 and 0.106 ms, and maximum amplitudes of 3.5, 24.5, 10.5, 24.5, 24.5, 35, 5.6, 6.3, 35 and 35 G/cm. During ^{15}N evolution times, ^{2}H decoupling with a 1 kHz RF field strength is used.

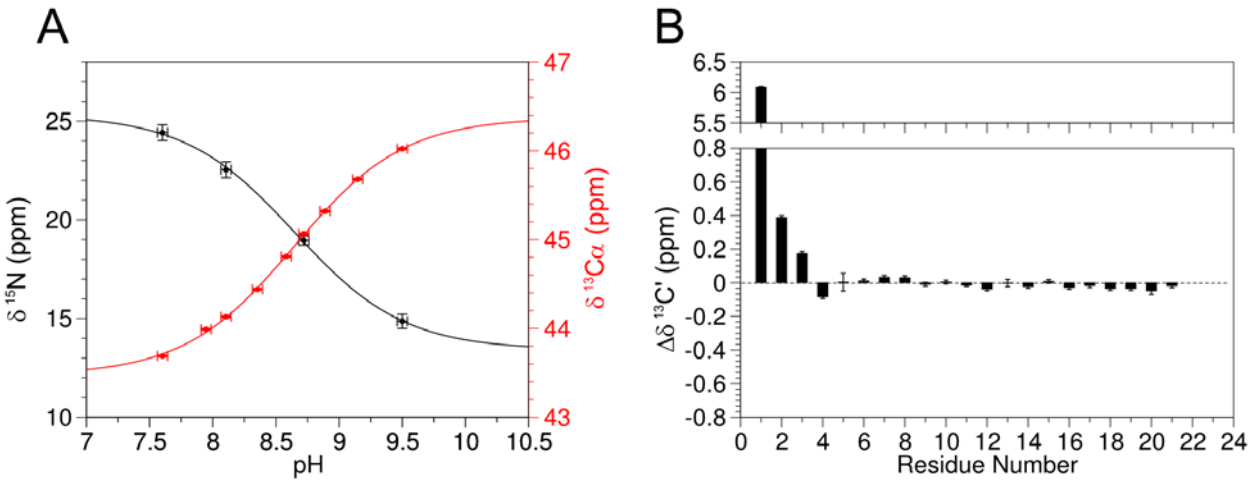

Figure S3. Chemical shift titration curves for (A) the Gly^1 N-terminus of the truncated HAfp20 construct, and (B) chemical shift changes between pH 9.5 and 7.6 of the $^{13}C'$ resonances in the same sample. Fitted values are: pK = 8.64 ± 0.02 ($^{13}C^{\alpha}$) and 8.6±0.2 (^{15}N), with ^{15}N and $^{13}C^{\alpha}$ chemical shifts of 25.4 ± 0.7 ppm and 43.47 ± 0.05 ppm, respectively, for the protonated form (NH_3^+), and 13.8 ± 1.6 ppm and 46.4 ± 0.1 ppm, respectively, for the deprotonated form (NH_2).

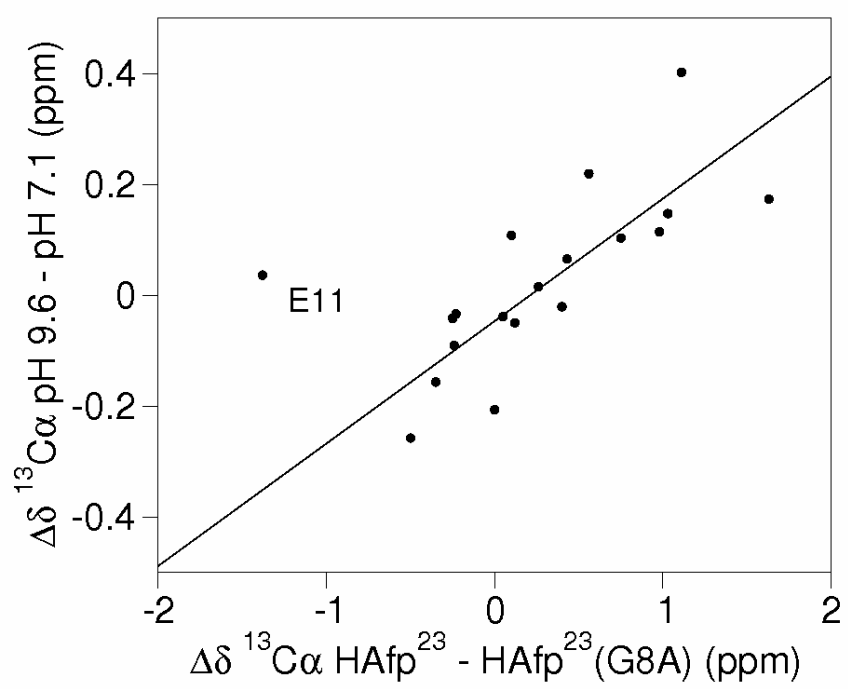

Figure S4. A plot for the $^{13}C^{\alpha}$ chemical shift difference between the wild type HAfp23 and its G8A mutant (x-axis) versus the shift difference between the wild type HAfp23 at pH 9.6 and pH 7.1 (y-axis). Plotted are the values for residues Phe^{3} to Tyr^{22}. The linear regression parameters are (excluding E11 from the fit): slope = 0.22, intercept = -0.05, R^2 = 0.65.

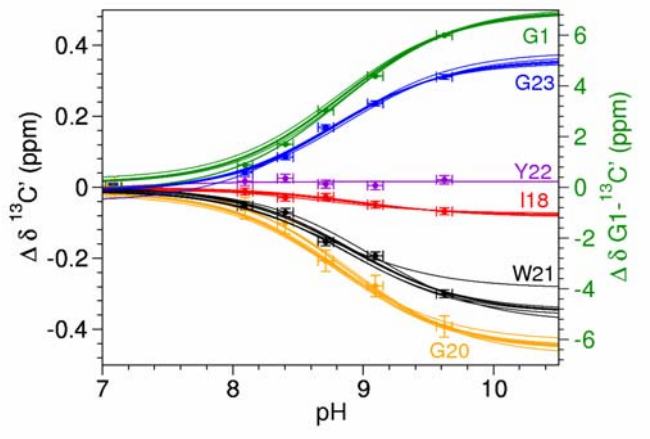

Figure S5. Jack-knifing fits of the pH dependence of Gly^{1} ^{13}C' (green, right axis) and ^{13}C' chemical shifts of various residues near the end of helix 2. Traces show each of the jack-knife fits by leaving out one data point at a time. The resulting ranges are: $\Delta\delta$ = [7.04, 7.39] ppm and pK = [8.80, 8.87] for Gly^{1}; $\Delta\delta$ = [-0.084, -0.074] ppm and pK = [8.67, 9.05] for Ile^{18}; $\Delta\delta$ = [-0.473, -0.432] ppm and pK = [8.81, 8.93] for Gly^{20}; $\Delta\delta$ = [-0.363, -0.284] ppm and pK = [8.71, 9.07] for Trp^{21}; $\Delta\delta$ = [0.351, 0.382] ppm and pK = [8.69, 8.86] for Gly^{23}.

References

(1) Han, X.; Tamm, L. K. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 13097-13102.
(2) Lorieau, J. L.; Louis, J. M.; Bax, A. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 11341-11346.
(3) Bundi, A.; Wuthrich, K. *Biopolymers* **1979**, *18*, 285-297.
(4) Kay, L. E.; Keifer, P.; Saarinen, T. *J. Am. Chem. Soc.* **1992**, *114*, 10663-10665.
(5) Geen, H.; Freeman, R. *J. Magn. Reson.* **1991**, *93*, 93-141.
(6) Vuister, G. W.; Bax, A. *J. Magn. Reson.* **1992**, *98*, 428-435.