Supplementary Material

The pK_a values of the catalytic residues in the retaining glycoside hydrolase T26H mutant of T4 lysozyme

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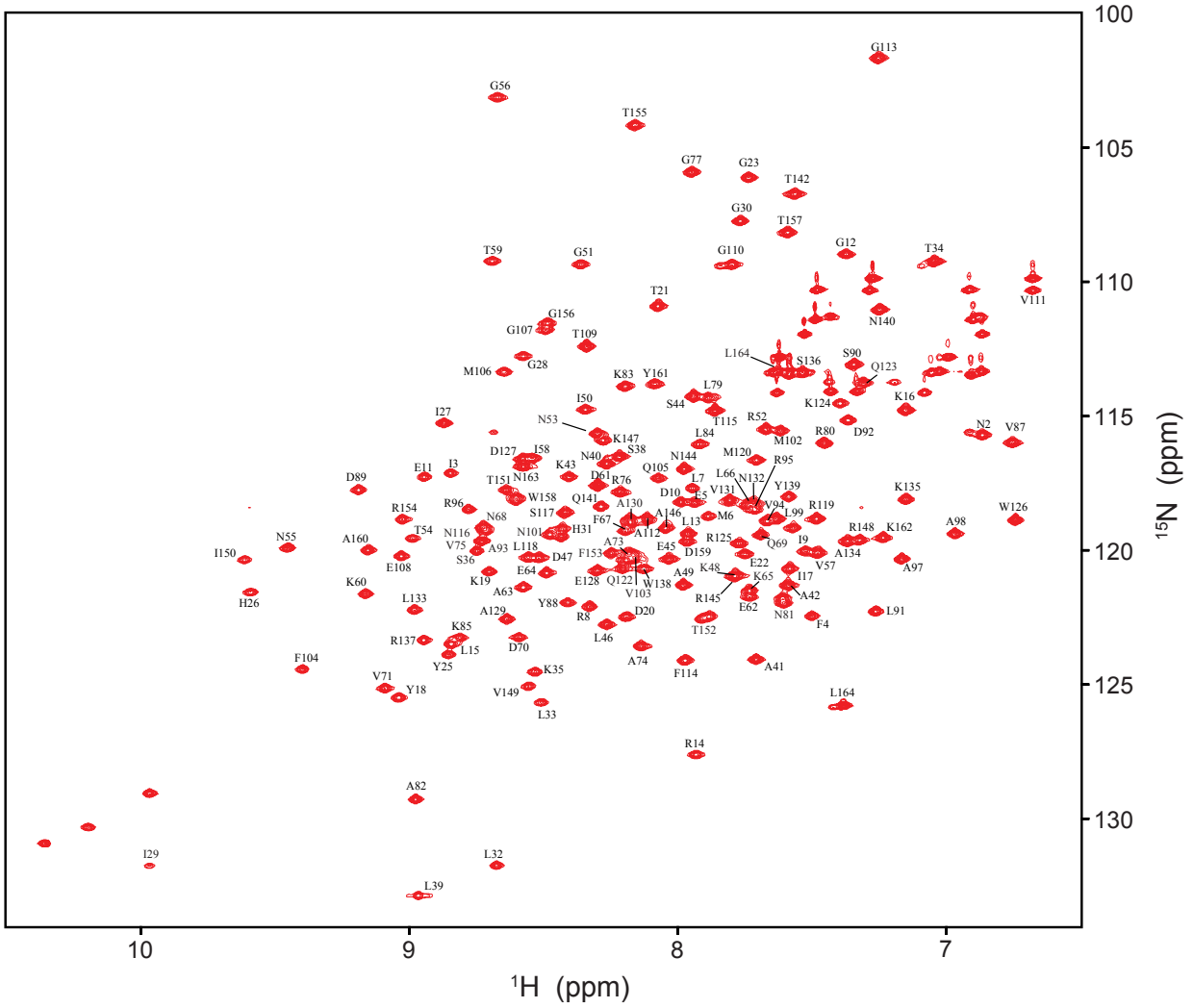
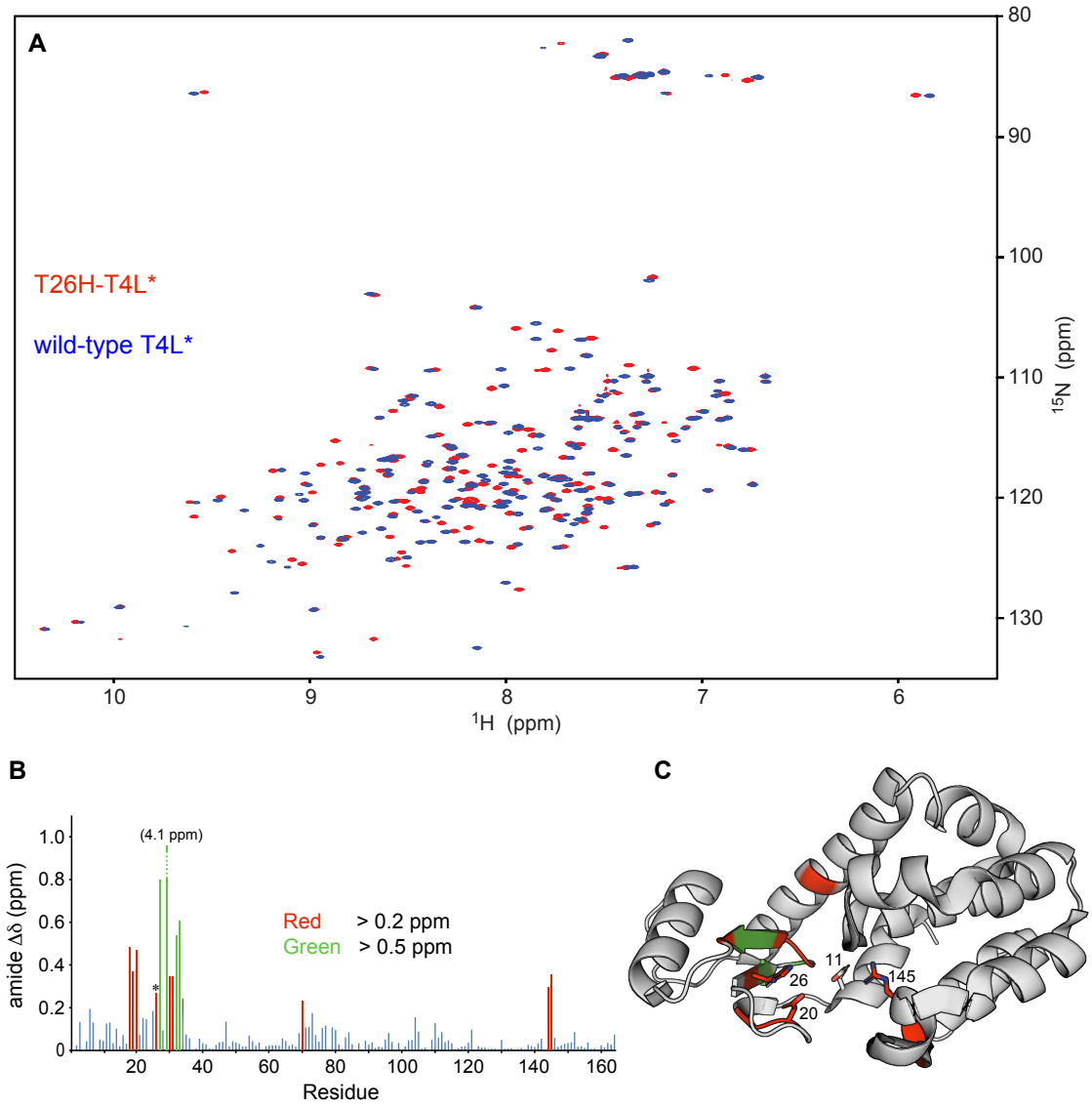
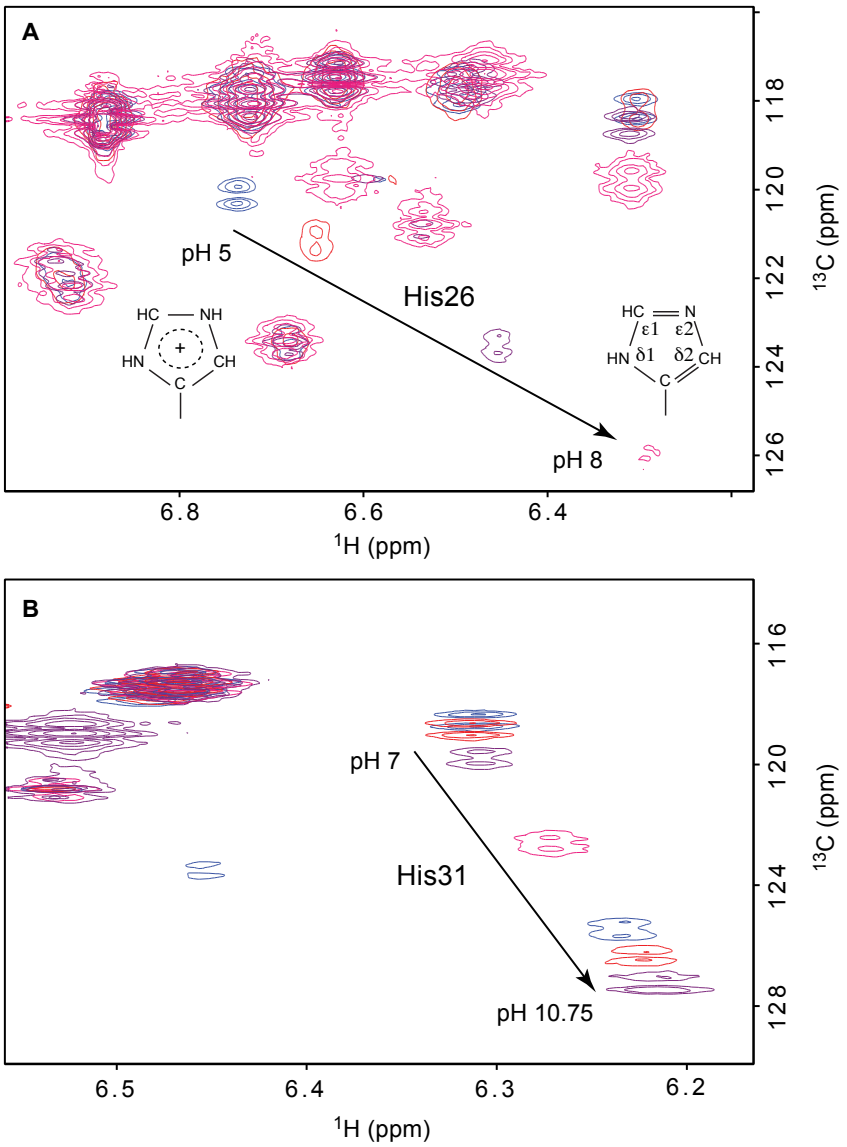


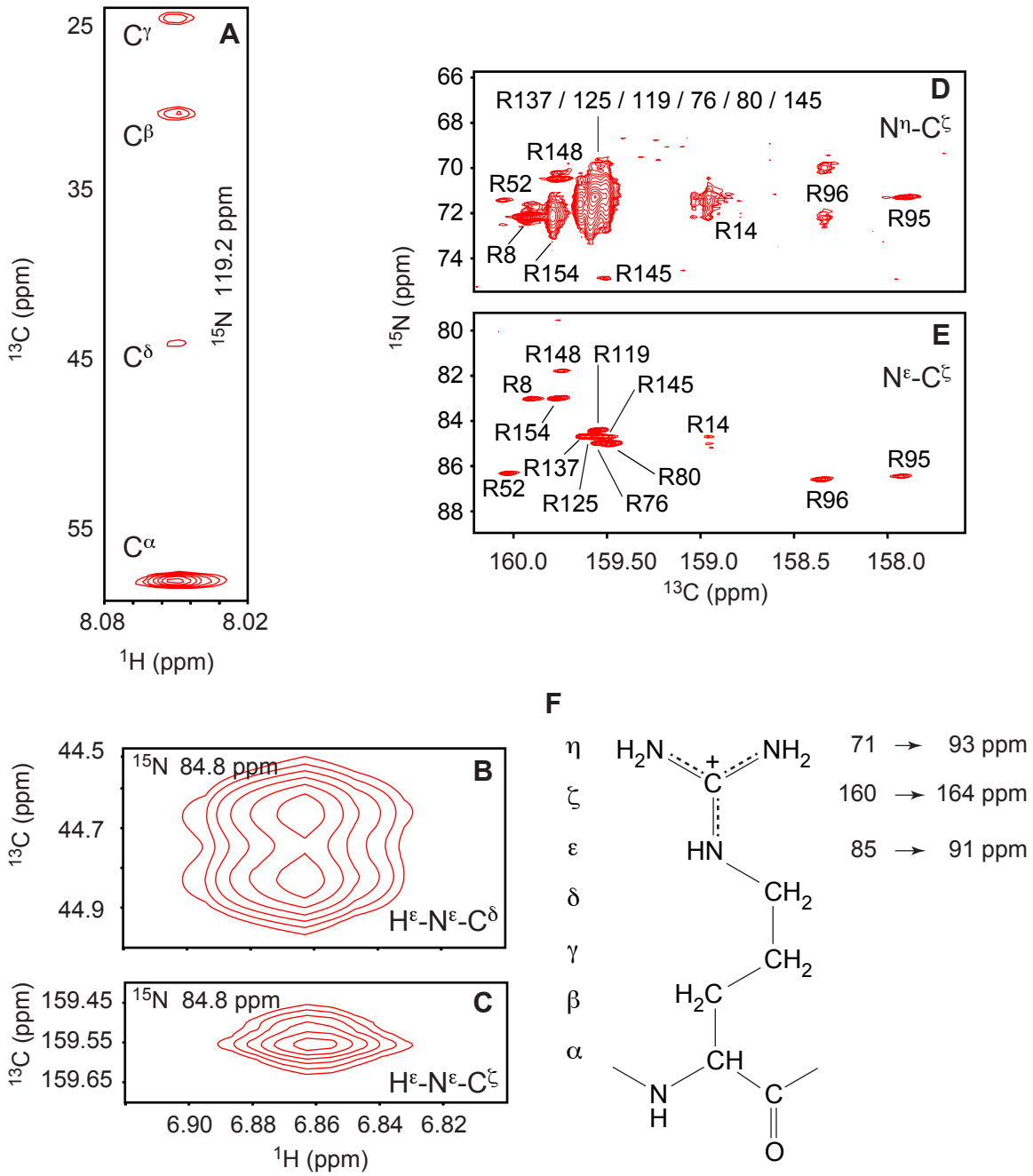
Supporting Information Figure S1. The ^{15}N-HSQC spectrum of T26H-T4L* in 100 mM KCl, 30 mM potassium phosphate, and 5% D_2O at pH 5.5 and 25 ^{o}C. Assigned mainchain amide $^{1}H^{N}$-^{15}N signals are labeled.



Supporting Information Figure S2. (A) Overlaid ^{15}N-HSQC spectra of T26H-T4L* (red) and wild-type T4L* (blue) with both samples at pH 5.5 and 25 °C. Signals from amides and arginine sidechains (~ 85 ppm in ^{15}N) are shown. (B) Histogram of amide chemical shift differences between corresponding amides in the two proteins, calculated[1] as $\Delta\delta = \{(\Delta\delta_H)^2 + \{(0.14\Delta\delta_N)^2\}^{1/2}$. Blank values are for prolines and residues for which assignments were not obtained. (C) When mapped on the structure of T26H-T4L* (1QT8.pdb), amides with the largest perturbed chemical shifts cluster around position 26 (*).



Supporting Information Figure S3. Superimposed ^{15}N-decoupled ^{13}C-HSQC spectra of uniformly ^{13}C/^{15}N-labeled T26H-T4L* showing the ^{1}H$^{\delta 2}$-^{13}C$^{\delta 2}$ signals of (A) His26 and (B) His31 as the protein was titrated between pH 5 and 11 at 25 ^{o}C. The ^{13}C$^{\delta 2}$ signals are doublets due to $^{1}J_{CC}$ coupling with the adjacent ^{13}C$^{\gamma}$. For clarity, only representative titration points are shown, and all data are presented in Fig. 2B. Both neutral histidines adopt the N$^{\delta 1}$H tautomer (structure shown) based on fit plateau ^{13}C$^{\delta 2}$ chemical shifts of 126.7 ppm (His26) and 127.2 ppm (His31).



Supporting Information Figure S4. Assignment of the signals from the guanidinium sidechain of Arg145 in uniformly $^{13}C/^{15}N$-labeled T26H-T4L* at pH 6.5 and 25 °C. (A) The ^{1}H-^{13}C plane from a 3D C(CCO)-TOCSY-NH spectrum taken at the ^{15}N shift of Ala146 (119.2 ppm). This

provided assignments for the $^{13}C^{\alpha}$ (58.8 ppm), $^{13}C^{\beta}$ (31.2 ppm), $^{13}C^{\gamma}$ (25.5 ppm) and $^{13}C^{\delta}$ (44.8 ppm) of Arg145. (B) The ^{1}H-^{13}C plane at the indicated ^{15}N shift (84.8 ppm) of a 3D $H^{\varepsilon}N^{\varepsilon}C^{\delta}$ spectrum[2] yielded the assignment of the $^{1}H^{\varepsilon}$ (6.86 ppm) and $^{15}N^{\varepsilon}$ (84.8 ppm) signals of Arg145 by virtue of a scalar correlation to the $^{13}C^{\delta}$ (see also Fig. 5). (C) The $^{13}C^{\zeta}$ (159.5 ppm) was then assigned from the corresponding ^{1}H-^{13}C plane of a 3D $H^{\varepsilon}N^{\varepsilon}C^{\zeta}$ spectrum.[3] (D, E) Finally, $^{15}N^{\eta}$ signals at 75 ppm and ~ 71 ppm were identified from a 2D $N^{\varepsilon/\eta}$–C^{ζ} correlation spectrum.[4,5] Panels D and E are from the same ^{13}C-detected spectrum, but E is displayed at a higher contour level for clarity. These assignments are similar to those reported for wild-type T4L*.[4-7] (F) The cartoon summarizes the expected $^{15}N^{\eta}$, $^{13}C^{\zeta}$, and $^{15}N^{\varepsilon}$ chemical shifts of an arginine sidechain upon titration from its positively charged to neutral form.[8,9] Under these conditions (pH 6.5), all thirteen arginines in T26H-T4L* have chemical shifts diagnostic of a fully protonated guanidinium group.

Supplementary References

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