Supplementary Materials

AT-Hook-Like Motif Is the Common DNA Binding Element of H-NS Family Proteins: Insights from the Solution Structure of *E. coli* H-NS$^{85–137}$/DNA Complex

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Supplementary Figures

Figure S1: (A) The 2D ^{1}H-^{15}N HSQC spectrum of free H-NS$^{85-137}$ in 50 mM phosphate buffer (pH 5.7) with 50 mM NaCl, with peak assignments indicated. (B) Overlay of the 2D ^{1}H-^{15}N HSQC spectra for Top1 DNA titration. Assignments of the NH signals with large chemical shift perturbations are labeled. The arrowed lines indicate titration traces. Protein/DNA ratios and the corresponding colors are indicated above the spectra.

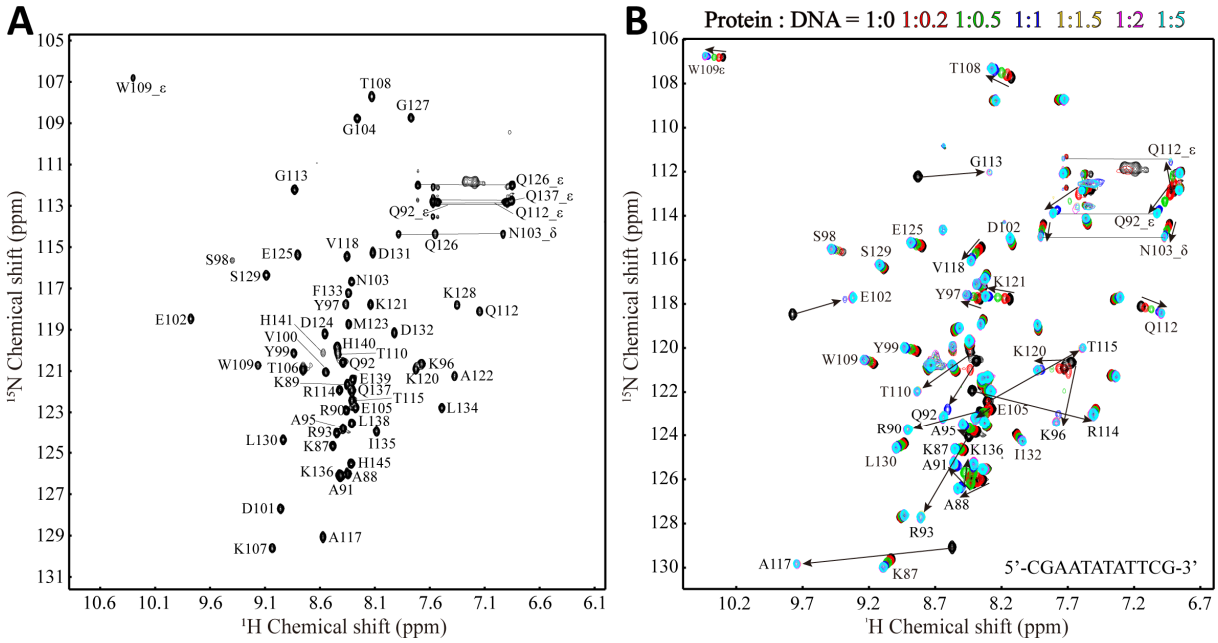

Figure S2: (A) Combined ^{1}H and ^{15}N chemical shift differences between free H-NS$^{85-137}$ and that with 5-fold of Top1 DNA. (B) Representative strips of 3D F1-$^{15}N/^{13}C$ filtered, F2-^{13}C edited NOESY-HSQC spectrum, with the corresponding inter-molecular NOE assignments labeled.

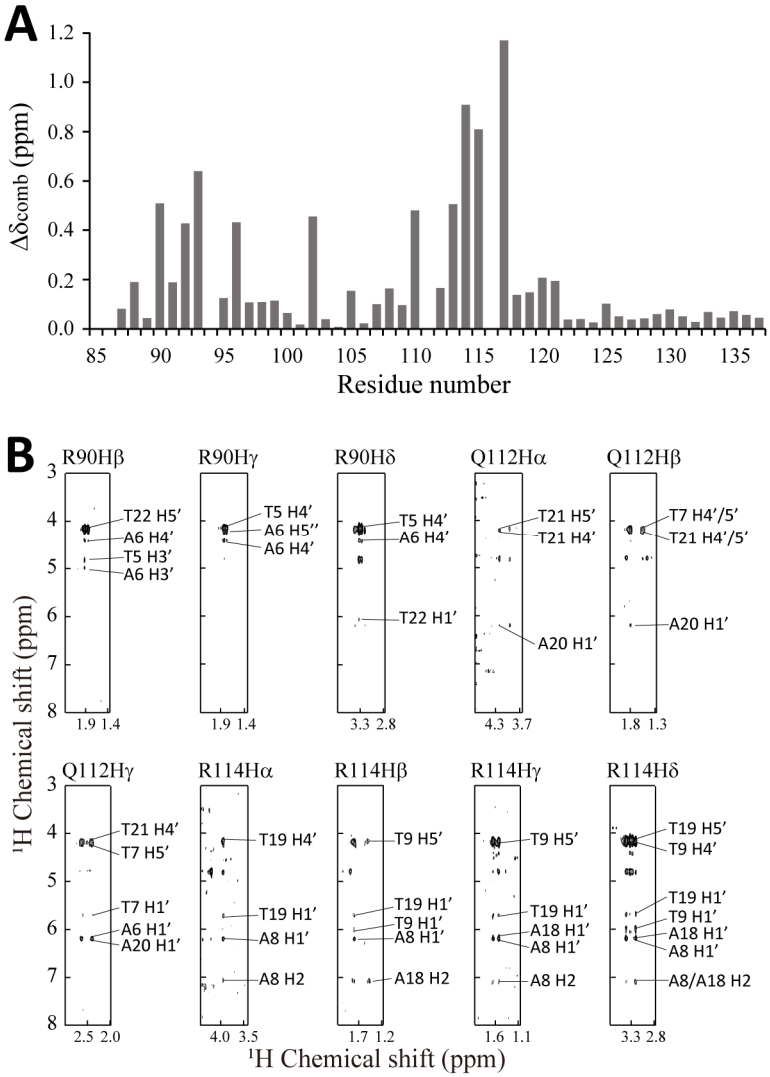

Figure S3: (A) Structure of H-NS$^{85-137}$ in complex with DNA, with side-chains of residues involved in hydrophobic core packing shown in magenta sticks. (B) Electrostatic surface of H-NS$^{85-137}$ in complex with DNA.

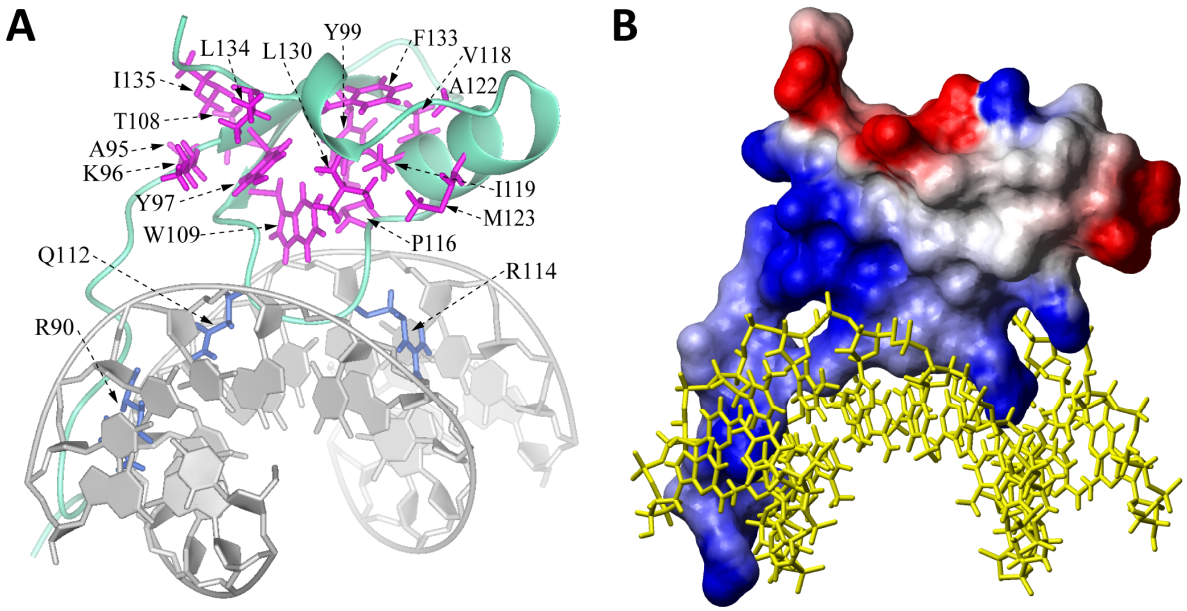

Figure S4: (A) Plots of ^{1}H-^{15}N heteronuclear NOE values of H-NS$^{85-137}$ in free (black square) and 10-fold Top1 DNA bound (red circle) forms. Magenta shaded bars indicate the linker and loop L2 regions with increased heteronuclear NOE values upon DNA binding; the cyan shaded bar indicates loop L1, whose values decrease upon DNA binding. (B) Structure of H-NS$^{85-137}$ in complex with Top1 DNA with the linker and loop L1 and L2 regions indicated by corresponding colors in A. Side-chains of negatively charged residues D101, E102 and E105 (red), nearby positively charged residues K107 and K121 (blue), and DNA contacting residues R90, R93, Q112, and R114 (green), are shown.

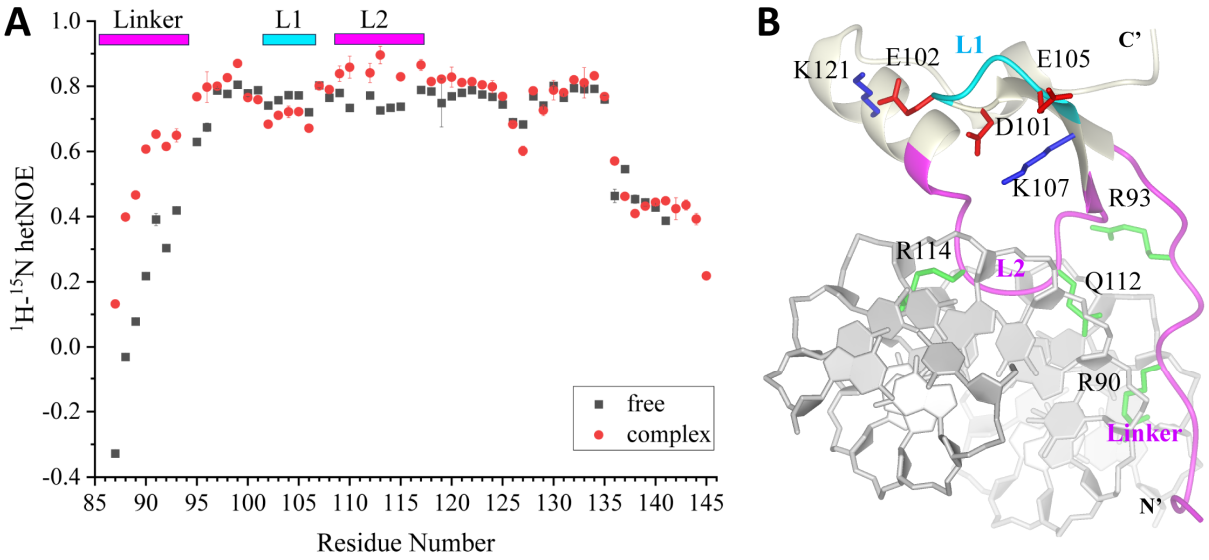

Figure S5: (A) Overlay of ribbon display of the structure of *E. coli* H-NS$^{85\text{-}137}$ (light blue) with that of free DNA binding domain structure of *Salmonella* H-NS (PDB ID 2L93) (pink), with the β-sheet regions (β1-L1-β2) highlighted in blue and red, respectively. Residues that differ between the two are indicated by corresponding blue and red balls at C$^{\alpha}$ positions. (B) Comparison of the hydrogen bonds (cyan stick) patterns of β-sheets in the structure of DNA bound *E. coli* H-NS$^{85\text{-}137}$ (complex, without β-bulge) and that in 2L92 (free, with a β-bulge). Inter proton distance (magenta broken stick) ranges between T106-H^{N} and T105-H$^{\alpha}$, K107-H^{N}, and V100-H$^{\alpha}$, are indicated. (C) Strips of 3D ^{1}H-^{15}N-edited NOESY-HSQC spectra of H-NS$^{85\text{-}137}$ in free and Top1 DNA bound forms, showing NOEs of T106-H^{N}, with assignments labeled.

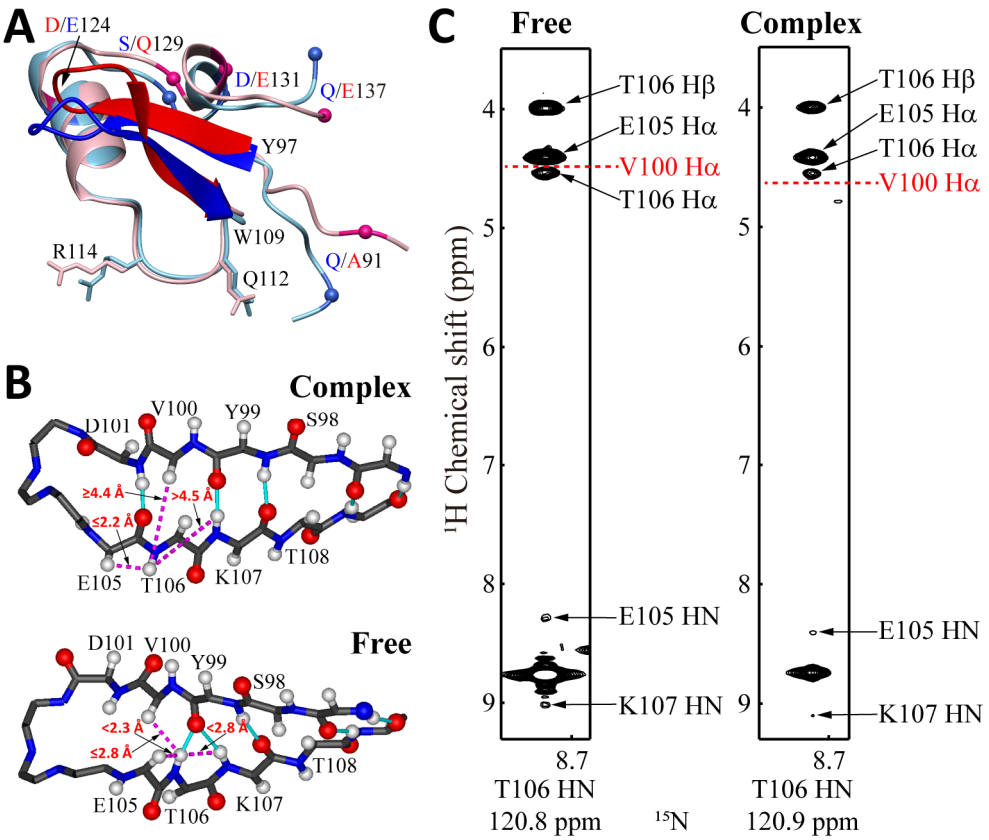

Figure S6: NMR titration of H-NS$^{85-137}$ mutants R114A (A) and R114K (B) with Top1 DNA. Left panel: overlay of 2D ^{1}H-^{15}N HSQC spectra of mutant at indicated protein/DNA ratios. Right panel: overlay of 2D ^{1}H-^{15}N HSQC spectra for WT and mutant at protein/DNA ratio 1:5. Assignments are indicated for significantly deviated NH signals between WT and mutant.

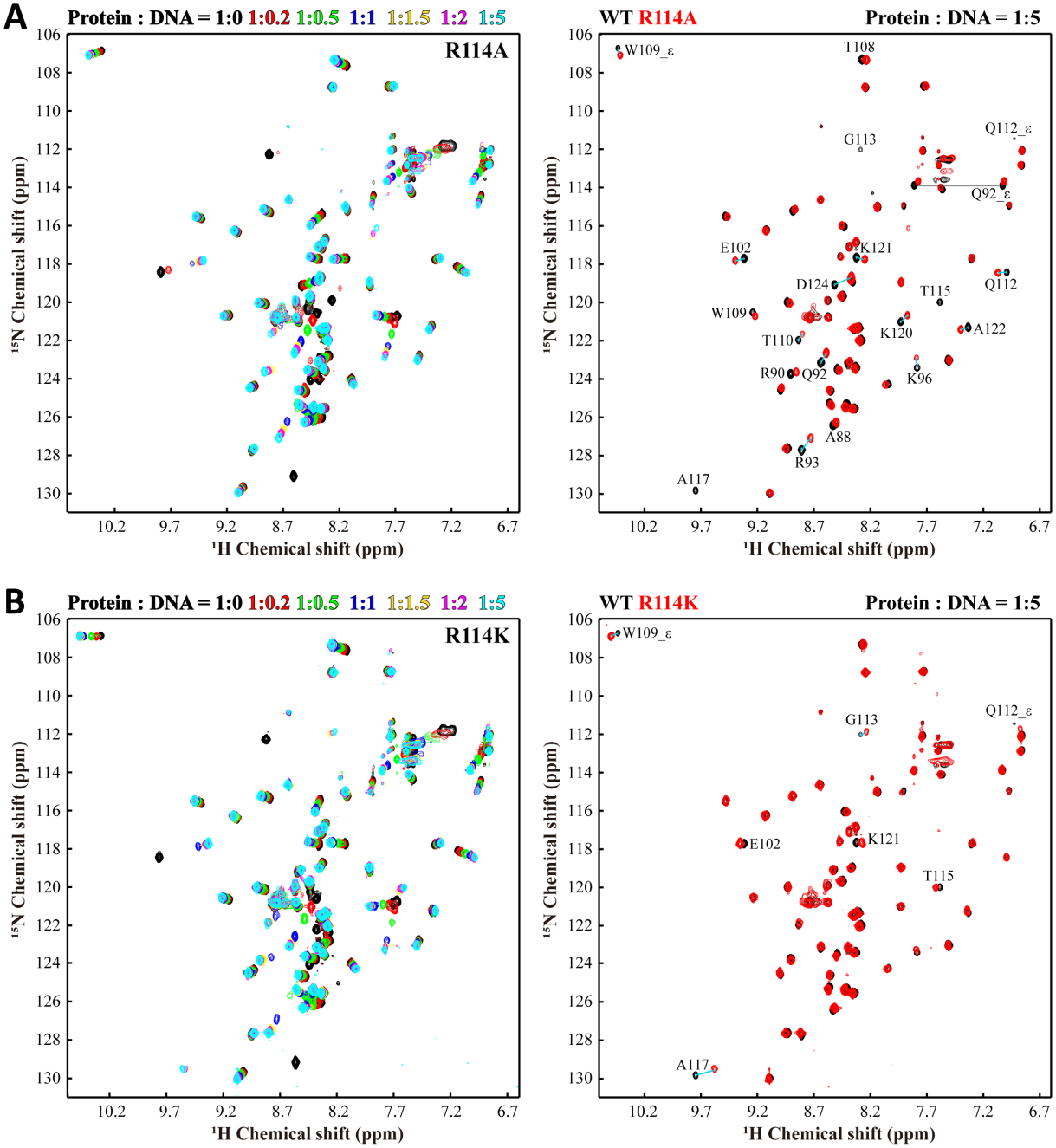

Figure S7: Overlay of 2D ^{1}H-^{15}N HSQC spectra between free WT H-NS$^{85-137}$ and mutants R114A (A), R114K (B), R90A (C), and R90K (D). Assignments are indicated for significantly deviated NH signals between WT and mutant.

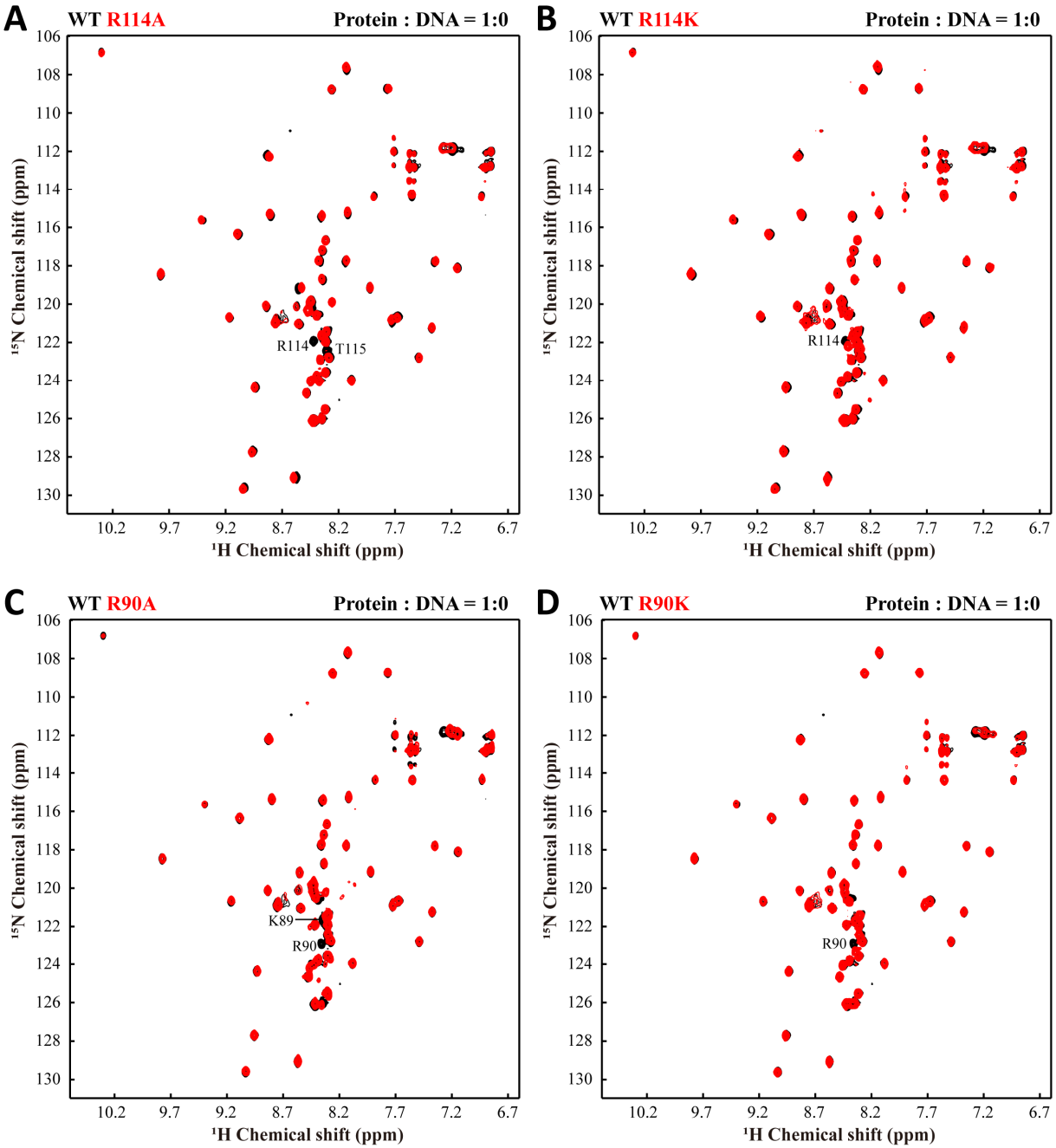

Figure S8: NMR titration of H-NS$^{85-137}$ mutants Q112A (A) and Q112R (B) with Top1 DNA. Left panel: overlay of 2D ^{1}H-^{15}N HSQC spectra of mutant at indicated protein/DNA ratios. Right panel: overlay of 2D ^{1}H-^{15}N HSQC spectra for WT and mutant at protein/DNA ratio 1:5. Assignments are indicated for significantly deviated NH signals between WT and mutant.

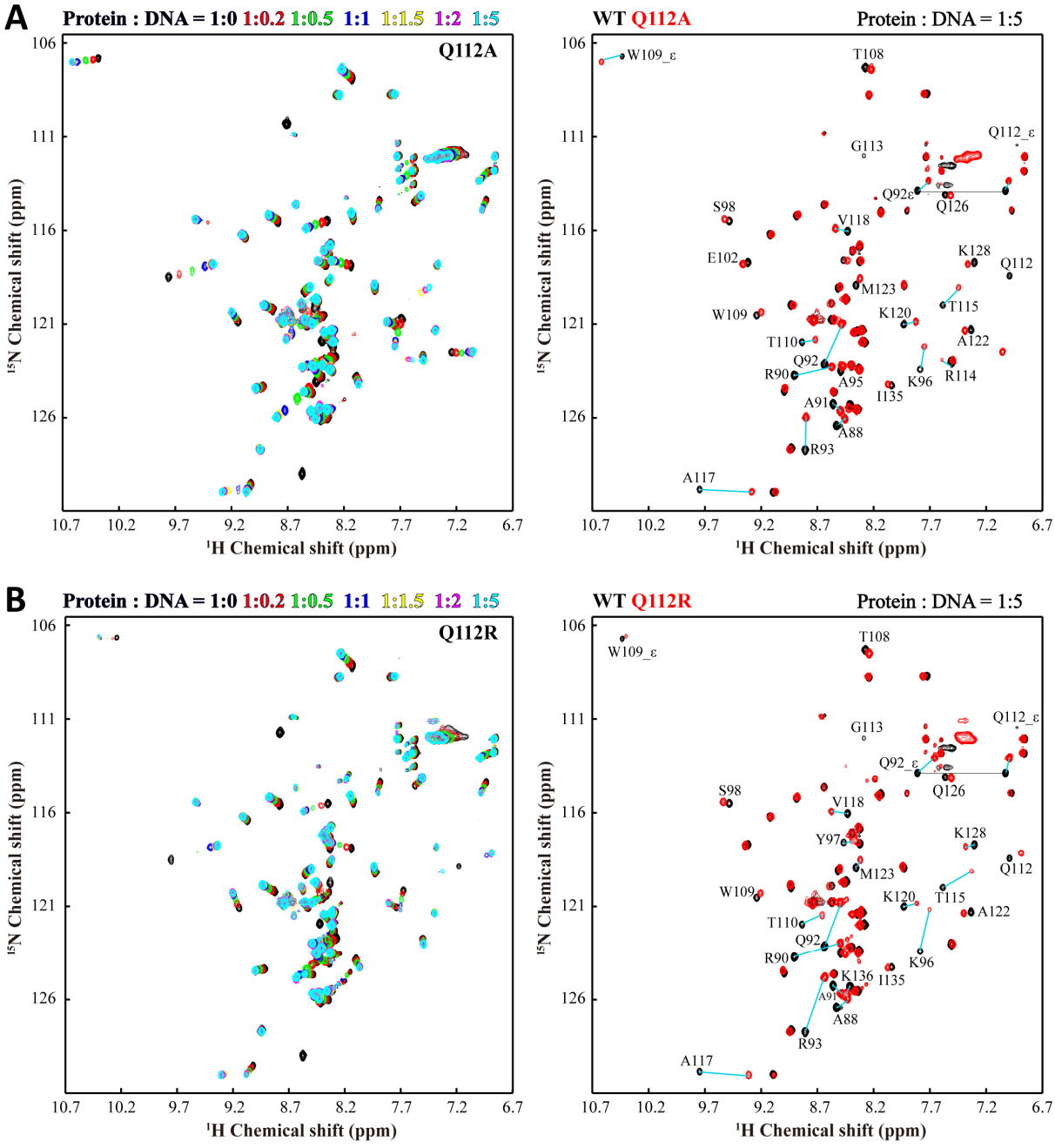

Figure S9: NMR titration of H-NS$^{85-137}$ mutants Q112N (A) and Q112V (B) with Top1 DNA. Left panel: overlay of 2D ^{1}H-^{15}N HSQC spectra of mutant at indicated protein/DNA ratios. Right panel: overlay of 2D ^{1}H-^{15}N HSQC spectra for WT and mutant at protein/DNA ratio 1:5. Assignments are indicated for significantly deviated NH signals between WT and mutant.

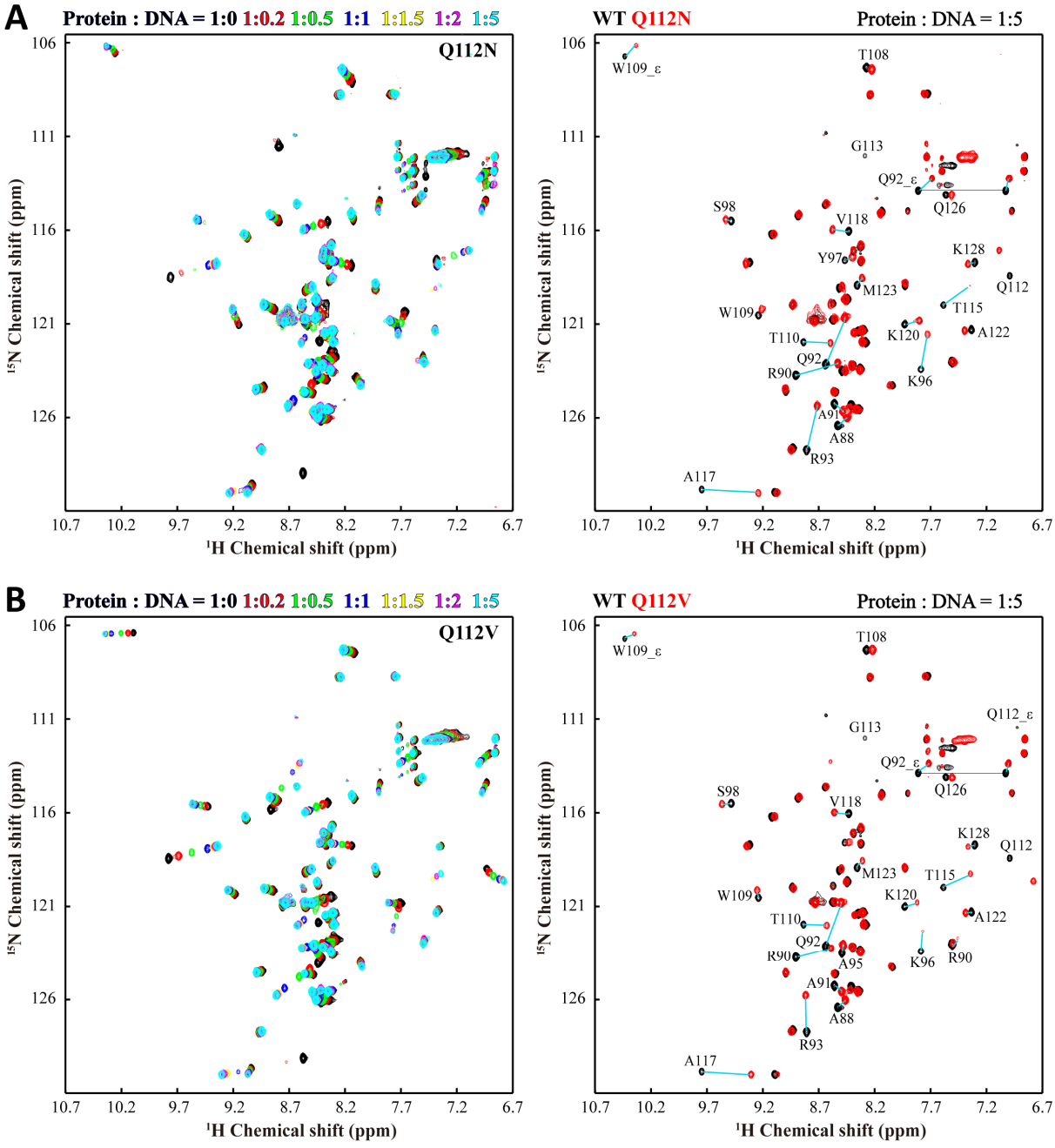

Figure S10: Overlay of 2D ^{1}H-^{15}N HSQC spectra between free WT H-NS$^{85-137}$ and mutants Q112A (A), Q112R (B), Q112N (C), and Q112V (D). Assignments are indicated for significantly deviated NH signals between WT and mutant.

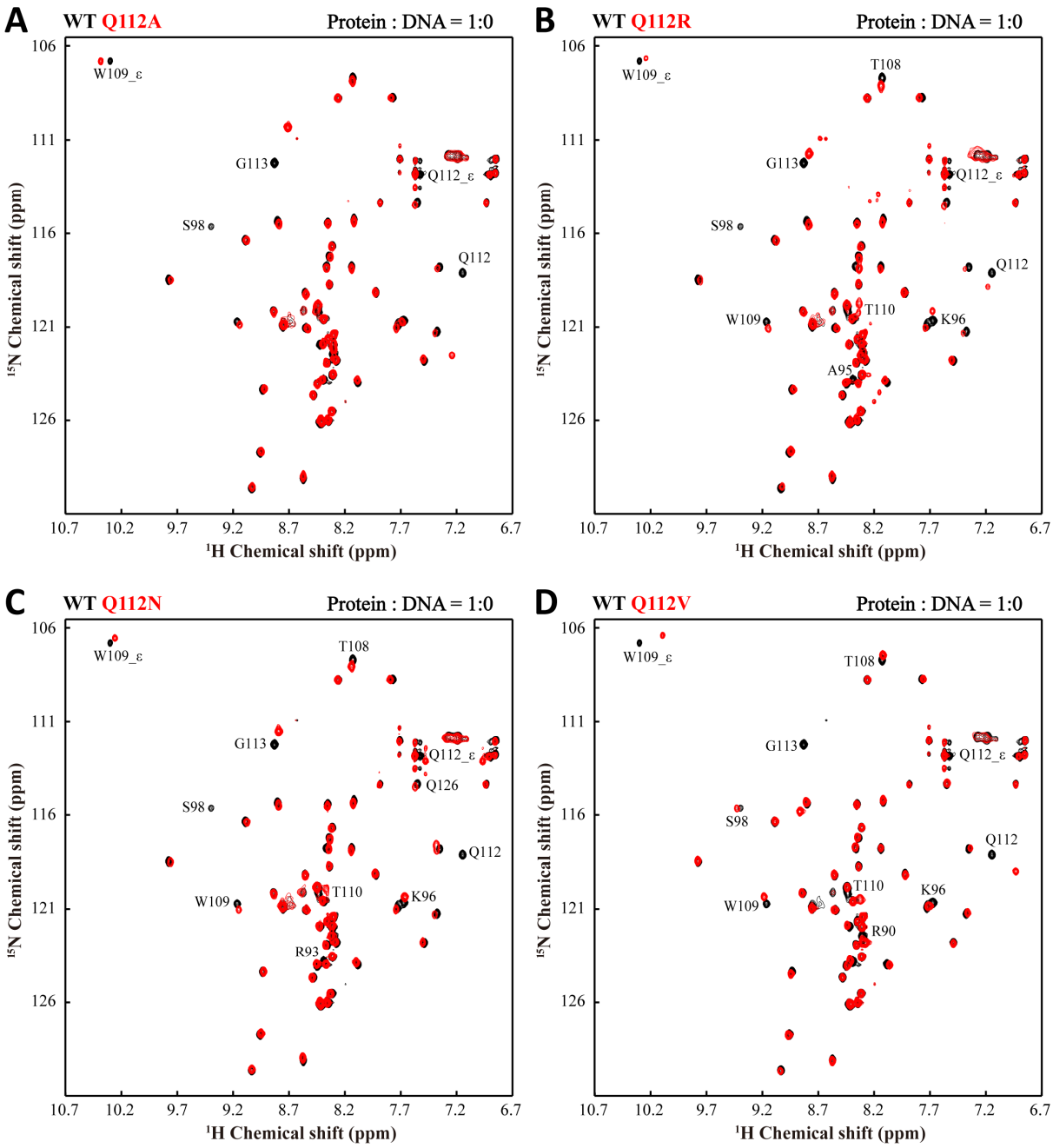

Figure S11: NMR titration of H-NS$^{85-137}$ mutants R90A (A) and R90K (B) with Top1 DNA. Left panel: overlay of 2D ^{1}H-^{15}N HSQC spectra of mutant at indicated protein/DNA ratios. Right panel: overlay of 2D ^{1}H-^{15}N HSQC spectra for WT and mutant at protein/DNA ratio 1:5. Assignments are indicated for significantly deviated NH signals between WT and mutant.

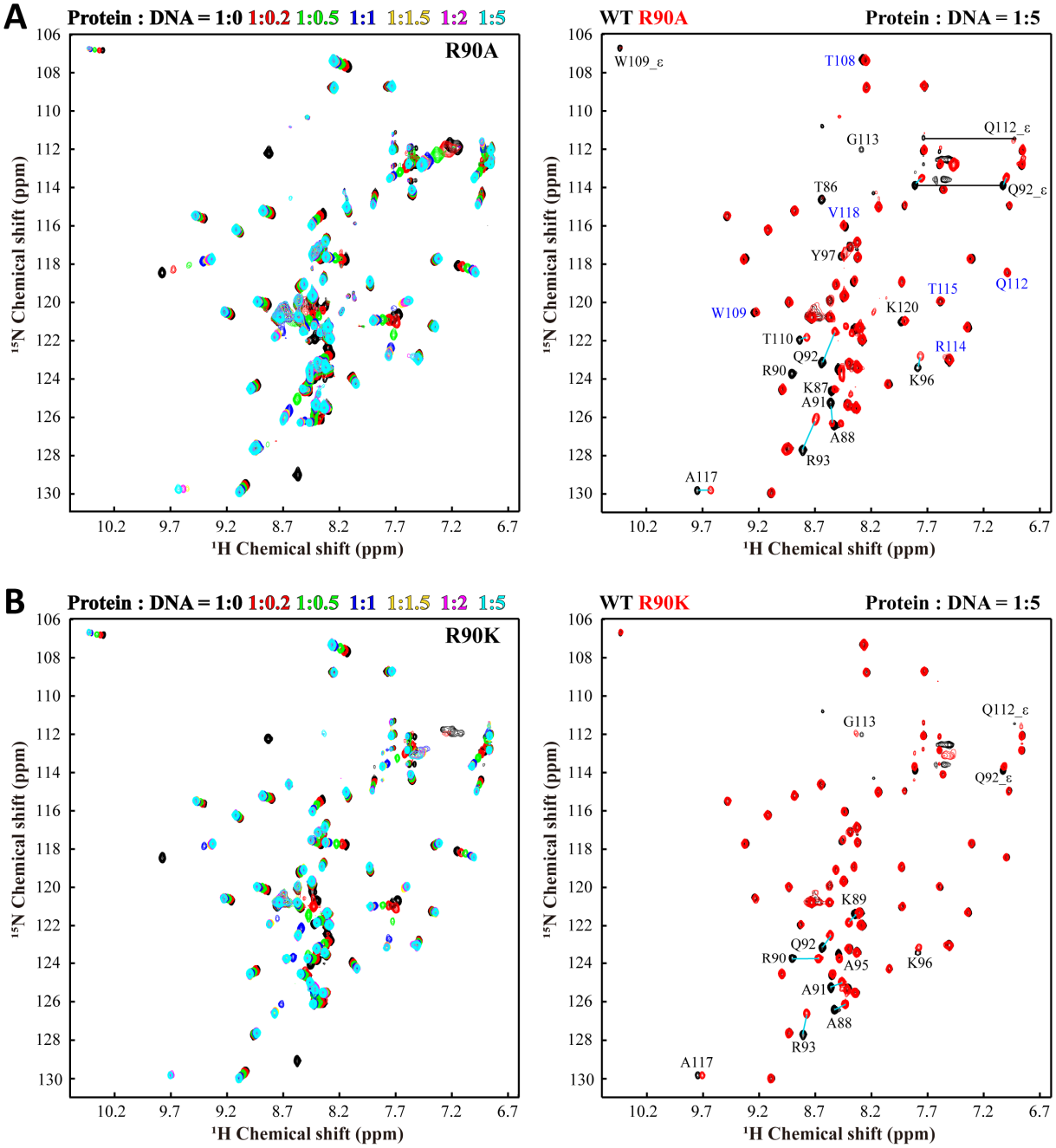

Figure S12: Overlay of 2D ^{1}H-^{15}N HSQC spectra between free WT H-NS$^{85-137}$ and mutants R93A (A), R93K (B), T110A (C), and T110S (D). Assignments are indicated for significantly deviated NH signals between WT and mutant.

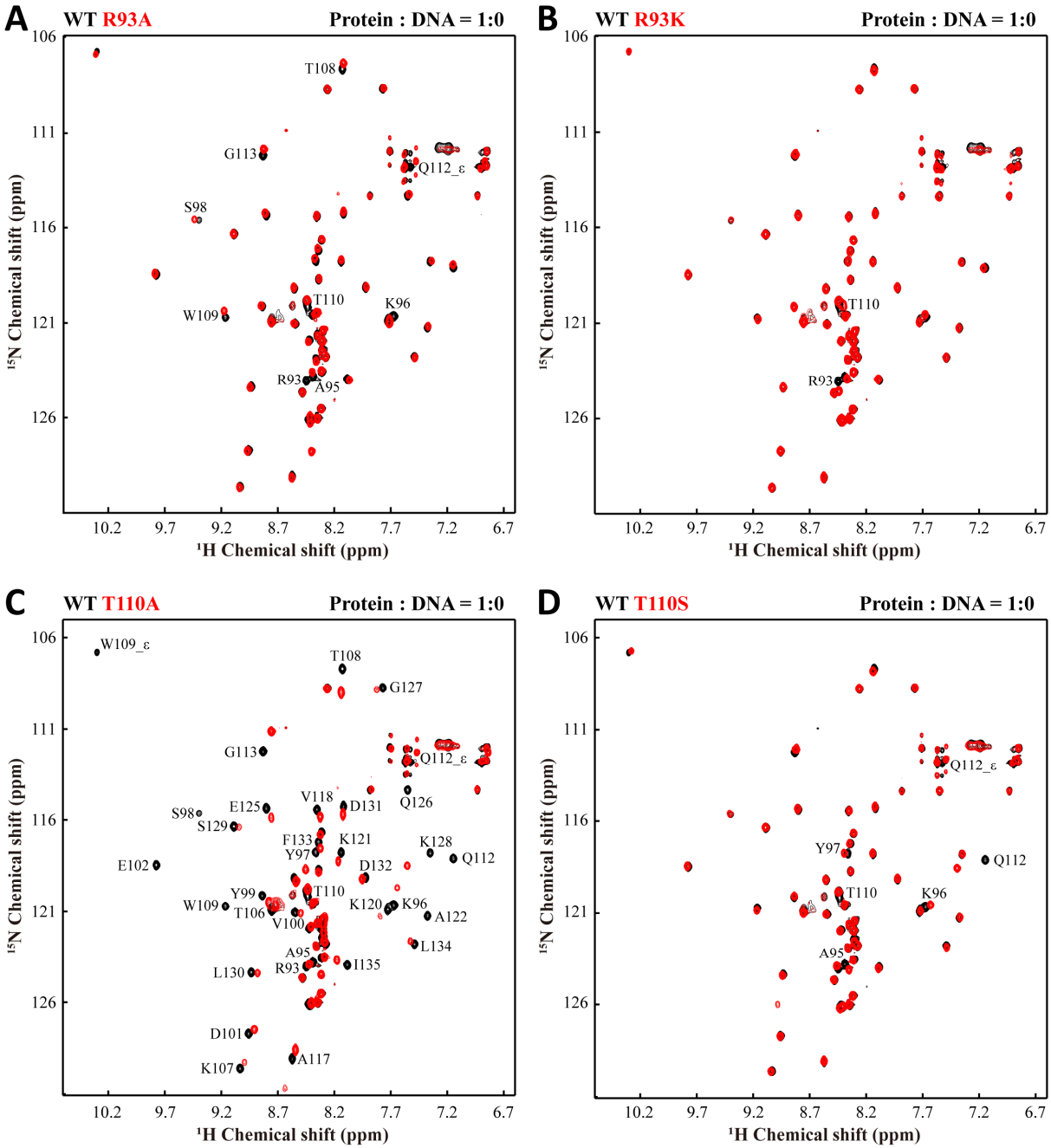

Figure S13: NMR titration of H-NS$^{85\text{-}137}$ mutants R93A (A) and R93K (B) with Top1 DNA. Left panel: overlay of 2D ^{1}H-^{15}N HSQC spectra of mutant at indicated protein/DNA ratios. Right panel: overlay of 2D ^{1}H-^{15}N HSQC spectra for WT and mutant at protein/DNA ratio 1:5. Assignments are indicated for significantly deviated NH signals between WT and mutant.

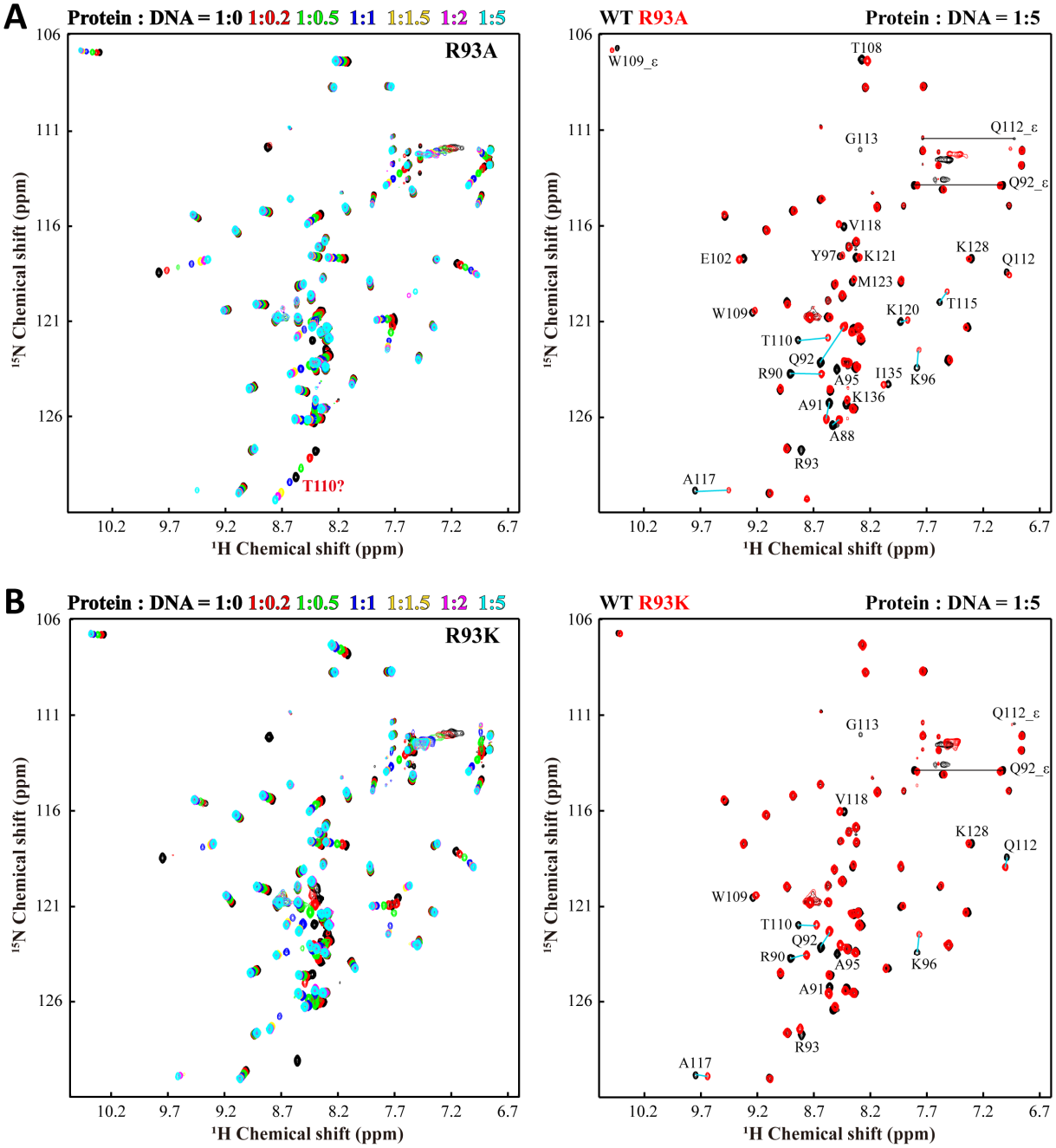

Figure S14: NMR titration of H-NS$^{85-137}$ mutants T110A (A) and T110S (B) with Top1 DNA. Left panel: overlay of 2D ^{1}H-^{15}N HSQC spectra of mutant at indicated protein/DNA ratios. Right panel: overlay of 2D ^{1}H-^{15}N HSQC spectra for WT and mutant at protein/DNA ratio 1:5. Assignments are indicated for significantly deviated NH signals between WT and mutant.

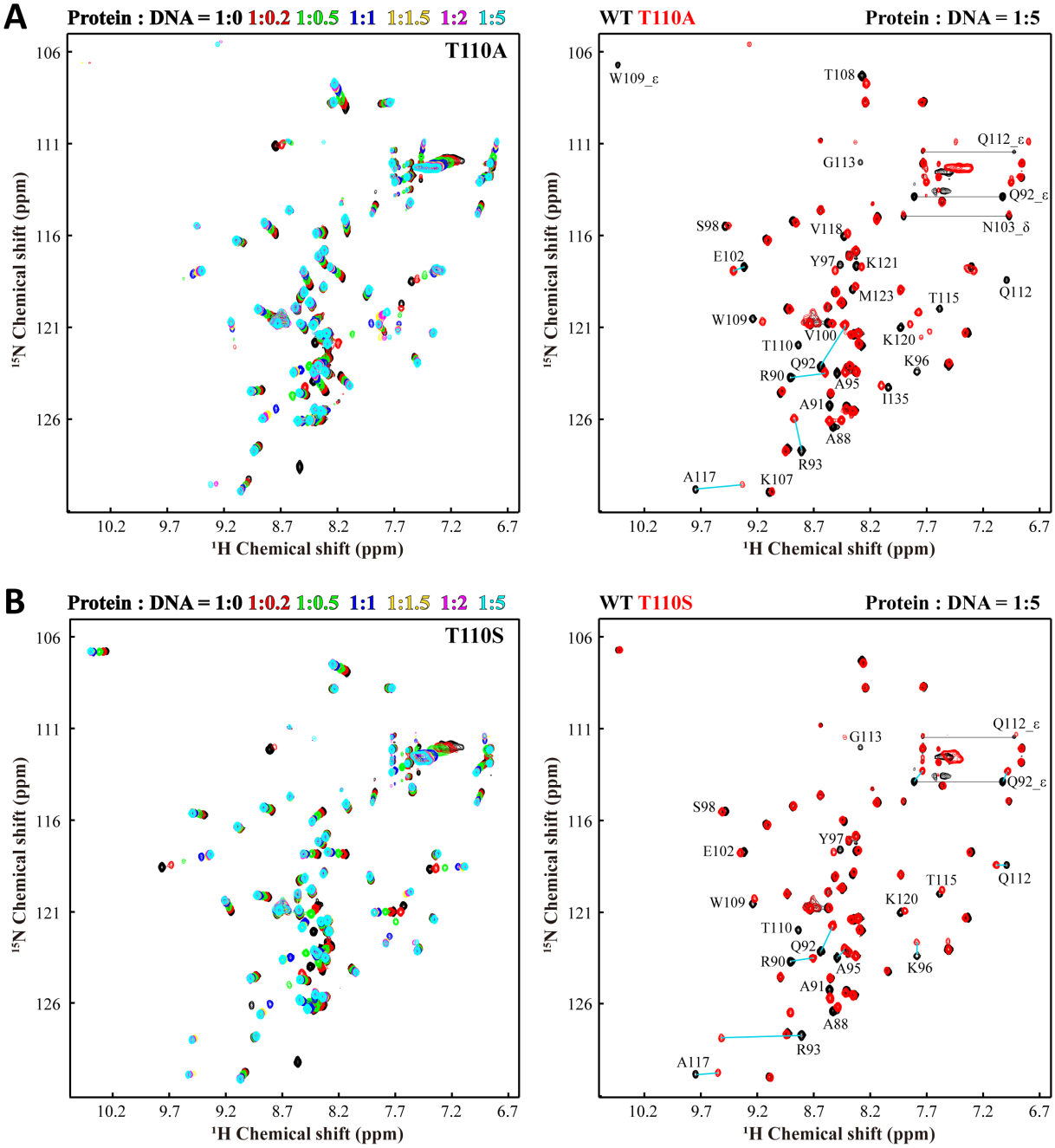

Figure S15: Overlay of 2D ^{1}H-^{15}N HSQC spectra between free WT H-NS$^{85-137}$ and mutants K87A (A), K89A (B), K120A (C), and K107A (D). Assignments are indicated for significantly deviated NH signals between WT and mutant.

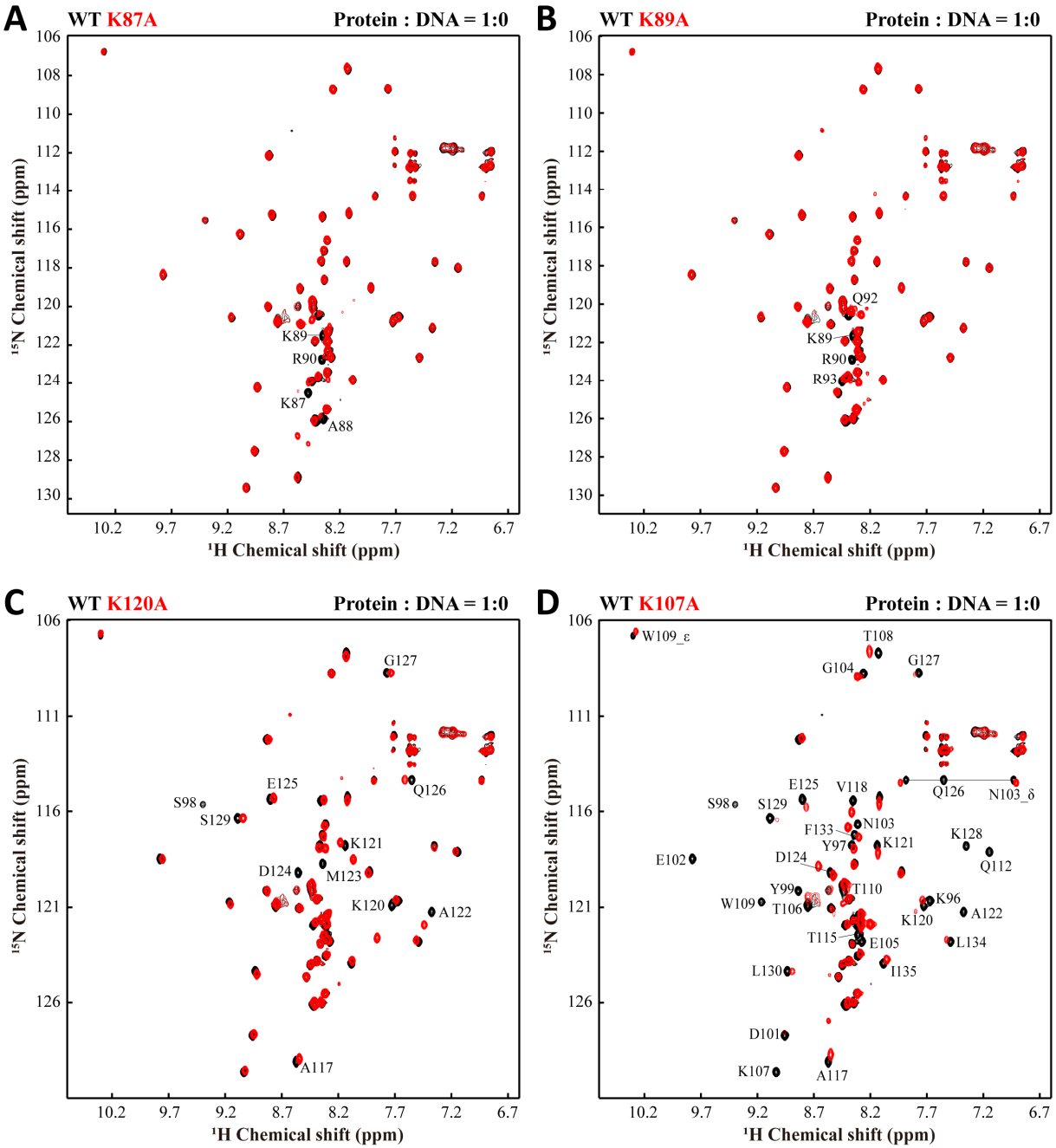

Figure S16: NMR titration of H-NS$^{85\text{-}137}$ mutants K87A (A) and K89A (B) with Top1 DNA. Left panel: overlay of 2D ^{1}H-^{15}N HSQC spectra of mutant at indicated protein/DNA ratios. Right panel: overlay of 2D ^{1}H-^{15}N HSQC spectra for WT and mutant at protein/DNA ratio 1:5. Assignments are indicated for significantly deviated NH signals between WT and mutant.

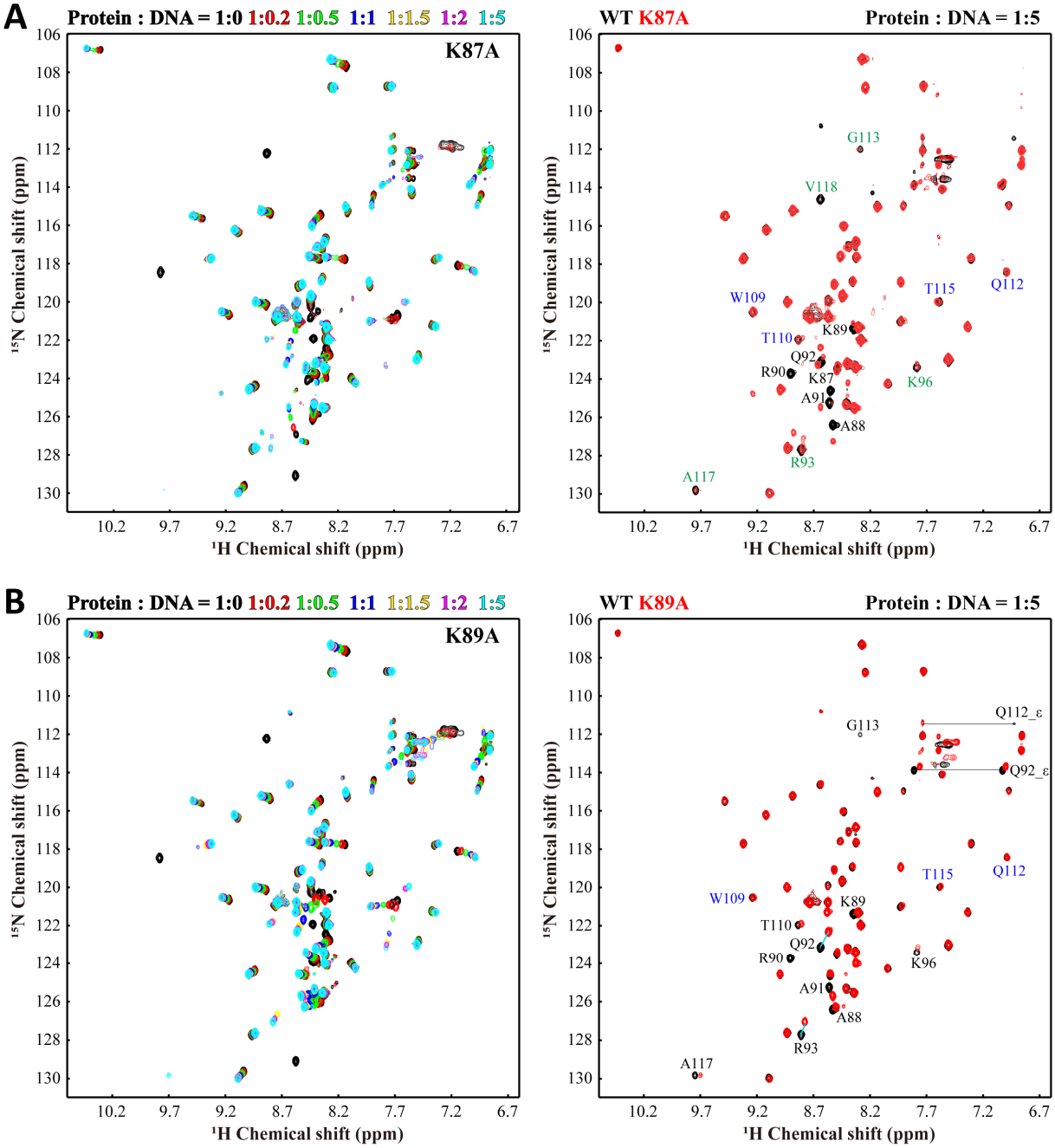

Figure S17: NMR titration of H-NS$^{85-137}$ mutants K120A (A) and K107A (B) with Top1 DNA. Left panel: overlay of 2D ^{1}H-^{15}N HSQC spectra of mutant at indicated protein/DNA ratios. Right panel: overlay of 2D ^{1}H-^{15}N HSQC spectra for WT and mutant at protein/DNA ratio 1:5. Assignments are indicated for significantly deviated NH signals between WT and mutant.

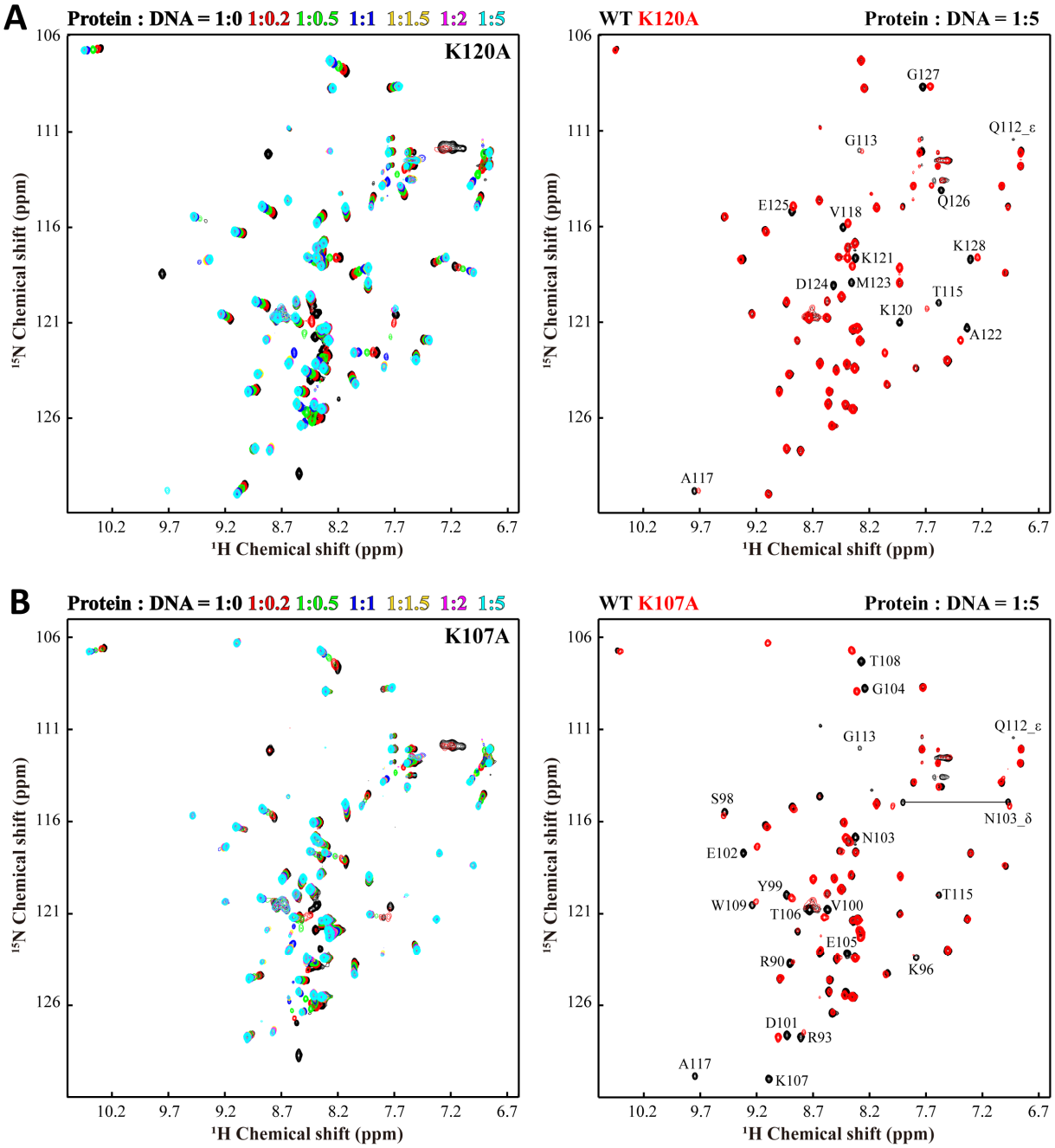

Figure S18: Overlay of 2D ^{1}H-^{15}N HSQC spectra between WT H-NS$^{85-137}$ and mutants K96A (A), K96M (B), K121A (C), and K121Q (D). Assignments are indicated for significantly deviated NH signals between WT and mutant.

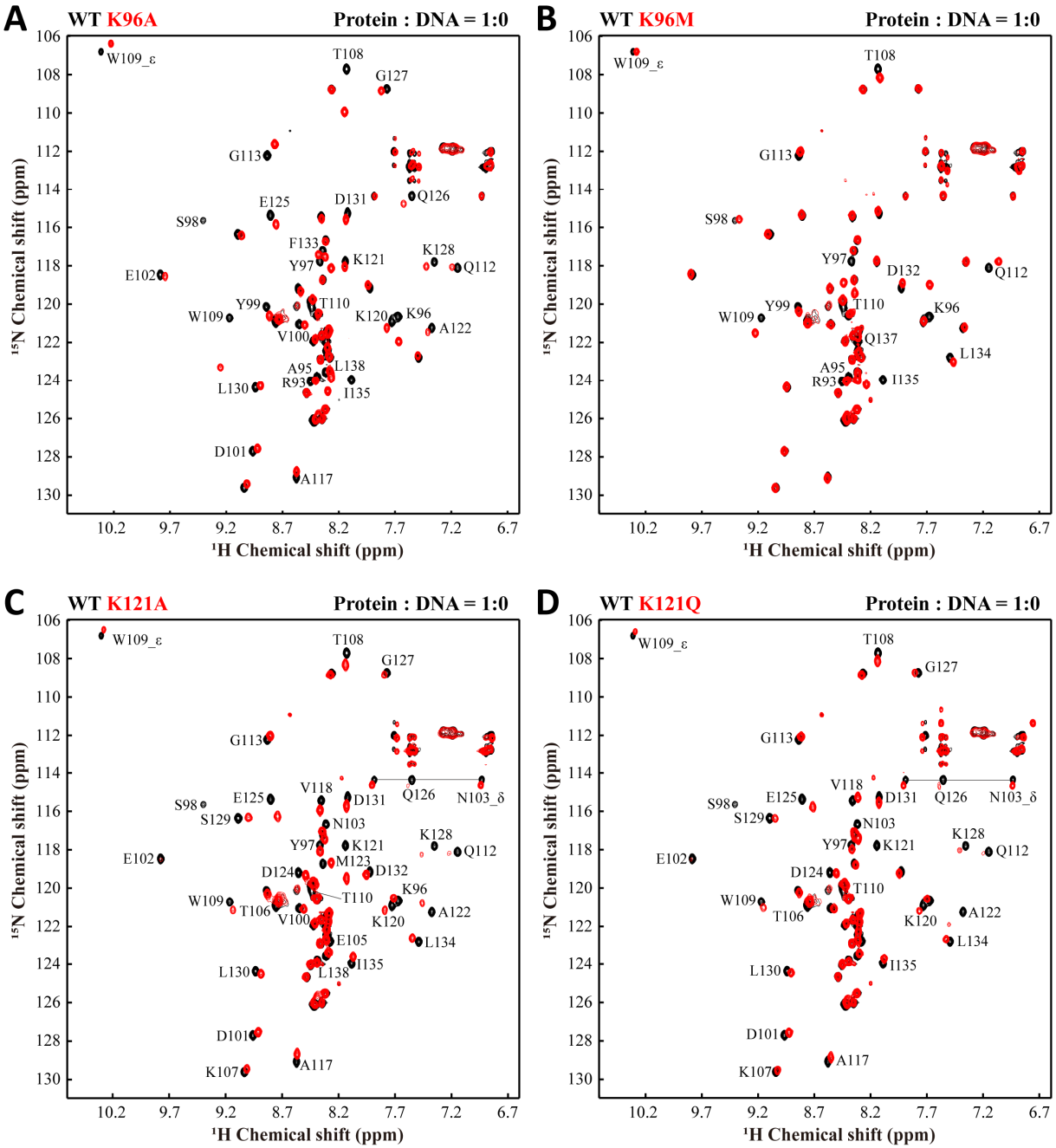

Figure S19: NMR titration of H-NS$^{85\text{-}137}$ mutants K96A (A) and K96M (B) with Top1 DNA. Left panel: overlay of 2D ^{1}H-^{15}N HSQC spectra of mutant at indicated protein/DNA ratios. Right panel: overlay of 2D ^{1}H-^{15}N HSQC spectra for WT and mutant at protein/DNA ratio 1:5. Assignments are indicated for significantly deviated NH signals between WT and mutant.

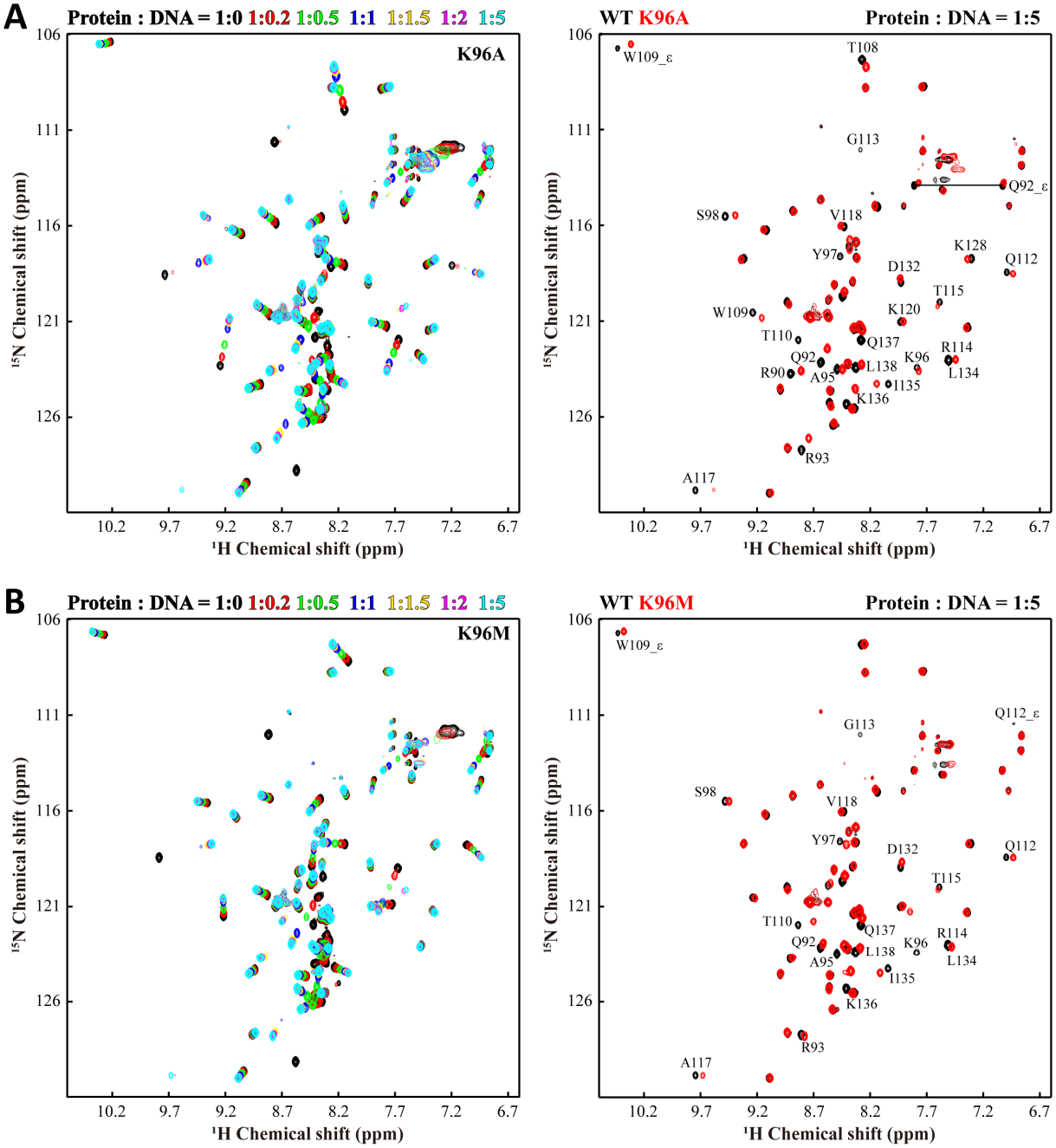

Figure S20: NMR titration of H-NS$^{85-137}$ mutants K121A (A) and K121Q (B) with Top1 DNA. Left side: overlay of 2D ^{1}H-^{15}N HSQC spectra of mutant at indicated protein/DNA ratios. Right side: overlay of 2D ^{1}H-^{15}N HSQC spectra for WT and mutant at protein/DNA ratio 1:5. Assignments are indicated for significantly deviated NH signals between WT and mutant.

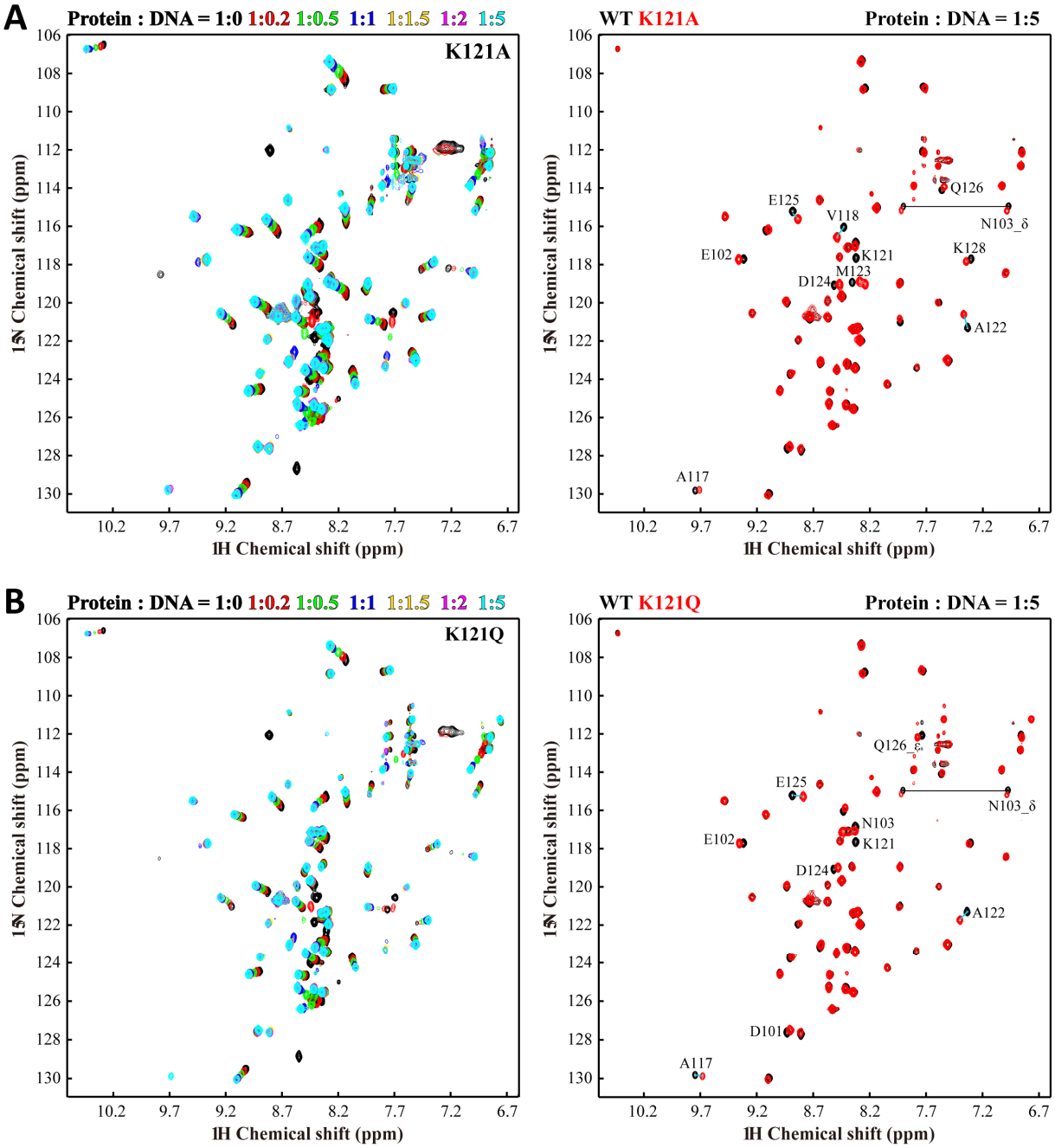

Figure S21: The RMSD plot for H-NS-bound (A) and Free (B) Top1 DNA during the 100 ns MD simulation. Structures were selected for every 0.1 ns.

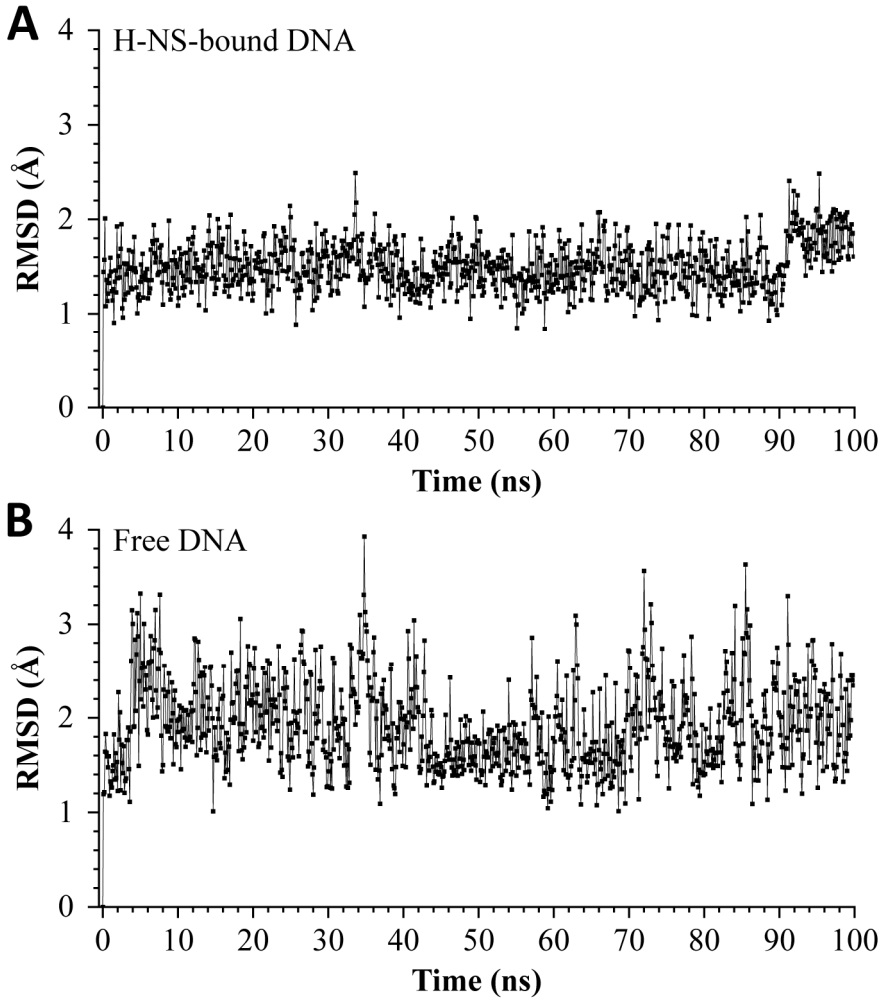

Figure S22: NMR titration of H-NS$^{85-137}$ with 3-AT (A) and 3-TA (B) DNA. Left panel: overlay of 2D ^{1}H-^{15}N HSQC spectra of H-NS$^{85-137}$ with indicated protein/DNA ratios. Right panel: overlay of 2D ^{1}H-^{15}N HSQC spectra of H-NS$^{85-137}$ with Top1 DNA and the specified DNA, at protein/DNA ratio 1:5. Assignments are indicated for significantly deviated NH signals between the two DNAs.

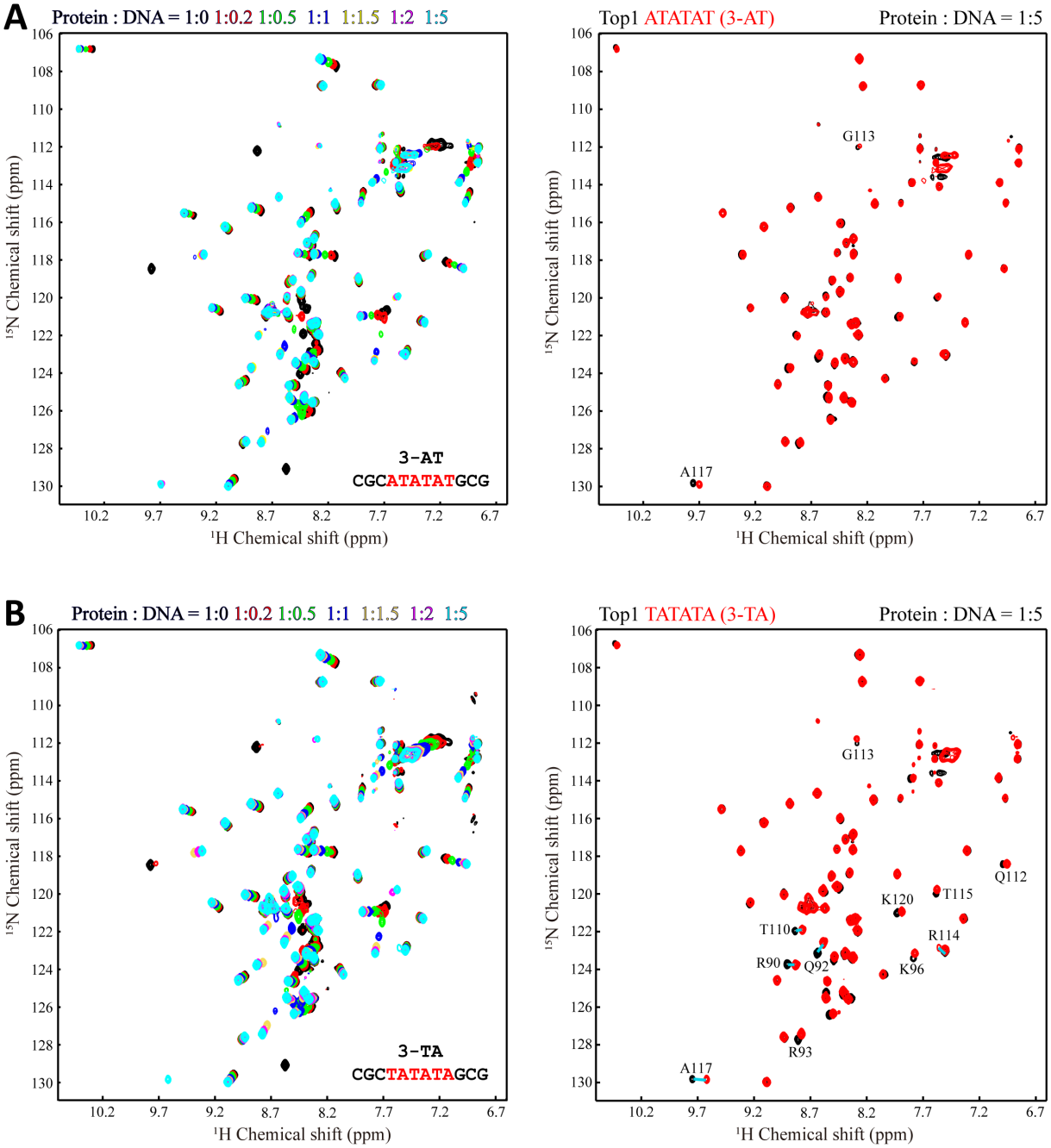

Figure S23: NMR titration of H-NS$^{85-137}$ with 2-TA (A) and 3ATAT (B) DNA. Left panel: overlay of 2D ^{1}H-^{15}N HSQC spectra of H-NS$^{85-137}$ with indicated protein/DNA ratios. Right panel: overlay of 2D ^{1}H-^{15}N HSQC spectra of H-NS$^{85-137}$ with Top1 DNA and the specified DNA, at protein/DNA ratio 1:5. Assignments are indicated for significantly deviated NH signals between the two DNAs.

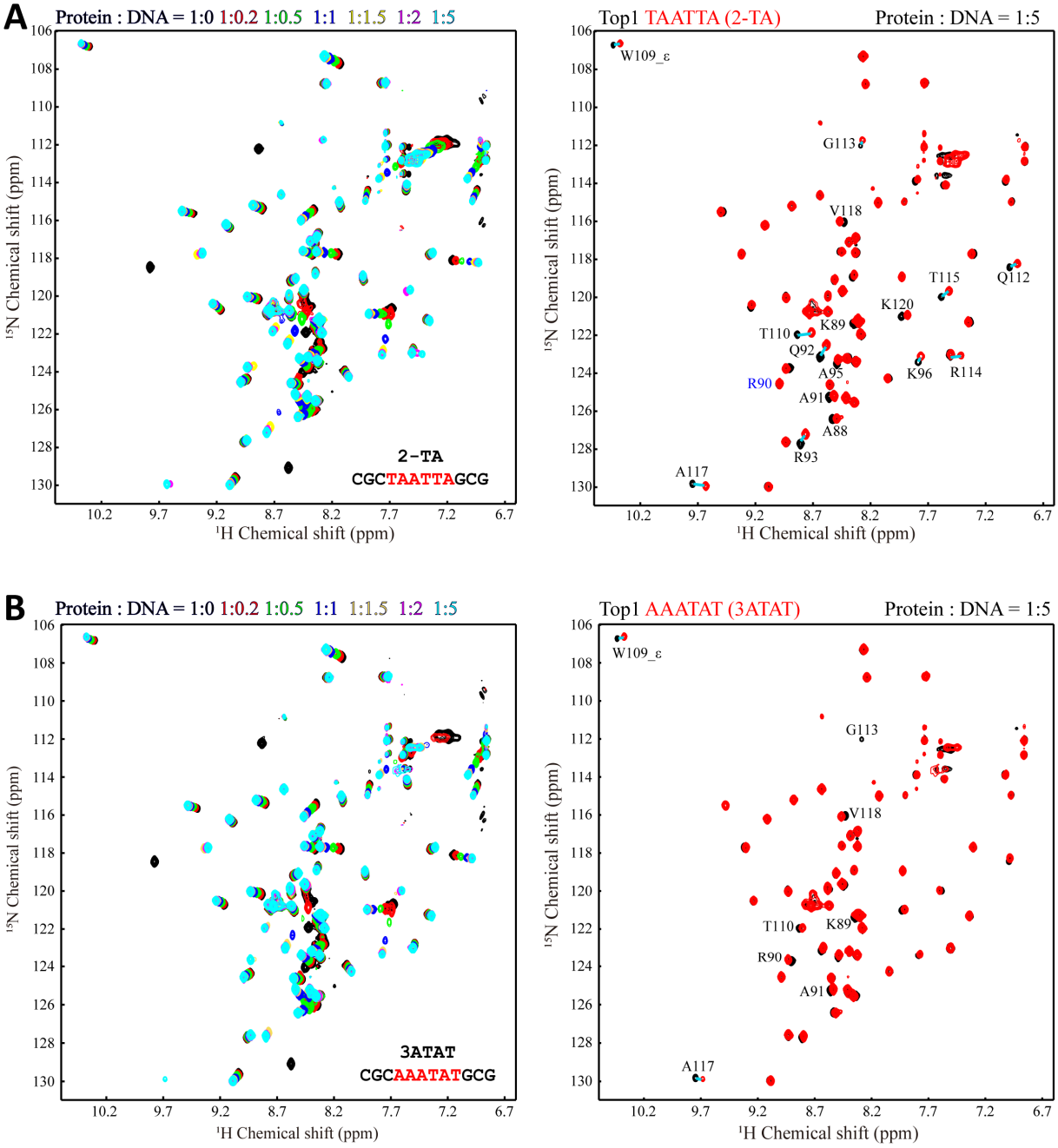

Figure S24: NMR titration of H-NS$^{85-137}$ with 3A2TA DNA (A) and 6A DNA (B). Left panel: overlay of 2D ^{1}H-^{15}N HSQC spectra of H-NS$^{85-137}$ with indicated protein/DNA ratios. Right panel: overlay of 2D ^{1}H-^{15}N HSQC spectra of H-NS$^{85-137}$ with Top1 DNA and the specified DNA, at protein/DNA ratio 1:5. Assignments are indicated for significantly deviated NH signals between the two DNAs.

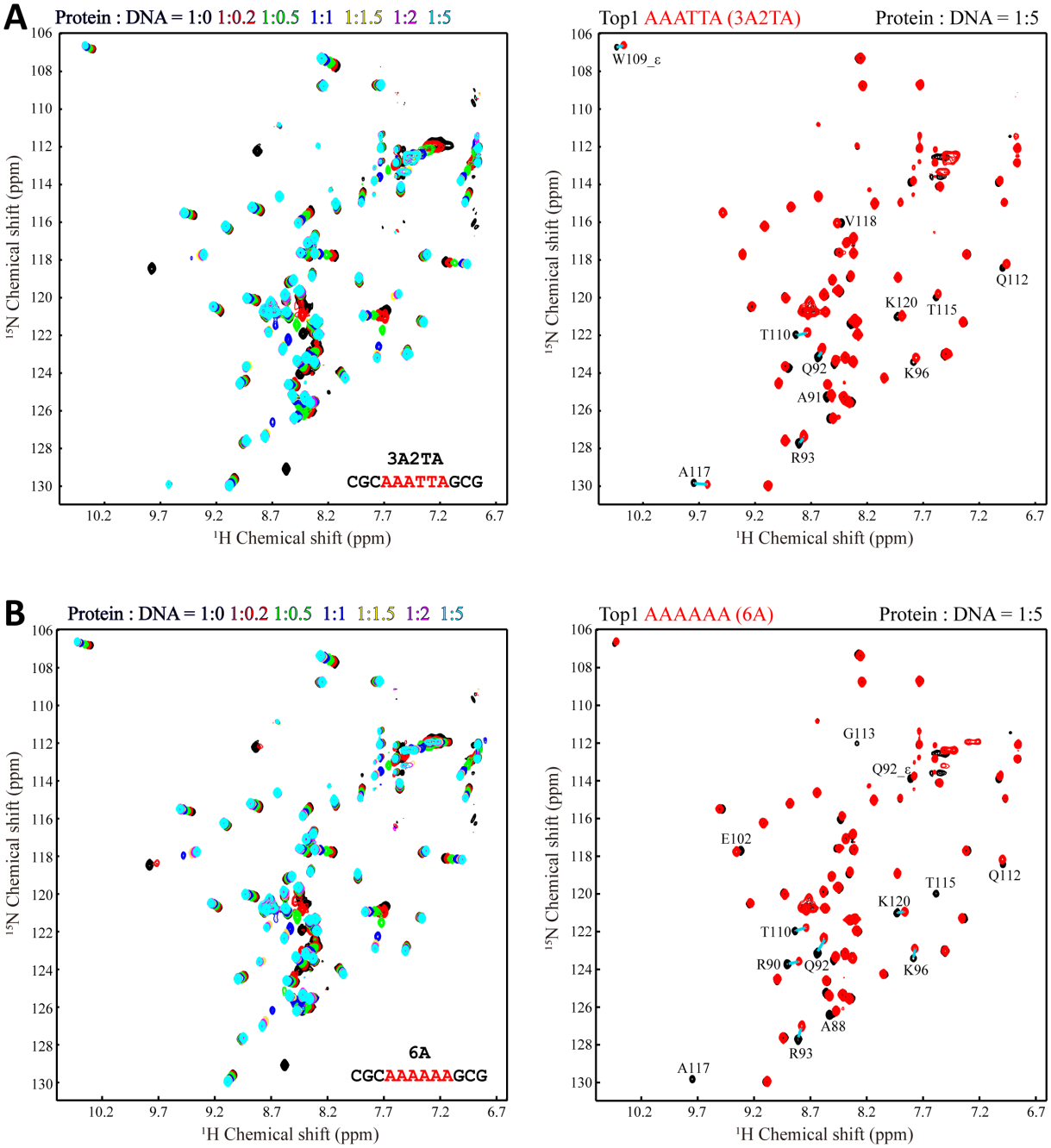

Figure S25: NMR titration of H-NS$^{85-137}$ with 3A3T DNA (A) and 4A2T DNA (B). Left panel: overlay of 2D ^{1}H-^{15}N HSQC spectra of H-NS$^{85-137}$ with indicated protein/DNA ratios. Right panel: overlay of 2D ^{1}H-^{15}N HSQC spectra of H-NS$^{85-137}$ with Top1 DNA and the specified DNA, at protein/DNA ratio 1:5. Assignments are indicated for significantly deviated NH signals between the two DNAs.

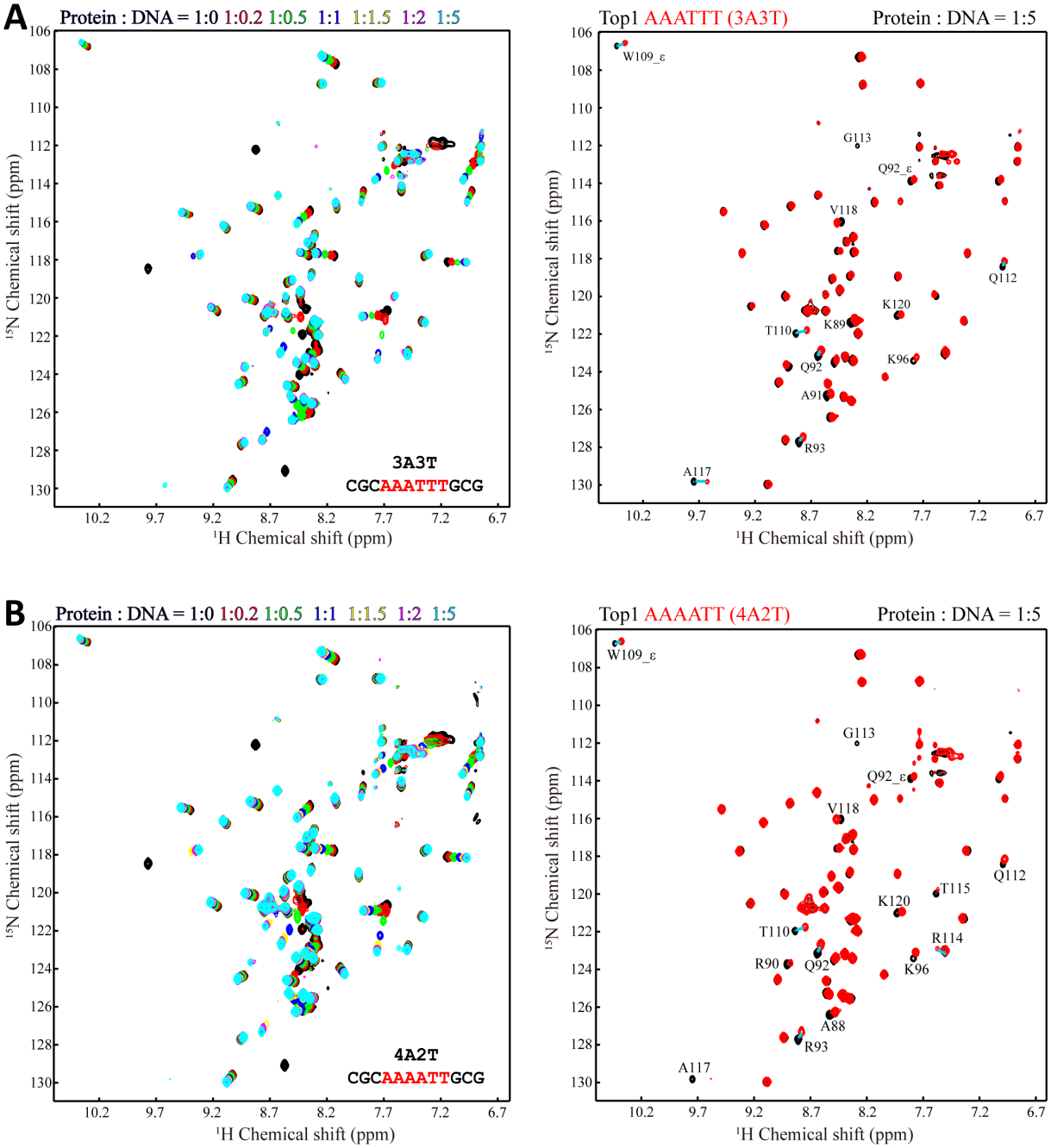

Figure S26: (A) Sequence logo of the DNA binding domains of H-NS family proteins with core tripeptide sequences of HAR (241), WAR (78), and RAR (36). (B) Sequence logo of H-NS family proteins with two consecutive DNA binding domains (1165). AT-hook-like motif is indicated by blue shaded box above, where "KY" and "FLI" signatures are indicated by red and green shaded boxes above, respectively. The number in the parentheses following each core tripeptide indicates the number of non-redundant sequences used in generating the logos.

