Building a stable RNA U-turn with a protonated cytidine

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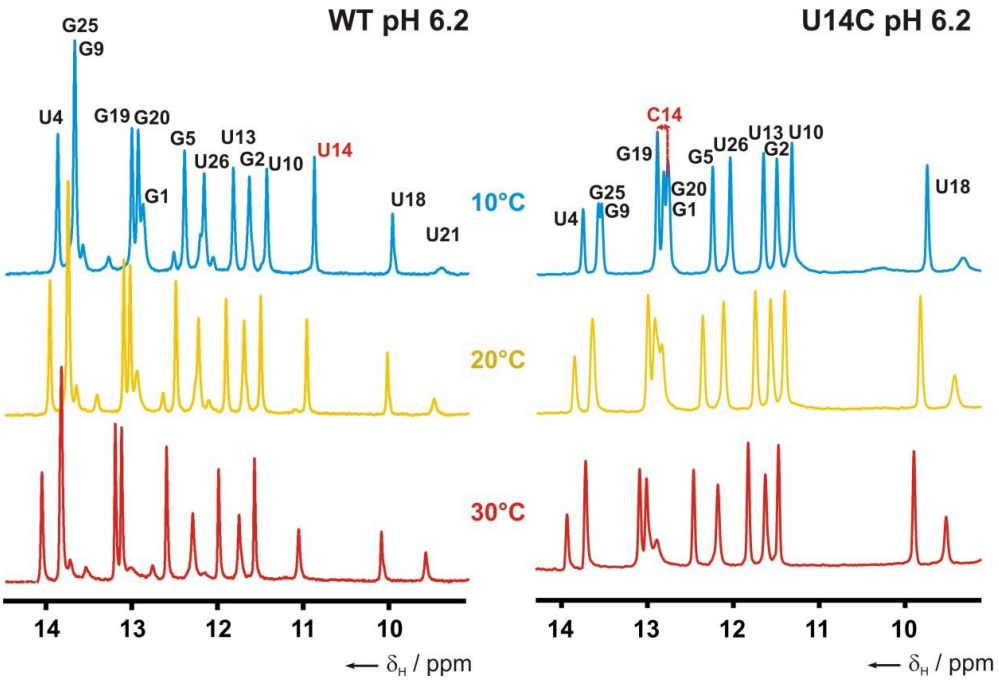
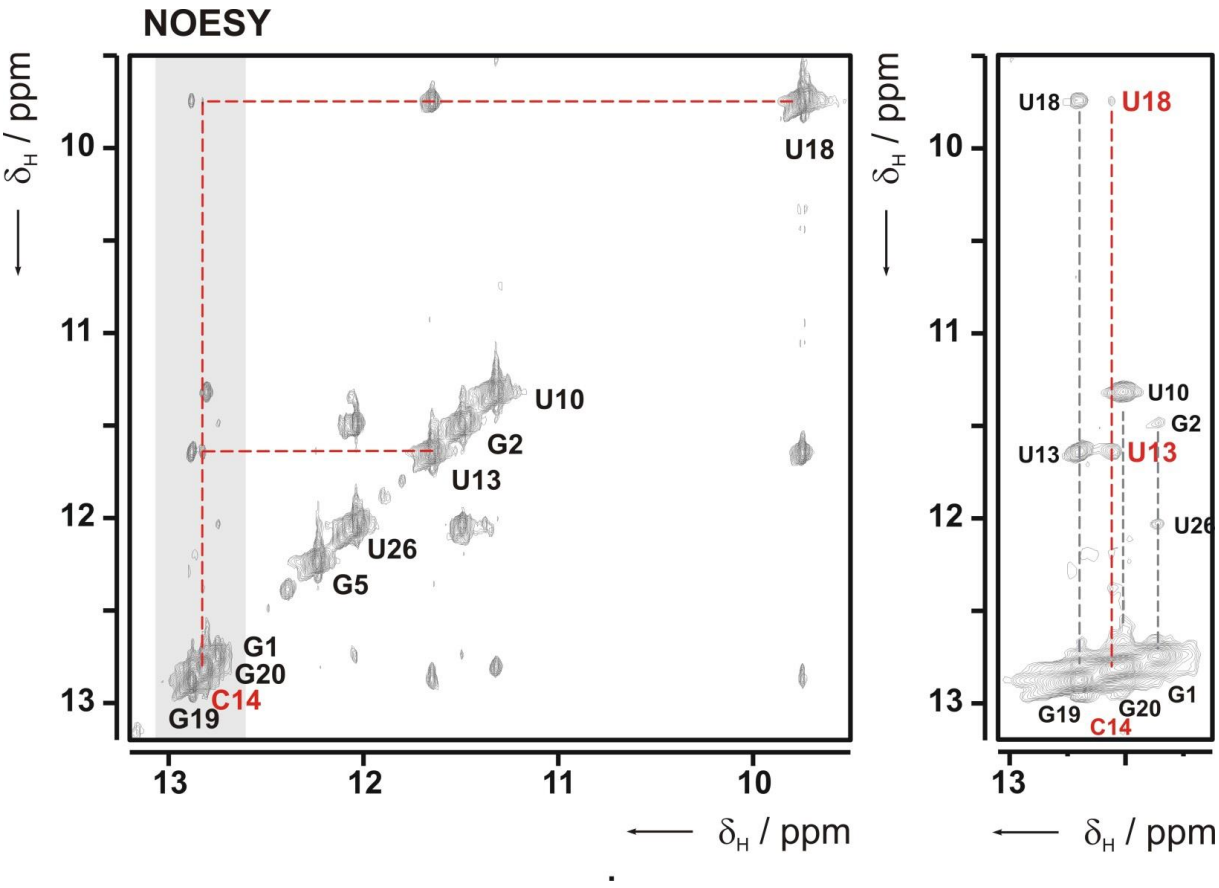
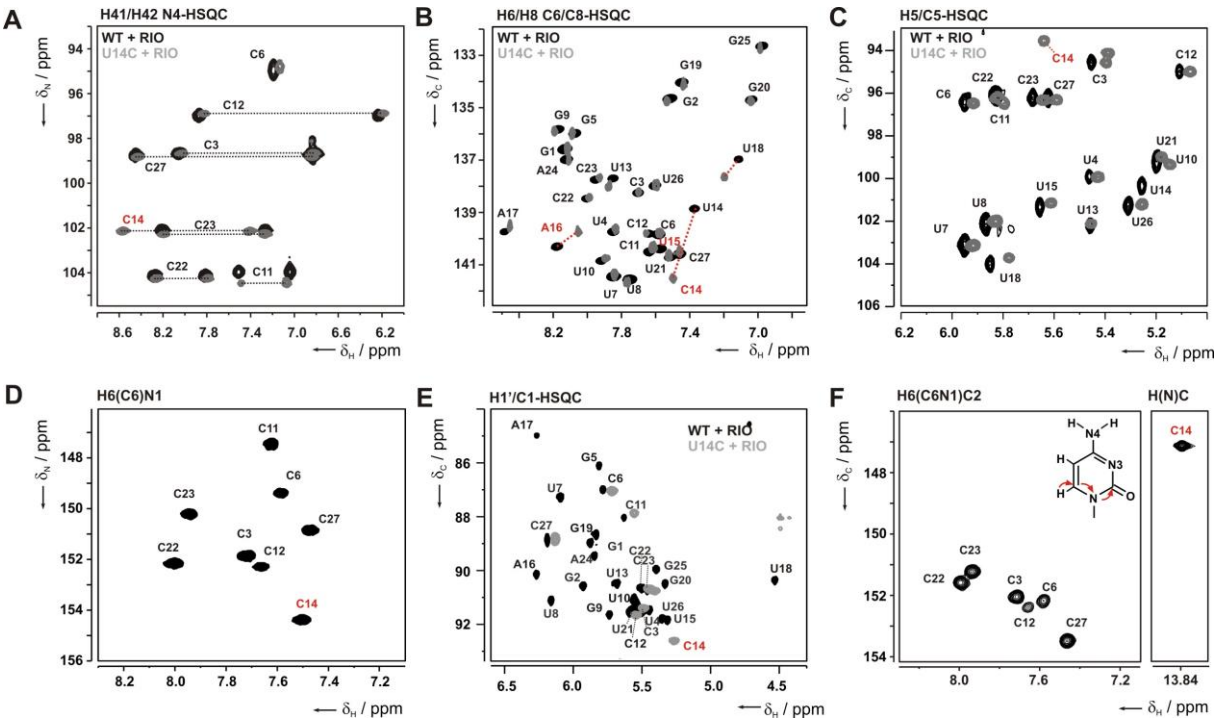
Supplementary figures and figure captions



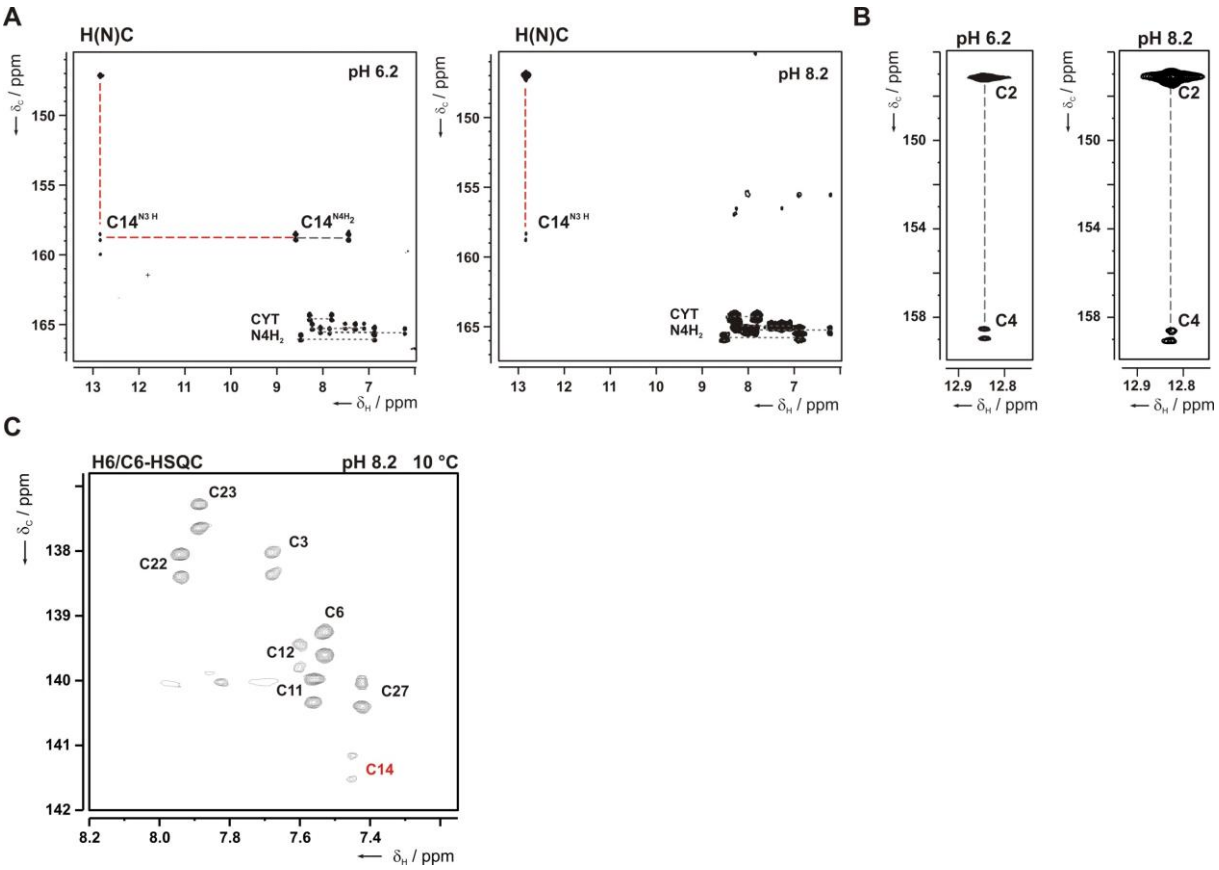
Supplementary Figure 1. Comparison of the temperature dependence of imino proton 1D-^{1}H-NMR-spectra of the WT and the U14C-mutant riboswitch in complex with ribostamycin at pH 6.2. Spectra were recorded with an unlabelled WT-sample and a ^{15}N-cytidine labeled U14C-mutant RNA. Signal assignments are indicated. The imino proton signals for U14 in the WT and C14 in the mutant are highlighted in red. The imino proton of the protonated C14 in the mutant is overlapping with the imino proton signals of G1, G19 and G20. We therefore intentionally did not decouple ^{15}N so that in these spectra a doublet is observed for the C14 imino resonance. One component of the C14 imino proton doublet is then observable with half of its nominal intensity on the right side next to the G1, G19 and G20 overlapping signals (red dashed line).



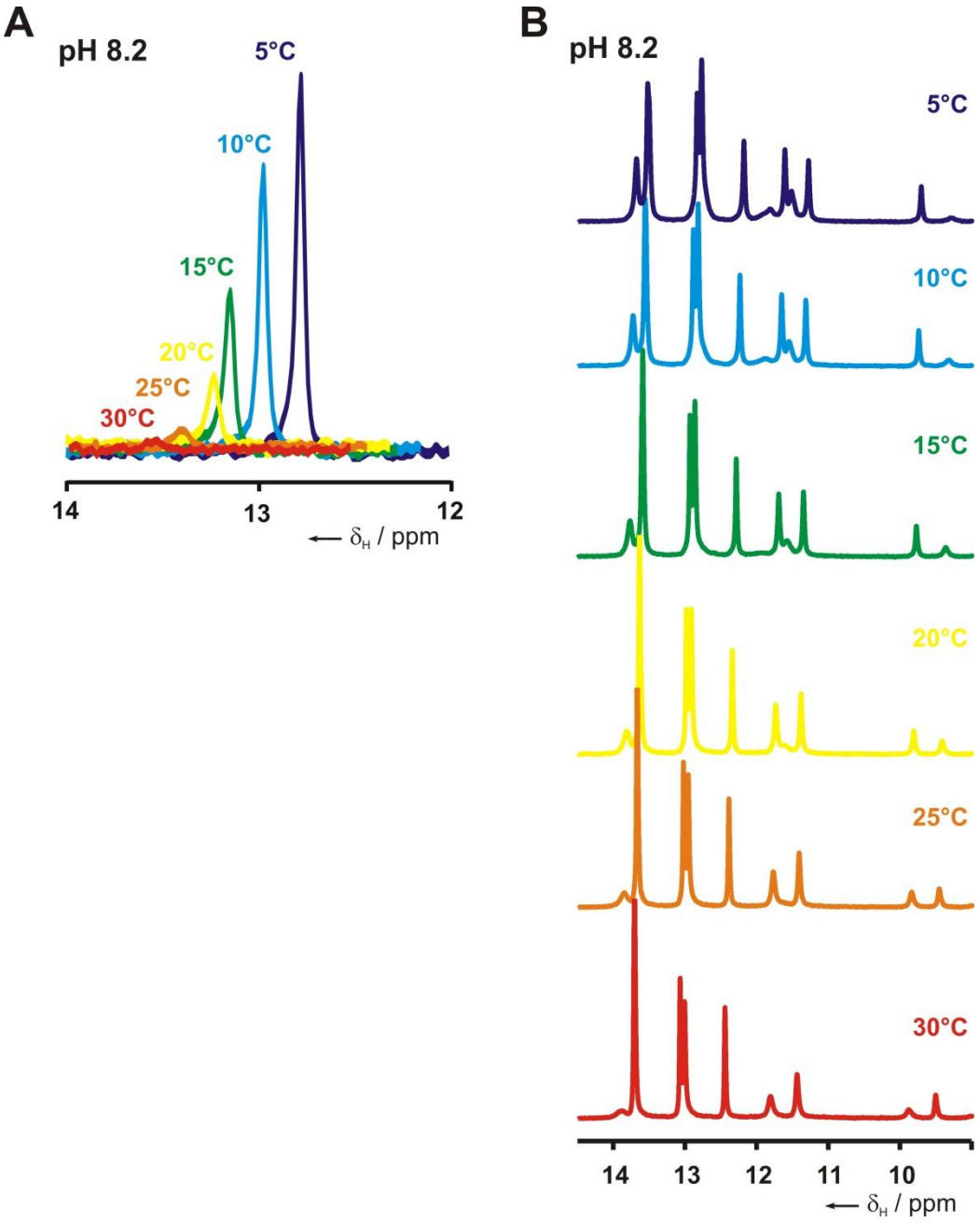
Supplementary Figure 2. Region of the imino proton cross peaks of the 2D-^{1}H,^{1}H-NOESY-spectrum recorded for the unlabeled U14C-mutant RNA in complex with ribostamycin at 10°C and pH 6.2 and plotted at a lower contour level than in Figure 2 to also show low-intensity NOE cross peaks. The area underlined in grey which contains the NOEs to the overlapping imino proton resonances of G1, G19, G20 and C14 is shown as a close-up to the right. All relevant cross peak assignments are given. Assignments of cross peaks involving the C14 imino proton are highlighted in red.



Supplementary Figure 3. Chemical shift finger print of C14 protonation. (A) $^{1}H,^{15}N$-HSQC-spectrum for the amino groups of the WT (black) and the U14C-mutant (grey) RNA in complex with ribostamycin at 10°C. Assignments are given. The N4 nitrogen and the protons of the protonated C14 show canonical chemical shifts. (B) Aromatic region of a $^{1}H,^{13}C$-HSQC-spectrum of the WT (black) and the U14C-mutant (grey) in complex with ribostamycin. Assignments are given. Large chemical shift differences are indicated by dashed red lines. (C) H5/C5 region of a $^{1}H,^{13}C$-HSQC-spectrum of the WT (black) and the U14C-mutant (grey). Assignments are given. (D) 2D-H6(C6)N1-spectrum of the U14C-mutant recorded using the selectively $^{13}C,^{15}N$-cytidine-labeled sample. Assignments are given. The N1 resonance of the protonated cytidine is shifted downfield compared to the other cytidine resonances in the U14C-mutant RNA. (E) Aliphatic region of a $^{1}H,^{13}C$-HSQC of the WT (black) and the U14C-mutant (grey) using the selectively $^{13}C,^{15}N$-cytidine-labeled sample. Assignments are given. (F) H6(C6N1)C2-experiment recorded for the selectively $^{13}C/^{15}N$-cytidine labeled U14C mutant (black) at 25°C (left). Assignments are given. C2 resonances of C11 and of the protonated C14 are missing. The 2D-H(N)C-experiment recorded at 10°C for the U14C mutant yields the C2 chemical shift of C14 (right).



Supplementary Figure 4. Comparison of the 2D-H(N)C-spectra of the U14C mutant in complex with ribostamycin at pH 6.2 and 8.2. (A) 2D-H(N)C-spectrum of the U14C mutant at pH 6.2 (left) and pH 8.2 (right) at 10°C. Correlations involving the C2 and C4 signals of C14 are indicated by dashed red lines. Signals belonging to the same amino group are indicated by dashed black lines. Amino proton signals of C14 are absent at pH 8.2. (B) Close-up of the C14 imino proton correlations with C2 and C4 from the $^{1}H,^{13}C$-H(N)C-spectrum of the U14C mutant at pH 6.2 (left) and pH 8.2 (right) at 10°C. C2 and C4 chemical shifts for C14 are virtually identical at the two pH values. (C) H6/C6 region of an $^{1}H,^{13}C$-HSQC-spectrum recorded with the $^{13}C/^{15}N$-cytidine-labeled sample at pH 8.2 and 10°C. Only one set of H6/C6 correlations can be observed with chemical shifts very similar to those at pH 6.2 (Supplementary Figure 3B).



Supplementary Figure 5. Temperature dependence of imino proton spectra of the U14C RNA in complex with ribostamycin at pH 8.2. (A) Temperature dependence of the signal intensity for C14 imino proton signal at pH 8.2 from 1D-^{1}H,^{15}N-HSQC-experiments recorded with a ^{13}C,^{15}N-cytidine labeled RNA. (B) Imino proton 1D-^{1}H-NMR-spectra of the unlabeled U14C-RNA in complex with ribostamycin at pH 8.2 and recorded at the indicated temperatures.