

Supplementary Materials

Physicochemical characterization of the catalytic unit of hammerhead ribozyme and its relationship with the catalytic activity

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Contents.

Figure S1. The four-base-paired RNA duplex derived from the 1YFV RNA duplex (A).

Figure S2. The ¹H-¹H NOESY spectrum of the RNA duplex 1 at pH 8.18 in the absence of CdCl₂.

Figure S3. The natural abundance ¹H-¹³C HSQC spectrum of the RNA duplex 1 at pH 8.18.

Figure S4. The 1D ¹H NMR spectra of duplex 2 under pH 10.9, 10.4 and 10.9.

Figure S5. The natural abundance ¹H-¹³C HSQC spectrum of the RNA duplex 1 at pH 4.40.

Table S1. The chemical shielding calculated for H8 atom in G in ppm.

Table S2. The chemical shift perturbation of H8 atom in G due to N1-deprotonation.

Supplemental Figure

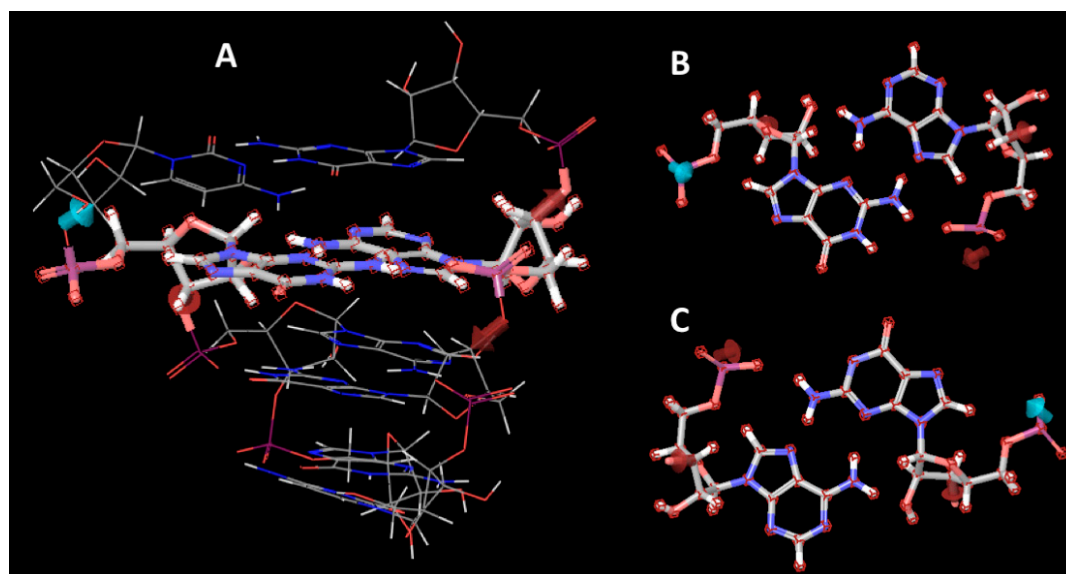


Figure S1. The four-base-paired RNA duplex derived from the 1YFV RNA duplex (A). The RNA was geometry optimized with the QM/MM (B3LYP/6-31G++*:OPLS2005) method as implemented in the Jaguar 8.2, Impact 6.1 and Qsite 6.1 computational programs [S1-S3]. In this calculation, the QM part included the sheared-type G-A pair and the MM included rest of the tetramer. (B) The QM part: The structure of the sheared-type G-A pair (the neutral form). (C) The QM part: The structure of the sheared-type G-A pair (the N1 deprotonated form). The bonds bridging QM and MM parts were indicated with the arrows.

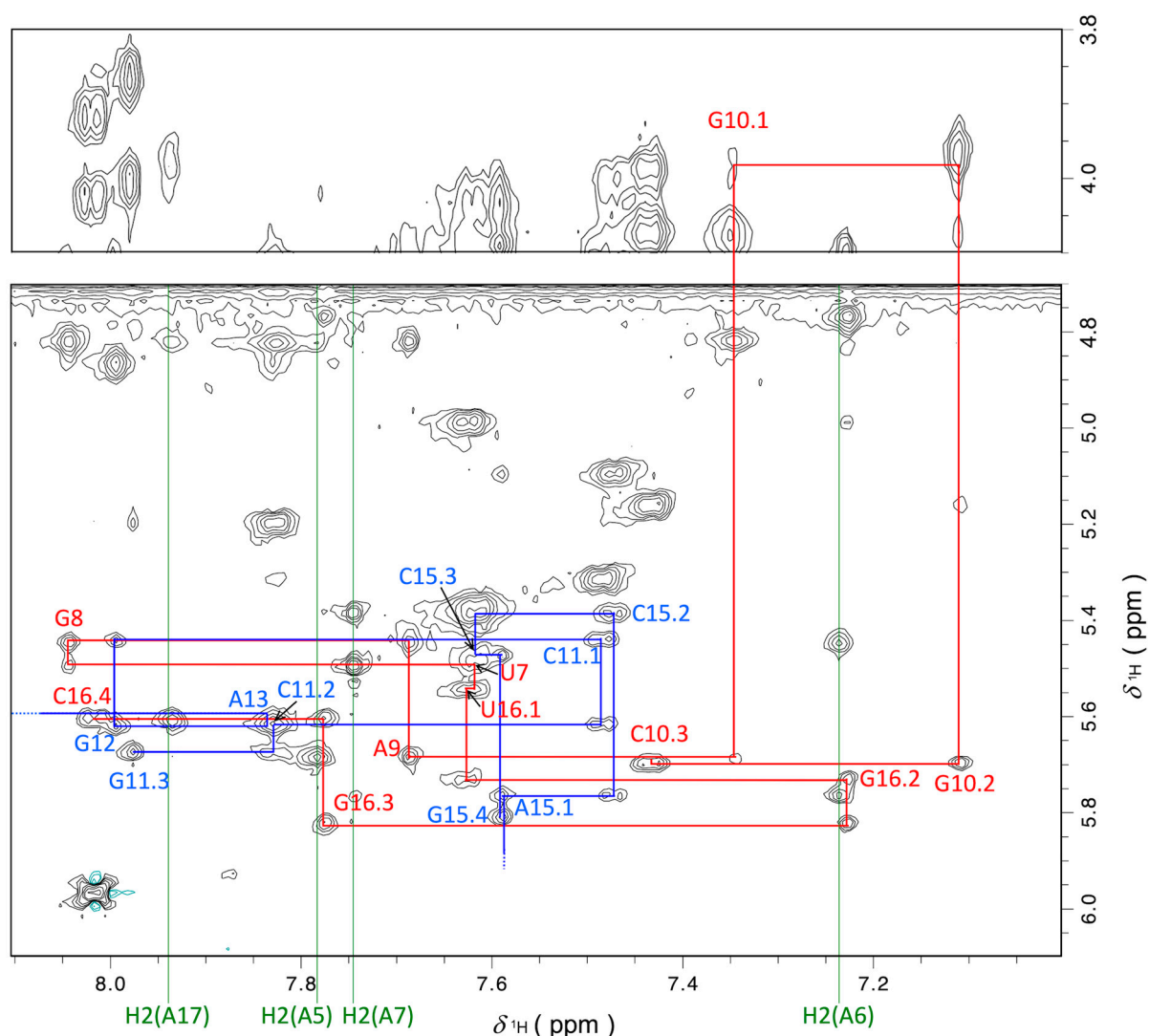


Figure S2. The ^1H - ^1H NOESY spectrum of the RNA duplex **1** at pH 8.18 in the absence of CdCl_2 . Sequential NOE walks of the two strands are drawn as red and blue lines, respectively. Intra-residue cross peaks are labeled with their residue numbers. The chemical shifts of H2 resonances of adenosine residues are shown in green lines, with their labels. The signals of the A14* residue were not observed under the 500 MHz frequency due to the exchange as reported [33]. The chemical shift of H1' of G10.1* was shifted upfield as observed previously [33, 37-39, 55]. The ^1H - ^1H NOESY spectrum was recorded at pH 8.18 during the pH-titration experiment with the following conditions. Spectral width: 6009.615 * 4997.324 Hz, Points: 4096 * 512 complex points, Number of scans: 32 times, Temperature: 303 K, Hardware: Bruker Avance III HD 500 MHz spectrometer equipped with a BBO cryogenic probe.

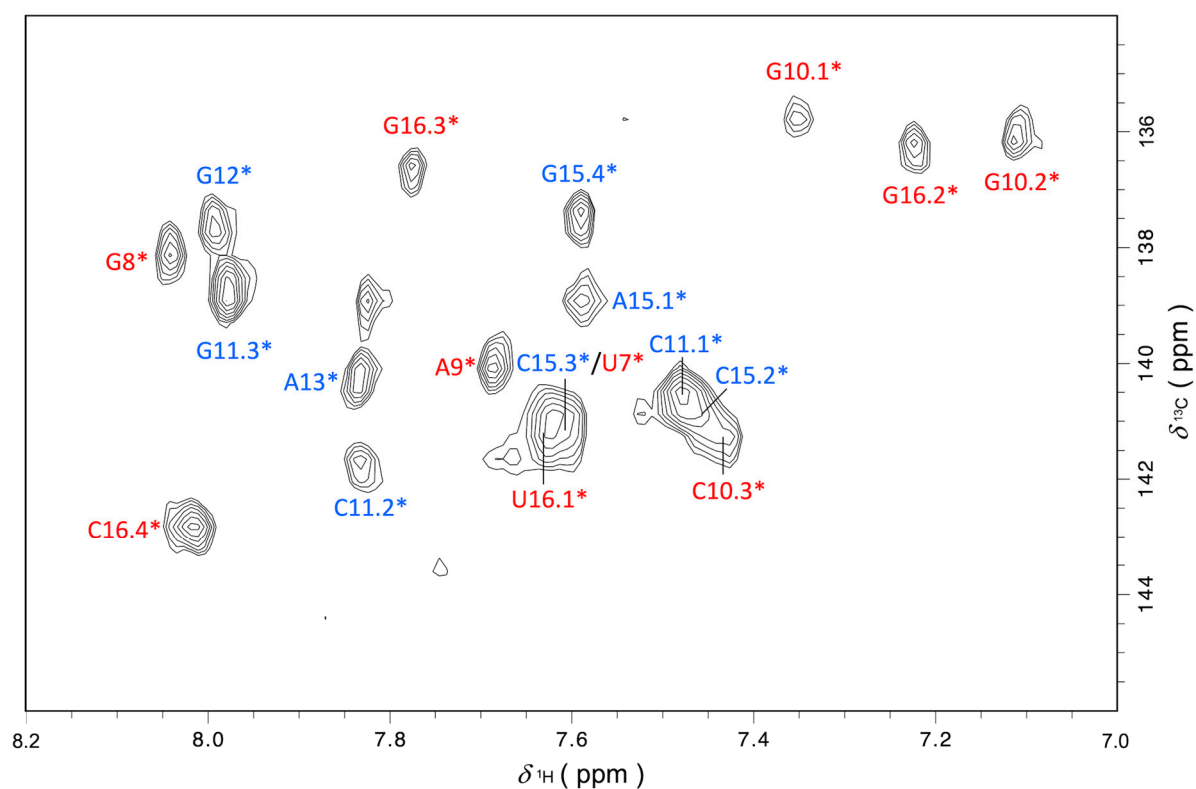


Figure S3. The natural abundance ^1H - ^{13}C HSQC spectrum of the RNA duplex 1 at pH 8.18. The respective intra-residue cross peaks are labeled with their residue numbers. The ^1H - ^{13}C HSQC spectrum in the absence of CdCl_2 was recorded at pH 8.18 during the pH-titration experiment with the following conditions. Spectral width: 7978.724 * 3141.776 Hz, Points: 2048 * 64 complex points, Number of scans: 128 times, Temperature: 303 K, Hardware: Bruker Avance III HD 500 MHz spectrometer equipped with a BBO cryogenic probe.

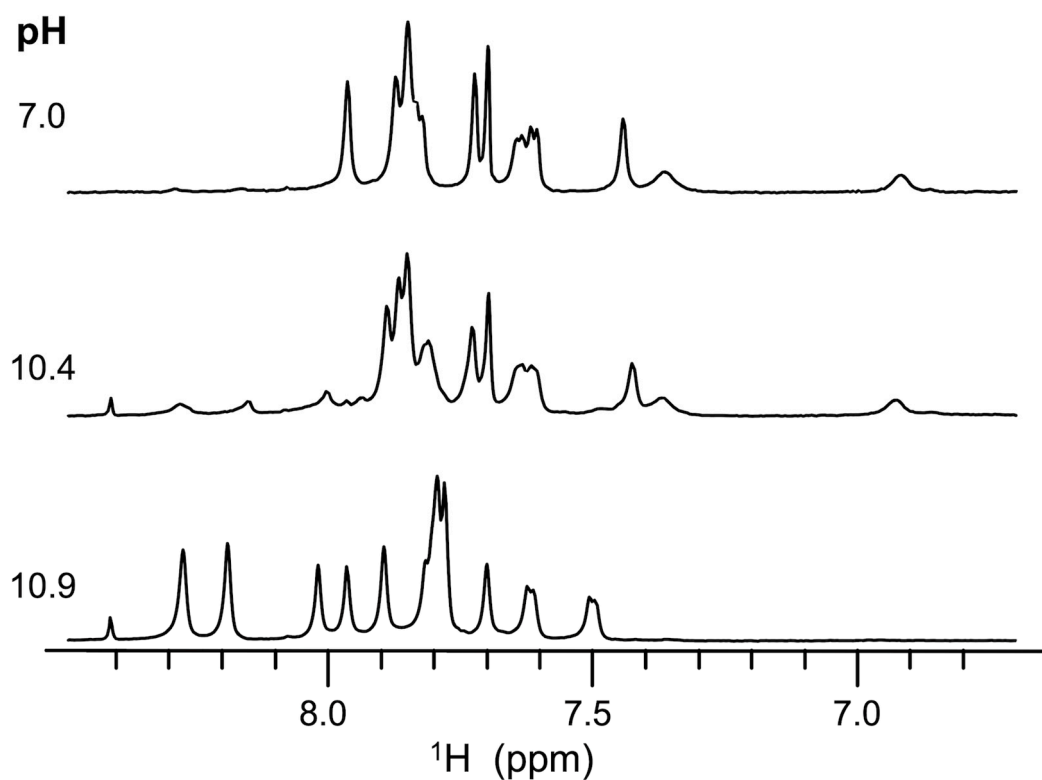


Figure S4. The 1D ^1H NMR spectra of duplex **2** under pH 10.9, 10.4 and 10.9. At pH 10.9, a totally different spectrum from those at other pH conditions was observed, which indicates the denaturation of the RNA duplex **2**. The conditions of spectrum measurements are the same as shown in the **Materials and Methods** section.

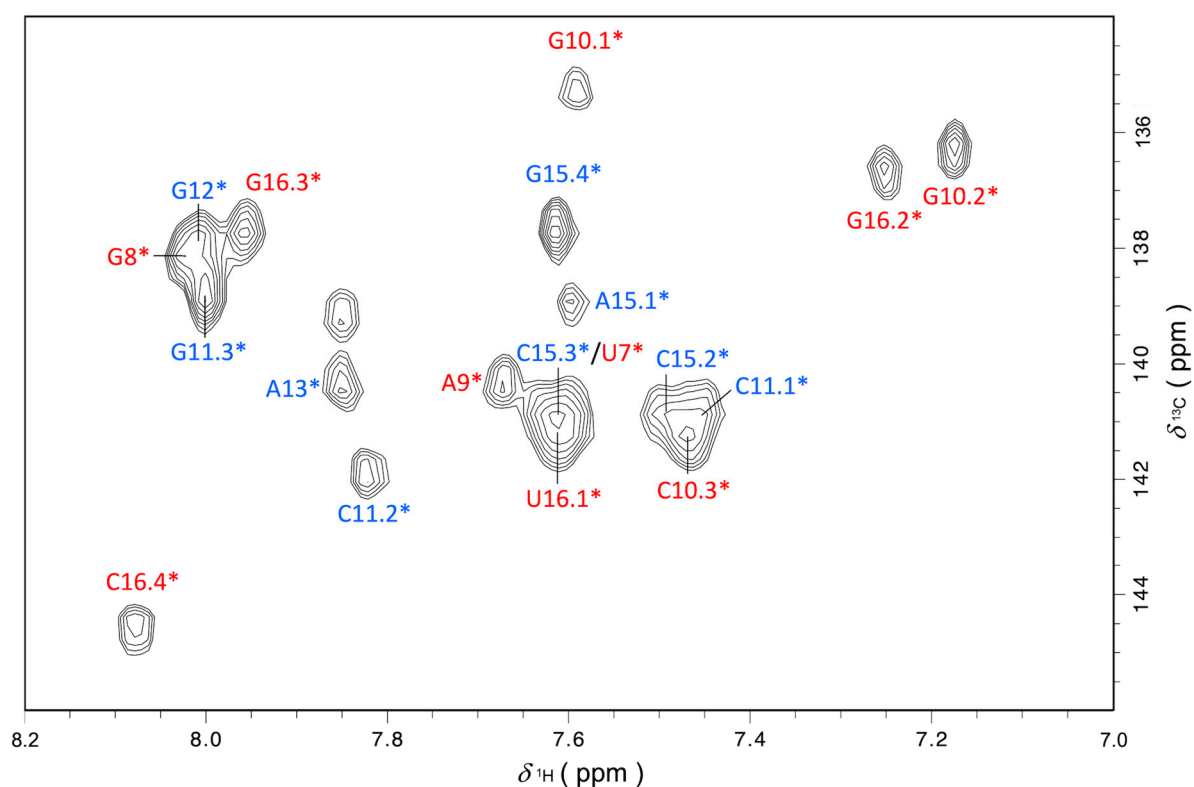


Figure S5. The natural abundance ^1H - ^{13}C HSQC spectrum of the RNA duplex **1** at pH 4.40. The respective intra-residue cross peaks are labeled with their residue numbers. The ^1H - ^{13}C HSQC spectrum in the absence of CdCl_2 was recorded at pH 8.18 during the pH-titration experiment with the following conditions. Spectral width: 7978.724 * 3141.776 Hz, Points: 2048 * 64 complex points, Number of scans: 192 times, Temperature: 303 K, Hardware: Bruker Avance III HD 500 MHz spectrometer equipped with a BBO cryogenic probe.

Supplemental Tables

Table S1. The chemical shielding calculated for H8 atom in G in ppm.

Molecule	State of G	$\sigma(\text{H8})$ (ppm)
G-A	neutral	23.4549
G-A	N1-deprotonated	23.6348
5'-GMP	neutral	23.6407
5'-GMP	N1-deprotonated	23.8413

G-A: the sheared-type G-A pair. 5'-GMP: guanosine 5'-monophosphate.

Table S2. The $\Delta\delta(\text{H8})$ chemical shift perturbation of H8 atom in G calculated due to N1-deprotonation in ppm.

Molecule	$\Delta\delta(\text{H8})$ (ppm)
G-A	− 0.17
5'-GMP	− 0.20

G-A: the sheared-type G-A pair. 5'-GMP: guanosine 5'-monophosphate. The $\Delta\delta(\text{H8})$ was calculated as $\Delta\delta(\text{H8}) = \delta(\text{H8 in N1-deprotonated G}) - \delta(\text{H8 in neutral G}) \approx \sigma(\text{H8 in neutral G}) - \sigma(\text{H8 in N1-deprotonated G})$.