**Supporting Information**

**1H, 13C and 15N chemical shift assignment of the stem-loops 5b + c from the 5'-UTR of SARS-CoV-2**

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**SI Figure 1: A** Predicted secondary structures of RNAs used for the divide-and-conquer approach of element SL5b+c. **B** Comparison of 1H,15N-imino regions of SL5b+c to SL5b\_GC and SL5c to verify consistent secondary structure by chemical shift similarity of sub-elements at 283 K (for experimental data see **Table 1 I, SI Table 1 I, and SI Table 2 I**).

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**SI Figure 2** **A** 1H,15N-TROSY, **B** 1H,1H-NOESY and **C** HNN-COSY spectra for imino-proton correlation of SL5b\_GC at 283K. Positive contours are given in blue, negative contours in red. The imino-proton correlations are annotated using the genomic numbering. Imino-proton correlations in **B** of closing base pairs are shown in grey, \* denote crosspeak visible at lower contour setting. Included in **C** is the overall experimentally observed secondary structure of SL5b\_GC with genomic numbering. Additional closing base pairs are annotated with ‘±x’. For experimental data see **SI Table 1** I-III.



**SI Figure 3** 31P 1D spectrum of the SL5c GAAA tetraloop at 298 K in buffer (100% D2O). Highlighted by arrows are typical GAAA tetraloop shift patterns for 31P as described in the literature (Legault & Pardi, 1994).



**SI Figure 4** 1H,15N-TROSY-based comparison of SL5b+c measured between 275 K and 298 K showing temperature response of the 15 imino signals (experimental information of spectra in **Table 1 I**). The signals of SL5c G256, G260, and G261 are not detectable at 298 K.

**SI Table 1** List of NMR experiments for SL5b\_GC conducted at BMRZ at temperatures a: 278 K, b: 283 K or c: 298 K. Spectra were recorded in NMR buffer with A: 95% H2O/ 5% D2O or B: 100% D2O. Experimental parameters and experiment-specific parameters are given. ns = number of scans, sw = spectral width, aq = acquisition time, o1/2/3 = carrier frequencies on channels 1/2/3, rel. delay = relaxation delay, CT = constant time, JR = jump-return, fw = forward.

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| **#** | **NMR experiment** | **Experimental parameters** | **Characteristic parameters** |
| **I** | **1H,15N-TROSYBMRZ** | **A** a 700 MHz, ns: 16, sw(f2): 21.0 ppm, sw(f1): 24.6 ppm, aq(f2): 68.6 ms, aq(f1): 73.1 ms, o1(1H): 4.7 ppm, o2(13C): 101 ppm, o3(15N): 153 ppm, rel. delay: 0.3 s, time: 30 min**A** b 600 MHz, ns: 8, sw(f2): 24.0 ppm, sw(f1): 25.3 ppm, aq(f2):62.3 ms, aq(f1): 83.2 ms, o1(1H): 4.7 ppm, o2(13C): 101 ppm, o3(15N): 153 ppm, rel. delay: 0.3 s, time: 15 min**A** c 800 MHz, ns: 16, sw(f2): 21.0 ppm, sw(f1): 24.6 ppm, aq(f2): xx ms, aq(f1): 60.0 ms, o1(1H): 4.7 ppm, o2(13C): 101 ppm, o3(15N): 153 ppm, rel. delay: 0.3 s, time: 1 h |  |
| **II** | **1H,15N-HNN-COSYBMRZ** | **A** b 600 MHz, ns: 16, sw(f3,1H): 8.75 ppm, sw(f2,15N): 90 ppm, sw(f1,15N): 120 ppm, aq(f3): 62.7 ms, aq(f2): 0.09 ms, aq(f1): 13.1 ms, o1(1H): 4.7 ppm, o2(13C): 185 ppm, o3(15N): 185 ppm, rel. delay: 0.3 s, time: 2 h**A** c 600 MHz, ns: 64, sw(f3,1H): 19.8 ppm, sw(f2,15N): 30 ppm, sw(f1,15N): 100 ppm, aq(f3): 105 ms, aq(f2): 0.03 ms, aq(f1): 31.7 ms, o1(1H): 4.7 ppm, o2(13C): 183 ppm, o3(15N): 183 ppm, rel. delay: 0.3 s, time: 3 h 35 min |  |
| **III** | **1H,1H-NOESYBMRZ**jump-return water suppression | **A** b 600 MHz, ns: 128, sw(f2): 20.8 ppm, sw(f1): 11.9 ppm, aq(f2): 31.4 ms, aq(f1): 63.28 ms, o1(1H): 4.7 ppm, o2(13C): 105 ppm, o3(15N): 153 ppm, rel. delay: 1.0 s, time: 19 h 40 min**A** c 800 MHz, ns: 224, sw(f2): 21.1 ppm, sw(f1): 11.9 ppm, aq(f2): 60.6 ms, aq(f1): 18.5 ms, o1(1H): 4.7 ppm, o2(13C): 120 ppm, o3(15N): 153 ppm, rel. delay: 1.0 s, time: 5 h | **b** NOE mixing time 150 ms, JR-delay 50 µs**c** NOE mixing time 250 ms, JR-delay 39 µs |
| **IV** | **1H,15N-HSQCBMRZ**Amino(Mori et al. 1995) | **A** a 700 MHz, ns: 8, sw(f2): 9.8 ppm, sw(f1): 32.9 ppm, aq(f2): 74.5 ms, aq(f1): 41.1 ms, o1(1H): 4.7 ppm, o2(13C): 101 ppm, o3(15N): 86 ppm, rel. delay: 0.8 s, time: 23 min**A** b 600 MHz, ns: 32, sw(f2): 10 ppm, sw(f1): 30.8 ppm, aq(f2): 85.2 ms, aq(f1): 68.2 ms, o1(1H): 4.7 ppm, o2(13C): 101 ppm, o3(15N): 86.5 ppm, rel. delay: 0.8 s, time: 2 h | INEPT transfer time 2.2 ms (1JNH 110 Hz) |
| **V** | **1H,1H-xf-NOESYBMRZ** xf: selectively filtering N-bond protons, watergate water suppression(Ikura and Bax 1992; Piotto et al. 1992; Sklenáŕ et al. 1993) | **A** c 800 MHz, ns: 128, sw(f2): 10 ppm, sw(f1): 6.25 ppm, aq(f2): 64 ms, aq(f1): 49.6 ms, o1(1H): 4.7 ppm, o2(13C): 115 ppm, o3(15N): 86 ppm, rel. delay: 1.0 s, time: 27 min | NOE mixing time 250 ms |
| **VI** | **1H,1H-TOCSYBMRZ** excitation sculpting water suppression(Shaka et al. 1988; Hwang and Shaka 1995) | **A** c 800 MHz, ns: 32, sw(f2): 8.75 ppm, sw(f1): 6.25 ppm, aq(f2): 99.8 ms, aq(f1): 51.2 ms, o1(1H): 4.7 ppm, o2(13C): 101 ppm, o3(15N): 86 ppm, rel. delay: 1.0 s, time: 5 h 32 min | CC-TOCSY mixing time 40 ms |
| **VII.1** | **1H,13C-HSQCBMRZ** aromatic region, H-C2/6/8 | **A** c 800 MHz, ns: 4, sw(f2): 8.3 ppm, sw(f1): 24.0 ppm, aq(f2): 76.8 ms, aq(f1): 39.8 ms, o1(1H): 4.7 ppm, o2(13C): 143 ppm, o3(15N): 150 ppm, rel. delay: 1.0 s, time: 40 min | INEPT transfer time 2.5 ms (1JCH 200 Hz), off-resonant Q3 shaped pulse for C5 decoupling at 99 ppm with 15 ppm bandwidth |
| **VII.2** | **1H,13C-HSQCBMRZ** aromatic region, H-C5 | **A** c 800 MHz, ns: 4, sw(f2): 9.0 ppm, sw(f1): 20.0 ppm, aq(f2): 79.8 ms, aq(f1): 63.6 ms, o1(1H): 4.7 ppm, o2(13C): 99 ppm, o3(15N): 185 ppm, rel. delay: 1.0 s, time: 40 min | INEPT transfer time 2.8 ms (1JCH 180 Hz), off-resonant Q3 shaped pulse for C2/6/8 decoupling at 150 ppm with 40 ppm bandwidth |
| **VII.3** | **1H,13C-HSQCBMRZ** ribose region, H-C1’ | **A** c 800 MHz, ns: 4, sw(f2): 9.0 ppm, sw(f1): 20.0 ppm, aq(f2): 79.8 ms, aq(f1): 63.6 ms, o1(1H): 4.7 ppm, o2(13C): 90 ppm, o3(15N): 158.5 ppm, rel. delay: 1.0 s, time: 40 min | INEPT transfer time 2.9 ms (1JCH 170 Hz), off-resonant Q3 shaped pulse for C2’ decoupling at 72 ppm with 12 ppm bandwidth |
| **VIII** | **1H,13C-ct-HSQCBMRZ**full (Vuister and Bax 1992) | **A** c 600 MHz, ns: 8, sw(f2): 8.33 ppm, sw(f1): 37.88 ppm, aq(f2): 102.4 ms, aq(f1): 44.8 ms, o1(1H): 4.7 ppm, o2(13C): 77 ppm, o3(15N): 155 ppm, rel. delay: 1.0 s, time: 1 h 20 min | INEPT transfer time 1.5 ms (1JCH 165 Hz), CT period 25 ms (1JCC 40 Hz) |
| **IX** | **(H)C(CCN)HBMRZ** imino-to-aromatics(Piotto et al. 1992; Sklenář et al. 1996) | **A** c 600 MHz, ns: 336, sw(f3): 20.9 ppm, sw(f2): 9.9 ppm, aq(f3): 90.2 ms, aq(f2): 42.6 ms, o1(1H): 4.7 ppm, o2(13C): 137 ppm, o3(15N): 154 ppm, rel. delay: 1.8 s, time: 1 d 33 min | CC-TOCSY mixing time 28 ms |
| **X** | **H(N)COBMRZ**imino-to-carbon (Favier and Brutscher 2011; Solyom et al. 2013) | **A** c 600 MHz, ns: 512, sw(f3): 21.0 ppm, sw(f1): 22.1 ppm, aq(f3): 62.6 ms, aq(f1): 19.2 ms, o1(1H): 4.7 ppm, o2(13C): 159 ppm, o3(15N): 153 ppm, rel. delay: 0.3 s, time: 8 h 31 min | NC-INEPT transfer time 18 ms (1JCN 28 Hz) |
| **XI** | **3D 13C-NOESY-HSQCBMRZ** aromatics and ribose(Piotto et al. 1992; Sklenáŕ et al. 1993) | **A** c 800 MHz, ns: 16, sw(f3,1H): 8.75 ppm, sw(f2,13C): 21.06 ppm, sw(f1,1H): 6.25 ppm, aq(f3): 73.1 ms, aq(f2): 9.4 ms, aq(f1): 20.0 ms, o1(1H): 4.7 ppm, o2(13C): 137 ppm, o3(15N): 154 ppm, rel. delay: 0.9 s, time: 22 h (NUS) | NOE mixing time 200 ms, HSQC transfer time 1.4 ms (1JCH 180 Hz) |
| **XII** | **3D TROSY-HCCH-COSYBMRZ** adenine C2-to-C8(Simon et al. 2001) | **B** c 600 MHz, ns: 16, sw(f3,1H): 8.75 ppm, sw(f2,13C): 22.1 ppm, sw(f1,13C): 58.47 ppm, aq(f3): 97 ms, aq(f2): 9.5 ms, aq(f1): 7.25 ms, o1(1H): 4.7 ppm, o2(13C): 142.5 ppm, o3(15N): 150 ppm, rel. delay: 1.0 s, time: 1 d 20 h | Bruker standard parameter set |
| **XIII.1** | **3D HCCH-TOCSYBMRZ** ribose C1’-to-C2’(Kay et al. 1993; Richter et al. 2010) | **A** c 800 MHz, ns: 8, sw(f3,1H): 8.56 ppm, sw(f2,13C): 9.47 ppm, sw(f1,13C): 35.5 ppm, aq(f3): 74.7 ms, aq(f2): 21.0 ms, aq(f1): 8.96 ms, o1(1H): 4.7 ppm, o2(13C): 76.5 ppm, o3(15N): 153 ppm, rel. delay: 1.0 s, time: 1 d 3 h | CC-TOCSY mixing time 5.4 ms |
| **XIII.2** | **3D HCCH-TOCSYBMRZ** ribose C1’-to-C5’(Kay et al. 1993; Richter et al. 2010) | **A** c 800 MHz, ns: 8, sw(f3,1H): 8.56 ppm, sw(f2,13C): 9.47 ppm, sw(f1,13C): 35.5 ppm, aq(f3): 74.7 ms, aq(f2): 21.0 ms, aq(f1): 11.2 ms, o1(1H): 4.7 ppm, o2(13C): 76.5 ppm, o3(15N): 153 ppm, rel. delay: 0.97 s, time: 1 d 9 h 20 min | CC-TOCSY mixing time 16 ms |
| **XIV** | **3D HCNBMRZ** C6/8-N1/9-C1’(Sklenář et al. 1993) | **A** c 600 MHz, ns: 32, sw(f3,1H): 8.33 ppm, sw(f2,13C): 13.25 ppm, sw(f1,15N): 41.1 ppm, aq(f3): 102.4 ms, aq(f2): 14.0 ms, aq(f1): 25.6 ms, o1(1H): 4.7 ppm, o2(13C): 115 ppm, o3(15N): 158 ppm, rel. delay: 1.0 s, time: 22 h (NUS) |  |
| **XV** | **3D fw directed HCC-TOCSY-CCHBMRZ** ribose H1’-to-H3’(Schwalbe et al. 1995; Marino et al. 1995; Glaser et al. 1996) | **A** c 600 MHz, ns: 8, sw(f3,1H): 8.33 ppm, sw(f2,13C): 38.54 ppm, sw(f1,1H): 4.17 ppm, aq(f3): 102.4 ms, aq(f2): 8.25 ms, aq(f1): 35.2 ms, o1(1H): 4.7 ppm, o2(13C): 77 ppm, o3(15N): 155 ppm, rel. delay: 1.0 s, time: 1 d 22 h | CC-TOCSY mixing time 9.2 ms |

**SI Table 2:** List of NMR experiments for SL5c conducted at BMRB at 283 K if not noted otherwise. Spectra were recorded in NMR buffer with A: 95% H2O/ 5% D2O or B: 100% D2O. Experimental parameters and experiment-specific parameters are given. ns = number of scans, sw = spectral width, aq = acquisition time, o1/2/3 = carrier frequencies on channels 1/2/3, rel. delay = relaxation delay, JR = jump-return.

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| **#** | **NMR experiment** | **Experimental parameters** | **Characteristic parameters** |
| **I** | **1H,15N-sfHMQCBMRZ**(Schanda and Brutscher 2005) | **A** 600 MHz, ns: 4096, sw(f2): 23.9 ppm, sw(f1): 25.3 ppm, aq(f2): 71.2 ms, aq(f1): 41.5 ms, o1(1H): 4.7 ppm, o2(13C): 101 ppm, o3(15N): 153 ppm, rel. delay: 0.3 s, time: 11.5 h |  |
| **II** | **1H,1H-NOESYBMRZ**jump-return water suppression | **A** iminos: 800 MHz, ns: 208, sw(f2): 21.0 ppm, sw(f1): 11.9 ppm, aq(f2): 60 ms, aq(f1): 31 ms, o1(1H): 4.7 ppm, o2(13C): 150 ppm, o3(15N): 153 ppm, rel. delay: 1.0 s, time: 18 h**B** aromatics: 600 MHz, ns: 64, sw(f2): 14 ppm, sw(f1): 6 ppm, aq(f2): 122 ms, aq(f1): 83 ms, o1(1H): 4.7 ppm, o2(13C): 99 ppm, o3(15N): 153 ppm, rel. delay: 1.5 s, time: 19 h 45 min | **A**: NOE mixing time 150 ms, JR-delay 200 µs**B**: NOE mixing time 150 ms, JR-delay 200 µs |
| **III** | **1H,1H-TOCSYBMRZ** excitation sculpting water suppression (Shaka et al. 1988; Hwang and Shaka 1995) | **A** 800 MHz, ns: 32, sw(f2): 9.0 ppm, sw(f1): 6.2 ppm, aq(f2): 71 ms, aq(f1): 38 ms, o1(1H): 4.7 ppm, o2(13C): 101 ppm, o3(15N): 86 ppm, rel. delay: 1.0 s, time: 4 h**B** 600 MHz, ns: 16, sw(f2): 9.0 ppm, sw(f1): 9.0 ppm, aq(f2): 308 ms, aq(f1): 47 ms, o1(1H): 4.7 ppm, rel. delay: 1.5 s, time: 4 h 40 min | **A** CC-TOCSY mixing time 40 ms**B** CC-TOCSY mixing time 80 ms |
| **IV** | **1H,13C-HSQCBMRZ** full (Bodenhausen and Ruben 1980) | **B** 600 MHz, ns: 256, sw(f2): 10.0 ppm, sw(f1): 106 ppm, aq(f2): 85 ms, aq(f1): 14 ms, o1(1H): 4.7 ppm, o2(13C): 108 ppm, o3(15N): 154.5 ppm, rel. delay: 1.0 s, time: 11 h 15 min | INEPT transfer time 1.47 ms (1JCH 170 Hz) |

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