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Supporting Information

The Extended Hadamard Transform: Sensitivity-Enhanced NMR Experiments Among Labile and Non-Labile ^1H s of SARS-CoV-2-derived RNAs

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Supplementary Information

Table S1. Parameters used for conventional, SMT, HMT, HETMAT and Hadamard HETMAT NOESY experiments on the RNA fragments presented in the main text.

RNA	5 SL5b+c							5 SL8		
Parameters	HMT NOESY	SMT NOESY	Sofast NOESY	Conv. NOESY	Hadamard HETMAT NOESY	HETMAT NOESY	HMQC NOESY	Hadamard HETMAT NOESY	HETMAT NOESY	HMQC NOESY
Temperature (K)	283	283	283	283	283	283	283	283	283	283
SW (ppm)	23.8	23.8	23.8	23.8	23.8	23.8	23.8	23.8	23.8	23.8
TD	3072	3072	3072	3072	3072	3072	3072	3072	3072	3072
SW ₁ (ppm)	/	/	5	11.5	/	/	24	/	/	24
TD ₁	8 or 16	12	256	768	8 or 16	13	384	8 or 32	21	384
NS	128	512	128	32	128 or 64	512	28	128	512	24
DS	32	32	32	32	32	32	32	32	32	32
d ₁ (s) [*]	0.3 or 1.5	0.3 or 1.5	0.3	1.0	1.5	1.5	1.5	1.5	1.5	1.5
RG	101	101	101	101	101	101	101	101	101	101
O ₁ (F ₂ , ppm)	4.697	4.693	4.693	4.693	4.693	4.693	4.693	4.693	4.693	4.693
O ₁ (F ₁ , ppm)	/	/	153	153	116	116	116	116	116	116
d ₈ (NOE, ms)	800	800	175	175	125	a: 125 b: 30	175	80	a: 80 b: 50	150
$\gamma B_1/2\pi$ saturation	8	8 - 30	/	/	/	/	/	/	/	/
n ^{**} (number of loops)	/	/	/	/	7	a: 7 b: 20	/	10	a: 10 b: 17	/
RF CP field [*] $\omega_1/2\pi$ (Hz)	/	/	/	/	20	a: 50 b: 75	/	20	a: 50 b: 75	/

^{*} d₁ = 0.3 and 1.5 sec were used for the selective SE and WG-based HMT/SMT, respectively.

^{**} a and b indicate the different parameters applied for narrow and broad peaks, respectively.

Selective SE-based Extended HMT (62 min)
WG-based Extended HMT (124 min)

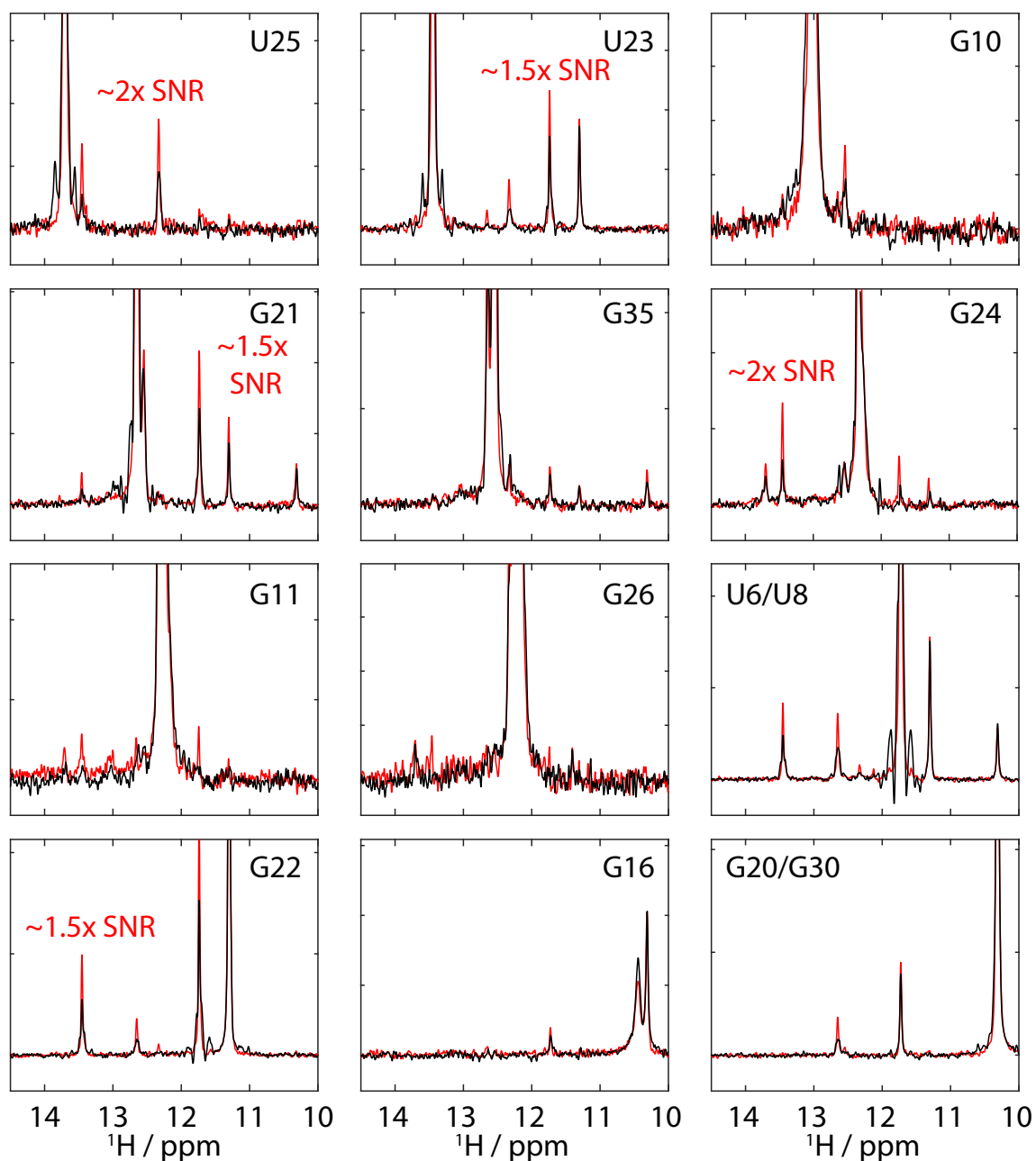


Figure S1. Comparison between selective spin-echo (SE) based- and WATERGATE (WG) based extended HMT NOESY for the 5_SL5b+c fragment. Note that ~2 times SNR enhancements and ~3 times SNRT enhancements are observed in selective SE-based compared to WG-based experiments.

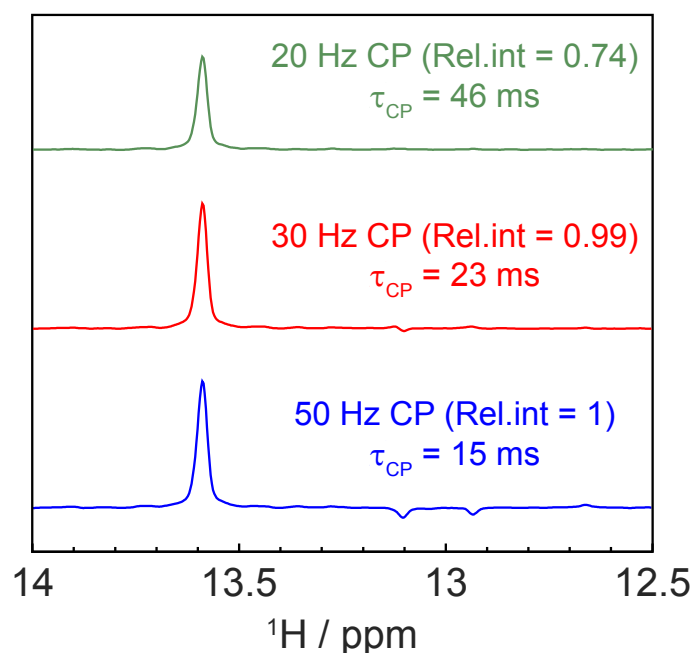


Figure S2. Assessing the performance of selective CP in Hadamard HETMAT experiments, by comparing 1D spectra measured with 1 loop and $\tau_{NOE} = 0$ ms but with different RF CP fields $\omega_1/2\pi$. Unlike the regular HETMAT experiment, where 50 Hz fields show sufficient selectivity at 1 GHz ^1H frequencies, 1D slices measured with 50 Hz and 30 Hz CP contain artifacts arising from the absence of a reference scan subtraction (see main text).

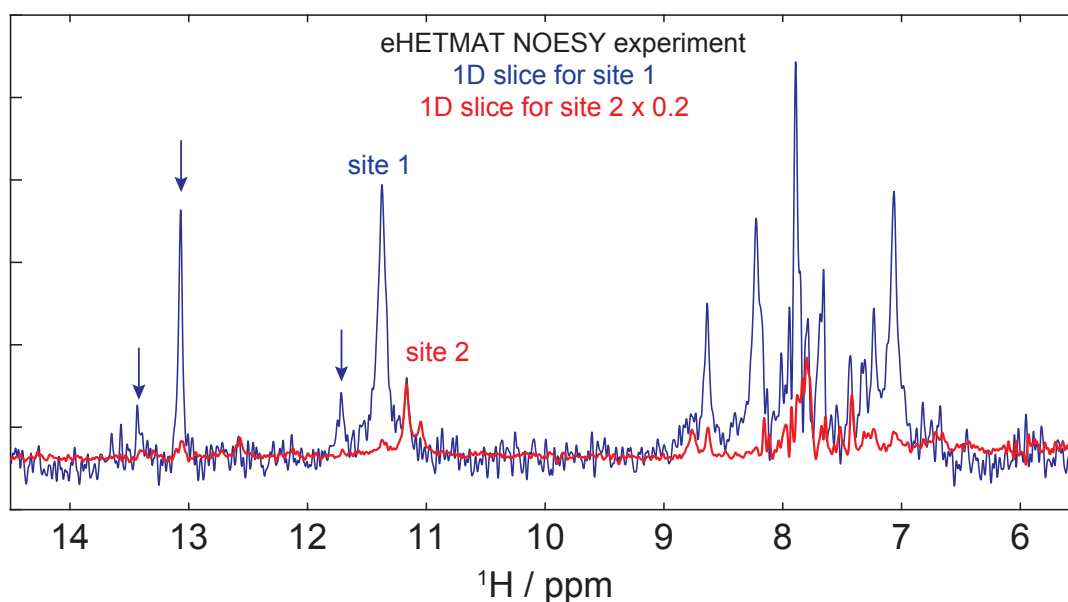


Figure S3. Illustrating the discrimination between genuine cross-peaks arising from site 1 and cross-talk-derived peaks arising from site 2. Arrows indicate the genuine imino-imino cross-peaks for site 1. For the amino region, multiple –but not all– peaks are genuine site 1 cross-peaks (not labeled with arrows).

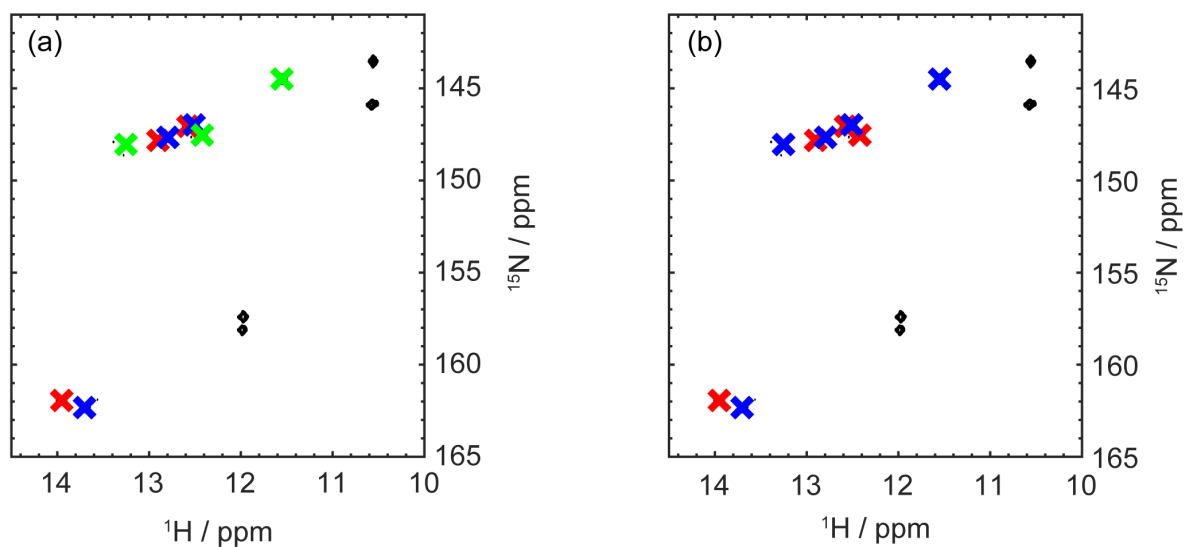


Figure S4. [^{15}N , ^1H]-HSQC spectra of the 5_SL5 fragment with peaks marked as selected for the extended HETMAT NOESY implementations with Hadamard matrices of dimension: (a) 8x8 (E_4); (b) 16x16 (E_8). Peaks encoded and decoded in the same set of experiments are marked with the same color.

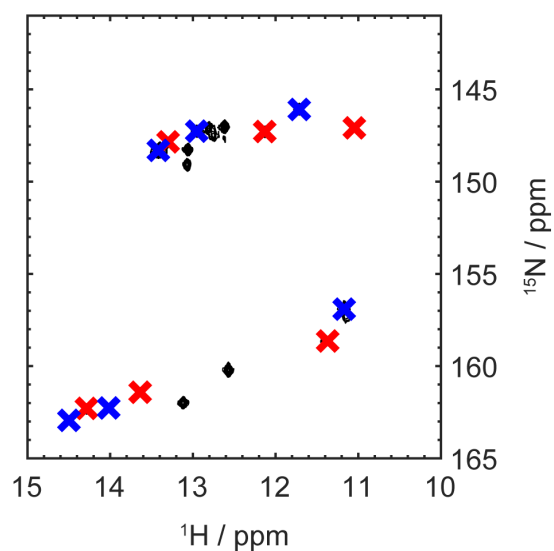


Figure S5. Idem as Figure S4, but for 5_SL8. An extended Hadamard matrix of dimension 16x16 (E_8) was used in these cases.

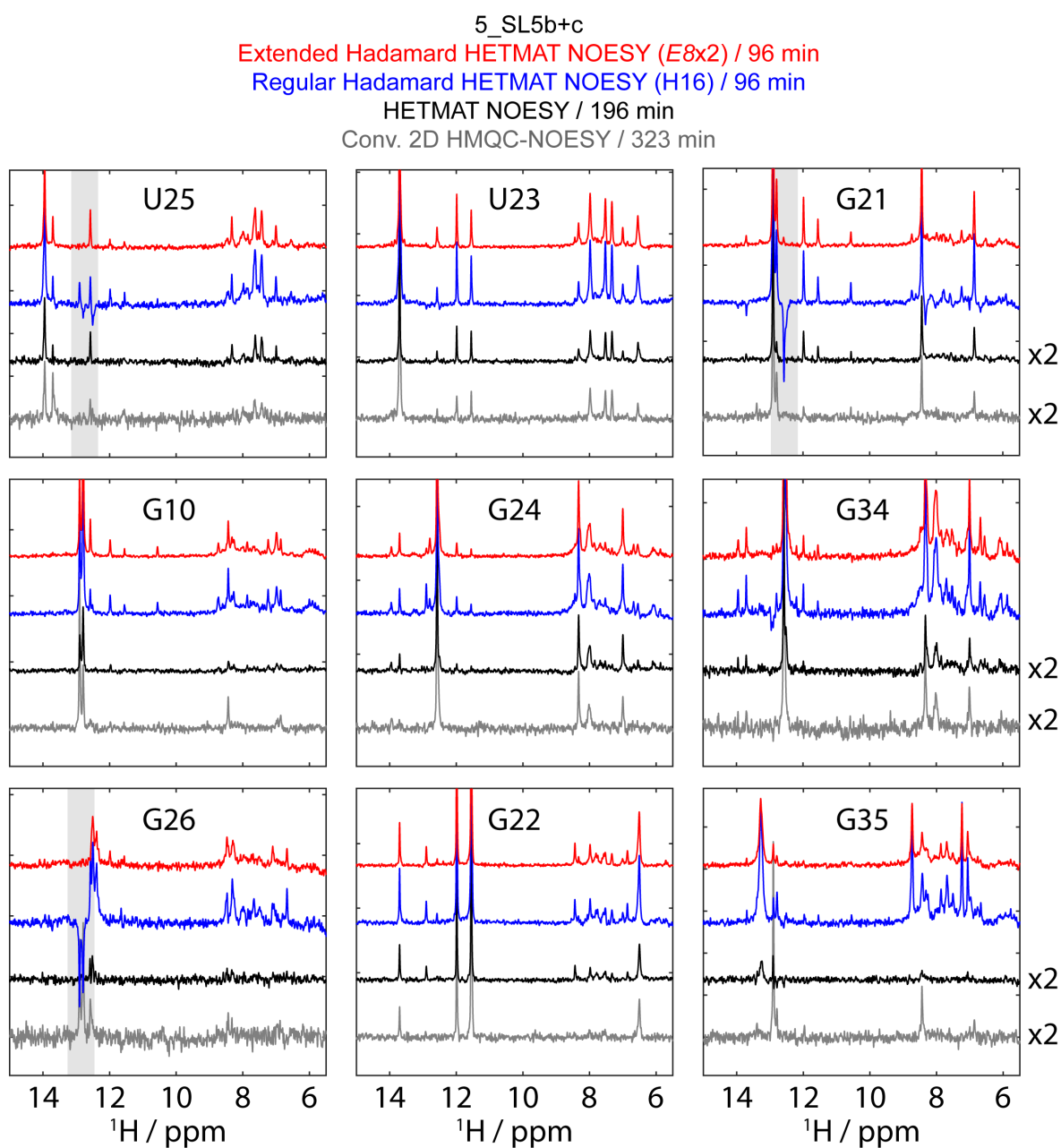


Figure S6. Comparison between conventional 2D HMQC NOESY (gray), HETMAT (black), regular Hadamard HETMAT (blue), and extended Hadamard HETMAT NOESY spectra acquired on 5_SL5b+c RNA fragment. Conditions are as shown in Figure 5 of the main text, but now all cross sections are displayed in the panels.

5_SL8
Extended Hadamard HETMAT NOESY (E8x2) / 96 min
Regular Hadamard HETMAT NOESY (H16) / 95 min
HETMAT NOESY / 262 min
Conv. 2D HMQC-NOESY / 277 min

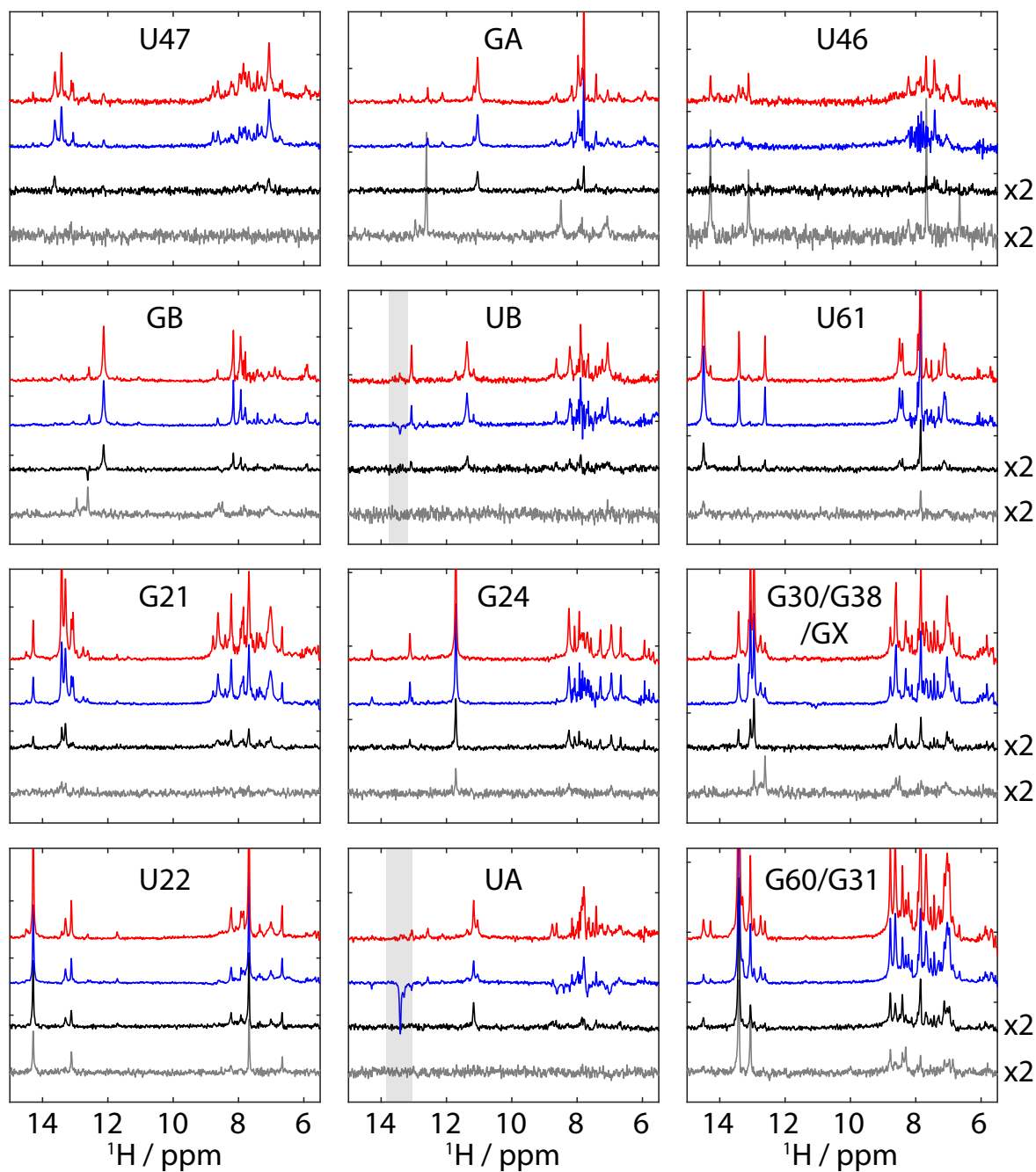


Figure S7. Idem as Figure S5, but for the 5_SL8 RNA fragment. Acquisition parameters for these data are as detailed in Figure 6 of the main text.

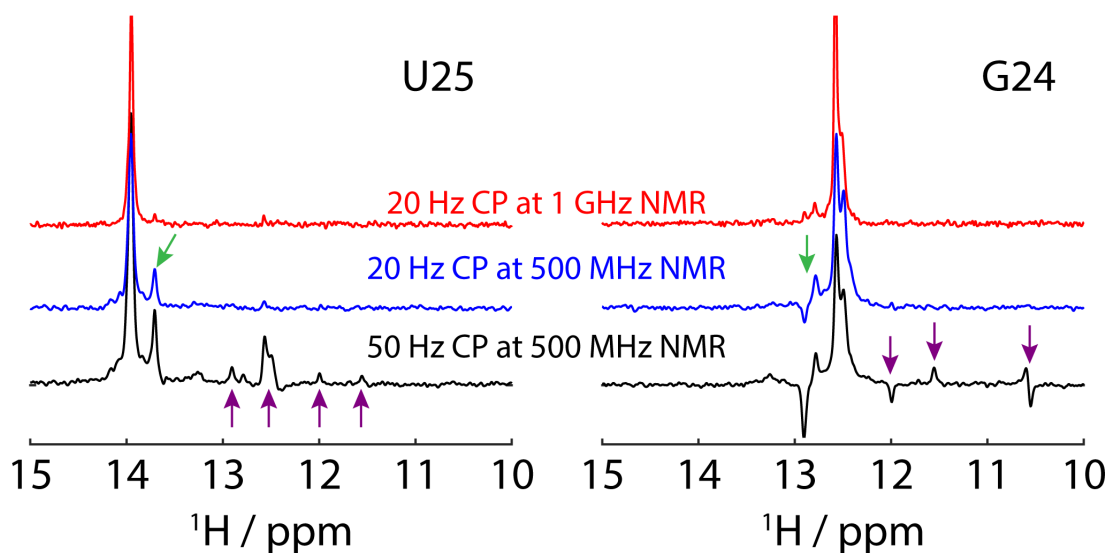


Figure S8. Assessing the selectivity of CP in Hadamard HETMAT experiments performed at 1 GHz (red) and at 500 MHz (blue and black) ^1H NMR frequencies on the indicated 5SL5b+c residues. Spectra were measured as described for Figure S4. Note that artifacts (purple arrows) arising due to the overlapping RF fields clearly visible in the black spectra. These cross-talk artifacts disappear when CP is done at 20 Hz matching fields, but other artifacts are still observed in the 500 MHz data (green arrow). These cross-talks disappear as sites are further separated at 1 GHz, but at 500 MHz they can lead to fake positive/negative cross-peak patterns after the Hadamard processing.