Instructions for HETMAT imino experiment

1. Select imino ^{15}N-^{1}H peaks from 2D HSQC/HMQC spectra using peak picking – and save it – automatic peak picking also works great but mind the threshold

2. Create new HETMAT experiment (use parameter set, not prosol compatible)

3. Based on the ^{15}N and ^{1}H chemical shifts, you would need to update frequency list for F1 and F3

4. Roughly divide the peaks based on the broadness and available spectral resolution – choose appropriate nutation field accordingly – rule of thumb 50 Hz for sharper and 75 Hz for broaded imino resonance – you would need to update pldb8 / pldb9 and pldb10 / pldb11 according to chosen γB_1 nutation field.

5. Choose duration of heteronuclear encoding according to choose CP power: p7=10400 for 75 Hz and p8=14500 for 50 Hz.

6. Choose d8 and d9 – standard options are d8= 30 – 50 ms and d9= 80 – 125 ms

7. Update vclist – number of loops used – by default use 6-8 loops for slower exchanging and 8-14 loops for faster exchanging peaks. Order loops according to the peak list (assign value for loops in the order of peak list according to your division into sharp and broad)

8. Once acquired, extract the data and use MATBAL processing scripts to obtain the pseudo 3D spectra

Here we show a table of acquisition parameters we used in our study:

RNA	5_SL5b+c		5_SL8b+c		5_SL8b+c	
Parameters	HMQC NOESY	HETMAT NOESY	HMQC NOESY	HETMAT NOESY	HMQC NOESY	HETMAT NOESY
Temperature (K)	283	283	283	283	298	298
SW (ppm)	23.8	23.8	23.8	23.8	23.8	23.8
TD	3072	3072	3072	3072	3072	3072
SW_1 (ppm)	24	/	24	/	24	/
TD_1	384	13	384	21	384	17
NS	28	512	32	480	32	512
DS	32	32	32	32	32	32
d_1 (s)	1.5	1.5	1.5	1.5	1.5	1.5
RG	101	101	101	101	101	101
O_1 (F_2, ppm)	4.697	4.697	4.694	4.694	4.698	4.698
O_1 (F_1, ppm)	116	116	116	116	116	116
d_8 (NOE, ms)[*]	175	a: 125 b: 30	150	a: 80 b: 50	200	a: 125 b: 50
n[*] (number of loops)	/	a: 7 b: 20	/	a: 10 b: 17	/	a: 7 b: 17
RF CP field[*] $\omega_1/2\pi$ (Hz)	/	a: 50 b: 75	/	a: 50 b: 75	/	a: 50 b: 75

*Choice for NOE mixing time, number of loops and CP RF field according to the broadness of the peak