Supplementary Material

Sugar-to-base correlation in nucleic acids with a 5D APSY-HCNCH or two 3D APSY-HCN experiments

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NMR spectroscopy

Setup of APSY experiments

The APSY NMR experiments were carried out on a Bruker Avance III 500 MHz spectrometer and on a Bruker Avance I 900 MHz spectrometer, which are operated with the software Topspin 2.1 (Bruker, Karlsruhe, Germany). The APSY series is started from a parent data set which is setup like for a conventional 5D experiment. The frequency axes of the five dimensions of the 5D APSY-HCNCH are defined as F1 for H1′, F2 for C1′, F3 for N1/9, F4 for C6/8, and F5 for H6/8 in the acquisition dimension; for both versions of the intra-base 3D APSY-HCN as F1 for N1/9, F2 for C6/8 and F3 for H6/8 in the acquisition dimension; for the sugar-to-base 3D APSY-HCN as F1 for N1/9, F2 for C1′, and F3 for H1′ in the acquisition dimension. The automated setup of the projections is carried out with an AU program (provided on www.apsy.ch), which first proposes an angle file based on the sweep widths, and subsequently creates the 2-dimensional data sets for the projection experiments accordingly. In comparison to the number of scans in the setup of a direct projection (with one type of nucleus in the indirect dimension), the number of scans is usually automatically doubled for each additional projected frequency axis, in order to compensate for the sensitivity differences of √2. In our case, the number of scans was kept constant, since the signal-to-noise ratio (S/N) was sufficient in all projections. Further acquisition parameters of the measurements on RNA SL23 are summarized in Tables S1 to S6. These parameters can serve as guidelines for further applications of the experiment. The projections which are set up and measured first are usually those which include the frequencies of only one nucleus type in the indirect dimension ("direct" projections), and therefore correspond to conventional two-dimensional spectra, which are for the 5D APSY-HCNCH experiment four spectra with

the acquisition dimension H6/8 and with either H1′, C1′, N1/9 or C6/8 as indirect dimension (Fig. 1). Correspondingly, the direct projections for the intra-base 3D APSY-HCN are the N1/9–H6/8 and the C6/8–H6/8 spectra; and for the sugar-to-base 3D APSY-HCN the N1/9–H1′and the C1′–H1′spectra. These projections can be used to estimate the specific sensitivity and the dispersion of the resonances of a particular protein, and to refine the sweep widths and number of scans accordingly. Details are described in (Hiller et al. 2005; Hiller et al. 2008).

The shaped pulses with two inversion frequencies are field dependent; their creation is straightforward and fast with the spectrometer software. E.g., with Topspin use Shape Tool and select an Iburp2 pulse; in the "Manipulate" menu choose "phase Modulation acc. to Offset", and specify length, number of frequencies (=2), and set the frequencies (e.g. to 0 and 8803 for pulse (*c*) in Fig. 2 with the corresponding "spoffs" set to 0), and save the resulting shape.

The setup program can optionally create a set of angles for optimal indirect acquisition times: each possible combination of nuclear frequencies in the indirect dimension has one angle for which both acquisition times become maximal with adjusted number of increments. This is particularly advantageous for the 5D APSY-HCNCH, where all evolution periods are of constant-time type.

Data analysis

Automated serial processing of the spectra was performed with Prosa 6.4 (Güntert et al. 1992), for which the input files were created by the AU program which also generated the APSY projections in the software Topspin. For all spectra, 1k complex data points were measured in the acquisition dimension, zero-filled to 2k points and multiplied with a 75°-shifted sine-bell function. The same window function was applied in the indirect dimensions after zero-filling to 512 complex data points. More acquisition and processing parameters can be found in the Tables S1 and S2 for the 5D APSY-HCNCH experiment, and in the Tables S3–S6 for the 3D APSY-HCN experiments. The subsequent analysis and calculation of the multidimensional peak list was accomplished with GAPRO (available on www.apsy.ch); parameters specified below for the GAPRO processing are only indications, the outcome is robust with respect to a wide range of values. The results were evaluated by overlaying the spectra with the back-projected multidimensional peak list in XEASY (Bartels et al. 1995; Eccles et al. 1991) as implemented in CARA 1.5.5 (cara.nmr.ch) as subroutine NEASY, and by comparing and analyzing the results with different MATLAB (R2010b, The MathWorks, Natick, MA, USA) scripts.

Parameters of APSY experiments with a SL23 RNA

5D APSY-HCNCH sugar-to-base experiment (H1′–C1′–N1/9–C6/8–H6/8)

Spectrometer: Bruker Avance III 500 MHz with cryogenic probe

Temperature: 25°C / 18°C (two data sets with the same parameters)

Total experiment time: 1 h 28 min

Nr. of projections: 16

Interscan delay: 1 s

GAPRO parameters: $S_{min,1} = S_{min,2} = 10$, $R_{min} = 26$ Hz, $\Delta\upsilon = 5.7$ pt, S/N = 3.6 (Hiller et al. 2005)

Table S1 Acquisition parameters of the 5D APSY-HCNCH experiment with a SL23 RNA

Dimension	Nucleus	Sweep width [ppm]	Sweep width [Hz]	Carrier frequency [ppm]
ω_1	H1′	3.4	1700	5.5
ω_2	C1′	9.0	1132	90.5
ω_3	N1/9	35.0	1774	160
ω_4	C6/8	11.0	1384	139.5
ω_5	H6/8	17.0	8503	7.5

Table S2 Projection angles and parameters of the 5D APSY-HCNCH experiment with a SL23 RNA[§].

α [°]	β [°]	γ [°]	Time [min]	# of complex points in indirect dimension	Maximal acquisition time [ms]	Nr. of scans	Indirect dimension frequencies
0	0	0	2	23	16.6	2	C6/8
0	0	90	5	58	34.1	2	H1′
0	90	0	4	38	33.6	2	C1′
90	0	0	5	60	33.8	2	N1/9
0	90	±45.6	6	69	34.0, 33.3	2	H1′,C1′
90	0	±45.6	7	83	34.1, 33.4	2	H1′, N1/9
90	±44.9	0	6	70	33.2, 33.3	2	C1′, N1/9
0	0	±70.3	6	62	33.8, 16.8	2	H1′,C6/8
±62.7	0	0	6	62	32.4, 16.7	2	N1/9, C6/8
0	±62.8	0	4	44	32.9, 16.9	2	C1′, C6/8

3D APSY-NCH (N1/9–C1′–H1′)

Spectrometer: Bruker Avance III 500 MHz with cryogenic probe

Temperature: 25°C

Total experiment time: 1 h 23 min

Nr. of projections: 32

Interscan delay: 0.7 s

GAPRO parameters: $S_{min,1} = S_{min,2} = 11$, $R_{min} = 7.0$ Hz, $\Delta\upsilon = 2.1$ pt, S/N = 3.6 (Hiller et al. 2005)

Table S3 Acquisition parameters of the 3D APSY-HCN sugar-to-base experiment with a SL23 RNA

Dimension	Nucleus	Sweep width [ppm]	Sweep width [Hz]	Carrier frequency [ppm]
ω_1	N1/9	35.0	1774	160.0
ω_2	C1′	9.0	1132	90.5
ω_3	H1′	17.0	8503	4.7

Table S4 Projection angles and parameters of the 3D APSY-HCN sugar-to-base experiment with a SL23 RNA

α [°]	Time [min]	# of complex points in indirect dimension	Maximal acquisition time [ms]	Nr. of scans	Indirect dimension frequencies
0	1	22	19.4	1	C1′
±19.3	1	24	18.6, 6.5	1	C1′, N1/9
±35.5	1	32	18.8, 13.4	1	C1′, N1/9
±38.9	1	34	18.6, 15.0	1	C1′, N1/9
±40.5	1	36	19.0, 16.3	1	C1′, N1/9
±50.3	2	46	19.0, 22.9	1	C1′, N1/9
±57.5	2	58	19.3, 30.3	1	C1′, N1/9
±63.1	3	70	19.0, 37.5	1	C1′, N1/9
±65.7	3	78	19.1, 42.2	1	C1′, N1/9
±73.1	4	100	16.8, 55.3	1	C1′, N1/9
±75.5	4	100	14.4, 55.6	1	C1′, N1/9
±77.9	4	100	12.0, 55.8	1	C1′, N1/9
±79.2	4	100	10.7, 56.0	1	C1′, N1/9
±83.2	4	100	6.7, 56.2	1	C1′, N1/9
±85.4	4	100	4.5, 56.3	1	C1′, N1/9
±89.1	4	100	0.9, 56.4	1	C1′, N1/9
90	4	100	56.4	1	N1/9

3D APSY-NCH (N1/9-C6/8- H6/8)

Spectrometer: Bruker Avance III 500 MHz (or Avance I 900 MHz) with cryogenic probe

Temperature: 25°C

Total experiment time: 43 min

Nr. of projections: 20

Interscan delay: 0.7 s

GAPRO parameters: $S_{min,1} = S_{min,2} = 11$, $R_{min} = 7.0$ Hz, $\Delta\upsilon = 2.1$ pt, S/N = 3.6 (Hiller et al. 2005)

Table S5 Acquisition parameters of the TROSY or MQ 3D APSY-HCN intra-base experiment with a SL23 RNA

Dimension	Nucleus	Sweep width [ppm]	Sweep width [Hz]	Carrier frequency [ppm]
ω_1	N1/9	31.0	1571	158.0
ω_2	C6/8	11.0	1384	139.5
ω_3	H1′	17.0	8503	4.7

Table S6 Projection angles and parameters of the TROSY or MQ 3D APSY-HCN intra-base experiment with a SL23 RNA

α [°]	Time [min]	# of complex points in indirect dimension	Maximal acquisition time [ms]	Nr. of scans	Indirect dimension frequencies
0	1	28	20.2	1	C6/8
±27.9	1	32	19.1, 10.1	1	C6/8, N1/9
±40.5	1	40	19.5, 16.7	1	C6/8, N1/9
±52.0	2	50	18.8, 24.1	1	C6/8, N1/9
±59.3	2	62	18.8, 31.7	1	C6/8, N1/9
±68.9	3	82	17.1, 44.3	1	C6/8, N1/9
±75.3	3	80	11.6, 44.2	1	C6/8, N1/9
±79.0	3	80	8.7, 44.6	1	C6/8, N1/9
±84.4	3	78	4.3, 43.8	1	C6/8, N1/9
±87.1	3	78	2.2, 43.9	1	C6/8, N1/9
90	3	78	44.0	1	N1/9

The parameters of the experiment on the 900 MHz Avance I spectrometer were set accordingly, i.e. with identical sweep widths (in ppm) and maximal evolution/acquisition times. The C6/8 constant-time evolution limit is slightly lower (~17.1 ms) for the MQ version due to the proton inversion pulses.

References

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