Supporting Information

Measuring radiofrequency fields in NMR spectroscopy using offset-dependent nutation profiles

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Supporting Figures

Figure S1. CONDENZ profiles (row 1) acquired on the anomeric carbon of sucrose at various B₁ fields ranging from 1 - 2000 Hz. Solid lines are fits of the data to the single-spin-1/2 Bloch equations (main manuscript, Eq. 9). The χ^2_{red} surface for each fit, evaluated by keeping the B₁ field fixed at various values during the fitting routine and plotted as the difference in χ^2_{red} from the best fit value, is shown in row 2. The bootstrap distribution for each fit is shown in row 3. Rows 4 and 5 are the χ^2_{red} surfaces and bootstrap distributions for the inhomogeneity in each B₁ field. The B₁ fields and errors obtained from the bootstrap distributions are indicated above each panel in row 1, while the corresponding I_{B1} magnitudes and errors are indicated above panels in row 4. I_{B1} values were obtained only for B₁ fields larger than 5 Hz.









Figure S2. Overlay of simulated CONDENZ profiles for a single ¹³C spin (open blue circles) and an AMX spin system (A = ${}^{13}C$, M = X = ${}^{1}H$, filled red squares) for B₁ field strengths ranging from 0.5 - 10 Hz applied on-resonance to the ¹³C spin in both cases. For the AMX spin system, a 4 kHz DIPSI-2 composite pulse decoupling train is applied on-resonance to the M spin during t_{nut} , while the X spin is off-resonance by 1.3 kHz. Scalar coupling constants within the AMX spin system were set to ${}^{1}J_{AM} = 169$ Hz, ${}^{2}J_{AX} = 7$ Hz, and ${}^{3}J_{MX} = 4$ Hz. The parameters for the AMX spin system were chosen based on the assignment of the sucrose anomeric ¹³C as the A spin. The parameters common to both simulations are: $t_{nut} = 0.4$ s, R₁ = 1.7 s⁻¹, $R_2 = 2.0$ s⁻¹ and $I_{B1} = 10$ %. It can be seen clearly from the CONDENZ profiles that the single spin-1/2 (open blue circles) and AMX data (filled red squares) overlay perfectly, demonstrating that the AMX spin system in the presence of DIPSI-2 composite pulse decoupling can be very well approximated to a single-spin-1/2 system for the purposes of quantifying CONDENZ data. The B₁ field strengths recovered by fitting the AMX CONDENZ profiles to the single-spin-1/2 Bloch equations (main manuscript, Eq. 9) are tabulated in Table S3 and agree excellently with the input values, consistent with the perfect overlay between the single spin and AMX three-spin data shown in this figure.



Figure S3. CONDENZ profiles (row 1) acquired on ¹⁵N^ε-labeled Trp at various B₁ fields ranging from 1 - 50 Hz. Solid lines are fits of the data to the single-spin-1/2 Bloch equations (main manuscript, Eq. 9). The χ^2_{red} surface for each fit, plotted as the difference in χ^2_{red} from the best fit value, evaluated by keeping the B₁ field fixed at various values during the fitting routine, is shown in row 2, while the bootstrap distribution for each fit is shown in row 3. Rows 4 and 5 are the χ^2_{red} surfaces and bootstrap distributions for the inhomogeneity in each B₁ field. The B₁ fields and errors obtained from the bootstrap distributions are indicated above each panel in row 1, while the corresponding I_{B1} magnitudes and errors are indicated above panels in row 4. I_{B1} values were determined only for B₁ fields larger than 5 Hz.







Supporting Tables

B ₁ setting	<i>t</i> _{nut}	Sweep width	Offset spacing
(Hz)	(ms)	(Hz)	(Hz)
1		-15.5 to 15.5	0.25
2		-25 to 25	0.5
3		-45 to 45	1
5		-70 to 70	2
7.5	400	-90 to 90	2
10	400	-90 to 90	2
15		-200 to 200	5
20		-280 to 280	5
25		-260 to 260	6
30		-280 to 280	5
100	50	-200 to 200	5
500		-2000 to 2000	50
750	5	-2000 to 2000	50
1000		-2000 to 2000	50
1500	2.5	-5000 to 5000	100
2000	2.3	-5000 to 5000	100

Table S1. The offset sweep width, offset spacing and nutation period (t_{nut}) which were used to acquire the CONDENZ profiles in Figure 1 and Figure S1.

B _{1 setting} (Hz)	R ₁ (s ⁻¹) ^a	$R_2(s^{-1})^{b}$	Chemical shift (Hz) ^c
1	1.749 ± 0.002	2.36 ± 0.03	0.70 ± 0.003
2	1.713 ± 0.003	2.05 ± 0.05	0.70 ± 0.02
3	1.719 ± 0.002	2.01 ± 0.05	0.70 ± 0.01
5	1.722 ± 0.005	2.0 ± 0.2	0.69 ± 0.05
7.5	1.750 ± 0.003	2.0 ± 0.2	0.68 ± 0.02
10	1.730 ± 0.003	2.01 ± 0.05	0.75 ± 0.02
15	1.726 ± 0.003	2.01 ± 0.08	0.71 ± 0.03
20	1.731 ± 0.004	1.96 ± 0.09	0.75 ± 0.03
25	1.718 ± 0.003	1.89 ± 0.04	0.74 ± 0.02
30	1.711 ± 0.003	1.84 ± 0.06	0.73 ± 0.02
100	1.38 ± 0.09	3.6 ± 0.2	0.69 ± 0.04
500	2.5 ± 0.3	5 ± 1	0.8 ± 0.2
750	3.6 ± 0.6	3 ± 2	0.2 ± 0.3
1000	0.3 ± 0.6	11 ± 2	0.4 ± 0.5
1500	7.9 ± 0.7	22 ± 3	0.2 ± 0.5
2000	0.1 ± 0.5	17 ± 6	2 ± 6

Table S2: Longitudinal (R_1) and transverse (R_2) relaxation rate constants and chemical shifts obtained by fitting CONDENZ profiles at various B_1 fields to equation 9 (main manuscript).

^a R₁ values obtained at B₁ fields between 1 - 30 Hz agree very well to within ~ 2 % of each other. R₁ values obtained by fitting higher B₁ field CONDENZ profiles are unreliable for two reasons. First, t_{nut} is much smaller (2.5 - 50 ms) for B₁ fields between 100 - 2000 Hz and 400 ms for 1 - 30 Hz, as these values were optimized to detect offset-dependent modulations for reliably obtaining B₁ fields (Table S1). As a consequence, decay due to longitudinal relaxation at higher fields is much less (0.5 - 10 %) compared to 1 - 30 Hz (50 %). It, therefore, follows that R₁ values cannot be reliably extracted from such data. Second, CONDENZ profiles at lower fields spanned offset values sufficiently large to include regions of baseline on either edge, while profiles at higher fields lack these baseline regions. Since R₁ extraction from the fit depends primarily on baseline data points, R₁ values at higher B₁ fields are unreliable.

^b R_2 values at lower B_1 fields (1 - 30 Hz) agree much better with each other than with values extracted from CONDENZ profiles at higher B_1 fields. The reason for this is again similar as for R_1 values. Transverse relaxation during the acquisition of CONDENZ profiles occurs because magnetization is present in the transverse plane for approximately half the nutation time. Since t_{nut} values are much smaller at higher B₁ fields (Table S1), there is very little decay due to transverse relaxation during t_{nut} and hence R₂ values cannot be fit reliably at higher B₁ fields.

 $^{\rm c}$ The errors in chemical shift values obtained from the fitting routine increase with B₁ field primarily because the spacing between offsets (Table S1) increase at higher B₁ fields, decreasing the effective 'resolution' of the CONDENZ profile and simultaneously, the ability to precisely locate the on-resonance chemical shift offset.

Table S3: RF field amplitudes (B₁), B₁ inhomogeneities (I_{B1}), R₁ and R₂ estimated by fitting CONDENZ data simulated for an AMX spin system (A = ¹³C, M = X = ¹H) with scalar coupling values ${}^{1}J_{AM} = 169$ Hz, ${}^{2}J_{AX} = 7$ Hz, and ${}^{3}J_{MX} = 4$ Hz. The A and M spins are on-resonance to the CW and 4 kHz DIPSI-2 RF fields respectively, while the X spin is 1.3 kHz off-resonance. Input values used were: R_{1 input} = 1.7 s⁻¹, R_{2 input} = 2.0 s⁻¹, I_{B1 input} = 10 %. The CW B₁ field was varied in the simulations over a range of values from 0.5 - 10 Hz. The simulated data were then modeled using the Bloch equations for a single-spin-1/2 system (main manuscript, Eq. 9) to derive the fit parameters. The simulated data are shown in Figure S2.

B1 input (Hz)	B _{1 fit} (Hz)	I _{B1 fit} (%)	$R_{1 \text{ fit}} (s^{-1})$	$R_{2\rm fit}(s^{\text{-}1})$
0.5	0.500	9.45	1.70	2.01
0.75	0.750	9.45	1.70	2.01
1.0	0.999	9.94	1.70	2.01
2.0	1.999	9.99	1.70	2.01
3.0	2.998	10.01	1.70	2.01
5.0	4.997	10.00	1.70	2.01
7.5	7.495	10.00	1.70	2.01
10	9.994	10.00	1.70	2.00

Table S4. ¹⁵N RF fields and inhomogeneities measured using ¹⁵N^{ε}-tryptophan. All CONDENZ profiles were measured using a *t_{nut}* of 200 ms, and the offset frequency and spacing were similar to the values used for sucrose (Table S1).

B _{1 setting} (Hz)	B ₁ CONDENZ (Trp _{int} , Hz)	I _{B1} (%)
1	0.99 ± 0.06	ND
2	2.09 ± 0.01	ND
5	5.17 ± 0.02	ND
7.5	7.79 ± 0.01	6.0 ± 0.4
10	10.39 ± 0.01	5.3 ± 0.2
15	15.65 ± 0.01	4.77 ± 0.09
20	20.87 ± 0.01	4.40 ± 0.06
30	31.35 ± 0.02	4.0 ± 0.1
40	41.87 ± 0.03	3.41 ± 0.04
50	52.27 ± 0.03	3.03 ± 0.03

ND: Not determined

Table S5: A comparison of RF fields measured on ¹⁵N nuclei using a) the on-resonance nutation experiment on the Gln62 amide resonance of U-¹⁵N ubiquitin, b) the CONDENZ approach with ¹⁵N^{ε}-tryptophan as an internal standard in the sample containing U-¹⁵N ubiquitin, c) CONDENZ profiles with ¹⁵N^{ε}-tryptophan as an external standard in a separate sample but without changing the tuning and matching capacitor settings after measuring the B₁ field on the U-¹⁵N ubiquitin sample, and d) CONDENZ data on a separate ¹⁵N^{ε}-tryptophan sample after tuning and matching. B₁ _{nutation} values are reported to the same number of decimal places as the B₁ _{CONDENZ} values to facilitate comparison.

B _{1 nutation} (ubiquitin, Hz)	B ₁ CONDENZ (Trp _{int} , Hz)	B ₁ CONDENZ (Trp _{ext} , Hz)	B1 CONDENZ (Trpext, after tuning/matching, Hz)
0.86	0.99 ± 0.06	1.03 ± 0.03	1.08 ± 0.01
2.08	2.09 ± 0.01	2.07 ± 0.02	2.22 ± 0.02
5.19	5.17 ± 0.02	5.19 ± 0.01	5.24 ± 0.02
10.47	10.39 ± 0.01	10.44 ± 0.01	10.51 ± 0.01
15.67	15.65 ± 0.01	15.70 ± 0.01	15.80 ± 0.01
20.93	20.87 ± 0.01	20.99 ± 0.02	21.12 ± 0.01
31.36	31.35 ± 0.02	31.42 ± 0.02	31.69 ± 0.02
41.82	41.87 ± 0.03	41.99 ± 0.01	42.26 ± 0.02

Table S6: Summary of RF fields measured using the CONDENZ method on the methyl carbon of ¹³CH₃ α -ketobutyric acid ($\varpi = 6.5$ ppm), the anomeric ¹³C of sucrose ($\varpi = 92.2$ ppm), the aromatic ¹³C of benzaldehyde ($\varpi = 135.5$ ppm, the carbon at the ortho position to the aldehyde group) and the aldehydic ¹³C of benzaldehyde ($\varpi = 196.5$ ppm).

B _{1 setting} (Hz)	B_1 (Hz) ${}^{13}C^{\delta} = 6.5$ ppm	B_1 (Hz) ${}^{13}C^{\delta} = 92.2 \text{ ppm}$	B_1 (Hz) ${}^{13}C^{\delta} = 135.5$ ppm	B_1 (Hz) ${}^{13}C^{\delta} = 196.5$ ppm
2	1.72 ± 0.02	2.1 ± 0.2	2.03 ± 0.01	1.98 ± 0.01
5	5.11 ± 0.01	5.13 ± 0.02	5.11 ± 0.01	5.09 ± 0.01
10	10.18 ± 0.02	10.32 ± 0.03	10.21 ± 0.02	10.17 ± 0.01
20	20.31 ± 0.01	20.63 ± 0.02	20.43 ± 0.01	20.21 ± 0.03