^{15}N and ^{13}C- SOFAST-HMQC editing enhances 3D-NOESY sensitivity in highly deuterated, selectively [^{1}H,^{13}C]-labeled proteins.

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Supporting Information

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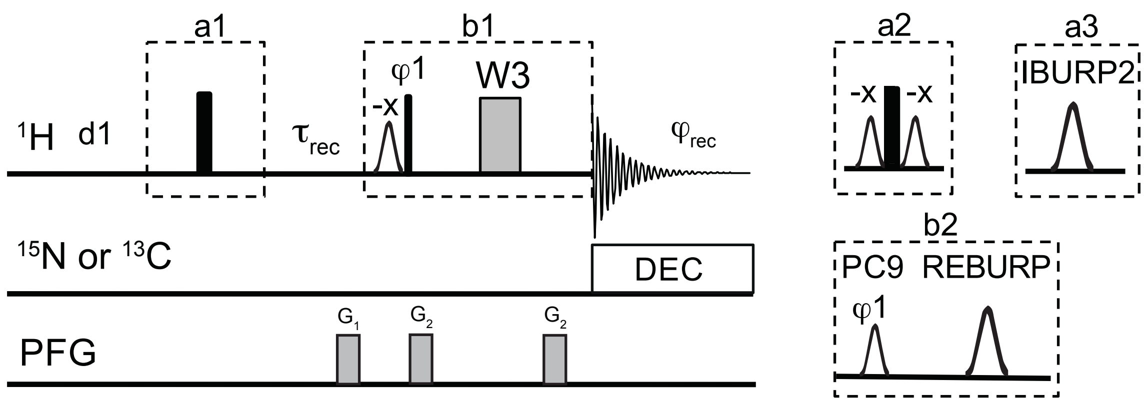
Table S1. Measurement of selective T_1 recovery in ^{15}N, [^{1}H,^{13}C]-Ile$_{\delta1}$, [50%-^{1}H,^{13}C]-Leu$_{\delta1,\delta2}$, and Val$_{\gamma1,\gamma2}$ MBP at 32 °C.*

Variant of pulse sequence	$^{1}H_N$ T_1 (s)	$^{1}H_M$ T_1 (s)
A 180° hard pulse (a1 + b1)	0.79	0.89
A 180° hard pulse with water flip-back 90° pulses (a2 + b1)	0.62	0.84
A 180° shaped IBURP2 pulse (a3 + b1)	0.40	0.58
A 180° shaped IBURP2 pulse (a3 + b2)	0.40	0.60

*Inversion recovery measurements were conducted using the pulse sequence below with the a1, a2, a3, b1 and b2 variants. Faster recovery (shorter T_1) is observed when pulsing selectively on either amides or methyls ^{1}H's and keeping water magnetization at equilibrium.



The pulse sequence of inversion recovery for the measurement of longitudinal relaxation time T_1. The narrow and wide bars represent 90° and 180° hard pulse. 1 ms long ^{1}H 90° water flip-back shaped pulse is of Sinc profile. Other ^{1}H shaped pulses are of 1.69 ms PC9, 1.0 ms IBURP2 and 1.15 ms REBURP (Geen and Freeman 1991), respectively. The phase cycling is: φ1 = (x, -x, -x, x), φ_{rec} = (x, -x, -x, x). GARP is used for ^{15}N or ^{13}C-decoupling. The durations and strengths of the gradients are G1 = (1 ms, 15 G/cm), G2 = (1 ms, 5 G/cm). Interscan delay (d1) is 4 s, and τ is variable delay setting to 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.2, 1.6, 2.0 s, respectively, for each 1D spectrum. Different combinations are used to measure the T_1 to evaluate the impact of water exchange transfer and isolate the effect of each individual moiety on overall relaxation. Inset a2 or a3 may replace a1, and inset b2 may replace b1. When measuring $^{1}H_N$'s T_1, ^{15}N-decoupling with its carrier offset at 118 ppm is used, and the offsets of the shaped pulses PC9, IBURP2 and REBURP are set to 8.5 ppm. When measuring $^{1}H_M$'s T_1, ^{13}C-decoupling with its carrier offset at 17 ppm is used, and the offsets of the shaped pulses PC9, IBURP2 and REBURP are set to 0.9 ppm.

Supplementary Table S2* List of SOFAST-NOESY sequences.

Index	SOFAST Sequence	Acquisition Dimension			No. of spectra	Flip Angle (α)	Excitation shaped pulse	Refocusing shaped pulse	Reference Sequence	Test Sample
		F1(t_1)	F2(t_2)	F3(t_3)						
1	$C_MNH_MH_N$ (CN-HMQC)	N, C_M	H_N, H_M		1	120°	PC9_4	Reburp		a, b
	3D HMQC-NOESY-HMQC									
2	N-C_MH_M	N	C_M	H_M	1	90-95°	PC9_4	Reburp	HMQC-NOESY-HMQC for N-CH	b
3	C_M-NH_N	C_M	N	H_N	1	90-95°	PC9_4	Reburp		b
4	N-NH_N	N	N	H_N	1	110°	PC9_4	Reburp	HMQC-NOESY-HMQC for N-NH	b
5	C_M-C_MH_M	C_M	C_M	H_M	1	110°	PC9_4	Reburp	HMQC-NOESY-HMQC for C-CH	a
6	C_{Aro}-C_MH_M	C_{Aro}	C_M	H_M	1	90-95°	PC9_4	Reburp	HSQC-NOESY-HMQC for C-CH	a,c
7	C_{Aro}-NH_N	C_{Aro}	N	H_N	1	90-95°	PC9_4	Reburp		a
8	NC_M-$NC_MH_NH_M$	N, C_M	N, C_M	H_N, H_M	4	110° (90°)	Cos-PC9_4	W5'	Time Shared (TS) H-CN-H HSQC-NOESY-HSQC§	b
	3D NOESY-HMQC									
9	H_NH_{Aro}-C_MH_M	H_N, H_{Aro}	C_M	H_M	1	90-95°	PC9_4	Reburp	NOESY-HMQC	a
10	H_M-C_MH_M	H_M	C_M	H_M	1	110°	PC9_4	Reburp	HMQC-NOESY-HMQC for H-CH	a
11	H_M-$C_{Aro}H_{Aro}$	H_M	C_{Aro}	H_{Aro}	1	90-95°	PC9_4	Reburp		b
12	H_NH_{Aro}-NH_N	H_N, H_{Aro}	N	H_N	1	110°	PC9_4	Reburp		a
13	H_NH_M-C_MH_M	H_N, H_M	C_M	H_M	2	90-95°	PC9_4	Reburp		b
14	H_N-$NC_MH_NH_M$	H_N	N, C_M	H_N, H_M	2	110° (90°)	PC9_4	W5'		b
15	H_NH_M-$NC_MH_NH_M$	H_N, H_M	N, C_M	H_N, H_M	4	120° (90°)	cosine and sine-modulated PC9_4	W5'		b

* Entries are divided into the 3D-HMQC-NOESY-HMQC and 3D NOESY-HMQC groups. The underlined entries correspond to experiments described in full in the main text. The value of flip angle in parentheses give artifact-free spectra. Experiments 8, 13, 15 are intended for ^{15}N, methyl labeled proteins with no aromatic residues. Test samples: FliT-FliJ fusion ^{15}N-methyl/aromatic labeled (a); ^{15}N-methyl MBP (b). FliT-FliJ fusion double labeled (c).

§See Xia and coworkers for the 'Time Shared' (TS) CN-CN-H HSQC-NOESY-HSQC and H-CN-H NOESY-HSQC experiments.(Xia et al. 2003)

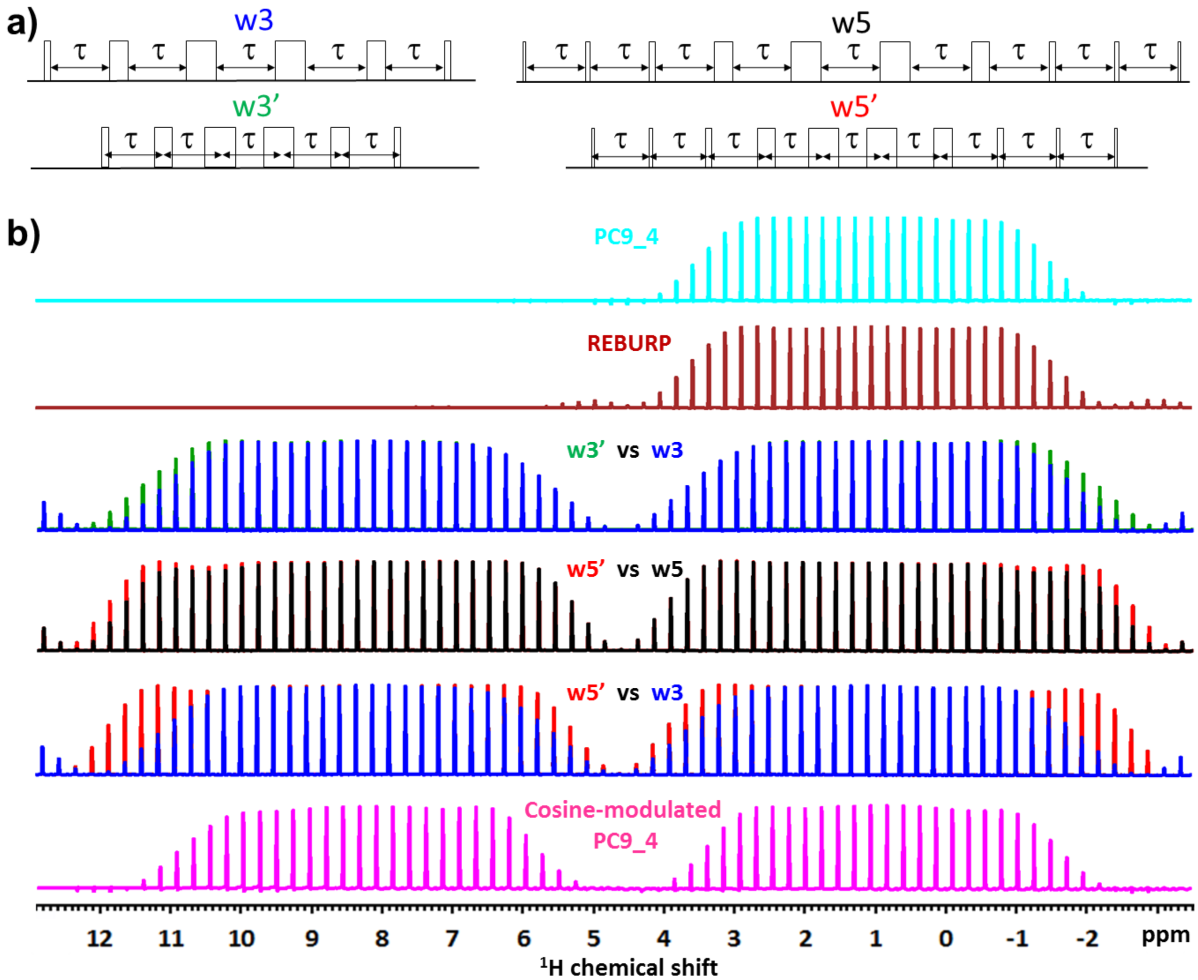


Figure S1. Experimental characterization of selective pulses excitation profiles. Pulse sequences of w3, w3', w5, w5' (a) and observed excitation profiles (b). In the pulse sequences w3 and w3', flip angles (α) and phases were 20.8°$_{x}$, 62.2°$_{x}$, 131.6°$_{x}$, 131.6°$_{-x}$, 62.2°$_{-x}$, and 20.8°$_{-x}$, respectively. In the pulse sequences w5 and w5', flip angles and phases were 7.8°$_{x}$, 18.5°$_{x}$, 37.2°$_{x}$, 70.0°$_{x}$, 134.2°$_{x}$, 134.2°$_{-x}$, 70.0°$_{-x}$, 37.2°$_{-x}$, 18.5°$_{-x}$, 7.8°$_{-x}$, respectively. τ was 188 μs (= 1/d, d is the distance in Hz between center and next null). To get the observing profiles of the PC9_4, REBURP, w3, w3', w5 and w5', a spin echo pulse sequence (90°$_{x}$ + PFG +200 μs + 180°$_{x}$ + PFG +200 μs + observe) was used. For PC9_4 profile, the 90°$_{x}$ was replaced with the PC9_4 shaped pulse and the 180° pulse was a hard pulse. For REBURP, w3, w3', w5, w5's profiles, the 90°$_{x}$ was a hard pulse and the 180° pulse was replaced with one of the REBURP, w3, w3', w5 and w5', respectively. The bandwidths of PC9_4, REBURP and cosine-modulated PC9_4 all were 4,250 Hz (5 ppm on 850 MHz spectrometer). To get the profile of cosine-modulated PC9_4, the 2D NC-HMQC pulse sequence was used. A Bruker standard sample of 0.1 M ^{13}C-labeled methanol in DMSO was used for the tests. Frequency offset was swept from -7,000 to 7,000 Hz in 200 Hz steps. For each profile, a total of 71 1D spectra were acquired. Each profile pair, "**w3' vs w3**", "**w5' vs w5**", and "**w5' vs w3**" is color-coded and superposed. A cosine-modulated PC9(Kupce and Freeman 1994) shaped pulse and a W5-type inversion composite pulse(Liu et al. 1998) are used to excite and refocus both amide ^1H_N and methyl ^1H_M, respectively. The ^{15}N and ^{13}C spectral widths can be adjusted separately to their optimal values. W5', a modified version of W5(Liu et al. 1998) with the pulse spacing τ measured from the center of the pulse instead of the pulse edge (Supp. Fig. S1) provides more ample and uniform inversion profile. However, either 3-9-19-WATERGATE (W3)(Sklenar et al. 1993) or W5 give bandwidth sufficient to cover the narrower excitation profile of the PC9_4 selective pulse. The PC9_4 pulse with appropriate ^1H offset can have up to approximately 5ppm excitation bandwidth. Higher bandwidth settings reduce the water suppression effect. The cosine modulation produces a dual 5ppm excitation profile centered at the water resonance (^1H chemical shift ~4.7ppm).

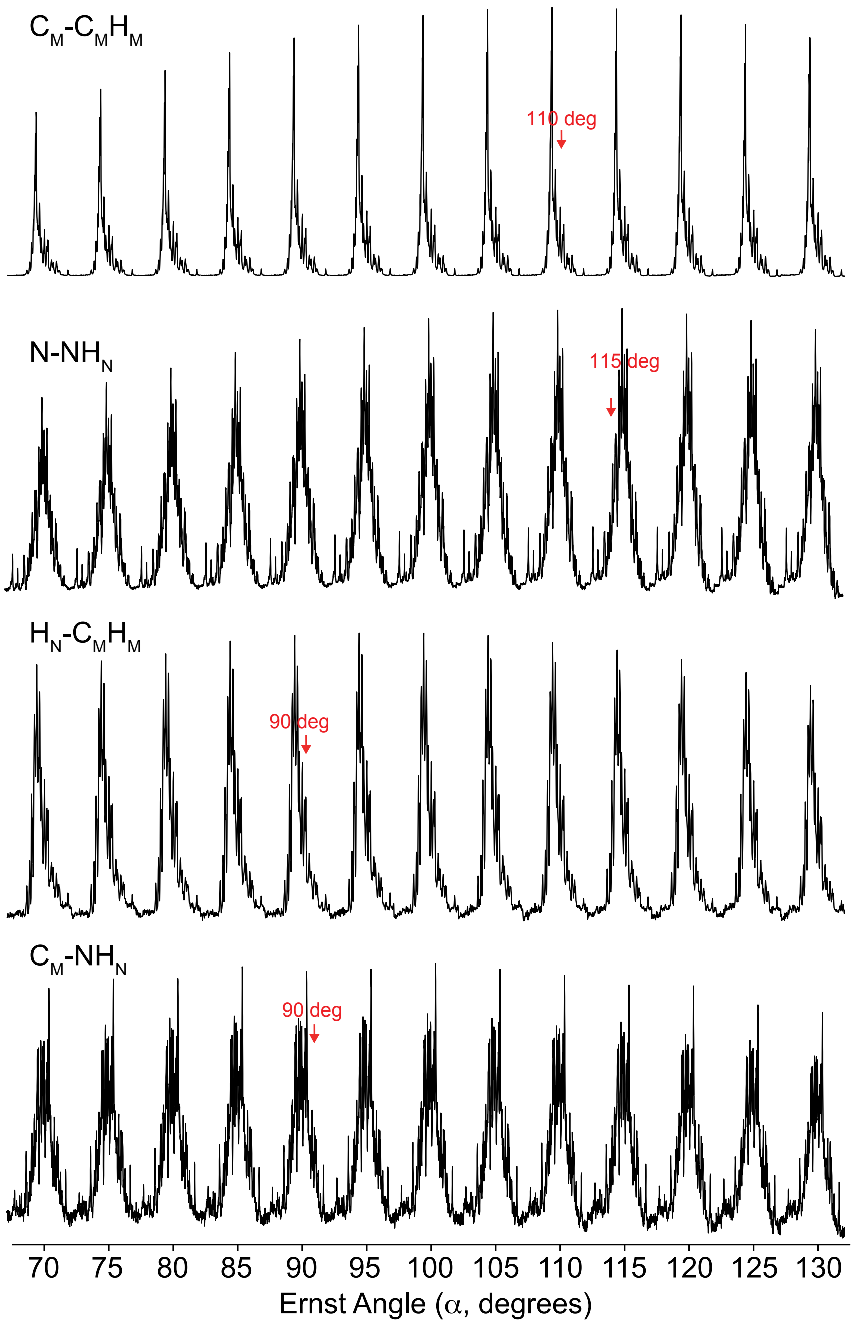


Figure S2. Ernst angle (α pulse) optimization for the four SOFAST 3D HMQC-NOESY-HMQC variants as labeled. The optimization was conducted with d1 = 0.2 s. The optimal α value is indicated in red on each spectrum. Compatible magnetization that can transfer between scans is present only for X-XH type experiments. Low to no effect (< 5%) is observed in X-YH type experiment since the magnetization cannot be transferred from scan to scan. In that X-YH the α pulse should be set to 90-95 degrees.

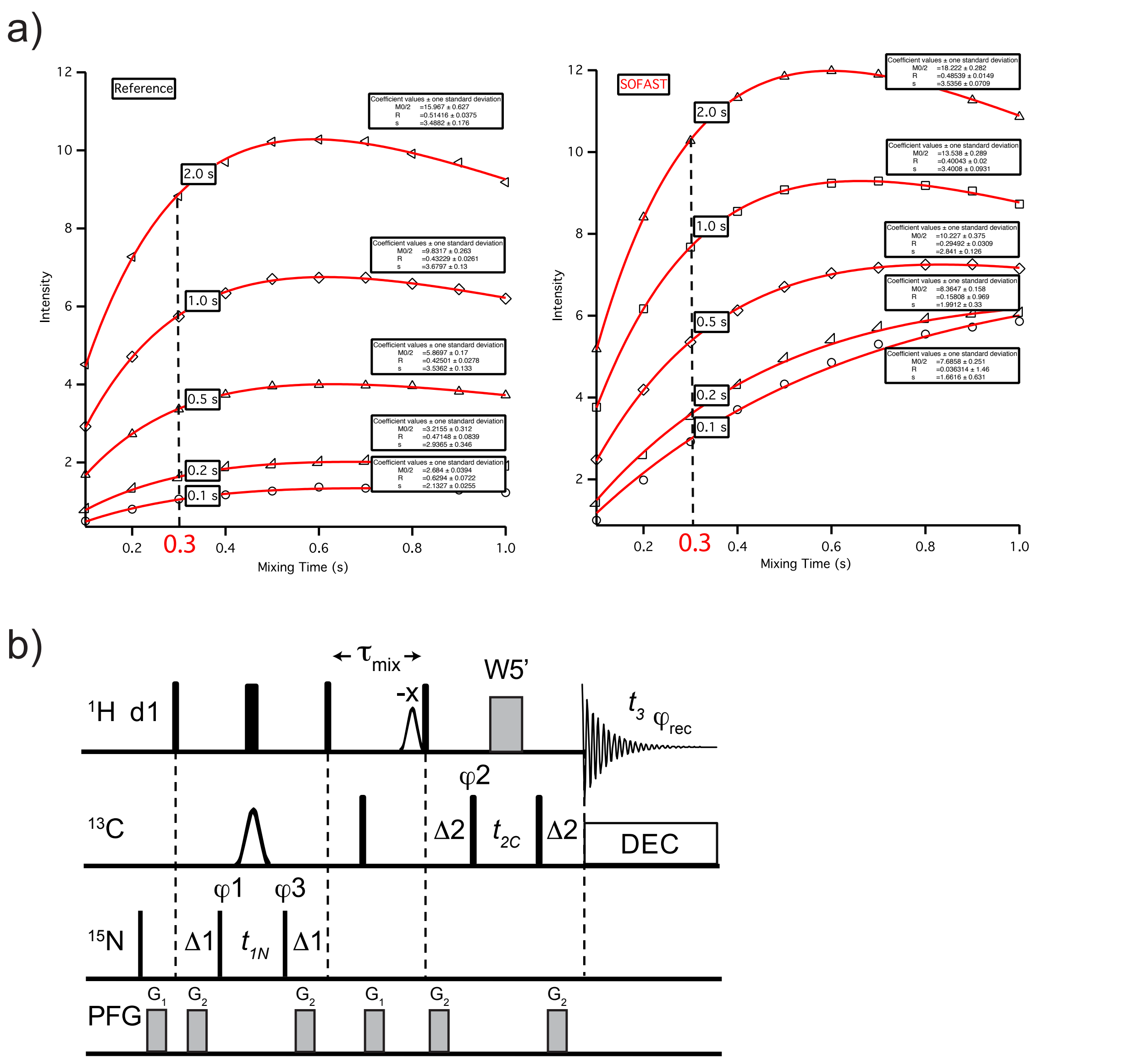


Figure S3. NOE buildup curves for MBP U-^{15}N, [1H, 13C]-Ile, Leu, and Val methyl sample at 32 °C (a). Comparison the of reference and SOFAST HMQC-NOESY-HMQC (main text Fig. 1d) NOE buildup curves obtained with as a series of 1D spectra acquired with 64 scans. Integrated intensities vs. mixing time were plotted at five different d1 values: 0.1, 0.2, 0.5, 1.0 and 2.0 s as labeled on the graph. Curves were fitted as described in the main text. Reference diagonal-free 3D HMQC-NOESY-HMQC (^{15}N(F_1)-^{13}C$_M$(F_2)-^{1}H$_M$(F_3)) with optimized W5' H_2O suppression (Liu et al. 1998) and flip-back pulse (b). The narrow and wide bars represent 90° and 180° hard pulse. The shaped pulse on ^{13}C channel represents a 500 μs long 180° smoothed CHIRP.(Hwang et al. 1997) The delays are: d1 = 0.2 sec, Δ1 = 5.2 ms, Δ2 = 4.0 ms, τ_{mix} = 0.3 sec. The phase cycling are: φ1 = (x, -x), φ2 = (x, -x , -x, x), φ3 = 4(x), 4(-x), φ_{rec} = (x, -x, -x, x, -x, x, x, -x). Bruker decoupling scheme bi_garp_2pl is used. The quadrature detections in $t1$ and $t2$ dimensions are acquired via States-TPPI of φ1 and φ2, respectively. The durations and strengths of the gradients are G1 = (1 ms, 15 G/cm), G2 = (1 ms, 5 G/cm).

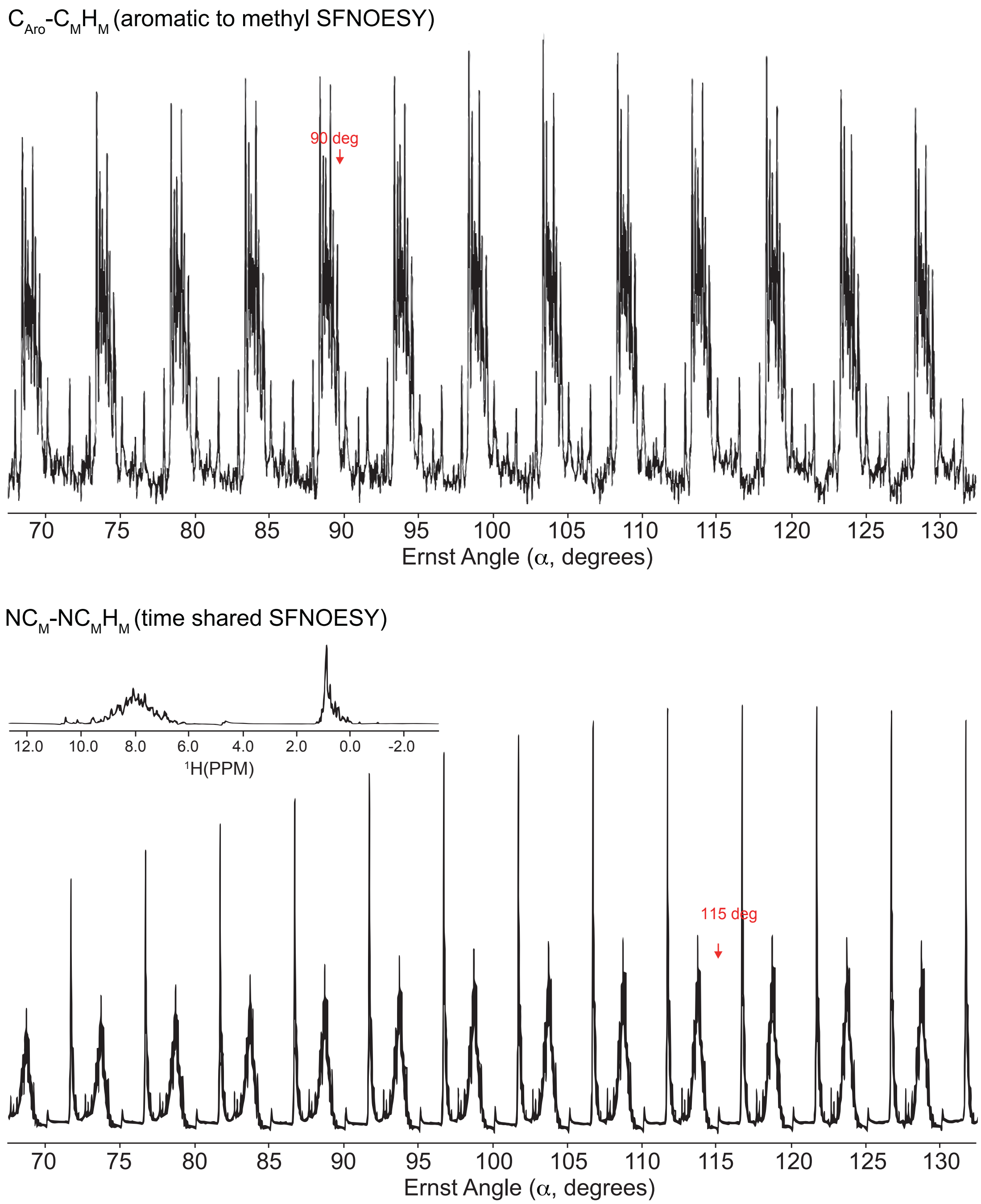


Figure S4. Ernst angle (α pulse) optimization for time-shared SOFAST 3D CN-HMQC-NOESY-CN-HMQC (upper) and for aromatic-methyl SOFAST 3D HMQC-NOESY-HMQC (lower) at fast pulsing regime (d1= 0.2 s). The optimal value for α was found to be 115 degrees, and 90 degrees respectively. Please note: the SOFAST 3D CN-HMQC-NOESY-CN-HMQC is more sensitive to the changes in α due to both amide and methyl magnetization sustaining the steady state polarization during fast pulsing. Lower effect (< 5%) is observed in X-YH-type aro-methyl experiment (b) as the magnetization cannot be transferred from scan to scan the pulse angle should be kept at 90-95°.

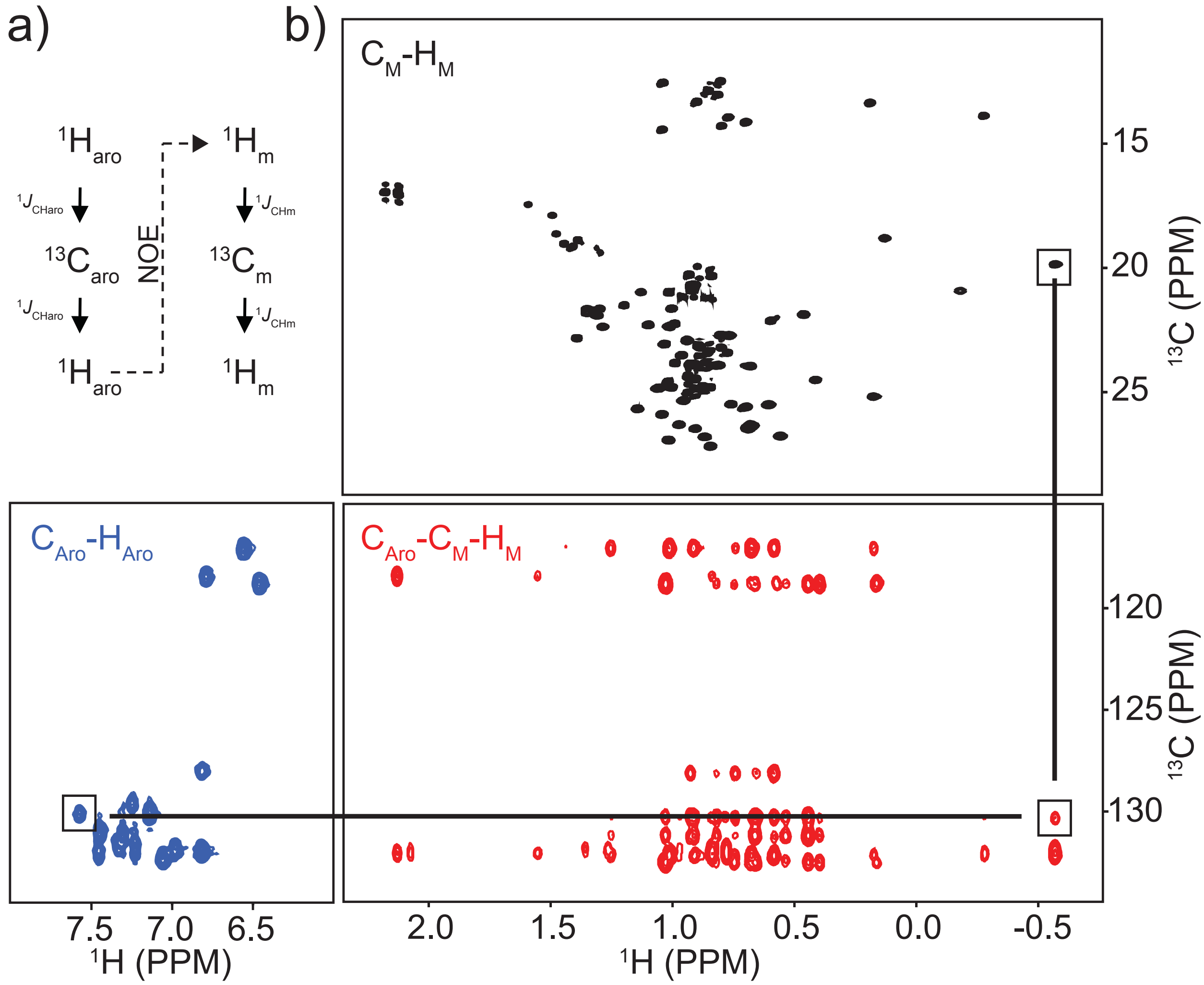


Figure S5. Panel a): schematic view of diagonal-free aromatic to methyl 3D-^{13}C-SFHMQC-NOESY-HMQC experiment. Panel b): reference 2D $^{13}C_{Aro}$-SFHMQC spectrum (lower left spectrum in blue), reference 2D $^{13}C_{M}$-SFHMQC (upper right in black), and 3D SFHMQC-NOESY-HMQC projection (lower right in red). The FliT-FliJ protein used for testing is *U*-[^{2}H,^{15}N] and contains ^{1}H-^{13}C on all methyl residues and *U*-[^{13}C,^{15}N] on Phe and Tyr. The d1 was 200 ms with 32 scans per point and 40 × 80 points and a total experiment time of 16 h. An example NOE crosspeak between Phe Hε and Val Cγ is marked on the spectrum and connected to the respective 2D reference dimensions.

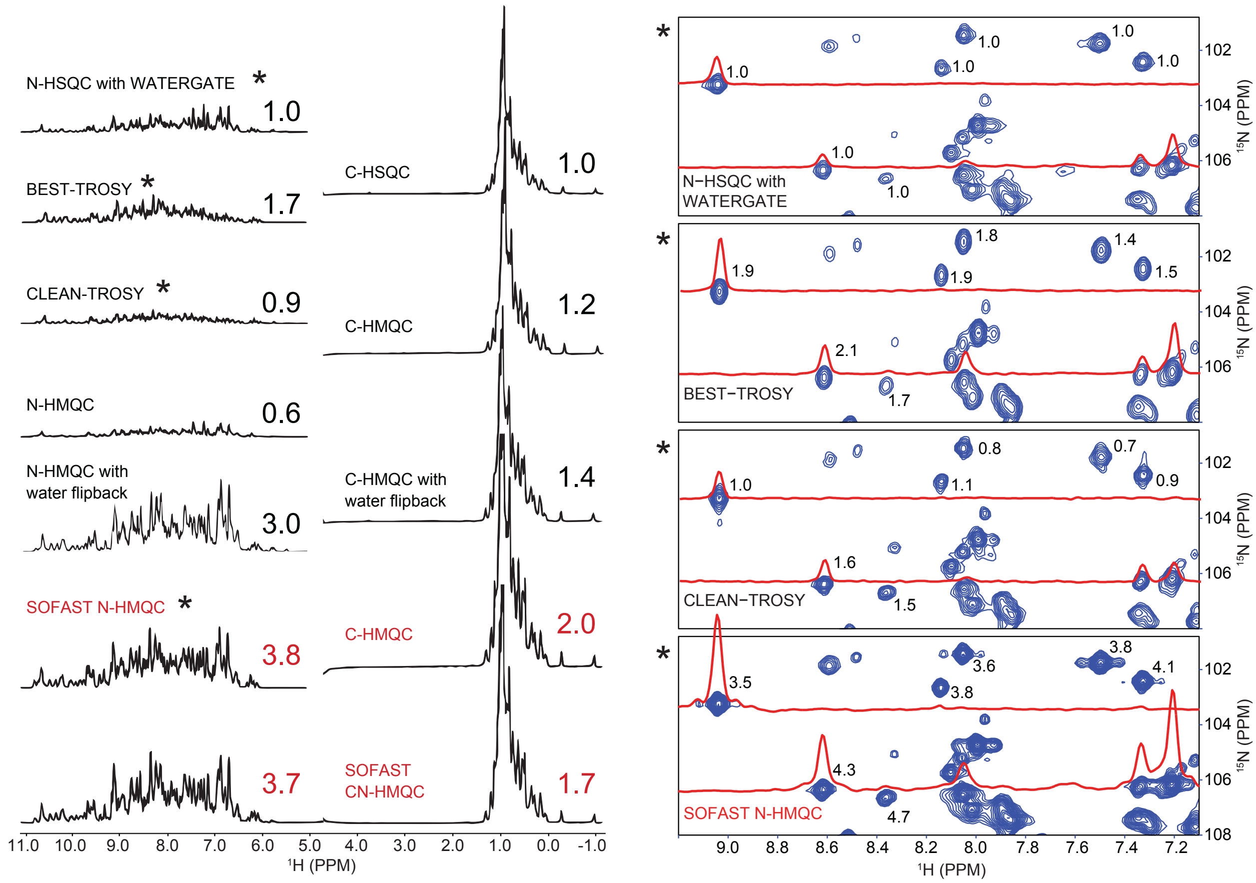


Figure S6. Comparison of the signal strength for ^{15}N and ^{13}C 2D HSQC, HMQC, SOFAST-HMQC and TROSY experiment labeled as indicated. All the ^{13}C correlation experiments use States-TPPI for phase sensitive 2D acquisition. All S/N figures indicated on the 1D strips on the left panel are derived from 2D data analysis. A sample of such analysis is shown on the right panel for selected ^{15}N based experiments marked with an asterisk. The ratios of signal strengths are scaled separately to the ^{15}N and ^{13}C HSQC that are taken as '1'. All experiments(Schanda et al. 2005; Schulte-Herbruggen and Sorensen 2000; Solyom et al. 2013) were performed with same parameters using an ^{15}N-methyl labeled MBP sample at 32 ºC. The recovery delay (d1) was set to 0.2 s. In addition to recapitulating the findings of Schanda and coworkers(Schanda et al. 2005), a few interesting points emerge from analysis of the results. (*i*) The ^{15}N-BEST-TROSY (Solyom et al. 2013) is 1.7× more sensitive with fast pulsing than the 'N-HSQC with WATERGATE' while the CLEAN-TROSY (Schulte-Herbruggen and Sorensen 2000) is weaker (0.9×) than the ^{15}N-BEST-TROSY due to more complicated pulse sequence and extra pulses that are used to remove anti-TROSY components. The main benefit of ^{15}N-TROSY is to select for the slow relaxing components and it does so at the expense of overall sensitivity, while the resolution and sensitivity can be improved by increasing both t_2 and t_1 acquisition times. Please note that the TROSY experiment uses Echo-AntiEcho (Rance-Kay) for phase sensitive acquisition and that HMQC uses States-TPPI. The former enhances sensitivity in 2D by a $\sqrt{2}$ factor.

(*ii*) Comparing the two spectra acquired with 'N-HMQC' and 'N-HMQC with water flip-back', the signal strength of the latter is 5.0× (3.0/0.6) that of the former. Here, the prodigious improvement is the result of the short d1 (d1 = 0.2 s) employed for the comparison. Under these conditions, the water magnetization recovery to steady state in the 'N-HMQC' is very minimal, and the water flip-back pulse in the 'N-HMQC with water flip-back' produces the observed boost in signal. The factor is reduced from 5.0 (d1 = 0.2 s) to 2.5 (5.5/2.2) with d1 set at 1 s as shown in Supp. Fig. S4a. We also added a water flip-back pulse in the C-HMQC sequence and found that it had a significant effect on the sensitivity (1.2 to 1.4) but lower than on the amide spectrum.

(*iii*) Between 'N-HMQC with water flip-back' and SOFAST N-HMQC, the signal ratio is only changed by approximately 1.3× (from 3.0 to 3.8). These experiments should have similar water suppression effects at longer d1, and the other differences between the two are that the SOFAST N-HMQC uses 120° flip angle and the methyl's magnetization, together with the ~5% spurious protonation present in the sidechains our deuterated sample, in SOFAST N-HMQC is not perturbed resulting in a shorter T_1 relaxation time.

(*iv*) The SOFAST CN-HMQC simultaneously acquires two HMQC with a 15% S/N sacrifice for its C-HMQC region and no L-optimized effect. At fast pulsing it manages to perform better than WATERGATE sequences with flip-back mainly due to Ernst angle effect. Presently, the N-HMQC spectral region in the SOFAST CN-HMQC does not use ^{13}C-decoupling pulse at the center of t_1 evolution. However, with its intended use for ^{15}N, and selectively [^{1}H-^{13}C]-methyl labeled samples this is no cause for concern. In these types of samples, CA and CO are not ^{13}C-labeled or expected to be. When running the experiment with *U*-^{13}C labeled samples, cosine-modulated SEDUCE1 can be used to decouple ^{13}CA/^{13}CO from ^{15}N (Mccoy and Mueller 1992). The S/N of N-HMQC part is very close to that of simple SOFAST N-HMQC (3.7 vs 3.8) but still better than simple N-HMQC with flip-back (3.0). However, the C-HMQC part in the SOFAST CN-HMQC has an additional delay compared to a regular C-HMQC. During the first FID, the additional delay is $2(\Delta_2 - \Delta_1)$ and for the last FID, the addtional delay becomes $TD\left(\frac{1}{2SW_N} - \frac{1}{2SW_C}\right) + 2(\Delta_2 - \Delta_1)$, where TD, SW_N, SW_C are time domain size (2× the number of increments), spectral widths of ^{15}N and ^{13}C, respectively. Given the normal values that are used for this experiment (TD = 148, SW_N = 3101 Hz, SW_C = 4383 Hz, Δ_2 = 5.2 ms, Δ_1 = 4.0 ms), the additional delay goes from 2.4 ms to 9.4 ms. This compromise results in a modest 15% (=0.3/2.0) signal loss. A small penalty in the signal strength of the C-HMQC part in the SOFAST CN-HMQC is paid, but in the end, the signal for the key methyl moieties is still 1.4 times (=1.7/1.2) of that of regular C-HMQC. In 3D SOFAST NOESY experiments based on the 2D CN-HMQC, the TD may be set to as low as 64 points. In that case, the range of additional delay from first to last FID will be lower (2.4 ms to 5.4 ms), and the signal decay should be lower than 15%.

(*v*) The signal of the C-HMQC is 1.2× that of the C-HSQC. This increment is due to the shorter pulse sequence of the C-HMQC versus the C-HSQC and the methyl-TROSY effect.(Tugarinov et al. 2003) Also, SOFAST C-HMQC brings substantial improvement (1.2× to 2.0×) by means the fast recovery time between experiments due to its shorter T_1 (L-optimized effect) and by Ernst Angle optimization (120° flip angle) during fast pulsing.

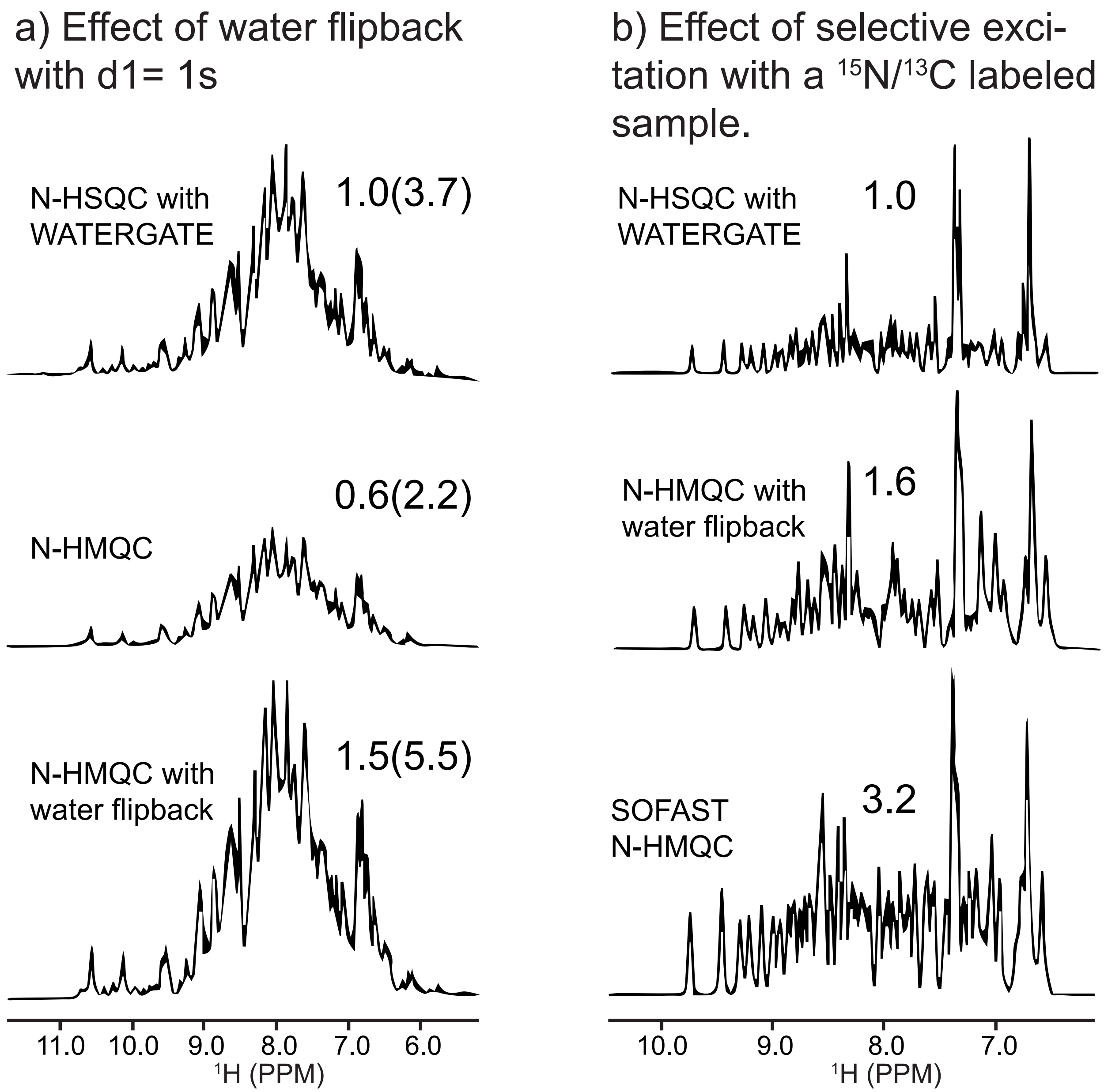


Figure S7. Comparison of the signal strength for the 2D HSQC, HMQC with and without water flip back, and SOFAST N-HMQC. The shown 1D spectra are from the positive projections of full 2D spectra. The ratios of signal strengths separately are scaled to the HSQC that are taken as ‘1’ for a) the values in parenthesis are scaled to the values in Fig. 4 for direct comparison. All experiments in panel a) or b) were performed with same parameters. In panel a), sample is an ^{15}N-methyl labeled MBP sample at 32 ºC, and the recovery delay (d1) was set to 1.0 sec. In panel b), sample is a uniformly ^{15}N/^{13}C-labeled protein (95 residues) run at 25 ºC (25 mM potassium phosphate, pH 6.5, 1 mM NaN_3), and the recovery delay (d1) was set to 0.2 sec.

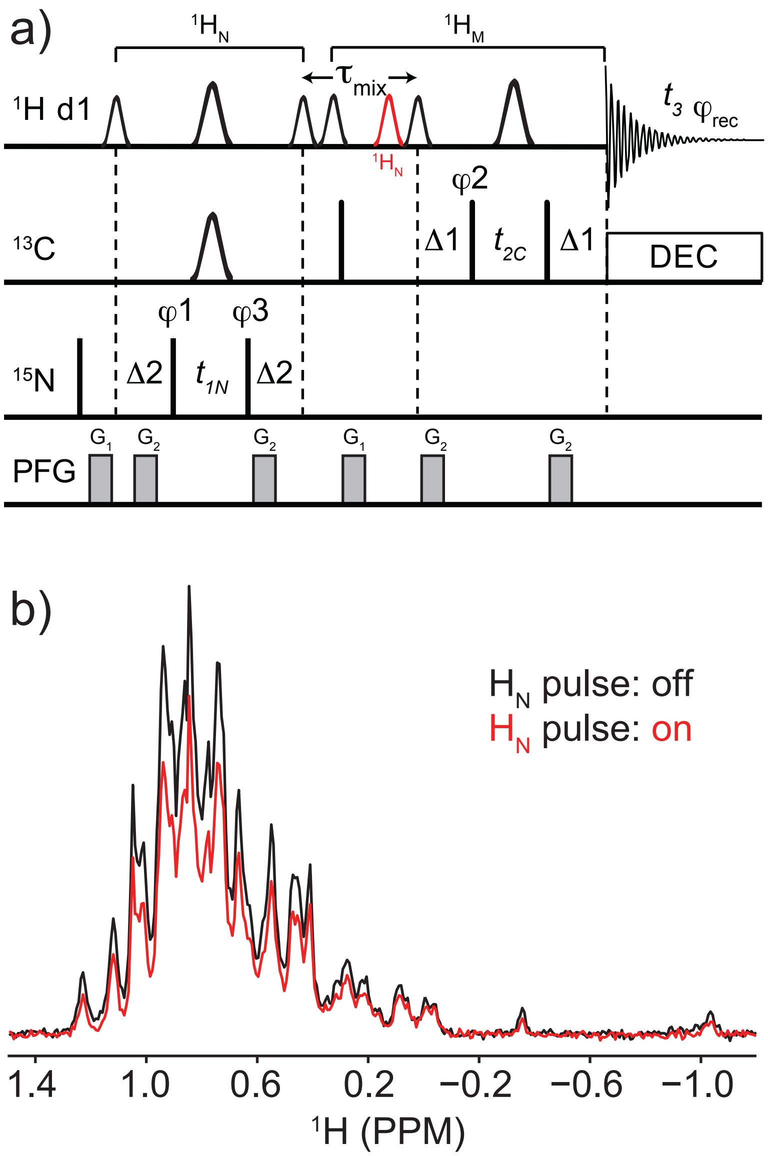
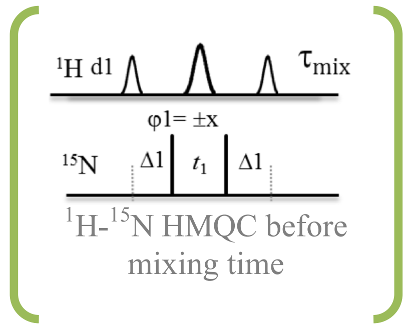


Figure S8. Characterization of the effect of residual $-I_z^X$ magnetization on signal intensity in X-YH-type experiments. The 'X' index refers to magnetization of protons attached to either C_M or N or C_{aro} in the case of frequency labeling of different moiety type during *first* and *second* HMQC editing in 3D HMQC-NOESY-HMQC. Panel a): pulse sequence as in Fig. 1d where 'X' = N. The shaped pulse in red labeled '^1H_N' is a test flip-down pulse that removes I_z^N magnetization recovered during mixing time τ (300 ms). Panel b): signal intensity with (black trace) or without (red trace) enhanced I_z^N recovery.

Appendix S1.

The following analysis is conducted to explain the two S/N mechanisms in the SOFAST-NOESY experiment: 1) the predominant effect of Ernst angle α in 3D SOFAST N(t1)-N(t2)H_N(t3) (or any X-XH type) and 2) the enhanced recovery mechanism in N(t1)-C_M(t2)H_M(t3) or (X-YH type) NOESY experiments.

For the ^{1}H-^{15}N HMQC before NOE, the evolution of signal based on spin operator is given below:



$$kI_z \xrightarrow{\frac{\pi}{2}I_x} -kI_y \quad (1a)$$

$$\xrightarrow{\frac{\pi}{2}2I_zS_z} 2kS_zI_x \quad (1b)$$

$$\xrightarrow{\pm\frac{\pi}{2}S_x} \mp 2kS_yI_x \quad (1c)$$

$$\xrightarrow{\Delta\omega_1 t_1 S_z + \pi I_x} \mp 2k[S_y \cos(\Delta\omega_1 t_1) - S_x sin\,(\Delta\omega_1 t_1)]I_x \quad (1d)$$

$$\xrightarrow{\frac{\pi}{2}S_x} \mp 2k[S_z \cos(\Delta\omega_1 t_1) - S_x sin\,(\Delta\omega_1 t_1)]I_x \quad (1e)$$

$$\xrightarrow{\frac{\pi}{2}2I_zS_z} \mp k(2I_z)^2 I_y \cos(\Delta\omega_1 t_1) \pm 2kS_xI_x sin\,(\Delta\omega_1 t_1) \quad (1f)$$

$$\xrightarrow{\frac{\pi}{2}I_x} \mp kI_z \cos(\Delta\omega_1 t_1) \pm 2kS_xI_x sin\,(\Delta\omega_1 t_1) \quad (1g)$$

$$\xrightarrow{gradient\ PFG\ during\ \tau} \mp kI_z \cos(\Delta\omega_1 t_1) \quad (1h)$$

In the above derivations, ^{1}H chemical shift is not included because ^{1}H π pulse in the middle of the HMQC refocuses the ^{1}H chemical shift evolution. After the initial $\frac{\pi}{2}$ (90°) pulse, ^{1}H polarization is changed to ^{1}H coherence I_y (eq. 1a). The scalar *J* coupling $^1J_{NH}$ ($\pi\ ^1J_{NH}\Delta_1 = \frac{\pi}{2}$) during the first Δ_1 period changes the ^{1}H's coherence I_y into antiphase coherence S_zI_x (eq. 1b). The first ^{15}N $\frac{\pi}{2}$ pulse of phase ±x changes the antiphase coherence into multiple quantum (zero and double quantum) coherence (S_yI_x) (eq. 1c). Please note that different phase (φ_1 = ±x) corresponds to the different sign (∓) of signal. During evolution t_1 period, the $^1J_{NH}$ coupling is refocused by the ^{1}H π pulse, and ^{15}N chemical shift and the ^{1}H π pulse are applied so that the coherence is frequency-labeled (eq. 1d). The second ^{15}N $\frac{\pi}{2}$ pulse changes the first term of multiple quantum coherence S_yI_x back into an antiphase coherence S_zI_x and leaves the second term S_xI_x unchanged (eq. 1e). The scalar J coupling $^1J_{NH}$ during the second Δ_1 period changes the antiphase coherence S_zI_x back into ^{1}H coherence I_y and leaves the second term still S_xI_x still unchanged (eq. 1f). Here please note that $(2I_z)^2 = 1$. Additionally, I_zS_z and I_xS_x commute ($[I_zS_z, I_xS_x] = 0$), i.e. $^1J_{NH}$ coupling does not affect multiple quantum coherence, therefore the second term S_xI_x is not changed. The second ^{1}H $\frac{\pi}{2}$ pulse changes the ^{1}H coherence I_y back into ^{1}H I_z polarization and leaves the second term unchanged (eq. 1g). Finally gradient during mixing time τ destroys the multiple quantum S_xI_x.

Appendix S2. Pulse program for 2D $C_MNH_MH_N$ SFHMQC (Bruker Topspin ver. 2.1 to 3.5).

```
;sfCNhmqcgpph19: sofast simultaneous 2D 15N/13C HMQC for HN and methyl
;Youlin Xia on 05/05/2016

;$CLASS=HighRes
;$DIM=3D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

prosol relations=<triple>

#include <Avance.incl>
#include <Delay.incl>
#include <Grad.incl>

"p4=p3*2"
"p19=300"
"p22=p21*2"
"d11=30m"
"d13=4u"
"d14=4.0m"   ;for 13C
"d15=5.2m"   ;for 15N

"d0=0u"
"d10=0u"
"in0 =inf1/2"                    ;cnst8 must be less than swC
"in10=1s/(2*cnst8)"              ;cnst8 = 30 * sfo3, 30 ppm of spectral width for 15N

"l1 = (d14-(1-0.638)*(p21-p3) -p19 -d16 -14u)*2/(in10-in0)+1"       ;TD(F1) <= L1
"l2 = l1"

"cnst2=5.0"
"p41=7.2/(cnst2*bf1/1000000)" /*  PC9  pulse length  */

"spw31=4*plw1*(pow((cnst3/90.0)*(p1/p41)/0.125,2))" /* PC9  power level  */
                ;"sp31=pl1-20*log(2*(p1/p41)/0.125)/log(10)" /* PC9  power level  */
"spoff31=0"  /*  PC9  offset */

"DELTA=p4"


1 d11 ze
2 d11 do:f2 do:f3
3 d1 pl0:f1 pl12:f2 pl3:f3 BLKGRAD
  10u UNBLKGRAD


  "d32 = d0*2 - p3*0.637*2"
  "if (d32 < 0) { d32 = 0; }"
  "d33 = d10*2 - p21*0.637*2"
  "if (d33 < 0) { d33 = 0; }"

  "d34 = d15-d14 + 0.5*(p22-p4+d33-d32)"           ;d34 > 0.5*p41
  "d35 = d34-p41*0.5"
  "d36 = d14-p19-d16-14u"
  "d37 = d15 -d34 - p19- d16 -14u"                                  ;d37 should be > 0
  "if (d32 == 0) { d37=d36-p21; d38=d37-p22-p4;}"  ;1st point

  (p21 ph2):f3
  p16:gp1
  200u


  4u cpd2:f2
  (p41:sp31 ph1):f1
  d35
  4u do:f2

  10u
  p19:gp2
  d16 pl1:f1 pl2:f2


  if "d32 > 0"
  {
  (center (p1*0.231 ph7 d19*2 p1*0.692 ph7 d19*2 p1*1.462 ph7 d19*2 p1*1.462 ph8 d19*2 p1*0.692 ph8
d19*2 p1*0.231 ph8):f1 (d36 p3 ph5 d32 p3 ph1 d36):f2 (d37 p21 ph5 d33 p21 ph8 d37):f3)
  }
  else
  {
  (center (p1*0.231 ph7 d19*2 p1*0.692 ph7 d19*2 p1*1.462 ph7 d19*2 p1*1.462 ph8 d19*2 p1*0.692 ph8
d19*2 p1*0.231 ph8):f1 (d36 p3 ph5 d32 p3 ph1 d36):f2 (d37 p21 ph5 DELTA p22 ph2 DELTA p21 ph8 d38):f3)
  }

  p19:gp2
  d16
  10u pl12:f2 pl16:f3

  4u cpd2:f2
  d34

  go=2 ph31 cpd3:f3
  d11 do:f2 do:f3 mc #0 to 2
     F1PH(ip5, id0 & id10)

4u BLKGRAD
exit

ph1 =0
ph2 =1
ph5 =0 2
ph7 =0
ph8 =2

ph31=0 2
```

```
;pl1 : f1 channel - power level for pulse (default)
;pl12: f2 channel - power level for pulse (default)
;pl16: f3 channel - power level for CPD/BB decoupling
;sp31: f1 channel - power level for shaped 90 degree pulse
;spnam31: Pc9_4_90.1000 with a consin-modulation
;p1 : f1 channel -  90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel -  90 degree high power pulse
;p4 : f2 channel - 180 degree high power pulse
;p16: homospoil/gradient pulse                         [1 msec]
;p19: homospoil/gradient pulse                         [0.3 msec]
;p41: f1 channel -   90 degree shaped pulse
;d0 : incremented delay (F1 in 3D)                     [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d2 : 1/((2J)XH)
;d8 : mixing time
;d11: delay for disk I/O                               [30 msec]
;d13: short delay                                      [4 usec]
;d16: delay for homospoil/gradient recovery
;d17: fixed delay
;d18: run time delay given by the pulse sequence
;d19: delay for binomial water suppression
;     d19 = (1/(2*d)), d = distance of next null (in Hz)
;d32: run time delay given by the pulse sequence
;d33: run time delay given by the pulse sequence
;d34: run time delay given by the pulse sequence
;d35: run time delay given by the pulse sequence
;d36: run time delay given by the pulse sequence
;d37: run time delay given by the pulse sequence
;d38: run time delay given by the pulse sequence
;cnst3: flipping angle [~120]
;cnst8: 15N spectral width in Hz [=36*sfo3]
;inf1: 1/SW(H) = 2 * DW(H)
;in0: 1/(2 * SW(H)) = DW(H)
;in20: 1s/(2 * cnst1) = DW(H)
;l1:   maximum TD1 value allowable
;NS: 2 * n
;DS: 2*n
;td1: number of experiments in F1
;FnMODE: States-TPPI (or TPPI) in F1
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence
;cpdprg2: f2 channel [bi_garp_2pl]
;cpd3: decoupling according to sequence defined by cpdprg3
;pcpd3: f3 channel - 90 degree pulse for decoupling sequence
;cpdprg3: f3 channel [bi_garp_2pl.2]


;for z-only gradients:
;gpz1: 20%
;gpz2: 60%

;use gradient files:
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.50
```

Appendix S3. Parameter set for 2D SOFAST $C_MNH_MH_N$ SFHMQC (Bruker Topspin ver. 2.1 to 3.5)

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Pulseprogram parameters of dataset:
C:/NMR/sofast/24/pdata/1

General		
PULPROG	sfCNhmqcgpph19	
TD	1356	
SWH [Hz, ppm]	13586.96	15.9789
AQ [sec]	0.0499008	
RG	128	
DW [µsec]	36.800	
DE [µsec]	25.00	
cnst2	5.000000	
CNST3	120.0000000	
CNST8	3101.8000488	
d0 [sec]	0	
D1 [sec]	0.20000000	
d10 [sec]	0	
d11 [sec]	0.03000000	
d13 [sec]	0.00000400	
d14 [sec]	0.00400000	
d15 [sec]	0.00520000	
D16 [sec]	0.00020000	
D19 [sec]	0.00007400	
D32 [sec]	0	
D33 [sec]	0	
D34 [sec]	0	
D35 [sec]	0	
D36 [sec]	0	
D37 [sec]	0	
D38 [sec]	0	
DELTA [sec]	0.00002240	
DS	16	
in0 [sec]	0.00011410	
in10 [sec]	0.00016120	
INF1 [µsec]	228.20	
l1	149	
l2	149	
NS	4	

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p4 [µsec]	22.40	
Channel f1		
SFO1 [MHz]	850.3039930	
O1 [Hz, ppm]	3993.01	4.696
NUC1	1H	
P1 [µsec]	10.50	
p41 [µsec]	1693.52	
PLW0 [W, -dBW]	0	1000.00
PLW1 [W, -dBW]	11.995	-10.79
SPNAM 31	Pc9_4_90.pw1693.5us.cos3230Hz	
SPOAL31	0.500	
spoffs31 [Hz]	0	
spw31 [W, -dBW]	0.20985	6.78
Channel f2		
SFO2 [MHz]	213.8116328	
O2 [Hz, ppm]	3527.83	16.500
NUC2	13C	
CPDPRG 2	bi_garp_2pl	
P3 [µsec]	11.20	
PCPD2 [µsec]	50.00	
PLW2 [W, -dBW]	180	-22.55
PLW12 [W, -dBW]	9.0317	-9.56
PLW30 [W, -dBW]	9.0317	-9.56
PLW31 [W, -dBW]	18.021	-12.56
Channel f3		
SFO3 [MHz]	86.1703179	
O3 [Hz, ppm]	10166.90	118.000
NUC3	15N	
CPDPRG 3	bi_garp_2pl.2	
P21 [µsec]	38.00	
p22 [µsec]	76.00	
PCPD3 [µsec]	240.00	
PLW3 [W, -dBW]	295	-24.70
PLW16 [W, -dBW]	7.3955	-8.69
PLW32 [W, -dBW]	7.3955	-8.69
PLW33 [W, -dBW]	14.756	-11.69
Gradient channel		
GPNAM 1	SMSQ10.100	

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GPNAM 2	SMSQ10.50
GPZ1 [%]	20.00
GPZ2 [%]	60.00
P16 [µsec]	1000.00
p19 [µsec]	300.00

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Appendix S4. Pulse program for 3D C_MN-$C_MNH_MH_N$ SFHMQC-NOESY-HMQC.

```
;sfCNhmqcnoeCNhmqc3d.NCm-NCmHnHm: simultaneous 3D CNhmqc-noesy-CNhmqc for  NCm(F1)-NCm(F2)HnHm(F3)
;Youlin Xia on 05/05/2016

/*
cnst8: =36*sfo3. cnst8 (for 15N)
TD(F2) <= L1

use "split ipap 2" to split the data, enter 1 for "scaling factor for AP spectrum", and enter 0 for
"splitting in F1(0) or F2(1)"


two sets of 3D data are acquired, one is for N(t1) + C(t1) and the other is for N(t1) - C(t1)
four sub 3D spectra will be obtained:

1: Cm(t1) NOE  N(t2)-Hn(t3)
2: Cm(t1) NOE Cm(t2)-Hm(t3)
3:  N(t1) NOE  N(t2)-Hn(t3)
4:  N(t1) NOE Cm(t2)-Hm(t3)

setting zgoptns -Dhard will use hard 90 degree pulse.

*/


;$CLASS=HighRes
;$DIM=3D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

prosol relations=<triple>

#include <Avance.incl>
#include <Delay.incl>
#include <Grad.incl>

"p4=p3*2"
"p19=500"
"p22=p21*2"
"d11=30m"
"d13=4u"
"d14=4.0m"   ;for 13C
"d15=5.2m"   ;for 15N

"d0=0u"
"d10=0u"
"in0 =inf1/2"                     ;cnst8 must be less than swC
"in10=1s/(2*cnst8)"               ;cnst8 = 30 * sfo3, 30 ppm of spectral width for 15N

"d20=0u"
"d30=0u"
"in20 =inf2/2"                    ;cnst8 must be less than swC
"in30=1s/(2*cnst8)"               ;cnst8 = 30 * sfo3, 30 ppm of spectral width for 15N

"l1 = (d14-(1-0.638)*(p21-p3) -p19 -d16 -8u)*2/(in10-in0)+1"        ;TD(F1) <= 2*L1, TD(F2) <= L1
"l2 = l1"

"cnst2=5.0"
"cnst3=90"
"p41=7.2/(cnst2*bf1/1000000)" /*  PC9  pulse length  */

"spw29=4*plw1*(pow((p1/p41)/0.125,2))" /* PC9  power level  */
                ;"sp29=pl1-20*log(2*(p1/p41)/0.125)/log(10)" /* PC9  power level  */
"spoff29=0"  /*  PC9  offset */

"spw31=4*plw1*(pow((cnst3/90.0)*(p1/p41)/0.125,2))" /* PC9  power level  */
                ;"sp31=pl1-20*log((cnst3*2/90.0)*(p1/p41)/0.125)/log(10)" /* PC9  power level  */
"spoff31=0"  /*  PC9  offset */

;"if ( p61 < 250u && d1 < 0.5 ) { p61 = 250u; } else { plw6=plw2*(pow((p3/p61),2)); }"
                /* 13C CPD power level */
;"plw26 =plw3*(pow((p21/pcpd3),2))"
                                                   /* 15N CPD  power level  */

"DELTA=p4"

"TAU=d8-p21-p16*3-0.5m-10u"
"l0=0"

aqseq 312

1 d11 ze
2 d11 do:f2 do:f3
3 20u pl12:f2 pl3:f3 BLKGRAD
  d1
  if "l0 % 2 == 0 && nsdone % ns == 0"
  {
  rp6
  }
  if "l0 % 2 == 1 && nsdone % ns == 0"
  {
  ip6*2
  }

  10u UNBLKGRAD


;for t1 dimension
  "d22 = d0*2 - p3*0.637*2"
  "if (d22 < 0) { d22 = 0; }"
  "d23 = d10*2 - p21*0.637*2"
  "if (d23 < 0) { d23 = 0; }"
  "d24 = d15-d14 + 0.5*(p22-p4+d23-d22)"                          ;d24 > 0.5*p41
  "d25 = d24-p41*0.5"
  "d26 = d14-p19-d16-8u"
  "d27 = d15 -d24 - p19- d16 -8u"                                 ;d27 should be > 0
  "if (d22 == 0) { d27=d26-p21; d28=d27-p22-p4;}"  ;1st point

;for t2 dimension
  "d32 = d20*2 - p3*0.637*2"
  "if (d32 < 0) { d32 = 0; }"
  "d33 = d30*2 - p21*0.637*2"
  "if (d33 < 0) { d33 = 0; }"

  "d34 = d15-d14 + 0.5*(p22-p4+d33-d32)"                          ;d34 > 0.5*p41
  "d35 = d34-p41*0.5"
  "d36 = d14-p19-d16-8u"
  "d37 = d15 -d34 - p19- d16 -8u"                                 ;d37 should be > 0
  "if (d32 == 0) { d37=d36-p21; d38=d37-p22-p4;}"  ;1st point

  (p21 ph2):f3
  p16:gp1
  200u

;1st CNHMQC
  4u cpds2:f2
#ifdef hard
  4u pl0:f1
  (p11:sp1 ph8):f1
  4u

  4u pl1:f1
  (p1 ph1):f1
  d24
#else
  4u pl0:f1
  (p41:sp29 ph1):f1
  d25
#endif
  4u do:f2

  4u
  p19:gp2
#ifdef hard
  d16 pl2:f2
#else
  d16 pl1:f1 pl2:f2
#endif

  if "d22 > 0"
  {
  (center (p1*0.231 ph7 d19*2 p1*0.692 ph7 d19*2 p1*1.462 ph7 d19*2 p1*1.462 ph8 d19*2 p1*0.692 ph8
d19*2 p1*0.231 ph8):f1 (d26 p3 ph3 d22 p3 ph4 d26):f2 (d27 p21 ph3 d23 p21 ph6 d27):f3)
  }
  else
  {
  (center (p1*0.231 ph7 d19*2 p1*0.692 ph7 d19*2 p1*1.462 ph7 d19*2 p1*1.462 ph8 d19*2 p1*0.692 ph8
d19*2 p1*0.231 ph8):f1 (d26 p3 ph3 d22 p3 ph4 d26):f2 (d27 p21 ph3 DELTA p22 ph2 DELTA p21 ph6 d28):f3)
  }

  p19:gp2
  d16
  4u pl12:f2

#ifdef hard
  4u cpds2:f2
  d24
  (p1 ph1):f1

  4u pl0:f1
  (p11:sp1 ph8):f1
  4u
#else
  4u pl0:f1 cpds2:f2
  d25
  (p41:sp29 ph1):f1
#endif
  4u do:f2

;NOESY
  TAU BLKGRAD
  10u UNBLKGRAD
  (p21 ph1):f3
  p16*3:gp3
  0.5m

;2nd CNHMQC
  4u cpd2:f2
#ifdef hard
  4u pl0:f1
  (p11:sp1 ph8):f1
  4u
  4u pl1:f1

  (p1 ph1):f1
  d34
#else
  (p41:sp31 ph1):f1
  d35
#endif
  4u do:f2
```

Appendix S4. (continued)

```
  4u
  p19:gp2
#ifdef hard
  d16 pl2:f2
#else
  d16 pl1:f1 pl2:f2
#endif

  if "d32 > 0"
  {
  (center (p1*0.231 ph7 d19*2 p1*0.692 ph7 d19*2 p1*1.462 ph7 d19*2 p1*1.462 ph8 d19*2 p1*0.692 ph8
d19*2 p1*0.231 ph8):f1 (d36 p3 ph5 d32 p3 ph1 d36):f2 (d37 p21 ph5 d33 p21 ph9 d37):f3)
  }
  else
  {
  (center (p1*0.231 ph7 d19*2 p1*0.692 ph7 d19*2 p1*1.462 ph7 d19*2 p1*1.462 ph8 d19*2 p1*0.692 ph8
d19*2 p1*0.231 ph8):f1 (d36 p3 ph5 d32 p3 ph1 d36):f2 (d37 p21 ph5 DELTA p22 ph2 DELTA p21 ph9 d38):f3)
  }

  p19:gp2
  d16
  4u pl12:f2 pl16:f3

  4u cpd2:f2
  d34

  go=2 ph31 cpd3:f3
  d11 do:f2 do:f3 mc #0 to 2
    ;for topspin2.0
     ;F1I(iu0, 2)
     ;F1PH(ip3, id0 & id10)
     ;F2PH(rd0 & rd10 & rp3 & ip5, id20 & id30)

    ;for topspin3.0
     F1I(iu0, 2)
     F1PH(calph(ph3, +90), caldel(d0, +in0) & caldel(d10, +in10))
     F2PH(calph(ph5, +90), caldel(d20, +in20) & caldel(d30, +in30))

4u BLKGRAD
exit



ph1 =0
ph2 =1
ph3 =0 0 2 2
ph4 =0 0 0 0 2 2 2 2
ph6 =0 0 0 0 2 2 2 2
ph5 =0 2
ph7 =0
ph8 =2
#ifdef hard
ph9 =0
#else
ph9 =2
#endif

ph31=0 2 2 0 2 0 0 2


;pl1 : f1 channel - power level for pulse (default)
;pl12: f2 channel - power level for pulse (default)
;pl16: f3 channel - power level for CPD/BB decoupling
;sp29: f1 channel - power level for shaped 90 degree pulse
;sp31: f1 channel - power level for shaped 90 degree pulse
;spnam29: Pc9_4_90.1000 with a consin-modulation
;spnam31: Pc9_4_90.1000 with a consin-modulation
;p1 : f1 channel -  90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel -  90 degree high power pulse
;p4 : f2 channel - 180 degree high power pulse
;p16: homospoil/gradient pulse                         [1 msec]
;p19: homospoil/gradient pulse                         [0.3 msec]
;p41: f1 channel -   90 degree shaped pulse
;d0 : incremented delay (F1 in 3D)                     [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d2 : 1/((2J)XH)
;d8 : mixing time
;d11: delay for disk I/O                               [30 msec]
;d13: short delay                                      [4 usec]
;d16: delay for homospoil/gradient recovery
;d17: fixed delay
;d18: run time delay given by the pulse sequence
;d19: delay for binomial water suppression
;     d19 = (1/(2*d)), d = distance of next null (in Hz)
;d22: run time delay given by the pulse sequence
;d23: run time delay given by the pulse sequence
;d24: run time delay given by the pulse sequence
;d25: run time delay given by the pulse sequence
;d26: run time delay given by the pulse sequence
;d27: run time delay given by the pulse sequence
;d28: run time delay given by the pulse sequence
;d32: run time delay given by the pulse sequence
;d33: run time delay given by the pulse sequence
;d34: run time delay given by the pulse sequence
;d35: run time delay given by the pulse sequence
;d36: run time delay given by the pulse sequence
;d37: run time delay given by the pulse sequence
;d38: run time delay given by the pulse sequence
```

```
;cnst2: excitation bandwidth [5ppm]
;cnst3: flipping angle [~110]
;cnst8: 15N spectral width in Hz [=36*sfo3]
;inf1: 1/SW(H) = 2 * DW(H)
;in0: 1/(2 * SW(H)) = DW(H)
;in20: 1s/(2 * cnst1) = DW(H)
;l1:   maximum TD2 value allowable
;NS: 8*n
;DS: 8*n
;td1: number of experiments in F1 * 2
;td2: number of experiments in F2
;FnMODE: States-TPPI (or TPPI) in F1 & F2
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence
;cpdprg2: f2 channel [bi_garp_2pl]
;cpd3: decoupling according to sequence defined by cpdprg3
;pcpd3: f3 channel - 90 degree pulse for decoupling sequence
;cpdprg3: f3 channel [bi_garp_2pl.2]


;for z-only gradients:
;gpz1: 25%
;gpz2: 35%
;gpz3: 30%

;use gradient files:
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.50
;gpnam3: SMSQ10.100
```

Appendix S5. Parameter set for 3D C_MN-$C_MNH_MH_N$ SFHMQC-NOESY-HMQC.

Wed Jul 20 15:25:55 CDT 2016

Pulseprogram parameters of dataset:
C:/NMR/sofast/20/pdata/1

General		
PULPROG	sfCNhmqcnoeCNhmqc3d.NCm-NCmHnHm	
TD	2048	
SWH [Hz, ppm]	13586.96	15.9789
AQ [sec]	0.0753664	
RG	128	
DW [μsec]	36.800	
DE [μsec]	25.00	
cnst2	5.000000	
cnst3	90.000000	
CNST8	3101.8000488	
d0 [sec]	0	
D1 [sec]	0.20000000	
D8 [sec]	0.30000001	
d10 [sec]	0	
d11 [sec]	0.03000000	
d13 [sec]	0.00000400	
d14 [sec]	0.00400000	
d15 [sec]	0.00520000	
D16 [sec]	0.00020000	
D19 [sec]	0.00007400	
d20 [sec]	0	
D22 [sec]	0	
D23 [sec]	0	
D24 [sec]	0	
D25 [sec]	0	
D26 [sec]	0	
D27 [sec]	0	
D28 [sec]	0	
d30 [sec]	0	
D32 [sec]	0	
D33 [sec]	0	
D34 [sec]	0	
D35 [sec]	0	
D36 [sec]	0	

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D37 [sec]	0	
D38 [sec]	0	
DELTA [sec]	0.00002240	
DS	16	
in0 [sec]	0.00011410	
in10 [sec]	0.00016120	
in20 [sec]	0.00011410	
in30 [sec]	0.00016120	
INF1 [μsec]	228.20	
INF2 [μsec]	228.20	
l0	0	
l1	140	
l2	140	
NS	8	
p4 [μsec]	22.40	
TAU [sec]	0.29645202	
ZGOPTNS		
Channel f1		
SFO1 [MHz]	850.3039930	
O1 [Hz, ppm]	3993.01	4.696
NUC1	1H	
P1 [μsec]	10.60	
p41 [μsec]	1693.52	
PLW0 [W, -dBW]	0	1000.00
PLW1 [W, -dBW]	12	-10.79
SPNAM 29	Pc9_4_90.pw1693.5us.cos3230Hz	
SPOAL29	0.500	
spoffs29 [Hz]	0	
spw29 [W, -dBW]	0.12035	9.20
SPNAM 31	Pc9_4_90.pw1693.5us.cos3230Hz	
SPOAL31	0.500	
spoffs31 [Hz]	0	
spw31 [W, -dBW]	0.12035	9.20
Channel f2		
SFO2 [MHz]	213.8116328	
O2 [Hz, ppm]	3527.83	16.500
NUC2	13C	
CPDPRG 2	bi_garp_2pl	

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Appendix S5. (continued)

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P3 [µsec]	11.20	
PCPD2 [µsec]	50.00	
PLW2 [W, -dBW]	180	-22.55
PLW12 [W, -dBW]	9.0317	-9.56
PLW30 [W, -dBW]	9.0317	-9.56
PLW31 [W, -dBW]	18.021	-12.56
Channel f3		
SFO3 [MHz]	86.1703179	
O3 [Hz, ppm]	10166.90	118.000
NUC3	15N	
CPDPRG 3	bi_garp_2pl.2	
P21 [µsec]	38.00	
p22 [µsec]	76.00	
PCPD3 [µsec]	240.00	
PLW3 [W, -dBW]	295	-24.70
PLW16 [W, -dBW]	7.3955	-8.69
PLW32 [W, -dBW]	7.3955	-8.69
PLW33 [W, -dBW]	14.756	-11.69
Gradient channel		
GPNAM 1	SMSQ10.100	
GPNAM 2	SMSQ10.50	
GPNAM 3	SMSQ10.100	
GPZ1 [%]	25.00	
GPZ2 [%]	35.00	
GPZ3 [%]	30.00	
P16 [µsec]	1000.00	
p19 [µsec]	500.00	

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Appendix S6. Pulse program for 3D C_{Aro}-C_MH_M SFHMQC-NOESY-HMQC (Topspin ver. 2.1 to 3.5).

```
;sfhmqcnoesyhmqc3d.Ca-CmHm: 3D HMQC-NOESY-HMQC for Caro(F1)-Cm(F2)Hm(F3)
;H-C(t1)-H -NOE -H-C(t2)-H(t3): C(t1) for aromatic 13C, C(t2) and H(t3) are for methyl
;Youlin Xia on 05/05/2016

prosol relations=<triple>

#include <Avance.incl>
#include <Delay.incl>
#include <Grad.incl>

"d11=30m"


"in0 =inf1/2"
"in10=inf2/2"
"d0=0u"
"d10=0u"


"TAU=d8-p3-p16-0.5m-5u"

"d14=1s/(cnst4*2)"                ;cnst4=125
"d15=1s/(cnst5*2)"                ;cnst5=160

"p41=7.2/(cnst2*bf1/1000000)" /*  PC9  pulse length  */
"p42=4.875/(cnst2*bf1/1000000)" /* REBURP pulse length  */

"spw25=plw1*(pow((p1/p41)/0.125,2))" /* PC9  power level  */
"spoff25=bf1*(cnst21/1000000)-o1"                               /*  PC9  offset */

"spw26=plw1*(pow((p1*2/p42)/0.0798,2))"   /* REBURP power level  */
"spoff26=spoff25"                                 /* REBURP offset */

"spw27=plw1*(pow((p1*(cnst3/90)/p41)/0.125,2))"
"spoff27=bf1*(cnst1/1000000)-o1"  /*  PC9  offset */

"spw28=plw1*(pow((p1*2/p42)/0.0798,2))"
"spoff28=spoff27"                                /*  PC9  offset */

"DELTA1=d14-p16-d16-p41*0.5"
"DELTA2=p41*0.5-de-4u"

"DELTA3=d15-p16-d16-p41*0.5"
"DELTA4=p41*0.5"

aqseq 321


1 d11 ze
2 d11 do:f2
3 d1 pl0:f1 pl2:f2

  10u UNBLKGRAD

  5u fq=cnst22(bf ppm):f2  ;set 13C to center of 13C freq of aromatci ring [125ppm]
  (p3 ph1):f2
  p16:gp3
  200u

  "d20 = d0*2 - p3*0.637*2"
  "if (d20 < 0) { d20 = 0; }"

  "d21 = d10*2 - p3*0.637*2"
  "if (d21 < 0) { d21 = 0; }"

  ;HMQC1
  (p41:sp25 ph1):f1
  p16:gp1
  d16

  (center (p42:sp26 ph5):f1 (DELTA3 p3 ph3 d20 p3 ph4 DELTA3):f2 )

  p16:gp1
  d16

  DELTA4 pl1:f1
  (p1 ph1):f1
  ;(p41:sp25 ph1):f1



  ;mixing
  TAU
  5u fq=0:f2     ;return back to o2p [16ppm]
  (p3 ph1):f2
  p16:gp3
  0.5m pl0:f1

  ;HMQC2

  (p41:sp27 ph1):f1
  p16:gp1
  d16

  (center (p42:sp28 ph8):f1 (DELTA1 p3 ph6 d21 p3 ph7 DELTA1):f2 )


  DELTA2
  p16:gp1
  d16 pl12:f2
  4u BLKGRAD
go=2 ph31 cpd2:f2
  d11 do:f2 mc #0 to 2
     ;for topspin2.0
     F1PH(rd10 & rp6 & ip3, id0)
     F2PH(ip6, id10)

     ;for topspin3.0
     ;F1PH(calph(ph3, +90), caldel(d0, +in0))
     ;F2PH(calph(ph6, +90), caldel(d10, +in10))
exit


ph1 =0
ph2 =1
ph3 =0 2
ph4 =0 0 2 2
ph5 =0 0 0 0 1 1 1 1
ph6 =0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2
ph7 =0
ph8 =0
ph31=0 2 2 0 2 0 0 2 2 0 0 2 0 2 2 0



;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;pl12: f2 channel - power level for CPD/BB decoupling
;sp25: f1 channel - power level for shaped  90 degree pulse
;sp26: f1 channel - power level for shaped 180 degree pulse
;sp27: f1 channel - power level for shaped  90 degree pulse
;sp28: f1 channel - power level for shaped 180 degree pulse
;spnam25: Pc9_4_90.1000
;spnam26: Reburp.1000
;spnam27: Pc9_4_90.1000
;spnam28: Reburp.1000
;p1 : f1 channel -  90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel -  90 degree high power pulse
;p4 : f2 channel - 180 degree high power pulse
;p16: homospoil/gradient pulse                            [1 msec]
;p41: f1 channel -   90 degree shaped pulse
;p42: f1 channel -  180 degree shaped pulse
;d0 : incremented delay (F1 in 3D)                        [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d2 : 1/((2J)XH)
;d8 : mixing time
;d10: incremented delay (F2 in 3D)                        [3 usec]
;d11: delay for disk I/O                                  [30 msec]
;d14: 1/(2JCH)                                            [4.0 msec]
;d16: delay for homospoil/gradient recovery
;d20: run time delay given by the pulse sequence
;d21: run time delay given by the pulse sequence
;cnst1: center of methyl 1H in ppm [0.9]
;cnst2: 1H excitation bandwidth [5]
;cnst3: flipping angle of the 2nd HMQC[110]
;cnst4: 1JCH of methyl [125]
;cnst5: 1JCH of aromatic ring [160]
;cnst21: center of aromatic 1H  chemical shifts [8.5]
;cnst22: center of aromatic 13C chemical shifts [125]
;o1p:    center of methyl 1H  chemical shifts [4.7]
;o2p:    center of methyl 13C chemical shifts [16]

;inf1: 1/SW(H) = 2 * DW(H)
;inf2: 1/SW(X) = 2 * DW(X)
;in0: 1/(2 * SW(H)) = DW(H)
;nd0: 2
;in10: 1/(2 * SW(X)) = DW(X)
;nd10: 2
;NS: 8 * n
;DS: 16
;td1: number of experiments in F1
;td2: number of experiments in F2
;FnMODE: States-TPPI (or TPPI) in F1
;FnMODE: States-TPPI (or TPPI) in F2
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence
;cpdprg2: f2 channel - bi_garp_2pl


;for z-only gradients:
;gpz1:  25%
;gpz3:  30%

;use gradient files:
;gpnam1: SMSQ10.100
;gpnam3: SMSQ10.100
```

Appendix S7. Parameter set for 3D C_{Aro}-C_MH_M SFHMQC-NOESY-HMQC (Bruker Topspin ver. 2.1 to 3.5).

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Pulseprogram parameters of dataset:
C:/NMR/sofast/109/pdata/1

General		
PULPROG	sfhmqcnoesyhmqc3d.am.CCH	
TD	2048	
SWH [Hz, ppm]	13736.26	16.1545
AQ [sec]	0.0745472	
RG	90.5	
DW [µsec]	36.400	
DE [µsec]	25.00	
CNST1	1.0000000	
CNST2	4.0000000	
CNST4	125.0000000	
CNST5	160.0000000	
CNST21	7.5000000	
d0 [sec]	0	
D1 [sec]	1.00000000	
D8 [sec]	0.30000001	
d10 [sec]	0	
d11 [sec]	0.03000000	
D16 [sec]	0.00020000	
D20 [sec]	0	
D21 [sec]	0.00180000	
DELTA1 [sec]	0.00174155	
DELTA2 [sec]	0.00102945	
DELTA3 [sec]	0.00086655	
DELTA4 [sec]	0.00105845	
DS	32	
in0 [sec]	0.00009350	
in10 [sec]	0.00011690	
INF1 [µsec]	187.00	
INF2 [µsec]	233.80	
NS	16	
P1 [µsec]	14.50	
TAU [sec]	0.29848382	
dCH [sec]	0.00400000	
dCH2 [sec]	0.00312500	

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Channel f1		
SFO1 [MHz]	850.3039982	
O1 [Hz, ppm]	3998.21	4.702
NUC1	1H	
p41 [µsec]	2116.90	
p42 [µsec]	1433.32	
PLW0 [W, -dBW]	0	1000.00
PLW1 [W, -dBW]	12	-10.79
SPNAM 25	Pc9_4_90.1000	
SPOAL25	0.500	
spoffs25 [Hz]	2379.04	
spw25 [W, -dBW]	0.036757	14.35
SPNAM 26	Reburp.1000	
SPOAL26	0.500	
spoffs26 [Hz]	2379.04	
spw26 [W, -dBW]	0.74844	1.26
SPNAM 27	Pc9_4_90.1000	
SPOAL27	0.500	
spoffs27 [Hz]	-3147.91	
spw27 [W, -dBW]	0.036757	14.35
SPNAM 28	Reburp.1000	
SPOAL28	0.500	
spoffs28 [Hz]	-3147.91	
spw28 [W, -dBW]	0.74844	1.26
Channel f2		
SFO2 [MHz]	213.8118466	
O2 [Hz, ppm]	3741.64	17.500
NUC2	13C	
CNST22	122.0000000	
CPDPRG 2	bi_garp_2pl	
P3 [µsec]	11.20	
PCPD2 [µsec]	50.00	
PLW2 [W, -dBW]	180	-22.55
PLW12 [W, -dBW]	9.0317	-9.56
PLW30 [W, -dBW]	9.0317	-9.56
PLW31 [W, -dBW]	36.127	-15.58
Gradient channel		
GPNAM 1	SMSQ10.100	

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GPNAM 3	SMSQ10.100
GPZ1 [%]	11.00
GPZ3 [%]	30.00
P16 [µsec]	1000.00

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Appendix S8. Pulse program for 3D H_NH_{Aro}-C_MH_M SFNOESY-HMQC (Bruker Topspin ver. 2.1 to 3.5).

```
;sfnoesyhmqc3d.HnHa-CmHm: NOESY-HMQC for HnHa(F1)-Cm(F2)Hm(F3)
;H(t1) -NOE -H-C(t2)-H(t3): H(t1) for both amide and aromatic 1H, C(t2) and H(t3) are for methyl
;Youlin Xia on 05/05/2016

;note: if setting cnst21 to 1 and cnst22 to 16, the pulse program can also apply for methyl-methyl NOESY


;$CLASS=HighRes
;$DIM=3D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

prosol relations=<triple>

#include <Avance.incl>
#include <Delay.incl>
#include <Grad.incl>

"d11=30m"


"in0 =inf1/2"
"in10=inf2/2"
"d0=3u"
"d10=0u"
"d14=1s/(cnst4*2)"                ;cnst4=125

"TAU=d8-p3-p16-0.5m"

"p41=7.2/(cnst2*bf1/1000000)" /*  PC9  pulse length  */
"p42=4.875/(cnst2*bf1/1000000)" /* REBURP pulse length  */

"spw25=plw1*(pow((p1/p41)/0.125,2))"
"spoff25=0"  /*  PC9  offset */

"spw26=plw1*(pow((p1*2/p42)/0.0798,2))"
"spoff26=0"                       /*  PC9  offset */

"spw27=plw1*(pow((p1*(cnst3/90)/p41)/0.125,2))"
"spoff27=bf1*(cnst1/1000000)-o1"  /*  PC9  offset */

"spw28=plw1*(pow((p1*2/p42)/0.0798,2))"
"spoff28=spoff27"                                /*  PC9  offset */

"DELTA1=d14-p16-d16-p41*0.5"
"DELTA2=p41*0.5-de-4u"

aqseq 321


1 d11 ze
2 d11 do:f2
3 d1 pl0:f1 pl3:f3

  10u UNBLKGRAD

  "d21 = d10*2 - p3*0.637*2"
  "if (d21 < 0) { d21 = 0; }"

  ;NOESY
  p16:gp3
  d16

  4u fq=cnst21(bf ppm):f1
  4u fq=cnst22(bf ppm):f2

  4u pl12:f2
  5u cpds5:f2
  (p41:sp25 ph3):f1
  d0
  (p22 ph1):f3
  d0
  (p42:sp26 ph5):f1
  3u
  (p22 ph1):f3
  3u
  (p41:sp25 ph4):f1
  5u do:f2
  4u fq=0:f2

  ;mixing
  TAU pl2:f2 fq=0:f1              ;0 Hz offset
  (p3 ph1):f2
  p16:gp3
  0.5m

  ;HMQC2

  (p41:sp27 ph1):f1
  p16:gp1
  d16

  (center (p42:sp28 ph8):f1 (DELTA1 p3 ph6 d21 p3 ph7 DELTA1):f2 )


  DELTA2
  p16:gp1
  d16 pl12:f2
  4u BLKGRAD

  go=2 ph31 cpd2:f2
  d11 do:f2 mc #0 to 2
     ;for topspin2.0
     F1PH(rd10 & rp6 & ip3, id0)
     F2PH(ip6, id10)

     ;for topspin3.0
     ;F1PH(calph(ph3, +90), caldel(d0, +in0))
     ;F2PH(calph(ph6, +90), caldel(d10, +in10))
exit


ph1 =0
ph2 =1
ph3 =0 2
ph4 =0
ph5 =0 0 0 0 1 1 1 1
ph6 =0 0 2 2
ph7 =0
ph8 =0
ph31=0 2 2 0 2 0 0 2



;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;pl12: f2 channel - power level for CPD/BB decoupling
;sp25: f1 channel - power level for shaped  90 degree pulse
;sp26: f1 channel - power level for shaped 180 degree pulse
;sp27: f1 channel - power level for shaped  90 degree pulse
;sp28: f1 channel - power level for shaped 180 degree pulse
;spnam25: Pc9_4_90.1000
;spnam26: Reburp.1000
;spnam27: Pc9_4_90.1000
;spnam28: Reburp.1000
;p1 : f1 channel -  90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel -  90 degree high power pulse
;p4 : f2 channel - 180 degree high power pulse
;p16: homospoil/gradient pulse                         [1 msec]
;p41: f1 channel -   90 degree shaped pulse
;p42: f1 channel -  180 degree shaped pulse
;d0 : incremented delay (F1 in 3D)                     [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d2 : 1/((2J)XH)
;d8 : mixing time
;d10: incremented delay (F2 in 3D)                     [3 usec]
;d11: delay for disk I/O                               [30 msec]
;d14: 1/(2JCH)                                         [3.7 msec]
;d16: delay for homospoil/gradient recovery
;d21: run time delay given by the pulse sequence
;cnst1: center of methyl 1H in ppm [0.9]
;cnst2: 1H excitation bandwidth [5]
;cnst3: flipping angle of the 2nd HMQC[110]
;cnst4: 1JCH of methyl [125]
;cnst5: 1JCH of aromatic ring [160]
;cnst21: center of aromatic 1H  chemical shifts [8.5]
;cnst22: center of aromatic 13C chemical shifts [125]
;o1p:    center of methyl 1H  chemical shifts [1]
;o2p:    center of methyl 13C chemical shifts [16]

;inf1: 1/SW(H) = 2 * DW(H)
;inf2: 1/SW(X) = 2 * DW(X)
;in0: 1/(2 * SW(H)) = DW(H)
;nd0: 2
;in10: 1/(2 * SW(X)) = DW(X)
;nd10: 2
;NS: 4 * n
;DS: 8
;td1: number of experiments in F1
;td2: number of experiments in F2
;FnMODE: States-TPPI (or TPPI) in F1
;FnMODE: States-TPPI (or TPPI) in F2
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence
;cpdprg2: f2 channel - bi_garp_128


;for z-only gradients:
;gpz1:  25%
;gpz3:  30%

;use gradient files:
;gpnam1: SMSQ10.100
;gpnam3: SMSQ10.100
```

Appendix S9. Parameter set for 3D H_NH_{Aro}-C_MH_M SFNOESY-HMQC.

Wed Jul 20 15:34:13 CDT 2016

Pulseprogram parameters of dataset:
C:/NMR/sofast/308/pdata/1

General		
PULPROG	sfnoesyhmqc3d.Am.HCH	
TD	1356	
SWH [Hz, ppm]	13586.96	15.9789
AQ [sec]	0.0499008	
RG	90.5	
DW [μsec]	36.800	
DE [μsec]	25.00	
CNST1	1.0000000	
CNST2	4.0000000	
CNST3	90.0000000	
CNST4	125.0000000	
d0 [sec]	0.00000300	
D1 [sec]	0.20000000	
D8 [sec]	0.30000001	
d10 [sec]	0	
d11 [sec]	0.03000000	
d14 [sec]	0.00400000	
D16 [sec]	0.00020000	
D21 [sec]	0.00180000	
DELTA1 [sec]	0.00174155	
DELTA2 [sec]	0.00102945	
DS	8	
in0 [sec]	0.00013070	
in10 [sec]	0.00011690	
INF1 [μsec]	261.40	
INF2 [μsec]	233.80	
NS	8	
P1 [μsec]	14.50	
TAU [sec]	0.29848883	
Channel f1		
SFO1 [MHz]	850.3039982	
O1 [Hz, ppm]	3998.21	4.702
NUC1	1H	
CNST21	8.6000004	

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p41 [μsec]	2116.90	
p42 [μsec]	1433.32	
PLW0 [W, -dBW]	0	1000.00
PLW1 [W, -dBW]	12	-10.79
SPNAM 25	Pc9_4_90.1000	
SPOAL25	0.500	
spoffs25 [Hz]	0	
spw25 [W, -dBW]	0.036033	14.43
SPNAM 26	Reburp.1000	
SPOAL26	0.500	
spoffs26 [Hz]	0	
spw26 [W, -dBW]	0.77141	1.13
SPNAM 27	Pc9_4_90.1000	
SPOAL27	0.500	
spoffs27 [Hz]	-3147.91	
spw27 [W, -dBW]	0.036033	14.43
SPNAM 28	Reburp.1000	
SPOAL28	0.500	
spoffs28 [Hz]	-3147.91	
spw28 [W, -dBW]	0.77141	1.13
Channel f2		
SFO2 [MHz]	213.8118466	
O2 [Hz, ppm]	3741.64	17.500
NUC2	13C	
CNST22	122.0000000	
CPDPRG 2	bi_garp_2pl	
CPDPRG 5	garp	
P3 [μsec]	11.20	
PCPD2 [μsec]	50.00	
PLW2 [W, -dBW]	180	-22.55
PLW12 [W, -dBW]	9.0317	-9.56
PLW30 [W, -dBW]	9.0317	-9.56
PLW31 [W, -dBW]	36.127	-15.58
Channel f3		
SFO3 [MHz]	86.1703179	
O3 [Hz, ppm]	10166.90	118.000
NUC3	15N	
P22 [μsec]	64.00	

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PLW3 [W, -dBW]	250	-23.98
Gradient channel		
GPNAM 1	SMSQ10.100	
GPNAM 3	SMSQ10.100	
GPZ1 [%]	11.00	
GPZ3 [%]	30.00	
P16 [μsec]	1000.00	

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Appendix S10. Pulse program for 3D H_M-C_MH_M SFNOESY-HMQC.

```
;sfnoesyhmqc3d.Hm-CmHm: sofast 3D NOESY-HMQC for Hm(F1)-Cm(F2)Hm(F3)
;H(t1) -NOE -H-C(t2)-H(t3): H(t1) for methyl 1H, C(t2) and H(t3) are for methyl
;Youlin Xia on 05/05/2016

;$CLASS=HighRes
;$DIM=3D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

prosol relations=<triple>

#include <Avance.incl>
#include <Delay.incl>
#include <Grad.incl>

"d11=30m"


"in0 =inf1/2"
"in10=inf2/2"
"d0=3u"
"d10=0u"
"d14=1s/(cnst4*2)"                ;cnst4=125

"TAU=d8-p3-p16-0.5m"

"p41=7.2/(cnst2*bf1/1000000)" /*  PC9  pulse length  */
"p42=4.875/(cnst2*bf1/1000000)" /* REBURP pulse length  */

"spw25=plw1*(pow((p1/p41)/0.125,2))"
"spoff25=0"  /*  PC9  offset */

"spw26=plw1*(pow((p1*2/p42)/0.0798,2))"
"spoff26=0"                      /*  PC9  offset */

"spw27=plw1*(pow((p1*(cnst3/90)/p41)/0.125,2))"
"spoff27=bf1*(cnst1/1000000)-o1"  /*  PC9  offset */

"spw28=plw1*(pow((p1*2/p42)/0.0798,2))"
"spoff28=spoff27"                                  /*  PC9  offset */

"DELTA1=d14-p16-d16-p41*0.5"
"DELTA2=p41*0.5-de-4u"

aqseq 321


1 d11 ze
2 d11 do:f2
3 d1 pl0:f1 pl2:f2

  10u UNBLKGRAD

  ;(p3 ph1):f2
  p16:gp3*1.2
  200u

  "d21 = d10*2 - p3*0.637*2"
  "if (d21 < 0) { d21 = 0; }"

  ;NOESY
  4u fq=cnst1(bf ppm):f1

#   ifdef cpd
  4u pl12:f2
  5u cpds5:f2
  (p41:sp25 ph3):f1
  d0
  d0
  (p42:sp26 ph5):f1
  6u
  (p41:sp25 ph4):f1
  5u do:f2
  4u pl2:f2
#   else
  (p41:sp25 ph3):f1
  d0
  (p4 ph1):f2
  d0
  (p42:sp26 ph5):f1
  3u
  (p4 ph1):f2
  3u
  (p41:sp25 ph4):f1
#   endif
  4u

  ;mixing
  TAU fq=0:f1    ;0 Hz offset
  (p3 ph1):f2
  p16:gp3
  0.5m

  ;HMQC2

  (p41:sp27 ph1):f1
  p16:gp1
  d16

  (center (p42:sp28 ph8):f1 (DELTA1 p3 ph6 d21 p3 ph7 DELTA1):f2 )


  DELTA2
  p16:gp1
  d16 pl12:f2
  4u BLKGRAD

  go=2 ph31 cpd2:f2
  d11 do:f2 mc #0 to 2
     ;for topspin2.0
     F1PH(rd10 & rp6 & ip3, id0)
     F2PH(ip6, id10)

     ;for topspin3.0
     ;F1PH(calph(ph3, +90), caldel(d0, +in0))
     ;F2PH(calph(ph6, +90), caldel(d10, +in10))
exit


ph1 =0
ph2 =1
ph3 =0 2
ph4 =0
ph5 =0 0 0 0 1 1 1 1
ph6 =0 0 2 2
ph7 =0
ph8 =0
ph31=0 2 2 0 2 0 0 2



;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;pl12: f2 channel - power level for CPD/BB decoupling
;sp25: f1 channel - power level for shaped  90 degree pulse
;sp26: f1 channel - power level for shaped 180 degree pulse
;sp27: f1 channel - power level for shaped  90 degree pulse
;sp28: f1 channel - power level for shaped 180 degree pulse
;spnam25: Pc9_4_90.1000
;spnam26: Reburp.1000
;spnam27: Pc9_4_90.1000
;spnam28: Reburp.1000
;p1 : f1 channel -  90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel -  90 degree high power pulse
;p4 : f2 channel - 180 degree high power pulse
;p16: homospoil/gradient pulse                        [1 msec]
;p41: f1 channel -   90 degree shaped pulse
;p42: f1 channel -  180 degree shaped pulse
;d0 : incremented delay (F1 in 3D)                    [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d2 : 1/((2J)XH)
;d8 : mixing time
;d10: incremented delay (F2 in 3D)                    [3 usec]
;d11: delay for disk I/O                              [30 msec]
;d14: 1/(2JCH)                                        [3.7 msec]
;d16: delay for homospoil/gradient recovery
;d21: run time delay given by the pulse sequence
;cnst1: center of methyl 1H in ppm [0.9]
;cnst2: 1H excitation bandwidth [5]
;cnst3: flipping angle of the 2nd HMQC[110]
;cnst4: 1JCH of methyl [125]
;cnst5: 1JCH of aromatic ring [160]
;o1p:    center of methyl 1H  chemical shifts [4.7]
;o2p:    center of methyl 13C chemical shifts [16]

;inf1: 1/SW(H) = 2 * DW(H)
;inf2: 1/SW(X) = 2 * DW(X)
;in0: 1/(2 * SW(H)) = DW(H)
;nd0: 2
;in10: 1/(2 * SW(X)) = DW(X)
;nd10: 2
;NS: 4 * n
;DS: 8
;td1: number of experiments in F1
;td2: number of experiments in F2
;FnMODE: States-TPPI (or TPPI) in F1
;FnMODE: States-TPPI (or TPPI) in F2
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence
;cpdprg2: f2 channel - bi_garp_2pl


;for z-only gradients:
;gpz1:  25%
;gpz3:  30%

;use gradient files:
;gpnam1: SMSQ10.100
;gpnam3: SMSQ10.100
```

Appendix S11. Parameter set for 3D H_M-C_MH_M NOESY-HMQC.

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Pulseprogram parameters of dataset:
C:/NMR/sofast/220/pdata/1

General		
PULPROG	sfnoesyhmqc3d.mm.HCH	
TD	1356	
SWH [Hz, ppm]	13586.96	15.9789
AQ [sec]	0.0499008	
RG	90.5	
DW [µsec]	36.800	
DE [µsec]	25.00	
CNST2	4.0000000	
CNST3	110.0000000	
CNST4	125.0000000	
d0 [sec]	0.00000300	
D1 [sec]	0.20000000	
D8 [sec]	0.30000001	
d10 [sec]	0	
d11 [sec]	0.03000000	
d14 [sec]	0.00400000	
D16 [sec]	0.00020000	
D21 [sec]	0.00180000	
DELTA1 [sec]	0.00174155	
DELTA2 [sec]	0.00102945	
DS	8	
in0 [sec]	0.00016800	
in10 [sec]	0.00011690	
INF1 [µsec]	336.00	
INF2 [µsec]	233.80	
NS	8	
P1 [µsec]	14.50	
TAU [sec]	0.29848883	
ZGOPTNS		
Channel f1		
SFO1 [MHz]	850.3039982	
O1 [Hz, ppm]	3998.21	4.702
NUC1	1H	
CNST1	1.0000000	

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p41 [µsec]	2116.90	
p42 [µsec]	1433.32	
PLW0 [W, -dBW]	0	1000.00
PLW1 [W, -dBW]	12	-10.79
SPNAM 25	Pc9_4_90.1000	
SPOAL25	0.500	
spoffs25 [Hz]	0	
spw25 [W, -dBW]	0.036033	14.43
SPNAM 26	Reburp.1000	
SPOAL26	0.500	
spoffs26 [Hz]	0	
spw26 [W, -dBW]	0.77141	1.13
SPNAM 27	Pc9_4_90.1000	
SPOAL27	0.500	
spoffs27 [Hz]	-3147.91	
spw27 [W, -dBW]	0.053827	12.69
SPNAM 28	Reburp.1000	
SPOAL28	0.500	
spoffs28 [Hz]	-3147.91	
spw28 [W, -dBW]	0.77141	1.13
Channel f2		
SFO2 [MHz]	213.8118466	
O2 [Hz, ppm]	3741.64	17.500
NUC2	13C	
CPDPRG 2	bi_garp_2pl	
P3 [µsec]	11.20	
P4 [µsec]	22.40	
PCPD2 [µsec]	50.00	
PLW2 [W, -dBW]	180	-22.55
PLW12 [W, -dBW]	9.0317	-9.56
PLW30 [W, -dBW]	9.0317	-9.56
PLW31 [W, -dBW]	36.127	-15.58
Gradient channel		
GPNAM 1	SMSQ10.100	
GPNAM 3	SMSQ10.100	
GPZ1 [%]	11.00	
GPZ3 [%]	30.00	
P16 [µsec]	1000.00	

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