Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information for:

Perfecting Band Selective Homo-Decoupling for decoupling two signals coupled within the Same Band

Ajay Verma[a,b], Subrato Bhattacharya[b], Bikash Baishya[a]*

[a]*Centre of Biomedical Research (Formerly Centre of Biomedical Magnetic Resonance), SGPGIMS Campus, Raebareli Road, Lucknow, 226014, India*

[b]*Department of Chemistry, Faculty of Science, Banaras Hindu University, Varanasi -221005, India*

1.1 Pulse Sequence Description:

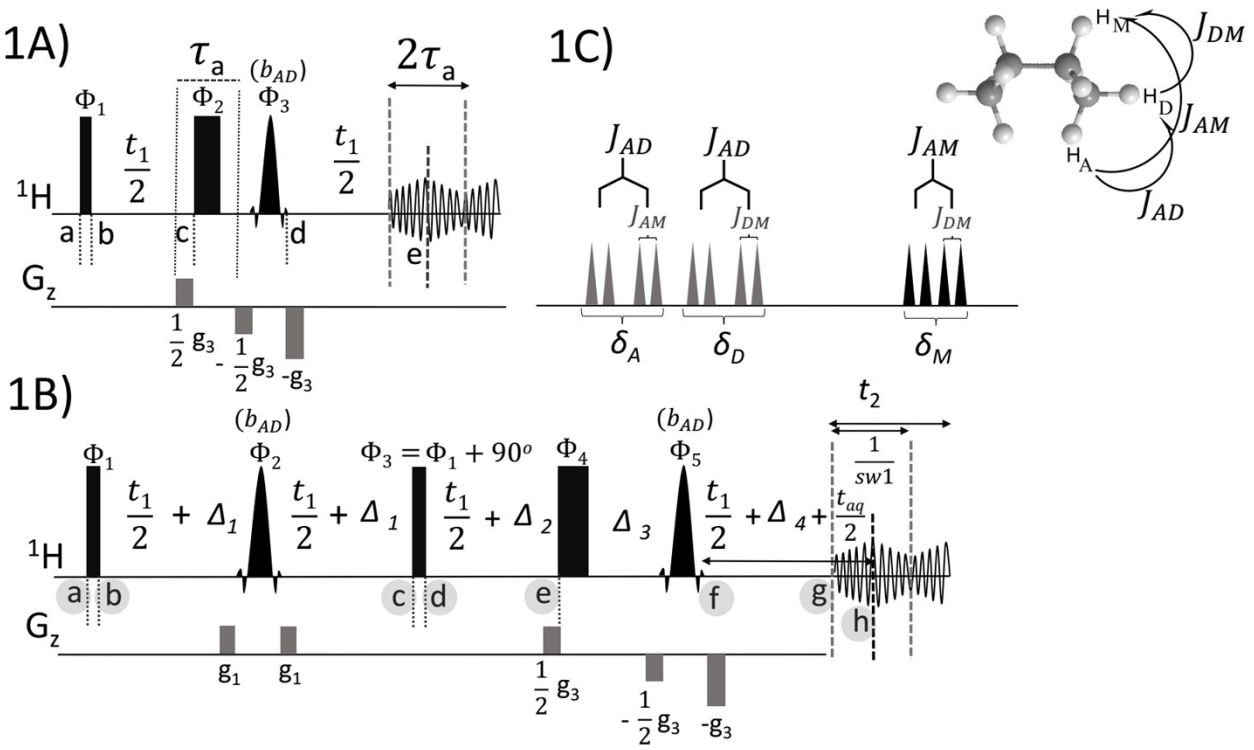

Figure S1. Pulse sequence for recording data chunking based a) Band Selective Homonuclear Decoupling (BASHD), b) Perfect echo-Band Selective Homonuclear Decoupling (PE-BASHD) experiment.

Pulse sequence for the Band Selective Homonuclear Decoupling (BASHD), and Perfect echo-Band Selective Homonuclear Decoupling (PE-BASHD) is displayed in Figure 1(in manuscript). Perfect echo sequence is modified via substitution of hard 180° pulse with BASH block. Narrow and wide rectangles represent hard 90° and 180° pulses, respectively. Selective pulses are represented by black shapes. For selective refocusing, ReBurp pulse of 1.55 ms was used for cyclosporine A. The gradients G_1 and G_3 are used for coherence selection with a gradient amplitude ratio of 35: 90. All gradients are squared smoothed shaped (SMSQ) with 600 µs duration and 200 µs recovery delay. An interferogram-based acquisition (also called pseudo 2D mode) is used and the duration of the data chunks is equal to twice the t_1 increment $(2SW_1)^{-1}$ in the homodecoupling dimension.

The delays Δ_1 to Δ_4 are $\Delta_1 = (2SW_1)^{-1} + p_{16} + 2d_{16}$, $\Delta_2 = (4SW_1)^{-1}$, $\Delta_3 = (4SW_1)^{-1} + p_{16} + 2d_{16}$, and $\Delta_4 = p_{16} + 2d_{16}$, where p_{16} is the length of the gradient and d_{16} is a recovery delay. Phase cycling is: Φ_1 = x −x, Φ_2 = y, Φ_3 = y y −y −y and Φ_{rec} = x −x for the BASHD experiment in (a) and Φ_1 = x, Φ_2 = y y −x −x −y −y x x, Φ_3 = y −y, $\Phi_4 = \Phi_5$ = x and Φ_{rec} = −x −x x x for the PE-BASHD experiment in (b).

Total delay duration between the pulses in pulse sequence in figure S1b is:

$$\Delta_1 = \frac{t1}{2} + \frac{t1}{2} + 2\Delta_2 + \Delta_4 = \tau_A$$

$$\frac{t1}{2} + \Delta_2 = \tau_B$$

$$\Delta_3 = \Delta_2 + \Delta_4$$

$$\frac{t1}{2} + \Delta_4 + \frac{tAq}{2} = \tau_c$$

1.2 Experimental Parameters of Band Selective Homonuclear Decoupling (BASHD) and Perfect Echo- Band Selective Homonuclear Decoupling (PE-BASHD) for Cyclosporine A recorded in pseudo 2D made

a) For H^{α} Region Protons

For Figure 3D and 3E: All the experiments were recorded on 800 MHz NMR spectrometer equipped with CPTCI cryoprobe and z- axis gradient only at 298K temperature. For H^{α} region signals, ReBurp refocusing pulse of 5.5 ms with 1.2 KHz bandwidth was used. All the spectra in figure (3D, and 3E) were acquired with t_2 and t_1 acquisition times of 350 ms and 500 ms in F_2 and F_1 dimensions respectively with 4 transients per t_1 increment for a total of 80 increments, TD and TD*1* were set to 5608, and 80 respectively. Recycle delay was 1.5 seconds. SW and SW*1* were set to 8 KHz, and 80 Hz in F_2 and F_1 respectively. Carrier frequency was set at 5.36 ppm. FID resolution was 1.42 Hz in F_2 and 1.00 Hz in F_1 dimension, respectively. Data chunk duration was 12.5 ms. Both spectra were processed with zero filled to 8*K* and 256 data point in F_2 and F_1 dimension respectively with unshifted sine bell (SSB=0) window function and line broadening of 0.3 Hz. Total experiment time to record the spectra in 3D was 15 minutes and 38 seconds, and for 3E was 12 minutes 53 seconds.

b) For H^{α} and H^{N} (amide) Region Protons

For H^{α} and H^{N} region signals in figure 4A and 4B, ReBurp refocusing pulse of 1.55 ms with 4.0 KHz bandwidth was used. All the acquisition and processing parameters for the H^{α} and H^{N} region protons were kept same as in figure 3D, and 3E. Carrier frequency was set at 6.51 ppm. Total experiment time to record the spectra in 4B was 13 minute, and for 4C was 15 minute 44 seconds.

c) FID Reconstruction from *Pseudo* 2D spectra

For both H^{α}, H^{α} and H^{N} region protons in figure 2c-f, 3D, 3E, 4A, 4B (in main manuscript), FID was reconstructed using *pshift* macro from the Manchester NMR methodology Group web pages (http://nmr.chemistry.manchester.ac.uk), which was zero filled to 32768 complex data points and multiplied with exponential window function (1.0 Hz line broadening) before Fourier transformations.

d) NMR Simulation and Experiment NMR Spectra of 2, 3-Dibromothiophene

For the simulation 2, 3-Dibromothiophene (50 µL) were used in a 5:4 mixture of C_6D_6 and $CDCl_3$ solvent (450 µL). Spectra (6b in manuscript) were recorded on 400 MHz NMR spectrometer equipped with BBFO smart probe and z- axis gradient. In Figure 6b, spectra were recorded as a function of temperature from 300K to 265K. For two doublet signals of two different protons (6.57 and 6.61 ppm), RSnob pulse of 9.5 ms with 247 Hz bandwidth was used. In Figure 6b, Spectra were acquired with t_1 and t_2 acquisition times of 411 ms and 250 ms in F_2 and F_1 respectively with 4 transients per t_1 increment for a total of 40 increments, TD and TD*1* were set to 2548, and 40 respectively. Recycle delay was 1.5 seconds. SW and SW*1* were set to 8 KHz, and 80 Hz in F_2 and F_1 respectively. Carrier frequency was set at 6.611 ppm (at the center of two doublets). FID resolution was 2.43 Hz in F_2 and 4.00 Hz in F_1 dimensions, respectively. Data chunk duration was 12.5 ms. Both spectra were processed with zero filled to 4*K* and 128 data point in F_2 and F_1 dimension respectively with Gaussian window function with (SSB=2) and line broadening (LB) of -1.0 Hz. Total experiment time to record the spectra in 6b was 7 minutes and 7 seconds.

In Figure 6a, NMR simulation was carried out in NMRSIM programme in BRUKER TOPSPIN 3.5 software with same experimental parameters as in Figure 6b.

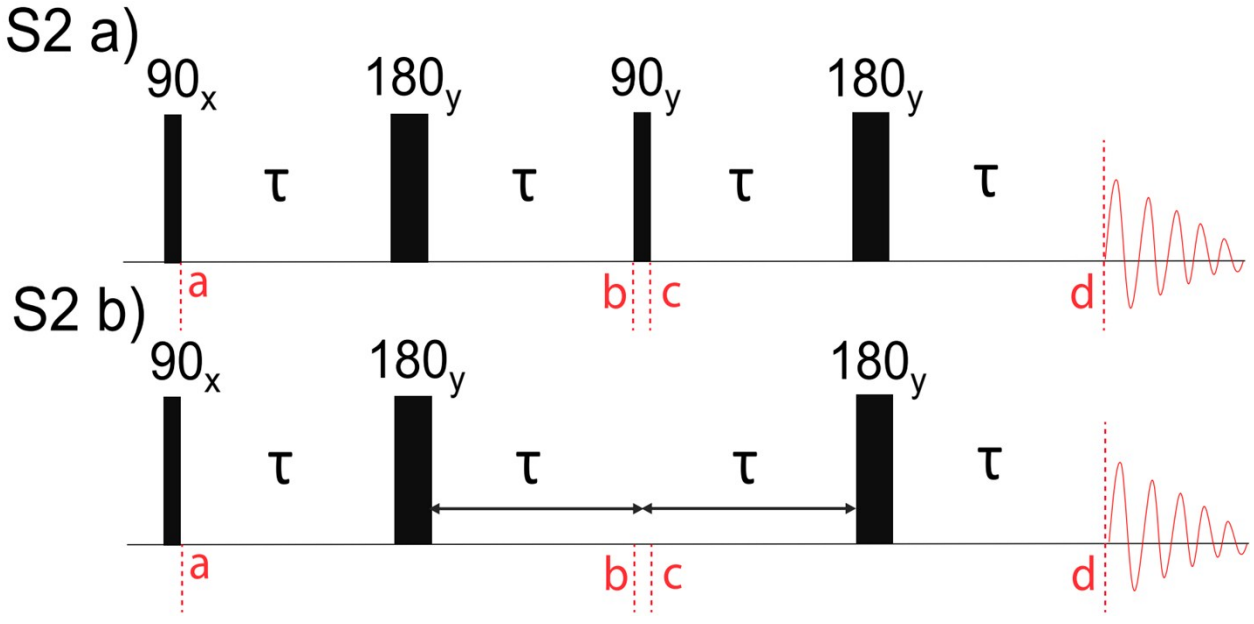

Figure S2: (a) Perfect echo pulse sequence (b) The double spin echo pulse sequence.

1.3 Line width issues of perfect echo spectrum for weakly coupled case:

The perfect echo sequence refocuses the effect of homonuclear scalar couplings via exchange of the antiphase terms. The method works ideally in two spin system with equal initial magnetizations for the two signals. Due to the exchange of antiphase coherences the relaxation of the signals are not independent throughout the sequence. Below we show the product operator calculations for a two spin system and mono exponential relaxation of these operators.

The perfect echo pulse sequence is displayed in Figure S2a. A spin system of the form H_1-C-C-H_2 is considered for discussion. Product operator calculation is discussed below where ^{1}H-^{1}H *J*-coupling is denoted as J_H, and chemical shift of spin 1 and 2 as Ω_1 and Ω_2. Since, weak homonuclear *J*-coupling interaction commutes with chemical shift interaction, and evolution of chemical shift is refocused by the refocusing pulses, only homonuclear scalar coupling evolution is considered here.

The operators present at time point 'a' and 'b' are:

$$-I_{1Y} - I_{2Y} \quad \text{and}$$

$$[-I_{1Y}\cos(\pi J_H 2\tau)exp(-R_{2(I1ip)}2\tau) + 2I_{1X}I_{2Z}\sin(\pi J_H 2\tau)exp(-R_{2(I1ap)}2\tau) - I_{2Y}\cos(\pi J_H 2\tau)(-R_{2(I2ip)}2\tau) + 2I_{2X}I_{1z}\sin(\pi J_H 2\tau)(-R_{2(I2ap)}2\tau)]$$

----------(1)

Where $R_{2(I1ip)}$ is the in-phase transverse relaxation rate constant of spin I_1, $R_{2(I1ap)}$ is the anti-phase transverse relaxation rate constant of spin I_1, $R_{2(I2ip)}$ is the in-phase transverse relaxation rate constant of spin I_2, $R_{2(I2ap)}$ is the anti-phase transverse relaxation rate constant of spin I_2

The operators at time point 'd' just after the *J*-refocusing 90_y^0 pulse can be written as:

$$[-I_{1Y}\cos(\pi J_H 2\tau)exp(-R_{2(I1ip)}2\tau) - 2I_{1Z}I_{2X}\sin(\pi J_H 2\tau)exp(-R_{2(I1ap)}2\tau) - I_{2Y}\cos(\pi J_H 2\tau)exp(-R_{2(I2ip)}2\tau) - 2I_{2Z}I_{1X}sin(\pi J_H 2\tau)exp(-R_{2(I2ap)}2\tau)]$$

---------- (2)

The exchange of antiphase states by *J*-refocusing 90^O_y pulse is evident. Now we look at the evolution of the operators during the 2nd half of the perfect echo and their relaxation behaviour

$$[-I_{1Y}\cos^2(\pi J_H 2\tau)\exp(-R_{2(I1ip)}4\tau) + 2I_{1X}I_{2Z}\cos(\pi J_H 2\tau)\exp(-R_{2(I1ip)}2\tau)\sin(\pi J_H 2\tau)\exp(-R_{2(I1ap)}2\tau) - 2I_{1Z}I_{2X}\sin(\pi J_H 2\tau)\exp(-R_{2(I1ap)}2\tau)\cos(\pi J_H 2\tau)exp(-R_{2(I2ap)}2\tau) - I_{2Y}\sin^2(\pi J_H 2\tau)\exp(-R_{2(I1ap)}2\tau)\exp(-R_{2(I2ip)}2\tau) - I_{2Y}\cos^2(\pi J_H 2\tau)exp(-R_{2(I2ip)}4\tau)$$

$$+ 2I_{2X}I_{1Z}\cos(\pi J_H 2\tau)\exp(-R_{2(I2ip)}2\tau)\sin(\pi J_H 2\tau)\exp(-R_{2(I2ap)}2\tau) - 2I_{2Z}I_{1X}\sin(\pi J_H 2\tau)\exp(-R_{2(I2ap)}2\tau)\cos(\pi J_H 2\tau)\exp(-R_{2(I1ap)}2\tau)$$

$$- I_{1Y}\sin^2(\pi J_H 2\tau)\exp(-R_{2(I2ap)}2\tau)\exp(-R_{2(I1ip)}2\tau)] \quad \text{----------(3)}$$

A careful analysis of the above eight terms reveals that refocusing to initial inphase single quantum coherences $-I_{1Y} - I_{2Y}$ will be complete provided $R_{2(I1ap)} = R_{2(I2ip)}$ and $R_{2(I1ip)} = R_{2(I2ap)}$ which is often not the case for a real system. If these relaxation rate constants are close in magnitude than refocused I_{1y} and I_{2y} coherences with uniform decay rate and hence similar line width will be observed.

For $R_{2(I1ap)} \neq R_{2(I2ip)}$ and $R_{2(I1ip)} \neq R_{2(I2ap)}$ refocusing to in-phase coherences $-I_{1Y} - I_{2Y}$ are not complete and small amount of antiphase coherences will also survive for both the signals. For in such a case 2nd and 7th term will not completely cancel out. Also 3rd and 6th terms will not cancel out completely. This will generate small amount of antiphase coherences.

Since refocusing of I_1 depends on how much antiphase coherence of I_2 exists just before the *J*-refocusing 90˚ pulse (at time point 'b') an average of the two decay rate $R_{2(I1ip)}$ *and* $R_{2(I2ap)}$will decide the linewidth of I_{1y}. The lines will be broader due to the contribution of faster decaying antiphase terms for a longer duration of 4τ relative to 2τ in a spin echo or regular BASHD experiment. Similarly, refocusing of I_2 depends on how much antiphase coherence of I_1 exists just before the *J*-refocusing 90˚ pulse. An average of the two decay rate $R_{2(I2ip)}$ *and* $R_{2(I1ap)}$will decide the linewidth of I_{2y}. This situation can be visualized by plugging $2\tau = \frac{1}{2J_H}$ in equation 3 above. And this visualization remains valid as equal role is played by in-phase and anti-phase coherences in the perfect echo sequence for the range of 2τ values that extends up to many hundreds of milliseconds. Comparison of Figures 2c, 2d (regular BASHD) vs 2e (PE-BASHD) in the main manuscript for strychnine reveals that line width of the PE-BASHD spectral lines are broader by a factor of 1.69 (=1.66/0.98) and 1.6 (=1.92/1.2) times. Not only two times longer transverse relaxation delay but also the faster relaxation of the antiphase coherence for a prolonged period of time determines the line width and hence affect sensitivity.

1.4: Full Hamiltonian and full propagator used for Matlab simulation of the the modulation of the in-phase SQ coherence I_Y (displayed in Figure 5 of the manuscript) as a function of the interval τ in the perfect echo sequence and double spin echo sequence.

$$H_{AB} = \omega_I I_Z + \omega_S S_Z + 2\pi J I.S$$

$$= \omega_I I_Z + \omega_S S_Z + 2\pi J(I_X S_X + I_Y S_Y + I_Z S_Z)$$

For perfect echo sequence of Figure S2a

$$\rho(4\tau) = e^{-H_{AB}\tau_1}\ e^{-i\pi F_y} e^{-H_{AB}\tau_1}\ e^{-i\frac{\pi}{2}F_y}\ e^{-H_{AB}\tau_1}\ e^{-H_{AB}\tau_1}$$

$$e(O)\ \ e^{H_{AB}\tau_1}\ e^{i\pi F_y}\ e^{H_{AB}\tau_1}\ e^{i\frac{\pi}{2}F_y}\ e^{H_{AB}\tau_1}\ e^{i\pi F_y}\ e^{H_{AB}\tau_1}$$

For the double spin echo sequence of Figure S2b

$$\rho(4\tau) = e^{-H_{AB}\tau_1}\ e^{-i\pi F_y} e^{-H_{AB}\tau_1}\ e^{-H_{AB}\tau_1}\ e^{-i\pi}$$

$$e(O)\ \ e^{H_{AB}\tau_1}\ e^{i\pi F_y}\ e^{H_{AB}\tau_1}\ e^{H_{AB}\tau_1}\ e^{i\pi F_y}\ e^{H_{AB}\tau_1}$$

1.5 Pulse Sequence code for Perfect Echo-Band Selective Homonuclear Decoupling (PE-BASHD) in BRUKER format

```
;avance-version
;Ajay Verma & Bikash Baishya
;using gradients
;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=
;Topspin 2.1 version

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

```
#include <De.incl>
#include <Delay.incl>

define delay tauA
define delay tauB
define delay tauC

;"p2=p1*2"
"d11=30m"
"d12=20u"
"in0=inf1/2"
"tauA=in0+(dw*2*cnst4)+de"
"tauB=in0/2-p16-d16-10u"
"tauC=in0/2+10u+(dw*2*cnst4)+de"

1 ze
2 d11
3 d12 pl1:f1
  d1
  50u UNBLKGRAD
4 p1 ph1                    ; First hard 90 degree pulse
  10u
  d0
  tauA pl0:f1
  p16:gp1
  d16
  d16
  (p10:sp0 ph2):f1          ; First selective 180 degree pulse
  d16
  p16:gp1
  d16
  d0
  tauA pl1:f1

5 p1 ph3                    ; Second hard 90 degree pulse
   =========  ZS Block Start  =========
  d0
  tauB
  10u
  p16:gp2*0.5
  d16
  p2 ph4
  tauC pl0:f1
  p16:gp2*-0.5
  d16
  d16
```

```
  (p11:sp1 ph5):f1        ; Second Selective 180 degree pulse
  d16
  p16:gp2*-1
  d16
  10u BLKGRAD
  d0
  ==========  ZS Block Ends  ========

   ========== FID Collection  ========
6 go=2 ph31
  30u
  d11 mc #0 to 2 F1QF(id0)
exit

   ph1=0
   ph2=1 1 2 2 3 3 0 0
   ph3=1 3
   ph4=0
   ph5=0
   ph31=2 2 0 0 2 2 0 0


;pl1 : f1 channel - power level for pulse (default)
;pl9 : f1 channel - power level for presaturation
;p1 : f1 channel -  90 degree high power pulse
;p2 : f1 channel -  180 degree high power pulse
;p16: homospoil/gradient pulse                  [1 msec]
;d0 : incremented delay (2D)                    [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d11: delay for disk I/O                        [30msec]
;d12: delay for power switching                 [20 usec]
;d16: delay for homospoil/gradient recovery
;inf1: 1/w, w = max. width of multiplet
;in0: 1/(2 * w), w = max. width of multiplet
;nd0: 2
;NS: 8 * n
;DS: 16
;td1: number of experiments
;FnMODE: QF
;for z-only gradients:
                        gp1 = 35%
                        gp2 = 90%
;use gradient files:
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
```

1.6 Pulse Sequence code for pseudo 2D Band Selective Homonuclear Decoupling (BASHD) in BRUKER format

```
;avance-version

;phase sensitive
;using gradients
;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

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

define delay tauA
define delay tauB
define delay tauC

;"p2=p1*2"
"d11=30m"
"d12=20u"
"in0=inf1/2"

"tauA=in0/2-p16-d16-50u"
"tauB=in0-p16-d16"
"tauC=in0/2-p16-d16-50u-(dw*2)-(dw*2*cnst4)-de"



1 ze
2 d11
3 d12 pl1:f1
  d1
4 p1 ph1
  d0
  tauA
  50u UNBLKGRAD
  p16:gp1*0.5
  d16
  p2 ph2
  tauB
  p16:gp1*-0.5
```

```
  d16
  (p11:sp1 ph3):f1
  p16:gp1*-1.0
  d16
  50u BLKGRAD
  tauC
  d0
  go=2 ph31
  d11 mc #0 to 2 F1QF(id0)
exit

ph1=0 2 0 2 1 3 1 3
ph2=0 0 0 0 0 0 0 0
ph3=0 0 1 1 0 0 1 1
ph31=0 2 2 0 1 3 3 1

;pl1 : f1 channel - power level for pulse (default)
;pl9 : f1 channel - power level for presaturation
;p1 : f1 channel -  90 degree high power pulse
;p2 : f1 channel -  180 degree high power pulse
;p16: homospoil/gradient pulse                    [1 msec]
;d0 : incremented delay (2D)                     [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d11: delay for disk I/O                         [30msec]
;d12: delay for power switching                  [20 usec]
;d16: delay for homospoil/gradient recovery
;inf1: 1/w, w = max. width of multiplet
;in0: 1/(2 * w), w = max. width of multiplet
;nd0: 2
;NS: 8 * n
;DS: 16
;td1: number of experiments
;FnMODE: QF

;for z-only gradients:
                              ;gpz1: 35%
;use gradient files:
;gpnam1: SINE.100
;gpnam2: SINE.100
```