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Supplementary information for *Spatially encoded 2D and 3D diffusion-ordered spectroscopy*

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CONTENTS

A. Discussion of models for SPEN DOSY

1. Introduction

Classic DOSY data processing relies on a model of the form:

$$S_i = S_0 \exp(-DK_i^2\Delta'), \tag{1}$$

where S_i is the attenuated intensity for the ith gradient increment, Δ' is an effective diffusion delay that accounts for the finite width of the gradient pulses, and K_i is the area of the diffusion-encoding gradient. For example, for the standard spin-echo experiment, using square gradients:

$$K_i = \gamma G_i \delta, \tag{2}$$

and

$$\Delta' = \Delta - \frac{\delta}{3}. \tag{3}$$

where G_i is the ith gradient intensity and δ is the duration of the gradient pulses.

For SPEN DOSY experiments, a convenient approach to fit the data would rely on a model of a form analogous to Eq. 1:

$$S(z) = S_0 \exp(-D\left(K_{\text{eff}}(z)\right)^2\Delta'), \tag{4}$$

where $K(z)$ is the effective gradient area at position z. Several equations of this form have been described in the literature.[1–3]

In this appendix, we will give a brief outline of the derivation of existing models and introduce an alternative model.

2. General expression

The attenuation caused by translational diffusion during a series of spin dynamics events is:

$$S(z,t) = S_0 \exp\left(-D\int_0^t \left(K(z,t')\right)^2 dt'\right), \tag{5}$$

where

$$K(z,t) = \frac{\partial\phi(z,t)}{\partial z} \tag{6}$$

is the first spatial derivative of the spins' phase. For the SPEN DOSY encoding block described in Fig. 1a of the main text, this may be rewritten as:

$$S(z,t) = S_0 \exp\left(-\left(b_{\text{chirp1}} + b_{\text{delay}} + b_{\text{chirp2}}\right)D\right), \tag{7}$$

where

$$b_{\text{chirp1}} = \int_0^\tau \left(K_{\text{chirp1}}(z,t')\right)^2 dt', \tag{8}$$

$$b_{\text{delay}} = (\Delta - \tau)\left(K_{\text{chirp1}}(z,\tau)\right)^2, \tag{9}$$

$$b_{\text{chirp2}} = \int_0^\tau \left(K_{\text{chirp2}}(z,t')\right)^2 dt'. \tag{10}$$

The term b_{chirp1} and b_{chirp2} correspond to the attenuation during the first and second chirp and gradient pair, respectively; the term b_{delay} corresponds to attenuation during the diffusion delay; $\tau = T_p + T_e$ is the duration of the chirp and gradient pair.

3. Analytical calculation

A common approximation to describe frequency swept pulses consists of assuming that spins flip instantaneously at the time t_Ω when the frequency of the pulse matches that of the spins.[2,3] This description may be adapted to the present case, in which an additional gradient is used after the chirp. Under this approximation:

$$K_{\text{chirp1}}(z,t) = \gamma G_e t \quad \text{when} \quad 0 < t < t_\Omega, \tag{11}$$
$$K_{\text{chirp1}}(z,t) = \gamma G_e (t - 2t_\Omega) \quad \text{when} \quad t_\Omega < t < \tau, \tag{12}$$
$$K_{\text{chirp2}}(z,t) = \gamma G_e (t + \tau - 2t_\Omega) \quad \text{when} \quad 0 < t < t_\Omega, \tag{13}$$
$$K_{\text{chirp2}}(z,t) = \gamma G_e (t - \tau) \quad \text{when} \quad t_\Omega < t < \tau. \tag{14}$$

For a pulse that sweeps linearly over a region of length L in a duration T_e, spins flip at a time:

$$t_\Omega = T_e \left(\frac{z}{L} + \frac{1}{2} \right). \tag{15}$$

Additional offset terms may also be included in Eq. 15. Combing Eqs 7 to 15, an analytical expression is obtained for $S(z)$:

$$S(z) = S_0 \exp\left(-D \left(\gamma G_e T_e\right)^2 \left(\frac{(T_e + T_p)^3}{6T_e^2} + \left(\frac{2z}{L} - \frac{T_p}{T_e} \right)^2 \left(\Delta - \frac{T_e + T_p}{2} \right) \right) \right) \tag{16}$$

which may be identified with Eq. 5 with the correspondance:

$$K_{\text{eff}}^{(a)} = -\gamma G_e T_e \left(\frac{2z}{L} - \frac{T_p}{T_e} \right), \tag{17}$$

and

$$\Delta'^{(a)} = \Delta - \frac{T_e + T_p}{2}. \tag{18}$$

The expression for $K_{\text{eff}}^{(a)}$ is also the effective gradient area for the chirp and gradient pair,[4] given in Eq. 2 of the main text.

4. Numerical calculation

Alternatively, the effective gradient area imparted by a chirp pulse can be calculated exactly as:[1]

$$K_{\text{eff}}^{(n)} = \int_0^{T_e} \frac{d}{dz} \left(\sqrt{\left(\Omega_0 + \gamma G_e z - \omega_{rf}(t)\right)^2 + \omega_1(t)^2} \right) dt + \gamma G_e T_p \tag{19}$$

where ω_{rf} and ω_1 are, respectively, the frequency and the amplitude of the chirp pulse as a function of time. Equation 19 may be evaluated numerically, as in the work of Keeler and co-workers. The duration of the chirp and gradient pair is assumed to be negligible. As a result, $b_{\text{chirp1}} \simeq 0$, $b_{\text{chirp2}} \simeq 0$ and

$$\Delta'^{(n)} = \Delta. \tag{20}$$

5. *Proposed model*

In the present work, we use a model that combines the exact calculation of the phase imparted by the chirp with an effective diffusion time. The former avoids the limitation of the "instantaneous-flip" approximation, and the latter captures the effect of the finite duration of the chirp and gradient pair. As a result:

$$K_{\text{eff}} = K_{\text{eff}}^{(n)} \tag{21}$$

and

$$\Delta' = \Delta - \frac{T_e + T_p}{2}. \tag{22}$$

The resulting model makes it possible to reduce systematic errors, as validated with numerical simulations (see Fig. 1c of the main text).

B. Materials and methods

1. *Sample preparation*

The mixture used to carry out nD SPEN DOSY experiments is composed of L-valine (7.2 mg), methanol (2.4 μL), ethanol (3.4 μL) and n-propanol (4.5 μL) solubilized in 590 μL of D_2O. The solution was shaken until complete dissolution of valine and filtered to remove any solid particles. The concentration is near 100 mM for each compound. The sample was then introduced in a standard 5 mm tube.

The same mixture was prepared for conventional nD DOSY, using instead a 5 mm Shigemi tube with a sample length of 10 mm.

A sample of 600 μL D_2O was prepared in a standard 5mm tube, to record a reference profile used for lineshape corrections with the residual H_2O signal.

2. *NMR spectroscopy*

All the experiments were carried out on a Bruker spectrometer operating at a Larmor frequency of 600.13 MHz, equipped with a triple-axis gradient probe. The temperature was set at a nominal value of 298 K and the diffusion time was 100 ms for all the DOSY experiments.

Conventional 2D spectra were recorded using a stimulated echo sequence with bipolar gradient pulses (stebpgp1s Bruker sequence with additional lock stabilisation gradients). Conventional 3D spectra were recorded using diffusion measurement using a stimulated echo sequence (ledbpgpco2s3d Bruker sequence with additional lock stabilisation). The diffusion-encoding gradient values consisted of a linear ramp ranging from 0.0065 T/m to 0.455 T/m. The duration of the gradient pulses was 800 μs. The 2D spectra were acquired with 16384 points in the direct dimension, a spectral width of 6000 Hz, a relaxation delay of 2.5 s and 16 gradient steps, resulting in a total experiment duration of 17 min. These parameters were transposed to the 3D experiment. The number of points was set to 128 in the additional indirect dimension, with a spectral width of 2600 Hz, resulting in total experiment duration of 14 h.

For the SPEN experiments, the diffusion-encoding parameters were chosen so that the minimum and maximum values of the gradient area would match those used in conventional DOSY, with a pulse sweeping a region of 10 mm. The resulting parameters are a chirp bandwidth of 110 000 Hz, an encoding gradient of 0.2535 T/m, a 1.5 ms duration for the chirp, and a 1.6 ms duration for the post-chirp gradient. Acquisition parameters were a 0.195 T/m acquisition gradient strength with 256 gradients loops of 256 points each and a 0.75 μs dwell time. This results in a spectral width of 2600 Hz. The gradient strength for coherence selection around the first chirp pulse were $a = 0.065$ T/m, $a + c = 0.1495$ T/m with a duration of 800 μs. The gradient strength for coherence selection around the second chirp pulse were $b = 0.1300$ T/m, $b + c = 0.2145$ T/m with a duration of 800 μs. The gradient strength for the spoiler during longitudinal storage was $f = -0.3716$ T/m and its counterpart $g_1 = -f$ with a duration of 800 μs. Balancing pulses for lock signal retention were $g_2 = (2a + c)/2$ and $g_3 = (2b + c)/2$ with a duration of 1600 μs. The prephasing gradient, g_4, was set to 0.0975 T/m.

The 2D experiment was recorded in a single scan of less than 250 ms. The SPEN DOSY COSY was recorded with 128 increments in the indirect dimensions and 8 dummy scans, with an inter-scan delay of 5 s, resulting in a total experimental time of 11 min.

The reference profile was acquired with a double-spin echo imaging pulse sequence using the same chirp parameters as for the SPEN DOSY experiments. The acquisition parameters were a 0.195 T/m acquisition gradient strength with 256 points and a 0.75 μs dwell time, which corresponds to a single echo of the SPEN DOSY acquisition.

3. Data processing

The data from conventional DOSY experiments were processed with the DOSY Toolbox, which is an open source programme for PFG diffusion NMR data processing.[5] For the DOSY-COSY and all SPEN experiments, the data were processed in MATLAB (The Mathworks, Natick, U.S.A.) using home-written routines, adapted in part from the DOSY Toolbox. In short, the nD SPEN data was read from Bruker files, then sorted and reshaped in order to get a nD matrix. The data was multiplied by a sine function and zero-filled in the t_1 or (t_1, t_3) dimension(s). Only the odd echoes were retained, and Fourier transformation was performed along all dimensions. The spectra were processed in magnitude mode.

For SPEN 2D DOSY, peak maxima were selected manually and the spatial profile for each peak was obtained as a row of the (z, ω) data set. For the 3D data set, regions were selected manually in the (ω_1, ω_3) plane and the spatial profile was obtained by integration over each region. The spatial profiles were fitted with a Stejskal-Tanner equation for all resonances. The DOSY representation was obtained Gaussian lineshapes with maxima corresponding to the calculated D value and linewidths set by the error of the fit.

4. Numerical simulation

Numerical simulations were carried out with MATLAB, using the SPINACH library[6] (version 1.8.3420). The SPEN DOSY experiment was simulated with encoding parameters that mimic the ones used experimentally. Specifically, encoding gradients of 0.2535 T/m were applied combined with a chirp pulse of 110 kHz bandwidth, with a duration of 1.5 ms. The post-chirp gradient had a duration of 1.6 ms. The acquisition was performed with a gradient

amplitude of 0.52 T/m, a duration of 192 μs and 256 points. The spectrometer gradient-based coherence selection was replaced by analytical coherence selection that proceeds by simply zeroing the unwanted elements of the state vector. The sample was described with 3000 points over a length of 0.015 m. For the diffusion operator, periodic boundary conditions were used, with 7-point central finite difference operators. The simulated 2D FID was Fourier transformed and the resulting spatial profile fitted with a Stejskal-Tanner equation. Full numerical implementation details will be published in a more specialised journal in due course; the corresponding Spinach example files will be included in the next public release of the code.

C. Supplementary figures

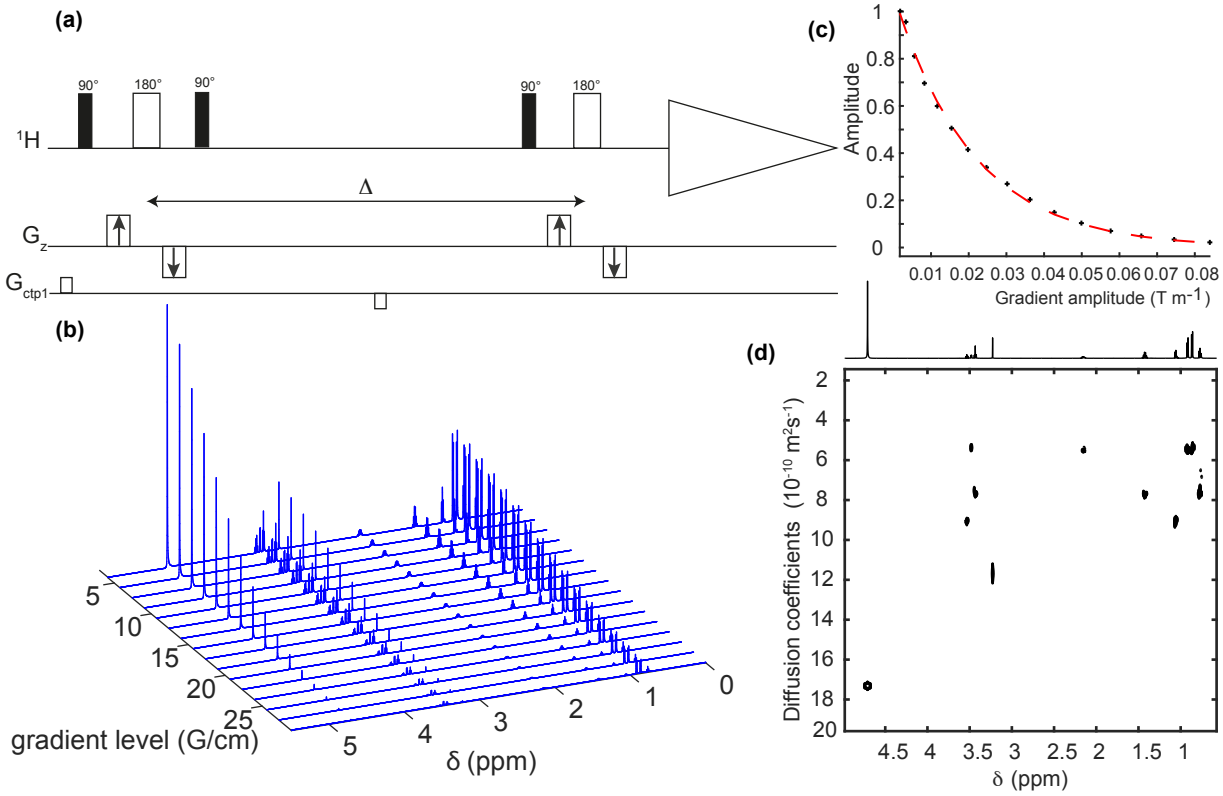

FIG. 1. 2D DOSY. (a) Pulse sequence for 2D DOSY with Z-filter and its compensating counterpart. (b) Series of decaying spectra obtained with the 2D DOSY experiment on a mixture of 3 alcohols (methanol, ethanol, propanol) and an amino-acid (L-valine) in D_2O. (c) Diffusion decay curve obtained from the data set shown in (b), for the methanol CH_3 resonances at 3.2 ppm. The best fit-curve for the modified Stejskal-Tanner equation is shown in red with D = 11.7 $m^2.s^{-1}$ (d) 2D DOSY display obtained from the data set shown in (b).

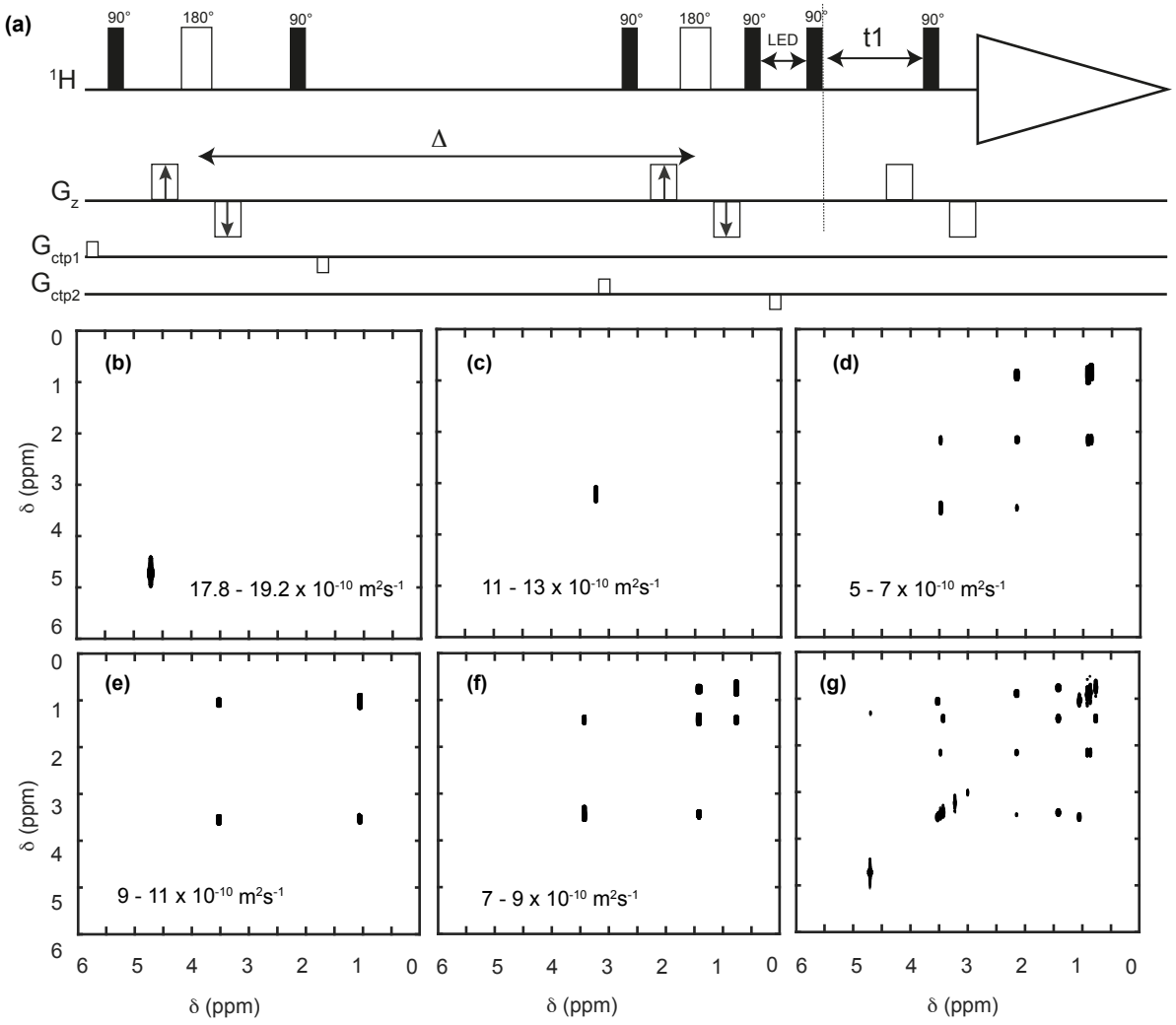

FIG. 2. 3D DOSY. (a) Pulse sequence for DOSY COSY. (b-f) COSY-type spectra obtained as slabs of the 3D (D, δ_{direct}, $\delta_{indirect}$) dataset resulting from DOSY processing. The selected range in D is shown in each panel. The COSY spectrum with the lowest diffusion gradient area is shown in (g).

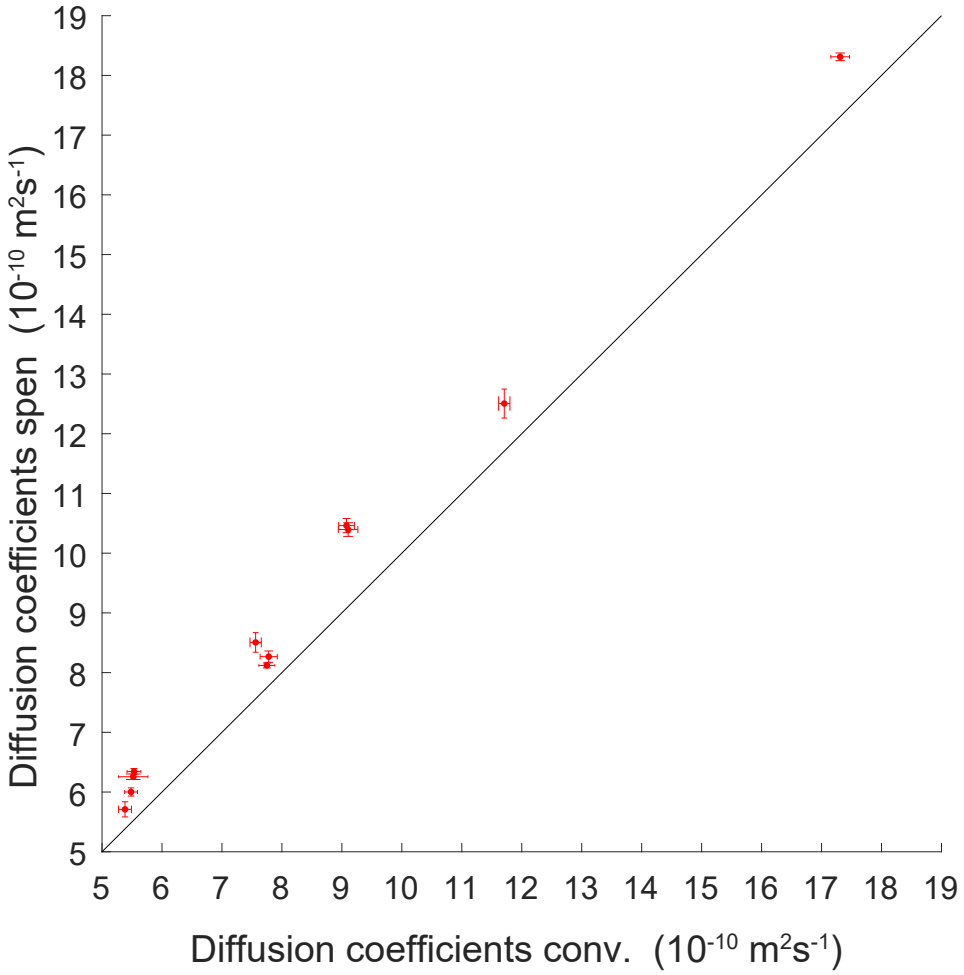

FIG. 3. Comparison of the diffusion coefficients obtained with conventional and SPEN 2D DOSY. The diagonal line is shown as a guide to the eye.

D. Pulse programs for SPEN DOSY

1. *SPEN 2D DOSY*

```
; Ludmilla Guduff, Carine van Heijenoort and Jean-Nicolas Dumez
; ICSN-CNRS
; spendosy
; 2D  sequence
; spatially encoded 2D diffusion-ordered spectroscopy

;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

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

"d20=(td*dw/(2*l3))-d6"
"d12=p11"
"DELTA1=d30-p11-2*d2-d11-2*p20-2*d16-2*p1-2*p21-2*d16-p22-d16-2*d11-2*d12-4*d2"
"p21=p20*2"

1 ze
  20u st0
  30m zd

2 30m
  d1
  50u UNBLKGRAD
  20u pl1:f1

  p22:gp24*-1
  d16

;excitation
  p1 ph1
  d5

;spatial encoding
  p20:gp20
  d16
  d2 gron0
```

```
  p11:sp1:f1 ph2
  d11
  d2 groff
  p20:gp21
  d16

;stimulated echo
  d5 pl1:f1
  p1 ph1
  p21:gp26*-1
  d16
  d2 gron1
  d12
  d11
  d2 groff
  p22:gp24
  d16
  DELTA1
  d2 gron1
  d12
  d11
  d2 groff
  p21:gp27*-1
  d16
  p1 ph1

;decoding period
  p20:gp22
  d16
  d2 gron0
  p11:sp1:f1 ph2
  d11
  d2 groff
  p20:gp23
  d16
  p25:gp25
  d16

;acquisition
  ACQ_START(ph30,ph31)
  1u DWELL_GEN:f1
3 d20 gron2
  d6 groff
  d20 gron3
  d6 groff
  lo to 3 times l3
  rcyc=2
```

```
  30m mc #0 to 2 F1QF(id2)
  100u BLKGRAD
exit

;phase cycling
ph1=0
ph2=0
ph3=0
ph29=0
ph30=0
ph31=0

;pl1 : f1 channel - power level for pulse (default)
;sp1: shaped pulse power level for selective detection
;p1 : f1 channel - 90 degree high power pulse
;p11: duration of the encoding chirp
;p20: crusher gradient
;p21: compensation gradient (2 x p20)
;p22: spoiler gradient
;p25: prephasing gradient
;spnam1 : shaped pulse for spatial encoding
;d1: relaxation delay; 1-5 * T1
;d2: ramping period for gradient pulse (25us)
;d5: 5us
;d11: post-chirp gradient
;d16: gradient recovery delay (200us)
;d30: diffusion time (big DELTA)
;d6: gradient ramp off during acquisition
;d20 + d6 : acquisition gradient duration
;GPZ0 : strength for encoding gradient [0-100]
;GPZ1 : compensation gradient GPZ1 = -GPZ0
;GPZ2 : strength for acquisition gradient [0-100]
;GPZ3 : strength for reversed acquisition gradient GPZ3 = -GPZ2
;GPZ20, GPZ21,  GPZ22 and GPZ23: crusher gradients
;GPZ24: spoiler gradient
;GPZ25: prephasing gradient
;GPZ26 and GPZ27: compensation gradient: GPZ26 = (GPZ20+GPZ21)/2 GPZ27=(GPZ22+GPZ23)/
;GPNAM20=GPNAM21=GPNAM22=GPNAM22=GPNAM24=GPNAM26=GPNAM27=SINE.100
;GPNAM25=RECT.1
;NS: 1
;l3=number of loops for acquisition
;IMPORTANT: set d20 + d6 = DW x TD(f3)/(2xL3)
```

```
; Ludmilla Guduff, Carine van Heijenoort and Jean-Nicolas Dumez
; ICSN-CNRS
; spencosydosy
; 3D  sequence
; spatially encoded 3D diffusion-ordered spectroscopy

;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

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

"d20=(td*dw/(2*l3))-d6"
"d12=p11"
"DELTA1=d30-p11-2*d2-d11-2*p20-2*d16-2*p1-2*p21-2*d16-p22-d16-2*d11-2*d12-4*d2"
"p21=p20*2"
"d0=3u"
"in0=inf1"

aqseq 321

1 ze
  20u st0
  30m zd

;;;;;;;;;;;;;;;;;; Beginning of dummy scans ;;;;;;;;;;;;;;;

21 30m
1u reset:f1

  d1
  50u UNBLKGRAD
  20u pl1:f1

  p22:gp24*-1
  d16

;excitation
  p1 ph1
```

```
  d5

;spatial encoding
  p20:gp20
  d16
  d2 gron0
  p11:sp1:f1 ph2
  d11
  d2 groff
  p20:gp21
  d16

;stimulated echo
  d5 pl1:f1
  p1 ph1
  p21:gp26*-1
  d16
  d2 gron1
  d12
  d11
  d2 groff
  p22:gp24
  d16
  DELTA1
  d2 gron1
  d12
  d11
  d2 groff
  p21:gp27*-1
  d16
  p1 ph4

;decoding period
  p20:gp22
  d16
  d2 gron0
  p11:sp1:f1 ph2
  d11
  d2 groff
  p20:gp23
  d16

;cosy encoding block
  d5 pl1:f1
  d0
  p28:gp28
  d16
```

```
  p0 ph2
  d13
  p28:gp28*-1
  d16
  p25:gp25
  d16

;acquisition
31 d20 gron2
  d6 groff
  d20 gron3
  d6 groff
  lo to 31 times l3
  100u BLKGRAD
  3m
  30m
  lo to 21 times l4


;;;;;;;;;;;;;;;;;;; End of dummy scans ;;;;;;;;;;;;;;;


2 30m
1u reset:f1
  d1
  50u UNBLKGRAD
  20u pl1:f1

  p22:gp24*-1
  d16

;excitation
  p1 ph1
  d5

;spatial encoding
  p20:gp20
  d16
  d2 gron0
  p11:sp1:f1 ph2
  d11
  d2 groff
  p20:gp21
  d16

;stimulated echo
  d5 pl1:f1
```

```
  p1 ph1
  p21:gp26*-1
  d16
  d2 gron1
  d12
  d11
  d2 groff
  p22:gp24
  d16
  DELTA1
  d2 gron1
  d12
  d11
  d2 groff
  p21:gp27*-1
  d16
  p1 ph4

;decoding period
  p20:gp22
  d16
  d2 gron0
  p11:sp1:f1 ph2
  d11
  d2 groff
  p20:gp23
  d16

;cosy encoding block
  d5 pl1:f1
  d0
  p28:gp28
  d16
  p0 ph2
  d13
  p28:gp28*-1
  d16
  p25:gp25
  d16

;acquisition
  ACQ_START(ph30,ph31)
  1u DWELL_GEN:f1
3 d20 gron2
  d6 groff
  d20 gron3
  d6 groff
```

```
  lo to 3 times l3
  100u BLKGRAD
  rcyc=2
  30m mc #0 to 2
  F1QF(id0)
  F2QF(id2)
  d17
exit

;phase cycling
ph1=0
ph2=0
ph3=0
ph4=0
ph29=0
ph30=0
ph31=0


;pl1 : f1 channel - power level for pulse (default)
;sp1: shaped pulse power level for selective detection
;p1 : f1 channel - 90 degree high power pulse
;p0: mixing pulse
;p11: duration of the encoding chirp
;p20: crusher gradient
;p21: compensation gradient (2 x p20)
;p22: spoiler gradient
;p25: prephasing gradient
;p28: coherence selection gradient for mixing
;spnam1 : shaped pulse for spatial encoding
;d1: relaxation delay; 1-5 * T1
;d2: ramping period for gradient pulse (25us)
;d5: 5us
;d11: post-chirp gradient
;d16: gradient recovery delay (200us)
;d30: diffusion time (big DELTA)
;d6: gradient ramp off during acquisition
;d20 + d6 : acquisition gradient duration
;GPZ0 : strength for encoding gradient [0-100]
;GPZ1 : compensation gradient GPZ1 = -GPZ0
;GPZ2 : strength for acquisition gradient [0-100]
;GPZ3 : strength for reversed acquisition gradient GPZ3 = -GPZ2
;GPZ20, GPZ21,  GPZ22 and GPZ23: crusher gradients
;GPZ24: spoiler gradient
;GPZ25: prephasing gradient
;GPZ26 and GPZ27: compensation gradient: GPZ26 = (GPZ20+GPZ21)/2 GPZ27=(GPZ22+GPZ23)/
;GPZ28: coherence selection gradient for mixing
```

```
;GPNAM20=GPNAM21=GPNAM22=GPNAM22=GPNAM24=GPNAM26=GPNAM27=GPNAM28=SINE.100
;GPNAM25=RECT.1
;NS: 1
;l3=number of loops for acquisition
;l4=number of dummy scans (DS does not work)
;IMPORTANT: set d20 + d6 = DW x TD(f3)/(2xL3)
```

---

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