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Supplementary information for *Ultrafast double-quantum NMR spectroscopy*

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Materials and Methods

Sample preparation

The 0.1 M sucrose sample was obtained by dissolving 34.2 mg of sucrose in 1 mL of D_2O. 600 µL of this solution were placed in a 5 mm NMR tube for analysis.

A mixture of metabolites was prepared from stock solutions containing each metabolite in H_2O/D_2O (90/10). These solutions were diluted to obtain a mixture with the following concentrations: alanine (13 mM), asparagine (20 mM), citrate (13 mM), glycerol (27 mM), lactate (18 mM), proline (24 mM) and serine (17 mM). 600 µL of this solution were placed in a 5 mm NMR tube for analysis. Traces of ethanol were present in the solution and thus ethanol signals were detected in most NMR spectra.

NMR spectroscopy

All spectra were recorded with a Bruker Avance I spectrometer operating at a ^1H Larmor frequency of 600.33 MHz, using a 5 mm TCI cryogenic probe. Presaturation during the interscan delay was added in all cases in order to suppress the residual water peak.

Conventional COSY spectra were acquired with the Bruker pulse sequence *cosygpprqf*, with 256 t_1 increments, and 1 scan. DQS and DQSsy spectra were acquired with the pulse sequence described in fig. S1, with 512 t_1 increments and 2 scans for sucrose, and 1024 t_1 increments and 4 scans for the mixture of metabolites. The inter-scan delay was 2.5 s for all conventional experiments. Presaturation was used during this delay for water suppression. The spectral width was 5 ppm in both dimensions for all experiments, except for the indirect dimension of the DQS spectrum, which was 20 ppm to avoid any possible folding of potential relay peaks. All spectra were processed with a non-shifted sinebell weighting function in both dimensions. In the DQS and DQSsy sequence, the final pulse had a flip angle of 120° in order to maximize the sensitivity for direct peaks;[1] The first gradient had half the area of the second gradient in order to select double-quantum coherence. The DQS spectrum with shearing was obtained by using the commend *ptilt1* on Topspin, with an Alpha value of 0.5 in F_1.

The total acquisition time was 12 min and 44 s for the COSY spectra, 50 min and 41 s for the DQS spectrum of sucrose, 3 h 23 min and 4 sec for the DQS spectrum of the metabolic mixture, 51 min and 47 s for the DQSsy spectrum of sucrose.

The ultrafast COSY pulse sequence has been described elsewhere.[2] Briefly, Chirp pulses of 15 ms and a sweep range of 10 kHz were applied for spatial encoding. The amplitude of the spatial encoding and coherence selection gradients was 1.33 G/cm and 42.4 G/cm, respectively. 64 gradients pairs were used during the acquisition, each one lasting 358.4 μs with an amplitude of 42.4 G/cm. Similar parameters were used for ultrafast DQS and DQSsy, and the final hard pulse was a 120° pulse, as in conventional experiments. The duration of the experiment was 2.2 s. For the single-scan spectra of sucrose, presaturation during 2 s was used for water suppression. For the mixture of metabolites, interleaving was used to double the spectral width in both dimensions,[3,4] which resulted in 4 times more scans to record the spectrum with an inter-scan delay of 5 s between scans. Presaturation was used during this delay for water suppression. For these spectra, 32 pairs of gradients were used during the acquisition, each one during 717.8 μs with amplitude of 42.4 G/cm. Purge gradients were added between scans in order to suppress any residual magnetisation. Four dummy scans were added for interleaved experiments, for an overall duration of 41 s. For all the ultrafast spectra, processing was done using an optimised Gaussian weighting function in the ultrafast dimension and a non-shifted sinebell weighting function in the conventional dimension. The ultrafast spectra were processed using a home-written routine in Topspin. All the pulse sequences and processing programs are available on demand.

Supplementary Figures

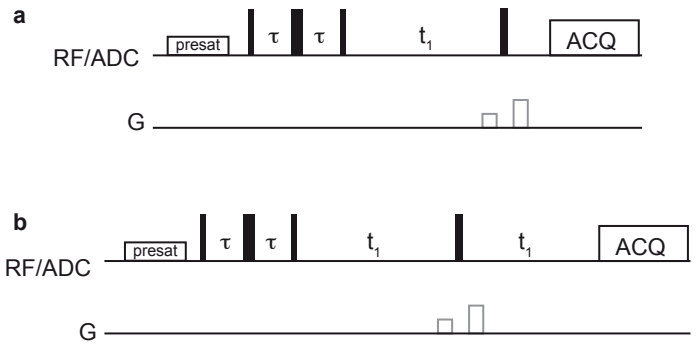

Fig. S1 Pulse sequences for conventional double-quantum NMR spectroscopy with **a** classic or **b** delayed acquisition. Hard 90° and 180° pulses are shown with thin and thick vertical bars, respectively. The last (mixing) pulse has a duration corresponding to a 120° flip angle. The delay τ corresponds the build-up of anti-phase magnetisation. The presaturation module is optional and is used here for water suppression.

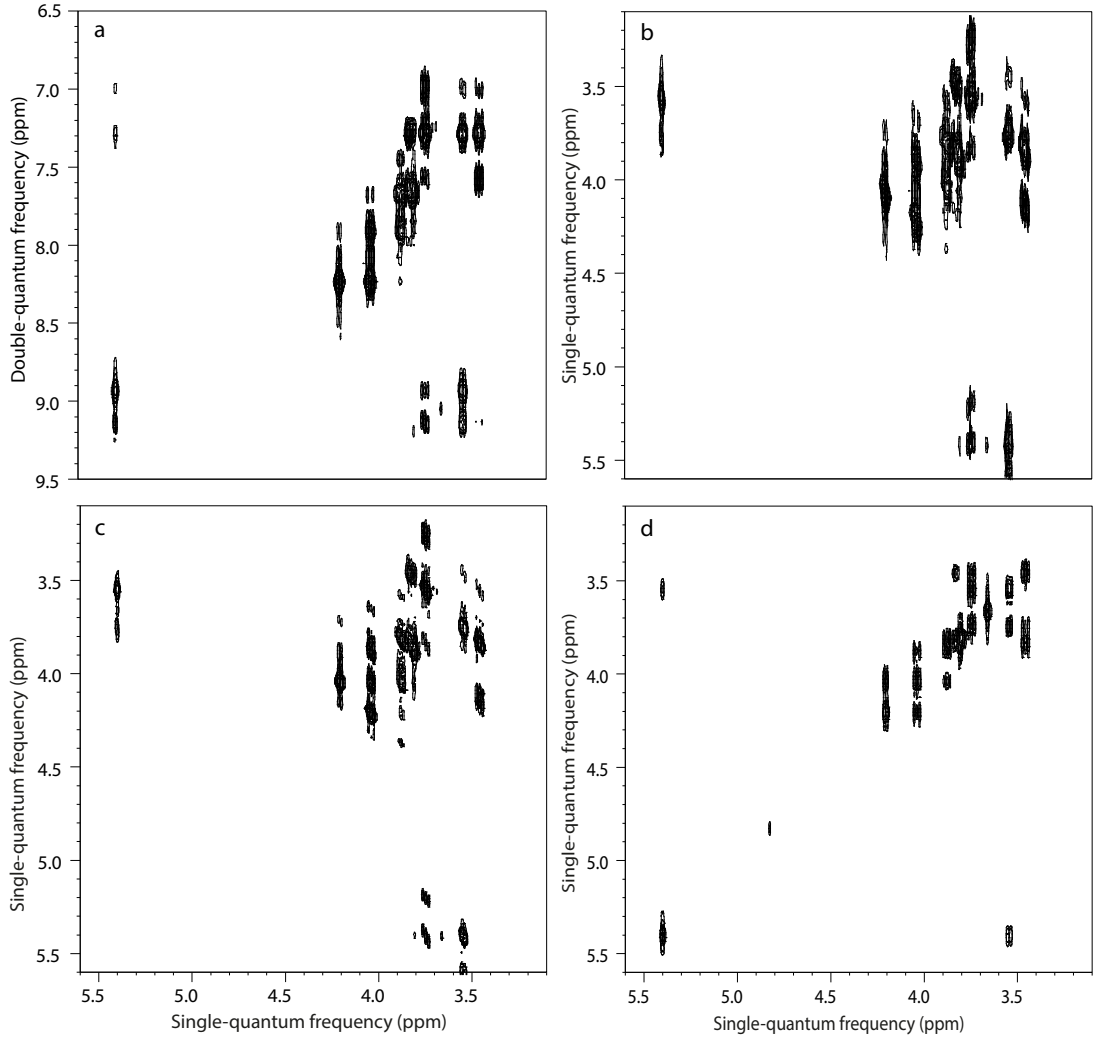

Fig. S2. Conventional 2D NMR spectra of a 0.1 M solution of sucrose in D_2O. (a) DQS (sequence shown in Fig. S1a) with 512 t_1 increments of 2 scans each and an inter-scan delay of 2.5s, using a double-quantum build-up delay τ = 25 ms; (b) spectrum (a) after shearing; (c) DQSsy (sequence shown in Fig. S1b) with 512 t_1 increments of 2 scans each and an inter-scan delay of 2.5s and (d) COSY with 256 t_1 increments of 1 scan each and an inter-scan delay of 2.5s

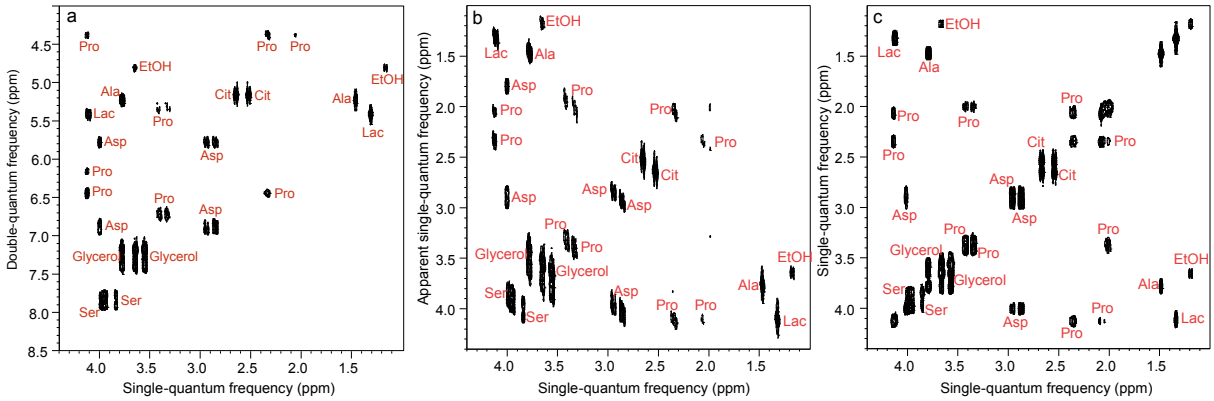

Fig. S3. Conventional NMR spectra of a mixture of 7 metabolites in H_2O/D_2O (90/10). (a) DQS (sequence shown in Fig. S1a) with 1024 t_1 increments of 4 scans each and an inter-scan delay of 2.5s, using a double-quantum build-up delay τ = 25 ms; (b) spectrum (a) after shearing; (c) COSY with 256 t_1 increments of 1 scan each and an inter-scan delay of 2.5s.

Supplementary Tables

The pSNR was calculated using the "sino" command in the Topspin software, in traces along the ultrafast (F_2) dimension. For the ufDQS spectrum, the pSNR is calculated using the non-sheared spectrum; to facilitate the comparison the peaks are referenced in the table using their position in the sheared spectrum.

Table S1: pSNR of selected well-resolved cross-peaks in the sucrose sample, labelled by their chemical shift (in ppm) along the ultrafast and conventional dimension.

	3.75, 3.55	3.53, 5.4	4.04, 4.2	4.04, 3.87
ufCOSY	357	38	356	215
ufDQS	264	59	263	66
ufDQSsy	138	124	367	70

Table S2: pSNR of selected well-resolved cross-peaks in the metabolic mixture, labelled by the metabolite name and by the chemical shift (in ppm) along the ultrafast and conventional dimension.

	Lac: 1.32, 4.1	Pro: 3.37, 3.32	Gly: 3.55, 3.7	Ala: 3.75, 1.47	Asp: 2.84, 3.98
ufCOSY	60	39	67	39	17
ufDQS	32	15	22	52	13

Pulse sequence for ultrafast double-quantum NMR spectroscopy

```
; Adrien le Guennec, Patrick Giraudeau, Stefano Caldarelli and Jean-Nicolas Dumez
; ICSN-CNRS
; dqsufprf3
; 2D ultrafast sequence
; Ultrafast double-quantum NMR spectroscopy

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

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

"p2=2*p1"
"d4=1s/(cnst3*4)"
"d0=3u"
"d11=30m"
"d12=20u"
"d13=4u"
"d20=(td*dw/(2*l3))-d6"
"TAU=p20"

1 ze
  20u st0
  20u pl9:f3

  30m zd
2 30m
  d1 cw:f3 ph29
  2u do:f3
  100u UNBLKGRAD
  20u pl1:f1

; excitation
  p1 ph1

; buildup of multi-spin coherences
  d4
  p2 ph2
  d4

; excitation of multiple-quantum coherences
  p1 ph2
```

```
; spatial encoding with crushers
  p20:gp21
  10u gron0
  p11:sp1:f1 ph2
  10u groff
  TAU
  p20:gp20
  10u gron1
  p11:sp1:f1 ph4
  10u groff
  p20:gp22

;pre-mixing gradient
  10u
  p24:gp24
  10u

;mixing
  p23:gp23
  10u pl1:f1
  p10 ph2
  10u
  p23:gp26

;post-mixing gradient
  10u
  p24:gp25
  10u

;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
  d17
exit

ph1=0 2
ph2=0
ph3=0
ph4=0
ph29=0
ph30=0
ph31=0 2
```

;pl1 : f1 channel - power level for pulse (default)
;pl9 : f3 channel - power level for presaturation
;sp1 : shaped pulse power level for spatial encoding
;p1 : f1 channel - 90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p10 : f1 channel - final high power pulse, can be 90 degree or 120 degree
;p11 : f1 channel - shaped pulse for spatial encoding
;p20 : coherence-selection gradients for spatial encoding
;p23 : coherence-selection gradients for mixing
;p24 : gradients for folding in the ultrafast dimension
;spnam1 : shaped pulse for spatial encoding
;d1 : relaxation delay; 1-5 * T1
;d6 : delay between acquisition gradients
;d20 + d6 : acquisition gradient duration
;d24 : pre-mixing gradient duration
;d25 : post-mixing gradient duration
;d17 : delay after experiment (not necessary)
;cnst3: = J(HH)
;GPZ0 : strength for excitation gradient [0-100]
;GPZ1 : strength for reversed excitation gradient GPZ1 = -GPZ0
;GPZ2 : strength for acquisition gradient [0-100]
;GPZ3 : strength for reversed acquisition gradient GPZ3 = -GPZ2
;GPZ20, GPZ21 and GPZ22: coherence-selection gradients for spatial encoding (GPZ20=GPZ21+GPZ22)
;GPZ23 and GPZ26: coherence-selection gradients for mixing (GPZ26=-2*GPZ23)
;GPZ24 and GPZ25: pre- and post-mixing gradients
;GPNAM20=GPNAM21= GPNAM22= SMSQ10.100
;GPNAM23=GPNAM26=SMSQ10.100
;GPNAM24=GPNAM25=RECT.1
;NS: 1 (or more if necessary)
;l3=number of loops for acquisition
;IMPORTANT: set d20 + d6 = DW x TD(f3)/(2xL3)

References

1(a) R. R. Ernst, G. Bodenhausen and A. Wokaun, *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*, Oxford University Press, Oxford, 1990; (b) C. Dalvit and J.-M. Bohlen, in *Annual Reports on NMR Spectroscopy*, ed. G. A. Webb, Academic Press, 1999, pp. 203-271; (c) A. D. Bain, D. W. Hughes, C. K. Anand, Z. Nie and V. J. Robertson, *Magn. Reson. Chem.*, 2010, **48**, 630.

2(a) M. Pathan, S. Akoka, I. Tea, B. Æ. Charrier and P. Giraudeau, *Analyst*, 2011, **136**, 3157; (b) A. Le Guennec, I. Tea, I. Antheaume, E. Martineau, B. Charrier, M. Pathan, S. Akoka and P. Giraudeau, *Anal. Chem.*, 2012, **84**, 10831.

3 L. Frydman, A. Lupulescu and T. Scherf, *J. Am. Chem. Soc.*, 2003, **125**, 9204.

4 L. Rouger, B. Charrier, M. Pathan, S. Akoka and P. Giraudeau, *J. Magn. Reson.*, 2014, **238**, 87.