SUPPORTING INFORMATIONS

Pushing the limits of signal resolution to make coupling measurement easier

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1. Comparison of BSD SERF with J-resolved spectra

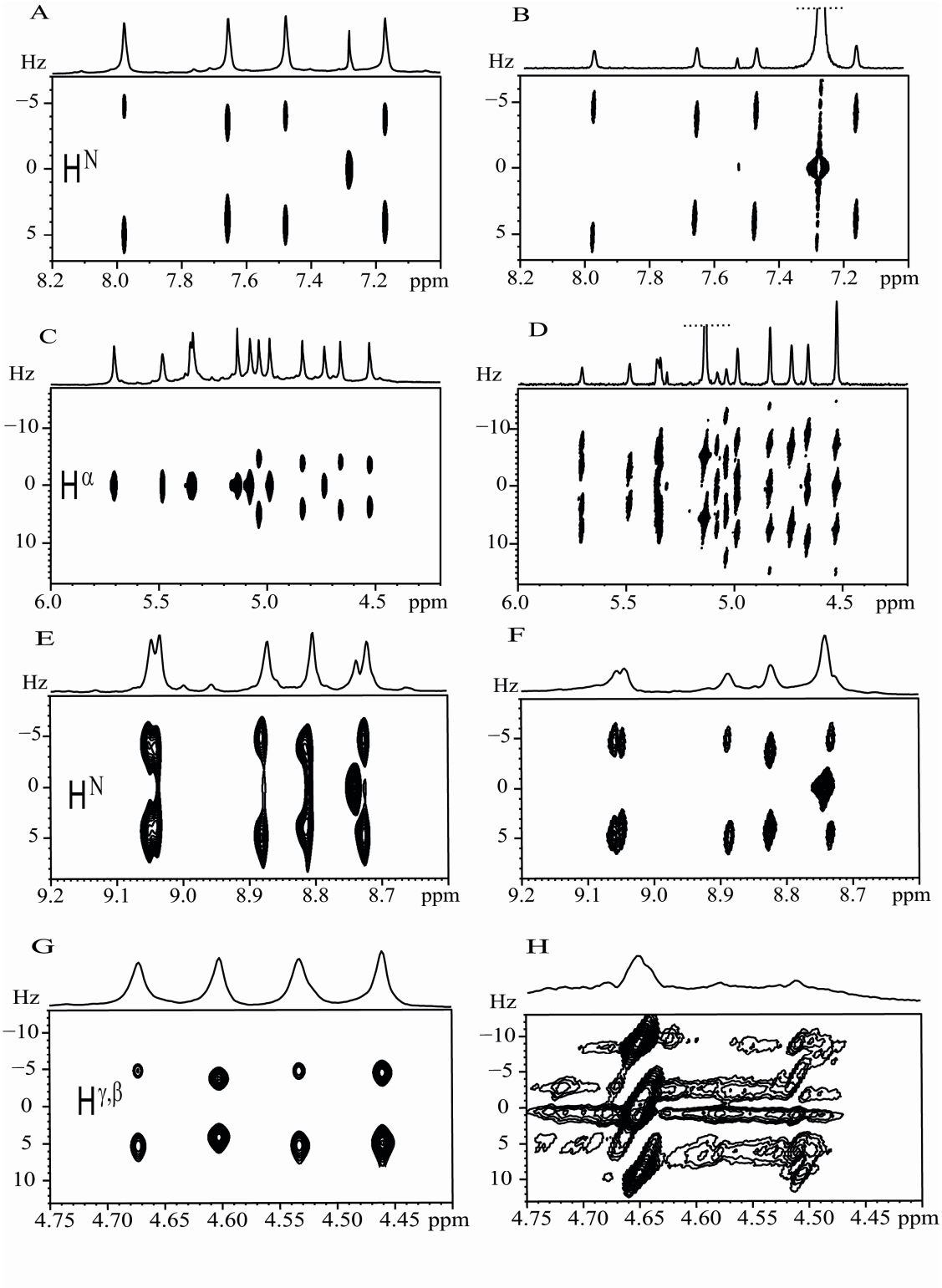

Figure S1: A, B, C, D cyclosporine A and E, F, G, H the heptapeptide. A, C, E, G the BSD SERF maps on H^N-Hα, Hα–H^N, H^N-Hγ/β, Hγ/β-H^N respectively and B, D, F, H, the J-resolved after 45° tilting maps on H^N-Hα, Hα–H^N, H^N-Hγ/β, Hγ/β-H^N respectively.

2. Comparison of BSD SERF and SERF for J_{HH} extraction

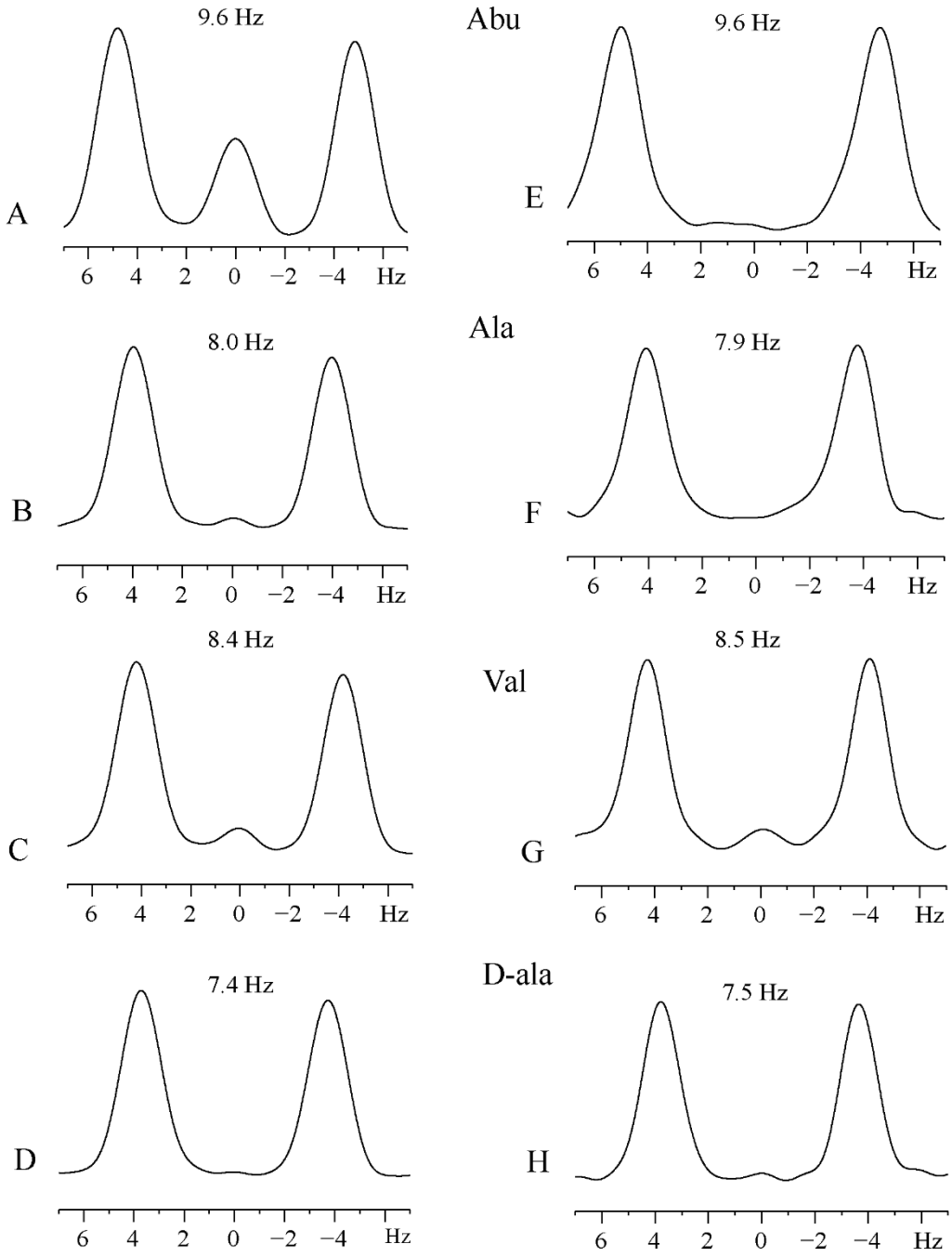

Figure S2: Extracted cross sections: A,B,C,D from 2D SERF (see A, on Figure 3) and E,F,G,H from 2D SERF pure shift (see B on Figure 3) for the residue Abu (A, E), Ala (B, F), Val (C, G) and D-Ala (D, H).

3. Precautions to select a given spins family

The Band Selective Decoupled pure shift techniques like BSD [1], BASH [2], implemented either in indirect or direct dimensions, and HOBS [3] in real time acquisition, use selective pulses to homodecouple ^{1}H spectra. As an example, the Methyls'area (0.8 ppm to 1.4 ppm) has to be carefully chosen: if the band of interest is overlapped with ^{1}H of another band like Hβ / Hγ (1.2 ppm to 1.6 ppm), the Methyls signal will not be completely ^{1}H decoupled: this is the case for Methyls of Mle4, Mle6 and Mle10 and Abu residues (see Figure S3 B). For obtaining the best homodecoupling, the first solution is to reduce the selectivity (400 to 200 Hz) by only considering the area from 0.8 to 1.1 ppm. Under such conditions, the BSD SERF applied on the restricted Methyl's area allows a very good vanishing of all ^{1}H-^{1}H scalar couplings (see Figure S3 C).

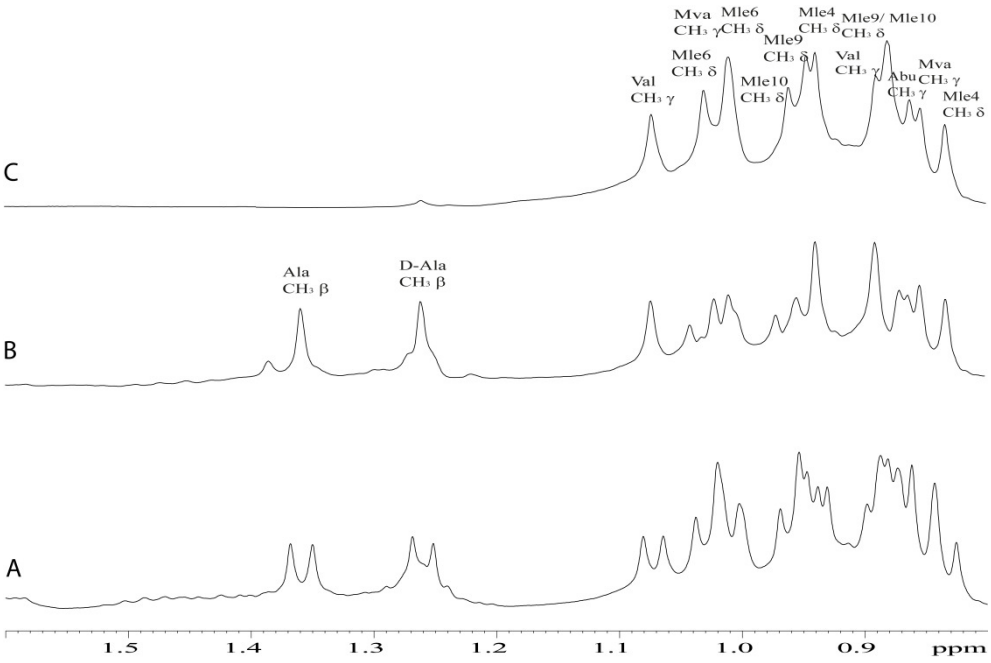

Figure S3: A) standard ^{1}H 1D spectrum, Band selective homodecoupling 1D by replacing the SERF block by its first EBURP 90° pulse (see Figure 1 of the main text) B) using 10 ms (400 Hz) EBURP / REBURP pulses, and C) using 20 ms (200 Hz) EBURP / REBURP pulses.

In case of overlapped areas another solution is the broadband homodecoupling pure shift or Zangger - Sterk technique [4]. However, this technique gives rise to sensitivity losses due to the slicing of the sample by the way of a simultaneous selective 180° pulse with an encoding gradient.

4. General experimental NMR part

NMR spectra were performed at 9.4 T at 300 K on a Bruker DRX 400 spectrometer using a $^{1}H/^{13}C/X$ Triple Broad Band Inverse probe equipped with a *z* field gradient coil and a standard variable-temperature unit (BVT 3000). All 3D spectra were obtained by recording 4096 x 32 x 16 matrices converted by the pure shift macro [5,6] to 2D 4096 x 32 points in the F_1 dimension. No apodization was applied in each dimension prior the double Fourier transform. Phasable 2D maps were obtained using the Quadrature Sequential Mode.

For the cyclosporine A, 6.7 ms (600 Hz) Burp pulses (EBURP-2 for excitation, REBURP for refocusing and a time reversal EBURP-2 for flip back) have been used in the SERF block for selecting H^N and Hα areas and 20 ms (200 Hz) for selecting HMe. In the band selective ^{1}H-^{1}H decoupled spectra (Figure 3/4 B, D), a REBURP selective pulse of 6.7 ms duration and bandwidth of 600 Hz for H^N ,Hα, Hβ and 20 ms duration (200 Hz) for the H^{Me}. The homodecoupled spectra (Figure 3/4 B,D) were acquired with number of t_2 increments (i.e., number of FID chunks) equal to 32, the duration of FID chunk is 19.2 ms, the number of complex data points of constructed FID in ^{1}H dimension is 4096, the relaxation delay is 1 s, and the number of scans is 4.

For the heptapeptide B, For the cyclosporine A, 6.7 ms (600 Hz) Burp pulses have been used in the SERF block for selecting H^N and Hγ,β areas. In the band selective ^{1}H-^{1}H decoupled spectra (Figure 5 B, D), a REBURP selective pulse of 6.7 ms duration and bandwidth of 600 Hz for H^N, Hγ,β. The homodecoupled spectra (Figure 5 B,D) were acquired with number of t_2 increments (i.e., number of FID chunks) equal to 32, the duration of FID chunk is 19.2 ms, the number of complex data points of constructed FID in ^{1}H dimension is 4096, the relaxation delay is 1 s, and the number of scans is 8.

5. References

[1] P. Király, M. Foroozandeh, L. Paudel, M. Nilsson, J. Clayden and G. A. Morris, *Chem. Commun*., 2014, **50**, 2514.

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[3] L. Castañar, P. Nolis, A. Virgili and T. Parella. *Chem. Eur. J.* 2013, **19**, 17286.

[4] K. Zangger, H. Sterk, *J. Magn. Reson.,* 1997, **124**, 486; N. H. Meyer, K. Zangger, *Chemphyschem.,* 2014, **15**, 49.

[5] J. A. Aguilar, S. Faulkner, M. Nilsson, G. A. Morris, *Angew. Chem. Int. Ed.*, 2010, **49**, 3901.

[6] http://nmr.chemistry.manchester.ac.uk/?q=node/256

6. Pulse program code for Bruker NMR spectrometers

```
;BSD-SERFph
; avance-version
;
; Selective refocussing experiment with a z filter for phase sensitive
version
; and a band selective element (BS) for pure shift along the direct
dimension
;based on:
; J. A. Aguilar, S. Faulkner, M. Nilsson, G. A. Morris, Angew. Chem. Int.
;Ed., 2010, 49, 3901
;
;The authors do not provide any guarantee as to the usability of the
;program.
;Use of this program may lead to serious damage to your spectrometer.
;The authors are not responsible for any damage resulting from the use of
this program.
;Note that all the parameters need to be properly adjusted for the program
;to work.
;This program may be freely copied and modified as long as the whole of
;this header
;is included in any copied or modified version.
; The resulting 3D dataset can be restructured using the pshift macro from
;the G. Morris group
;
;
;$CLASS=HighRes
;$DIM=3D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

define delay DELAY1
define delay DELAY2
define delay DELAY3

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

aqseq 312               ;define order of increments for 3D seq


"d0=3u"                  ; set initial value of pure shift incremented
delay
"in0=inf1/2"            ; set pure shift chunk size
"p2=p1*2"               ; 180 pulse width = 2 x 90 pulse width

;Homodecoupling delays
"DELAY1=in0/2-p16-d16-50u"
                        ; set delay before 1st 180 to in0/2
"DELAY2=in0-p16-d16"
                        ; set delay between 180 pulses to in0
"DELAY3=in0/2-p16-d16-(dw*2)-(dw*2*cnst4)-de"
                        ; set delay after 2nd 180 to in0/2 and...
                        ;   ...start acquisition L4 points early

;SERF delays
"in10=inf2/4"              ; set SERF increment QSEQ quadrature
```

```
"d10=3u"                ; set initial value of SERF incremented delay

1  ze
2   d11                  ; initialise
    d1 pl0:f1           ; set power level in f1 channel
   50u UNBLKGRAD        ; gradient blanking pulse
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;; SERF element ;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;

3 (p1 ph10):f1              ;virtual pulse at pl0=120dB for quadrature
despite z-filter
  (p11:sp1 ph1:r) ; A spin
  d10
  p19:gp0
  d19
  (p12:sp2 ph2:r)  ; X spin
  4u
  (p13:sp3 ph6:r)  ; A spin
   p19:gp0
  d19
  d10
  (p12:sp2 ph2:r) ;X spin
  4u
  (p14:sp4 ph4:r) ;A spin
  p20:gp1
  d20
  (p15:sp5 ph5:r); A spin

;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;; Band Selective Homodecoupling element ;;;;;;;;;;;;;;;;
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
4  d0                   ; incremented (pure shift) delay
   DELAY1               ;
   50u
   p16:gp3*0.5          ; CTP gradient pulse
   d16 pl1:f1                ; gradient stabilisation delay
   p2 ph0               ; 180 pulse
   DELAY2               ;
   p16:gp3*-0.5         ; CTP gradient pulse
   d16 pl0:f1                 ; gradient stabilisation delay

   p17:sp7:f1 ph3       ; selective BS 180


   p16:gp3*-1.0         ; CTP gradient
   d16                  ; gradient stabilisation delay
   50u BLKGRAD          ; gradient blanking pulse
   DELAY3               ;
   d0                   ; incremented (pure shift) delay

5  go=2 ph31            ; acquisition macro
   40u
   d11 mc #0 to 2       ; write data macro

       F1QF(id0)        ; BS loop
       F2PH(rd0 & ip10, id10)
       exit

; Tables for phase cycling
ph0=0
```

```
ph3=0 0 0 0 1 1 1 1
ph1=0 2 0 2 0 2 0 2
ph2=0 0 0 0
ph6=0 0 0 0
ph4=0 0 0 0
ph5=0 0 2 2 0 0 2 2
ph10=0
ph31=0 2 2 0 2 0 0 2

;Descriptions of the acquisition parameters
;p16 : CTP gradient pulse width (1 ms)

;d0  : incremented (pure shift) delay
;d1  : relaxation delay; 1-5 * T1
;d10  : incremented delay (SERF)
;d16 : gradient stabilisation delay [100-200 usec]
;d19 : gradient stabilisation delay [100-200 usec]
;d20 : gradient stabilisation delay [100-200 usec]

;cnst4  : Number of points to start the acquisition early
;pl0 : f1 channel - high power level pulse off (120 dB)
;pl1 : f1 channel - high power level hard pulse (default)

;NS  : 4 * n
;sp1: soft power for 90° selective pulse on A nuclei
;sp2: soft power for 180° selective pulse on X nuclei
;sp3: soft power for 180° selective pulse on A nuclei
;sp4: soft power for 90°flip back selective pulse on A nuclei
;sp5: soft power for 90° selective pulse on A nuclei
;sp7: soft power for 180° selective pulse on A nuclei for BS Homodecoupling
;p11: 90° shaped pulse on A nuclei
;p12: 180° shaped pulse on X nuclei
;p13: 180° shaped pulse on A nuclei
;p14: 90° shaped flip back pulse on A nuclei
;p15: 90° shaped pulse on A nuclei
;p17 : 180 selective pulse width for BSD
;d1 : relaxation delay; 1-5 * T1
;d0 : incremented delay; 3usec
;d11 : delay for disk I/O  [30 msec]
;NS: 2 * n
;p16=1000us
;p19=500us
;p20=1000us
;gpz0=15%
;gpz1=50%
;gpz3=80%
;on the A - X multiplets to be excited
;1 td : number of BS chunks to acquire      (F1)
;2 td : number of SERF increments            (F2)
;3 td : number of data points in time domain (F3)
;1 FnMODE: QF
;2 FnMODE: QSEQ
;for z-only gradients:
;use gradient files:
;gpnam0: SINE.100
;gpnam1: SINE.100
;gpnam3: SINE.100
;Copyright Université Paris Sud
```