CSSF-CLIP-HSQMBC: Measurement of heteronuclear couplings in severely crowded spectral regions

Aitor Moreno,¹ Kine Østnes Hansen² and Johan Isaksson*³

¹Bruker BioSpin AG, Application Science department, CH-8117 Fällanden, Switzerland
²Marbio, UiT - The Arctic University of Norway, Breivika, NO-9037 Tromsø, Norway
³Department of Chemistry, UiT - The Arctic University of Norway, Breivika, NO-9037 Tromsø, Norway

SUPPORTING INFORMATION
Figure S1: Stacked plot of a representative crosspeak \( ^2J_{3'\text{H}2} \) displaying scaling with diminishing block size (d62, bottom to top). Splittings for this and two more peaks are plotted in Figure S2. Parameters: constant acquisition time = 0.85 s, acquisition block length (# of loops) = 142 (3), 107 (4), 85 (5), 61 (7), 53 (8), 43 (10), 36 (12), 28 (15), 21 (20), 18 (24) ms. Selective pulse duration = 40 ms.

Figure S2: Measured splitting in the real-time band-selective homodecoupled CLIP-HSQMBC spectrum as a function of the block length and the duration of the selective bshd pulse for
(a) the $^2\text{J}_{\text{C}3'\text{H}2'}$, (b) the $^3\text{J}_{\text{C}8'\text{H}2'}$ and (c) the $^3\text{J}_{\text{C}4'\text{H}2'}$ couplings. (d) All three splittings from the 40ms gaussian series normalized on the true coupling, showing that they scale uniformly. Parameters: constant acquisition time = 0.85 s, acquisition block length (# of loops) = 142 (3), 107 (4), 85 (5), 71 (6), 61 (7), 53 (8), 43 (10), 36 (12), 28 (15), 21 (20) and 18 (24) ms. Selective pulse duration = 5, 20 and 40 ms.

**Figure S3** One representative splitting ($^3\text{J}_{\text{C}4'\text{H}2'}$) as a function of cumulative pulse length of the selective bshd pulses at a constant acquisition time. Parameters: constant acquisition time = 0.85 s, acquisition block length (# of loops) = 142 (3), 107 (4), 85 (5), 71 (6), 61 (7), 53 (8), 43 (10), 36 (12), 28 (15), 21 (20) and 18 (24) ms. Selective pulse duration = 5, 20 and 40 ms.
**Figure S4** All three splittings normalized on the true coupling using two different total acquisition times (red: 0.85 s, blue: 1.7 s), showing a dependence relative to the acquisition block size, and not the total number of applied pulses. Parameters: acquisition time = 0.85 and 1.7 s, acquisition block length (# of loops) = 142 (3), 107 (4), 85 (5), 71 (6), 61 (7), 53 (8), 43 (10), 36 (12), 28 (15), 21 (20) and 18 (24) ms for 0.85 s acquisition and doubled number of loops to achieve the same block lengths for 1.7 s acquisition time. Constant selective pulse duration = 20 ms.

**Bruker pulse sequences of the CSSF-CLIP-HSQMBC experiment**

```plaintext
;cssf_clip_hsqmbc
;advance-version (12/01/11)
;CSSF-CLIP-HSQMBC
;2D proton-selective CSSF-CLIP-HSQMBC experiment
;using selective refocussing with a shaped pulse
;with chemical shift selective filter (CSSF)
;single echo
;for an easy measurement of long-range proton-carbon coupling constants
;phase sensitive using Echo/Antiecho-TPPI gradient selection
;using shaped pulses for all 180degree pulses on f2 - channel

;P.T.Robinson, T.N. Pham & D. Uhrin, J. Magn. Reson. 170, 97-103 (2004)
;S.J. Duncan, R. Lewis, M.A. Bernstein & P. Sandor, Magn. Reson. Chem. 45, 283-288 (2007)
;J. Sauri, T. Parella, JF. Espinosa, Org. Biomol. Chem. 11, 4473-4478 (2013)

;CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
```
"p2=p1*2"
"d4=1s/(cnst2*4)"
"d11=30m"

"d0=3u"
"in0=inf1/2"

"DELTA1=p16+d16+50u"
"DELTA2=d4-liarger(p12,p14)/2-p16-d16-50u"
"DELTA=p16+d16+50u+p2+d0*2+50u"

"d20=3u"
"in20=1s/(cnst20*td0*2)"
"spoff2=0"

"acq0=0"
baseopt_echo

1 2e
d11
2 30m
20u p1:f1 BLKGRAD
d1
50u UNBLKGRAD
p1 ph1
d20
p16 gp1
d16
(center (p12:sp2 ph12) (p14:sp3 ph6):f2 )
2u
2u p1:f1
(p2 ph1)
p16 gp1*-1
d16
4u
d20
50u
p16 gp3
d16
DELTA2
4u
(center (p12:sp2 ph1) (p14:sp3 ph6):f2 )
4u
DELTA2 p2:f2 p1:f1
50u
p16 gp3
d16
(center (p1 ph2):f1 (p3 ph3):f2 )
d0
(p2 ph5)
d0
50u
p16 gp1*EA
d16
50u
(p24:sp7 ph4):f2
DELTA p2:f2
(p3 ph4):f2
50u
p16 gp4
d16
(p1 ph1):f1
50u
p16 gp5
d16
DELTA2
(center (p12:sp2 ph1) (p14:sp3 ph1):f2 )
DELTA2 p12:f2 p11:f1
50u
p16:gp5
4u
p16:gp2
d16 BLKGRAD
(p3 ph1):f2
go=2 ph31
30m mc #0 to 2
F0(d20 & xd)
F1EA(exec(r20) & calgrad(EA), caldel(d0, +in0) & calph(ph3, +180) & calph(ph6, +180) & calph(ph31, +180))

exit

ph1=0
ph2=1
ph3=0 2
ph4=0
ph5=0
ph6=0
ph12=0
ph31=0 2

;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;sp2 : f1 channel - shaped pulse
;sp3 : f2 channel - shaped pulse (180degree inversion)
;spnam3 : Crp60,0.5,20.1
;sp7 : f2 channel - shaped pulse (180degree refocussing)
;spnam7 : Crp60comp.4
;p1 : f1 channel - 90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel - 90 degree high power pulse
;p12 : f1 channel - 180 degree shaped pulse
;p14 : f2 channel - 180 degree shaped pulse for inversion
; = 500usec for Crp60,0.5,20.1
;p16 : homospoil/gradient pulse [1 msec]
p24 : f2 channel - 180 degree shaped pulse for refocussing
; = 2msec for Crp60comp.4
;d0 : incremented delay (2D) [3 usec]
d1 : relaxation delay; 1-5 * T1
;d4 : 1/(4J)XH
;d11 : delay for disk I/O [30 msec]
d16 : delay for homospoil/gradient recovery
d20 : incremented delay [3 usec]
cnst2 = J(XH)
cnst20 : distance (in Hz) to next multiplet (to be suppressed)
jn20 : 1/(cnt20*td0*2)
jnf1 : 1/SW(X) = 2 * DW(X)
jn0 : 1/(2 * SW(X)) = DW(X)
n00 : 2
ns : 2 * n, total number of scans: NS * TD0
ds : >= 16
td0 : TD0 = number of steps for suppression of undesired signals [8-16]
td1 : number of experiments

use gradient ratio: gp 1 : gp 2 : gp 3 : gp 4 : gp5
; 80 : 20.1 : 33 : 50 : 17 for C-13

;for z-only gradients:
gp21 : 80%
gp22 : 20.1% for C-13
gp3 : 33%
gp4 : 50%
gp5 : 17%

use gradient files:
CSSF-CLIP-HSQMBC experiment using selective refocussing with a shaped pulse. 
With chemical shift selective filter (CSSF) single echo for an easy measurement of long-range proton-carbon coupling constants. 
Phase sensitive using Echo/Antiecho-TPPI gradient selection using shaped pulses for all 180° pulses on f2 - channel with bandselective homodecoupling.

P. T. Robinson, T. N. Pham & D. Uhrin, J. Magn. Reson. 170, 97-103 (2004)
(S. J. Duncan, R. Lewis, M. A. Bernstein & P. Sandor, Magn. Reson. Chem. 45, 283-288 (2007))
J. Sauri, T. Parella, JF. Espinosa, Org. Biomol. Chem. 11, 4473-4478 (2013)
L. Castanar, P. Nolis A. Virgili & T. Parella, Chem. Eur. J. 19, 17283-17286 (2013)
J. Ying, J. Roche & A. Bax, J. Magn. Reson. 241, 97-102 (2014)

;FILE="cssf_clip_hsqmbc_bshd (Avance NEO version)"
;cssf_clip_hsqmbc_bshd_NEO
;advance-version (12/01/11)
;CSSF-CLIP-HSQMBC
;2D proton-selective CSSF-CLIP-HSQMBC experiment
;using selective refocussing with a shaped pulse
;with chemical shift selective filter (CSSF)
;single echo
;for an easy measurement of long-range proton-carbon coupling constants
;phase sensitive using Echo/Antiecho-TPPI gradient selection
;using shaped pulses for all 180° pulses on f2 - channel
;with bandselective homodecoupling

;P. T. Robinson, T. N. Pham & D. Uhrin, J. Magn. Reson. 170, 97-103 (2004)
;S. J. Duncan, R. Lewis, M. A. Bernstein & P. Sandor, Magn. Reson. Chem. 45, 283-288 (2007)
;J. Sauri, T. Parella, JF. Espinosa, Org. Biomol. Chem. 11, 4473-4478 (2013)
;L. Castanar, P. Nolis A. Virgili & T. Parella, Chem. Eur. J. 19, 17283-17286 (2013)
;J. Ying, J. Roche & A. Bax, J. Magn. Reson. 241, 97-102 (2014)

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

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

"p2=p1*2"
"d4=1s/(cnst2*4)"
"d11=30m"
"p29=300u"
"d0=3u"
"in0=inf1/2"

"DELTA1=p16+d16+50u"
"DELTA2=d4-larger(p12,p14)/2-p16-d16-50u"
"DELTA=p16+d16+50u+p2+d0*2+50u"

"d20=3u"
"in20=1s/(cnst20*td0*2)"

"spoff2=0"

"d62=aq/(l0*2)"
"d63=d62/2"
"acqt0=0"
baseopt_echo

1 2e
d11
2 30m
20u pl1:f1 BLKGRAD
p29:gp7
d16
5u
(p12:sp2 ph8):f1
5u
p29:gp7
d16
0.1u REC_UNBLK
0.05u DWELL_RELEASE
d62
0.05u DWELL_HOLD
0.1u REC_BLK
10u
p29:gp6
d16 pl1:f1
(p2 ph7):f1
p29:gp6
d16
10u
p29:gp7
d16
5u
(p12:sp2 ph8):f1
5u
p29:gp7
d16
0.1u REC_UNBLK
0.05u DWELL_RELEASE
d62
0.05u DWELL_HOLD
0.1u REC_BLK
lo to 4 times l0
d62
rcyc=2
30m mc #0 to 2
F0(id20 & zd)
F1EA(exec(rd20) & calgrad(EA), caldele(d0, +in0) & calph(ph3, +180) & calph(ph6, +180) & calph(ph31, +180))
4u BLKGRAD
exit
ph1=0
ph2=1
ph3=0 2
ph4=0
ph5=0
ph6=0
ph7=0
ph8=2
ph12=0
ph30=0
ph31=0 2

;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;sp2: f1 channel - shaped pulse
;sp3: f2 channel - shaped pulse (180degree inversion)
;spnam3: Crp60,0.5,20.1
;sp7: f2 channel - shaped pulse (180degree refocussing)
;spnam7: Crp60comp.4
;p1 : f1 channel - 90 degree high power pulse
cssfClip_hsqmbc_bshd (Avance III version)

use gradient ratio: gp 1 : gp 2 : gp 3 : gp 4 : gpz5 : gpz6 : gpz7

; for z-only gradients:
; gpz1: 80%
; gpz2: 20.1% for C-13
; gpz3: 33%
; gpz4: 50%
; gpz5: 17%
; gpz6: 3%
; gpz7: 5%

use gradient files:
; gpnam1: SMSQ10.100
; gpnam2: SMSQ10.100
; gpnam3: SMSQ10.100
; gpnam4: SMSQ10.100
; gpnam5: SMSQ10.100
; gpnam6: SMSQ10.100
; gpnam7: SMSQ10.100

; $Id: hsqcetgpsisp2.2,v 1.8 2012/01/31 17:49:26 - Exp $

P.T.Robinson, T.N. Pham & D. Uhrin, J. Magn. Reson. 170, 97-103 (2004)
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J. Ying, J. Roche & A. Bax, J. Magn. Reson. 241, 97-102 (2014)


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

"p2=p1*2"
"d4=1s/(cnst2*4)"
"d11=30m"
"p29=300u"
"d0=3u"
"in0=inf1/2"

"DELTA1=p16+d16+50u"
"DELTA2=d4-larger(p12,p14)/2-p16-d16-50u"
"DELTA=p16+d16+50u+p2+d0*2+50u"

"d20=3u"
"in20=1s/(cnst20*td0*2)"

"spoff2=0"
"d62=aq/(l0*2)"
"d63=d62/2"

"acq0=0"
baseopt_echo
dwellmode explicit

1 ze
d11
2 30m
20u p1:f1 BLKGRAD
d1
50u UNBLKGRAD
p1 ph1
d20
p16 gp1
d16
(center (p12:sp2 ph12) (p14:sp3 ph6):f2 )
2u
2u p1:f1
(p2 ph1)
p16 gp1*-1
d16
4u
d20
50u
p16 gp3
d16
DELTA2
4u
(center (p12:sp2 ph1) (p14:sp3 ph6):f2 )
4u
DELTA2 p12:f2 p11:f1
50u
p16 gp3
```
d16
(center (p1 ph2):f1 (p3 ph3):f2 )
d0
(p2 ph5)
d0
50u
p16 gp1*EA
d16
50u
(p24:sp7 ph4):f2
DELTAP p12:f2
(p3 ph4):f2
50u
p16 gp4
d16
(p1 ph1):f1
50u
p16 gp5
d16
DELTAP2
(center (p12:sp2 ph1) (p14:sp3 ph1):f2 )
DELTAP2 p12:f2 p11:f1
50u
p16 gp5
4u
p16 gp2
d16
(p3 ph1):f2
10u
ACQ_START(ph30, ph31)
0.1u REC_UNBLK
0.05u DWL_CLK_ON
d63
0.05u DWL_CLK_OFF
0.1u REC_BLK
4 10u
p29 gp6
d16 p11:f1
(p2 ph7):f1
p29 gp6
d16
10u
p29 gp7
d16
5u
(p12:sp2 ph8):f1
5u
p29 gp7
d16
0.1u REC_UNBLK
0.05u DWL_CLK_ON
d62
0.05u DWL_CLK_OFF
0.1u REC_BLK
10u
p29 gp6
d16 p11:f1
(p2 ph7):f1
p29 gp6
d16
10u
p29 gp7
d16
5u
(p12:sp2 ph8):f1
5u
p29-gp7
d16

0.1u REC_UNBLK
0.05u DWL_CLK_ON
d62
0.05u DWL_CLK_OFF
0.1u REC_BLK

lo to 4 times lo

d62

cyc=2

30m mc #0 to 2
F0(d20 & zd)
F1E(exec(d20)) & calgrad(EA), caldel(d0, +in0) & calph(ph3, +180) & calph(ph6, +180) & calph(ph31, +180))

4u BLKGRAD

exit

ph1=0
ph2=1
ph3=0 2
ph4=0
ph5=0
ph6=0
ph7=0
ph8=2
ph12=0
ph30=0
ph31=0 2

;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;sp2: f1 channel - shaped pulse
;sp3: f2 channel - shaped pulse (180degree inversion)
;spnam3: Crp60,0.5,20.1
;sp7: f2 channel - shaped pulse (180degree refocussing)
;spnam7: Crp60comp.4

;pl1 : f1 channel - 90 degree high power pulse
;pl2 : f1 channel - 180 degree high power pulse
;pl3 : f2 channel - 90 degree high power pulse
;pl4 : f2 channel - 180 degree shaped pulse for inversion
;pl16: homospoil/gradient pulse [1 msec]
;pl24: f2 channel - 180 degree shaped pulse for refocussing

;cnst2: = J(XH)
;cnst20: distance (in Hz) to next multiplet (to be suppressed)
in0: 1s/(cnst20*tdd*2)

;jn1: 1/(5W(X)) = z * DW(X)
jn0: 1/(2 * SW(X)) = DW(X)
nd0: 2
ns: 2 * n, total number of scans: NS * T0

dsc: >= 16
td0: T0 = number of steps for suppression of undesired signals [8-16]
td1: number of experiments

FnMODE: echo-antiecho
;use gradient ratio: gp 1 : gp 2 : gp 3 : gp 4 : gpz5 : gpz6 : gpz7
; 80 : 20.1 : 33 : 50 : 17 : 3 : 5 for C-13

;for z-only gradients:
;gpz1: 80%
gpz2: 20.1% for C-13
gpz3: 33%
gpz4: 50%
gpz5: 17%
gpz6: 3%
gpz7: 5%

;use gradient files:
gpnam1: SMSQ10.100
gpnam2: SMSQ10.100
gpnam3: SMSQ10.100
gpnam4: SMSQ10.100
gpnam5: SMSQ10.100
gpnam6: SMSQ10.100
gpnam7: SMSQ10.100

; $Id: hsqcetgpsisp2.2,v 1.8 2012/01/31 17:49:26 - Exp $