Accurate measurement of $^{3}J_{HNH\alpha}$ couplings in small or disordered proteins from WATERGATE-optimized TROSY spectra

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SUPPLEMENTARY MATERIAL

Table S1: $^{3}J_{HNH\alpha}$ values measured for GB3 (Hz)

Q2	7.55
Y3	9.77
K4	9.52
L5	9.34
V6	9.55
I7	9.46
N8	9,89
G9	12.96
K10	4.70
T11	10.06
L12	7.84
K13	9.34
E15	8.23
T16	6.58
T17	8.88
T18	6.62
K19	9.38
A20	7.41
V21	3.38
D22	6.27
A23	3.42
E24	3.82
A26	4.32
E27	5.14
K28	3.78
A29	4.52
F30	4.11
K31	3.96
Q32	4.68
Y33	3.43
A34	3.74
N35	3.64
D36	3.50
N37	9.33
G38	12.05
V39	9.12
D40	8.39
G41	9.28
V42	8.81
W43	8.70
T44	8.81
Y45	8.92
D46	9.91
D47	3.52
A48	4.28
T49	9.86
K50	7.17
T51	10.07
F52	9.47
T53	9.46
V54	9.81
T55	9.95
E56	8.49

Table S2: $^{3}J_{HNH\alpha}$ measured for $A\beta^{1-40}$ (Hz)

E3	6.30
F4	7.02
R5	7.25
D7	6.86
S8	5.86
G9	12.69
Y10	6.38
E11	6.29
V12	6.78
H13	7.25
Q15	6.26
K16	6.22
L17	6.69
V18	8.30
F19	7.70
F20	7.74
A21	5.60
E22	5.95
D23	6.64
V24	6.82
G25	11.92
S26	6.51
N27	7.32
K28	6.46
G29	12.06
A30	5.49
I31	7.65
I32	7.45
G33	11.91
L34	6.72
M35	7.25
V36	7.49
G37	11.96
G38	11.97
V39	7.94
V40	8.62

Bruker pulse program of the ^{1}H-^{15}N TROSY-HSQC experiment

```
#include <bits.jfy>

"in0=inf1*0.5"
"in10=inf1*0.25"

"l3=td1*0.5"

"d25=p22+97u+de+p1*0.36"

"d0=3u"
"d10=3u"
"d11=50m"
"d21=2.7m-p21"
"d22=2.7m-p21-p10"
"d23=2.1m-p2-14u"  ;adjust the delay empirically to minimize the anti-
TROSY 15N component (see Schulte-Herbruggen and Sorensen, JMR 2000,
144, 123-128)
"d24=p24+d0+d10*2+p1*5.33+p14+50u-p23-p7*1.27"


1       ze
        1m
2       d11 do:N
        1.2m LOCKH_OFF
3       3m
4       3m
5       3m
6       1m do:N
7       10u
        d1 BLKGRAD
        1m UNBLKGRAD
        10u pl1:H
        10u pl7:N
;------- start 90-degree on hn -----------------------
       (p1 ph0):H
        3u
        p21:gp1
        d21
        300u
       (center (p1*2 ph0):H (p7*2 ph0):N)
        d21
        p21:gp1
        303u
       (p1 ph1):H                ;INEPT to 15N
        3u
        3u pl12:H
       (p2 ph11:r):H ;1m rectangular water flipback pulse at 800 MHz
;goto 999 ;read out here and adjust pldb12 and phcor11 for minimal
residual water at rg=1
        6u
        4u pl0:H
        p20:gp0
        200u
```

```
 ;****************************************
if "l1==1"
   {
       (p7 ph7):N
   }
else
   {
       (p7 ph17):N
   }

        16u
        p23:gp5*EA ;first encoding gradient pulse, 1.1m at -59%
        d24

if "l1==1"
   {
       (p7*2 ph7):N
   }
else
   {
       (p7*2 ph17):N
   }

        d0  ;t1/2

       (p14:sp4 ph0 3u 3u pl1 p1 ph0 p1*2.33 ph1 p1 ph0):H ;p14=1.1m,
sp4=IBURP2 pulse at an offset of 2900Hz at 800MHz

        p24:gp6*EA ;second encoding gradient pulse, 0.9ms at 59%
        50u
        d10 gron10     ;t1/4, weak gradient (1.3%) to keep water at -z
        5u groff
        d10 gron10*-1  ;t1/4, weak gradient (1.3%) to keep water at -z
        5u groff

if "l1==1"
   {
       (p1 ph2):H
        3u
        3u pl0:H
       (p2:sp2 ph12:r):H  ;90 degree 1m sinc1 water flipback pulse,
zero offset
   }
else
   {
       (p1 ph3):H
        3u
        3u pl0:H
       (p2:sp3 ph13:r):H  ;90 degree 1m sinc1 water flipback pulse,
zero offset
   }
;goto 999 ;read out here to adjust spdb2 and phcor12 with l1=1; then
spdb3 and phcor13 with l1 changed to 2; to minimize residual water;
make sure l1 is set back to 1

        6u gron2
        d23 pl1:H
```

```
        2u groff
       (center (p1*2 ph0):H (p7*2 ph0):N)
        3u gron2
        d23 pl0:H
        3u groff
       (p2:sp1 ph0:r):H ;90 degree 1m sinc1 water flipback pulse, zero
offset
        6u
        2u pl1:H
if "l1==1"
   {
       (p1 ph0):H (p7 ph14):N    ;DOUBLE 90
   }
else
   {
       (p1 ph0):H (p7 ph4):N     ;DOUBLE 90
   }
;goto 999 ;read out here to adjust spdb1 and phcor0 to minimize
residual water

        5u
        p21:gp3
        d22 pl0:H

       (center
         (d25 3u fq=cnst4 p10:sp10 ph0 3u 2u pl1 p1*2 ph10 3u 2u pl0
p10:sp10 ph0):H ;at 800 MHz, p10=0.6m, sp10 is a 90 degree sine1 water
flipback pulse, spoffs10=0 but the carrier may be shifted upfield by
cnst4=-250Hz for abeta
         (p7*2 ph0):N
       )
;goto 999 ;read out here to adjust spdb10 to minimize residual water

        3u
        2u fq=0:H ;shift the carrier back to water in case cnst4 is
used to shift the carrier upfield
        p21:gp3
        d22 pl1:H

       (p7 ph0):N
        p22:gp4         ;decoding gradient, 202u at 59%
999     95u
        5u BLKGRAMP
       go=2 ph31
        100u do:N
        1m LOCKH_OFF
        d11 wr #0 if #0 zd
        50u iu1
        50u igrad EA
        lo to 3 times 2
        1m id0
        1m id10
        1m ru1
        lo to 4 times l3
        10u rd0
        10u rd10
1m do:N
```

```
1m
exit

ph0=0
ph1=1
ph2=1
ph3=3
ph4=3
ph7=1 0 3 2
ph10=2
ph11=0
ph12=1
ph13=3
ph14=1
ph17=1 2 3 0
ph31=1 2 3 0
```