Supplementary Material

Improved accuracy of ^{15}N-^{1}H scalar and residual dipolar couplings from gradient-enhanced IPAP-HSQC experiments on protonated proteins

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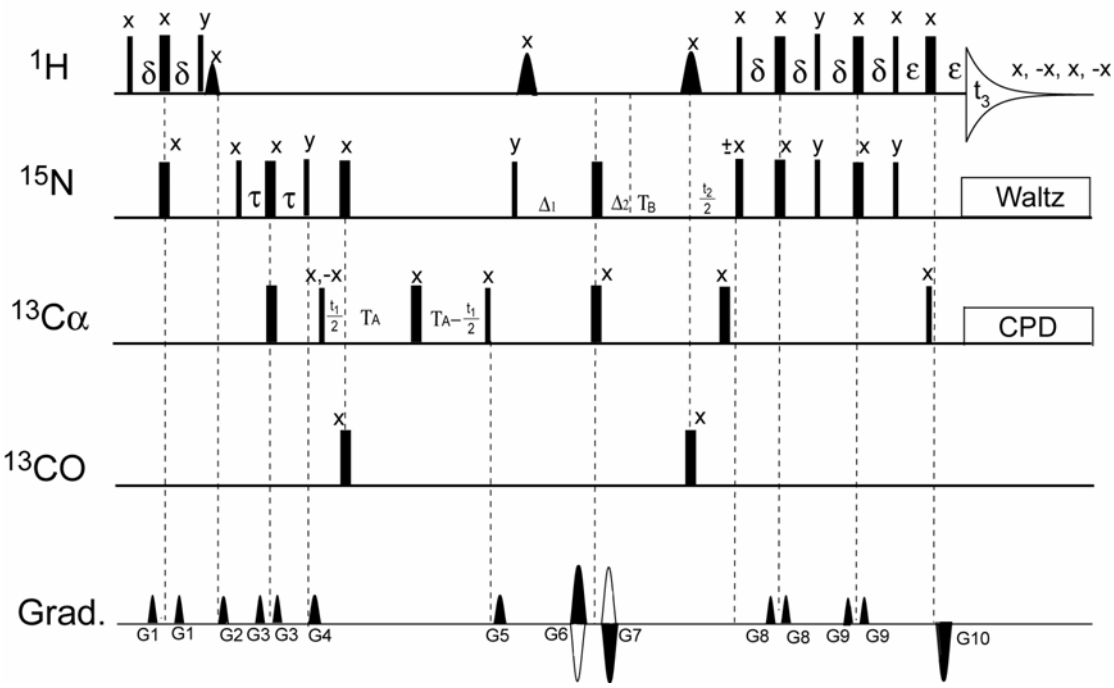

Figure S1. Pulse scheme of the 3D HNCA experiment. Narrow and wide pulses correspond to 90º and 180º flip angles, respectively. The ^{1}H 90º water flip back pulse is sine-bell shaped and has a duration of 1.5 ms. A ReBURP pulse is employed to decouple H^N during ^{15}N evolution with a duration of 1.6 ms at 600 MHz, and centered at 8.5 ppm. Quadrature detection in the t_1 dimension is by the States-TPPI method and in the t_2 dimension by the Rance-Kay method, alternating the phase of the ^{15}N 90º pulse after G_7 between *x* and *–x*, in concert with the polarity of G_6 and G_7. Mixed-time t_2 evolution (Ying JF, Chill JH, Louis JM and Bax A J. Biomol. NMR 37: 195-204 (2007)) is used to improve resolution in this dimension. Delays: T_A = 14 ms and T_B = 11.6 ms; $\Delta_1 = \max(0, T_B - t_2/2)$; $\Delta_2 = \max(0, t_2/2 - T_B)$. All pulsed field gradients are sine-bell shaped with maximum gradient strengths at their midpoints of 1.2 G/cm for G_1, 4.2 G/cm for G_2, 1.2 G/cm for G_3, 6.6 G/cm for G_4, 9.0 G/cm for G_5, 22.2 G/cm for G_6, –22.2 G/cm for G_7, 5.4 G/cm for G_8, 7.8 G/cm for G_9, 22.2 G/cm for G_{10}, and durations $G_{1,2,3,4,5,6,7,8,9,10}$ = 1.5, 1.3, 0.33, 0.5, 1.1, 1.2, 1.2, 2.6, 2.6, 0.244 ms. All gradients are applied along z-axis.

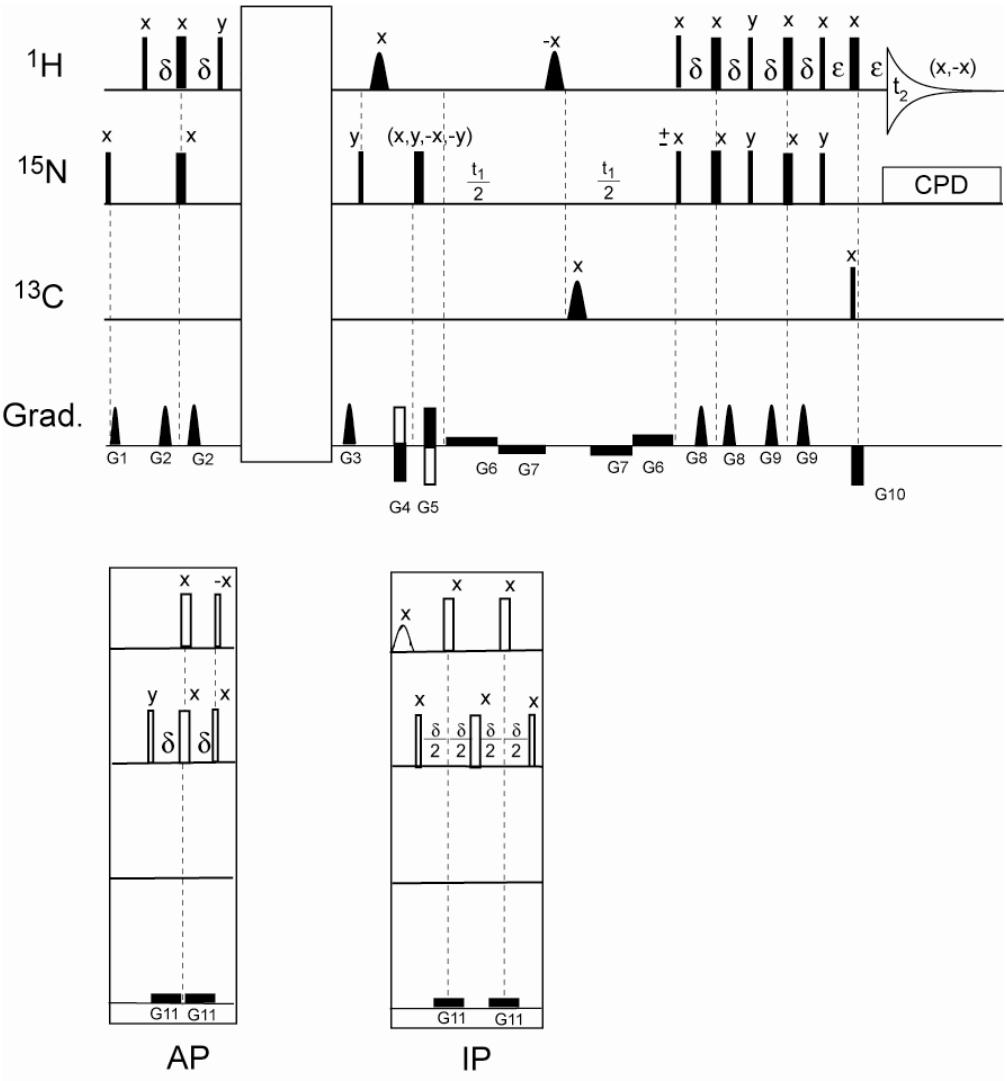

Figure S2. Pulse scheme of the modified gradient-enhanced 2D BSD-IPAP HSQC experiment. The pulses in the IP (AP) box are executed for in-phase spectrum (antiphase spectrum). Narrow and wide pulses correspond to 90º and 180º flip angles respectively. The ^{1}H 90º water flip back pulse is sine-bell shaped, has a duration of 1.5 ms. Solid shaped ^{1}H pulses are of the IBURP2 type (Geen and Freeman 1991) and serve to decouple $H^{\alpha,\beta}$ from ^{15}N. They are centered at 2.4 ppm, with a duration adjusted to invert ±2.8 ppm. A 600 μs 180º hyperbolic secant shaped pulse (Silver et al. 1984), centered at 116 ppm, is used to decouple C^{α} and C'. Rance-Kay t_1 quadrature detection is used by alternating the phase of the ^{15}N 90º pulse after G_6 between *x* and *–x*, in concert with alternating the polarity of G_4 and G_5 (Kay et al. 1992). Pulsed field gradients $G_{1,2,3,8,9}$ are sine-bell shaped 1.8 (G_{1x}), 6.6 (G_{1y}), 10.2 (G_{2y}), 9.0 (G_{2z}), 22.2 (G_{3z}), 9.0 (G_{8z}), 33.0 (G_{9x}), and 14.1 (G_{9z}) G/cm. G_4, G_5, G_6, G_7, G_{10} and G_{11} are rectangular with strengths of 16.2 (G_{4x}), 16.2 (G_{4y}), 16.2 (G_{4z}), -16.2 (G_{5x}), -16.2 (G_{5y}), -16.2 (G_{5z}), 0.6 (G_{6z}), -0.6 (G_{7z}), 16.2 (G_{10x}), 16.2 (G_{10y}), 16.2 (G_{10z}) and 7.8 (G_{11y}) G/cm. Gradient durations: $G_{1,2,3,4,5,6,7,8,9,10,11}$ = 0.7, 0.7, 1.7, 1.0, 1.0, t_1/4, t_1/4, 1.1, 1.3, 0.203, 2.65 ms. Delay durations: δ = 2.65 ms; ε = 0.55 ms.

Table S1. $^{2}J_{NH\alpha}$ couplings measured in the K4AK19EV42E GB3 mutant

Residue	$^{2}J_{NH\alpha}$ (Hz)
Q2	1.092
Y3	0.494
A4	0.366
L5	1.128
V6	1.001
I7	1.153
N8	1.535
K10	0.78
T11	1.265
L12	1.06
K13	0.907
E15	0.201
T16	1.595
T17	-0.178
T18	1.397
E19	0.896
A20	0.965
D22	1.071
A23	1.008
E24	0.834
T25	0.321
A26	0.648
E27	1.013
K28	0.265
A29	0.577
F30	0.826
K31	0.746
Y33	0.706
A34	0.618
N35	0.794
D36	0.246
N37	1.055
V39	1.156
D40	1.36
E42	1.073
W43	0.255
T44	1.235
Y45	1.048
D46	1.171
D47	1.041

A48	0.466
T49	1.059
K50	0.415
T51	0.554
F52	0.054
T53	0.751
V54	1.061
T55	1.231
E56	0.632

Table S2. $^{1}J_{NH}$ splittings measured for the protonated mutant K4AK19EV42E at 500 and 750 MHz.

Residue	$^{1}J_{NH}$	
	750 MHz	500 MHz
Q2	-93.27	-93.27
Y3	-93.10	-93.22
A4	-92.86	-93.22
L5	-91.89	-92.19
V6	-92.63	-93.01
I7	-92.94	-93.12
N8	-93.74	-93.89
G9	-94.43	-94.59
K10	-92.45	-92.41
T11	-91.74	-91.71
L12	-92.53	-92.68
K13	-92.20	-92.50
G14	-94.22	-94.46
E15	-92.05	-92.31
T16	-93.42	-93.70
T17	-92.84	-93.08
T18	-93.32	-93.47
E19	-92.69	-92.84
A20	-94.37	-94.35
V21	-91.91	-91.86
D22	-92.55	-92.77
A23	-92.36	-92.62
E24	-92.97	-93.19
A26	-93.57	-93.80
K28	-93.41	-93.63
A29	-93.55	-93.73
F30	-94.05	-94.33
K31	-93.97	-94.20
Q32	-93.43	-93.61

Y33	-92.93	-93.13
A34	-93.48	-93.83
N35	-93.81	-94.03
D36	-93.78	-93.99
N37	-92.08	-92.35
G38	-95.01	-94.94
V39	-93.21	-93.27
D40	-92.83	-92.86
G41	-93.63	-93.57
E42	-92.49	-92.45
W43	-92.64	-92.75
T44	-93.51	-93.88
Y45	-92.13	-92.45
D46	-93.21	-93.52
D47	-93.85	-94.09
A48	-93.44	-93.56
T49	-91.70	-91.83
K50	-93.45	-93.70
T51	-92.54	-92.73
F52	-91.97	-92.29
T53	-92.89	-93.25
V54	-93.34	-93.55
T55	-92.57	-92.73
E56	-92.52	-92.63

HNCA sequence code of Figure S1; recorded on a Bruker Avance Topspin2.1 system with cryoprobe and z-axis gradients.

"d3=3u"
"d4=14m"
"d5=d3+p7*2+d4-p4-3u"
"d6=11.5m"
"d10=3u"
"d14=3u"
"d15=3u"
"d0=d6+d10+p9*2+d14-97u+d15"
"d9=p9*2"
"d11=50m"
"d12=200u"
"d18=11.5m-3u-p27"
;"d20=in20-p7-p5*0.636"
"d21=2.6m-p21-203u"
"d23=2.6m"
"d24=2.6m"
"d25=p9*2+p25+120u"
"in14=in0"
"in10=in0"
"in15=in0"
"in3=in5"

"cnst21=54"
"cnst22=176"

1 ze
1m
1m
2 d11 do:f3
d12
10u
3 d12*3
4 d12*4
5 d12*3
6 10u setnmr3^0 setnmr0^34^32^33 ctrlgrad 7
1m do:f2
1m do:f3
1m setnmr3^0
10u fq=cnst21(bf ppm):f2
d1

```
            1m setnmr3|0
            10u setnmr3|0 setnmr0|34|32|33 ctrlgrad 0
            1m
            10u pl1:f1
            10u pl7:f3
;------- start 90-degree on hn ----------------------
           (p1 ph0):f1
            3u
            p21:gp21
            d21
            200u
            (center (p1*2 ph0):f1 (p7*2 ph0):f3)
            d21
            p21:gp21
            203u
            (p1 ph1):f1                    ;INEPT to 15N
            3u pl2:f1
            (p2:sp2 ph0:r):f1
            3u
            p20:gp20
            200u
;---------start INEPT to Ca -------------------------------
            (p7 ph0):f3
            3u pl5:f2
            p27:gp21
            d18
            (center (p7*2 ph0):f3 (p9*2 ph0):f2)
            3u
            p27:gp21
            d18
            (p7 ph1):f3
            5u
            p28:gp28
            200u pl15:f2
;----------- start Ca evolution --------------------------
            (p5 ph5):f2
            d3
            (center (p4:sp3 ph0):f2 (p7*2 ph0):f3)
            d4
            3u pl5:f2
            (p9*2 ph0):f2
            d5
            (p4:sp3 ph0):f2
            3u
```

```
              3u pl15:f2
              (p5 ph0):f2
              3u
              p22:gp22
              100u pl5:f2
              100u pl1:f1
;-------------start f3 N evolution ----------------------------
            (p7 ph7):f3
            3u
            (p8:sp8 ph2):f1
              d0
          if "l2==2" goto 27
              p26:gp26
              100u
            (center (p7*2 ph0):f3 (p9*2 ph0):f2)
            p26:gp27
              goto 26
27            p26:gp27
              100u
            (center (p7*2 ph0):f3 (p9*2 ph0):f2)
              p26:gp26
26            3u
              d6
              d10
              (center (p8:sp8 ph2):f1 (p4:sp3 ph0):f2)
              d14 pl1:f1
            (p9*2 ph0):f2
              d15
                3u
;-------------transfer back to f1 ----------------------------
            (center (p1 ph0):f1 (p7 ph4):f3)
              3u gron23
              d23
              3u groff
            (center (p1*2 ph0):f1 (p7*2 ph0):f3)
              3u gron23
              d23
              3u groff
            (center (p1 ph1):f1 (p7 ph1):f3)
              3u gron24
              d24
              3u groff
            (center (p1*2 ph0):f1 (p7*2 ph0):f3)
              3u gron24
```

```
            d24
            3u groff
           (center (p1 ph0):f1 (p7 ph1):f3)
           d25
           (p1*2 ph0):f1
            5u pl15:f2
           (p9 ph0):f2
            5u
           (p9:sp11 ph0):f2
            p25:gp25
999         50u pl30:f3
            50u fq=cnst22(bf ppm):f2
            5u pl19:f2
            5u setnmr3^0 setnmr0^34^32^33 ctrlgrad 7
            go=2 ph31 cpd3:f3 cpds2:f2
            10u do:f3 do:f2
            d11 wr #0 if #0 zd




            d12 ip4*2
            d12 iu2
            lo to 3 times 2
            if "d0 - 1u < in0"
            {
              d12 id15
              d12 id10
            }
            else
            {
            d12 id14
            d12 dd0
            }
              d12 ru2;ip31*2
            lo to 4 times l3



d12 rd0
d12 rd10
d12 rd14
d12 rd15
```

```
        d12    ip5
        lo to 5 times 2
        d12 id3
        d12 dd5
        d12 ip31*2
        lo to 6 times l4



1m
1m do:f2
1m do:f3
1m setnmr3^0
1m
1m
exit

ph0=0
ph1=1
ph2=2
ph3=3
ph4=0
ph5=0 2
ph7=1
ph11=2
ph31=0 2
```

IPAP HSQC sequence code of Figure 3 for a Bruker Avance system with Topspin 2.1

```
"d6=12.5m"
"d10=3u"
"d11=50m"
"d12=200u"
"d20=3u"
"d7=d20*4+p14-63u"

"d21=2.65m"
"d23=2.65m"
"d24=2.65m"
"d25=p25+p9+110u+p9+10u"

1       ze
        1m ;RESET
        1m
2       d11 do:f3 do:f2
        d12
4       d12*2
5       d12*3
6       10u
        1m ;setnmr3^0
        10u
        1m
        1m do:f2
        1m do:f3
        d1
        1m ;setnmr3|0
        10u pl1:f1
        10u pl7:f3
        10u setnmr3|0 setnmr0|34|32|33 ctrlgrad 0
        (p7 ph1):f3
        p21:gp28
        100u
;------- start 90-degree on hn ----------------------
        (p1 ph0):f1
        3u gron21
        d21
        3u groff
        (center (p1*2 ph0):f1 (p7*2 ph0):f3)
        3u gron21
        d21
```

```
            3u groff
            (p1 ph8):f1                    ;INEPT to 15N
            3u pl2:f1
            (p10:sp3 ph0:r):f1
            6u
            3u pl1:f1
if "l1==1" goto 111
           (p7 ph1):f3
            3u gron22
            d21
            3u groff
           (center (p1*2 ph0):f1 (p7*2 ph0):f3)
            3u gron22
            d21
            3u groff
            (center(p1 ph0):f1 (p7 ph0):f3)
              3u
            (p10:sp5 ph4:r):f1
            6u
111         1m
            p20:gp20
            200u pl16:f2
            5u
            3u pl2:f1
;---------start f3 evolution --------------------------------
           (p7 ph7):f3
           d7
           (p13:sp13 ph11):f1
           if "l2==2" goto 27
            p26:gp26
            100u
            (p7*2 ph6):f3
            p26:gp27
            goto 26
27          p26:gp27
            100u
            (p7*2 ph6):f3
            p26:gp26
26          3u gron5
            d20
            3u groff
            5u
            3u gron3
            d20
```

```
  3u groff
 (p13:sp13 ph12):f1
 (p14:sp14 ph0):f2          ;hsec 13C dec
  3u gron3
  d20
  3u groff
  5u
  3u gron5
  d20 pl1:f1
  3u groff
 (center (p1 ph0):f1 (p7 ph5):f3)
  3u gron23
  d23
  3u groff
 (center (p1*2 ph0):f1 (p7*2 ph0):f3)
  3u gron23
  d23
  3u groff
 (center (p1 ph1):f1 (p7 ph1):f3)
  3u gron24
  d24
  3u groff
 (center (p1*2 ph0):f1 (p7*2 ph0):f3)
  3u gron24
  d24
  3u groff
 (center (p1 ph0):f1 (p7 ph1):f3)
 d25
 (p1*2 ph9):f1
  5u pl17:f2
 (p9 ph0):f2
  5u
 (p9:sp11 ph0):f2
  p25:gp25
999  100u pl30:f3
  5u pl19:f2
  5u setnmr3^0 setnmr0^34^32^33 ctrlgrad 7
  go=2 ph31 cpd3:f3 cpds2:f2
  10u do:f3 do:f2
  1m ;setnmr3^0
  d11 wr #0 if #0 zd
```

```
        d12    iu1
        lo to 4 times 2
        d12    ru1
        d12*0.5 ip5*2
        d12*0.5    iu2
        lo to 5 times 2
        d12*0.5 ru2
        d12*0.5 id20
        d12 ip7*2
        d12 ip31*2
        lo to 6 times l4



1m
1m do:f2
1m do:f3
1m ;setnmr3^0
1m ;RESET
1m
exit

ph0=0
ph1=1
ph2=2
ph3=3
ph4=0
ph5=0
ph6=0 1 2 3
ph7=0
ph8=1
ph9=0 0 0 0
ph10=2
ph11=0 0
ph12=2 2
ph31=0 2
```

The following sequence is written to achieve equal intensity of IP and AP components and was tested on a Bruker Avance 750 MHz spectrometer, Topspin 2.1, equipped with a three-axes gradient, triple resonance, room temperature probe. This sequence produces almost equal intensity, with AP spectrum slightly more intense (~1-2%) than the IP spectrum (for GB3; tc ~3.3 ns) caused by the slightly faster relaxation of antiphase operator 2NxHz than that of inphase operator Ny.

IPAP HSQC sequence for more equal intensity of IP/AP components (Figure S2).

```
"d6=12.5m"
"d10=3u"
;"d14=3u"
;"d0=d6+d10+p4*4+p9*2+d14*2+3u"
"d11=50m"
"d12=200u"
;"d18=12.5m-3u-p26"
;"d19=50ms"
"d20=10u"
;"d7=d20*4+p9*4+37u"
"d7=d20*4+p14-63u"

"d21=2.6m-p21-203u-p7"
"d23=2.6m-p23"
"d24=2.6m-p24"
;"d25=p25+110u"
"d25=p25+p9+110u+p9+10u"

1       ze
        1m
        1m
2       d11 do:f3 do:f2
        d12
4       d12*2
5       d12*3
6       10u
        1m
        10u
        1m
        1m do:f2
        1m do:f3
        d1
        1m
        10u pl1:f1
        10u pl7:f3
        10u setnmr3|0 setnmr0|34|32|33 ctrlgrad 0
```

```
            (p7 ph1):f3
            p21:gp28
            100u
;------- start 90-degree on hn ----------------------
            (p1 ph0):f1
            3u
            p21:gp21
            d21
            200u
            (center (p1*2 ph0):f1 (p7*2 ph0):f3)
            d21
            p21:gp21
            203u
            (p1 ph8):f1                    ;INEPT to 15N
            3u pl2:f1
            6u
            3u pl1:f1
if "l1==1" goto 110
          (p7 ph1):f3
            3u gron22
            2.7m
            3u groff
          (center (p1 ph0 p1*2.2 ph1 p1 ph0):f1 (p7*2 ph0):f3)
            3u gron22
            2.7m
            3u groff
            (center(p1 ph0):f1 (p7 ph0):f3)
            goto 111
110         3u
            (p10:sp5 ph4:r):f1
            6u
            (p7 ph0):f3
            3u gron22
            1.35m
            3u groff
            (p1 ph0 p1*2.2 ph1 p1 ph0):f1
            3u gron22
            1.35m
            3u groff
            (p7*2 ph0):f3
            3u gron22
            1.35m
            3u groff
          (p1 ph0 p1*2.2 ph1 p1 ph0):f1
```

```
            3u gron22
            1.35m
            3u groff
            (p7 ph0):f3
111         5u
            p20:gp20
            200u pl16:f2
            5u
            3u pl2:f1
;---------start f3 evolution --------------------------------
           (p7 ph7):f3
            d7
            (p13:sp13 ph11):f1
           if "l2==2" goto 27
            p26:gp26
            100u
            (p7*2 ph9):f3
            p26:gp27
            goto 26
27          p26:gp27
            100u
            (p7*2 ph9):f3
            p26:gp26
26          3u gron5
            d20
            3u groff
            5u
            3u gron3
            d20
            3u groff
           (p13:sp13 ph12):f1
           (p14:sp14 ph0):f2          ;hsec 13C dec
            3u gron3
            d20
            3u groff
            5u
            3u gron5
            d20 pl1:f1
            3u groff
           (center (p1 ph0):f1 (p7 ph5):f3)
            p23:gp23
            d23
           (center (p1*2 ph0):f1 (p7*2 ph0):f3)
            p23:gp23
```

```
          d23
         (center (p1 ph1):f1 (p7 ph1):f3)
          p24:gp24
          d24
         (center (p1*2 ph0):f1 (p7*2 ph6):f3)
          p24:gp24
          d24
         (p1 ph0):f1
          d25
         (p1*2 ph0):f1
          5u pl17:f2
         (p9 ph0):f2
          5u
         (p9:sp11 ph0):f2
          p25:gp25

999       100u pl30:f3
          5u pl19:f2
          5u setnmr3^0 setnmr0^34^32^33 ctrlgrad 7
          go=2 ph31 cpd3:f3
          10u do:f3 do:f2
          1m
          d11 wr #0 if #0 zd



          d12    iu1
          lo to 4 times 2
          d12    ru1
          d12*0.5 ip5*2
          d12*0.5    iu2
          lo to 5 times 2
          d12*0.5 ru2
          d12*0.5 id20
          d12 ip7*2
          d12 ip31*2
          lo to 6 times l4



1m
1m do:f2
1m do:f3
1m ;setnmr3^0
```

1m ;RESET
1m
exit

ph0=0
ph1=1
ph2=2
ph3=3
ph4=0
ph5=0
ph6=0
ph7=0
ph8=1
ph9=0 1 2 3
ph10=2
ph11=0 0
ph12=2 2
ph31=0 2 0 2

The FID processing script for IPAP-HSQC experiment

Note that even though the data are acquired with Rance-Kay quadrature in t1, the script below indicates "Complex", so that the COADD routine can first separate the interleaved data, and subsequently the bruk_ranceY.M macro does the Rance-Kay rearrangement to generate complex data in t1.

```
#!/bin/csh

bruk2pipe -in ./ser    \
   -bad 0.0 -aswap -DMX -decim 2000 -dspfvs 20 -grpdly 67.9862518310547    \
   -xN                 1536    -yN                  800    \
   -xT                  704    -yT                  400    \
   -xMODE               DQD    -yMODE           Complex    \
   -xSW           10000.000    -ySW            2631.579    \
   -xOBS            747.761    -yOBS             75.778    \
   -xCAR              4.823    -yCAR            117.628    \
   -xLAB                 1H    -yLAB                15N    \
   -ndim                 2    -aq2D             States    \
   -out ./test.fid -verb -ov

nmrPipe -in test.fid \
|nmrPipe -fn COADD -cList 0.95 1.0 -axis Y -time \
|nmrPipe -fn MAC -macro $NMRTXT/bruk_ranceY.M -noRd -noWr    \
-verb -ov -out test1.fid

nmrPipe -in test.fid \
|nmrPipe -fn COADD -cList 0.95 -1 -axis Y -time \
|nmrPipe -fn MAC -macro $NMRTXT/bruk_ranceY.M -noRd -noWr    \
-verb -ov -out test2.fid
```

The corresponding Fourier transformation script

```
#!/bin/csh

nmrPipe -in test1.fid \
| nmrPipe    -fn SOL                                          \
| nmrPipe    -fn SP -off 0.4 -end 0.98 -pow 2 -c 0.5       \
| nmrPipe    -fn ZF -size 8192                                  \
| nmrPipe    -fn FT                                          \
| nmrPipe    -fn EXT -x1 12ppm -xn 5.5ppm -sw -verb     \
| nmrPipe    -fn PS -p0 167.2 -p1 -31.4 -di                \
#| nmrPipe    -fn PS -p0 -141.1 -p1 58.0 -di                \
| nmrPipe    -fn TP                                          \
| nmrPipe    -fn SP -off 0.5 -end 0.99 -pow 1 -c 0.5       \
| nmrPipe    -fn ZF -size 4096                                  \
```

```
| nmrPipe    -fn FT -neg                                                \
| nmrPipe    -fn PS -p0 0 -p1 0                          \
#| nmrPipe    -fn POLY -auto -ord 0                                \
| nmrPipe    -fn TP                                                  \
| nmrPipe    -fn POLY -auto                                \
      -verb -ov -out test1.ft2


nmrPipe -in test2.fid \
| nmrPipe    -fn SOL                                                  \
| nmrPipe    -fn SP -off 0.4 -end 0.98 -pow 2 -c 0.5       \
| nmrPipe    -fn ZF -size 8192                                        \
| nmrPipe    -fn FT                                                  \
| nmrPipe    -fn EXT -x1 12ppm -xn 5.5ppm -sw -verb      \
| nmrPipe    -fn PS -p0 167.2 -p1 -31.4 -di                \
#| nmrPipe    -fn PS -p0 -141.1 -p1 58.0 -di                 \
| nmrPipe    -fn TP                                                  \
| nmrPipe    -fn SP -off 0.5 -end 0.99 -pow 1 -c 0.5       \
| nmrPipe    -fn ZF -size 4096                                        \
| nmrPipe    -fn FT -neg                                              \
| nmrPipe    -fn PS -p0 0 -p1 0                          \
#| nmrPipe    -fn POLY -auto -ord 0                                \
| nmrPipe    -fn TP                                                  \
| nmrPipe    -fn POLY -auto                                \
      -verb -ov -out test2.ft2



exit 0
```