Supporting Material

Rapid proton-detected NMR assignment for proteins with fast magic angle spinning

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S1. Sample preparation

SH3

The chicken alpha-spectrin SH3 domain was expressed as discussed previously in (Akbey *et al*. 2010) in *E. coli* BL21-DE3 grown in triple labeled (^{2}H, ^{13}C and ^{15}N) M9 medium; deuterated water; 3 g/L of d_7-^{13}C-glucose; 1 g/L ^{15}N-ammonium chloride, and 60 mg/L carbenicillin. Cells from 0.2 L of overnight culture in $^{1}H_2O$ were used to inoculate 1 L of expression culture. Cells were grown to an $OD_{600\ nm}$ of 0.7 at 37 °C and then induced at 20 °C with 1 mM IPTG overnight. After purification of the tagless protein by anion exchange chromatography (Q-Sepharose FF) and gel filtration (Superdex 75), the protein was lyophilized and redissolved in 100% $^{1}H_2O$ and an equal volume of 200 mM ammonium sulfate and stored at 4 °C for at least 3 days to ensure complete exchange of protons. Crystallization was initiated via a pH shift to 7.5 +/- 0.5 in an ammonia atmosphere. Microcrystals were packed into a 1.3 mm rotor using a table-top centrifuge (Eppendorf 5417-F).

B2m

Wild type beta-2 microglobulin (wt β2m) was expressed in triple labeled (^{2}H, ^{13}C and ^{15}N) M9 medium; deuterated water, fully labeled d_7-^{13}C-glucose and ^{15}N-ammonium chloride were used. Culture of *E. coli* BL21 strain expressing wt β2m was induced by IPTG at 37 °C overnight. The subsequent refolding and purification steps were performed in non-deuterated water according to (Esposito *et al*. 2000), allowing the full back exchange of all protein amides. Triple labeled wt β2m was then crystallized at a concentration of 10 mg/ml using the sitting drop vapor diffusion technique (crystallization solution: 27% PEG 4000, 20% glycerol, 100mM Na acetate, pH 5.0). After several weeks wt β2m microcrystals were harvested and loaded into a 1.3mm NMR rotor by centrifugation at 14000 r.p.m.

AP205

The recombinant AP205 coat protein (Klovins *et al*., 2002) was expressed in *E.coli* using a modified pETDuet vector (Novagen). Bacteria were grown in 100% D_2O medium enriched with ^2H,^{13}C-glucose (2 g/L) and ^{15}N-labeled ammonium chloride (1 g/L) until they reached OD_{600}=0.7. IPTG was added to 1 mM final concentration and cells were grown for 4 more hours before being centrifuged and frozen. Lysis buffer (40 mM tris-HCl, pH 8.0, 300 mM NaCl, 1 mg/ml lysozyme, 10 ug/ml DNase, 10 mM $MgCl_2$,) was added (3 mL per gram of cells), and cells were further lysed by sonication. The resulting solution was centrifuged, and the supernatant was loaded on a sepharose CL-4B (GE Healthcare) column. The resulting fractions containing the capsids were further purified by Fractogel (Merck) ion-exchange column. For proton exchange of the amide deuterons, the capsids were soaked for 1 hour in 2M guanidinium hydrochloride at room temperature, followed by dialysis against 10 mM HEPES pH 7.5. The resulting solution was mixed to a solution containing 10 mM HEPES pH 7.5, 0.1M NaCl and 10% PEG w/v 4000, and centrifuged at 165,000×*g* for 15 h at 12°C directly into the NMR rotor using a device provided by Bruker (Bertini et al, 2012), similar to one previously described (Böckmann et al, 2009).

M2

M2 was expressed in *E. Coli* (BL21DE3) as an N-terminal fusion to a His-9 tagged TrpLE to target the protein to inclusion bodies as previously described (Schnell and Chou, 2008). A fresh 50 uL transformation was grown for 2-3 hours in 1 mL of SOC media at 37 °C with Kanamycin antibiotic (50 mg/L) with shaking at 220 rpm. Following antibiotic selection, the 1 mL culture was transfered to 50 mL of fresh media comprised of 95 % D_2O M9 and 5% SOC and grown overnight at 37 °C in a 250 mL Erlenmeyer flask at 220 rpm in an incubator shaker. Cells were then pelleted for 30 minutes at 4 °C, and transferred to 1 L of deuterated M9 media and grown in a 2.8 L Fernbach flask (no baffles) at 37 °C and 190 rpm, until the OD_{600} reached 0.55 at which point the temperature was reduced to 22 °C, and grown until an OD_{600} of 0.8 to 1.0. Protein expression was then induced with 150 uM of IPTG, and cells were harvested 24 to 36 hours later. The doubling time in D_2O M9 and 37 °C was about 120 minutes.

The protein was purified on a nickel column in 6 M guanidine buffer, and precipitated by dialysis against water in 10 kD cutoff dialysis tubing. The precipitated protein was spun down, then dissolved in 5-10 mL of 70 % formic acid, and cleaved with cyanogen bromide (0.75 g, 1 hour at room temperature in the dark with a nitrogen purge). The solution was then dialyzed 2x30 minutes against water in a 3.5 kD cutoff dialysis cassette (Pearce) to remove cyanogen bromide, 20 to 40 mL water was added, and the protein was lyophilized. Final purification by HPLC was achieved by dissolving the lyophilized protein in 2:1:1 hexafluoro-2-propanol (HFIP):formic acid:water (HFIP was added 20 minutes prior to formic acid and water) and injecting onto a preparative C4 column equilibrated in buffer A (95 % water, 5% acetonitrile, 0.1% trifluoroacetic acid (TFA)) and running a gradient to 100% buffer B (57% isopropyl alcohol, 37% acetonitrile, 5% water, 0.1% TFA) over about 40 minutes at 5 mL per minute. Cleaved TrpLe eluted first, followed by uncleaved protein, and pure M2. The eluted protein was diluted in 50% acetonitrile for a good lyophilization. The pure protein yield was 2 to 5 mg per liter.

M2 samples were prepared for MAS NMR similarly to previous reports (Andreas *et al*., 2010). Deuterated DPhPC lipid (FB reagents) and lyophilized $M2_{18-60}$ were dissolved in semi-denaturing buffer (2M guanidine, 40 mM phosphate, 30 mM glutamate, 3 mM sodium azide, pH 7.8, ≥33mg/mL octyl glucoside (OG) detergent) with a lipid to protein ratio of 1:1 by weight. Buffer components were purchased from Sigma, and detergent from Affymetrix. The resulting solution was then dialyzed in a 3.5kD cutoff dialysis cassette (Thermo) against 1 L of sample buffer (40 mM phosphate, 30 mM glutamate, 3 mM sodium azide, pH 7.8) for at least 5 days with 1 to 2 dialysis buffer changes per day. Lipid/M2 bilayers formed a white precipitate after approximately 24 hours. Solid material was pelleted by centrifugation at ~100 000 x g in a Beckmann ultracentrifuge and finally packed into a Bruker 1.3 mm rotor using an ultracentrifuge packing tool (Böckmann et al, 2009).

OmpG

OmpG was expressed in deuterated medium as described in (Linser et al. 2011), with the exception that all refolding and reconstitution steps were performed in buffers containing 100% H2O, to obtain a sample that is fully protonated in the exchangeable sites.

S2. HSQC acquisition parameters

Table S1 gives the information pertaining to the sizes of the datasets in both the frequency and time domains, the number of increments and scans per increment, and the total experimental time of acquisition. Recycle delay is the inter-scan delay time; ns is the number of scans per increment; sw HxN gives the spectral widths in the ^{1}H, and ^{15}N dimensions of the frequency domain; incs is the number of increments that were acquired in the ^{15}N dimension of the time domain; aq HxN gives maximum times out to which the ^{1}H, and ^{15}N dimensions in the time domain were sampled; S/N the signal-to-noise ratio of the total integrated signal in the first free-induction decay (FID); and expt is the total experiment time.

parameter	SH3	β2m	AP205	M2	OmpG
recycle delay / s	1.0	1.0	1.0	1.5	1.0
ns	8	8	16	8	8
sw HxN / ppm	100.0 x 39.4	100.0 x 49.3	100.0 x 42.3	100.0 x 49.3	100.0 x 39.4
incs	400	400	256	200	160
aq HxN / ms	20.0 x 50.0	20.0 x 40.0	20.0 x 29.9	20.0 x 32.0	20.0 x 20.0
S/N (first FID)	100	81	61	53	56
expt	**1 h**	**1 h**	**1 h 30 m**	**1h**	**25'**

Table S1. Experimental parameters for the 2D HSQC spectra.

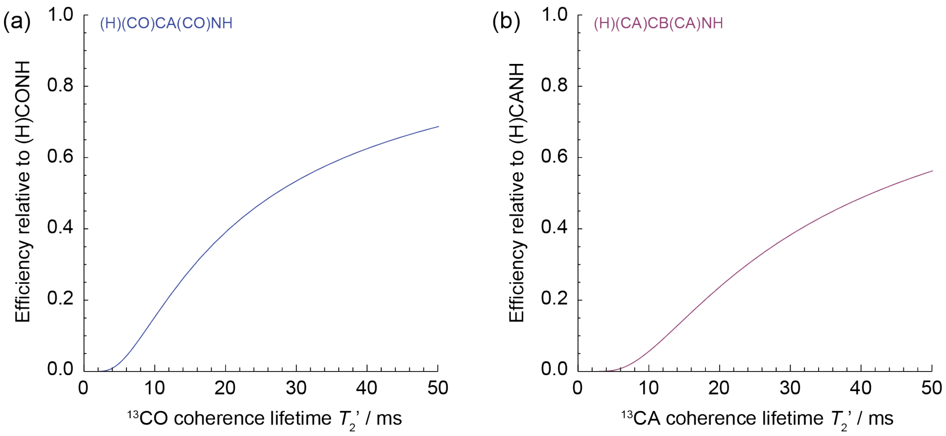

Figure S1. Simulated efficiency for (a) 3D (H)(CO)CA(CO)NH and (b) 3D (H)(CA)CB(CA)NH spectra as a function of the ^{13}C coherence lifetimes T_2', relative to (H)CONH and (H)CANH, respectively. In the two experiments, the half-delays of the spin echoes for the out-and-back CO-CA and CA-CB transfers were set to 4.7 ms and 7.2 ms, respectively.

S3. References

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S4. List of assigned peaks

All shifts are reported in ppm and referenced with respect to DSS.

SH3

Residue	Number	HN	N	C	CA	CB
LYS	6			176.2		
GLU	7	8.13	120.7	174.6	55.5	30.9
LEU	8	7.72	120.5	177.0	52.9	44.9
VAL	9	8.79	111.2	172.7	57.5	35.0
LEU	10	8.84	123.8	176.6	52.5	45.7
ALA	11	9.11	123.8	178.0	52.4	18.8
LEU	12	9.04	123.8	175.0	55.7	42.1
TYR	13	6.82	123.8	173.8	54.9	42.9
ASP	14	8.21	123.8	176.5	54.5	41.4
TYR	15	8.42	118.9	172.8	59.9	42.8
GLN	16	7.46	126.7	174.1	53.9	28.5
GLU	17	7.60	122.4	175.8	56.0	30.0
LYS	18	8.54	119.4	174.6	54.6	33.4
SER	19	7.07	111.2	173.1	56.5	63.2
PRO	20			177.0		
ARG	21	7.71	112.1	176.7	55.4	28.5
GLU	22	7.54	122.4	174.7	55.8	32.8
VAL	23	7.36	111.9	172.0	59.2	34.3
THR	24	6.44	116.5	174.3	61.8	71.4
MET	25	9.25	121.2	173.5	53.8	35.6
LYS	26	8.90	124.7	175.1	53.3	33.4
LYS	27	8.89	122.0	177.9	58.2	31.5
GLY	28	8.73	116.2	173.7	44.7	
ASP	29	8.29	122.0	175.2	55.1	41.7
ILE	30	8.49	120.0	176.7	58.2	35.1
LEU	31	9.38	128.4	176.6	53.0	41.7
THR	32	8.05	118.8	173.6	62.8	69.1
LEU	33	8.73	130.1	174.5	54.7	42.2
LEU	34	8.79	125.6	177.5	54.8	42.0
ASN	35	7.33	113.9	174.6	54.1	40.1
SER	36	9.11	124.8	173.7	56.8	61.6
THR	37	8.05	112.7	175.9	64.8	70.3
ASN	38	9.14	125.7	174.9	54.1	41.1
LYS	39	8.42	121.3	176.3	58.2	31.9

ASP	40	7.81	115.2	177.1	55.5	43.7
TRP	41	8.25	122.8	174.3	55.9	32.0
TRP	42	9.07	123.8	174.4	53.7	31.2
LYS	43	8.73	123.4	175.5	54.9	33.8
VAL	44	9.33	122.2	173.3	58.8	35.9
GLU	45	8.67	119.9	175.2	54.6	33.0
VAL	46	8.70	125.4		60.0	32.4
ASP	48			175.3	54.6	
ARG	49	7.93	119.4	174.6	55.2	33.0
GLN	50	8.17	116.7	176.0	53.2	30.6
GLY	51	8.53	106.8	170.6	45.5	
PHE	52	9.02	118.7	175.5	58.9	41.8
VAL	53	8.72	110.4	172.6	58.1	32.8
PRO	54			177.6	61.7	29.2
ALA	55	7.33	128.8	178.8	54.1	14.9
ALA	56	7.79	113.2	177.9	52.8	17.3
TYR	57	7.17	113.4	174.2	54.7	36.7
VAL	58	7.26	110.8	173.8	57.8	34.7
LYS	59	8.55	119.5	176.3	53.7	36.1
LYS	60	9.07	126.5	176.5	58.1	32.2
LEU	61	8.03	125.6	175.5	53.9	40.6
ASP	62	7.72	127.9		55.8	42.0

b2m

Residue	Number	1H	15N	13C	13CA	13CB
ILE	1	8.88	123.0	174.9	60.1	41.3
GLN	2	8.85	122.2	174.9	54.5	30.5
ARG	3	9.83	124.7	174.7	54.4	34.0
THR	4	9.45	123.6	173.5	59.9	69.8
LYS	6	8.65	123.4	176.5	55.6	31.1
ILE	7	8.56	123.5	175.2	60.9	41.0
GLN	8	9.03	126.1	173.9	54.5	32.8
VAL	9	9.06	123.9	175.4	60.5	33.5
TYR	10	8.45	122.9	173.0	56.0	38.8
SER	11	10.54	119.7	174.9	56.0	66.1
ARG	12	8.60	120.5	174.1	53.2	35.2
HIS	13	8.93	119.1	172.6	51.0	27.8
ASN	21	8.87	123.2	173.7	51.6	41.4
PHE	22	9.16	122.0	173.9	55.5	40.5
LEU	23	8.51	126.4	173.8	53.1	38.5
ASN	24	8.42	121.1	172.8	51.7	40.9
CYS	25	9.49	121.4	171.5	53.9	41.3
TYR	26	9.67	128.5	174.3	54.3	39.4
VAL	27	9.66	132.8	175.1	60.8	31.5
SER	28	9.57	119.3	174.7	56.8	66.4
GLY	29	6.68	110.2	174.0	46.7	
PHE	30	7.66	112.4	174.7	54.6	41.4

HIS	31	8.62	117.7	174.7	57.7	31.6
SER	33	8.77	114.4		59.1	60.4
ASP	34	8.01	121.1	175.2	54.7	40.6
ILE	35	8.32	121.8	171.3	62.3	38.9
GLU	36	7.86	124.4	174.8	54.5	32.0
VAL	37	8.47	124.4	173.6	60.4	34.8
ASP	38	9.14	123.2	175.4	53.3	45.7
LEU	39	8.68	120.2	175.0	53.5	43.8
LEU	40	9.25	120.4	175.6	52.9	44.7
LYS	41	9.04	120.9	176.6	54.1	33.3
ASN	42	9.89	128.2	175.8	54.3	36.5
GLY	43	8.98	102.8	173.2	45.0	
GLU	44	7.91	120.8	176.0	54.1	30.9
ARG	45	8.88	123.9	176.8	57.3	29.4
ILE	46	8.90	126.6	176.1	61.8	37.0
GLU	47	8.69	127.7		57.9	
LEU	54	9.21	129.9	175.5	53.7	44.0
SER	55	9.79	122.2	172.1	57.6	66.7
PHE	56	8.92	113.3	174.8	55.7	40.1
SER	57	9.07	115.5	174.9	56.0	65.1
PHE	62	8.24	123.0	174.4	57.3	40.4
TYR	63	8.96	116.2	174.1	57.6	40.5
LEU	64	9.87	125.7	173.6	53.6	46.2
LEU	65	9.14	129.2		53.9	43.5
TYR	66	8.86	132.1	173.9	55.8	41.0
TYR	67	8.58	122.2	173.0	55.5	41.3
THR	68	8.12	111.5	171.6	59.9	70.7
GLU	69	8.60	128.2	176.0	56.9	29.0
PHE	70	9.26	125.6	173.1	55.0	39.7
THR	71	8.37	114.7	173.0	57.8	69.6
THR	73	8.30	112.6	176.2	60.2	71.3
GLU	74	9.03	120.3	177.0	58.9	29.0
LYS	75	7.77	114.0	176.8	56.5	32.3
ASP	76	7.31	118.5	175.1	54.7	43.7
GLU	77	8.60	121.0	175.4	54.7	31.9
TYR	78	9.59	124.5	175.5	56.5	41.5
ALA	79	8.92	121.3	174.1	50.8	23.2
CYS	80	9.30	119.5	171.5	52.7	43.4
ARG	81	9.46	127.8	174.6	53.6	32.9
VAL	82	9.19	128.4	173.5	60.3	35.0
ASN	83	9.17	123.4	173.4	51.5	41.0
HIS	84	8.06	124.0	175.4	57.0	35.2
VAL	85	8.40	123.7	173.9	64.7	30.6
THR	86	7.39	106.6	176.0	62.3	69.2
LEU	87	8.36	123.6	176.9	53.2	41.5
SER	88	8.79	118.5		59.2	61.5
GLN	89	7.32	117.3	172.4	52.8	29.4
LYS	91	8.83	125.7	174.9	55.0	34.1
ILE	92	8.62	125.6	176.0	60.2	37.9

VAL	93	9.15	129.6	175.5	61.2	32.9
LYS	94	8.92	127.5	176.6	56.3	32.7
TRP	95	8.95	123.2	175.1	56.8	27.8
ASP	96	8.74	132.9	175.2	52.9	41.0

AP205

Residue	Number	H	N	C	CA	CB
MET	6	8.19	118.2	173.3	54.8	36.6
GLN	7	8.95	117.7	173.9	52.8	32.0
VAL	17	8.97	124.4	172.8	61.6	35.1
TRP	18	10.33	128.6	172.2	58.7	33.1
SER	19	9.27	113.0	173.9	56.2	66.5
ASP	20	7.43	126.0	172.6	50.7	41.8
PRO	21			171.1	64.8	32.0
THR	22	8.64	105.5	172.8	61.3	69.9
ARG	23	8.21	126.4	174.0	57.2	28.3
LEU	24	8.72	126.6	170.4	57.3	42.1
SER	25	7.57	109.0	173.2	58.2	63.0
THR	26	8.63	122.7	173.5	61.9	69.1
THR	27	8.45	115.4	173.6	59.2	73.4
PHE	28	9.03	122.9		56.9	41.6
SER	29	9.10	122.5	176.2	55.7	66.3
ALA	30	9.07	125.7	173.2	49.8	23.6
SER	31	8.46	114.9	174.5	55.8	65.7
LEU	32	8.50	121.6	172.2	53.4	44.5
LEU	33	8.77	123.7	173.8	54.0	44.2
ARG	34	8.69	127.0	172.8	60.4	30.5
ILE	41	8.18	120.4	172.3	61.5	38.4
ALA	42	8.21	125.0	172.5	51.2	20.4
GLU	43	8.25	122.0	172.5	55.1	31.3
LEU	44	8.95	124.8	172.5	52.7	43.9
ASN	45	10.68	124.3	173.9	53.2	39.7
ASN	46	8.92	122.8	172.6	55.0	40.9
VAL	47	8.28	128.2	173.2	62.0	33.4
SER	48	8.68	121.5	173.1	55.5	63.7
GLY	49	9.72	117.2	177.2	44.5	
GLN	50	8.77	123.7		53.9	30.9
TYR	51	9.71	128.3		55.8	40.3
CYS	65	8.24	118.8	174.8	55.1	40.2
ALA	66	9.12	133.7	171.6	50.8	19.2
ASP	67	6.87	119.8	170.4	54.4	42.8
ALA	68	9.05	131.4	169.6	53.7	18.8
CYS	69	9.15	112.2	173.8	54.0	41.3
VAL	70	7.18	121.2	173.3	62.9	31.0
ILE	71	8.30	128.9	172.4	60.7	38.1
PRO	73			173.4	61.8	31.5
ASN	74	8.31	114.8	172.2	52.2	40.9
GLU	75	9.22	119.3	174.0	53.5	32.2
ASN	76	8.81	120.3	172.6	52.5	39.2
GLN	77	9.20	125.4	172.0	55.6	29.2
SER	78	9.11	116.9	175.1	56.4	66.6
ILE	79	9.05	123.0	173.8	59.9	42.0
ARG	80	9.23	126.3	172.5	53.6	34.0

THR	81	10.11	125.1		61.8	72.0
VAL	82	9.93	127.6	172.0	59.9	34.0
ILE	83	9.35	130.2	172.9	61.1	39.9
SER	84	9.38	122.2	175.2	56.2	65.2
GLY	85	8.15	111.4	174.3	45.2	
SER	86	10.54	121.7	172.2	59.4	64.5
ALA	87	9.15	123.2	166.5	53.4	19.3
GLU	88	9.61	118.9	169.7	61.6	27.7
ASN	89	7.84	117.2	172.7	51.6	37.9
LEU	90	7.41	121.6	170.6	58.0	42.0
ALA	91	8.80	118.2	166.0	56.0	18.1
THR	92	7.56	106.0	170.7	63.5	69.3
LEU	93	8.05	129.2	167.9	57.7	41.1
LYS	94	9.05	117.6	167.7	60.9	32.6
ALA	95	7.18	123.1	166.8	55.0	17.4
GLU	96	8.68	120.8	167.9	58.2	27.7
TRP	97	8.79	124.6	171.4	59.4	30.9
GLU	98	7.87	117.5	168.2	59.1	28.8
THR	99	7.95	116.4	173.7	66.7	68.6
HIS	100	9.04	123.8	170.9	60.4	
LYS	101	8.12	115.3	170.6	61.2	32.1
ARG	102	6.96	115.9	169.7	58.7	29.6
ASN	103	8.25	119.3	171.3	55.3	37.2
VAL	104	8.41	120.2	168.9	66.7	30.7
ASP	105	9.35	121.8	168.6	57.4	39.2
THR	106	8.41	118.2	172.6	66.4	67.7
LEU	107	7.19	117.5	171.3	56.7	42.6
PHE	108	9.27	117.6	173.4	59.5	40.9
ALA	109	8.24	122.0	168.5	55.1	19.3
SER	110	8.53	110.5	173.2	58.1	63.7
GLY	111	6.18	108.0	173.9	44.3	
ASN	112	8.85	115.5	170.3	52.8	39.2
ALA	113	8.88	126.4	166.7	56.1	19.4
GLY	114	10.13	107.2	174.1	46.1	
LEU	115	7.41	114.2	171.7	54.0	43.3
GLY	116	7.82	101.3	175.3	44.4	
PHE	117	7.53	118.6	172.6	56.8	40.9
LEU	118	8.94	123.9	171.9	53.5	44.0
ASP	119	7.72	122.1		50.2	41.9
PRO	120			170.2	64.2	31.0
THR	121	8.65	110.4	173.3	61.5	68.9
ALA	122	7.44	125.3	170.8	52.6	20.0
ALA	123	8.33	125.4	173.0	51.5	16.9
ILE	124	7.85	128.6	171.5	57.1	36.3
VAL	125	8.32	119.0	173.5	58.7	35.3
SER	126	8.79	113.2		55.1	63.4

M2

Residue	Number	H	N	C	CA	CB
PRO	25			177.2	65.3	
LEU	26	8.69	115.7	178.1	58.1	40.8
VAL	27	7.49	119.3	178.4	66.1	31.8
VAL	28	8.13	119.7	178.1	66.7	31.6
ALA	29	8.38	118.1	178.1	55.2	18.9
ALA	30	8.35	118.0	177.0	55.3	18.9
ASN	31	7.67	110.9	174.8	54.9	41.5
ILE	32	7.70	110.0	177.6	61.3	41.5
ILE	33	8.20	121.2	175.0	64.4	38.0
GLY	34	9.11	109.5	175.7	47.7	
ILE	35	7.60	121.3	176.7	64.3	37.7
LEU	36	8.42	118.3	177.3	57.7	41.3
HIS	37	8.53	116.3	175.2	59.5	31.1
LEU	38	7.93	116.1	178.6	58.5	42.5
ILE	39	8.19	114.0	175.8	60.6	42.7
LEU	40	8.45	125.7	178.3	58.6	41.6
TRP	41	9.74	120.8	178.3	60.9	28.4
ILE	42	8.34	116.4	178.1	64.2	38.1
LEU	43	8.79	118.9	179.6	57.9	41.7
ASP	44	9.41	120.8	178.2	58.0	43.6
ARG	45	8.61	116.2	178.2	58.0	29.2
LEU	46	8.06	113.9	177.0	56.5	43.4
PHE	47	7.89	112.4	175.5	58.7	42.2
PHE	48	7.72	116.4	175.6	61.0	40.1
LYS	49	10.23	120.0	175.4	56.8	30.9
SER	50	9.55	110.8	174.2	60.9	67.9
ILE	51	10.35	125.6	177.2	65.8	38.5
LEU	26’	8.61	116.2	177.9	58.1	40.8
VAL	27’	7.38	119.1	177.6	66.7	31.9
VAL	28’	7.97	118.5	178.5	65.8	31.7
ALA	29’	8.50	119.5	178.5	55.5	18.9
ALA	30’	8.33	117.5	178.1	55.3	18.9
ASN	31’	8.66	115.5	177.3	58.2	41.1
ILE	32’	8.63	118.6	177.2	65.6	36.9
ILE	33’	8.43	119.8	177.4	66.1	37.6
GLY	34’	8.67	106.5	174.9	47.9	
ILE	35’	8.58	121.6	177.0	66.1	38.1
LEU	36’	8.78	119.6	177.1	58.2	41.6
HIS	37’	8.74	118.5	177.2	62.3	32.0
LEU	38’	8.26	120.1	178.1	58.4	40.7
ILE	39’	8.90	118.0	177.2	65.7	37.9
LEU	40’	8.64	118.7	179.0	58.2	40.9
TRP	41’	8.35	122.3	177.4	62.7	27.9
ILE	42’	9.01	119.3	177.9	65.7	38.2
LEU	43’	8.83	117.6	179.7	58.1	41.9

OmpG

Residue	Number	H	N	C	CA	CB
ILE	11				59.6	43.3
GLY	12	7.82	110.3	170.4	47.0	
ALA	13	8.53	116.6	176.1	51.0	24.1
TYR	36				57.0	42.2
PHE	37	9.20	118.7	174.5	56.4	45.1
ASN	38	9.37	122.0	172.1	53.1	42.7
ALA	39	9.11	120.4	175.4	51.1	25.0
ALA	40	9.02	123.5	176.0	51.2	23.9
ASN	41	8.10	118.4		53.1	39.0
GLY	42	8.90	116.8	174.2	45.4	
PRO	43			176.4	63.7	32.4
TRP	44	8.03	122.5	177.6	57.7	33.4
ARG	45	9.25	121.7	175.0	56.5	34.7
ILE	46	9.77	127.1	173.6	60.9	42.1
ALA	47	9.58	128.9	175.5	51.6	24.0
LEU	48	9.60	122.8	176.6	54.3	46.9
ALA	49	9.21	122.7	175.5	52.3	24.4
TYR	50	9.07	118.3	172.1	60.1	42.2
TYR	51	6.13	128.2		55.4	43.4
GLU	73	9.68	122.0		55.4	34.7
VAL	74	9.35	120.2	173.6	61.0	35.9
HIS	75	9.78	125.2	172.7	54.6	32.6
TYR	76	8.30	125.4	173.3	56.8	42.6
GLN	77	7.91	128.9	173.6	54.0	28.2
GLY	87			170.2	45.4	
LEU	88	7.95	117.1	174.2	54.7	49.3
THR	89	8.52	121.8	174.6	62.2	71.4
GLY	90	9.60	113.6	172.9	44.6	
GLY	91	9.44	109.2	170.4	46.4	
PHE	92	9.21	120.6	173.3	57.3	44.3
ARG	93	7.63	124.0		54.4	37.2
TYR	95			173.3	56.7	42.8
GLY	96	8.63	114.0	170.9	44.4	
TYR	97	8.17	118.9		58.3	42.4
ALA	108	8.10	120.5	175.7	52.2	24.2
ASN	109	8.57	111.2	175.7	57.5	39.0
ILE	115			174.7	59.8	42.3
ALA	116	9.11	125.7	176.8	50.4	24.7
PRO	117			176.1	62.8	34.7
ASP	118	8.80	116.8	173.8	54.1	45.1
TRP	119	8.28	115.2	175.4	57.5	33.5
LEU	123			177.6	56.4	42.3
THR	124	8.68	109.3	172.8	59.8	72.3
ARG	128				55.0	34.6
PHE	129	9.35	122.1	173.6	55.0	42.4

ASN	130	9.00	123.8	174.0	51.2	42.5
GLY	131	7.18	105.1	169.1	45.7	
TRP	132	6.93	113.3	175.0	55.1	32.0
LEU	133	8.73	124.9	173.7	55.3	45.5
SER	134	10.25	118.9	172.9	57.3	67.3
MET	135	8.82	124.3			
ASP	141			177.8		
LEU	142	8.26	116.5	181.3	58.1	40.4
ASN	143	8.93	115.8	177.1	55.7	37.5
THR	144	7.62	116.2	175.2	65.7	69.2
THR	145	8.57	110.2	177.4	62.9	69.9
GLY	146	8.26	108.9	174.1	46.1	
TYR	147	7.08	120.3		58.4	39.3
ALA	148	8.19	122.8	179.0	51.4	18.4
ASP	149	8.25	115.5	176.8	56.9	42.1
THR	150	8.43	118.0	173.0	62.4	71.4
ARG	151	8.94	124.8	174.4	54.9	32.0
VAL	152			173.6	59.9	35.7
GLU	153	9.13	127.6	173.7	54.0	36.4
THR	154	9.00	122.1	169.3	60.0	70.1
GLU	155	5.50	123.8	176.0	56.2	31.2
THR	156	8.89	122.9	171.8	60.2	70.9
GLY	157	8.71	113.7	172.1	48.0	
LEU	158	8.52	118.2	176.1	53.0	46.6
GLN	159	9.06	120.2	174.8	54.1	33.0
TYR	160	9.88	130.7	174.7	56.5	42.3
THR	161	8.33	124.4	174.1	63.2	70.1
PHE	162	8.34	125.8	176.2	61.4	39.6
ASN	163	8.23	111.0	173.9	52.8	39.3
GLU	164	8.93	114.6	176.6	59.4	29.7
THR	165	8.76	116.0	173.9	64.6	70.3
VAL	166	7.96	119.4	173.9	61.5	35.3
ALA	167	8.19	128.7	172.8	50.9	23.4
LEU	168	9.03	116.7	175.7	54.2	48.2
ARG	169	9.40	124.7	175.8	54.8	34.7
VAL	170	8.92	122.5	173.0	63.1	34.6
ASN	171	9.64	121.8	174.7	51.2	42.9
TYR	172	9.67	122.3	173.1	57.5	41.9
TYR	173	8.51	127.8	172.1	55.9	43.1
LEU	174	7.60	126.5	174.0	52.9	46.1
ILE	194			175.4	59.5	39.3
ARG	195	9.47	126.3	173.5	55.1	33.3
ALA	196	8.55	124.4	175.5	49.9	22.2
TYR	197	9.60	117.4	174.6	56.2	43.1
LEU	198	8.36	123.6	171.1	51.7	45.2
PRO	199			177.6	64.5	32.4
LEU	200	9.49	127.0	179.1	53.3	43.9
THR	201	9.57	122.0	174.0	61.9	70.2
LEU	202	8.45	128.2	176.4	52.4	43.3

GLY	203	8.53	111.7	176.0	47.6	
ASN	204	9.36	126.7	174.4	54.7	39.0
HIS	205	8.54	121.2	176.2	55.8	33.3
SER	206	9.39	120.4	173.1	57.8	65.1
VAL	207	8.73	124.3	175.2	61.3	35.1
THR	208	9.96	121.8	173.5	59.4	71.4
PRO	209			176.0	62.4	33.2
TYR	210	9.07	118.8		57.5	42.5
THR	211	8.98	111.5		59.4	71.3
SER	250			173.1	57.3	67.0
VAL	251	8.93	115.9	175.2	59.3	36.5
SER	252	9.46	119.3	172.8	57.2	67.8
LEU	253	9.67	121.7	175.7	54.6	47.5
GLU	254	9.74	122.0		56.1	
ALA	273			175.6	51.4	24.2
GLY	274	8.92	105.3	172.8	46.4	
VAL	275			173.6	59.7	36.0
GLY	276	9.28	111.8	172.0	46.1	

S5. Bruker code of the pulse sequences

^{15}N-^{1}H HSQC

```
;15N-1H dipolar HSQC
;Avance III version
;parameters:
;p1 : 1H 90 pulse @ plw1
;pl1 : 1H power for 90 pulse
;p15 : contact time for H->N CP (typically 1500u)
;pl2 : 15N power for H->N CP
;sp0 : 1H power for H->N CP
;spnam0 : Ramp90.100
;spoffs0 : 0
;spoal0: 0.5
;p17 : contact time for N->H CP (typically 400u to 800u)
;pl20 : 15N power for N->H CP
;sp10 : 1H power for N->H CP
;spnam10 : Ramp100.90
;spoffs10 : 0
;spoal10: 0.5
;p7 : 15N 90 pulse @ plw7
;pl7 : 15N power for 90 pulse
;p3 : 13C 90 pulse @ plw11
;pl11 : 13C power for 90 pulse
;cpdprg1 : 1H decoupling (sltppm for 1Hprot, waltz16 for 2Hprot)
;cpdprg4 : Water suppression with cwX @ 15kHz
;cpdprg5 : Water suppression with cwY @ 15kHz
;cpdprg2 : 15N decoupling (waltz16_pl16)
;cpdprg3 : 13C decoupling (waltz16_pl17)
;pl12 : 1H power for water suppression(waltz16)
;pl13: 1H decoupling power (sltppm or waltz16)
;pl16: 15N decoupling power (waltz16)
;pl17: 13C decoupling power (waltz16)
;pcpd1 : 25u (waltz16 10 kHz) - 33.33u (sltppm 15kHz)
;pcpd2 : 25u (waltz16 10 kHz)
;pcpd3 : 25u (waltz16 10 kHz)

#include <Avancesolids.incl>

;cnst11 : to adjust t=0 for acquisition, if digmod = baseopt
"acqt0=1u*cnst11"
"in0=inf1"
"in30=inf1"

1 ze

2 d1 do:f2 do:f3
#include <p15_prot.incl>
#include <aq_prot.incl>

  (p1  pl1 ph1):f1

  (p15 pl2 ph2):f2 (p15:sp0 ph10):f1

  1u cpds1:f1
   (center (d0) (p3*2 ph0 pl11):f3)
  1u do:f1

;;;;;;;;;;;;;;; water suppression block starts
  (p7 pl7 ph5):f2
 1u cpds4:f1
  d30*0.25
 1u do:f1
 1u cpds5:f1
  d30*0.25
 1u do:f1

 1u cpds4:f1
  d30*0.25
 1u do:f1

 1u cpds5:f1
  d30*0.25
 1u do:f1
  (p7 pl7 ph6):f2
;;;;;;;;;;;;;;; water suppression block ends

  (p17 pl20 ph7):f2 (p17:sp10 ph11):f1

  1u cpds2:f2 cpds3:f3
  go=2 ph31
  1m do:f2         do:f3

  10m mc #0 to 2 F1PH(ip2,id0&dd30)

HaltAcqu, 1m      ;jump address for protection files
exit                                                    ;quit

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

^{13}C-^{15}N-^{1}H (H)CANH

```
;13C-15N-1H hCANH experiment

;Avance III version
;parameters:
;p1 : 1H 90 pulse @ plw1
;pl1 : 1H power for 90 pulse
;p15 : contact time for H->C CP (typically between 2 and 6ms)
;sp29 : 13C power for H->C CP
;sp1 : 1H power for H->C CP
;spnam1 : Ramp90.100
;spoffs1 : 0
;spnam29 : Rectangle
;spoffs29 : 0 (if offs3 set on CA)
;spoal29: 1 (end of the CP)
;p16 : contact time for C->N CP (typically up to 10ms)
;sp2 : 15N power for C->N CP
;sp9 : 13C power for C->N CP
;spnam2 : tangential ramp
;spoffs2 : 0
;spoal2: 1
;spnam9 : Rectangle
;spoffs9 : 0 (if offs3 set on CA)
;spoal9: 0 (beginning of the CP)
;p17 : contact time for N->H CP (typically 400u to 800u)
;pl20 : 15N power for N->H CP
;sp10 : 1H power for N->H CP
;spnam10 : Ramp100.90
;spoffs10 : 0
;spoal10: 0.5
;p7 : 15N 90 pulse @ plw7
;pl7 : 15N power for 90 pulse
;p3 : 13C 90 pulse @ plw11
;pl11 : 13C power for 90 pulse
;p18 : CO selective 180 pulse @ spw18
;sp18 : 13C power for selective 180 pulse
;spnam18 : Q3
;spoffs18 : + xxx Hz (set the offset of the pulse on CO)
;spoal18: 0.5
;p19 : CA selective 180 pulse @ spw19
;sp19 : 13C power for selective 180 pulse
;spnam19 : Q3
;spoffs19 : 0 (if offs3 is on CA)
;spoal19: 0.5
;cpdprg1 : 1H decoupling (sltppm for 1Hprot, waltz16 for 2Hprot)
;cpdprg4 : Water suppression with cwX @ 15kHz
;cpdprg5 : Water suppression with cwY @ 15kHz
;cpdprg2 : 15N decoupling (waltz16_pl16)
;cpdprg3 : 13C decoupling (waltz16_pl17)
;pl12 : 1H power for water suppression(waltz16)
;pl13: 1H decoupling power (sltppm or waltz16)
;pl16: 15N decoupling power (waltz16)
;pl17: 13C decoupling power (waltz16)
;pcpd1 : 25u (waltz16 10 kHz) - 33.33u (sltppm 15kHz)
;pcpd2 : 25u (waltz16 10 kHz)
;pcpd3 : 25u (waltz16 10 kHz)

#include <Avancesolids.incl>

;cnst11 : to adjust t=0 for acquisition, if digmod = baseopt
"acqt0=1u*cnst11"
"in0=inf1"
"in30=inf1"
"in10=inf2/2"
aqseq 312

1 ze

2 d1 do:f2 do:f3
#include <p15_prot.incl>
#include <aq_prot.incl>

  (p1 pl1 ph1):f1

  (p15:sp29 ph15):f3 (p15:sp1 ph20):f1

  1u cpds1:f1
  (p18:sp18 ph17):f3
  1u
  (p19:sp19 ph19):f3
  1u
  d10
  (center (p7*2 ph0 pl7):f2 (p18:sp18 ph17):f3)
  d10
  1u do:f1

  (p16:sp9 ph10):f3 (p16:sp2 ph2):f2

  1u cpds1:f1 cpds3:f3
  d0
  1u do:f1 do:f3

;;;;;;;;;;;;;;; water suppression block starts
  (p7 pl7 ph5):f2
 1u cpds4:f1
  d30*0.25
 1u do:f1
 1u cpds5:f1
```

```
  d30*0.25
 1u do:f1

 1u cpds4:f1
  d30*0.25
 1u do:f1

 1u cpds5:f1
  d30*0.25
 1u do:f1
  (p7 pl7 ph6):f2
;;;;;;;;;;;;;;; water suppression block ends

  (p17 pl20 ph7):f2 (p17:sp10 ph11):f1

  1u cpds2:f2 cpds3:f3
  go=2 ph31
  1m do:f2         do:f3

  10m mc #0 to 2
  F1PH(calph(ph2, +90), caldel(d0, +in0))
  F2PH(calph(ph15, +90), caldel(d10, +in10))

HaltAcqu, 1m      ;jump address for protection files
exit                                                 ;quit

ph0=0
ph1  = 0 2
ph20 = 1
ph15 = 0
ph10 = 0
ph2 =  1
ph5 =  0
ph6 =  0 0 2 2
ph12 = 1 1 1 1
ph17=0
ph19=0
ph7 =  1
ph11 = 1 1 1 1 3 3 3 3
ph31 = 1 3 3 1 3 1 1 3
```

^{13}C-^{15}N-^{1}H (H)CONH

```
;13C-15N-1H hCONH experiment

;Avance III version
;parameters:
;p1 : 1H 90 pulse @ plw1
;pl1 : 1H power for 90 pulse
;p15 : contact time for H->C CP (typically between 2 and 6ms)
;sp28 : 13C power for H->C CP
;sp1 : 1H power for H->C CP
;spnam1 : Ramp90.100
;spoffs1 : 0
;spnam28 : Rectangle
;spoffs28 : 0 (if offs3 set on CO)
;spoal18: 1 (end of the CP)
;p16 : contact time for C->N CP (typically up to 10ms)
;sp2 : 15N power for C->N CP
;sp8 : 13C power for C->N CP
;spnam2 : tangential ramp
;spoffs2 : 0
;spoal2: 1
;spnam8 : Rectangle
;spoffs8 : 0 (if offs3 set on CO)
;spoal8: 0 (beginning of the CP)
;p17 : contact time for N->H CP (typically 400u to 800u)
;pl20 : 15N power for N->H CP
;sp10 : 1H power for N->H CP
;spnam10 : Ramp100.90
;spoffs10 : 0
;spoal10: 1
;p7 : 15N 90 pulse @ plw7
;pl7 : 15N power for 90 pulse
;p3 : 13C 90 pulse @ plw11
;pl11 : 13C power for 90 pulse
;p18 : CO selective 180 pulse @ spw18
;sp18 : 13C power for selective 180 pulse
;spnam18 : Q3
;spoffs18 : 0 (if offs3 set on CO)
;spoal18: 0.5
;p19 : CA selective 180 pulse @ spw19
;sp19 : 13C power for selective 180 pulse
;spnam19 : Q3
;spoffs19 : - xxx Hz (set the offset of the pulse on CA)
;spoal19: 0.5
;cpdprg1 : 1H decoupling (sltppm for 1Hprot, waltz16 for 2Hprot)
;cpdprg4 : Water suppression with cwX @ 15kHz
;cpdprg5 : Water suppression with cwY @ 15kHz
;cpdprg2 : 15N decoupling (waltz16_pl16)
;cpdprg3 : 13C decoupling (waltz16_pl17)
;pl12 : 1H power for water suppression(waltz16)
;pl13: 1H decoupling power (sltppm or waltz16)
;pl16: 15N decoupling power (waltz16)
;pl17: 13C decoupling power (waltz16)
;pcpd1 : 25u (waltz16 10 kHz) - 33.33u (sltppm 15kHz)
```

```
;pcpd2 : 25u (waltz16 10 kHz)
;pcpd3 : 25u (waltz16 10 kHz)

#include <Avancesolids.incl>

;cnst11 : to adjust t=0 for acquisition, if digmod = baseopt
"acqt0=1u*cnst11"
"in0=inf1"
"in30=inf1"
"in10=inf2/2"
aqseq 312

1 ze

2 d1 do:f2 do:f3
#include <p15_prot.incl>
#include <aq_prot.incl>

  (p1 pl1 ph1):f1

  (p15:sp28 ph15):f3 (p15:sp1 ph20):f1

  1u  cpds1:f1
  (p19:sp19 ph17):f3
  1u
  (p18:sp18 ph19):f3
  1u
  d10
  (center (p7*2 ph0 pl7):f2 (p19:sp19 ph17):f3)
  d10
  1u do:f1

  (p16:sp8 ph10):f3 (p16:sp2 ph2):f2

  1u cpds1:f1 cpds3:f3
  d0
  1u do:f1 do:f3

;;;;;;;;;;;;;;; water suppression block starts
  (p7 pl7 ph5):f2
 1u cpds4:f1
  d30*0.25
 1u do:f1
 1u cpds5:f1
  d30*0.25
 1u do:f1

 1u cpds4:f1
  d30*0.25
 1u do:f1

 1u cpds5:f1
  d30*0.25
 1u do:f1
  (p7 pl7 ph6):f2
;;;;;;;;;;;;;;; water suppression block ends

  (p17 pl20 ph7):f2 (p17:sp10 ph11):f1

  1u cpds2:f2 cpds3:f3
  go=2 ph31
  1m do:f2          do:f3

  10m mc #0 to 2
  F1PH(calph(ph2, +90), caldel(d0, +in0))
  F2PH(calph(ph15, +90), caldel(d10, +in10))

HaltAcqu, 1m      ;jump address for protection files
exit                                            ;quit

ph0=0
ph1  = 0 2
ph20 = 1
ph15 = 0
ph10 = 0
ph2 =  1
ph5 =  0
ph6 =  0 0 2 2
ph12 = 1 1 1 1
ph17=0
ph19=0
ph7 =  1
ph11 = 1 1 1 1 3 3 3 3
ph31 = 1 3 3 1 3 1 1 3
```

^{13}C-^{15}N-^{1}H (H)CO(CA)NH

```
;13C-15N-1H hCOcaNH experiment

;Avance III version
;parameters:
;p1 : 1H 90 pulse @ plw1
;pl1 : 1H power for 90 pulse
;p15 : contact time for H->C CP (typically between 2 and 6ms)
;sp28 : 13C power for H->C CP
```

```
;sp1 : 1H power for H->C CP
;spnam1 : Ramp90.100
;spoffs1 : 0
;spnam28 : Rectangle
;spoffs28 : + x Hz (if offs3 set between CA and CO, to set the offset on CO)
;spoal28: 1 (end of the CP)
;p16 : contact time for C->N CP (typically up to 10ms)
;sp2 : 15N power for C->N CP
;sp9 : 13C power for C->N CP
;spnam2 : tangential ramp
;spoffs2 : 0
;spoal2: 1
;spnam9 : Rectangle
;spoffs9 : - x Hz (if offs3 set between CA and CO, to set the offset on CA)
;spoal9: 0 (beginning of the CP)
;p17 : contact time for N->H CP (typically 400u to 800u)
;pl20 : 15N power for N->H CP
;sp10 : 1H power for N->H CP
;spnam10 : Ramp100.90
;spoffs10 : 0
;spoal10: 0.5
;p7 : 15N 90 pulse @ plw7
;pl7 : 15N power for 90 pulse
;p3 : 13C 90 pulse @ plw11
;pl11 : 13C power for 90 pulse
;p18 : CO selective 180 pulse @ spw18
;sp18 : 13C power for selective 180 pulse
;spnam18 : Q3
;spoffs18 : + x Hz (if offs3 set between CA and CO, to set the offset of the pulse on CO)
;spoal18: 0.5
;p19 : CA selective 180 pulse @ spw19
;sp19 : 13C power for selective 180 pulse
;spnam19 : Q3
;spoffs19 : - x Hz (if offs3 set between CA and CO, to set the offset of the pulse on CA)
;spoal19: 0.5
;d11 : first J-evolution CO-CA (usually set to 4.7ms)
;d12 : second J-evolution CO-CA (usually set to 4.7ms, but can be shorter in case of short T2'(CA))
;cpdprg1 : 1H decoupling (sltppm for 1Hprot, waltz16 for 2Hprot)
;cpdprg4 : Water suppression with cwX @ 15kHz
;cpdprg5 : Water suppression with cwY @ 15kHz
;cpdprg2 : 15N decoupling (waltz16_pl16)
;cpdprg3 : 13C decoupling (waltz16_pl17)
;pl12 : 1H power for water suppression(waltz16)
;pl13: 1H decoupling power (sltppm or waltz16)
;pl16: 15N decoupling power (waltz16)
;pl17: 13C decoupling power (waltz16)
;pcpd1 : 25u (waltz16 10 kHz) - 33.33u (sltppm 15kHz)
;pcpd2 : 25u (waltz16 10 kHz)
;pcpd3 : 25u (waltz16 10 kHz)

#include <Avancesolids.incl>

;cnst11 : to adjust t=0 for acquisition, if digmod = baseopt
"acqt0=1u*cnst11"
"in0=inf1"
"in30=inf1"
"in10=inf2/2"
aqseq 321

1 ze

2 d1 do:f2 do:f3
#include <p15_prot.incl>
#include <aq_prot.incl>

  (p1 pl1 ph1):f1

  (p15:sp28 ph15):f3 (p15:sp1 ph20):f1

  1u cpds1:f1
  (p19:sp19 ph17):f3
  1u
  (p18:sp18 ph19):f3
  1u
  d10
  (center (p7*2 ph0 pl7):f2 (p19:sp19 ph17):f3)
  d10
  1u do:f1

  1u cpds1:f1
  d11
  (p3*2 pl11 ph14):f3
  d11
  (p3 pl11 ph16):f3
  (p18:sp18 ph19):f3
  d12
  (p19:sp19 ph17):f3
  (p18:sp18 ph19):f3
  d12
  1u do:f1

  (p16:sp9 ph10):f3 (p16:sp2 ph2):f2

  1u cpds1:f1 cpds3:f3
  d0
  1u do:f1 do:f3

 ;;;;;;;;;;;;;;; water suppression block starts
  (p7 pl7 ph5):f2
 1u cpds4:f1
  d30*0.25
```

```
 1u do:f1
 1u cpds5:f1
  d30*0.25
 1u do:f1

 1u cpds4:f1
  d30*0.25
 1u do:f1

 1u cpds5:f1
  d30*0.25
 1u do:f1
  (p7 pl7 ph6):f2
;;;;;;;;;;;;;;;; water suppression block ends

  (p17 pl20 ph7):f2 (p17:sp10 ph11):f1

  1u cpds2:f2 cpds3:f3
  go=2 ph31
  1m do:f2         do:f3

  10m mc #0 to 2
  F1PH(calph(ph2, +90), caldel(d0, +in0))
  F2PH(calph(ph15, +90), caldel(d10, +in10))

HaltAcqu, 1m      ;jump address for protection files
exit                                                    ;quit

ph0=0
ph1  = 0 2
ph20 = 1
ph15 = 0
ph10 = 0
ph2 =  1
ph5 =  0
ph6 =  0 0 2 2
ph14= {0}*4 {1}*4
ph16= 0
ph17={0}*8 {1}*8
ph19=0
ph12 = 1
ph7 =  1
ph11 = 1 1 1 1
ph31 =  3 1 1 3 1 3 3 1 1 3 3 1 3 1 1 3
```

^{13}C-^{15}N-^{1}H (H)(CO)CA(CO)NH

```
;13C-15N-1H hcoCAcoNH experiment

;Avance III version
;parameters:
;p1 : 1H 90 pulse @ plw1
;pl1 : 1H power for 90 pulse
;p15 : contact time for H->C CP (typically between 2 and 6ms)
;sp28 : 13C power for H->C CP
;sp1 : 1H power for H->C CP
;spnam1 : Ramp90.100
;spoffs1 : 0
;spoal1: 0
;spnam28 : Rectangle
;spoffs28 : + x Hz (if offs3 set between CA and CO, to set the offset on CO)
;spoal28: 1 (end of the CP)
;p16 : contact time for C->N CP (typically up to 10ms)
;sp2 : 15N power for C->N CP
;sp8 : 13C power for C->N CP
;spnam2 : tangential ramp
;spoffs2 : 0
;spoal2: 1
;spnam8 : Rectangle
;spoffs8 : + x Hz (if offs3 set between CA and CO, to set the offset on CO)
;spoal8: 0 (beginning of the CP)
;p17 : contact time for N->H CP (typically 400u to 800u)
;pl20 : 15N power for N->H CP
;sp10 : 1H power for N->H CP
;spnam10 : Ramp100.90
;spoffs10 : 0
;spoal10: 0.5
;p7 : 15N 90 pulse @ plw7
;pl7 : 15N power for 90 pulse
;p3 : 13C 90 pulse @ plw11
;pl11 : 13C power for 90 pulse
;p18 : CO selective 180 pulse @ spw18
;sp18 : 13C power for selective 180 pulse
;spnam18 : Q3
;spoffs18 : + x Hz (if offs3 set between CA and CO, to set the offset of the pulse on CO)
;spoal18: 0.5
;p19 : CA selective 180 pulse @ spw19
;sp19 : 13C power for selective 180 pulse
;spnam19 : Q3
;spoffs19 : - x Hz (if offs3 set between CA and CO, to set the offset of the pulse on CA
;spoal19: 0.5
;d28 : J-evolution CO-CA (usually set to 4.7ms)
;cpdprg1 : 1H decoupling (sltppm for 1Hprot, waltz16 for 2Hprot)
;cpdprg4 : Water suppression with cwX @ 15kHz
;cpdprg5 : Water suppression with cwY @ 15kHz
;cpdprg2 : 15N decoupling (waltz16_pl16)
```

```
;cpdprg3 : 13C decoupling (waltz16_pl17)
;pl12 : 1H power for water suppression(waltz16)
;pl13: 1H decoupling power (sltppm or waltz16)
;pl16: 15N decoupling power (waltz16)
;pl17: 13C decoupling power (waltz16)
;pcpd1 : 25u (waltz16 10 kHz) - 33.33u (sltppm 15kHz)
;pcpd2 : 25u (waltz16 10 kHz)
;pcpd3 : 25u (waltz16 10 kHz)

#include <Avancesolids.incl>

;cnst11 : to adjust t=0 for acquisition, if digmod = baseopt
"acqt0=1u*cnst11"
"in0=inf1"
"in30=inf1"
"in10=inf2/2"
aqseq 321

1 ze

2 d1 do:f2 do:f3
#include <p15_prot.incl>
#include <aq_prot.incl>

  (p1 pl1 ph1):f1

  (p15:sp28 ph2):f3 (p15:sp1 ph0):f1

  d28 cpds1:f1
  (p3*2 pl11 ph3):f3
  d28
  (p3 pl11 ph4):f3

  1u  cpds2:f2
  d10
  (p18:sp18 ph20):f3
  d10
  1u
  (p19:sp19 ph20):f3
  1u
  (p18:sp18 ph20):f3
  1u  do:f2

  (p3 pl11 ph5):f3
  d28
  (p3*2 pl11 ph20):f3
  d28


  1u do:f1
  (p16:sp8 ph7):f3 (p16:sp2 ph8):f2

  1u cpds1:f1 cpds3:f3
  d0
  1u do:f1 do:f3

;;;;;;;;;;;;;;; water suppression block starts
  (p7 pl7 ph9):f2
 1u cpds4:f1
  d30*0.25
 1u do:f1
 1u cpds5:f1
  d30*0.25
 1u do:f1
 1u cpds4:f1
  d30*0.25
 1u do:f1
 1u cpds5:f1
  d30*0.25
 1u do:f1
  (p7 pl7 ph10):f2
;;;;;;;;;;;;;;; water suppression block ends

  (p17 pl20 ph11):f2 (p17:sp10 ph12):f1

  1u cpds2:f2 cpds3:f3
  go=2 ph31
  1m do:f2          do:f3

  10m mc #0 to 2
  F1PH(calph(ph8, +90), caldel(d0, +in0))
  F2PH(calph(ph2, +90) & calph(ph4, +90), caldel(d10, +in10))

HaltAcqu, 1m       ;jump address for protection files
exit                                                    ;quit

ph0 = 0
ph1 = 1 3
ph2 = 1 1 3 3
ph3 = 0
ph4 = 1
ph5 = 1
ph6 = 0
ph7 = {1}*4 {3}*4
ph8 = {0}*8 {2}*8
ph9 = 3
ph10= 1
ph11= 0
ph12= 0
ph20= 0
ph31= 0 2 2 0 2 0 0 2 2 0 0 2 0 2 2 0
```

^{13}C-^{15}N-^{1}H (H)(CA)CB(CA)NH

```
;13C-15N-1H hcaCBcaNH experiment

;Avance III version
;parameters:
;p1 : 1H 90 pulse @ plw1
;pl1 : 1H power for 90 pulse
;p15 : contact time for H->C CP (typically between 2 and 6ms)
;sp29 : 13C power for H->C CP
;sp1 : 1H power for H->C CP
;spnam1 : Ramp90.100
;spoffs1 : 0
;spoal1: 0
;spnam29 : Rectangle
;spoffs29 : + x Hz (if offs3 set between CA and CB, to set the offset on CA)
;spoal29: 1 (end of the CP)
;p16 : contact time for C->N CP (typically up to 10ms)
;sp2 : 15N power for C->N CP
;sp9 : 13C power for C->N CP
;spnam2 : tangential ramp
;spoffs2 : 0
;spoal2: 1
;spnam9 : Rectangle
;spoffs9 : + x Hz (if offs3 set between CA and CB, to set the offset on CA)
;spoal9: 0 (beginning of the CP)
;p17 : contact time for N->H CP (typically 400u to 800u)
;pl20 : 15N power for N->H CP
;sp10 : 1H power for N->H CP
;spnam10 : Ramp100.90
;spoffs10 : 0
;spoal10: 1
;p7 : 15N 90 pulse @ plw7
;pl7 : 15N power for 90 pulse
;p3 : 13C 90 pulse @ plw11
;pl11 : 13C power for 90 pulse
;p19 : 13C aliphatic 180 pulse @ spw19 (typically 200u)
;sp19 : 13C power for aliphatic 180 pulse
;spnam19 : Q3
;spoffs19 : 0 (if offs3 set between CA and CB)
;spoal19: 0.5
;d28 : J-evolution CB-CA (usually set to 7.2ms)
;d8 : z-filter (typically 1 to 5ms)
;cpdprg1 : 1H decoupling (sltppm for 1Hprot, waltz16 for 2Hprot)
;cpdprg4 : Water suppression with cwX @ 15kHz
;cpdprg5 : Water suppression with cwY @ 15kHz
;cpdprg2 : 15N decoupling (waltz16_pl16)
;cpdprg3 : 13C decoupling (waltz16_pl17)
;pl12 : 1H power for water suppression(waltz16)
;pl13: 1H decoupling power (sltppm or waltz16)
;pl16: 15N decoupling power (waltz16)
;pl17: 13C decoupling power (waltz16)
;pcpd1 : 25u (waltz16 10 kHz) - 33.33u (sltppm 15kHz)
;pcpd2 : 25u (waltz16 10 kHz)
;pcpd3 : 25u (waltz16 10 kHz)

#include <Avancesolids.incl>

;cnst11 : to adjust t=0 for acquisition, if digmod = baseopt
"acqt0=1u*cnst11"
"in0=inf1"
"in30=inf1"
"in10=inf2"
aqseq 321

1 ze

2 d1 do:f2 do:f3
#include <p15_prot.incl>
#include <aq_prot.incl>

  (p1 pl1 ph1):f1

  (p15:sp29 ph2):f3 (p15:sp1 ph0):f1

  d28 cpds1:f1
  (p19:sp19 ph3):f3
  d28

  (p3 pl11 ph4):f3

  1u  cpds2:f2
  d10
  1u  do:f2


  (p3 pl11 ph5):f3

  d28
  (p19:sp19 ph20):f3
  d28

  1u do:f1
  (p3 pl11 ph6):f3
  d8
```

```
  (p3 pl11 ph6):f3
  1u
  (p16:sp9 ph7):f3 (p16:sp2 ph8):f2

  1u cpds1:f1 cpds3:f3
  d0
  1u do:f1 do:f3

;;;;;;;;;;;;;;;; water suppression block starts
  (p7 pl7 ph9):f2
 1u cpds4:f1
  d30*0.25
 1u do:f1
 1u cpds5:f1
  d30*0.25
 1u do:f1
 1u cpds4:f1
  d30*0.25
 1u do:f1
 1u cpds5:f1
  d30*0.25
 1u do:f1
  (p7 pl7 ph10):f2
;;;;;;;;;;;;;;;; water suppression block ends

  (p17 pl20 ph11):f2 (p17:sp10 ph12):f1

  1u cpds2:f2 cpds3:f3
  go=2 ph31
  1m do:f2         do:f3

  10m mc #0 to 2
  F1PH(calph(ph8, +90), caldel(d0, +in0))
  F2PH(calph(ph2, +90) & calph(ph4, +90), caldel(d10, +in10))

HaltAcqu, 1m      ;jump address for protection files
exit                                                   ;quit

ph0 = 0
ph1 = 1 3
ph2 = 1 1 3 3
ph3 = 0
ph4 = 1
ph5 = 1
ph6 = 0
ph7 = {1}*4 {3}*4
ph8 = {0}*8 {2}*8
ph9 = 3
ph10= 1
ph11= 0
ph12= 0
ph20= 0
ph31= 0 2 2 0 2 0 0 2 2 0 0 2 0 2 2 0
```

^{13}C-^{15}N-^{1}H (H)(CA)CB(CACO)NH

```
;13C-15N-1H hcaCBcacoNH experiment

;Avance III version
;parameters:
;p1 : 1H 90 pulse @ plw1
;pl1 : 1H power for 90 pulse
;p15 : contact time for H->C CP (typically between 2 and 6ms)
;sp29 : 13C power for H->C CP
;sp1 : 1H power for H->C CP
;spnam1 : Ramp90.100
;spoffs1 : 0
;spoal1: 0
;spnam29 : Rectangle
;spoffs29 : + x Hz (if offs3 set between CA and CB, to set the offset on CA)
;spoal29: 1 (end of the CP)
;p16 : contact time for C->N CP (typically up to 10ms)
;sp2 : 15N power for C->N CP
;sp8 : 13C power for C->N CP
;spnam2 : tangential ramp
;spoffs2 : 0
;spnam8 : Rectangle
;spoffs8 : + y Hz (if offs3 + cnst33 set between CA and CO, to set the offset on CO)
;spoal8: 0 (beginning of the CP)
;p17 : contact time for N->H CP (typically 400u to 800u)
;pl20 : 15N power for N->H CP
;sp10 : 1H power for N->H CP
;spnam10 : Ramp100.90
;spoffs10 : 0
;spoal10: 1
;p7 : 15N 90 pulse @ plw7
;pl7 : 15N power for 90 pulse
;p3 : 13C 90 pulse @ plw11
;pl11 : 13C power for 90 pulse
;p39 : 13C aliphatic 180 pulse @ spw19 (typically 200u)
;sp39 : 13C power for aliphatic 180 pulse
;spnam39 : Q3
;spoffs39 : 0 (if offs3 set between CA and CB, cnst32 = 0)
;spoal39: 0.5
;p18 : CO selective 180 pulse @ spw18
;sp18 : 13C power for selective 180 pulse
```

```
;spnam18 : Q3
;spoffs18 : + x Hz (if offs3 set between CA and CO using cnst33, to set the offset of the pulse on CO)
;spoal18: 0.5
;p19 : CA selective 180 pulse @ spw19
;sp19 : 13C power for selective 180 pulse
;spnam19 : Q3
;spoffs19 : - x Hz (if offs3 set between CA and CO using cnst33, to set the offset of the pulse on CA)
;spoal19: 0.5
;d28 : J-evolution CB-CA (usually set to 7.2ms)
;d8 : z-filter (typically 1 to 5ms)
;d12 : first J-evolution CO-CA (usually set to 4.7ms, but can be shorter in case of short T2'(CA))
;d11 : second J-evolution CO-CA (usually set to 4.7ms)
;cnst32 : usually 0 (if offs3 between CA and CB)
;cnst33 : positive offset to put the carrier between CA and CO
;cpdprg1 : 1H decoupling (sltppm for 1Hprot, waltz16 for 2Hprot)
;cpdprg4 : Water suppression with cwX @ 15kHz
;cpdprg5 : Water suppression with cwY @ 15kHz
;cpdprg2 : 15N decoupling (waltz16_pl16)
;cpdprg3 : 13C decoupling (waltz16_pl17)
;pl12 : 1H power for water suppression(waltz16)
;pl13: 1H decoupling power (sltppm or waltz16)
;pl16: 15N decoupling power (waltz16)
;pl17: 13C decoupling power (waltz16)
;pcpd1 : 25u (waltz16 10 kHz) - 33.33u (sltppm 15kHz)
;pcpd2 : 25u (waltz16 10 kHz)
;pcpd3 : 25u (waltz16 10 kHz)

#include <Avancesolids.incl>

;cnst11 : to adjust t=0 for acquisition, if digmod = baseopt
"acqt0=1u*cnst11"
"in0=inf1"
"in30=inf1"
"in10=inf2"
aqseq 321

1 ze

2 d1 do:f2 do:f3
#include <p15_prot.incl>
#include <aq_prot.incl>
  1u fq=cnst32:f3
  (p1 pl1 ph1):f1

  (p15:sp29 ph2):f3 (p15:sp1 ph0):f1

  d28 cpds1:f1
  (p39:sp39 ph3):f3
  d28

  (p3 pl11 ph4):f3

  1u  cpds2:f2
  d10
  1u  do:f2

  (p3 pl11 ph5):f3

  d28
  (p39:sp39 ph20):f3
  d28

  1u do:f1
  (p3 pl11 ph6):f3
  d8 fq=cnst33:f3
  (p3 pl11 ph16):f3
  1u cpds1:f1

  (p18:sp18 ph19):f3
  d12
  (p19:sp19 ph17):f3
  (p18:sp18 ph19):f3
  d12

  (p3 pl11 ph18):f3
  d11
  (p3*2 pl11 ph14):f3
  d11
  1u do:f1

  (p16:sp8 ph27):f3 (p16:sp2 ph8):f2

  1u cpds1:f1 cpds3:f3
  d0
  1u do:f1 do:f3

;;;;;;;;;;;;;;;; water suppression block starts
  (p7 pl7 ph9):f2
 1u cpds4:f1
  d30*0.25
 1u do:f1
 1u cpds5:f1
  d30*0.25
 1u do:f1
 1u cpds4:f1
  d30*0.25
 1u do:f1
 1u cpds5:f1
  d30*0.25
 1u do:f1
  (p7 pl7 ph10):f2
```

```
;;;;;;;;;;;;;;;; water suppression block ends

  (p17 pl20 ph11):f2 (p17:sp10 ph12):f1

  1u cpds2:f2 cpds3:f3
  go=2 ph31
  1m do:f2          do:f3

  10m mc #0 to 2
  F1PH(calph(ph8, +90), caldel(d0, +in0))
  F2PH(calph(ph2, +90) & calph(ph4, +90), caldel(d10, +in10))

HaltAcqu, 1m       ;jump address for protection files
exit                                                    ;quit

ph0 = 0
ph1 = 1 3
ph2 = 1 1 3 3
ph3 = 0
ph4 = 1
ph5 = 1
ph6 = 0
ph8 = {0}*16 {2}*16
ph9 = 3
ph10= 1
ph11= 0
ph12= 0
ph20= 0
ph16=3
ph18=0
ph17={0}*4 {1}*4
ph19=0
ph14= {0}*8 {1}*8
ph27 = 0
ph31= 0 2 2 0 2 0 0 2 2 0 0 2 0 2 2 0 2 0 0 2 0 2 2 0 0 2 2 0 2 0 0 2
```