Supporting Information

Protein NMR resonance assignment without spectral analysis: 5D solid-state automated projection spectroscopy (SO-APSY)

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Contents:

A. NMR sample preparation

GB1. Uniformly ^{13}C,^{15}N-labelled GB1 was overexpressed, purified, dialysed against phosphate buffer and and precipitated to microcrystals in form A according to published protocol.[1-2] The crystals were packed into a 0.7 mm Bruker MAS rotor.

Cu(I),Zn-SOD. Uniformly ^{2}H,^{13}C,^{15}N-labelled and completely back-exchanged human Cu^{I},Zn-loaded superoxide dismutase (SOD) was obtained as previously reported,[3-4] crystallised using the sitting drop method,[5] and packed into a 1.3 mm Bruker MAS rotor.

$\beta2m_{D76N}$. Expression of ^{2}H,^{13}C,^{15}N-labelled $\beta2m_{D76N}$ was performed as described earlier, with purification steps, including refolding, performed in non-deuterated water to allow proton back-exchange at all amide sites.[6] A solution of purified (unlabeled) $\beta2m_{D76N}$ (100 µM β2m, 25 mM sodium phosphate, pH 7.4) was then incubated at 37°C with vigorous shaking for 5 days, resulting in a mother generation of fibrils (G_0).[7] A second generation G_1 was obtained in the same conditions with the addition of 1.5% v/v of G_0 and, in order to limit polymorphism, the same procedure was iterated for seven generations. Homogeneity of the samples was tested with transmission electron-microscopy and MAS NMR. The final sample (G_8) was generated from triple-labelled $\beta2m_{D76N}$.

Microcrystals (GB1 and SOD) and fibrils ($\beta2m_{D76N}$) were packed by ultracentrifugation at 165,000×g at 12°C directly into the NMR rotor using a device provided by Giotto Biotech, similar to those described in literature.[8-9]

B. Pulse sequence description

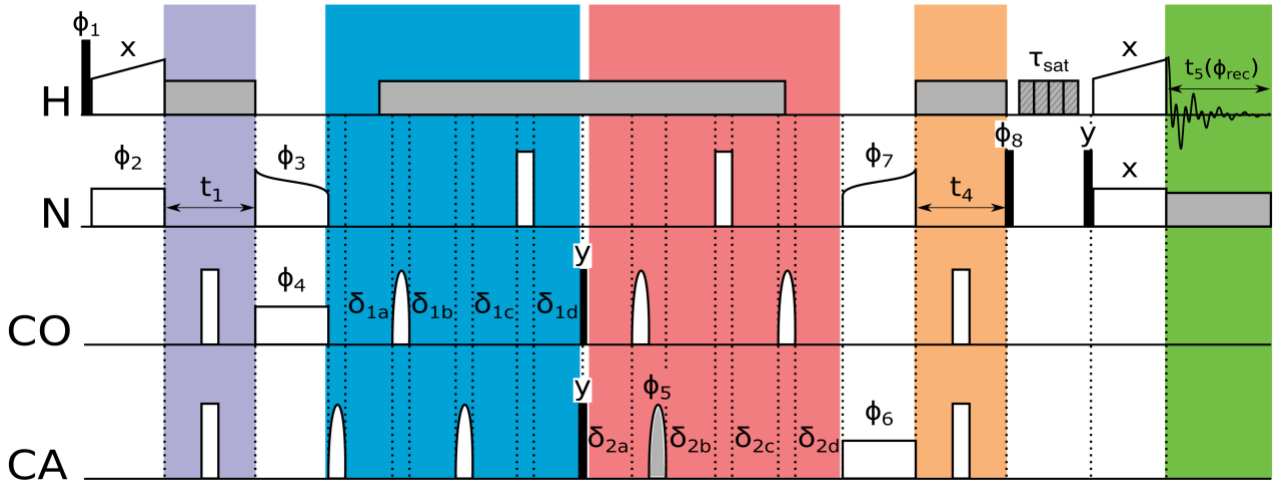

Figure S1. Pulse sequence for 5D (H)NCOCANH experiment. Heteronuclear cross-polarisation (CP) transfers are shown as wide open shapes simultaneously on two channels. RF strengths, durations and amplitude shapes used for CP transfers are summarised in Table S3. Heteronuclear decoupling is shown in grey. Swept-TPPM[10-11] or WALTZ-16[12] ^{1}H decoupling with r.f. field of 10 kHz and pulse length of 25 µs was used for GB1 and SOD/$\beta2M_{D76N}$, respectively. WALTZ-16 with r.f. field of 10 kHz and pulse length of 25 µs was used for ^{15}N decoupling during acquisition. Open pulses represent a flip-angle of 180°, while solid pulses are 90°. Bell shapes on carbon channels represent C' or Cα selective shaped pulses. All shaped pulses were Q3,[13] with the exception of the Cα refocussing pulse (shown in grey), where a REBURP was used.[14] The shaped pulse duration and max. r.f. field strength were 144.3 µs and 22.9 kHz ($^{13}C'$-selective Q3), 129.1 µs and 25.6 kHz ($^{13}C\alpha$ selective Q3), and 638.0 µs and 9.82 µs ($^{13}C\alpha$ selective ReBURP). The MISSISSIPPI water suppression,[15] with duration τ_{sat} of 150 (for GB1) or 100 ms (SOD, β2m) and r.f. strength of either 53 kHz (GB1), 34 kHz (SOD) or 39 kHz (β2m), is shown in hatched grey. The Cα-C' coherence transfer delays are maximally exploited for the either constant-time or shared-time ("semi-constant time") chemical shift evolution of $^{13}C'$ (t_2) and $^{13}C\alpha$ (t_3). The respective

formulas for delays δ_{1a}, δ_{1b}, δ_{1c}, δ_{1d} (or δ_{2a}, δ_{2b}, δ_{2c}, δ_{2d}) vary depending on whether t_2 (or t_3) exceeds the relaxation-optimised transfer delay $2\delta_{CA/CO}{}^{opt} = \tan^{-1}(\pi J T_2{}'^{CA/CO} / (\pi J))$, or the relaxation-independent transfer delay $2\delta = (2\ {}^1J_{CA\text{-}CO})^{-1}$. They are determined as follows:

$\delta_{1a} = \max(\delta_{CO}{}^{opt} + 2pw_{90}{}^{N} - \frac{1}{2}\ t_2, 0)$,

$\delta_{1b} = \max(\frac{1}{2}\ t_2 - \delta, 0)$,

$\delta_{1c} = \max(\delta_{CO}{}^{opt}, \min(\frac{1}{2}\ t_2, \delta), 0)$,

$\delta_{1d} = \frac{1}{2}\ t_2$,

$\delta_{2a} = \max(\delta_{CA}{}^{opt} + 2pw_{90}{}^{N} - \frac{1}{2}\ t_3, 0)$,

$\delta_{2b} = \max(\delta_{CA}{}^{opt}, \frac{1}{2}\ t_3)$,

$\delta_{2c} = \max(\frac{1}{2}\ t_3, \delta)$,

$\delta_{2d} = \max(\frac{1}{2}\ t_3 - \delta_{CA}{}^{opt}, 0)$.

The coupling constant ${}^1J_{CA\text{-}CO}$ of 55 Hz was assumed, and the bulk ^{13}C refocussed coherence lifetimes are summarised in Table S5. ^{15}N offset was set to 117.5 (for GB1), 115.6 (SOD) or 119.5 ($\beta 2M_{D76N}$) ppm while ^{1}H offset was set on resonance with H_2O line. The carbon carrier frequency was set to the centre of the Cα/C' spectrum at 114 ppm for the entire experiment. Shaped pulses and CP transfer offsets were set to -60 and +60 ppm for the Cα and C' channels respectively while the hard 90° pulse was applied at zero offset. Stepwise phase modulation was used to approximate the off-resonance effect of these pulses, and zero phase was enforced (aligned) at the end and at the beginning of the ^{13}C pulse for ^{15}N→^{13}C' and ^{13}Cα→^{15}N CP transfers, respectively. A time-dependent phase increment was applied to carbon refocussing pulses to control the centre of the respective carbon dimension (Cα or C') and was calculated in degrees as 180*Ω*t where Ω is the carbon offset from the carrier in Hz and t is the time increment. Re-equilibration delay of 1.0, 0.5 and 1.18 s was used for GB1, Cu(I),Zn-SOD and $\beta 2M_{D76N}$, respectively. The phases cycle was $\phi_1 = \{y, -y\}$, $\phi_2 = y$, $\phi_3 = \{y, y, -y, -y\}$, $\phi_4 = y$, $\phi_5 = \{4(x), 4(-x)\}$, $\phi_6 = y$, $\phi_7 = x$, $\phi_8 = \{8(y), 8(-y)\}$ and $\phi_{rec} = \{x, -x, -x, x, -x, x, x, -x, -x, x, x, -x, x, -x, -x, x\}$. Quadrature detection in the indirect dimensions was accomplished by incrementing ϕ_2 (in ω_1), decrementing ϕ_4 (in ω_2), decrementing ϕ_6 (in ω_3), or incrementing ϕ_7 (in ω_4), simultaneously with t_1, t_2, t_3, and t_4 time incrementation.

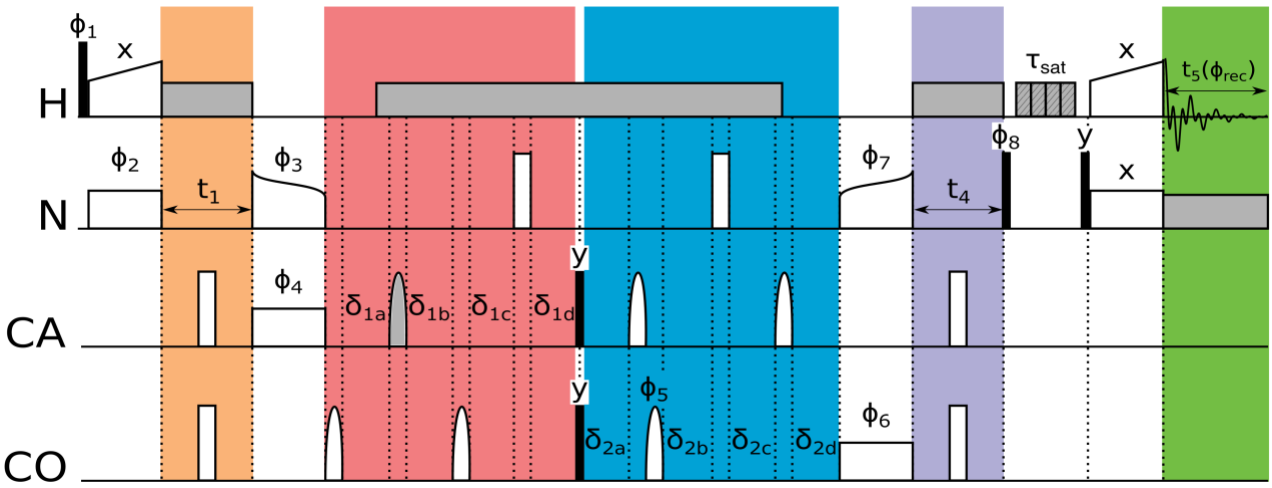

Figure S2. Pulse sequence for 5D (H)NCACONH experiment. The description of Figure S1 remains valid with a few exceptions that follow: delays δ_{1a} and δ_{1c} are determined with the use of $\delta_{CA}{}^{opt}$, while delays δ_{2a}, δ_{2b} and δ_{2d} depend on $\delta_{CO}{}^{opt}$. The zero phase is enforced (aligned) at the end of ^{13}Cα, and at the beginning of the ^{13}C' pulse for the ^{15}N→^{13}Cα and ^{13}C'→^{15}N CP transfers, respectively. ^{13}Cα and ^{13}C' shaped pulses are exchanged to preserve their context, i.e. the ^{13}Cα refocussing pulse (ReBURP) is performed between δ_{1a} and δ_{1b}, etc.

C. NMR data acquisition details

Table S1: (H)NCACONH experiment acquisition parameters

Sample	Max. evolution time, ms in dimension					Spectral widths, ppm[a] in dimension					MAS rate, kHz	Scans	Efficiency[e]	Number of projec-tions	Time, h
	ω_1 N	ω_2 Cα	ω_3 C′	ω_4 N	ω_5 H	ω_1 N	ω_2 Cα	ω_3 C′	ω_4 N	ω_5 H					
GB1	18.1	10.0	13.9	18.1	20.5[b]	30	25	12	30	100	110	32	0.21	24	40.5
SOD	13.1	9.1	9.9	13.1	24.8[c]	37	28	12	37	100	60	80	0.09	32	72.0
$\beta2M_{D76N}$	9.9	7.0	8.0	9.9	20.5[d]	30	30	10	30	100	55	48	0.09	32	57.5

[a] at the static field used (23.5 T) 1 ppm ^{1}H = 1000.3 Hz, 1 ppm ^{13}C = 251.5 Hz, and 1 ppm ^{15}N = 101.4 Hz
[b] truncated to 10.0 ms during data processing
[c] truncated to 9.9 ms during data processing
[d] truncated to 7.2 ms during data processing
[e] the signal magnitude with respect to the first FID of (H)NH dipolar correlation experiment

Table S2: (H)NCOCANH experiment acquisition parameters

Sample	Max. evolution time, ms in dimension					Spectral widths, ppm [a] in dimension					MAS rate, kHz	Scans	Efficiency[e]	Number of projec-tions	Time, h
	ω_1 N	ω_2 C′	ω_3 Cα	ω_4 N	ω_5 H	ω_1 N	ω_2 C′	ω_3 Cα	ω_4 N	ω_5 H					
GB1	18.1	13.9	9.9	18.1	20.5[b]	30	12	26	30	100	110	32	0.18	24	41.5
SOD	13.1	9.9	9.1	13.1	24.8[c]	37	12	28	37	100	60	80	0.11	32	71.0
$\beta2M_{D76N}$	9.9	8.0	7.0	9.9	20.5[d]	30	10	30	30	55	55	48	0.09	32	58.0

[a] at the static field used (23.5 T) 1 ppm ^{1}H = 1000.3 Hz, 1 ppm ^{13}C = 251.5 Hz, and 1 ppm ^{15}N = 101.4 Hz
[b] truncated to 10.0 ms during data processing
[c] truncated to 9.9 ms during data processing
[d] truncated to 7.2 ms during data processing
[e] the signal magnitude with respect to the first FID of (H)NH dipolar correlation experiment

Table S3: Details of cross-polarization transfers

CP transfer no		Sample		
		GB1	SOD	$\beta2M_{D76N}$
(H)NCACONH				
1 (^{1}H→^{15}N)	contact time, ms	2.3	1.25	1.0
	^{1}H r.f. max. field, kHz	149.1	103.5	95.7
	^{1}H r.f. amplitude ramp[a]	+10%	+10%	+10%
	^{15}N r.f. field (const), kHz	33.4	35.2	35.0
2 (^{15}N→^{13}Cα)	contact time, ms	19.0	19.0	18.0
	^{15}N r.f. max. field, kHz [b]	42.8	27.0	30.9
	^{15}N r.f. average field, kHz [b]	37.2	23.5	26.8
	^{15}N r.f. modulation depth, kHz [b]	5.6	3.5	4.0
	^{15}N r.f. effective coupling, Hz [b]	1359	858	980
	^{13}Cα r.f. field (const), kHz	70.0	43.5	25.0
3 (^{13}C′→^{15}N)	contact time, ms	19.0	14.0	16.0
	^{13}C′ r.f. field (const), kHz	70.0	43.9	25.0
	^{15}N r.f. max. field, kHz [b]	43.3	27.0	31.3
	^{15}N r.f. average field, kHz [b]	37.7	23.5	27.2
	^{15}N r.f. modulation depth, kHz [b]	–5.7	–3.5	4.1
	^{15}N r.f. effective coupling, Hz [b]	1376	858	993
4 (^{1}H→^{15}N)	contact time, ms	0.7	0.6	0.4
	^{15}N r.f. field (const), kHz	33.4	35.2	35.0
	^{1}H r.f. max. field, kHz	143.9	100.7	94.5
	^{1}H r.f. amplitude ramp [a]	+10%	+10%	+10%
(H)NCOCANH				
1 (^{1}H→^{15}N)	as for (H)NCACONH			
2 (^{15}N→^{13}C′)	contact time, ms	19.0	14.0	16.0
	^{15}N r.f. max. field, kHz [b]	42.8	27.0	31.1
	^{15}N r.f. average field, kHz [b]	37.2	23.5	27.0

Table S3: Details of cross-polarization transfers (cont.)

CP transfer no		Sample		
		GB1	SOD	β2M$_{D76N}$
	^{15}N r.f. modulation depth, kHz [b]	5.6	3.5	4.1
	^{15}N r.f. effective coupling, Hz [b]	1359	858	986
	^{13}C' r.f. field (const), kHz	70.0	44.4	25.0
3 (^{13}Cα→^{15}N)	contact time, ms	19.0	17.0	18.0
	^{13}Cα r.f. field (const), kHz	70.0	44.3	25.0
	^{15}N r.f. max. field, kHz [b]	43.3	27.0	30.9
	^{15}N r.f. average field, kHz [b]	37.7	23.5	26.8
	^{15}N r.f. modulation depth, kHz [b]	–5.7	–3.5	4.0
	^{15}N r.f. effective coupling, Hz [b]	1376	858	980
4 (^{1}H→^{15}N)	as for (H)NCACONH			

[a] An x % ramp means a linear amplitude modulation from (1-x/100) to 1.0

[b] The tangential shapes (for adiabatic CP[16]) are defined by 4 empirical parameters: the average r.f. field ω_{avg} (close to the Hartmann-Hahn matching condition), the effective dipolar coupling d^{eff} (averaged over crystal orientations and decreased due to internal motions), the modulation depth Δ, and the contact time τ, according to the following equation:

$$\omega(t) = \omega_{avg} + d^{eff} \tan\left(\frac{2}{\tau}\text{atan}\left(\frac{\Delta}{d_{NC}^{eff}}\right)\left(\frac{\tau}{2} - t\right)\right)$$

Table S4: Projection angles used in APSY series

No	α [a]	β [a]	γ [a]	Number of projections	Number of time points[b]
GB1: (H)NCACONH					
1	0.0	0.0	0.0	1	55x2
2	0.0	0.0	90.0	1	55x2
3	0.0	90.0	0.0	1	63x2
4	90.0	0.0	0.0	1	42x2
5-6	± 37.6	0.0	0.0	2	69x2x2
7-8	0.0	± 29.0	0.0	2	79x2x2
9-10	0.0	0.0	± 45.0	2	78x2x2
11-12	90.0	± 35.8	0.0	2	71x2x2
13-14	90.0	0.0	± 52.4	2	69x2x2
15-16	0.0	90.0	± 61.0	2	79x2x2
17-20	± 37.6	± 23.7	0.0	4	89x2x2x2
21-24	± 37.6	0.0	± 38.4	4	89x2x2x2
Total				24	3634
GB1: (H)NCOCANH					
1	0.0	0.0	0.0	1	55x2
2	0.0	0.0	90.0	1	55x2
3	0.0	90.0	0.0	1	42x2
4	90.0	0.0	0.0	1	75x2
5-6	± 28.8	0.0	0.0	2	80x2x2
7-8	0.0	± 37.6	0.0	2	69x2x2
9-10	0.0	0.0	± 45.0	2	78x2x2
11-12	90.0	± 54.5	0.0	2	72x2x2
13-14	90.0	0.0	± 61.2	2	80x2x2
15-16	0.0	90.0	± 52.4	2	69x2x2
17-20	± 28.8	± 34.0	0.0	4	90x2x2x2
21-24	± 28.8	0.0	± 41.2	4	96x2x2
Total				24	3350

Table S4: Projection angles used in APSY series (cont.)

No	α [a]	β [a]	γ [a]	Number of projections	Number of time points [b]
SOD: (H)NCACONH					
1	0.0	0.0	0.0	1	49x2
2	0.0	0.0	90.0	1	49x2
3	0.0	90.0	0.0	1	64x2
4	90.0	0.0	0.0	1	30x2
5-6	± 37.3	0.0	0.0	2	57x2x2
7-8	0.0	± 34.8	0.0	2	77x2x2
9-10	0.0	0.0	± 45.0	2	70x2x2
11-12	90.0	± 42.4	0.0	2	66x2x2
13-14	90.0	0.0	± 52.7	2	57x2x2
15-16	0.0	90.0	± 55.2	2	77x2x2
17-20	± 37.3	± 29.0	0.0	4	81x2x2x2
21-24	± 37.3	0.0	± 38.5	4	75x2x2x2
25-28	0.0	± 34.8	± 39.4	4	91x2x2x2
29-32	90.0	± 42.4	± 44.1	4	81x2x2x2
Total				32	4624
SOD: (H)NCOCANH					
1	0.0	0.0	0.0	1	49x2
2	0.0	0.0	90.0	1	49x2
3	0.0	90.0	0.0	1	30x2
4	90.0	0.0	0.0	1	64x2
5-6	± 34.8	0.0	0.0	2	77x2x2
7-8	0.0	± 37.3	0.0	2	57x2x2
9-10	0.0	0.0	± 45.0	2	70x2x2
11-12	90.0	± 47.6	0.0	2	66x2x2
13-14	90.0	0.0	± 55.2	2	77x2x2
15-16	0.0	90.0	± 52.7	2	57x2x2
17-20	± 34.8	± 32.0	0.0	4	81x2x2x2
21-24	± 34.8	0.0	± 39.4	4	91x2x2x2
25-28	0.0	± 37.3	± 38.5	4	75x2x2x2
29-32	90.0	± 47.6	± 44.1	4	81x2x2x2
Total				32	4624
$\beta 2M_{D76N}$: (H)NCACONH					
1	0.0	0.0	0.0	1	30x2
2	0.0	0.0	90.0	1	30x2
3	0.0	90.0	0.0	1	53x2
4	90.0	0.0	0.0	1	20x2
5-6	± 38.9	0.0	0.0	2	36x2x2
7-8	0.0	± 35.4	0.0	2	55x2x2
9-10	0.0	0.0	± 45.0	2	43x2x2
11-12	90.0	± 41.5	0.0	2	50x2x2
13-14	90.0	0.0	± 51.1	2	36x2x2
15-16	0.0	90.0	± 54.6	2	55x2x2
17-20	± 38.9	± 29.0	0.0	4	57x2x2x2
21-24	± 38.9	0.0	± 37.9	4	47x2x2x2
25-28	0.0	± 35.4	± 39.2	4	62x2x2x2
29-32	90.0	± 41.5	± 42.9	4	57x2x2x2
Total				32	3150

Table S4: Projection angles used in APSY series (cont.)

No	α [a]	β [a]	γ [a]	Number of projections	Number of time points[b]
β2M$_{D76N}$: (H)NCOCANH					
1	0.0	0.0	0.0	1	30x2
2	0.0	0.0	90.0	1	30x2
3	0.0	90.0	0.0	1	20x2
4	90.0	0.0	0.0	1	53x2
5-6	± 35.4	0.0	0.0	2	55x2x2
7-8	0.0	± 38.9	0.0	2	36x2x2
9-10	0.0	0.0	± 45.0	2	43x2x2
11-12	90.0	± 48.5	0.0	2	50x2x2
13-14	90.0	0.0	± 54.6	2	55x2x2
15-16	0.0	90.0	± 51.1	2	36x2x2
17-20	± 35.4	± 33.3	0.0	4	57x2x2x2
21-24	± 35.4	0.0	± 39.2	4	62x2x2x2
25-28	0.0	± 38.9	± 37.9	4	47x2x2x2
29-32	90.0	± 48.5	± 42.9	4	57x2x2x2
Total				32	3150

[a] Time increments in the indirect dimensions are determined by angles α, β and γ in the following way:

$$\Delta_1 = (sw_1)^{-1} \sin(\gamma) \quad (1)$$

$$\Delta_2 = (sw_2)^{-1} \sin(\beta) \cos(\gamma) \quad (2)$$

$$\Delta_3 = (sw_3)^{-1} \sin(\alpha) \cos(\beta) \cos(\gamma) \quad (3)$$

$$\Delta_4 = (sw_4)^{-1} \cos(\alpha) \cos(\beta) \cos(\gamma) \quad (4)$$

[b] The number of time increments is followed by the number of quadratures ("x2") depending on the type of projection. Details on data handling that leads to actual projections are given in section E.

D. Amide proton and heteronuclear refocussed coherence lifetimes

Table S5: Bulk $^{1}H^{N}$, ^{15}N, $^{13}C\alpha$, and $^{13}C'$ refocussed coherence lifetimes under fast MAS and low-power decoupling

Sample	MAS rate, kHz	Refocussed coherence lifetime, ms			
		$^{13}C'$	$^{13}C\alpha$	^{15}N	$^{1}H^{N}$
GB1	110	89 [a]	71 [a]	120 [a,b]	4.7
SOD	60	36 [c]	22 [c]	42 [c,d]	9.0 [d]
β2M$_{D76N}$	55	36 [c]	23 [c]	30	4.7

[a] Under 10 kHz swept-TPPM decoupling[10-11]

[b] Reported by Andreas et al[17] for an equivalent sample of GB1, at identical MAS rate and B_0 field

[c] Under 10 kHz WALTZ-16 decoupling[12]

[d] Reported by Knight et al[3] for an equivalent sample of SOD-Cu^{I},Zn at identical MAS rate and B_0 field

E. Performance of interresidue amide proton transfer experiments

In solution two equivalent experiments have been proposed to record interresidue amide proton and nitrogen correlations: 5D HN(CA)CONH[18] and 6D APSY HNCOCANH.[19] In the following discussion we will not consider two other experiments, 4D HN(CA)NH[20] and 3D TROSY-(H)N(CA)NH[21], since their performance is in general inferior to the experiments passing through CO.

5D HN(CA)CONH provides two kinds of correlations: interresidue H(i-1)N(i-1)CO(i-1)N(i)H(i), and intraresidue H(i)N(i)CO(i)N(i)H(i). 6D HNCOCANH performs the same coherence transfer but in the opposite direction, yielding interresidue H(i)N(i)CO(i-1)CA(i-1)N(i-1)H(i-1) and an intraresidue peak H(i)N(i)CO(i-1)CA(i-1)N(i)H(i). Since both experiment provide basically the same information, we will focus here on the analysis of the latter experiment only.

We will calculate stepwise the transfer function f for the entire experiment, using the original naming convention of experiment steps ("s", "t", "u", "v", "w"):[19]

1) $H^{N(i)}_z \rightarrow 2H^{N(i)}_zN^{(i)}_z$ (up to point "s"):

$$f_1 = \sin(\pi J_{NH}\tau_1)\exp(-\overline{R}_{2,H}\tau_1)$$

where $\overline{R}_{2,H}$ is the average relaxation rate of the in-phase ($H^{\pm}$) and anti-phase SQ coherences ($2H^{\pm}N_z$). The optimal transfer is obtained for $\tau_1 = (\pi J_{NH})^{-1}\mathrm{atan}\left(\pi J_{NH}/\overline{R}_{2,H}\right)$ and equals to:

$$f_1^{max} = \frac{\eta}{\sqrt{1+\eta^2}}\exp\left(-\frac{1}{\eta}\mathrm{atan}\,\eta\right)$$

where $\eta = \pi J_{NH}/\overline{R}_{2,H}$.

2) $2H^{N(i)}_zN^{(i)}_z \rightarrow 2N^{(i)}_zC'^{(i-1)}_z$ (from point "s" to "t"):

$$f_2 = \sin\left(\pi\,{}^1J_{NC'}\tau_2\right)\sin(\pi J_{NH}\tau_{NH})\exp(-R_{2,N}\tau_2)$$

where $R_{2,N}$ is the relaxation rate of the in-phase SQ ^{15}N coherence ($N^{\pm}$). The optimal delay τ_{NH}=$(2J_{NH})^{-1}$ and $\tau_2 = \left(\pi\,{}^1J_{NC'}\right)^{-1}\mathrm{atan}\left(\pi\,{}^1J_{NC'}/R_{2,N}\right)$, hence the optimal transfer is:

$$f_2^{max} = \frac{\zeta}{\sqrt{1+\zeta^2}}\exp\left(-\frac{1}{\zeta}\mathrm{atan}\,\zeta\right)$$

where $\zeta = \pi\,{}^1J_{NC'}/R_{2,N}$.

3) $2N^{(i)}_zC'^{(i-1)}_z \rightarrow 4N^{(i)}_zC'^{(i-1)}_zC^{\alpha(i-1)}_z$ (from point "t" to "u"):

$$f_2 = \sin\left(\pi\,{}^1J_{C\alpha C'}\tau_3\right)\exp(-R_{2,C'}\tau_3)$$

where $R_{2,C'}$ is the relaxation rate of the in-phase SQ $^{13}C'$ coherence. The optimal transfer is obtained for $\tau_3 = \left(\pi\,{}^1J_{C\alpha C'}\right)^{-1}\mathrm{atan}\left(\pi\,{}^1J_{C\alpha C'}/R_{2,C'}\right)$ and equals to:

$$f_3^{max} = \frac{\kappa}{\sqrt{1+\kappa^2}}\exp\left(-\frac{1}{\kappa}\mathrm{atan}\,\kappa\right)$$

where $\kappa = \pi\,{}^1J_{C\alpha C'}/R_{2,C'}$.

4) $4N^{(i)}_zC'^{(i-1)}_zC^{\alpha(i-1)}_z \rightarrow 2N^{(i-1)}_zC^{\alpha(i-1)}_z$ or $2N^{(i)}_zC^{\alpha(i-1)}_z$ (from point "u" to "v"): the active evolution of both 2- and 1-bond N–C^{α} couplings leads to splitting of the coherence pathway into the desired inter- and undesired intraresidue correlations ("back transfer"). Their transfer functions are as follows:

$$f_{4inter} = \sin\left(\pi\,{}^2J_{NC\alpha}\tau_4\right)\sin\left(\pi\,{}^1J_{NC\alpha}\tau_4\right)\cos\left(\pi\,{}^1J_{C\alpha C\beta}\tau_4\right)\sin\left(\pi\,{}^1J_{C\alpha C'}\tau_{C\alpha C'}\right)\exp(-R_{2,C\alpha}\tau_4)$$

$$f_{4intra} = \cos\left(\pi\,{}^2J_{NC\alpha}\tau_4\right)\cos\left(\pi\,{}^1J_{NC\alpha}\tau_4\right)\cos\left(\pi\,{}^1J_{C\alpha C\beta}\tau_4\right)\sin\left(\pi\,{}^1J_{C\alpha C'}\tau_{C\alpha C'}\right)\exp(-R_{2,C\alpha}\tau_4)$$

with the exception of *i-1* glycine residues where C^{α} does not dephase due to ${}^1J_{C\alpha C\beta}$. The middle term $\cos\left(\pi\,{}^1J_{C\alpha C\beta}\tau_4\right)$ can be omitted for all *i-1* residues except TSNDLFY, for which the evolution under ${}^1J_{C\alpha C\beta}$ can be eliminated with a suitably selective C^{α} refocusing pulse. $R_{2,C\alpha}$ stands for relaxation rate of SQ C^{α} coherence, and delay $\tau_{C\alpha C'}$=$\left(2\,{}^1J_{C\alpha C'}\right)^{-1}$ can be exactly matched to maximize the transfer. The best choice of the delay τ_4 depends on whether the back transfer is suppressed (as in the original implementation of Fiorito et al) or not. In such a case, $\tau_4 \approx 2(J_{\alpha\beta})^{-1}$ approximately maximizes the $\sin\left(\pi\,{}^2J_{NC\alpha}\tau_4\right)\sin\left(\pi\,{}^1J_{NC\alpha}\tau_4\right)$ while suppressing the $\cos\left(\pi\,{}^2J_{NC\alpha}\tau_4\right)\cos\left(\pi\,{}^1J_{NC\alpha}\tau_4\right)$ term, due to coincidence of coupling constant values. The best value of τ_4 can be found only numerically, e.g. by iterating the formula:

$$\tau_{4,i+1} = \frac{k}{J_{\alpha\beta}} + \frac{1}{\pi^{\alpha\beta}_J}\mathrm{atan}\left(\frac{1}{\pi J_{\alpha\beta}}\left(\frac{\pi\,{}^1J_{NC\alpha}}{\tan\left(\pi\,{}^1J_{NC\alpha}\tau_{4,i}\right)} + \frac{\pi\,{}^2J_{NC\alpha}}{\tan\left(\pi\,{}^2J_{NC\alpha}\tau_{4,i}\right)} - R_{2,C\alpha}\right)\right)$$ with k = 2.

For relaxation rates above $R_{2,C\alpha} \approx 30\ \mathrm{Hz}$ the optimum transfer of intraresidue coherences occurs at lower value of $\tau_4 \approx (J_{\alpha\beta})^{-1}$, and the precise value can be found with a numerical scheme as above but

with *k* = 1. At this condition the intraresidue coherences are not suppressed. For residues for which the evolution under ${}^1J_{C\alpha C\beta}$ is absent or nearly suppressed, the approximate optimum value of f_{4inter} is found at delay $\tau_4 = 2R_{2,C\alpha}\xi^{-1}\,\mathrm{atan}\,\xi$, where $\xi = \pi\left({}^2J_{NC\alpha} + {}^1J_{NC\alpha}\right)/R_{2,C\alpha}$, and the precise one can be found using an iterative scheme:

$$\tau_{4,i+1} = \frac{1}{\pi\,{}^1J_{NC\alpha}}\mathrm{atan}\left(\frac{\pi\,{}^2J_{NC\alpha}}{R_{2,C\alpha}}\frac{\tan\left(\pi\,{}^1J_{NC\alpha}\tau_{4,i}\right)}{\tan\left(\pi\,{}^2J_{NC\alpha}\tau_{4,i}\right)} + \frac{\pi\,{}^1J_{NC\alpha}}{R_{2,C\alpha}}\right)$$

In practice, one rather chooses the best condition for residues TSNDLFY (affected by ${}^1J_{C\alpha C\beta}$) since the best transfer conditions for other residues are significantly broader.

5) $2N^{(i-1)}{}_zC^{\alpha(i-1)}{}_z \rightarrow 2H^{N(i-1)}{}_zN^{(i-1)}{}_z$ or $2N^{(i)}{}_zC^{\alpha(i-1)}{}_z \rightarrow 2H^{N(i)}{}_zN^{(i)}{}_z$ (from point "v" to "w"):

$$f_{5inter} = \sin\left(\pi\,{}^1J_{NC\alpha}\tau_5\right)\cos\left(\pi\,{}^2J_{NC\alpha}\tau_5\right)\sin(\pi J_{NH}\tau_{NH})\exp\left(-R_{2,N}\tau_5\right)$$

$$f_{5intra} = \sin\left(\pi\,{}^2J_{NC\alpha}\tau_5\right)\cos\left(\pi\,{}^1J_{NC\alpha}\tau_5\right)\sin(\pi J_{NH}\tau_{NH})\exp\left(-R_{2,N}\tau_5\right)$$

Here again two- and one-bond N–C$^\alpha$ couplings are in competition, and the optimal delay τ_5 cannot be derived in the analytic form. However, since the magnitude of these couplings is similar, one usually approximates $\sin\left(\pi\,{}^1J_{NC\alpha}\tau_5\right)\cos\left(\pi\,{}^2J_{NC\alpha}\tau_5\right) \approx \frac{1}{2}\sin\left(\pi\left({}^2J_{NC\alpha} + {}^1J_{NC\alpha}\right)\tau_5\right)$. Effectively, the optimal transfer is given by the formula:

$$f_5^{max} = \frac{\lambda}{2\sqrt{1+\lambda^2}}\exp\left(-\frac{1}{\lambda}\mathrm{atan}\,\lambda\right)$$

where $\lambda = \pi\left({}^2J_{NC\alpha} + {}^1J_{NC\alpha}\right)/R_{2,N}$ for both inter- and intraresidue correlations. The precise value of optimal delay for intraresidue transfer function can be found using a series:

$$\tau_{5,i+1} = \frac{1}{\pi\,{}^1J_{NC\alpha}}\mathrm{atan}\left(\frac{\pi\,{}^1J_{NC\alpha}}{R_{2,N} + \pi\,{}^2J_{NC\alpha}\tan\left(\pi\,{}^2J_{NC\alpha}\tau_{5,i}\right)}\right)$$

6) $2H^{N(i-1)}{}_zN^{(i-1)}{}_z \rightarrow H^{N(i-1)}{}_{x/y}$ or $2H^{N(i)}{}_zN^{(i)}{}_z \rightarrow H^{N(i)}{}_{x/y}$ (from point "w" to acquisition): Fiorito et al implemented this transfer as a simple INEPT, however, it is fair to assume that the sensitivity-enhancement scheme (as used by Kazimierczuk et al) can be used to optimize the transfer, at least for proteins with MW up to ~25 kDa. The transfer function is roughly approximated by an average of efficiency of two transfer pathways:

$$f_6 = \frac{\sqrt{2}}{2}\left(f_{MQ,SQ} + f_{SQ,z}\right)$$

$$f_{MQ,SQ} = \exp\left(-R_{2MQ}\tau_{6a}\right)\exp\left(-\overline{R}_{2,H}\tau_{6b}\right)\sin(\pi J_{NH}\tau_{6b})$$

$$f_{SQ,z} = \exp\left(-\overline{R}_{2,N}\tau_{6a}\right)\exp\left(-R_{1,H}\tau_{6b}\right)\sin(\pi J_{NH}\tau_{6a})$$

where R_{2MQ} stands for relaxation rate of $2H_yN_x$ term, i.e. a mixture of ZQ and DQ H–N coherences. The factor $\sqrt{2}$ is not detectable in a 1D measurement, but was introduced here to reflect a higher efficiency of the multidimensional experiment.

For the TROSY version of the experiment (on a deuterated protein, protonated only at labile amide proton sites), the above considerations remain valid, but the relaxation rate of $^{15}N^{\pm}H\beta$ (TROSY) coherence is used at steps 2 and 5. Also, ST2-ST2 TROSY detection block (analogous to step 6 above) is characterized by the following transfer function:

$$f_6^{TR} = \frac{\sqrt{2}}{2}\cdot\frac{1}{4}\left(f_{SQ,SQ} + f_{MQ,MQ} + f_{SQ,MQ} + f_{MQ,SQ}\right)$$

Here again the factor $\sqrt{2}$ accounts for the increased sensitivity in multidimensional experiment due to preservation of equivalent pathways after ^{15}N evolution ($^{15}N^{\pm}$), the factor ½ reflects the loss due to selection of TROSY coherence, and the factor ¼ which averages four pathways necessary to transfer TROSY coherence, for which the efficiencies are as follows:

$$f_{SQ,SQ} = \exp\left(-\overline{R}_{2,N}\tau_{6a}\right)\exp\left(-\overline{R}_{2,H}\tau_{6b}\right)$$

$$f_{MQ,MQ} = \exp\left(-R_{2MQ}\tau_{6a}\right)\exp\left(-R_{2MQ}\tau_{6b}\right)$$
$$f_{SQ,MQ} = \exp\left(-\overline{R}_{2,N}\tau_{6a}\right)\exp\left(-R_{2MQ}\tau_{6b}\right)$$
$$f_{MQ,SQ} = \exp\left(-R_{2MQ}\tau_{6a}\right)\exp\left(-\overline{R}_{2,H}\tau_{6b}\right)$$

For simplicity, we assume here the relaxation unbalanced implementation of ST2ST2, where $\tau_{6a} = \tau_{6b} = (2J_{NH})^{-1}$.

The following values of scalar coupling were used: ${}^{1}J_{NH} = 93$ Hz, ${}^{1}J_{C\alpha N} = 11$ Hz, ${}^{2}J_{C\alpha N} = 7$ Hz, ${}^{1}J_{NC'} = 15$ Hz, ${}^{1}J_{C\alpha C'} = 55$ Hz, ${}^{1}J_{C\alpha C\beta} = 35$ Hz.

Additionally, we took into account the nonideal response of coherences to RF pulses. The implementation of Fiorito et al incorporates 10 refocussing, 13 inversion and 15 excitation or flip-back pulses. We assumed a realistic 0.99 performance of each π/2 pulse, 0.98 for inversion, and 0.95 for refocussing pulses, giving rise to RF pulse factor, f_{RF}.

Overall, the experiment sensitivity in the liquid-state (LS) for the interresidue pathway is the following product:
$$f_{LS} = f_{RF}f_1^{max}f_2^{max}f_3^{max}f_{4inter}^{max}f_5^{max}f_6^{max}$$

We assumed (i) isotropic tumbling of a spherical rotor, and (ii) aqueous solution with viscosity at T=300 K, thus approximately a correlation time of $\tau_C \approx q\,MW$ where q = 1 ns / 2.6 kDa, and spectral density in a form: $J(\omega) = \frac{\tau_C}{1+\omega^2\tau_C{}^2}$. The transverse relaxation rates of coherences relevant for the current discussion were:

a) amide proton SQ coherence relaxation rate, $\overline{R}_{2,H}$, with a contribution of dipolar interactions to nearby proton, nitrogen and carbon (and, optionally, deuterium) spins, and CSA of $\Delta\sigma_H = -16$ ppm:
$$\overline{R}_{2,H} = \frac{1}{2}\left(R_2^{NH}(H) + R_2^{NH}(2HN_z) + R_1^{CSA}(N)\right) + R_2^{HH}(H) + R_2^{CH}(H) + R_2^{DH}(H) + R_2^{CSA}(H)$$

b) amide nitrogen SQ coherence relaxation rate, $\overline{R}_{2,N}$, with a contribution of dipolar interactions only to nearby proton and carbon spins, and CSA of $\Delta\sigma_N = 164.5$ ppm:
$$\overline{R}_{2,N} = \frac{1}{2}\left(R_2^{NH}(N) + R_2^{NH}(2NH_z) + R_1^{HH}(H) + R_1^{CSA}(H)\right) + R_2^{NC}(N) + R_2^{CSA}(N)$$
and, for purely in-phase (^{1}H-decoupled) SQ coherence:
$$R_{2,N} = R_2^{NH}(N) + R_2^{NC}(N) + R_2^{CSA}(N)$$

c) carbonyl carbon SQ coherence relaxation rate, $R_{2,C'}$, with a contribution of dipolar interactions to nearby proton, nitrogen and carbon (and, optionally, deuterium) spins, and CSA of $\Delta\sigma_C = -119.5$ ppm:
$$R_{2,C'} = R_2^{C'H}(C') + R_2^{C'N}(C') + R_2^{C'C}(C') + R_2^{C'D}(C') + R_2^{CSA}(C')$$

d) alpha carbon SQ coherence relaxation rate, $R_{2,C\alpha}$, with a contribution of dipolar interactions to nearby proton, nitrogen and carbon (and, optionally, deuterium) spins, and CSA of $\Delta\sigma_{CA} = 25$ ppm:
$$R_{2,C\alpha} = R_2^{C\alpha H}(C\alpha) + R_2^{C\alpha N}(C\alpha) + R_2^{C\alpha C}(C\alpha) + R_2^{C'D}(C') + R_2^{CSA}(C\alpha)$$

e) amide proton-nitrogen interconverted zero- and double quantum coherence relaxation rate, with a contribution of dipolar interactions to remote proton and carbon (and, optionally, deuterium) spins:
$$R_{2MQ} = \frac{1}{2}\left(R_2^{NH}\left(N^{\pm}H^{\mp}\right) + R_2^{NH}(N^{\pm}H^{\pm})\right) + R_2^{HH}(H) + R_2^{DH}(H) + R_2^{CH}(H) + R_2^{CSA}(H) + R_2^{CSA}(N)$$

f) amide-proton and amide-nitrogen TROSY coherence relaxation rates:
$$R_{2,H(N\beta)} = \overline{R}_{2,H} + \eta_{xy}^{NH,H}(H)$$
$$R_{2,N(H\beta)} = \overline{R}_{2,N} + \eta_{xy}^{NH,N}(N)$$

The respective terms were calculated according to well-known formulas for dipolar relaxation:[22]

$$R_2^{NH}(H) = \frac{d_{NH}^2}{20}\left(4J(0) + J(\omega_H - \omega_N) + 3J(\omega_H) + 6J(\omega_N) + 6J(\omega_H + \omega_N)\right)$$
$$R_2^{NH}(2HN_z) = \frac{d_{NH}^2}{20}\left(4J(0) + J(\omega_H - \omega_N) + 3J(\omega_H) + 6J(\omega_H + \omega_N)\right)$$

$$R_2^{HH}(H) = \frac{1}{20}\sum_{K\neq H} d_{HK}^2\left(5J(0) + 9J(\omega_H) + 6J(2\omega_H)\right)$$
$$R_1^{HH}(H) = \frac{1}{10}\sum_{K\neq H} d_{HK}^2\left(J(0) + 3J(\omega_H) + 6J(2\omega_H)\right)$$
$$R_2^{CH}(H) = \frac{1}{20}\sum_{C} d_{HC}^2\left(4J(0) + J(\omega_H - \omega_C) + 3J(\omega_H) + 6J(\omega_C) + 6J(\omega_H + \omega_C)\right)$$
$$R_2^{DH}(H) = \frac{1}{20}\sum_{D} d_{HD}^2\left(4J(0) + J(\omega_H - \omega_D) + 3J(\omega_H) + 6J(\omega_D) + 6J(\omega_H + \omega_D)\right)$$
$$R_2^{NH}(N) = \frac{1}{20}\left(d_{NH}^2 + \sum_{K} d_{NK}^2\right)\left(4J(0) + J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H) + 6J(\omega_H + \omega_N)\right)$$
$$R_2^{NH}(2NH_z) = R_2^{NH}(N) - \frac{d_{NH}^2}{20}6J(\omega_H)$$
$$R_2^{NC}(N) = \frac{1}{20}\sum_{C} d_{CN}^2\left(4J(0) + J(\omega_C - \omega_N) + 3J(\omega_N) + 6J(\omega_C) + 6J(\omega_C + \omega_N)\right)$$
$$R_2^{NH}\left(N^{\pm}H^{\mp}\right) = \frac{1}{20}\left(2J(\omega_H - \omega_N) + 3J(\omega_H) + 3J(\omega_N)\right)$$
$$R_2^{NH}(N^{\pm}H^{\pm}) = \frac{1}{20}\left(12J(\omega_H + \omega_N) + 3J(\omega_H) + 3J(\omega_N)\right)$$
$$R_2^{C`H}(C') = \frac{1}{20}\left(d_{C`H}^2 + \sum_{K} d_{C`K}^2\right)\left(4J + J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H) + 6J(\omega_H + \omega_C)\right)$$
$$R_2^{C`N}(C') = \frac{d_{NC`}^2}{20}\left(4J(0) + J(\omega_C - \omega_N) + 3J(\omega_C) + 6J(\omega_N) + 6J(\omega_C + \omega_N)\right)$$
$$R_2^{C`C}(C') = \frac{1}{20}\sum_{C\neq C`} d_{CC`}^2\left(5J(0) + 9J(\omega_C) + 6J(2\omega_C)\right)$$
$$R_2^{C`D}(C') = \frac{1}{20}\sum_{D} d_{C`D}^2\left(4J(0) + J(\omega_C - \omega_D) + 3J(\omega_C) + 6J(\omega_D) + 6J(\omega_C + \omega_D)\right)$$

(the last four equations can readily be transformed for $C\alpha$ by a substitution of $C\alpha$ and $C`$). In the above equations "H" refers exclusively to the amide proton, "K" stands for remote (aliphatic) protons, and dipolar constant for spins *I* and *S*, d_{IS} is given by formula:

$$d_{IS} = \frac{\mu_0 \hbar \gamma_I \gamma_S}{4\pi\ r_{IS}^3}$$

For the dipolar proton-proton interactions, we assumed a distance cut-off of 5 Å, and included the following contacts, depending on the kind of secondary structure: d($^1H^N$, $^1H^N$ *i+1*) = 4.3 Å, d($^1H^N$, $^1H^N$ *i–1*) = 4.3 Å, d($^1H^N$, $^1H^N$ cross-strand) = 4.3 Å, d($^1H^N$, $^1H\alpha$) = 2.9 Å, d($^1H^N$, $^1H\alpha$ i–1) = 2.2 Å, d($^1H^N$, $^1H\alpha$ cross-strand) = 3.9 Å, d($^1H^N$, $^1H\beta_2$) = 2.5 Å, d($^1H^N$, $^1H\beta_3$) = 2.5 Å in the β-sheets, and the following ones in α-helices: d($^1H^N$, $^1H^N$ *i+1*) = 2.8 Å, d($^1H^N$, $^1H^N$ *i–1*) = 2.8 Å, d($^1H^N$, $^1H^N$ *i+2*) = 4.2 Å, d($^1H^N$, $^1H^N$ *i–2*) = 4.2 Å, d($^1H^N$, $^1H^N$ *i+3*) = 4.8 Å, d($^1H^N$, $^1H^N$ *i–3*) = 4.8 Å, d($^1H^N$, $^1H\alpha$) = 2.9 Å, d($^1H^N$, $^1H\alpha$ i+1) = 3.5 Å, d($^1H^N$, $^1H\alpha$ i–1) = 3.5 Å, d($^1H^N$, $^1H\alpha$ i+2) = 4.4 Å, d($^1H^N$, $^1H\alpha$ i–2) = 4.4 Å, d($^1H^N$, $^1H\alpha$ i+3) = 4.4 Å, d($^1H^N$, $^1H\alpha$ i–3) = 4.4 Å, d($^1H^N$, $^1H\alpha$ i+4) = 4.4 Å, d($^1H^N$, $^1H\alpha$ i–4) = 4.4 Å, d($^1H^N$, $^1H\beta_2$) = 2.5 Å, d($^1H^N$, $^1H\beta_3$) = 2.5 Å, d($^1H^N$, $^1H\beta_2$ *i+1*) = 3.0 Å, d($^1H^N$, $^1H\beta_3$ *i+1*) = 3.0 Å. For heteronuclei, we took into account the following spatial proximities: d(^{15}N, $^1H^N$) = 1.02 Å, d($^{13}C\alpha$, $^1H\alpha$) = 1.09 Å, d($^{13}C\alpha$, $^1H^N$ *i*) = 2.1 Å, d($^{13}C\alpha$, $^1H^N$ *i+1*) = 2.5 Å, d($^{13}C\alpha$, $^1H\beta_2$) = 2.1 Å, d($^{13}C\alpha$, $^1H\beta_3$) = 2.1 Å, d(^{15}N, $^{13}C\alpha$) = 1.47 Å, d(^{15}N, $^{13}C'$) = 1.32 Å, d($^{13}C'$, $^{13}C\alpha$) = 1.54 Å, d($^{13}C\boldsymbol{\beta}$, $^{13}C\alpha$) = 1.54 Å, d($^{13}C'$, $^1H^N$) = 2.8 Å, d($^{13}C'$, $^1H\alpha$) = 2.2 Å, and additionally, in beta-sheets: d($^{13}C'$, $^1H^N$ *i+1*) = 2.0 Å, d($^{13}C'$, $^1H^N$ *cross-strand*) = 3.0 Å, d($^{13}C'$, $^1H\alpha$ *i+1*) = 2.5 Å, d($^{13}C'$, $^1H\alpha$ *i–1*) = 4.6 Å, d($^{13}C'$, $^1H\alpha$ *cross-strand*) = 3.0 Å. In the case of a deuterated protein, a 100% replacement of side-chain protons ("K" indices) to deuterons was assumed. The relaxation rates were averaged equally between α-helices and β-sheets.

The contributions originating from CSA mechanism are as follows:

$$R_2^{CSA}(I) = \frac{1}{45}(\Delta\sigma_I\omega_I)^2(4J(0) + 3J(\omega_I))$$

$$R_1^{CSA}(I) = \frac{2}{15}(\Delta\sigma_I\omega_I)^2 J(\omega_I)$$

where *I* = H, N, Cα, or C', and $\Delta\sigma_I$ stands for the difference of axial and perpendicular principal components of the axially symmetric chemical shift tensor of spin *I*.

The interference terms crucial for the favorable relaxation of amide H-N TROSY coherences are:

$$\eta_{xy}^{NH,H}(H) = -(3\cos(\Theta)^2 - 1)\frac{d_{NH}}{2\sqrt{5}} \cdot \frac{\Delta\sigma_H\omega_H}{5\sqrt{3}}(4J(0) + 3J(\omega_H))$$

$$\eta_{xy}^{NH,N}(N) = -(3\cos(\Theta)^2 - 1)\frac{d_{NH}}{2\sqrt{5}} \cdot \frac{\Delta\sigma_N\omega_N}{5\sqrt{3}}(4J(0) + 3J(\omega_N))$$

where $\Theta = 17°$ is the assumed angle between the N–H vector and the principal axis of CSA (H or N) tensor.

We neglected here the effects of chemical exchange (with solvent protons) and internal dynamics.

In solids coherence transfer efficiencies do not exhibit any dependence on molecular weight. We assumed the following performance of four dipolar-based (CP) transfers, according to values reported in the literature for microcrystalline proteins at fast MAS frequencies (90-100 kHz)[17, 23]:

$$f_1(CP: H \rightarrow N) = 0.9$$
$$f_2(CP: N \rightarrow C') = 0.57$$
$$f_4(CP: C\alpha \rightarrow N) = 0.39$$
$$f_5(CP: N \rightarrow H) = 0.58$$

The efficiency of in-phase ^{13}C' to in-phase ^{13}Cα transfer, f_3, which is here implemented as INEPT, depends on the ^{13}C' and ^{13}Cα coherence lifetimes. As a representative case we assumed *Acinetobacter phage* coat protein 205 for which the following values were reported in the literature,[17, 24] as a function of the spinning rate and level of deuteration:

Labelling	MAS rate, kHz	Refocussed coherence lifetime, ms	
		^{13}C'	^{13}Cα
U-^{1}H^{N},^{2}H, ^{13}C, ^{15}N	60	40	25
U-^{1}H, ^{13}C, ^{15}N	60	26	8.6
U-^{1}H, ^{13}C, ^{15}N	100	27	22

The performance obtained for a deuterated protein spun at 60 kHz, and a fully protonated protein at 100 kHz MAS was virtually identical, and was thus reported in Figure 1 simply as "MAS NMR". Similarly to the solution NMR case, we accounted for the limited performance of RF pulses (5 excitation/flip-back, 2 refocussing and 6 relevant inversion pulses). The total transfer function for the solid-state NMR case was calculated as:

$$f_{ssNMR} = f_{RF} f_1 f_2 f_3 f_4 f_5$$

F. Fourier data processing and GAPRO parameters

The orthogonal projections are Fourier transformed as the conventional data (details can be found in Table S6). The skewed projections are acquired with a total number of 2^{K+1} quadrature components where *K* is the number of projection angles that are neither 0 nor 90 degrees ("non-orthogonal"). Such data is treated in the following way: the components are collected in the 2^K groups of two, Fourier transformed and stored separately in the interim locations. Subsequently, their linear 2^K combinations (either sums or differences) are calculated and stored as final projections. These projections differ by the sign of the (scaled) chemical shift offsets of the co-evolved shifts, which is taken into account by GAPRO. Data splitting, regrouping and final linear combinations are performed by Topspin AU programs transparently for the user.

Table S6: Fourier processing parameters of the 2D projections

Sample [a]	Direct dimension		Indirect dimension	
	N [b]	Window [c]	N [b]	Window [c]

GB1	8192	QSINE 3	1024	QSINE 3
SOD	8192	QSINE 3	1024	QSINE 3
$\beta2M_{D76N}$	8192	QSINE 4	1024	QSINE 3

[a] Identical processing parameters were used for forward and backward 5D experiments

[b] Number of points in the real part of the Fourier Transformed signal

[c] The window function in the time domain: QSINE*n* – a squared, shifted sine bell function $w(t) = \sin(\pi(1 - 1/n)(t / t_{max}) + \pi/n)^2$ (where *n* = 3 and 4 for QSINE3 and QSINE4, respectively)

Table S7: GAPRO peak picking and geometric analysis parameters

Sample [a]	$S_{min1,2}$ [b]	$\Delta\nu_{min}$ [c] Hz	r_{min} [d] Hz	S/N [e]	^{1}H range, ppm
GB1	7	50	40	6	[5.6, 11.7]
SOD	7	100	80	6	[5.4, 10.9]
$\beta2M_{D76N}$	6	160	60	6	[6.9, 12.4]

[a] Identical parameters were used for forward and backward 5D experiments

[b] Minimal support = the number of projections confirming a 5D peak coordinates

[c] Peak matching tolerance in the directly detected dimension

[d] Peak matching tolerance in the indirectly detected dimensions

[e] Minimum signal-to-noise ratio of a peak

G. 5D peak lists for GB1, SOD and $\beta2m_{D76N}$

Tables S8-13 show the FLYA output peak list with automated assignment encoded in the last 6 columns. In the FLYA input files these columns contain zeros. They were directly copied from APSY experiment data folders, and completed with a respective header to allow the proper interpretation of columns by FLYA.

Table S8: Peak list from (H)NCACONH APSY experiment on GB1

```
# Number of dimensions 5
#FORMAT xeasy5D
#INAME 1 M
#INAME 2 A
#INAME 3 O
#INAME 4 N
#INAME 5 H
#SPECTRUM hNCACONH M A O N H
   1 118.574  57.264 179.310 121.493   9.169 1 U   5.500E+00  0.000E+00 a 0 N.35    CA.35    C.35    N.36    H.36 #MAP    35
   2 119.040  53.939 178.949 104.311   7.173 1 U   5.500E+00  0.000E+00 a 0 N.48    CA.48    C.48    N.49    H.49 #MAP    48
   3 121.473  56.001 175.749 115.192   7.437 1 U   5.500E+00  0.000E+00 a 0 N.36    CA.36    C.36    N.37    H.37 #MAP    36
   4 117.660  60.452 178.483 118.937   7.652 1 U   5.500E+00  0.000E+00 a 0 N.28    CA.28    C.28    N.29    H.29 #MAP    28
   5 123.477  54.600 179.685 121.003   8.253 1 U   5.500E+00  0.000E+00 a 0 N.23    CA.23    C.23    N.24    H.24 #MAP    23
   6 108.461  46.905 173.811 122.099   8.368 1 U   5.500E+00  0.000E+00 a 0 N.38    CA.38    C.38    N.39    H.39 #MAP    38
   7 118.196  58.673 172.346 124.291   8.529 1 U   5.500E+00  0.000E+00 a 0 N.54    CA.54    C.54    N.55    H.55 #MAP    54
   8 122.768  56.172 179.354 118.584   8.577 1 U   5.500E+00  0.000E+00 a 0 N.34    CA.34    C.34    N.35    H.35 #MAP    34
   9 118.979  66.575 178.264 118.734   8.829 1 U   5.500E+00  0.000E+00 a 0 N.29    CA.29    C.29    N.30    H.30 #MAP    29
  10 121.408  53.933 173.975 115.465   9.013 1 U   5.500E+00  0.000E+00 a 0 N.15    CA.15    C.15    N.16    H.16 #MAP    15
  11 125.888  54.342 175.545 125.852   9.531 1 U   5.500E+00  0.000E+00 a 0 N.19    CA.19    C.19    N.20    H.20 #MAP    19
  12 109.971  44.673 172.976 121.323  10.048 1 U   5.500E+00  0.000E+00 a 0 N.9     CA.9     C.9     N.10    H.10 #MAP     9
  13 112.569  62.586 174.085 130.370  10.832 1 U   5.500E+00  0.000E+00 a 0 N.51    CA.51    C.51    N.52    H.52 #MAP    51
  14 115.161  53.624 173.987 108.497   8.066 1 U   5.500E+00  0.000E+00 a 0 N.37    CA.37    C.37    N.38    H.38 #MAP    37
  15 116.599  59.232 177.694 117.657   7.169 1 U   5.500E+00  0.000E+00 a 0 N.27    CA.27    C.27    N.28    H.28 #MAP    27
  16 122.084  61.888 174.888 131.415   9.208 1 U   5.500E+00  0.000E+00 a 0 N.39    CA.39    C.39    N.40    H.40 #MAP    39
  17 126.485  59.966 174.990 127.365   9.385 1 U   5.500E+00  0.000E+00 a 0 N.6     CA.6     C.6     N.7     H.7 #MAP     6
  18 108.949  61.003 173.763 118.966   9.422 1 U   5.500E+00  0.000E+00 a 0 N.44    CA.44    C.44    N.45    H.45 #MAP    44
  19 125.899  50.765 177.515 116.084   8.773 1 U   5.500E+00  0.000E+00 a 0 N.20    CA.20    C.20    N.21    H.21 #MAP    20
  20 116.573  61.471 170.984 125.880   7.959 1 U   5.500E+00  0.000E+00 a 0 N.18    CA.18    C.18    N.19    H.19 #MAP    18
  21 130.374  56.658 175.668 112.147   9.354 1 U   5.500E+00  0.000E+00 a 0 N.52    CA.52    C.52    N.53    H.53 #MAP    52
  22 116.009  63.673 174.863 115.664   7.445 1 U   5.500E+00  0.000E+00 a 0 N.21    CA.21    C.21    N.22    H.22 #MAP    21
  23 115.437  60.245 171.702 116.726   8.484 1 U   5.500E+00  0.000E+00 a 0 N.16    CA.16    C.16    N.17    H.17 #MAP    16
  24 108.687  45.185 172.589 119.624   8.748 1 U   5.500E+00  0.000E+00 a 0 N.41    CA.41    C.41    N.42    H.42 #MAP    41
  25 105.936  45.002 171.106 121.414   8.997 1 U   5.500E+00  0.000E+00 a 0 N.14    CA.14    C.14    N.15    H.15 #MAP    14
  26 118.974  57.937 171.691 126.731   7.565 1 U   5.500E+00  0.000E+00 a 0 N.45    CA.45    C.45    N.46    H.46 #MAP    45
  27 121.160  61.713 178.379 122.794   9.303 1 U   5.500E+00  0.000E+00 a 0 N.33    CA.33    C.33    N.34    H.34 #MAP    33
  28 124.266  61.396 173.906 131.230   8.035 1 U   5.500E+00  0.000E+00 a 0 N.55    CA.55    C.55    N.56    H.56 #MAP    55
  29 124.148  55.188 177.116 116.578   8.818 1 U   5.500E+00  0.000E+00 a 0 N.26    CA.26    C.26    N.27    H.27 #MAP    26
```

30 131.420 52.750 174.924 108.675 8.113 1 U 5.500E+00 0.000E+00 a 0 N.40 CA.40 C.40 N.41 H.41 #MAP 40
31 121.032 54.580 181.278 117.642 8.580 1 U 5.500E+00 0.000E+00 a 0 N.24 CA.24 C.24 N.25 H.25 #MAP 24
32 125.105 50.706 176.241 109.934 8.357 1 U 5.500E+00 0.000E+00 a 0 N.8 CA.8 C.8 N.9 H.9 #MAP 8
33 125.531 57.672 176.730 108.979 9.074 1 U 5.500E+00 0.000E+00 a 0 N.43 CA.43 C.43 N.44 H.44 #MAP 43
34 120.840 60.241 179.570 121.672 8.092 1 U 5.500E+00 0.000E+00 a 0 N.31 CA.31 C.31 N.32 H.32 #MAP 31
35 112.134 60.377 171.802 118.211 8.290 1 U 5.500E+00 0.000E+00 a 0 N.53 CA.53 C.53 N.54 H.54 #MAP 53
36 116.713 60.330 173.994 116.567 9.319 1 U 5.500E+00 0.000E+00 a 0 N.17 CA.17 C.17 N.18 H.18 #MAP 17
37 125.937 55.919 174.813 123.961 9.169 1 U 5.500E+00 0.000E+00 a 0 N.2 CA.2 C.2 N.3 H.3 #MAP 2
38 119.579 55.230 177.803 125.531 9.399 1 U 5.500E+00 0.000E+00 a 0 N.42 CA.42 C.42 N.43 H.43 #MAP 42
39 121.652 58.950 177.347 121.160 8.806 1 U 5.500E+00 0.000E+00 a 0 N.32 CA.32 C.32 N.33 H.33 #MAP 32
40 127.352 53.045 174.644 126.487 9.111 1 U 5.500E+00 0.000E+00 a 0 N.5 CA.5 C.5 N.6 H.6 #MAP 5
41 119.334 55.465 175.223 112.582 7.543 1 U 5.500E+00 0.000E+00 a 0 N.50 CA.50 C.50 N.51 H.51 #MAP 50
42 117.710 67.475 175.553 124.172 7.458 1 U 5.500E+00 0.000E+00 a 0 N.25 CA.25 C.25 N.26 H.26 #MAP 25
43 126.746 51.011 176.061 123.898 8.866 1 U 5.500E+00 0.000E+00 a 0 N.46 CA.46 C.46 N.47 H.47 #MAP 46
44 123.835 54.465 176.991 119.081 8.675 1 U 5.500E+00 0.000E+00 a 0 N.47 CA.47 C.47 N.48 H.48 #MAP 47
45 115.620 52.533 175.006 123.497 9.416 1 U 5.500E+00 0.000E+00 a 0 N.22 CA.22 C.22 N.23 H.23 #MAP 22
46 106.735 62.099 173.207 128.187 7.533 1 U 5.500E+00 0.000E+00 a 0 N.11 CA.11 C.11 N.12 H.12 #MAP 11
47 123.248 54.947 173.113 127.352 9.317 1 U 5.500E+00 0.000E+00 a 0 N.4 CA.4 C.4 N.5 H.5 #MAP 4
48 121.283 59.304 178.889 106.695 9.132 1 U 5.500E+00 0.000E+00 a 0 N.10 CA.10 C.10 N.11 H.11 #MAP 10
49 123.682 53.445 175.579 105.951 8.740 1 U 5.500E+00 0.000E+00 a 0 N.13 CA.13 C.13 N.14 H.14 #MAP 13
50 127.383 54.826 174.829 125.140 9.151 1 U 5.500E+00 0.000E+00 a 0 N.7 CA.7 C.7 N.8 H.8 #MAP 7
51 124.006 57.150 174.726 123.213 9.358 1 U 5.500E+00 0.000E+00 a 0 N.3 CA.3 C.3 N.4 H.4 #MAP 3
52 104.291 60.567 175.472 119.337 8.077 1 U 5.500E+00 0.000E+00 a 0 N.49 CA.49 C.49 N.50 H.50 #MAP 49
53 118.361 54.506 171.226 125.974 8.571 1 U 5.500E+00 0.000E+00 a 0 N.1 CA.1 C.1 N.2 H.2 #MAP 1
54 128.201 54.612 173.630 123.726 9.004 1 U 5.500E+00 0.000E+00 a 0 N.12 CA.12 C.12 N.13 H.13 #MAP 12

Table S9: Peak list from (H)NCOCANH APSY experiment on GB1

Number of dimensions 5
#FORMAT xeasy5D
#INAME 1 N
#INAME 2 O
#INAME 3 A
#INAME 4 M
#INAME 5 J
#SPECTRUM hNCOCANH N O A M J
1 121.460 179.344 57.305 118.587 8.561 1 U 5.500E+00 0.000E+00 a 0 N.36 C.35 CA.35 N.35 H.35 #MAP 34
2 116.007 177.541 50.845 125.856 9.520 1 U 5.500E+00 0.000E+00 a 0 N.21 C.20 CA.20 N.20 H.20 #MAP 19
3 127.336 175.029 60.017 126.488 9.095 1 U 5.500E+00 0.000E+00 a 0 N.7 C.6 CA.6 N.6 H.6 #MAP 5
4 118.689 178.300 66.607 118.981 7.644 1 U 5.500E+00 0.000E+00 a 0 N.30 C.29 CA.29 N.29 H.29 #MAP 28
5 125.860 175.590 54.415 125.897 7.944 1 U 5.500E+00 0.000E+00 a 0 N.20 C.19 CA.19 N.19 H.19 #MAP 18
6 119.590 172.631 45.242 108.664 8.094 1 U 5.500E+00 0.000E+00 a 0 N.42 C.41 CA.41 N.41 H.41 #MAP 40
7 117.645 181.319 54.644 121.012 8.238 1 U 5.500E+00 0.000E+00 a 0 N.25 C.24 CA.24 N.24 H.24 #MAP 23
8 124.251 172.390 58.744 118.227 8.277 1 U 5.500E+00 0.000E+00 a 0 N.55 C.54 CA.54 N.54 H.54 #MAP 53
9 121.395 171.123 45.071 105.957 8.722 1 U 5.500E+00 0.000E+00 a 0 N.15 C.14 CA.14 N.14 H.14 #MAP 13
10 115.445 173.990 53.993 121.416 8.978 1 U 5.500E+00 0.000E+00 a 0 N.16 C.15 CA.15 N.15 H.15 #MAP 14
11 109.929 176.299 50.765 125.148 9.134 1 U 5.500E+00 0.000E+00 a 0 N.9 C.8 CA.8 N.8 H.8 #MAP 7
12 112.087 175.694 56.680 130.373 10.815 1 U 5.500E+00 0.000E+00 a 0 N.53 C.52 CA.52 N.52 H.52 #MAP 51
13 108.467 174.022 53.680 115.180 7.421 1 U 5.500E+00 0.000E+00 a 0 N.38 C.37 CA.37 N.37 H.37 #MAP 36
14 115.152 175.768 56.053 121.517 9.153 1 U 5.500E+00 0.000E+00 a 0 N.37 C.36 CA.36 N.36 H.36 #MAP 35
15 118.988 178.531 60.523 117.664 7.157 1 U 5.500E+00 0.000E+00 a 0 N.29 C.28 CA.28 N.28 H.28 #MAP 27
16 122.784 178.421 61.768 121.137 8.787 1 U 5.500E+00 0.000E+00 a 0 N.34 C.33 CA.33 N.33 H.33 #MAP 32
17 118.555 179.391 56.226 122.813 9.291 1 U 5.500E+00 0.000E+00 a 0 N.35 C.34 CA.34 N.34 H.34 #MAP 33
18 121.282 172.988 44.753 109.948 8.345 1 U 5.500E+00 0.000E+00 a 0 N.10 C.9 CA.9 N.9 H.9 #MAP 8
19 116.573 174.001 60.398 116.729 8.467 1 U 5.500E+00 0.000E+00 a 0 N.18 C.17 CA.17 N.17 H.17 #MAP 16
20 121.151 177.380 58.996 121.683 8.067 1 U 5.500E+00 0.000E+00 a 0 N.33 C.32 CA.32 N.32 H.32 #MAP 31
21 104.302 179.014 53.991 119.101 8.659 1 U 5.500E+00 0.000E+00 a 0 N.49 C.48 CA.48 N.48 H.48 #MAP 47
22 116.575 177.170 55.240 124.160 7.446 1 U 5.500E+00 0.000E+00 a 0 N.27 C.26 CA.26 N.26 H.26 #MAP 25
23 123.952 174.841 55.989 125.943 8.554 1 U 5.500E+00 0.000E+00 a 0 N.3 C.2 CA.2 N.2 H.2 #MAP 1
24 125.098 174.845 54.846 127.362 9.369 1 U 5.500E+00 0.000E+00 a 0 N.8 C.7 CA.7 N.7 H.7 #MAP 6
25 130.342 174.119 62.639 112.603 7.524 1 U 5.500E+00 0.000E+00 a 0 N.52 C.51 CA.51 N.51 H.51 #MAP 50
26 131.401 174.937 61.942 122.119 8.358 1 U 5.500E+00 0.000E+00 a 0 N.40 C.39 CA.39 N.39 H.39 #MAP 38
27 124.165 175.633 67.528 117.624 8.556 1 U 5.500E+00 0.000E+00 a 0 N.26 C.25 CA.25 N.25 H.25 #MAP 24
28 125.519 177.821 55.309 119.629 8.735 1 U 5.500E+00 0.000E+00 a 0 N.43 C.42 CA.42 N.42 H.42 #MAP 41
29 126.743 171.746 57.969 118.956 9.404 1 U 5.500E+00 0.000E+00 a 0 N.46 C.45 CA.45 N.45 H.45 #MAP 44
30 112.539 175.255 55.514 119.331 8.053 1 U 5.500E+00 0.000E+00 a 0 N.51 C.50 CA.50 N.50 H.50 #MAP 49
31 118.155 171.828 60.407 112.142 9.340 1 U 5.500E+00 0.000E+00 a 0 N.54 C.53 CA.53 N.53 H.53 #MAP 52
32 123.469 175.049 52.596 115.632 7.426 1 U 5.500E+00 0.000E+00 a 0 N.23 C.22 CA.22 N.22 H.22 #MAP 21
33 118.917 173.793 61.051 108.987 9.061 1 U 5.500E+00 0.000E+00 a 0 N.45 C.44 CA.44 N.44 H.44 #MAP 43
34 106.723 178.968 59.344 121.352 10.038 1 U 5.500E+00 0.000E+00 a 0 N.11 C.10 CA.10 N.10 H.10 #MAP 9
35 116.705 171.736 60.291 115.467 9.001 1 U 5.500E+00 0.000E+00 a 0 N.17 C.16 CA.16 N.16 H.16 #MAP 15
36 125.815 171.022 61.530 116.576 9.306 1 U 5.500E+00 0.000E+00 a 0 N.19 C.18 CA.18 N.18 H.18 #MAP 17
37 131.181 173.940 61.446 124.299 8.515 1 U 5.500E+00 0.000E+00 a 0 N.56 C.55 CA.55 N.55 H.55 #MAP 54

38	117.643	177.711	59.252	116.594	8.812	1	U	5.500E+00	0.000E+00	a	0	N.28	C.27	CA.27	N.27	H.27 #MAP	26
39	108.664	174.926	52.818	131.398	9.193	1	U	5.500E+00	0.000E+00	a	0	N.41	C.40	CA.40	N.40	H.40 #MAP	39
40	115.631	174.869	63.711	116.100	8.766	1	U	5.500E+00	0.000E+00	a	0	N.22	C.21	CA.21	N.21	H.21 #MAP	20
41	119.017	177.050	54.537	123.892	8.848	1	U	5.500E+00	0.000E+00	a	0	N.48	C.47	CA.47	N.47	H.47 #MAP	46
42	128.220	173.242	62.123	106.683	9.120	1	U	5.500E+00	0.000E+00	a	0	N.12	C.11	CA.11	N.11	H.11 #MAP	10
43	123.842	176.099	51.062	126.753	7.544	1	U	5.500E+00	0.000E+00	a	0	N.47	C.46	CA.46	N.46	H.46 #MAP	45
44	127.331	173.163	55.028	123.346	9.335	1	U	5.500E+00	0.000E+00	a	0	N.5	C.4	CA.4	N.4	H.4 #MAP	3
45	108.945	176.782	57.736	125.547	9.383	1	U	5.500E+00	0.000E+00	a	0	N.44	C.43	CA.43	N.43	H.43 #MAP	42
46	120.986	179.744	54.644	123.491	9.404	1	U	5.500E+00	0.000E+00	a	0	N.24	C.23	CA.23	N.23	H.23 #MAP	22
47	119.279	175.507	60.585	104.318	7.157	1	U	5.500E+00	0.000E+00	a	0	N.50	C.49	CA.49	N.49	H.49 #MAP	48
48	105.907	175.596	53.495	123.715	8.999	1	U	5.500E+00	0.000E+00	a	0	N.14	C.13	CA.13	N.13	H.13 #MAP	12
49	122.079	173.851	46.954	108.497	8.054	1	U	5.500E+00	0.000E+00	a	0	N.39	C.38	CA.38	N.38	H.38 #MAP	37
50	126.430	174.699	53.080	127.415	9.303	1	U	5.500E+00	0.000E+00	a	0	N.6	C.5	CA.5	N.5	H.5 #MAP	4
51	120.771	178.950	57.623	118.780	8.816	1	U	5.500E+00	0.000E+00	a	0	N.31	C.30	CA.30	N.30	H.30 #MAP	29
52	123.243	174.728	57.139	124.012	9.164	1	U	5.500E+00	0.000E+00	a	0	N.4	C.3	CA.3	N.3	H.3 #MAP	2
53	121.471	179.604	60.293	120.888	9.163	1	U	5.500E+00	0.000E+00	a	0	N.32	C.31	CA.31	N.31	H.31 #MAP	30
54	123.640	173.681	54.587	128.156	7.515	1	U	5.500E+00	0.000E+00	a	0	N.13	C.12	CA.12	N.12	H.12 #MAP	11

Table S10: Peak list from (H)NCACONH APSY experiment on Cu^{I},Zn-SOD

```
# Number of dimensions 5
#FORMAT xeasy5D
#INAME 1 M
#INAME 2 A
#INAME 3 O
#INAME 4 N
#INAME 5 H
#SPECTRUM hNCACONH M A O N H
```

1	125.420	60.900	173.510	120.950	7.993	1	U	5.500000E+00	0.000000E+00	e	0	N.118	CA.118	C.118	N.119	H.119 #MAP	108
2	118.370	55.420	173.210	112.490	7.168	1	U	5.500000E+00	0.000000E+00	e	0	N.70	CA.70	C.70	N.71	H.71 #MAP	62
3	123.770	61.150	175.450	126.570	8.907	1	U	5.500000E+00	0.000000E+00	e	0	N.7	CA.7	C.7	N.8	H.8 #MAP	7
3	123.770	61.150	175.450	126.570	8.907	1	U	5.500000E+00	0.000000E+00	e	0	N.91	CA.91	C.91	N.92	H.92 #MAP	81
4	106.730	45.530	171.130	124.390	8.451	1	U	5.500000E+00	0.000000E+00	e	0	N.147	CA.147	C.147	N.148	H.148 #MAP	137
5	97.700	45.410	173.630	121.320	7.145	1	U	5.500000E+00	0.000000E+00	e	0	N.82	CA.82	C.82	N.83	H.83 #MAP	72
5	97.700	45.410	173.630	121.320	7.145	1	U	5.500000E+00	0.000000E+00	e	0	N.138	CA.138	C.138	N.139	H.139 #MAP	128
6	118.430	54.340	174.330	112.660	6.665	1	U	5.500000E+00	0.000000E+00	e	0	N.115	CA.115	C.115	N.116	H.116 #MAP	105
7	112.830	44.670	172.790	118.440	7.433	1	U	5.500000E+00	0.000000E+00	e	0	N.114	CA.114	C.114	N.115	H.115 #MAP	104
8	117.130	60.190	174.530	121.530	7.578	1	U	5.500000E+00	0.000000E+00	e	0	N.112	CA.112	C.112	N.113	H.113 #MAP	102
9	115.470	53.380	174.720	121.120	7.744	1	U	5.500000E+00	0.000000E+00	e	0	N.122	CA.122	C.122	N.123	H.123 #MAP	112
10	126.540	55.730	172.120	115.820	8.130	1	U	5.500000E+00	0.000000E+00	e	0	N.45	CA.45	C.45	N.46	H.46 #MAP	41
11	124.370	62.360	175.350	127.430	8.618	1	U	5.500000E+00	0.000000E+00	e	0	N.148	CA.148	C.148	N.149	H.149 #MAP	138
12	118.660	60.950	176.650	131.350	9.690	1	U	5.500000E+00	0.000000E+00	e	0	N.94	CA.94	C.94	N.95	H.95 #MAP	84
13	120.860	61.450	177.150	129.450	10.173	1	U	5.500000E+00	0.000000E+00	e	0	N.47	CA.47	C.47	N.48	H.48 #MAP	43
14	126.010	50.290	174.940	123.770	9.100	1	U	5.500000E+00	0.000000E+00	e	0	N.6	CA.6	C.6	N.7	H.7 #MAP	6
15	121.030	51.600	176.360	122.320	10.152	1	U	5.500000E+00	0.000000E+00	e	0	N.123	CA.123	C.123	N.124	H.124 #MAP	113
16	128.970	66.620	176.670	97.730	8.463	1	U	5.500000E+00	0.000000E+00	e	0	N.81	CA.81	C.81	N.82	H.82 #MAP	71
17	121.640	59.630	171.090	125.990	9.177	1	U	5.500000E+00	0.000000E+00	e	0	N.5	CA.5	C.5	N.6	H.6 #MAP	5
18	123.250	50.270	175.430	121.630	9.558	1	U	5.500000E+00	0.000000E+00	e	0	N.4	CA.4	C.4	N.5	H.5 #MAP	4
19	113.740	42.780	173.270	120.570	8.282	1	U	5.500000E+00	0.000000E+00	e	0	N.41	CA.41	C.41	N.42	H.42 #MAP	37
19	113.740	42.780	173.270	120.570	8.282	1	U	5.500000E+00	0.000000E+00	e	0	N.56	CA.56	C.56	N.57	H.57 #MAP	52
20	127.430	61.910	175.140	116.740	9.290	1	U	5.500000E+00	0.000000E+00	e	0	N.149	CA.149	C.149	N.150	H.150 #MAP	139
21	126.030	57.180	176.170	113.770	8.749	1	U	5.500000E+00	0.000000E+00	e	0	N.40	CA.40	C.40	N.41	H.41 #MAP	36
21	126.030	57.180	176.170	113.770	8.749	1	U	5.500000E+00	0.000000E+00	e	0	N.55	CA.55	C.55	N.56	H.56 #MAP	51
22	118.940	53.940	175.340	118.380	8.329	1	U	5.500000E+00	0.000000E+00	e	0	N.79	CA.79	C.79	N.80	H.80 #MAP	69
23	123.040	55.980	177.540	114.410	7.362	1	U	5.500000E+00	0.000000E+00	e	0	N.144	CA.144	C.144	N.145	H.145 #MAP	134
24	115.800	52.020	175.680	120.910	9.402	1	U	5.500000E+00	0.000000E+00	e	0	N.46	CA.46	C.46	N.47	H.47 #MAP	42
25	119.630	54.820	171.540	119.010	6.990	1	U	5.500000E+00	0.000000E+00	e	0	N.78	CA.78	C.78	N.79	H.79 #MAP	68
26	129.590	53.180	174.320	125.460	8.931	1	U	5.500000E+00	0.000000E+00	e	0	N.117	CA.117	C.117	N.118	H.118 #MAP	107
27	111.200	46.460	172.910	118.690	7.798	1	U	5.500000E+00	0.000000E+00	e	0	N.93	CA.93	C.93	N.94	H.94 #MAP	83
28	107.240	46.250	170.900	120.330	7.987	1	U	5.500000E+00	0.000000E+00	e	0	N.16	CA.16	C.16	N.17	H.17 #MAP	14
28	107.240	46.250	170.900	120.330	7.987	1	U	5.500000E+00	0.000000E+00	e	0	N.141	CA.141	C.141	N.142	H.142 #MAP	131
29	116.740	42.840	173.390	120.350	8.959	1	U	5.500000E+00	0.000000E+00	e	0	N.150	CA.150	C.150	N.151	H.151 #MAP	140
30	111.240	45.120	172.940	121.130	8.671	1	U	5.500000E+00	0.000000E+00	e	0	N.10	CA.10	C.10	N.11	H.11 #MAP	10
31	112.620	62.240	173.080	129.790	8.823	1	U	5.500000E+00	0.000000E+00	e	0	N.116	CA.116	C.116	N.117	H.117 #MAP	106
32	123.020	53.570	174.540	107.340	8.351	1	U	5.500000E+00	0.000000E+00	e	0	N.15	CA.15	C.15	N.16	H.16 #MAP	13
33	109.330	46.790	171.840	126.590	8.119	1	U	5.500000E+00	0.000000E+00	e	0	N.44	CA.44	C.44	N.45	H.45 #MAP	40
34	121.200	55.640	172.030	120.460	7.158	1	U	5.500000E+00	0.000000E+00	e	0	N.83	CA.83	C.83	N.84	H.84 #MAP	73
34	121.200	55.640	172.030	120.460	7.158	1	U	5.500000E+00	0.000000E+00	e	0	N.139	CA.139	C.139	N.140	H.140 #MAP	129
35	126.160	56.290	172.150	123.590	8.735	1	U	5.500000E+00	0.000000E+00	e	0	N.18	CA.18	C.18	N.19	H.19 #MAP	16
36	120.240	62.480	176.010	122.970	7.947	1	U	5.500000E+00	0.000000E+00	e	0	N.14	CA.14	C.14	N.15	H.15 #MAP	12
37	121.450	61.000	176.810	112.820	9.557	1	U	5.500000E+00	0.000000E+00	e	0	N.113	CA.113	C.113	N.114	H.114 #MAP	103
38	108.020	44.220	171.630	114.430	7.882	1	U	5.500000E+00	0.000000E+00	e	0	N.33	CA.33	C.33	N.34	H.34 #MAP	29
39	113.540	58.650	174.830	118.130	8.673	1	U	5.500000E+00	0.000000E+00	e	0	N.87	CA.87	C.87	N.88	H.88 #MAP	77

40 114.690 53.910 174.750 109.360 8.868 1 U 5.500000E+00 0.000000E+00 e 0 N.43 CA.43 C.43 N.44 H.44 #MAP 39
41 110.900 53.920 173.310 106.700 8.035 1 U 5.500000E+00 0.000000E+00 e 0 N.146 CA.146 C.146 N.147 H.147 #MAP 136
42 112.360 55.360 174.670 113.100 7.216 1 U 5.500000E+00 0.000000E+00 e 0 N.71 CA.71 C.71 N.72 H.72 #MAP 63
43 114.350 50.910 175.020 110.990 8.995 1 U 5.500000E+00 0.000000E+00 e 0 N.145 CA.145 C.145 N.146 H.146 #MAP 135
44 118.080 61.440 173.590 129.130 9.341 1 U 5.500000E+00 0.000000E+00 e 0 N.88 CA.88 C.88 N.89 H.89 #MAP 78
45 121.320 60.420 175.540 127.630 9.203 1 U 5.500000E+00 0.000000E+00 e 0 N.29 CA.29 C.29 N.30 H.30 #MAP 25
46 119.390 45.600 171.360 115.200 5.836 1 U 5.500000E+00 0.000000E+00 e 0 N.51 CA.51 C.51 N.52 H.52 #MAP 47
47 129.130 49.190 177.460 124.460 8.391 1 U 5.500000E+00 0.000000E+00 e 0 N.89 CA.89 C.89 N.90 H.90 #MAP 79
48 131.360 49.640 174.970 125.260 8.506 1 U 5.500000E+00 0.000000E+00 e 0 N.95 CA.95 C.95 N.96 H.96 #MAP 85
49 125.570 56.270 175.970 121.820 9.193 1 U 5.500000E+00 0.000000E+00 e 0 N.23 CA.23 C.23 N.24 H.24 #MAP 21
49 125.570 56.270 175.970 121.820 9.193 1 U 5.500000E+00 0.000000E+00 e 0 N.120 CA.120 C.120 N.121 H.121 #MAP 110
50 120.430 63.120 175.390 130.130 8.251 1 U 5.500000E+00 0.000000E+00 e 0 N.151 CA.151 C.151 N.152 H.152 #MAP 141
51 126.490 53.920 175.670 120.930 8.269 1 U 5.500000E+00 0.000000E+00 e 0 N.8 CA.8 C.8 N.9 H.9 #MAP 8
52 116.860 54.550 177.180 104.090 8.678 1 U 5.500000E+00 0.000000E+00 e 0 N.126 CA.126 C.126 N.127 H.127 #MAP 116
53 113.080 44.020 171.830 105.470 8.745 1 U 5.500000E+00 0.000000E+00 e 0 N.72 CA.72 C.72 N.73 H.73 #MAP 64
54 120.380 61.060 174.300 126.210 8.697 1 U 5.500000E+00 0.000000E+00 e 0 N.17 CA.17 C.17 N.18 H.18 #MAP 15
54 120.380 61.060 174.300 126.210 8.697 1 U 5.500000E+00 0.000000E+00 e 0 N.142 CA.142 C.142 N.143 H.143 #MAP 132
55 106.120 45.160 174.530 120.720 8.125 1 U 5.500000E+00 0.000000E+00 e 0 N.37 CA.37 C.37 N.38 H.38 #MAP 33
56 115.000 52.740 176.670 105.320 8.285 1 U 5.500000E+00 0.000000E+00 e 0 - - - - -
57 111.810 62.860 172.440 107.930 6.935 1 U 5.500000E+00 0.000000E+00 e 0 N.134 CA.134 C.134 N.135 H.135 #MAP 124
58 118.250 60.430 174.920 110.470 8.569 1 U 5.500000E+00 0.000000E+00 e 0 N.49 CA.49 C.49 N.50 H.50 #MAP 45
59 120.360 53.370 176.550 118.280 8.664 1 U 5.500000E+00 0.000000E+00 e 0 N.57 CA.57 C.57 N.58 H.58 #MAP 53
60 120.530 58.180 177.230 119.640 8.078 1 U 5.500000E+00 0.000000E+00 e 0 N.77 CA.77 C.77 N.78 H.78 #MAP 67
61 114.210 54.890 176.360 121.590 9.541 1 U 5.500000E+00 0.000000E+00 e 0 N.20 CA.20 C.20 N.21 H.21 #MAP 18
62 119.710 52.860 176.960 101.720 7.732 1 U 5.500000E+00 0.000000E+00 e 0 N.60 CA.60 C.60 N.61 H.61 #MAP 56
63 110.440 55.550 178.360 119.370 10.212 1 U 5.500000E+00 0.000000E+00 e 0 N.50 CA.50 C.50 N.51 H.51 #MAP 46
64 115.100 54.700 176.790 120.630 7.455 1 U 5.500000E+00 0.000000E+00 e 0 N.75 CA.75 C.75 N.76 H.76 #MAP 65
65 129.370 53.830 178.340 117.020 10.449 1 U 5.500000E+00 0.000000E+00 e 0 N.125 CA.125 C.125 N.126 H.126 #MAP 115
66 123.520 51.850 172.170 114.120 8.372 1 U 5.500000E+00 0.000000E+00 e 0 N.19 CA.19 C.19 N.20 H.20 #MAP 17
67 121.570 55.700 173.490 130.350 9.283 1 U 5.500000E+00 0.000000E+00 e 0 N.21 CA.21 C.21 N.22 H.22 #MAP 19
68 120.890 54.720 175.760 111.250 8.884 1 U 5.500000E+00 0.000000E+00 e 0 N.9 CA.9 C.9 N.10 H.10 #MAP 9
69 120.970 57.970 174.290 125.540 8.943 1 U 5.500000E+00 0.000000E+00 e 0 N.119 CA.119 C.119 N.120 H.120 #MAP 109
70 125.910 54.430 175.310 127.530 9.288 1 U 5.500000E+00 0.000000E+00 e 0 - - - - -
71 114.290 56.720 172.800 123.040 8.521 1 U 5.500000E+00 0.000000E+00 e 0 N.34 CA.34 C.34 N.35 H.35 #MAP 30
72 120.680 55.220 177.310 114.730 8.691 1 U 5.500000E+00 0.000000E+00 e 0 N.42 CA.42 C.42 N.43 H.43 #MAP 38
73 123.280 59.640 173.050 124.100 9.123 1 U 5.500000E+00 0.000000E+00 e 0 N.35 CA.35 C.35 N.36 H.36 #MAP 31
74 108.250 45.690 174.640 118.370 8.902 1 U 5.500000E+00 0.000000E+00 e 0 N.130 CA.130 C.130 N.131 H.131 #MAP 120
75 115.600 54.830 175.150 121.690 9.387 1 U 5.500000E+00 0.000000E+00 e 0 N.63 CA.63 C.63 N.64 H.64 #MAP 57
76 104.240 45.410 176.860 118.430 7.154 1 U 5.500000E+00 0.000000E+00 e 0 N.127 CA.127 C.127 N.128 H.128 #MAP 117
77 125.460 55.950 173.410 108.060 8.374 1 U 5.500000E+00 0.000000E+00 e 0 N.32 CA.32 C.32 N.33 H.33 #MAP 28
78 121.390 54.840 174.650 122.990 8.383 1 U 5.500000E+00 0.000000E+00 e 0 - - - - -
79 126.780 59.840 174.320 124.470 8.627 1 U 5.500000E+00 0.000000E+00 e 0 N.1 CA.1 C.1 N.2 H.2 #MAP 1
80 124.560 54.580 174.570 123.430 9.131 1 U 5.500000E+00 0.000000E+00 e 0 N.90 CA.90 C.90 N.91 H.91 #MAP 80
81 124.020 54.020 175.440 106.070 8.303 1 U 5.500000E+00 0.000000E+00 e 0 N.36 CA.36 C.36 N.37 H.37 #MAP 32
82 126.020 60.030 175.410 125.820 8.955 1 U 5.500000E+00 0.000000E+00 e 0 N.31 CA.31 C.31 N.32 H.32 #MAP 27
83 121.250 53.070 177.200 110.220 8.470 1 U 5.500000E+00 0.000000E+00 e 0 N.11 CA.11 C.11 N.12 H.12 #MAP 11
84 117.640 52.110 177.150 113.640 8.949 1 U 5.500000E+00 0.000000E+00 e 0 N.86 CA.86 C.86 N.87 H.87 #MAP 76
85 119.000 53.970 175.380 110.720 8.087 1 U 5.500000E+00 0.000000E+00 e 0 N.26 CA.26 C.26 N.27 H.27 #MAP 24
86 119.550 54.820 171.410 119.100 6.881 1 U 5.500000E+00 0.000000E+00 e 0 - - - - -
87 123.250 64.280 178.340 111.990 7.983 1 U 5.500000E+00 0.000000E+00 e 0 N.103 CA.103 C.103 N.104 H.104 #MAP 93
87 123.250 64.280 178.340 111.990 7.983 1 U 5.500000E+00 0.000000E+00 e 0 N.106 CA.106 C.106 N.107 H.107 #MAP 96
88 107.170 45.760 171.010 117.790 8.069 1 U 5.500000E+00 0.000000E+00 e 0 N.85 CA.85 C.85 N.86 H.86 #MAP 75
89 115.180 53.770 171.690 111.700 8.137 1 U 5.500000E+00 0.000000E+00 e 0 - - - - -
90 113.460 57.360 177.220 117.240 7.834 1 U 5.500000E+00 0.000000E+00 e 0 N.98 CA.98 C.98 N.99 H.99 #MAP 88
91 120.440 53.300 176.370 107.350 8.264 1 U 5.500000E+00 0.000000E+00 e 0 N.84 CA.84 C.84 N.85 H.85 #MAP 74
91 120.440 53.300 176.370 107.350 8.264 1 U 5.500000E+00 0.000000E+00 e 0 N.140 CA.140 C.140 N.141 H.141 #MAP 130
92 108.600 51.410 179.660 108.290 7.254 1 U 5.500000E+00 0.000000E+00 e 0 N.129 CA.129 C.129 N.130 H.130 #MAP 119

Table S11: Peak list from (H)NCOCANH APSY experiment on Cu^{I},Zn-SOD

Number of dimensions 5
#FORMAT xeasy5D
#INAME 1 N
#INAME 2 O
#INAME 3 A
#INAME 4 M
#INAME 5 J
#SPECTRUM hNCOCANH N O A M J
1 126.510 175.480 61.120 123.780 9.094 1 U 5.500000E+00 0.000000E+00 e 0 N.8 C.7 CA.7 N.7 H.7 #MAP 6
1 126.510 175.480 61.120 123.780 9.094 1 U 5.500000E+00 0.000000E+00 e 0 N.92 C.91 CA.91 N.91 H.91 #MAP 80
2 121.010 173.520 60.840 125.450 8.930 1 U 5.500000E+00 0.000000E+00 e 0 N.119 C.118 CA.118 N.118 H.118 #MAP 107

3 110.940 175.040 50.920 114.440 7.359 1 U 5.500000E+00 0.000000E+00 e 0 N.146 C.145 CA.145 N.145 H.145 #MAP 134
4 122.930 176.020 62.430 120.230 7.338 1 U 5.500000E+00 0.000000E+00 e 0 N.15 C.14 CA.14 N.14 H.14 #MAP 11
5 124.380 171.120 45.490 106.700 8.032 1 U 5.500000E+00 0.000000E+00 e 0 N.148 C.147 CA.147 N.147 H.147 #MAP 136
6 120.470 172.050 55.600 121.260 7.143 1 U 5.500000E+00 0.000000E+00 e 0 N.84 C.83 CA.83 N.83 H.83 #MAP 72
6 120.470 172.050 55.600 121.260 7.143 1 U 5.500000E+00 0.000000E+00 e 0 N.140 C.139 CA.139 N.139 H.139 #MAP 128
7 129.800 173.100 62.230 112.690 6.664 1 U 5.500000E+00 0.000000E+00 e 0 N.117 C.116 CA.116 N.116 H.116 #MAP 105
8 118.350 175.400 53.960 119.000 6.989 1 U 5.500000E+00 0.000000E+00 e 0 N.80 C.79 CA.79 N.79 H.79 #MAP 68
9 122.220 176.370 51.610 121.110 7.743 1 U 5.500000E+00 0.000000E+00 e 0 N.124 C.123 CA.123 N.123 H.123 #MAP 112
10 125.870 174.380 61.090 120.640 7.986 1 U 5.500000E+00 0.000000E+00 e 0 N.18 C.17 CA.17 N.17 H.17 #MAP 14
10 125.870 174.380 61.090 120.640 7.986 1 U 5.500000E+00 0.000000E+00 e 0 N.143 C.142 CA.142 N.142 H.142 #MAP 131
11 121.280 173.690 45.420 97.750 8.458 1 U 5.500000E+00 0.000000E+00 e 0 N.83 C.82 CA.82 N.82 H.82 #MAP 71
11 121.280 173.690 45.420 97.750 8.458 1 U 5.500000E+00 0.000000E+00 e 0 N.139 C.138 CA.138 N.138 H.138 #MAP 127
12 116.760 175.170 61.880 127.460 8.613 1 U 5.500000E+00 0.000000E+00 e 0 N.150 C.149 CA.149 N.149 H.149 #MAP 138
13 120.560 173.310 42.780 113.790 8.748 1 U 5.500000E+00 0.000000E+00 e 0 N.42 C.41 CA.41 N.41 H.41 #MAP 36
13 120.560 173.310 42.780 113.790 8.748 1 U 5.500000E+00 0.000000E+00 e 0 N.57 C.56 CA.56 N.56 H.56 #MAP 51
14 118.420 172.800 44.640 112.850 9.551 1 U 5.500000E+00 0.000000E+00 e 0 N.115 C.114 CA.114 N.114 H.114 #MAP 103
15 125.310 174.970 49.670 131.410 9.686 1 U 5.500000E+00 0.000000E+00 e 0 N.96 C.95 CA.95 N.95 H.95 #MAP 84
16 118.270 174.310 55.630 129.470 10.170 1 U 5.500000E+00 0.000000E+00 e 0 N.49 C.48 CA.48 N.48 H.48 #MAP 43
17 107.330 176.510 53.280 120.480 7.158 1 U 5.500000E+00 0.000000E+00 e 0 N.85 C.84 CA.84 N.84 H.84 #MAP 73
17 107.330 176.510 53.280 120.480 7.158 1 U 5.500000E+00 0.000000E+00 e 0 N.141 C.140 CA.140 N.140 H.140 #MAP 129
18 120.890 175.720 52.020 115.850 8.127 1 U 5.500000E+00 0.000000E+00 e 0 N.47 C.46 CA.46 N.46 H.46 #MAP 41
19 104.220 177.190 54.500 117.010 10.445 1 U 5.500000E+00 0.000000E+00 e 0 N.127 C.126 CA.126 N.126 H.126 #MAP 115
20 112.860 176.870 60.910 121.550 7.578 1 U 5.500000E+00 0.000000E+00 e 0 N.114 C.113 CA.113 N.113 H.113 #MAP 102
21 125.960 171.120 59.640 121.740 9.570 1 U 5.500000E+00 0.000000E+00 e 0 N.6 C.5 CA.5 N.5 H.5 #MAP 4
22 124.380 177.550 49.160 129.120 9.334 1 U 5.500000E+00 0.000000E+00 e 0 N.90 C.89 CA.89 N.89 H.89 #MAP 78
23 120.390 170.920 46.250 107.310 8.324 1 U 5.500000E+00 0.000000E+00 e 0 N.17 C.16 CA.16 N.16 H.16 #MAP 13
23 120.390 170.920 46.250 107.310 8.324 1 U 5.500000E+00 0.000000E+00 e 0 N.142 C.141 CA.141 N.141 H.141 #MAP 130
24 123.600 172.200 56.260 126.200 8.688 1 U 5.500000E+00 0.000000E+00 e 0 N.19 C.18 CA.18 N.18 H.18 #MAP 15
25 107.230 174.520 53.510 123.000 7.944 1 U 5.500000E+00 0.000000E+00 e 0 N.16 C.15 CA.15 N.15 H.15 #MAP 12
26 118.040 174.820 58.620 113.660 8.946 1 U 5.500000E+00 0.000000E+00 e 0 N.88 C.87 CA.87 N.87 H.87 #MAP 76
27 126.560 171.870 46.770 109.380 8.873 1 U 5.500000E+00 0.000000E+00 e 0 N.45 C.44 CA.44 N.44 H.44 #MAP 39
28 127.400 175.400 62.350 124.430 8.452 1 U 5.500000E+00 0.000000E+00 e 0 N.149 C.148 CA.148 N.148 H.148 #MAP 137
29 123.770 174.960 50.270 126.070 9.170 1 U 5.500000E+00 0.000000E+00 e 0 N.7 C.6 CA.6 N.6 H.6 #MAP 5
30 115.260 171.450 45.590 119.470 10.209 1 U 5.500000E+00 0.000000E+00 e 0 N.52 C.51 CA.51 N.51 H.51 #MAP 46
31 114.790 177.380 55.070 120.570 8.282 1 U 5.500000E+00 0.000000E+00 e 0 N.43 C.42 CA.42 N.42 H.42 #MAP 37
32 109.340 174.790 53.860 114.750 8.698 1 U 5.500000E+00 0.000000E+00 e 0 N.44 C.43 CA.43 N.43 H.43 #MAP 38
33 106.190 175.520 54.030 124.190 9.115 1 U 5.500000E+00 0.000000E+00 e 0 N.37 C.36 CA.36 N.36 H.36 #MAP 31
34 120.320 173.410 42.860 116.790 9.282 1 U 5.500000E+00 0.000000E+00 e 0 N.151 C.150 CA.150 N.150 H.150 #MAP 139
35 105.480 171.830 44.030 113.130 7.216 1 U 5.500000E+00 0.000000E+00 e 0 N.73 C.72 CA.72 N.72 H.72 #MAP 63
36 112.690 174.370 54.340 118.480 7.433 1 U 5.500000E+00 0.000000E+00 e 0 N.116 C.115 CA.115 N.115 H.115 #MAP 104
37 129.400 177.150 61.440 120.890 9.395 1 U 5.500000E+00 0.000000E+00 e 0 N.48 C.47 CA.47 N.47 H.47 #MAP 42
38 129.000 178.960 53.250 118.370 8.320 1 U 5.500000E+00 0.000000E+00 e 0 N.81 C.80 CA.80 N.80 H.80 #MAP 69
39 121.810 175.970 56.220 125.520 8.933 1 U 5.500000E+00 0.000000E+00 e 0 N.24 C.23 CA.23 N.23 H.23 #MAP 20
39 121.810 175.970 56.220 125.520 8.933 1 U 5.500000E+00 0.000000E+00 e 0 N.121 C.120 CA.120 N.120 H.120 #MAP 109
40 130.170 173.580 55.670 121.550 9.528 1 U 5.500000E+00 0.000000E+00 e 0 N.22 C.21 CA.21 N.21 H.21 #MAP 18
41 115.860 172.190 55.680 126.570 8.114 1 U 5.500000E+00 0.000000E+00 e 0 N.46 C.45 CA.45 N.45 H.45 #MAP 40
42 131.310 176.690 60.920 118.760 7.788 1 U 5.500000E+00 0.000000E+00 e 0 N.95 C.94 CA.94 N.94 H.94 #MAP 83
43 101.810 177.010 52.850 119.690 6.915 1 U 5.500000E+00 0.000000E+00 e 0 N.61 C.60 CA.60 N.60 H.60 #MAP 55
44 113.100 174.710 55.370 112.370 7.162 1 U 5.500000E+00 0.000000E+00 e 0 N.72 C.71 CA.71 N.71 H.71 #MAP 62
45 119.070 171.560 54.780 119.780 8.067 1 U 5.500000E+00 0.000000E+00 e 0 N.79 C.78 CA.78 N.78 H.78 #MAP 67
46 114.540 171.630 44.210 108.130 8.352 1 U 5.500000E+00 0.000000E+00 e 0 N.34 C.33 CA.33 N.33 H.33 #MAP 28
47 110.510 174.880 60.380 118.260 8.531 1 U 5.500000E+00 0.000000E+00 e 0 N.50 C.49 CA.49 N.49 H.49 #MAP 44
47 110.510 174.880 60.380 118.260 8.531 1 U 5.500000E+00 0.000000E+00 e 0 N.59 C.58 CA.58 N.58 H.58 #MAP 53
48 127.270 175.020 56.470 121.790 9.374 1 U 5.500000E+00 0.000000E+00 e 0 N.65 C.64 CA.64 N.64 H.64 #MAP 57
49 118.080 174.770 45.530 108.030 7.260 1 U 5.500000E+00 0.000000E+00 e 0 N.131 C.130 CA.130 N.130 H.130 #MAP 119
50 115.570 175.440 58.730 121.880 9.191 1 U 5.500000E+00 0.000000E+00 e 0 N.25 C.24 CA.24 N.24 H.24 #MAP 21
50 115.570 175.440 58.730 121.880 9.191 1 U 5.500000E+00 0.000000E+00 e 0 N.122 C.121 CA.121 N.121 H.121 #MAP 110
51 112.440 173.230 55.430 118.270 8.675 1 U 5.500000E+00 0.000000E+00 e 0 N.71 C.70 CA.70 N.70 H.70 #MAP 61
51 112.440 173.230 55.430 118.270 8.675 1 U 5.500000E+00 0.000000E+00 e 0 N.111 C.110 CA.110 N.110 H.110 #MAP 99
52 113.930 176.200 57.100 125.990 8.721 1 U 5.500000E+00 0.000000E+00 e 0 N.41 C.40 CA.40 N.40 H.40 #MAP 35
53 123.200 172.670 56.730 114.530 7.878 1 U 5.500000E+00 0.000000E+00 e 0 N.35 C.34 CA.34 N.34 H.34 #MAP 29
54 108.060 173.450 55.910 125.460 8.964 1 U 5.500000E+00 0.000000E+00 e 0 N.33 C.32 CA.32 N.32 H.32 #MAP 27
55 121.490 176.430 54.900 114.230 8.392 1 U 5.500000E+00 0.000000E+00 e 0 N.21 C.20 CA.20 N.20 H.20 #MAP 17
56 120.650 174.610 45.140 106.080 8.269 1 U 5.500000E+00 0.000000E+00 e 0 N.38 C.37 CA.37 N.37 H.37 #MAP 32
57 118.510 176.860 45.430 104.150 8.670 1 U 5.500000E+00 0.000000E+00 e 0 N.128 C.127 CA.127 N.127 H.127 #MAP 116
58 111.860 178.890 64.270 123.510 8.106 1 U 5.500000E+00 0.000000E+00 e 0 N.134 C.133 CA.133 N.133 H.133 #MAP 122
59 120.900 175.670 53.900 126.590 8.892 1 U 5.500000E+00 0.000000E+00 e 0 N.9 C.8 CA.8 N.8 H.8 #MAP 7
60 125.730 175.410 55.510 127.500 9.202 1 U 5.500000E+00 0.000000E+00 e 0 N.31 C.30 CA.30 N.30 H.30 #MAP 25
61 126.200 175.180 54.900 124.420 8.610 1 U 5.500000E+00 0.000000E+00 e 0 N.3 C.2 CA.2 N.2 H.2 #MAP 1
61 126.200 175.180 54.900 124.420 8.610 1 U 5.500000E+00 0.000000E+00 e 0 N.69 C.68 CA.68 N.68 H.68 #MAP 59
62 111.290 175.770 54.720 120.880 8.252 1 U 5.500000E+00 0.000000E+00 e 0 N.10 C.9 CA.9 N.9 H.9 #MAP 8
63 114.710 177.730 55.880 123.110 8.421 1 U 5.500000E+00 0.000000E+00 e 0 N.145 C.144 CA.144 N.144 H.144 #MAP 133
64 121.530 174.550 60.190 117.100 7.843 1 U 5.500000E+00 0.000000E+00 e 0 N.100 C.99 CA.99 N.99 H.99 #MAP 88

64	121.530	174.550	60.190	117.100	7.843	1	U	5.500000E+00	0.000000E+00	e	0	N.113	C.112	CA.112	N.112	H.112 #MAP	101
65	114.170	172.140	51.870	123.640	8.733	1	U	5.500000E+00	0.000000E+00	e	0	N.20	C.19	CA.19	N.19	H.19 #MAP	16
66	118.570	174.590	54.130	126.190	9.300	1	U	5.500000E+00	0.000000E+00	e	0	N.70	C.69	CA.69	N.69	H.69 #MAP	60
67	128.120	176.890	51.790	115.320	5.827	1	U	5.500000E+00	0.000000E+00	e	0	N.53	C.52	CA.52	N.52	H.52 #MAP	47
68	129.130	173.700	61.470	118.100	8.657	1	U	5.500000E+00	0.000000E+00	e	0	N.89	C.88	CA.88	N.88	H.88 #MAP	77
69	121.700	175.390	50.300	123.040	9.144	1	U	5.500000E+00	0.000000E+00	e	0	N.5	C.4	CA.4	N.4	H.4 #MAP	3
70	125.440	175.580	60.040	126.010	9.239	1	U	5.500000E+00	0.000000E+00	e	0	N.32	C.31	CA.31	N.31	H.31 #MAP	26
71	130.100	175.450	63.130	120.400	8.950	1	U	5.500000E+00	0.000000E+00	e	0	N.152	C.151	CA.151	N.151	H.151 #MAP	140
72	108.090	172.670	62.750	111.850	7.961	1	U	5.500000E+00	0.000000E+00	e	0	-	-	-	-	-	
73	125.420	174.420	53.200	129.800	8.835	1	U	5.500000E+00	0.000000E+00	e	0	N.118	C.117	CA.117	N.117	H.117 #MAP	106
74	121.050	174.700	53.410	115.530	8.476	1	U	5.500000E+00	0.000000E+00	e	0	N.123	C.122	CA.122	N.122	H.122 #MAP	111
75	124.270	174.500	59.750	125.980	9.424	1	U	5.500000E+00	0.000000E+00	e	0	N.68	C.67	CA.67	N.67	H.67 #MAP	58
76	120.540	175.910	54.100	120.610	7.462	1	U	5.500000E+00	0.000000E+00	e	0	N.77	C.76	CA.76	N.76	H.76 #MAP	65
77	106.650	173.310	53.840	110.970	9.004	1	U	5.500000E+00	0.000000E+00	e	0	N.147	C.146	CA.146	N.146	H.146 #MAP	135
78	119.710	177.320	58.150	120.730	8.323	1	U	5.500000E+00	0.000000E+00	e	0	N.78	C.77	CA.77	N.77	H.77 #MAP	66
79	125.480	174.360	57.940	121.000	7.990	1	U	5.500000E+00	0.000000E+00	e	0	N.120	C.119	CA.119	N.119	H.119 #MAP	108
80	127.530	175.570	60.430	121.290	9.015	1	U	5.500000E+00	0.000000E+00	e	0	N.30	C.29	CA.29	N.29	H.29 #MAP	24
81	124.280	172.980	59.660	123.080	8.520	1	U	5.500000E+00	0.000000E+00	e	0	N.36	C.35	CA.35	N.35	H.35 #MAP	30
82	117.030	178.350	53.830	129.420	10.009	1	U	5.500000E+00	0.000000E+00	e	0	N.126	C.125	CA.125	N.125	H.125 #MAP	114
83	118.710	172.970	46.470	111.140	8.432	1	U	5.500000E+00	0.000000E+00	e	0	N.94	C.93	CA.93	N.93	H.93 #MAP	82
84	121.230	172.960	45.120	111.340	8.909	1	U	5.500000E+00	0.000000E+00	e	0	N.11	C.10	CA.10	N.10	H.10 #MAP	9
85	120.420	176.930	54.700	115.210	8.600	1	U	5.500000E+00	0.000000E+00	e	0	N.76	C.75	CA.75	N.75	H.75 #MAP	64
86	126.160	176.240	61.020	110.610	8.460	1	U	5.500000E+00	0.000000E+00	e	0	-	-	-	-	-	
87	105.910	177.150	58.280	122.790	7.711	1	U	5.500000E+00	0.000000E+00	e	0	N.137	C.136	CA.136	N.136	H.136 #MAP	125
88	126.110	175.690	53.630	125.510	8.510	1	U	5.500000E+00	0.000000E+00	e	0	N.97	C.96	CA.96	N.96	H.96 #MAP	85
89	123.150	172.890	57.990	118.500	8.901	1	U	5.500000E+00	0.000000E+00	e	0	N.103	C.102	CA.102	N.102	H.102 #MAP	91
89	123.150	172.890	57.990	118.500	8.901	1	U	5.500000E+00	0.000000E+00	e	0	N.106	C.105	CA.105	N.105	H.105 #MAP	94
90	97.840	176.650	66.610	129.020	7.904	1	U	5.500000E+00	0.000000E+00	e	0	N.82	C.81	CA.81	N.81	H.81 #MAP	70
91	110.660	177.070	52.870	120.540	8.116	1	U	5.500000E+00	0.000000E+00	e	0	N.39	C.38	CA.38	N.38	H.38 #MAP	33
92	118.580	178.360	53.770	118.170	8.910	1	U	5.500000E+00	0.000000E+00	e	0	N.132	C.131	CA.131	N.131	H.131 #MAP	120
93	123.390	176.520	61.520	125.890	8.647	1	U	5.500000E+00	0.000000E+00	e	0	N.4	C.3	CA.3	N.3	H.3 #MAP	2
94	121.720	175.140	54.700	115.540	7.551	1	U	5.500000E+00	0.000000E+00	e	0	N.64	C.63	CA.63	N.63	H.63 #MAP	56
95	129.460	177.590	52.190	122.240	10.151	1	U	5.500000E+00	0.000000E+00	e	0	N.125	C.124	CA.124	N.124	H.124 #MAP	113

Table S12: Peak list from (H)NCACONH APSY experiment on $\beta 2m_{D76N}$

```
# Number of dimensions 5
#FORMAT xeasy5D
#INAME 1 M
#INAME 2 A
#INAME 3 O
#INAME 4 N
#INAME 5 H
#SPECTRUM hNCACONH M A O N H
```

1	126.300	53.200	175.740	108.970	7.345	1	U	5.500000E+00	0.000000E+00	e	0	N.38	CA.38	C.38	N.39	H.39 #MAP	17
2	116.730	55.310	173.160	125.050	7.687	1	U	5.500000E+00	0.000000E+00	e	0	N.61	CA.61	C.61	N.62	H.62 #MAP	40
3	121.140	55.820	175.870	112.460	7.932	1	U	5.500000E+00	0.000000E+00	e	0	N.40	CA.40	C.40	N.41	H.41 #MAP	19
4	109.010	44.790	170.590	121.210	9.202	1	U	5.500000E+00	0.000000E+00	e	0	N.29	CA.29	C.29	N.30	H.30 #MAP	10
5	129.230	59.430	174.500	112.510	8.173	1	U	5.500000E+00	0.000000E+00	e	0	N.56	CA.56	C.56	N.57	H.57 #MAP	35
6	130.400	59.600	174.330	113.600	6.961	1	U	5.500000E+00	0.000000E+00	e	0	N.67	CA.67	C.67	N.68	H.68 #MAP	46
7	113.880	52.430	172.190	107.270	8.599	1	U	5.500000E+00	0.000000E+00	e	0	N.42	CA.42	C.42	N.43	H.43 #MAP	21
8	115.140	57.960	174.540	113.750	9.121	1	U	5.500000E+00	0.000000E+00	e	0	N.58	CA.58	C.58	N.59	H.59 #MAP	37
9	119.760	56.580	174.420	126.330	8.399	1	U	5.500000E+00	0.000000E+00	e	0	N.37	CA.37	C.37	N.38	H.38 #MAP	16
10	108.970	53.170	176.860	121.260	8.571	1	U	5.500000E+00	0.000000E+00	e	0	N.24	CA.24	C.24	N.25	H.25 #MAP	5
10	108.970	53.170	176.860	121.260	8.571	1	U	5.500000E+00	0.000000E+00	e	0	N.39	CA.39	C.39	N.40	H.40 #MAP	18
11	112.530	59.440	172.120	115.160	7.736	1	U	5.500000E+00	0.000000E+00	e	0	N.57	CA.57	C.57	N.58	H.58 #MAP	36
12	113.730	52.330	174.480	127.650	8.950	1	U	5.500000E+00	0.000000E+00	e	0	N.59	CA.59	C.59	N.60	H.60 #MAP	38
13	116.650	52.960	173.230	127.290	9.252	1	U	5.500000E+00	0.000000E+00	e	0	N.63	CA.63	C.63	N.64	H.64 #MAP	42
14	112.510	55.450	173.570	113.970	8.291	1	U	5.500000E+00	0.000000E+00	e	0	N.41	CA.41	C.41	N.42	H.42 #MAP	20
15	127.590	54.980	172.860	130.980	9.677	1	U	5.500000E+00	0.000000E+00	e	0	N.69	CA.69	C.69	N.70	H.70 #MAP	48
16	125.020	59.880	174.680	116.590	8.865	1	U	5.500000E+00	0.000000E+00	e	0	N.62	CA.62	C.62	N.63	H.63 #MAP	41
17	128.570	54.510	174.030	119.830	9.166	1	U	5.500000E+00	0.000000E+00	e	0	N.36	CA.36	C.36	N.37	H.37 #MAP	15
18	126.500	54.000	174.250	122.310	8.629	1	U	5.500000E+00	0.000000E+00	e	0	N.47	CA.47	C.47	N.48	H.48 #MAP	26
19	127.070	50.830	177.050	116.720	8.057	1	U	5.500000E+00	0.000000E+00	e	0	-	-	-	-	-	
20	127.640	55.920	175.790	116.740	9.312	1	U	5.500000E+00	0.000000E+00	e	0	N.60	CA.60	C.60	N.61	H.61 #MAP	39
21	121.410	54.550	173.570	125.640	9.416	1	U	5.500000E+00	0.000000E+00	e	0	N.25	CA.25	C.25	N.26	H.26 #MAP	6
22	122.120	54.190	174.430	128.700	8.665	1	U	5.500000E+00	0.000000E+00	e	0	N.48	CA.48	C.48	N.49	H.49 #MAP	27
23	121.660	54.320	173.140	113.840	7.232	1	U	5.500000E+00	0.000000E+00	e	0	N.22	CA.22	C.22	N.23	H.23 #MAP	3
24	126.660	55.000	174.010	129.510	9.585	1	U	5.500000E+00	0.000000E+00	e	0	N.74	CA.74	C.74	N.75	H.75 #MAP	51
25	127.400	55.080	174.620	121.630	8.966	1	U	5.500000E+00	0.000000E+00	e	0	N.64	CA.64	C.64	N.65	H.65 #MAP	43
26	128.610	61.580	173.880	129.030	9.307	1	U	5.500000E+00	0.000000E+00	e	0	N.49	CA.49	C.49	N.50	H.50 #MAP	28
27	107.150	46.240	171.890	118.030	8.311	1	U	5.500000E+00	0.000000E+00	e	0	N.43	CA.43	C.43	N.44	H.44 #MAP	22
28	118.240	58.480	175.260	128.610	8.649	1	U	5.500000E+00	0.000000E+00	e	0	N.35	CA.35	C.35	N.36	H.36 #MAP	14

29	122.300	51.390	174.280	121.910	8.270	1	U	5.500000E+00	0.000000E+00	e 0 N.21	CA.21	C.21	N.22	H.22 #MAP	2
30	121.230	51.900	174.020	126.960	8.949	1	U	5.500000E+00	0.000000E+00	e 0 N.30	CA.30	C.30	N.31	H.31 #MAP	11
31	119.610	56.320	175.270	118.060	7.333	1	U	5.500000E+00	0.000000E+00	e 0 N.34	CA.34	C.34	N.35	H.35 #MAP	13
31	119.610	56.320	175.270	118.060	7.333	1	U	5.500000E+00	0.000000E+00	e 0 N.52	CA.52	C.52	N.53	H.53 #MAP	31
32	117.890	62.400	173.060	126.530	9.103	1	U	5.500000E+00	0.000000E+00	e 0 N.73	CA.73	C.73	N.74	H.74 #MAP	50
33	123.350	55.740	175.900	117.520	8.580	1	U	5.500000E+00	0.000000E+00	e 0 N.54	CA.54	C.54	N.55	H.55 #MAP	33
34	114.690	56.280	173.620	119.330	8.193	1	U	5.500000E+00	0.000000E+00	e 0 N.33	CA.33	C.33	N.34	H.34 #MAP	12
35	114.020	54.470	174.880	109.150	7.998	1	U	5.500000E+00	0.000000E+00	e 0 N.23	CA.23	C.23	N.24	H.24 #MAP	4
36	131.130	56.720	172.630	119.510	9.126	1	U	5.500000E+00	0.000000E+00	e 0 N.70	CA.70	C.70	N.71	H.71 #MAP	49
37	113.490	62.280	172.120	127.550	8.664	1	U	5.500000E+00	0.000000E+00	e 0 N.68	CA.68	C.68	N.69	H.69 #MAP	47
38	121.620	55.800	171.940	121.600	8.620	1	U	5.500000E+00	0.000000E+00	e 0 -	-	-	-	-	
39	119.560	54.440	173.850	124.400	9.103	1	U	5.500000E+00	0.000000E+00	e 0 N.45	CA.45	C.45	N.46	H.46 #MAP	24
40	119.920	54.330	175.310	109.030	8.680	1	U	5.500000E+00	0.000000E+00	e 0 N.28	CA.28	C.28	N.29	H.29 #MAP	9
41	124.350	59.930	174.560	126.260	8.710	1	U	5.500000E+00	0.000000E+00	e 0 N.46	CA.46	C.46	N.47	H.47 #MAP	25
42	124.230	55.770	171.780	124.610	9.049	1	U	5.500000E+00	0.000000E+00	e 0 -	-	-	-	-	
43	117.920	55.450	175.690	119.230	8.471	1	U	5.500000E+00	0.000000E+00	e 0 -	-	-	-	-	
44	126.640	59.480	172.730	119.380	8.545	1	U	5.500000E+00	0.000000E+00	e 0 N.51	CA.51	C.51	N.52	H.52 #MAP	30
45	127.870	57.380	172.950	123.400	8.691	1	U	5.500000E+00	0.000000E+00	e 0 -	-	-	-	-	

Table S13: Peak list from (H)NCOCANH APSY experiment on $\beta 2m_{D76N}$

```
# Number of dimensions 5
#FORMAT xeasy5D
#INAME 1 N
#INAME 2 O
#INAME 3 A
#INAME 4 M
#INAME 5 J
#SPECTRUM hNCOCANH N O A M J
```

1	121.066	176.934	53.197	108.954	7.337	1	U	5.500000E+00	0.000000E+00	e 0 N.40	C.39	CA.39	N.39	H.39 #MAP	18
2	116.630	174.682	59.862	125.089	7.680	1	U	5.500000E+00	0.000000E+00	e 0 N.63	C.62	CA.62	N.62	H.62 #MAP	41
3	113.819	173.586	55.462	112.428	7.930	1	U	5.500000E+00	0.000000E+00	e 0 N.42	C.41	CA.41	N.41	H.41 #MAP	20
4	108.942	175.787	53.217	126.371	8.388	1	U	5.500000E+00	0.000000E+00	e 0 N.39	C.38	CA.38	N.38	H.38 #MAP	17
5	127.630	174.509	52.390	113.723	9.125	1	U	5.500000E+00	0.000000E+00	e 0 N.60	C.59	CA.59	N.59	H.59 #MAP	38
6	121.123	170.598	44.830	109.061	8.663	1	U	5.500000E+00	0.000000E+00	e 0 N.30	C.29	CA.29	N.29	H.29 #MAP	10
7	116.667	175.804	55.933	127.642	8.951	1	U	5.500000E+00	0.000000E+00	e 0 N.61	C.60	CA.60	N.60	H.60 #MAP	39
8	119.730	174.037	54.543	128.545	8.642	1	U	5.500000E+00	0.000000E+00	e 0 N.37	C.36	CA.36	N.36	H.36 #MAP	15
9	127.511	172.130	62.243	113.506	6.953	1	U	5.500000E+00	0.000000E+00	e 0 N.69	C.68	CA.68	N.68	H.68 #MAP	47
10	113.698	174.564	57.977	115.146	7.732	1	U	5.500000E+00	0.000000E+00	e 0 N.59	C.58	CA.58	N.58	H.58 #MAP	37
11	115.099	172.099	59.469	112.468	8.170	1	U	5.500000E+00	0.000000E+00	e 0 N.58	C.57	CA.57	N.57	H.57 #MAP	36
12	121.600	174.572	55.107	127.237	9.238	1	U	5.500000E+00	0.000000E+00	e 0 N.65	C.64	CA.64	N.64	H.64 #MAP	43
13	119.366	175.493	53.836	129.452	9.604	1	U	5.500000E+00	0.000000E+00	e 0 N.76	C.75	CA.75	N.75	H.75 #MAP	52
14	107.248	172.149	52.457	113.956	8.288	1	U	5.500000E+00	0.000000E+00	e 0 N.43	C.42	CA.42	N.42	H.42 #MAP	21
15	125.061	173.156	55.308	116.697	9.297	1	U	5.500000E+00	0.000000E+00	e 0 N.62	C.61	CA.61	N.61	H.61 #MAP	40
16	112.587	175.984	55.879	121.389	8.579	1	U	5.500000E+00	0.000000E+00	e 0 N.41	C.40	CA.40	N.40	H.40 #MAP	19
17	126.312	174.449	56.605	119.823	9.160	1	U	5.500000E+00	0.000000E+00	e 0 N.38	C.37	CA.37	N.37	H.37 #MAP	16
18	127.210	173.291	52.944	116.616	8.865	1	U	5.500000E+00	0.000000E+00	e 0 N.64	C.63	CA.63	N.63	H.63 #MAP	42
19	112.551	174.491	59.425	129.279	8.355	1	U	5.500000E+00	0.000000E+00	e 0 N.57	C.56	CA.56	N.56	H.56 #MAP	35
20	118.159	175.237	56.094	119.454	8.203	1	U	5.500000E+00	0.000000E+00	e 0 N.35	C.34	CA.34	N.34	H.34 #MAP	13
20	118.159	175.237	56.094	119.454	8.203	1	U	5.500000E+00	0.000000E+00	e 0 N.77	C.76	CA.76	N.76	H.76 #MAP	53
21	128.825	173.961	61.553	128.806	8.671	1	U	5.500000E+00	0.000000E+00	e 0 N.50	C.49	CA.49	N.49	H.49 #MAP	28
22	128.506	175.293	58.332	118.227	7.327	1	U	5.500000E+00	0.000000E+00	e 0 N.36	C.35	CA.35	N.35	H.35 #MAP	14
23	126.466	173.320	62.406	117.963	8.762	1	U	5.500000E+00	0.000000E+00	e 0 N.74	C.73	CA.73	N.73	H.73 #MAP	50
24	119.721	172.693	56.925	131.013	9.690	1	U	5.500000E+00	0.000000E+00	e 0 N.71	C.70	CA.70	N.70	H.70 #MAP	49
25	119.392	175.926	59.191	118.095	8.296	1	U	5.500000E+00	0.000000E+00	e 0 N.45	C.44	CA.44	N.44	H.44 #MAP	23
25	119.392	175.926	59.191	118.095	8.296	1	U	5.500000E+00	0.000000E+00	e 0 N.78	C.77	CA.77	N.77	H.77 #MAP	54
26	129.317	174.112	55.003	126.598	9.091	1	U	5.500000E+00	0.000000E+00	e 0 N.75	C.74	CA.74	N.74	H.74 #MAP	51
27	122.455	172.874	55.741	119.471	8.620	1	U	5.500000E+00	0.000000E+00	e 0 -	-	-	-	-	
28	125.877	174.739	59.997	124.384	9.085	1	U	5.500000E+00	0.000000E+00	e 0 -	-	-	-	-	
29	109.275	174.748	54.424	113.720	7.228	1	U	5.500000E+00	0.000000E+00	e 0 N.24	C.23	CA.23	N.23	H.23 #MAP	4
30	122.107	174.276	53.994	126.323	8.677	1	U	5.500000E+00	0.000000E+00	e 0 N.48	C.47	CA.47	N.47	H.47 #MAP	26
31	116.865	173.791	60.499	116.552	8.076	1	U	5.500000E+00	0.000000E+00	e 0 -	-	-	-	-	
32	117.984	171.924	46.239	107.223	8.590	1	U	5.500000E+00	0.000000E+00	e 0 N.44	C.43	CA.43	N.43	H.43 #MAP	22
33	125.373	174.474	52.763	121.628	8.977	1	U	5.500000E+00	0.000000E+00	e 0 N.66	C.65	CA.65	N.65	H.65 #MAP	44
34	119.580	172.951	59.300	127.316	8.437	1	U	5.500000E+00	0.000000E+00	e 0 -	-	-	-	-	
35	117.163	175.847	55.645	123.239	9.374	1	U	5.500000E+00	0.000000E+00	e 0 N.55	C.54	CA.54	N.54	H.54 #MAP	33
36	125.699	173.522	54.594	121.320	8.505	1	U	5.500000E+00	0.000000E+00	e 0 N.26	C.25	CA.25	N.25	H.25 #MAP	6
37	129.312	173.085	54.420	129.249	9.330	1	U	5.500000E+00	0.000000E+00	e 0 -	-	-	-	-	
38	130.965	176.483	56.511	117.602	8.641	1	U	5.500000E+00	0.000000E+00	e 0 -	-	-	-	-	
39	128.977	172.754	56.128	121.767	8.630	1	U	5.500000E+00	0.000000E+00	e 0 -	-	-	-	-	
40	113.584	174.475	59.566	130.345	9.043	1	U	5.500000E+00	0.000000E+00	e 0 N.68	C.67	CA.67	N.67	H.67 #MAP	46
41	126.749	174.003	51.915	121.179	9.209	1	U	5.500000E+00	0.000000E+00	e 0 N.31	C.30	CA.30	N.30	H.30 #MAP	11
42	124.521	177.566	56.578	126.654	8.662	1	U	5.500000E+00	0.000000E+00	e 0 -	-	-	-	-	

```
43 119.970 172.978  59.557 127.501   8.573 1 U   5.500000E+00 0.000000E+00 e 0 N.28    C.27    CA.27    N.27    H.27 #MAP    8
44 113.881 173.171  54.373 121.446   8.243 1 U   5.500000E+00 0.000000E+00 e 0 N.23    C.22    CA.22    N.22    H.22 #MAP    3
45 139.727 176.887  70.693 119.311   6.969 1 U   5.500000E+00 0.000000E+00 e 0 -       -       -        -       -
46 117.741 180.406  54.265 104.333   7.095 1 U   5.500000E+00 0.000000E+00 e 0 -       -       -        -       -
```

H. Details of FLYA calculations

FLYA module implemented in CYANA 3.98 software package was used. The experiments were defined in an auxiliary CYANA library as follows:

```
SPECTRUM hNCACONH M A O N H
 0.980 H_AMI M:N_AMI A:C_ALI O:C_BYL N:N_AMI H:H_AMI

SPECTRUM hNCOCANH N O A M J
 0.980 H_AMI N:N_AMI O:C_BYL A:C_ALI M:N_AMI (C_BYL) J:H_AMI

SPECTRUM ssCBcaNH  HN N C
0.980  HN:H_AMI  N:N_AMI (C_BYL) C_ALI C:C_ALI
```

The above definitions discard proline residues as a start of magnetization pathway. In the case of $\beta 2M_{D76N}$ the amino acid sequence provided to FLYA was restricted to the structured fibril core (residues 20-81). Slightly modified definitions were used in this case to allow the magnetisation to originate from and be detected on (a non-N-terminal) residue S20 amide protons:

```
SPECTRUM ssCBcaNH  HN N C
0.980  HN:H_AMI  N:N_AMI C_ALI C:C_ALI

SPECTRUM hNCOCANH N O A M J
0.980  H_AMI N:N_AMI  O:C_BYL  A:C_ALI M:N_AMI J:H_AMI
```

In the case of GB1, FLYA results were compared to the reference resonance assignment reported by Andreas et al, and deposited in BMRB under accession number 30088.[17] For Cu^{I},Zn-SOD, we used an adapted resonance assignment obtained by Knight et al[3] (BMRB entry 18509). The shifts of $^{13}C'$ were recalibrated by +2.65 ppm to account for the difference in ^{13}C reference between DSS (assumed by FLYA) and TMS (as deposited in BMRB), while the shifts of $^{13}C\alpha$ were additionally increased by 0.66 ppm to roughly compensate ^{2}H-induced shifts. The reference resonance assignment of Cu^{I},Zn-SOD was then updated and completed based on four 3D experiments acquired on the same sample as the 5D datasets: (H)CANH, (H)(CO)CA(CO)NH, (H)CONH, (H)CO(CA)NH. The reference shifts for $\beta 2M_{D76N}$ were obtained independently based on a set of six $^{1}H^{N}$-detected experiments: (H)CANH, (H)(CO)CA(CO)NH, (H)CONH, (H)CO(CA)NH, (H)(CA)CB(CA)NH and (H)(CA)CB(CA)(CO)NH.

Table S14: FLYA parameters [a]

Sample	Assignment tolerance			Tolerance for comparison with the reference		Population size
	T_C, ppm ($^{13}C\alpha$, $^{13}C'$)	T_N, ppm (^{15}N)	T_H, ppm ($^{1}H^N$)	T_{CN}^{REF}, ppm	T_H^{REF}, ppm	N_{popul}
GB1	0.25	0.35	0.10	0.25	0.10	50
SOD	0.20	0.40	0.10	0.40	0.10	1000
$\beta 2M_{D76N}$	0.20	0.40	0.05	0.40	0.10	200

[a] The parameter naming convention (highlighted in *blue*) as used in the following FLYA batch script (*cya):

structurepeaks:=
assignpeaks:=hNCACONH,hNCOCANH
Assignment tolerances
assigncs_accH:= T_H
assigncs_accC:= T_C
assigncs_accN:= T_N
Tolerances for comparison with reference
tolerance:= T_H^{ref}, T_H^{ref}, T_{CN}^{ref}
shiftassign_population:=N_{popul}
shiftassign_iterations:=15000
analyzeassign_group := BB: N H CA C
randomseed := 3771
command select_atoms
 atom select "N H CA C"
end
read lib apsy_experiment_definitions.lib append
read seq sequence_file.seq
flya runs=20 assignpeaks=$assignpeaks structure= shiftreference=reference_shift_file.prot

Table S15: FLYA assignment summary

Protein	Nucleus	Number of "strong" assignments				
		correct [a]	incompatible [a]	additional [a]	all	Max
GB1	$^{1}H^N$	55	0	0	55	55 [b]
	^{15}N	55	0	1	56	56 [c]
	$^{13}C\alpha$	55	0	0	55	56
	$^{13}C'$	55	0	0	55	56
	all	220	0	1	221	223
SOD	$^{1}H^N$	65	7	3	75	127 [b, e]
	^{15}N	74	3	0	77	127 [c, e]
	$^{13}C\alpha$	71	6	1	78	133 [e]
	$^{13}C'$	70	6	1	77	133 [e]
	all	280	22	5	307	520 [e]
$\beta 2M_{D76N}$	$^{1}H^N$	20	1	0	21	57 [b, f]
	^{15}N	21	0	0	21	57 [c, f]
	$^{13}C\alpha$	20	1	0	21	59 [f]
	$^{13}C'$	26	3	0	29	59 [f]
	all	87	5	0	92	232 [f]

[a] With respect to the reference assignment
[b] The number of amide protons, i.e. excluding proline residues and N-terminus
[c] excluding proline ^{15}N atoms
[e] discounting dynamic residues 1-3, 23-27, 54, 59, 69, 107, 110-111, 128-130, 133, 139, 153
[f] within the fibril core (residues 20-81), discounting dynamic residues 25, 51-52

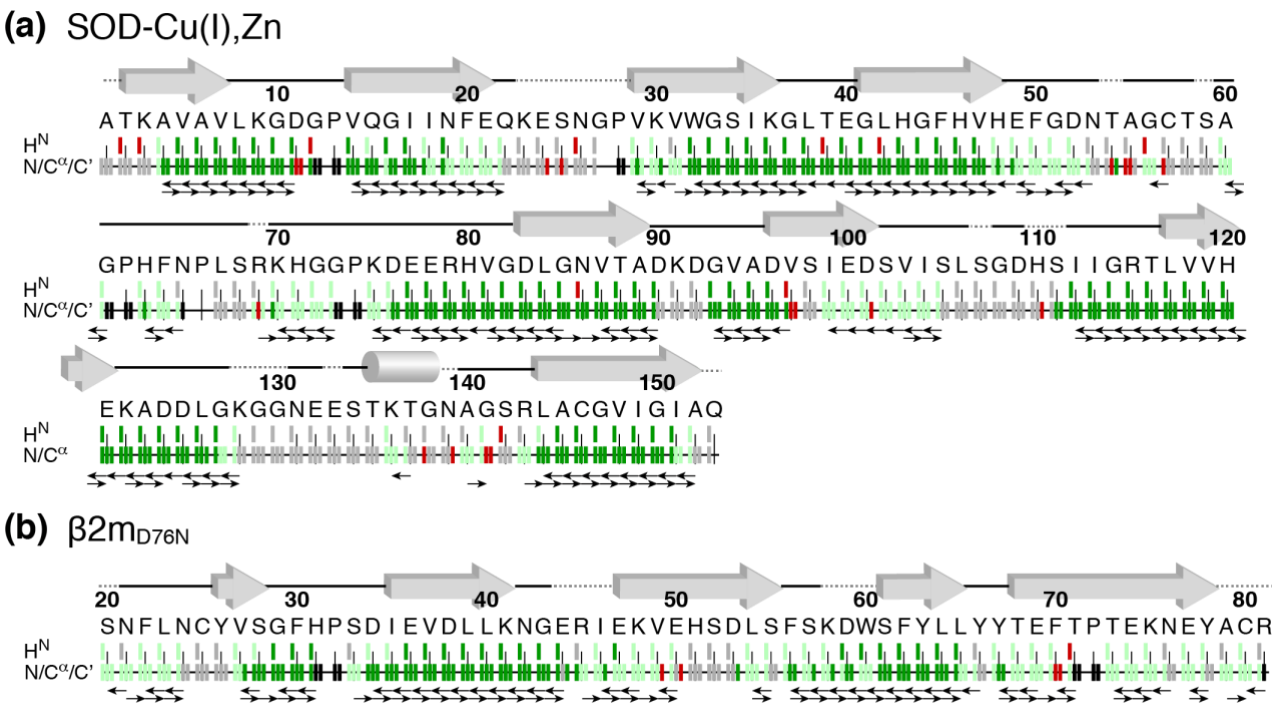

Figure S3. Graphical summary of FLYA automated resonance assignment of (a) Cu^{I},Zn-SOD and (b) $\beta 2M_{D76N}$ based on the pairs of 5D data sets (i.e. forward and backward correlations). The same conventions for secondary structure elements and color-coding of resonances as for the main text Figure 5 are used. Black arrows denote the observed 5D correlations and define the upper limit for (manual) resonance assignment based on the available spectral data.

I. FLYA results for SOD and $\beta 2m_{D76N}$ with additional Cβ information

The calculations were performed with exactly same parameters as specified in the Table S15, with the following 2 exceptions:

1) An unassigned intraresidue $^{13}C\beta$ (*i*) – ^{15}N (*i*) – $^{1}H^{N}$ (*i*) peak list was provided
assignpeaks:=hNCACONH,hNCOCANH,hcaCBcaNH

In the case of Cu^{I},Zn-SOD, a synthetic peak list with 111 entries was constructed from published Cβ shifts of Cu^{II},Zn-SOD,[5] and reference ^{15}N and $^{1}H^{N}$ shifts. In the case of $\beta 2m_{D76N}$ an experimental, unfiltered peak list from a 3D (H)(CA)CB(CA)NH experiment (62 peaks) was used.

2) The respective lines in the FLYA batch script were adapted to allow Cβ shifts in the spin system

```
analyzeassign_group := BB: N H CA CB C
command select_atoms
 atom select "N H CA CB C"
end
```

Table S16: FLYA assignment summary (the calculations with additional Cβ information)

Protein	Nucleus	Number of "strong" assignments				
		correct [a]	incompatible [a]	additional [a]	all	Max
SOD	$^{1}H^{N}$	90	3	3	96	127 [b, e]
	^{15}N	89	3	3	95	127 [c, e]
	$^{13}C\alpha$	84	3	2	89	133 [e]
	$^{13}C'$	83	4	3	90	133 [e]
	$^{13}C\beta$	71	6	1	78	107 [e]
	all-$^{13}C\beta$	346	13	11	370	520 [e]
	all	417	19	12	448	637 [e]
$\beta 2M_{D76N}$	$^{1}H^{N}$	43	2	1	46	57 [b, f]
	^{15}N	45	1	2	48	57 [c, f]
	$^{13}C\alpha$	39	0	2	41	59 [f]
	$^{13}C'$	39	2	0	41	59 [f]
	$^{13}C\beta$	41	0	2	43	55 [f]
	all-$^{13}C\beta$	166	5	5	176	232 [f]
	all	207	5	7	219	287 [f]

[a] With respect to the reference assignment

[b] The number of amide protons, i.e. excluding proline residues and N-terminus

[c] excluding proline ^{15}N atoms

[e] discounting dynamic residues 1-3, 23-27, 54, 59, 69, 107, 110-111, 128-130, 133, 139, 153

[f] within the fibril core (residues 20-81), discounting dynamic residues 25, 51-52

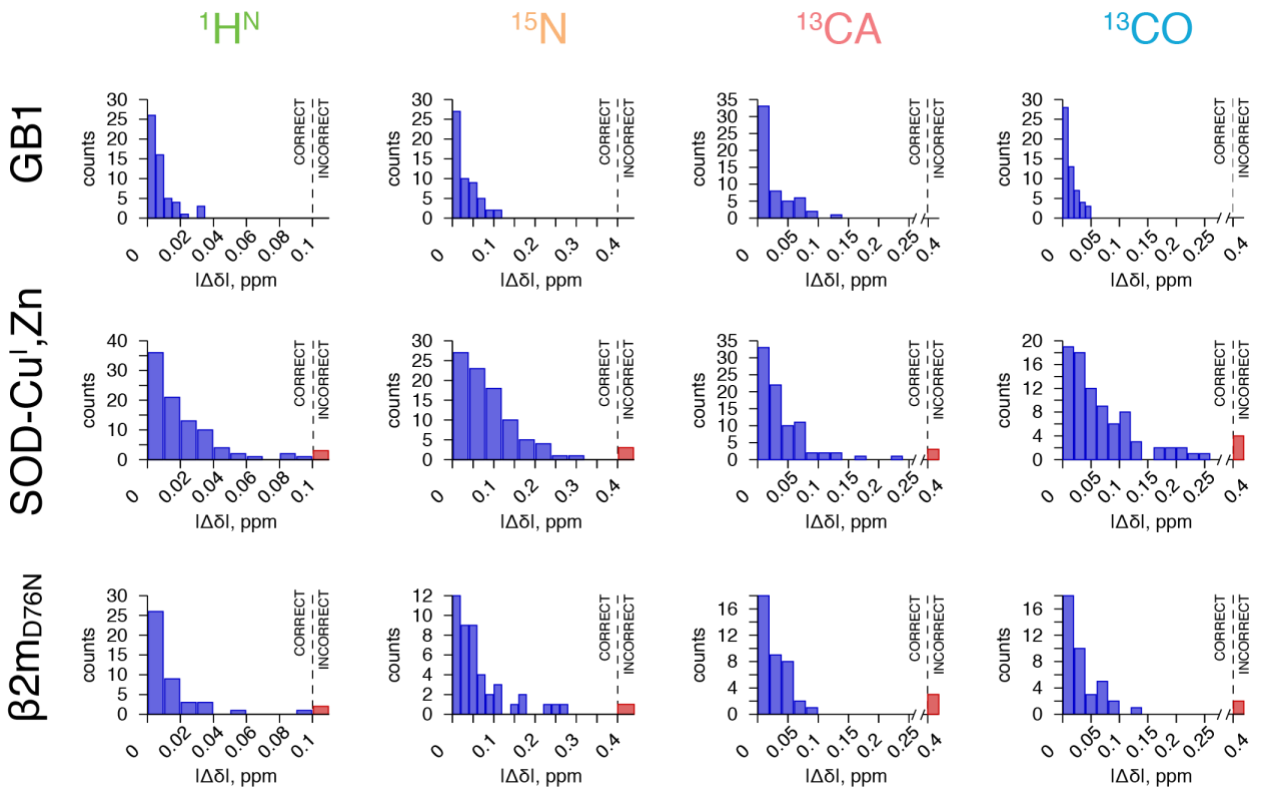

Figure S4. The histograms of consensus chemical shifts obtained by FLYA in function of their deviations from the reference shifts. The thresholds for classification of a shift as correct or incorrect are denoted by vertical dashed lines in each plot.

J. Pulse program code for Bruker spectrometers

Pulse sequence 1. (H)NCACONH

```
;hNCACONH5D.crmn
;5D pulse sequence for protein backbone assignment
;Forward transfer amide-detection
;with 15N and 13C decoupling
;written by Henry Orton and Jan Stanek

;Avance II+ version
;$COMMENT=5D hNCACONH
;$CLASS=Solids
;$DIM=5D
;$TYPE=cross polarisation
;$OWNER=CRMN

;p1 : 1H 90 pulse @ plw1
;pl1 : 1H power for 90 pulse
;p7 : 15N 90 pulse @ plw7
;pl7 : 15N power for 90 pulse
;p3 : 13C 90 pulse @ plw3
;pl11 : 13C power for 90 pulse

;p15 : contact time for H->N CP
;sp0 : 1H power for H->N CP
;pl2 : 15N power for H->N CP

;p17 : contact time for N->H CP
;sp10 : 1H power for N->H CP
;pl20 : 15N power for N->H CP

;spnam10 : Ramp100.90
;spoffs10 : 0

;p16 : contact time for N->CO CP
;p26 : contact time for CA->N CP

;spnam0 : Ramp90.100
;spoffs0 : 0

;p26 : N -> Co CP
;p27 : Ca -> N CP
;p18 : Co selective pi
;p19 : Ca selective pi

;cpdprg1 : 1H decoupling (sltppm_40pTr41 for 1Hprot, waltz16_pl12 for 2Hprot)
;cpdprg2 : 15N decoupling (waltz16_pl16)
;cpdprg3 : 13C decoupling (waltz16_pl17)

;pl12 : 1H decoupling power for (waltz16)
;pl18 : 1H decoupling for (cw_pl18)
;pl13: 1H decoupling (sltppm)
;pl16: 15N decoupling power (waltz16)
;pl17: 13C decoupling power (waltz16)

;pcpd1 : 25u (waltz16 10 kHz) - 33.33 (sltppm 15kHz)
;pcpd2 : 25u (waltz16 10 kHz)
;pcpd3 : 25u (waltz16 10 kHz)
```

```
;d1  : recycle delay
;d31  : N evolution (first)
;d32  : CA evolution
;d33  : CO evolution
;d34  : N evolution (second)

;$COMMENT=basic cp experiment, arbitrary contact and decoupling schemes
;$CLASS=Solids
;$DIM=1D
;$TYPE=cross polarisation
;$SUBTYPE=simple 1D
;$OWNER=Bruker

prosol relations=<solids_cp>
#include <Avancesolids.incl>

;cnst9 : J(CA-CO) scalar coupling constant (55 Hz)
;cnst10 : estimated bulk T2 of CA [sec]
;cnst11 : estimated bulk T2 of CO [sec]
;cnst21 : CO frequency effective offset, about 173.3 ppm (ppm)
;cnst22 : CA frequency effective offset, about 53.3 ppm (ppm)

"plw16=plw7*(pow(p7/25,2))"    ; 15N waltz 10kHz decoupling power level

;13C frequencies for center frequency calculation
"cnst20=(sfo3-bf3)*1000000/bf3" ;center frequency of 113.7 ppm

"plw11=plw3"

"spoal8=0.0"
"spoffs8=bf3*((cnst21 - cnst20)/1000000)"                                   ;CO frequency

"spoal9=1.0"
"spoffs9=bf3*((cnst22 - cnst20)/1000000)"                                   ;CA frequency

;CO selective Q3 pulse parameters (refocussing)
"p18=3.448/(95.0*bf3/1000000)"              ;95 ppm bandwidth (safe)
"spw18=plw3*pow((0.5/(p18*0.1515))/(0.25/p3),2)"   ;Q3 power level
"spoal18=0.5"                               ;default value
"spoff18=bf3*((cnst21 - cnst20)/1000000)"        ;CO frequency

;CA selective Q3 pulse parameters (inversion)
"p19=3.41/(105.0*bf3/1000000)"              ;105 ppm bandwidth (safe)
"spw19=plw3*pow((0.5/(p19*0.1515))/(0.25/p3),2)"   ;Q3 power level
"spoal19=0.5"                               ;default value
"spoff19=bf3*((cnst22 - cnst20)/1000000)"        ;CA frequency

;CO selective Q3 pulse parameters (inversion)
"p12=3.41/(95.0*bf3/1000000)"               ;95 ppm bandwidth (safe)
"spw12=plw3*pow((0.5/(p12*0.1515))/(0.25/p3),2)"   ;Q3 power level
"spoal12=0.5"                               ;default value
"spoff12=bf3*((cnst21 - cnst20)/1000000)"        ;CO frequency

;CA selective REBURP pulse parameters (refocussing)
"p11=4.814/(30*bf3/1000000)"                ;30 ppm bandwidth
"spw11=plw3*pow((0.5/(p11*0.0798))/(0.25/p3),2)"   ;ReBURP power level
"spoal11=0.5"                               ;default value
```

```
"spoff11=bf3*((cnst22 - cnst20)/1000000)"                    ;CA frequency

;Carbon scalar coupling transfer delays
"cnst9=55.0"                          ;J(CA-CO)=55
"d2=2*p7"                             ;15N 180 delay
"d3=0.25/cnst9"                       ;1/4J(CA-CO)
"d28=0.5*atan(cnst11*PI*cnst9)/(PI*cnst9)"    ;1/4J(CA-CO) relaxation CO optimised delay
"d29=0.5*atan(cnst10*PI*cnst9)/(PI*cnst9)"    ;1/4J(CA-CO) relaxation CA optimised delay

;increments for apsy
"in31=inf1"
"in32=inf2"
"in33=inf3"
"in34=inf4"

;define additional phase increments for off resonance evolution
"cnst25=360*(cnst22-cnst20)*bf3/2"                    ; an intemediate variable (necessary) FOR phase 4
"cnst26=360*(cnst21-cnst20)*bf3/2"                    ; an intemediate variable (necessary) FOR phase 4

;delays for apsy
"d31=0.0"
"d32=0.0"
"d33=0.0"
"d34=0.0"

define delay DELTA1a
define delay DELTA2a
define delay DELTA3a
define delay DELTA4a

define delay DELTA1b
define delay DELTA2b
define delay DELTA3b
define delay DELTA4b

1 ze
2 d1 do:f2 do:f3

#include <p15_prot.incl>
#include <aq_prot.incl>

  "DELTA1a= larger(d29+d2-d32*0.5, 0)"
  "DELTA2a= larger(0, d32*0.5-d3)"
  "DELTA3a= larger(-larger(-d32*0.5, -d3), d29)"
  "DELTA4a= d32*0.5"

  "DELTA1b= larger(d28+d2-d33*0.5, 0)"
  "DELTA2b= larger(d28, d33*0.5)"
  "DELTA3b= -larger(-d33*0.5, -d3)"
  "DELTA4b= larger(0, d33*0.5-d3)"

"cnst23=cnst25*d32" ; CA
"cnst24=cnst26*d33" ; CO

  1u ip7+cnst23      ; TPPI-like phase increment for offset evolution of CA
  1u ip11+cnst24     ; TPPI-like phase increment for offset evolution of CO

  1m fq=cnst20(bf ppm):f3                ;reset the 13C offset to CO/CA
```

```
  (p1  pl1 ph1):f1
  (p15 pl2 ph2):f2 (p15:sp0 ph3):f1          ;H -> N CP

  1u cpds1:f1

  ;N t1 evolution
  if "p3*2 > d31" {
    (center (d31) (p3*2 ph0 pl11):f3)
  } else {
    d31
  }

1u do:f1

  (p26:sp9 ph4):f3 (p26:sp3 ph5):f2          ;N -> CA CP
  2u cpds1:f1

;;CA-CO transfer

  (p12:sp12 ph6):f3                          ;CO Q3 (inversion)
  6u
  DELTA1a
  (p11:sp11 ph7):f3                          ;CA REBURP (refocusing)
  4u
  DELTA2a
  (p12:sp12 ph6):f3                          ;CO Q3 (inversion)
  DELTA3a
  (p7*2 ph0 pl7):f2                          ;N 180
  4u
  DELTA4a

   (p3 ph8 pl11):f3                         ;C hard 90

  9u
  DELTA1b
  (p19:sp19 ph10):f3                          ;CA Q3 (inversion)
            4u
  (p18:sp18 ph11):f3
                          ;CO Q3 (refocusing)
            4u
  DELTA2b
  (p7*2 ph0 pl7):f2                          ;N 180
  4u
  DELTA3b
  (p19:sp19 ph10):f3                          ;CA Q3 (inversion)
  4u
  DELTA4b
  1u do:f1
  (p16:sp8 ph12):f3 (p16:sp2 ph13):f2        ;CO -> N CP

;;end CA-CO transfer

  1u cpds1:f1

  ;N t4 evolution
  if "p3*2 > d34" {
    (center (d34) (p3*2 ph0 pl11):f3)
```

```
 } else {
   d34
 }

 1u do:f1

;;water suppression starts
 (p7 pl7 ph14):f2

 1u pl12:f1
 (d30*0.25 cw ph20):f1
 (d30*0.25 cw ph21):f1
 (d30*0.25 cw ph22):f1
 (d30*0.25 cw ph23):f1
 1u do:f1

 (p7 pl7 ph15):f2
;;water suppression ends

 (p17 pl20 ph16):f2 (p17:sp10 ph17):f1      ;N -> H CP

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

 10m mc #0 to 2
 F1PH(calph(ph2, +90), caldel(d31, +in31))
 F2PH(calph(ph4, -90), caldel(d32, +in32))                    ; CA
 F3PH(calph(ph12, -90), caldel(d33, +in33))                   ; CO
 F4PH(calph(ph13, +90), caldel(d34, +in34))

HaltAcqu, 1m
exit

ph0  = 0
ph1  = 1 3
ph2  = 1
ph3  = 0
ph4  = 1
ph5  = 1 1 3 3
ph6  = 0
ph7  = 0
ph8  = 1
ph10 = 1
ph11 = {0}*4 {1}*4
ph12 = 1
ph13 = 0
ph14 = {1}*8 {3}*8
ph15 = 1
ph16 = 0
ph17 = 0
ph20 = 0
ph21 = 1
ph22 = 2
ph23 = 3

ph31 = 0 2 2 0  2 0 0 2
     2 0 0 2  0 2 2 0
```

Pulse sequence 2. (H)NCOCANH

```
;hNCOCANH5D.crmn
;5D pulse sequence for protein backbone assignment
;Reverse transfer amide detection
;with 15N and 13C decoupling
;written by Henry Orton and Jan Stanek

;Avance II+ version
;$COMMENT=5D hNCOCANH
;$CLASS=Solids
;$DIM=5D
;$TYPE=cross polarisation
;$OWNER=CRMN

;p1 : 1H 90 pulse @ plw1
;pl1 : 1H power for 90 pulse
;p7 : 15N 90 pulse @ plw7
;pl7 : 15N power for 90 pulse
;p3 : 13C 90 pulse @ plw3
;pl11 : 13C power for 90 pulse

;p15 : contact time for H->N CP
;sp0 : 1H power for H->N CP
;pl2 : 15N power for H->N CP

;p17 : contact time for N->H CP
;sp10 : 1H power for N->H CP
;pl20 : 15N power for N->H CP

;spnam10 : Ramp100.90
;spoffs10 : 0

;p16 : contact time for N->CO CP
;p26 : contact time for CA->N CP

;spnam0 : Ramp90.100
;spoffs0 : 0

;p18 : Co selective pi
;p19 : Ca selective pi

;cpdprg1 : 1H decoupling (sltppm_40pTr41 for 1Hprot, waltz16_pl12 for 2Hprot)
;cpdprg2 : 15N decoupling (waltz16_pl16)
;cpdprg3 : 13C decoupling (waltz16_pl17)

;pl12 : 1H decoupling power for (waltz16)
;pl13: 1H decoupling (sltppm)
;pl16: 15N decoupling power (waltz16)
;pl17: 13C decoupling power (waltz16)

;pcpd1 : 25u (waltz16 10 kHz) - 33.33 (sltppm 15kHz)
;pcpd2 : 25u (waltz16 10 kHz)
;pcpd3 : 25u (waltz16 10 kHz)

;d1  : recycle delay
;d31  : N evolution (first)
;d32  : Co evolution
;d33  : Ca evolution
```

```
;d34  : N evolution (second)

;$COMMENT=basic cp experiment, arbitrary contact and decoupling schemes
;$CLASS=Solids
;$DIM=1D
;$TYPE=cross polarisation
;$SUBTYPE=simple 1D
;$OWNER=Bruker

prosol relations=<solids_cp>
#include <Avancesolids.incl>

;cnst9 : J(CA-CO) scalar coupling constant (55 Hz)
;cnst10 : estimated bulk T2 of CA [sec]
;cnst11 : estimated bulk T2 of CO [sec]
;cnst21 : CO frequency effective offset, about 173.3 ppm (ppm)
;cnst22 : CA frequency effective offset, about 53.3 ppm (ppm)

"spoal0=0.5"
"spoff0=0.0"
"spoal10=0.5"
"spoff10=0.0"

"spoal2=0.5"
"spoff2=0.0"
"spoal3=0.5"
"spoff3=0.0"
"plw16=plw7*(pow(p7/25,2))"    ; 15N waltz 10kHz decoupling power level

;13C frequencies for center frequency calculation
"cnst20=(sfo3-bf3)*1000000/bf3" ;center frequency of 113.7 ppm

"plw11=plw3"

"spoal8=1.0"
"spoffs8=bf3*((cnst21 - cnst20)/1000000)"                                   ;CO frequency

"spoal9=0.0"
"spoffs9=bf3*((cnst22 - cnst20)/1000000)"                                   ;CA frequency

;CO selective Q3 pulse parameters (refocussing)
"p18=3.448/(95.0*bf3/1000000)"              ;95 ppm bandwidth (safe)
"spw18=plw3*pow((0.5/(p18*0.1515))/(0.25/p3),2)"   ;Q3 power level
"spoal18=0.5"                       ;default value
"spoff18=bf3*((cnst21 - cnst20)/1000000)"       ;CO frequency

;CA selective Q3 pulse parameters (inversion)
"p19=3.41/(105.0*bf3/1000000)"              ;105 ppm bandwidth (safe)
"spw19=plw3*pow((0.5/(p19*0.1515))/(0.25/p3),2)"   ;Q3 power level
"spoal19=0.5"                       ;default value
"spoff19=bf3*((cnst22 - cnst20)/1000000)"       ;CA frequency

;CO selective Q3 pulse parameters (inversion)
"p12=3.41/(95.0*bf3/1000000)"               ;95 ppm bandwidth (safe)
"spw12=plw3*pow((0.5/(p12*0.1515))/(0.25/p3),2)"   ;Q3 power level
"spoal12=0.5"                       ;default value
"spoff12=bf3*((cnst21 - cnst20)/1000000)"       ;CO frequency
```

```
;CA selective REBURP pulse parameters (refocussing)
"p11=4.814/(30*bf3/1000000)"                ;30 ppm bandwidth
"spw11=plw3*pow((0.5/(p11*0.0798))/(0.25/p3),2)"   ;ReBURP power level
"spoal11=0.5"                               ;default value
"spoff11=bf3*((cnst22 - cnst20)/1000000)"                                   ;CA frequency

;Carbon scalar coupling delays
"cnst9=55.0"                                ;J(CA-CO)=55
"d2=2*p7"                                   ;15N 180 delay
"d3=0.25/cnst9"                             ;1/4J(CA-CO)
"d28=0.5*atan(cnst11*PI*cnst9)/(PI*cnst9)"       ;1/4J(CA-CO) relaxation CO optimised delay
"d29=0.5*atan(cnst10*PI*cnst9)/(PI*cnst9)"       ;1/4J(CA-CO) relaxation CA optimised delay

;increments for apsy
"in31=inf1"
"in32=inf2"
"in33=inf3"
"in34=inf4"

;define additional phase increments for off resonance evolution
"cnst25=360*(cnst21-cnst20)*bf3/2"                              ; an intemediate variable (necessary) FOR phase 4
"cnst26=360*(cnst22-cnst20)*bf3/2"                              ; an intemediate variable (necessary) FOR phase 4

;delays for apsy
"d31=0.0"
"d32=0.0"
"d33=0.0"
"d34=0.0"

define delay DELTA1a
define delay DELTA2a
define delay DELTA3a
define delay DELTA4a

define delay DELTA1b
define delay DELTA2b
define delay DELTA3b
define delay DELTA4b

1 ze
2 d1 do:f2 do:f3

#include <p15_prot.incl>
#include <aq_prot.incl>

  "DELTA1a= larger(d28+d2-d32*0.5, 0)"
  "DELTA2a= larger(0, d32*0.5-d3)"
  "DELTA3a= larger(-larger(-d32*0.5, -d3), d28)"
  "DELTA4a= d32*0.5"

  "DELTA1b= larger(d29+d2-d33*0.5, 0)"
  "DELTA2b= larger(d29, d33*0.5)"
  "DELTA3b= -larger(-d33*0.5, -d3)"
  "DELTA4b= larger(0, d33*0.5-d3)"

"cnst23=cnst25*d32" ; CO
"cnst24=cnst26*d33" ; CA
1u ip7+cnst23      ; TPPI-like phase increment for offset evolution of CO
```

```
1u ip11+cnst24      ; TPPI-like phase increment for offset evolution of CA

 1m fq=cnst20(bf ppm):f3                  ;reset the 13C offset to CO

 (p1  pl1 ph1):f1
 (p15 pl2 ph2):f2 (p15:sp0 ph3):f1          ;H -> N CP

 1u cpds1:f1
 ;N t1 evolution
 if "p3*2 > d31" {
  (center (d31) (p3*2 ph0 pl11):f3)
 } else {
  d31
 }

1u do:f1

 (p16:sp8 ph4):f3 (p16:sp2 ph5):f2          ;N -> CO CP
 2u cpds1:f1

;;CO-CA transfer

 (p19:sp19 ph6):f3                      ;CA Q3 (inversion)
 6u
 DELTA1a
 (p18:sp18 ph7):f3                      ;CO Q3 (refocusing)
 4u
 DELTA2a
 (p19:sp19 ph6):f3                      ;CA Q3 (inversion)
 DELTA3a
 (p7*2 ph0 pl7):f2                      ;N 180
 4u
 DELTA4a

  (p3 ph8 pl11):f3                     ;C hard 90

 9u
 DELTA1b
 (p12:sp12 ph10):f3                     ;CO Q3 (inversion)
          4u
 (p11:sp11 ph11):f3                     ;CA REBURP (refocusing)
          4u
 DELTA2b
 (p7*2 ph0 pl7):f2                      ;N 180
 4u
 DELTA3b
 (p12:sp12 ph10):f3                     ;CO Q3 (inversion)
 4u
 DELTA4b
 1u do:f1
 (p26:sp9 ph12):f3 (p26:sp3 ph13):f2        ;CA -> N CP

;;end CO-CA transfer

 1u cpds1:f1

 ;N t4 evolution
 if "p3*2 > d34" {
```

```
   (center (d34) (p3*2 ph0 pl11):f3)
  } else {
    d34
  }
    1u do:f1

;;water suppression starts
  (p7 pl7 ph14):f2,

  1u pl12:f1
  (d30*0.25 cw ph20):f1
  (d30*0.25 cw ph21):f1
  (d30*0.25 cw ph22):f1
  (d30*0.25 cw ph23):f1
  1u do:f1

  (p7 pl7 ph15):f2
;;water suppression ends

  (p17 pl20 ph16):f2 (p17:sp10 ph17):f1       ;N -> H CP

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

  10m mc #0 to 2
  F1PH(calph(ph2, +90), caldel(d31, +in31))
  F2PH(calph(ph4, -90), caldel(d32, +in32))  ; CO
  F3PH(calph(ph12, -90), caldel(d33, +in33)) ; CA
  F4PH(calph(ph13, +90), caldel(d34, +in34))

HaltAcqu, 1m
exit

ph0  = 0
ph1  = 1 3
ph2  = 1
ph3  = 0
ph4  = 1
ph5  = 1 1 3 3
ph6  = 0
ph7  = 0 ;{0}*4 {1}*4
ph8  = 1
ph10 = 1
ph11 = {0}*4 {1}*4
ph12 = 1
ph13 = 0
ph14 = {1}*8 {3}*8
ph15 = 1
ph16 = 0
ph17 = 0
ph20 = 0
ph21 = 1
ph22 = 2
ph23 = 3

ph31 = 0 2 2 0  2 0 0 2
       2 0 0 2  0 2 2 0
```

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