

## Supporting Information

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

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## A. NMR sample preparation

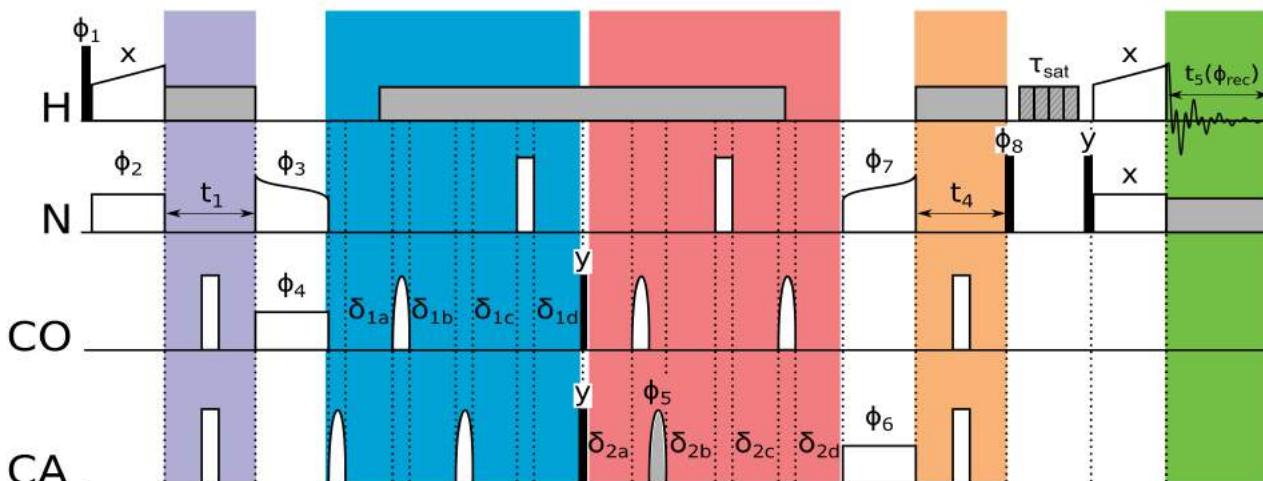
**GB1.** Uniformly  $^{13}\text{C}$ ,  $^{15}\text{N}$ -labelled GB1 was overexpressed, purified, dialysed against phosphate buffer and precipitated to microcrystals in form A according to published protocol.<sup>1-2</sup> The crystals were packed into a 0.7 mm Bruker MAS rotor.

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

**$\beta 2\text{m}_{\text{D76N}}$ .** Expression of  $^2\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ -labelled  $\beta 2\text{m}_{\text{D76N}}$  was performed as described earlier, with purification steps, including refolding, performed in non-deuterated water to allow proton back-exchange at all amide sites.<sup>6</sup> A solution of purified (unlabelled)  $\beta 2\text{m}_{\text{D76N}}$  (100  $\mu\text{M}$   $\beta 2\text{m}$ , 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 ( $G_0$ ).<sup>7</sup> 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  $\beta 2\text{m}_{\text{D76N}}$ .

Microcrystals (GB1 and SOD) and fibrils ( $\beta 2\text{m}_{\text{D76N}}$ ) 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.<sup>8-9</sup>

## 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<sup>10-11</sup> or WALTZ-16<sup>12</sup>  $^1\text{H}$  decoupling with r.f. field of 10 kHz and pulse length of 25  $\mu\text{s}$  was used for GB1 and SOD/ $\beta 2\text{M}_{\text{D76N}}$ , respectively. WALTZ-16 with r.f. field of 10 kHz and pulse length of 25  $\mu\text{s}$  was used for  $^{15}\text{N}$  decoupling during acquisition. Open pulses represent a flip-angle of 180°, while solid pulses are 90°. Bell shapes on carbon channels represent C' or C $\alpha$  selective shaped pulses. All shaped pulses were Q3,<sup>13</sup> with the exception of the C $\alpha$  refocussing pulse (shown in grey), where a REBURP was used.<sup>14</sup> The shaped pulse duration and max. r.f. field strength were 144.3  $\mu\text{s}$  and 22.9 kHz ( $^{13}\text{C}'$ -selective Q3), 129.1  $\mu\text{s}$  and 25.6 kHz ( $^{13}\text{C}\alpha$  selective Q3), and 638.0  $\mu\text{s}$  and 9.82  $\mu\text{s}$  ( $^{13}\text{C}\alpha$  selective ReBURP). The MISSISSIPPI water suppression,<sup>15</sup> with duration  $\tau_{\text{sat}}$  of 150 (for GB1) or 100 ms (SOD,  $\beta 2\text{m}$ ) and r.f. strength of either 53 kHz (GB1), 34 kHz (SOD) or 39 kHz ( $\beta 2\text{m}$ ), is shown in hatched grey. The C $\alpha$ -C' coherence transfer delays are maximally exploited for the either constant-time or shared-time (“semi-constant time”) chemical shift evolution of  $^{13}\text{C}'$

$(t_2)$  and  $^{13}\text{Ca}$  ( $t_3$ ). The respective formulas for delays  $\delta_{1a}, \delta_{1b}, \delta_{1c}, \delta_{1d}$  (or  $\delta_{2a}, \delta_{2b}, \delta_{2c}, \delta_{2d}$ ) vary depending on whether  $t_2$  (or  $t_3$ ) exceeds the relaxation-optimised transfer delay  $2\delta_{\text{CA/CO}}^{\text{opt}} = \tan^{-1}(\pi J_{\text{CA/CO}} / (\pi J))$ , or the relaxation-independent transfer delay  $2\delta = (2^{1}J_{\text{CA-CA}})^{-1}$ . They are determined as follows:

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

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

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

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

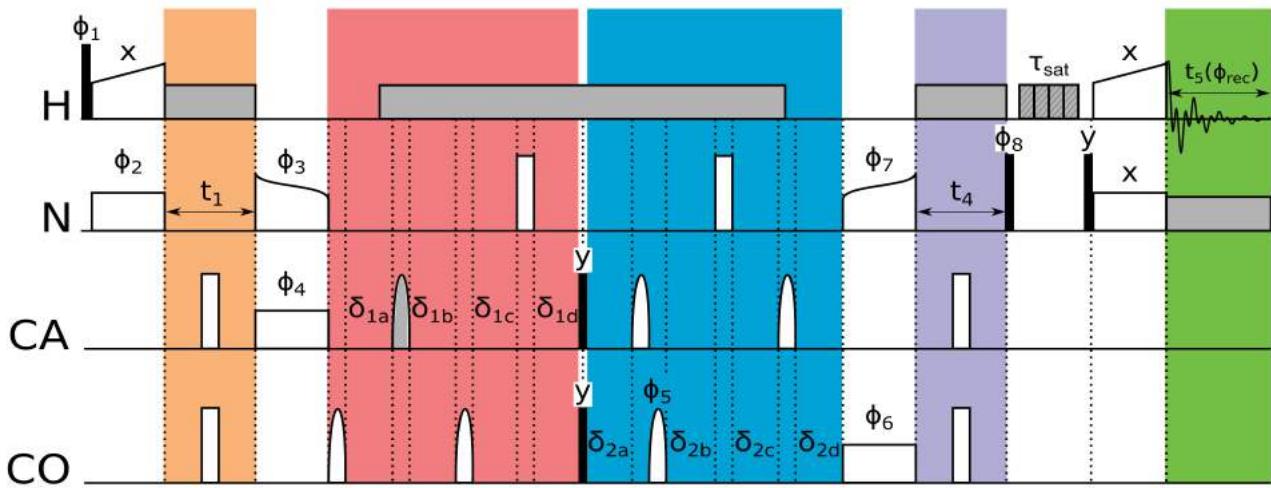
$$\delta_{2a} = \max(\delta_{\text{CA}}^{\text{opt}} + 2\text{pw}_{90}^{\text{N}} - \frac{1}{2}t_3, 0),$$

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

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

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

The coupling constant  $J_{\text{CA-CA}}$  of 55 Hz was assumed, and the bulk  $^{13}\text{C}$  refocussed coherence lifetimes are summarised in Table S5.  $^{15}\text{N}$  offset was set to 117.5 (for GB1), 115.6 (SOD) or 119.5 ( $\beta\text{2M}_{\text{D76N}}$ ) ppm while  $^1\text{H}$  offset was set on resonance with  $\text{H}_2\text{O}$  line. The carbon carrier frequency was set to the centre of the  $\text{Ca}/\text{C}'$  spectrum at 114 ppm for the entire experiment. Shaped pulses and CP transfer offsets were set to -60 and +60 ppm for the  $\text{Ca}$  and  $\text{C}'$  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  $^{13}\text{C}$  pulse for  $^{15}\text{N} \rightarrow ^{13}\text{C}'$  and  $^{13}\text{Ca} \rightarrow ^{15}\text{N}$  CP transfers, respectively. A time-dependent phase increment was applied to carbon refocussing pulses to control the centre of the respective carbon dimension ( $\text{Ca}$  or  $\text{C}'$ ) and was calculated in degrees as  $180^{\circ}\Omega^*t$  where  $\Omega$  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  $\beta\text{2M}_{\text{D76N}}$ , 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  $\phi_{\text{rec}} = \{x, -x, -x, x, -x, x, x, -x, -x, x, x, -x, x, -x, -x, x\}$ . Quadrature detection in the indirect dimensions was accomplished by incrementing  $\phi_2$  (in  $\omega_1$ ), decrementing  $\phi_4$  (in  $\omega_2$ ), decrementing  $\phi_6$  (in  $\omega_3$ ), or incrementing  $\phi_7$  (in  $\omega_4$ ), 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  $\delta_{1a}$  and  $\delta_{1c}$  are determined with the use of  $\delta_{\text{CA}}^{\text{opt}}$ , while delays  $\delta_{2a}$ ,  $\delta_{2b}$  and  $\delta_{2d}$  depend on  $\delta_{\text{CO}}^{\text{opt}}$ . The zero phase is enforced (aligned) at the end of  $^{13}\text{Ca}$ , and at the beginning of the  $^{13}\text{C}'$  pulse for the  $^{15}\text{N} \rightarrow ^{13}\text{C}'$  and  $^{13}\text{C}' \rightarrow ^{15}\text{N}$  CP transfers, respectively.  $^{13}\text{Ca}$  and  $^{13}\text{C}'$  shaped pulses are exchanged to preserve their context, i.e. the  $^{13}\text{Ca}$  refocussing pulse (ReBURP) is performed between  $\delta_{1a}$  and  $\delta_{1b}$ , etc.

## C. NMR data acquisition details

Table S1: (H)NCACONH experiment acquisition parameters

Sample	Max. evolution time, ms in dimension					Spectral widths, ppm <sup>a</sup> in dimension					MAS rate, kHz	Scans	Efficiency <sup>e</sup>	Number of projec- tions	Time, h
	$\omega_1$ N	$\omega_2$ $\text{C}\alpha$	$\omega_3$ $\text{C}'$	$\omega_4$ N	$\omega_5$ H	$\omega_1$ N	$\omega_2$ $\text{C}\alpha$	$\omega_3$ $\text{C}'$	$\omega_4$ N	$\omega_5$ H					
GB1	18.1	10.0	13.9	18.1	20.5 <sup>b</sup>	30	25	12	30	100	110	32	0.21	24	40.5
SOD	13.1	9.1	9.9	13.1	24.8 <sup>c</sup>	37	28	12	37	100	60	80	0.09	32	72.0
$\beta 2\text{M}_{\text{D}76\text{N}}$	9.9	7.0	8.0	9.9	20.5 <sup>d</sup>	30	30	10	30	100	55	48	0.09	32	57.5

<sup>a</sup> at the static field used (23.5 T) 1 ppm  $^1\text{H}$  = 1000.3 Hz, 1 ppm  $^{13}\text{C}$  = 251.5 Hz, and 1 ppm  $^{15}\text{N}$  = 101.4 Hz

<sup>b</sup> truncated to 10.0 ms during data processing

<sup>c</sup> truncated to 9.9 ms during data processing

<sup>d</sup> truncated to 7.2 ms during data processing

<sup>e</sup> 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 <sup>a</sup> in dimension					MAS rate, kHz	Scans	Efficiency <sup>e</sup>	Number of projec- tions	Time, h
	$\omega_1$ N	$\omega_2$ $\text{C}'$	$\omega_3$ $\text{Ca}$	$\omega_4$ N	$\omega_5$ H	$\omega_1$ N	$\omega_2$ $\text{C}'$	$\omega_3$ $\text{Ca}$	$\omega_4$ N	$\omega_5$ H					
GB1	18.1	13.9	9.9	18.1	20.5 <sup>b</sup>	30	12	26	30	100	110	32	0.18	24	41.5
SOD	13.1	9.9	9.1	13.1	24.8 <sup>c</sup>	37	12	28	37	100	60	80	0.11	32	71.0
$\beta 2\text{M}_{\text{D}76\text{N}}$	9.9	8.0	7.0	9.9	20.5 <sup>d</sup>	30	10	30	30	55	55	48	0.09	32	58.0

<sup>a</sup> at the static field used (23.5 T) 1 ppm  $^1\text{H}$  = 1000.3 Hz, 1 ppm  $^{13}\text{C}$  = 251.5 Hz, and 1 ppm  $^{15}\text{N}$  = 101.4 Hz

<sup>b</sup> truncated to 10.0 ms during data processing

<sup>c</sup> truncated to 9.9 ms during data processing

<sup>d</sup> truncated to 7.2 ms during data processing

<sup>e</sup> 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	$\beta 2\text{M}_{\text{D}76\text{N}}$	
<b>(H)NCACONH</b>				
1 ( $^1\text{H} \rightarrow ^{15}\text{N}$ )	contact time, ms	2.3	1.25	1.0
	$^1\text{H}$ r.f. max. field, kHz	149.1	103.5	95.7
	$^1\text{H}$ r.f. amplitude ramp <sup>a</sup>	+10%	+10%	+10%
	$^{15}\text{N}$ r.f. field (const), kHz	33.4	35.2	35.0
2 ( $^{15}\text{N} \rightarrow ^{13}\text{C}\alpha$ )	contact time, ms	19.0	19.0	18.0
	$^{15}\text{N}$ r.f. max. field, kHz <sup>b</sup>	42.8	27.0	30.9
	$^{15}\text{N}$ r.f. average field, kHz <sup>b</sup>	37.2	23.5	26.8
	$^{15}\text{N}$ r.f. modulation depth, kHz <sup>b</sup>	5.6	3.5	4.0
	$^{15}\text{N}$ r.f. effective coupling, Hz <sup>b</sup>	1359	858	980
	$^{13}\text{C}\alpha$ r.f. field (const), kHz	70.0	43.5	25.0
3 ( $^{13}\text{C}' \rightarrow ^{15}\text{N}$ )	contact time, ms	19.0	14.0	16.0
	$^{13}\text{C}'$ r.f. field (const), kHz	70.0	43.9	25.0
	$^{15}\text{N}$ r.f. max. field, kHz <sup>b</sup>	43.3	27.0	31.3
	$^{15}\text{N}$ r.f. average field, kHz <sup>b</sup>	37.7	23.5	27.2
	$^{15}\text{N}$ r.f. modulation depth, kHz <sup>b</sup>	-5.7	-3.5	4.1
	$^{15}\text{N}$ r.f. effective coupling, Hz <sup>b</sup>	1376	858	993
4 ( $^1\text{H} \rightarrow ^{15}\text{N}$ )	contact time, ms	0.7	0.6	0.4
	$^{15}\text{N}$ r.f. field (const), kHz	33.4	35.2	35.0
	$^1\text{H}$ r.f. max. field, kHz	143.9	100.7	94.5
	$^1\text{H}$ r.f. amplitude ramp <sup>a</sup>	+10%	+10%	+10%
<b>(H)NCOCANH</b>				
1 ( $^1\text{H} \rightarrow ^{15}\text{N}$ )	as for (H)NCACONH			
2 ( $^{15}\text{N} \rightarrow ^{13}\text{C}'$ )	contact time, ms	19.0	14.0	16.0
	$^{15}\text{N}$ r.f. max. field, kHz <sup>b</sup>	42.8	27.0	31.1
	$^{15}\text{N}$ r.f. average field, kHz <sup>b</sup>	37.2	23.5	27.0

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

CP transfer no		Sample		
		GB1	SOD	$\beta_2 M_{D76N}$
3 ( $^{13}\text{C}\alpha \rightarrow ^{15}\text{N}$ )	$^{15}\text{N}$ r.f. modulation depth, kHz <sup>b</sup>	5.6	3.5	4.1
	$^{15}\text{N}$ r.f. effective coupling, Hz <sup>b</sup>	1359	858	986
	$^{13}\text{C}'$ r.f. field (const), kHz	70.0	44.4	25.0
3 ( $^{13}\text{C}\alpha \rightarrow ^{15}\text{N}$ )	contact time, ms	19.0	17.0	18.0
	$^{13}\text{C}\alpha$ r.f. field (const), kHz	70.0	44.3	25.0
	$^{15}\text{N}$ r.f. max. field, kHz <sup>b</sup>	43.3	27.0	30.9
	$^{15}\text{N}$ r.f. average field, kHz <sup>b</sup>	37.7	23.5	26.8
	$^{15}\text{N}$ r.f. modulation depth, kHz <sup>b</sup>	-5.7	-3.5	4.0
	$^{15}\text{N}$ r.f. effective coupling, Hz <sup>b</sup>	1376	858	980
4 ( $^1\text{H} \rightarrow ^{15}\text{N}$ )	as for (H)NCACONH			

<sup>a</sup> An x % ramp means a linear amplitude modulation from (1-x/100) to 1.0

<sup>b</sup> The tangential shapes (for adiabatic CP<sup>16</sup>) are defined by 4 empirical parameters: the average r.f. field  $\omega_{avg}$  (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  $\Delta$ , and the contact time  $\tau$ , according to the following equation:

$$\omega(t) = \omega_{avg} + d^{eff} \tan \left( \frac{2}{\tau} \arctan \left( \frac{\Delta}{d^{eff}_N} \right) \left( \frac{\tau}{2} - t \right) \right)$$

**Table S4: Projection angles used in APSY series**

No	$\alpha^a$	$\beta^a$	$\gamma^a$	Number of projections	Number of time points <sup>b</sup>
<b>GB1: (H)NCACONH</b>					
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	$\pm 37.6$	0.0	0.0	2	69x2x2
7-8	0.0	$\pm 29.0$	0.0	2	79x2x2
9-10	0.0	0.0	$\pm 45.0$	2	78x2x2
11-12	90.0	$\pm 35.8$	0.0	2	71x2x2
13-14	90.0	0.0	$\pm 52.4$	2	69x2x2
15-16	0.0	90.0	$\pm 61.0$	2	79x2x2
17-20	$\pm 37.6$	$\pm 23.7$	0.0	4	89x2x2x2
21-24	$\pm 37.6$	0.0	$\pm 38.4$	4	89x2x2x2
Total				24	3634
<b>GB1: (H)NCOCANH</b>					
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	$\pm 28.8$	0.0	0.0	2	80x2x2
7-8	0.0	$\pm 37.6$	0.0	2	69x2x2
9-10	0.0	0.0	$\pm 45.0$	2	78x2x2
11-12	90.0	$\pm 54.5$	0.0	2	72x2x2
13-14	90.0	0.0	$\pm 61.2$	2	80x2x2
15-16	0.0	90.0	$\pm 52.4$	2	69x2x2
17-20	$\pm 28.8$	$\pm 34.0$	0.0	4	90x2x2x2
21-24	$\pm 28.8$	0.0	$\pm 41.2$	4	96x2x2
Total				24	3350

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

No	$\alpha^a$	$\beta^a$	$\gamma^a$	Number of projections	Number of time points <sup>b</sup>
<b>SOD: (H)NCACONH</b>					
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	$\pm 37.3$	0.0	0.0	2	57x2x2
7-8	0.0	$\pm 34.8$	0.0	2	77x2x2
9-10	0.0	0.0	$\pm 45.0$	2	70x2x2
11-12	90.0	$\pm 42.4$	0.0	2	66x2x2
13-14	90.0	0.0	$\pm 52.7$	2	57x2x2
15-16	0.0	90.0	$\pm 55.2$	2	77x2x2
17-20	$\pm 37.3$	$\pm 29.0$	0.0	4	81x2x2x2
21-24	$\pm 37.3$	0.0	$\pm 38.5$	4	75x2x2x2
25-28	0.0	$\pm 34.8$	$\pm 39.4$	4	91x2x2x2
29-32	90.0	$\pm 42.4$	$\pm 44.1$	4	81x2x2x2
Total				32	4624
<b>SOD: (H)NCOCANH</b>					
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	$\pm 34.8$	0.0	0.0	2	77x2x2
7-8	0.0	$\pm 37.3$	0.0	2	57x2x2
9-10	0.0	0.0	$\pm 45.0$	2	70x2x2
11-12	90.0	$\pm 47.6$	0.0	2	66x2x2
13-14	90.0	0.0	$\pm 55.2$	2	77x2x2
15-16	0.0	90.0	$\pm 52.7$	2	57x2x2
17-20	$\pm 34.8$	$\pm 32.0$	0.0	4	81x2x2x2
21-24	$\pm 34.8$	0.0	$\pm 39.4$	4	91x2x2x2
25-28	0.0	$\pm 37.3$	$\pm 38.5$	4	75x2x2x2
29-32	90.0	$\pm 47.6$	$\pm 44.1$	4	81x2x2x2
Total				32	4624
<b><math>\beta</math>2M<sub>D76N</sub>: (H)NCACONH</b>					
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	$\pm 38.9$	0.0	0.0	2	36x2x2
7-8	0.0	$\pm 35.4$	0.0	2	55x2x2
9-10	0.0	0.0	$\pm 45.0$	2	43x2x2
11-12	90.0	$\pm 41.5$	0.0	2	50x2x2
13-14	90.0	0.0	$\pm 51.1$	2	36x2x2
15-16	0.0	90.0	$\pm 54.6$	2	55x2x2
17-20	$\pm 38.9$	$\pm 29.0$	0.0	4	57x2x2x2
21-24	$\pm 38.9$	0.0	$\pm 37.9$	4	47x2x2x2
25-28	0.0	$\pm 35.4$	$\pm 39.2$	4	62x2x2x2
29-32	90.0	$\pm 41.5$	$\pm 42.9$	4	57x2x2x2
Total				32	3150

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

No	$\alpha^a$	$\beta^a$	$\gamma^a$	Number of projections	Number of time points <sup>b</sup>
<b><math>\beta_2M_{D76N}</math>: (H)NCOCANH</b>					
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	$\pm 35.4$	0.0	0.0	2	55x2x2
7-8	0.0	$\pm 38.9$	0.0	2	36x2x2
9-10	0.0	0.0	$\pm 45.0$	2	43x2x2
11-12	90.0	$\pm 48.5$	0.0	2	50x2x2
13-14	90.0	0.0	$\pm 54.6$	2	55x2x2
15-16	0.0	90.0	$\pm 51.1$	2	36x2x2
17-20	$\pm 35.4$	$\pm 33.3$	0.0	4	57x2x2x2
21-24	$\pm 35.4$	0.0	$\pm 39.2$	4	62x2x2x2
25-28	0.0	$\pm 38.9$	$\pm 37.9$	4	47x2x2x2
29-32	90.0	$\pm 48.5$	$\pm 42.9$	4	57x2x2x2
Total			32	3150	

<sup>a</sup> Time increments in the indirect dimensions are determined by angles  $\alpha$ ,  $\beta$  and  $\gamma$  in the following way:

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

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

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

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

<sup>b</sup> 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  $^1H^N$ ,  $^{15}N$ ,  $^{13}Ca$ , and  $^{13}C'$  refocussed coherence lifetimes under fast MAS and low-power decoupling

Sample	MAS rate, kHz	Refocussed coherence lifetime, ms			
		$^{13}C'$	$^{13}Ca$	$^{15}N$	$^1H^N$
GB1	110	89 <sup>a</sup>	71 <sup>a</sup>	120 <sup>a,b</sup>	4.7
SOD	60	36 <sup>c</sup>	22 <sup>c</sup>	42 <sup>c,d</sup>	9.0 <sup>d</sup>
$\beta_2M_{D76N}$	55	36 <sup>c</sup>	23 <sup>c</sup>	30	4.7

<sup>a</sup> Under 10 kHz swept-TPPM decoupling<sup>10-11</sup>

<sup>b</sup> Reported by Andreas et al<sup>17</sup> for an equivalent sample of GB1, at identical MAS rate and  $B_0$  field

<sup>c</sup> Under 10 kHz WALTZ-16 decoupling<sup>12</sup>

<sup>d</sup> Reported by Knight et al<sup>3</sup> for an equivalent sample of SOD-Cu<sup>1</sup>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<sup>18</sup> and 6D APSY HNCOCANH.<sup>19</sup> In the following discussion we will not consider two other experiments, 4D HN(CA)NH<sup>20</sup> and 3D TROSY-(H)N(CA)NH<sup>21</sup>, 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"):<sup>19</sup>

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

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

where  $\bar{R}_{2,H}$  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  $\tau_1 = (\pi J_{NH})^{-1} \tan(\pi J_{NH}/\bar{R}_{2,H})$  and equals to:

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

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

- 2)  $2H^{N(i)}_z N^{(i)}_z \rightarrow 2N^{(i)}_z C^{(i-1)}_z$  (from point "s" to "t"):

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

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

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

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

- 3)  $2N^{(i)}_z C^{(i-1)}_z \rightarrow 4N^{(i)}_z C^{(i-1)}_z C^{\alpha(i-1)}_z$  (from point "t" to "u"):

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

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

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

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

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

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

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

with the exception of *i*-1 glycine residues where C<sup>α</sup> does not dephase due to <sup>1</sup>J<sub>CαCβ</sub>. The middle term  $\cos(\pi^1 J_{C\alpha C\beta} \tau_4)$  can be omitted for all *i*-1 residues except TSNDLFY, for which the evolution under <sup>1</sup>J<sub>CαCβ</sub> can be eliminated with a suitably selective C<sup>α</sup> refocusing pulse. R<sub>2,Cα</sub> stands for relaxation rate of SQ C<sup>α</sup> coherence, and delay  $\tau_{C\alpha C'} = (2^1 J_{C\alpha C'})^{-1}$  can be exactly matched to maximize the transfer. The best choice of the delay  $\tau_4$  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  $\sin(\pi^2 J_{NC\alpha} \tau_4) \sin(\pi^1 J_{NC\alpha} \tau_4)$  while suppressing the  $\cos(\pi^2 J_{NC\alpha} \tau_4) \cos(\pi^1 J_{NC\alpha} \tau_4)$  term, due to coincidence of coupling constant values. The best value of  $\tau_4$  can be found only numerically, e.g. by iterating the formula:

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

For relaxation rates above  $R_{2,C\alpha} \approx 30$  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  $\tau_4 = 2R_{2,C\alpha}\xi^{-1} \text{atan } \xi$ , where  $\xi = \pi({}^2J_{NC\alpha} + {}^1J_{NC\alpha})/R_{2,C\alpha}$ , and the precise one can be found using an iterative scheme:

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

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

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

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

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

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

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

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

- 6)  $2H^{N(i-1)}_z N^{(i-1)}_z \rightarrow H^{N(i-1)}_{x/y}$  or  $2H^{N(i)}_z N^{(i)}_z \rightarrow H^{N(i)}_{x/y}$  (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  $\sim 25$  kDa. The transfer function is roughly approximated by an average of efficiency of two transfer pathways:

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

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

$$f_{SQ,Z} = \exp(-R_{2,N}\tau_{6a}) \exp(-R_{1,H}\tau_{6b}) \sin(\pi J_{NH}\tau_{6a})$$

where  $R_{2MQ}$  stands for relaxation rate of  $2H_N$  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  ${}^{15}N^\ddagger H\beta$  (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:

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

Here again the factor  $\sqrt{2}$  accounts for the increased sensitivity in multidimensional experiment due to preservation of equivalent pathways after  ${}^{15}N$  evolution ( ${}^{15}N^\ddagger$ ), the factor  $\frac{1}{2}$  reflects the loss due to selection of TROSY coherence, and the factor  $\frac{1}{4}$  which averages four pathways necessary to transfer TROSY coherence, for which the efficiencies are as follows:

$$\begin{aligned}
f_{SQ,SQ} &= \exp(-\bar{R}_{2,N}\tau_{6a}) \exp(-\bar{R}_{2,H}\tau_{6b}) \\
f_{MQ,MQ} &= \exp(-R_{2MQ}\tau_{6a}) \exp(-R_{2MQ}\tau_{6b}) \\
f_{SQ,MQ} &= \exp(-\bar{R}_{2,N}\tau_{6a}) \exp(-R_{2MQ}\tau_{6b}) \\
f_{MQ,SQ} &= \exp(-R_{2MQ}\tau_{6a}) \exp(-\bar{R}_{2,H}\tau_{6b})
\end{aligned}$$

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:  $^1J_{NH} = 93$  Hz,  $^1J_{C\alpha N} = 11$  Hz,  $^2J_{C\alpha N} = 7$  Hz,  $^1J_{NC'} = 15$  Hz,  $^1J_{C\alpha C'} = 55$  Hz,  $^1J_{C\alpha C\beta} = 35$  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  $\pi/2$  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:

$$f_{LS} = f_{RF} f_1^{max} f_2^{max} f_3^{max} f_4^{max} f_5^{max} f_6^{max}$$

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  $\tau_C \approx q \text{ MW}$  where  $q = 1 \text{ ns} / 2.6 \text{ kDa}$ , 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,  $\bar{R}_{2,H}$ , with a contribution of dipolar interactions to nearby proton, nitrogen and carbon (and, optionally, deuterium) spins, and CSA of  $\Delta\sigma_H = -16$  ppm:

$$\bar{R}_{2,H} = \frac{1}{2} \left( R_2^{NH}(H) + R_2^{NH}(2HN_z) + R_1^{CSA}(N) \right) + R_2^{HH}(H) + R_2^{CH}(H) + R_2^{DH}(H) + R_2^{CSA}(H)$$

- b) amide nitrogen SQ coherence relaxation rate,  $\bar{R}_{2,N}$ , with a contribution of dipolar interactions only to nearby proton and carbon spins, and CSA of  $\Delta\sigma_N = 164.5$  ppm:

$$\bar{R}_{2,N} = \frac{1}{2} \left( R_2^{NH}(N) + R_2^{NH}(2NH_z) + R_1^{HH}(H) + R_1^{CSA}(H) \right) + R_2^{NC}(N) + R_2^{CSA}(N)$$

and, for purely in-phase ( $^1H$ -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:

$$R_{2,C'} = R_2^{CH}(C') + R_2^{CN}(C') + R_2^{CC}(C') + R_2^{CD}(C') + R_2^{CSA}(C')$$

- 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:

$$R_{2,C\alpha} = R_2^{C\alpha H}(C\alpha) + R_2^{C\alpha N}(C\alpha) + R_2^{C\alpha C}(C\alpha) + R_2^{C\alpha D}(C') + R_2^{CSA}(C\alpha)$$

- 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:

$$R_{2MQ} = \frac{1}{2} \left( R_2^{NH}(N^\pm H^\mp) + R_2^{NH}(N^\pm H^\pm) \right) + R_2^{HH}(H) + R_2^{DH}(H) + R_2^{CH}(H) + R_2^{CSA}(H) + R_2^{CSA}(N)$$

- f) amide-proton and amide-nitrogen TROSY coherence relaxation rates:

$$R_{2,H(N\beta)} = \bar{R}_{2,H} + \eta_{xy}^{NH,H}(H)$$

$$R_{2,N(H\beta)} = \bar{R}_{2,N} + \eta_{xy}^{NH,N}(N)$$

The respective terms were calculated according to well-known formulas for dipolar relaxation:<sup>22</sup>

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

(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 / 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,  $d(^{13}Ca, ^1H\alpha) = 2.1$  Å,  $d(^{13}Ca, ^1H^N i+1) = 2.5$  Å,  $d(^{13}Ca, ^1H\beta_2) = 2.1$  Å,  $d(^{13}Ca, ^1H\beta_3) = 2.1$  Å,  $d(^{15}N, ^{13}Ca) = 1.47$  Å,  $d(^{15}N, ^{13}C') = 1.32$  Å,  $d(^{13}C', ^{13}Ca) = 1.54$  Å,  $d(^{13}C\beta, ^{13}Ca) = 1.54$  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  $I = H, N, C\alpha$ , 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  $\Theta = 17^\circ$  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)<sup>17, 23</sup>:

$$\begin{aligned} f_1(CP: H \rightarrow N) &= 0.9 \\ f_2(CP: N \rightarrow C') &= 0.57 \\ f_4(CP: C\alpha \rightarrow N) &= 0.39 \\ f_5(CP: N \rightarrow H) &= 0.58 \end{aligned}$$

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

Labelling	MAS rate, kHz	Refocussed coherence lifetime, ms	
		$^{13}C'$	$^{13}C\alpha$
U- $^1H$ , $^2H$ , $^{13}C$ , $^{15}N$	60	40	25
U- $^1H$ , $^{13}C$ , $^{15}N$	60	26	8.6
U- $^1H$ , $^{13}C$ , $^{15}N$	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 <sup>a</sup>	Direct dimension		Indirect dimension	
	N <sup>b</sup>	Window <sup>c</sup>	N <sup>b</sup>	Window <sup>c</sup>
GB1	8192	QSINE 3	1024	QSINE 3
SOD	8192	QSINE 3	1024	QSINE 3
$\beta 2M_{D76N}$	8192	QSINE 4	1024	QSINE 3

<sup>a</sup> Identical processing parameters were used for forward and backward 5D experiments

<sup>b</sup> Number of points in the real part of the Fourier Transformed signal

<sup>c</sup> 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 <sup>a</sup>	S <sub>min1,2</sub> <sup>b</sup>	$\Delta v_{\min}$ <sup>c</sup> Hz	r <sub>min</sub> <sup>d</sup> Hz	S/N <sup>e</sup>	<sup>1</sup> H range, ppm
GB1	7	50	40	6	[5.6, 11.7]
SOD	7	100	80	6	[5.4, 10.9]
$\beta 2M_{D76N}$	6	160	60	6	[6.9, 12.4]

<sup>a</sup> Identical parameters were used for forward and backward 5D experiments

<sup>b</sup> Minimal support = the number of projections confirming a 5D peak coordinates

<sup>c</sup> Peak matching tolerance in the directly detected dimension

<sup>d</sup> Peak matching tolerance in the indirectly detected dimensions

<sup>e</sup> Minimum signal-to-noise ratio of a peak

## G. 5D peak lists for GB1, SOD and $\beta 2M_{D76N}$

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 (<sup>1</sup>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	106.733	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<sup>1</sup>,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
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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<sup>1</sup>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</td						

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<sub>D76N</sub>

# 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<sub>D76N</sub>

# 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 hNCACNH 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<sub>D76N</sub> 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.<sup>17</sup> For Cu<sup>1</sup>Zn-SOD, we used an adapted resonance assignment obtained by Knight et al<sup>3</sup> (BMRB entry 18509). The shifts of <sup>13</sup>C were recalibrated by +2.65 ppm to account for the difference in <sup>13</sup>C reference between DSS (assumed by FLYA) and TMS (as deposited in BMRB), while the shifts of <sup>13</sup>C<sub>α</sub> were additionally increased by 0.66 ppm to roughly compensate <sup>2</sup>H-induced shifts. The reference resonance assignment of Cu<sup>1</sup>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<sub>D76N</sub> were obtained independently based on a set of six <sup>1</sup>H<sup>N</sup>-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 <sup>a</sup>

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

<sup>a</sup> The parameter naming convention (highlighted in blue) as used in the following FLYA batch script (\*cya):  
structurepeaks:=

assignpeaks:=hNCACONH,hNCOCANH

# Assignment tolerances

assignncs\_accH:=  $T_H$

assignncs\_accC:=  $T_C$

assignncs\_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				Max
		correct <sup>a</sup>	incompatible <sup>a</sup>	additional <sup>a</sup>	all	
GB1	$^1\text{H}^N$	55	0	0	55	55 <sup>b</sup>
	$^{15}\text{N}$	55	0	1	56	56 <sup>c</sup>
	$^{13}\text{C}\alpha$	55	0	0	55	56
	$^{13}\text{C}'$	55	0	0	55	56
	all	220	0	1	221	223
SOD	$^1\text{H}^N$	65	7	3	75	127 <sup>b, e</sup>
	$^{15}\text{N}$	74	3	0	77	127 <sup>c, e</sup>
	$^{13}\text{C}\alpha$	71	6	1	78	133 <sup>e</sup>
	$^{13}\text{C}'$	70	6	1	77	133 <sup>e</sup>
	all	280	22	5	307	520 <sup>e</sup>
$\beta 2\text{M}_{D76N}$	$^1\text{H}^N$	20	1	0	21	57 <sup>b, f</sup>
	$^{15}\text{N}$	21	0	0	21	57 <sup>c, f</sup>
	$^{13}\text{C}\alpha$	20	1	0	21	59 <sup>f</sup>
	$^{13}\text{C}'$	26	3	0	29	59 <sup>f</sup>
	all	87	5	0	92	232 <sup>f</sup>

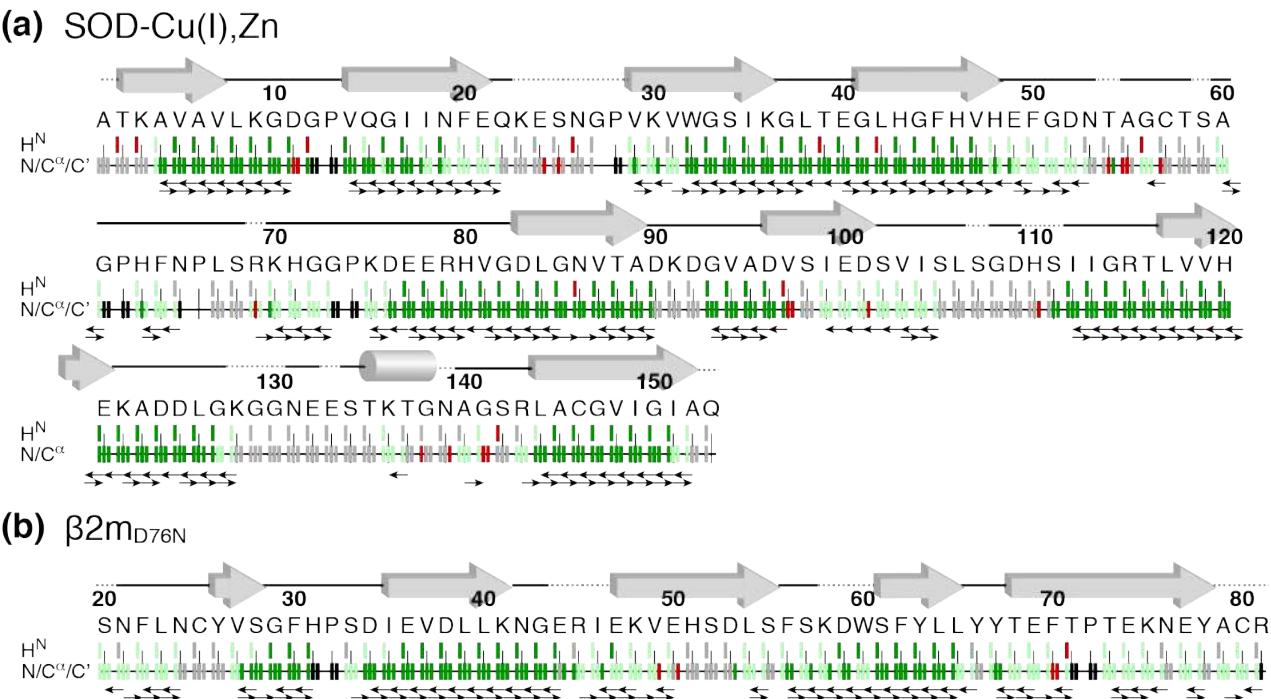
<sup>a</sup>With respect to the reference assignment

<sup>b</sup> The number of amide protons, i.e. excluding proline residues and N-terminus

<sup>c</sup> excluding proline  $^{15}\text{N}$  atoms

<sup>e</sup> discounting dynamic residues 1-3, 23-27, 54, 59, 69, 107, 110-111, 128-130, 133, 139, 153

<sup>f</sup> 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<sup>1</sup>Zn-SOD and (b) β2M<sub>D76N</sub> 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 C $\beta$ information

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

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

In the case of Cu<sup>I</sup>Zn-SOD, a synthetic peak list with 111 entries was constructed from published Cβ shifts of Cu<sup>II</sup>Zn-SOD,<sup>5</sup> and reference <sup>15</sup>N and <sup>1</sup>H<sup>N</sup> shifts. In the case of β2m<sub>D76N</sub> 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 C $\beta$  shifts in the spin system  
 analyzeassign\_group := BB; N H CA CB C

```
    ,      g _g -  
command select_atoms  
atom select "N H CA CB C"  
end
```

**Table S16: FLYA assignment summary (the calculations with additional C $\beta$  information)**

Protein	Nucleus	Number of “strong” assignments				Max
		correct <sup>a</sup>	incompatible <sup>a</sup>	additional <sup>a</sup>	all	
SOD	$^1\text{H}^{\text{N}}$	90	3	3	96	127 <sup>b, e</sup>
	$^{15}\text{N}$	89	3	3	95	127 <sup>c, e</sup>
	$^{13}\text{C}\alpha$	84	3	2	89	133 <sup>e</sup>
	$^{13}\text{C}'$	83	4	3	90	133 <sup>e</sup>
	$^{13}\text{C}\beta$	71	6	1	78	107 <sup>e</sup>
	all- $^{13}\text{C}\beta$	346	13	11	370	520 <sup>e</sup>
	all	417	19	12	448	637 <sup>e</sup>
$\beta 2\text{M}_{D76\text{N}}$	$^1\text{H}^{\text{N}}$	43	2	1	46	57 <sup>b, f</sup>
	$^{15}\text{N}$	45	1	2	48	57 <sup>c, f</sup>
	$^{13}\text{C}\alpha$	39	0	2	41	59 <sup>f</sup>
	$^{13}\text{C}'$	39	2	0	41	59 <sup>f</sup>
	$^{13}\text{C}\beta$	41	0	2	43	55 <sup>f</sup>
	all- $^{13}\text{C}\beta$	166	5	5	176	232 <sup>f</sup>
	all	207	5	7	219	287 <sup>f</sup>

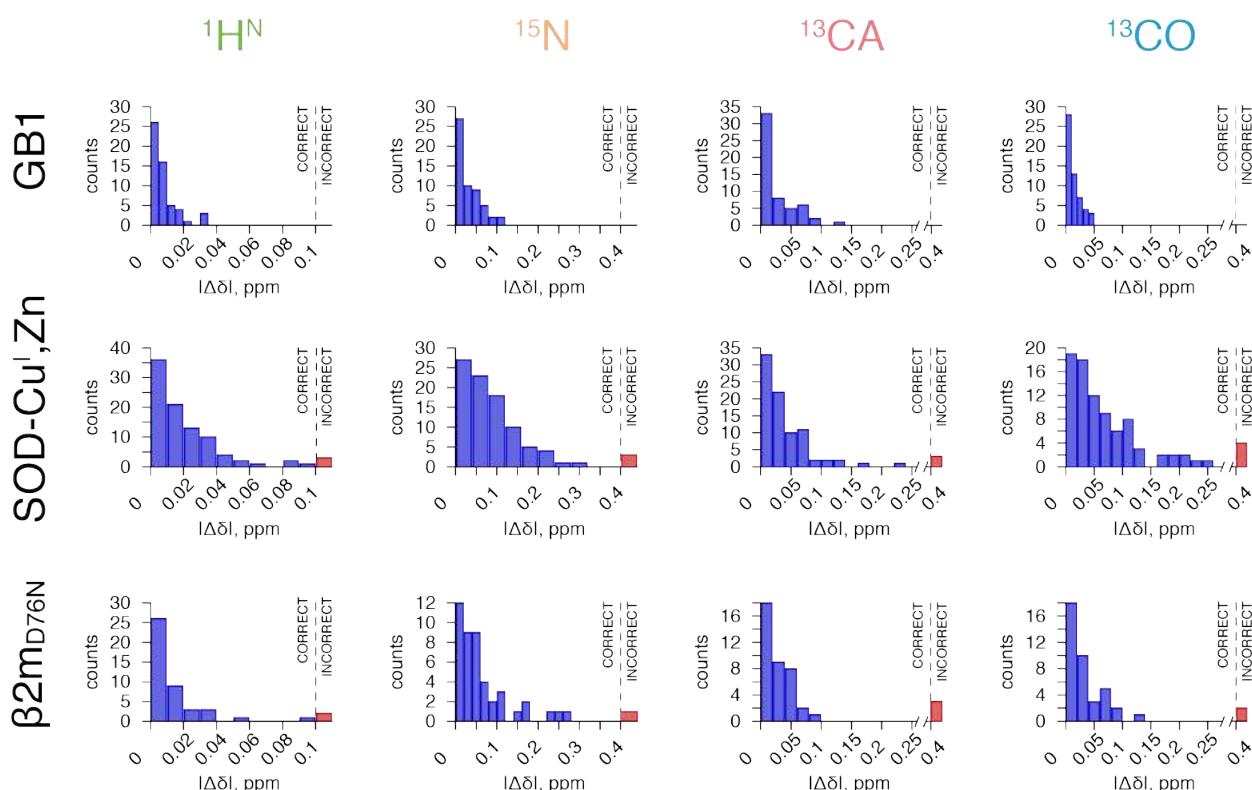
<sup>a</sup>With respect to the reference assignment

<sup>b</sup>The number of amide protons, i.e. excluding proline residues and N-terminus

<sup>c</sup>excluding proline  $^{15}\text{N}$  atoms

<sup>e</sup>discounting dynamic residues 1-3, 23-27, 54, 59, 69, 107, 110-111, 128-130, 133, 139, 153

<sup>f</sup>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) FOF phase 4
"cnst26=360*(cnst21-cnst20)*bf3/2" ; an intemediate variable (necessary) FOF 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  
DETA1a  
(p11:sp11 ph7):f3 ;CA REBURP (refocusing)  
4u  
DETA2a  
(p12:sp12 ph6):f3 ;CO Q3 (inversion)  
DETA3a  
(p7*2 ph0 pl7):f2 ;N 180  
4u  
DETA4a  
  
(p3 ph8 pl11):f3 ;C hard 90
```

```
9u  
DETA1b  
(p19:sp19 ph10):f3 ;CA Q3 (inversion)  
4u  
(p18:sp18 ph11):f3  
    ;CO Q3 (refocusing)  
4u  
DETA2b  
(p7*2 ph0 pl7):f2 ;N 180  
4u  
DETA3b  
(p19:sp19 ph10):f3 ;CA Q3 (inversion)  
4u  
DETA4b  
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))          ; CA
F2PH(calph(ph4, -90), caldel(d32, +in32))          ; CO
F3PH(calph(ph12, -90), caldel(d33, +in33))
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) FOF phase 4
"cnst26=360*(cnst22-cnst20)*bf3/2" ; an intemediate variable (necessary) FOF 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

```

## K. References

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