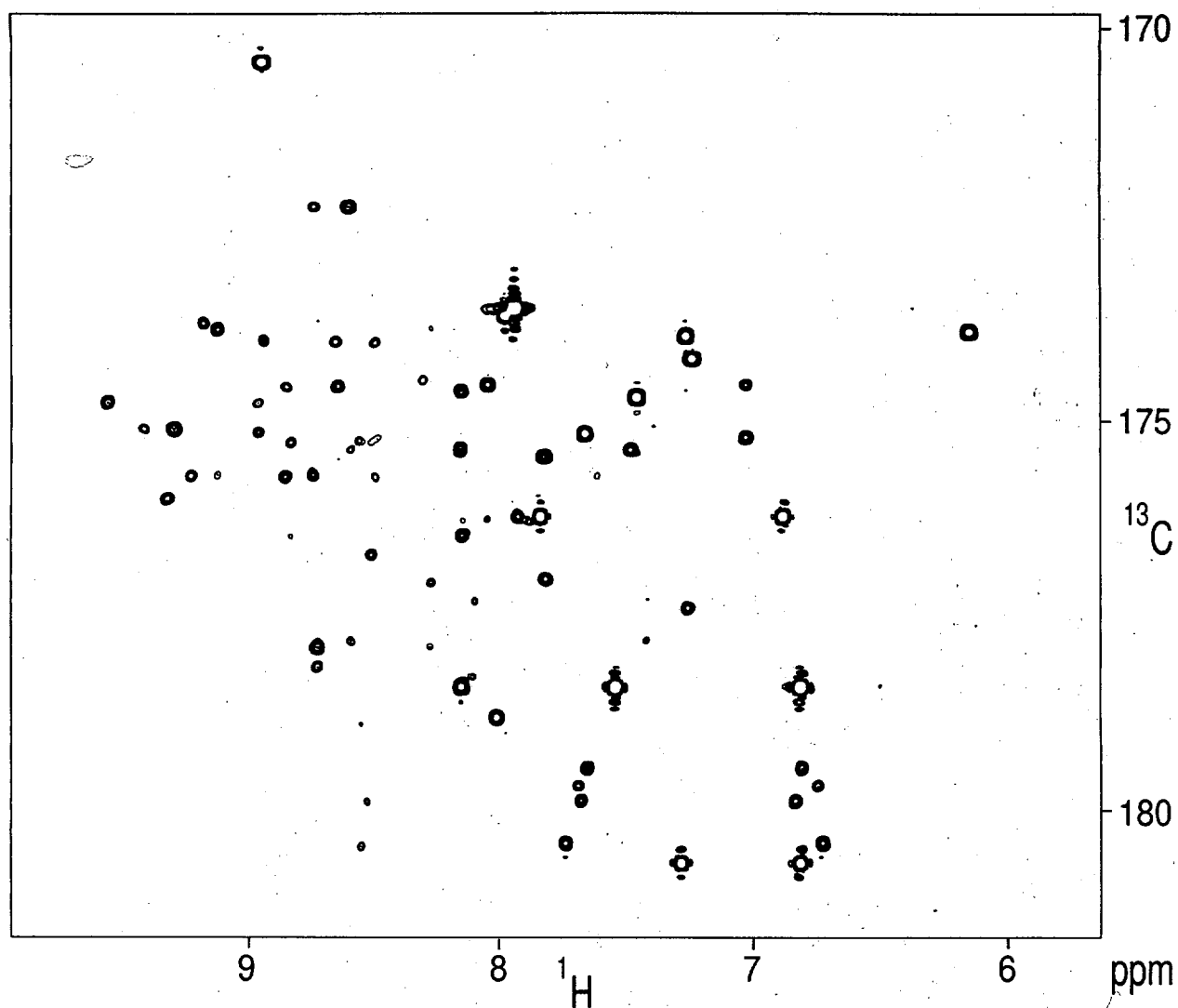


Supporting Information Figure 1. Pulse scheme of the 2D H(N)CO experiment, used to detect through-hydrogen bond J connectivities, for use with perdeuterated  $^{13}\text{C}/^{15}\text{N}$ -proteins with correlation times larger than 7 ns. Narrow and wide pulses correspond to flip angles of  $90^\circ$  and  $180^\circ$ , respectively. Hashed  $^1\text{H}$   $180^\circ$  pulses (for decoupling  $^2J_{\text{C}'\text{HN}}$ ) are only needed if very high  $^{13}\text{C}'$  resolution is required. All pulses phases are  $x$ , unless specified.  $^{13}\text{C}'$  pulses have the shape of the center lobe of a  $\text{sinc}/x$  function, and durations of  $150\ \mu\text{s}$ . Shaped  $^{13}\text{C}'$  pulses at the midpoint of the  $2T$  periods are  $180^\circ$ ; pulses bracketing the  $^{13}\text{C}'$  evolution period are of lower power and correspond to  $90^\circ$ . Water suppression is accomplished by means of a WATERGATE sequence in the final reverse INEPT transfer. Composite  $^{15}\text{N}$  decoupling during  $t_2$  used GARP modulation. The  $^{13}\text{C}^{\alpha/\beta}$   $180^\circ$  pulse duration (applied at 50 ppm) was adjusted such that it has a null at the  $^{13}\text{C}'$  frequency. Delay durations:  $\delta = 2.65\ \text{ms}$ ;  $T = 16.6, 50, \text{ or } 66.6\ \text{ms}$  (see text). Phase cycling:  $\phi_1 = y, -y$ ;  $\phi_2 = 2(x), 2(-x)$ ; Receiver =  $x, 2(-x), x$ . Gradients are sinebell shaped, with peak amplitudes of  $30\ \text{G/cm}$ , and durations  $G_{1,2,3,4,5} = 1.1, 0.5, 0.6, 0.7, 0.4\ \text{ms}$ , and directions  $xyz, y, x, -x, \text{ and } z$ , respectively. For proteins with substantial overlap in the  $^1\text{H}^{\text{N}}$  dimension, it may be advantageous to extend the experiment to a 3D scheme, where the second  $2T$  period is converted into a constant-time  $^{15}\text{N}$  evolution period, and Rance-Kay coherence selection can be used for retaining sensitivity comparable to the 2D experiment.

-S2-



Supporting Information Figure 2. 2D H(N)CO spectra of U- $^{13}\text{C}/^{15}\text{N}$  ubiquitin, recorded with dephasing delays optimized for small couplings (using  $2T = 2/|J_{\text{NC}}| = 133.2$  ms). The spectrum was recorded with the scheme shown in Supporting Information Figure 1. The spectrum results from a  $128^* \times 1024^*$  data matrices, with acquisition times of 51 ms ( $t_1$ ) and 60 ms ( $t_2$ ). The spectrum was recorded with 400 scans per complex  $t_1$  increment (total time 20 h).

- S3-

Supporting Information Table 1.  $J_{NC'}$  couplings measured in human ubiquitin.

		$^1J_{NC'}$ (Hz)
I3-N	L15-C'	0.43
F4-N	S65-C'	0.56
K6-N	L67-C'	0.54
T7-N	K11-C'	0.57
I13-N	V5-C'	0.66
L15-N	I3-C'	0.62
I23-N	R54-C'	0.50
V26-N	T22-C'	0.28
K27-N	I23-C'	0.50
A28-N	E24-C'	0.27
K29-N	N25-C'	0.25
I30-N	V26-C'	0.36
Q31-N	K27-C'	0.38
D32-N	A28-C'	0.28
E34-N	I30-C'	0.69
R42-N	V70-C'	0.50
I44-N	H68-C'	0.57
F45-N	K48-C'	0.52
L50-N	L43-C'	0.61
L56-N	D21-C'	0.52
S57-N	P19-C'	0.38
E64-N	Q2-C'	0.80
L67-N	F4-C'	0.63
H68-N	I44-C'	0.60
L69-N	K6-C'	0.49
V70-N	R42-C'	0.56
R72-N	Q40-C'	0.32
Intraresidue		$^2J_{NC'}$ (Hz)
V17		0.50
S20		0.57
D21		0.38
K33		0.37
E34		1.09
D39		0.21
I44		0.41
Q49		0.25
T55		0.36
S57		0.32
Y59		0.50
Q62		0.41
		$^3J_{NC'}$ (Hz)
D32-N	D32-C'	1.32
N60-N	N60-C'	1.28