Supporting Information

Accurate electron–nucleus distances from paramagnetic relaxation enhancements

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Sample preparation

Cloning

Mutations at two different sites were introduced into wild-type calbindin D_{9k} to simplify the NMR spectra and guard against protein degradation. The mutation Pro43Gly removes the *cis-trans* isomerization equilibrium otherwise present in the protein and the mutation Asn56Asp was made to prevent any backbone rearrangement.^{1–3} The gene of the double mutant P43G/N56D of calbindin D_{9k} was cloned into the vector pETMCSI.⁴ The mutant P43M has also been reported to suppress *cis-trans* isomerization⁵ and we used the double mutant P43M/N56D in earlier experiments that explored the use of RE-BURP pulses to refocus RDCs. These samples were prepared with identical expression, purification, and sample preparation protocols.

Calbindin D_{9k} expression and purification

A 15 mL overnight starter culture of transformed *E. coli* BL21(DE3) cells was used to inoculate 1.0 L of ampicillin supplemented LB-media and incubated at 37 °C with shaking. An OD₆₀₀ of 0.6 was achieved after 4 hours, after which the cells were pelleted and resuspended in 0.5 L of M9 minimal media supplemented with 0.6 g/L ¹⁵N ammonium chloride or 6.0 g/L ¹³C glucose. Following incubation at 37 °C for 1 hour, IPTG was added to 1 mM and incubation at 25 °C continued overnight. Cells were pelleted and resuspended in 50 mM HEPES (pH 7.5), 20 mM NaCl.

The purification of the calbindin D_{9k} samples followed an established protocol⁶ with modifications. Cells were lysed by a French Press, then heat-treated in an 80 °C water bath for 8 minutes. The lysate was then clarified by centrifugation at 30000 g for 1 hour. EDTA (pH 8.0) was added to the supernatant to 5 mM to increase affinity to a DEAE column. The samples were purified by a DEAE column using 50 mM Tris (pH 7.5) as running buffer, using a gradient from 0 to 0.5 M NaCl. Calbindin D_{9k} eluted at about 150 mM NaCl. Following concentration to 2.5 mL, calcium chloride (15 mM) was added, then the sample was loaded onto an S75 size exclusion column, and eluted with 50 mM Tris (pH 7.5), 400 mM NaCl to give 50 mg of purified uniformly ¹⁵N-labeled product.

Lanthanide substitution for NMR sample preparation

10 equivalents of lanthanide salt were added to an 0.2 mM stock solution of calbindin D_{9k}. This was dialysed overnight with 20 mM MES (pH 6.5), 400 mM NaCl. The dialysis buffer was then changed to 20 mM MES (pH 6.5) and dialysed overnight. The protein solution was concentrated to the desired concentration and 50 μ L deuterium oxide were added to make a 0.5 mL sample for NMR.

NMR measurements

PCS measurement and $\Delta\chi$ tensor fitting

NMR spectra were recorded of protein solutions in 20 mM MES buffer, pH 6.5, at 25 °C. The assignment of the amide resonances was confirmed for uniformly ¹⁵N-labeled diamagnetic CbCaLu using 3D NOESY-¹⁵N-HSQC and HNCO spectra by reference to the BMRB entry 16340.⁷ PCSs in paramagnetic samples were measured in [¹⁵N,¹H]-HSQC spectra using CbCaLu or CbCaY as the diamagnetic reference. (Spectra of CbCaLu or CbCaY were indistinguishable for amide protons not in the immediate vicinity of the lanthanide binding site.) The PCSs of the amide protons together with the crystal structure (PDB ID 4ICB²) were used to fit $\Delta\chi$ tensors using the software Numbat.⁸

Table S1. χ tensor parameters for calbindin D_{9k} P43G/N56D^a

Sample	$\chi_{\text{iso}}^{\text{b}}$	$\Delta\chi_{\text{ax}}^{\text{b}}$	$\Delta\chi_{\text{rh}}^{\text{b}}$	$\alpha/^{\circ}$	$\beta/^{\circ}$	$\gamma/^{\circ}$	$Q\text{-factor}^{\text{c}}$
CbCaTb	82.7	29.7	12.2	152	153	71	0.08
CbCaEr	80.4	-8.1	-4.9	126	142	42	0.05

^a The isotropic magnitudes of the χ tensors were calculated using Eq. 3 with parameters from Bleaney⁹ at 298 K. The anisotropy components were experimentally determined from PCSs of backbone amide protons by fitting $\Delta\chi$ tensors to the crystal structure 4ICB² while constraining the position of the lanthanide ion to the coordinates of the Ca²⁺ ion in site-II.

^b In units of 10⁻³² m³.

^c Quality factors were calculated as the root-mean-square deviation between experimental and back-calculated PCSs divided by the root-mean-square of the experimental PCSs.

Additional $\Delta\chi$ tensor determinations

$\Delta\chi$ tensors were also determined for the P43M/N56D mutant of calbindin D_{9k} loaded with Tb³⁺, Dy³⁺, Ho³⁺, Er³⁺, or Tm³⁺, and PCSs of backbone amides could be measured for 41, 40, 48, 50, and 53 residues, respectively. The resonance assignment was verified by a 3D NOESY-¹⁵N-HSQC spectrum of CbCaY and this sample was also used as the diamagnetic reference for PCS calculations. The fitted $\Delta\chi$ tensors are reported in Table S2.

Where both mutants had been measured with the same lanthanide ion (Tb³⁺ or Er³⁺), the $\Delta\chi$ tensors were practically indistinguishable, indicating conservation of the 3D structure and paramagnetic properties (Tables S1 and S2).

Table S2. χ tensor parameters for the calbindin D_{9k} mutants P43M/N56D^a

Sample	χ_{iso}	$\Delta\chi_{\text{ax}}$	$\Delta\chi_{\text{rh}}$	$\alpha/^{\circ}$	$\beta/^{\circ}$	$\gamma/^{\circ}$	Q -factor
CbCaTb	82.7	29.7	12.5	155	152	75	0.08
CbCaDy	99.2	22.4	8.0	148	150	106	0.06
CbCaHo	98.5	14.7	4.7	138	143	98	0.04
CbCaEr	80.4	-8.2	-4.7	126	142	42	0.04
CbCaTm	50.4	-20.4	-9.2	125	140	65	0.05

^a Tensor values reported in units of 10^{-32} m^3 . See footnotes of Table S1 for further details.

Pseudo-3D $R_2(^1\text{H})$ relaxation pulse sequence

Figure S1. [^{15}N , ^1H]-HSQC-type pulse sequence for the measurement of $R_2(^1\text{H})$ relaxation rates of amide protons. The sequence is that of Iwahara et al. (ref. 10) supplemented with water-selective pulses to retain the water magnetisation as longitudinal magnetisation during the relaxation delay T . The INEPT delay was set to $1/2J_{\text{HN}}$ assuming $J_{\text{HN}} = 95$ Hz. Open and filled shapes represent 180° and 90° pulses, respectively. All shaped pulses were applied on the water resonance as sinc-pulses of 1 ms duration. Pulsed field gradients were applied for 0.6 ms with the following magnitudes: $g_1 = 20$ G/cm; $g_2 = 15$ G/cm; $g_3 = 15$ G/cm; $g_4 = 25$ G/cm. Phase cycle: $\phi_1 = (-x, -x, x, x)_2$; $\phi_2 = -\phi_1$; $\phi_3 = y, y, y, y, -y, -y, -y, -y$; $\phi_4 = -\phi_3$; $\phi_5 = (x, -x)_4$; receiver = $(x, -x, -x, x)_2$. Experiments were conducted with a recycle delay of 1 s.

Control experiments

A large number of control experiments were conducted to establish the best experiments for PRE measurements and test the influence of RDCs and non-specific intermolecular PREs. Table S3 provides a summary. The designs and results of individual experiments are described below. The success of different experimental schemes was judged by the RMSD between PRE-derived distances and distances measured in the crystal structure 4ICB. The $R_2(^1\text{H})$ experiments A, F and G are discussed in the main text; experiment A provided the best correlations of distances derived from PREs versus distances measured in the crystal structure. $R_1(^1\text{H})$ experiments L and M are also discussed in the main text; they were conducted to compare the performance of $R_1(^1\text{H})$ PREs with $R_2(^1\text{H})$ PREs. All other control experiments are discussed below.

Table S3. Summary of relaxation experiments^a

Label	Sample ^b	Conc. (mM) ^c	Relaxation delays (ms)	Experiment ^d	Total time (hrs)	¹ H NMR frequency (MHz)	No. peaks	RMSD (Å)
A	Cb(NC)CaEr+Lu	5.0	0, 5, 10	<i>R</i> ₂ HNCO	36	600	47	0.9
B	Cb(N)CaEr+Lu	0.4	0, 5, 10	<i>R</i> ₂ HSQC	24	600	37	0.9
C	Cb(N)CaEr,Lu	0.2	0, 5, 10	<i>R</i> ₂ HSQC	24	600	45	1.5
D	Cb(N)CaEr,Lu	0.2	0, 5	<i>R</i> ₂ HSQC	24	800	43	1.7
E	Cb(N)CaTb,Lu	0.2	0, 5	<i>R</i> ₂ HSQC	24	800	33	4.8
F	Cb(N)CaEr,Lu	0.2	0, 5, 15, 30	<i>R</i> ₂ HSQC	24	800	43	1.8
G	Cb(N)CaTb,Lu	0.2	0, 5, 15, 30	<i>R</i> ₂ HSQC	24	800	33	5.2
H	Cb(N)CaHo,Y	0.2	0, 5, 10, 20	<i>R</i> ₂ HSQC-reb	24	600	48	1.6
I	Cb(N)CaDy,Y	0.2	0, 5, 10, 20	<i>R</i> ₂ HSQC-reb	24	600	40	1.9
J	Cb(N)CaTb,Y	0.2	0, 5, 10, 20	<i>R</i> ₂ HSQC-reb	24	600	41	3.3
K	Cb(N)CaTm,Y	0.2	0, 5, 10, 20	<i>R</i> ₂ HSQC-reb	24	600	53	3.6
L	Cb(N)CaTb,Lu	0.2	[20-100] ^e	<i>R</i> ₁ HSQC-sat	72	800	25	1.4
M	Cb(N)CaEr,Lu	0.2	[20-100] ^e	<i>R</i> ₁ HSQC-sat	72	800	33	1.1
N	Cb(N)CaEr+Lu	0.4	[20-100] ^e	<i>R</i> ₁ HSQC-sat	24	600	29	1.4

^a Parameters explored by specific experiments are highlighted in red.

^b Sample names encode the isotope labeling, lanthanoid ion, and diamagnetic reference. For example, Cb(NC)CaEr,Lu refers to uniformly ¹⁵N/¹³C labeled calbindin D_{9k} loaded with calcium in site-I and erbium in site-II of the paramagnetic protein; the diamagnetic relaxation rates were measured separately using lutetium in the diamagnetic reference. Cb(N)CaEr+Lu refers to ¹⁵N labeled protein and loading with erbium and lutetium in 1:1 ratio to provide an internal diamagnetic reference.

^c For separated diamagnetic and paramagnetic samples, this is the concentration of each individual sample. For samples with an internal diamagnetic reference, this is the total protein concentration.

^d *R*₂ HNCO refers to the pseudo-4D HNCO relaxation experiment. *R*₂ HSQC represents the pseudo-3D [¹⁵N,¹H]-HSQC experiment. *R*₂ HSQC-reb is the same experiment but with a REBURP pulse applied to the amide protons to refocus RDCs. *R*₁ HSQC-sat is the saturation-recovery experiment.

^e Six linearly spaced relaxation delays between 20 ms and 100 ms, and an additional delay at 15 s for complete recovery.

Control experiment B: PREs measured with an internal diamagnetic reference are independent of sample concentration

Experiment B was recorded with a total protein concentration of 0.4 mM in contrast to experiment A, which was performed with 5 mM protein concentration. The distance correlation plot yielded the same RMSD of 0.9 Å for the 37 non-overlapping amide cross-peaks that could be resolved in the pseudo-3D $R_2(^1\text{H})$ experiment (Figure S2). 10 additional amide cross-peaks could be evaluated in the pseudo-4D experiment A.

Figure S2. $R_2(^1\text{H})$ PRE data for mixture of CbCaEr and CbCaLu with corresponding distances. 0.4 mM total protein concentration. Acquired at 600 MHz sampling relaxation delays 0, 5 and 10 ms. In this figure as in all following figures, the experimental data are plotted versus the corresponding calculated data derived from the crystal structure 4ICB.² Experimental PREs were converted into metal-nuclear distances using the isotropic Curie-spin relaxation theory only (Eq. 2 of the main text). Error bars reflect the uncertainties in experimental R_2 values and represent 95% confidence intervals. The asymmetric error bars for distances were obtained by propagating R_2 values through the relevant PRE theory and represent 95% confidence intervals. (a) $R_2(^1\text{H})$ PREs. (b) Distances from the paramagnetic center derived from the $R_2(^1\text{H})$ PRE data versus the corresponding distances in the crystal structure.

Control experiment C: Effect of non-specific intermolecular PREs when paramagnetic and diamagnetic samples are measured separately

Even at low protein concentrations (in this case 0.2 mM), non-specific intermolecular PREs contribute when pseudo-3D R_2 HSQC relaxation experiments are recorded separately for the paramagnetic and diamagnetic samples. The effect is unimportant for nuclear spins close to the metal (between 10 and 15 Å), but results in too short distances for spins far from the metal center. The RMSD of the distance correlation increased to 1.5 Å (Figure S3). The improved spectral resolution in the absence of an internal reference allowed PRE measurement for 45 amide cross-peaks.

Figure S3. $R_2(^1\text{H})$ PRE data obtained from separate spectra recorded of 0.2 mM solutions of CbCaEr and CbCaLu (experiment C of Table S3: 600 MHz; relaxation delays 0, 5, and 10 ms). See legend of Figure S2 for further details. (a) $R_2(^1\text{H})$ PREs. (b) Corresponding distances.

Control experiments D and E: RDC artefacts at high magnetic field and dependence on magnetic susceptibility anisotropy

^1H - ^1H RDCs evolving during the spin-echo period of the $R_2(^1\text{H})$ relaxation delay result in signal attenuation in the paramagnetic but not in the diamagnetic protein. As RDCs increase with the square of the magnetic field, the effect is more detrimental at higher field strengths. The coupling evolutions are cosine modulated and therefore less pronounced for short relaxation delays. It is thus of interest to explore measurements at a higher magnetic field (800 MHz ^1H NMR frequency) conducted with a very short relaxation delay (0 and 5 ms; note that RDCs evolve during the INEPT delay even for $T = 0$, Figure S1). In addition, RDCs are expected to be less problematic for a lanthanide ion with a small rather than large $\Delta\chi$ tensor (Er^{3+} versus Tb^{3+}). Figure S4 compares $R_2(^1\text{H})$ PRE data for CbCaEr and CbCaTb recorded on a 800 MHz NMR spectrometer. For CbCaEr, the RMSD of the distance correlation increased to 1.7 Å. For CbCaTb, the apparent PREs were much larger than expected, leading to a very poor RMSD of the distance correlation (4.8 Å). For the $\Delta\chi$ tensor determined for CbCaTb (Table S1) and the crystal structure of calbindin $\text{D}_{9\text{k}}$, we predict that, on average, each amide proton has ^1H - ^1H RDCs of 15 Hz due to interactions with other protons (predominantly H^{N} and H^{α} protons). A 15 Hz RDC attenuates the signal for the relaxation delay $T = 5$ ms by 10%. This is a greater source of signal attenuation than small PREs, which are of the order of 2 s^{-1} at larger distances from the metal.

Figure S4. $R_2(^1\text{H})$ PRE data obtained from separate spectra recorded of CbCaEr, CbCaTb, and CbCaLu (experiments D and E of Table S3: 800 MHz; relaxation delays 0 and 5 ms; 0.2 mM protein concentration). See legend of Figure S2 for further details. (a) $R_2(^1\text{H})$ PREs. (b) Corresponding distances.

Control experiments F and G: Allowing RDC evolution during longer relaxation delays and correcting signal attenuations by predicted RDCs

Adding the data measured with longer relaxation delays (15 and 30 ms) to the data of experiments D and E resulted in larger apparent PREs, and the RMSDs of the distance correlations increased to 1.8 Å and 5.2 Å for CbCaEr and CbCaTb, respectively (Figure S4).

Figure S5. $R_2(^1\text{H})$ PRE data obtained from separate spectra recorded of CbCaEr, CbCaTb, and CbCaLu (experiments F and G of Table S3: 800 MHz; relaxation delays 0, 5, 15, and 30 ms; 0.2 mM protein concentration). See legend of Figure S2 for further details. (a) $R_2(^1\text{H})$ PREs. (b) Corresponding distances.

Knowledge of the $\Delta\chi$ tensors and 3D structure of the protein allows prediction of the ^1H - ^1H RDCs and compensation of their effect on signal attenuation during the R_2 relaxation delays. Using the crystal structure 4ICB, we calculated the RDCs of each amide proton with backbone amide and α -protons within a 10 Å radius (Eq. 7). To estimate the impact of the RDCs on the PRE measurements, the cross-peak intensities observed for paramagnetic calbindin D_{9k} samples, I^{para} , were fitted using Eq. S1 with R_2^{PRE} and a scaling factor a between the paramagnetic and diamagnetic spectra as fitting parameters,

$$I^{\text{para}}(T) = a I^{\text{dia}}(T) \exp(-R_2^{\text{PRE}}(T + \frac{1}{2})) \prod_i \cos(\pi J_i^{\text{RDC}} T) \quad (\text{S1})$$

where $I^{\text{dia}}(T)$ is the cross-peak intensity observed for the diamagnetic reference for the relaxation delay T and the index i of the product of cosine functions runs over all $^1\text{H}^{\text{N}}\text{-}^1\text{H}$ RDCs. The initial time point at $T = 0$ captures RDC evolution during the INEPT delay $\frac{1}{2J}$. Only data from experiments with relaxation delays $T = 0$ and 5 ms (the data of experiments D and E) were used for the fitting, as inclusion of the data recorded with $T = 15$ and 30 ms gave significantly poorer fits. Conversion of these corrected PREs into distances greatly improved the correlation between experimental distances and distances in the crystal structure for both CbCaTb and CbCaEr, giving RMSDs of 1.2 and 1.8 Å, respectively, and much better agreements particularly for longer distances (Figure S6). This indicates that RDC artefacts are the main source of error in PRE measurements with lanthanide ions that feature large $\Delta\chi$ tensors. In the case of CbCaTb, however, the RDC correction resulted in negative PRE values for some of the residues in the flexible regions of the protein and therefore were omitted from the correlation plots of Figure 6. It is well known that RDCs are averaged to smaller values in dynamic polypeptide segments. Therefore the negative PREs likely reflect overcompensation by too large RDCs predicted for a static protein structure.

Figure S6. Same as Figure S5, but correcting the apparent $R_2(^1\text{H})$ PRE data by the predicted RDC evolution during the relaxation delay. (a) $R_2(^1\text{H})$ PREs. (b) Corresponding distances.

Control experiments H–K: Refocussing of RDCs by a semi-selective RE-BURP pulse

In an attempt to refocus the RDCs between amide protons and protons with chemical shifts outside the amide region, we substituted the hard 180° refocussing pulse during the relaxation delay T by a semi-selective RE-BURP pulse covering the H^N region of the 1H NMR spectrum. (As the RE-BURP pulse did not excite the water resonance, we also removed the following water-selective flip-back pulse in the $R_2(^1H)$ HSQC pulse sequence of Figure S1.) The pulse sequence was tested on calbindin D_{9k} loaded with Tb^{3+} , Dy^{3+} , Ho^{3+} , or Tm^{3+} . As the RE-BURP pulse also refocused the scalar $^3J(H^N, H^\alpha)$ couplings, the same modified pulse sequence was also applied to the diamagnetic reference, CbCaY. Spectra were measured with relaxation delays of 0, 5, 10, and 20 ms at a 1H NMR frequency of 600 MHz. The double-mutant P43M/N56D was used for the experiments conducted with RE-BURP pulse.

The RMSDs of the distance correlations obtained with Ho^{3+} , Dy^{3+} , Tb^{3+} , and Tm^{3+} were 1.6 Å, 1.9 Å, 3.3 Å, and 3.6 Å, respectively (Figure S7). For CbCaTb, the use of the RE-BURP pulse yielded an improvement in RMSD of the distance correlation from 4.8 Å to 3.3 Å (Table S3). The improvement was limited because the RE-BURP pulse did not refocus RDCs between amide protons and some PREs were overestimated due to large PCSs shifting H^α resonances into the excitation range of the RE-BURP pulse, which were outside the RE-BURP excitation profile in the diamagnetic sample. This effect greatly affected the PREs determined for some of the amide protons in CbCaTm and CbCaTb.

Figure S7. $R_2(^1\text{H})$ PRE data obtained from separate spectra recorded of CbCaHo, CbCaTb, CbCaDy, CbCaTm, and CbCaY (experiments H–K of Table S3: 600 MHz; relaxation delays 0, 5, 10, and 20 ms; 0.2 mM protein concentration). See legend of Figure S2 for further details. (a) $R_2(^1\text{H})$ PREs. (b) Corresponding distances.

Control experiment N: Effect of non-specific intermolecular PREs on $R_1(^1\text{H})$ PREs

To assess the impact of non-specific intermolecular PREs on $R_1(^1\text{H})$ PRE data, the $R_1(^1\text{H})$ saturation–recovery experiment was recorded with a 1:1 mixture of CbCaEr and the diamagnetic reference CbCaLu. The resulting RMSD in distance correlation was 1.4 Å, which is no improvement when compared to the data obtained from separate spectra of diamagnetic and paramagnetic species (Figure S8; see experiments M and N in Table S3). The increase in RMSD can be attributed to a loss in precision due to a shorter total recording time used (24 versus 72 h).

Figure S8. $R_1(^1\text{H})$ PRE data for a 1:1 mixture of CbCaEr and CbCaLu (experiments M and N of Table S3: 600 MHz; relaxation delays 20 ms, 40 ms, 60 ms, 80 ms, 100 ms, and 15 s; 0.4 mM total protein concentration). (a) $R_1(^1\text{H})$ PREs. (b) Corresponding distances.

Cross-relaxation effects in $R_1(^1\text{H})$ measurements

Figure S9. Cross-peak intensities measured in the saturation–recovery experiment plotted versus the recovery time for the amides of Tyr13 and Gly42 in CbCaLu. The insert shows an enlarged view of the initial rate. The red lines indicate the best fits to a single exponential recovery. The fits yielded $R_1 = 0.43 \text{ s}^{-1}$ for Tyr13 and $R_1 = 9.13 \text{ s}^{-1}$ for Gly42. The data points of Gly42 do not conform to a model of single exponential relaxation.

Table S4: CbCaLu P43G/N56D assignment with uniform ^{15}N labeling

	H (ppm)	N (ppm)
1Lys	8.581	122.832
2Ser	9.180	120.324
4Glu	8.896	118.039
5Glu	8.126	122.367
6Leu	8.564	120.485
7Lys	8.286	121.152
8Gly	7.887	105.358
9Ile	8.105	123.006
10Phe	8.455	120.340
11Glu	8.541	115.346
12Lys	7.755	119.175
13Tyr	7.314	115.113
14Ala	8.298	119.475
15Ala	6.869	117.002
16Lys	7.165	120.224
17Glu	9.690	116.172
18Gly	9.018	113.168
19Asp	8.362	128.246
21Asn	9.052	114.946
22Gln	7.261	114.316
23Leu	9.629	125.318
24Ser	10.171	122.422
25Lys	8.846	122.197
26Glu	8.056	117.444
27Glu	7.818	120.355
28Leu	8.806	119.384
29Lys	8.455	120.110
30Leu	7.653	118.467
31Leu	8.191	124.340
32Leu	8.865	120.058
33Gln	8.621	114.496
34Thr	7.632	110.852
35Glu	8.422	116.180
36Phe	7.846	114.729
38Ser	8.413	113.842
39Leu	7.983	122.898
40Leu	7.764	117.022
41Lys	7.417	120.335
42Gly	8.179	108.614
43Gly	8.417	109.165
44Ser	8.044	117.328
45Thr	8.406	114.110
46Leu	8.806	122.368
47Asp	8.102	117.192
48Glu	7.788	119.742
49Leu	8.398	121.887
50Phe	9.025	119.341
51Glu	7.887	116.323
52Glu	7.723	118.700

53Leu	7.807	114.893
54Asp	8.317	120.829
55Lys	7.800	122.894
56Asp	7.708	114.362
57Gly	7.917	109.207
58Asp	8.088	117.995
59Gly	10.254	112.740
60Glu	7.539	117.306
61Val	11.028	133.032
62Ser	9.608	127.951
63Phe	9.640	123.482
64Glu	8.429	118.436
65Glu	8.093	121.240
66Phe	8.857	118.946
67Gln	7.629	115.807
68Val	7.125	118.094
69Leu	7.119	120.345
70Val	7.085	117.312
71Lys	7.496	118.217
72Lys	7.381	117.078
73Ile	7.482	114.444
74Ser	7.809	116.988
75Gln	7.617	126.275

Table S5: CbCaLu P43G/N56D assignment with uniform ^{15}N and ^{13}C labeling

	H (ppm)	N (ppm)	C (ppm)
1Lys	8.577	122.778	176.025
2Ser	9.169	120.236	179.669
4Glu	8.881	117.949	179.893
5Glu	8.114	122.281	180.201
6Leu	8.553	120.400	178.411
7Lys	8.277	121.047	177.916
8Gly	7.877	105.306	177.150
9Ile	8.094	122.898	176.683
10Phe	8.444	120.291	176.375
11Glu	8.532	115.257	179.238
12Lys	7.745	119.073	178.172
13Tyr	7.303	114.996	176.375
14Ala	8.293	119.374	178.948
15Ala	6.863	116.925	177.569
16Lys	7.155	120.113	177.148
17Glu	9.674	116.049	176.113
18Gly	9.007	113.067	174.089
19Asp	8.329	128.095	174.661
21Asn	9.033	114.851	174.005
22Gln	7.248	114.192	174.166
23Leu	9.618	125.244	176.798
24Ser	10.160	122.308	175.237
25Lys	8.832	122.103	178.077
26Glu	8.037	117.209	179.723

27Glu	7.803	120.245	178.994
28Leu	8.791	119.293	177.639
29Lys	8.441	120.019	177.638
30Leu	7.637	118.363	179.033
31Leu	8.176	124.222	179.335
32Leu	8.856	119.936	178.588
33Gln	8.612	114.400	177.507
34Thr	7.617	110.773	175.486
35Glu	8.409	116.072	176.833
36Phe	7.834	114.624	179.505
38Ser	8.403	113.779	176.514
39Leu	7.975	122.818	178.364
40Leu	7.749	116.921	176.971
41Lys	7.401	120.200	176.793
42Gly	8.174	108.525	174.762
43Gly	8.407	109.087	174.874
44Ser	8.043	117.378	174.484
45Thr	8.374	113.951	175.230
46Leu	8.798	122.328	178.166
47Asp	8.109	117.092	178.765
48Glu	7.769	119.588	179.545
49Leu	8.393	121.806	178.397
50Phe	9.016	119.260	176.494
51Glu	7.872	116.234	178.214
52Glu	7.721	118.619	177.982
53Leu	7.804	114.868	179.466
54Asp	8.298	120.665	177.575
55Lys	7.801	122.890	176.857
56Asp	7.697	114.289	177.340
57Gly	7.904	109.116	174.948
58Asp	8.085	117.944	177.114
59Gly	10.247	112.669	172.872
60Glu	7.535	117.176	176.107
61Val	11.017	132.968	174.431
62Ser	9.590	127.834	174.991
63Phe	9.627	123.405	177.696
64Glu	8.421	118.336	180.536
65Glu	8.076	121.140	178.786
66Phe	8.844	118.836	175.958
67Gln	7.616	115.716	177.440
68Val	7.110	117.967	177.838
69Leu	7.116	120.229	177.960
70Val	7.072	117.171	177.890
71Lys	7.482	118.143	177.952
72Lys	7.371	116.947	178.127
73Ile	7.469	114.389	176.360
74Ser	7.804	116.912	173.289
75Gln	7.605	126.178	NaN

Table S6: CbCaY P43M/N56D assignment with uniform ^{15}N labeling

	H(ppm)	N(ppm)
1Lys	8.570	122.750
2Ser	9.157	120.281
4Glu	8.879	118.019
5Glu	8.115	122.315
6Leu	8.557	120.457
7Lys	8.280	121.201
8Gly	7.888	105.398
9Ile	8.100	122.945
10Phe	8.461	120.409
11Glu	8.530	115.358
12Lys	7.755	119.179
13Tyr	7.312	115.112
14Ala	8.316	119.494
15Ala	6.868	117.046
16Lys	7.171	120.198
17Glu	9.684	116.175
18Gly	9.008	113.166
19Asp	8.350	128.152
21Asn	9.069	115.109
22Gln	7.257	114.469
23Leu	9.630	125.503
24Ser	10.156	122.341
25Lys	8.828	122.202
26Glu	8.051	117.341
27Glu	7.804	120.295
28Leu	8.774	119.402
29Lys	8.425	120.035
30Leu	7.656	118.494
31Leu	8.190	124.309
32Leu	8.805	120.036
33Gln	8.623	114.564
34Thr	7.643	110.882
35Glu	8.409	116.174
36Phe	7.862	114.873
38Ser	8.393	113.688
39Leu	7.962	122.687
40Leu	7.724	116.993
41Lys	7.452	120.405
42Gly	8.071	108.853
43Met	8.350	120.037
44Ser	8.245	115.167
45Thr	8.104	113.850
46Leu	8.678	122.502
47Asp	8.114	117.529
48Glu	7.788	119.570
49Leu	8.375	121.783
50Phe	8.979	119.345
51Glu	7.865	116.149
52Glu	7.709	118.673

53Leu	7.838	114.964
54Asp	8.289	120.566
55Lys	7.760	122.896
56Asp	7.697	114.282
57Gly	7.900	109.112
58Asp	8.075	118.108
59Gly	10.305	112.741
60Glu	7.550	117.376
61Val	10.936	132.617
62Ser	9.603	127.760
63Phe	9.618	123.415
64Glu	8.424	118.395
65Glu	8.081	121.161
66Phe	8.848	119.040
67Gln	7.630	115.895
68Val	7.111	118.045
69Leu	7.114	120.256
70Val	7.089	117.301
71Lys	7.494	118.196
72Lys	7.377	117.010
73Ile	7.476	114.398
74Ser	7.802	116.954
75Gln	7.614	126.264

Table S7: Data from experiment A (see Table S3)

	R2 (PRE)	Error-R2 (PRE)	PCS(H)	PCS(N)	PCS(C)
1Lys	1.76	0.05	0.043	-0.041	-0.013
2Ser	1.49	0.04	-0.061	-0.045	-0.259
4Glu	1.46	0.06	-0.194	-0.248	-0.197
5Glu	2.42	0.07	-0.172	-0.215	-0.210
6Leu	3.97	0.06	-0.178	-0.216	-0.304
7Lys	7.22	0.08	-0.372	-0.408	-0.573
8Gly	6.25	0.08	-0.379	-0.464	-0.376
9Ile	7.45	0.10	-0.300	-0.318	-0.325
10Phe	12.74	0.12	-0.459	-0.517	-0.879
11Glu	14.45	0.07	-0.770	-0.829	-0.687
12Lys	9.32	0.09	-0.510	-0.536	-0.448
13Tyr	15.68	0.12	-0.429	-0.388	-0.492
14Ala	31.08	0.17	-0.812	-0.754	-1.035
16Lys	14.94	0.15	-0.490	-0.459	-0.304
17Glu	15.06	0.08	-0.424	-0.334	-0.428
18Gly	10.61	0.12	-0.251	-0.319	-0.668
19Asp	76.21	1.06	-0.867	-0.945	-1.658
21Asn	95.47	0.71	-2.173	-2.093	5.382
26Glu	49.28	0.22	0.852	0.952	0.616
27Glu	48.05	0.45	0.589	0.527	0.592
28Leu	79.48	0.42	1.173	1.000	1.044
29Lys	28.54	0.11	1.026	0.991	0.589
30Leu	12.51	0.08	0.494	0.493	0.356
31Leu	12.55	0.13	0.422	0.366	0.426

32Leu	12.64	0.10	0.627	0.596	0.456
33Gln	4.14	0.09	0.434	0.439	0.244
34Thr	1.81	0.07	0.262	0.238	0.165
35Glu	1.79	0.09	0.232	0.201	0.175
36Phe	6.70	0.07	0.304	0.275	0.302
38Ser	1.67	0.19	0.270	0.230	0.370
39Leu	3.00	0.06	0.392	0.323	0.485
40Leu	4.56	0.07	0.435	0.449	0.401
41Lys	1.93	0.06	0.369	0.371	0.294
43Gly	1.81	0.15	0.289	0.275	0.339
44Ser	2.88	0.08	0.380	0.343	0.475
45Thr	7.84	0.08	0.492	0.528	0.746
46Leu	5.07	0.09	0.645	0.750	1.132
47Asp	11.55	0.10	0.705	0.903	0.938
48Glu	10.87	0.18	0.718	0.812	1.165
49Leu	27.11	0.11	1.180	1.313	2.675
50Phe	73.17	0.64	2.147	2.721	2.480
69Leu	78.17	0.50	1.365	1.290	0.750
70Val	25.81	0.13	0.608	0.589	0.360
71Lys	11.97	0.08	0.459	0.398	0.389
72Lys	7.77	0.08	0.553	0.488	0.379
73Ile	4.72	0.07	0.413	0.385	0.237
74Ser	2.45	0.06	0.261	0.281	0.122

Table S8: Data from experiment B (see Table S3)

	R2 (PRE)	Error-R2 (PRE)	PCS (H)	PCS (N)
1Lys	2.14	0.46	0.051	0.016
2Ser	1.10	0.43	-0.051	-0.039
5Glu	4.54	0.42	-0.163	-0.198
6Leu	4.45	0.43	-0.170	-0.189
7Lys	8.58	0.54	-0.362	-0.407
8Gly	6.30	0.57	-0.370	-0.420
9Ile	7.98	0.64	-0.290	-0.322
11Glu	15.12	0.62	-0.753	-0.830
12Lys	9.80	0.50	-0.496	-0.524
13Tyr	15.22	0.75	-0.428	-0.401
14Ala	25.19	0.69	-0.795	-0.775
15Ala	14.04	2.00	-0.758	-0.791
17Glu	14.03	0.52	-0.415	-0.333
18Gly	11.04	0.74	-0.249	-0.350
19Asp	91.05	3.87	-0.859	-0.908
24Ser	119.67	13.20	-0.109	0.169
27Glu	43.55	0.99	0.600	0.453
28Leu	70.58	1.62	1.168	0.993
29Lys	25.13	0.61	1.022	0.965
30Leu	13.01	0.50	0.496	0.479
31Leu	11.62	0.53	0.422	0.364
32Leu	14.58	0.51	0.625	0.544
35Glu	2.67	0.62	0.235	0.198
39Leu	3.06	0.42	0.392	0.364

40Leu	4.31	0.47	0.432	0.429
41Lys	2.83	0.38	0.368	0.339
43Gly	2.80	2.35	0.286	0.245
46Leu	5.39	0.48	0.640	0.741
49Leu	26.85	0.61	1.163	1.296
50Phe	65.99	1.59	2.118	2.693
51Glu	128.15	6.03	1.746	2.233
68Val	110.61	5.74	1.153	0.958
70Val	23.71	0.69	0.599	0.546
71Lys	10.58	0.48	0.454	0.414
72Lys	7.55	0.45	0.545	0.455
73Ile	5.85	0.41	0.407	0.393
75Gln	1.25	0.30	0.187	0.174

Table S9: Data from experiment C (see Table S3)

	R2 (PRE)	Error-R2 (PRE)	PCS (H)	PCS (N)
1Lys	2.42	0.84	0.054	0.027
2Ser	1.72	0.71	-0.048	-0.039
4Glu	3.05	0.65	-0.184	-0.218
5Glu	9.02	0.70	-0.162	-0.200
6Leu	7.43	0.85	-0.169	-0.186
7Lys	10.87	0.81	-0.360	-0.395
8Gly	9.88	0.80	-0.368	-0.424
9Ile	11.89	0.96	-0.288	-0.319
11Glu	16.05	1.14	-0.752	-0.831
12Lys	12.41	0.82	-0.494	-0.521
13Tyr	18.65	0.94	-0.413	-0.397
14Ala	30.35	0.92	-0.792	-0.775
15Ala	21.55	2.76	-0.756	-0.799
16Lys	16.22	0.79	-0.475	-0.460
17Glu	14.85	0.93	-0.419	-0.329
18Gly	8.97	1.49	-0.248	-0.363
19Asp	95.27	5.70	-0.862	-0.919
21Asn	91.12	3.38	-2.150	-2.065
24Ser	166.73	28.44	-0.108	0.173
27Glu	49.92	1.52	0.600	0.531
28Leu	65.58	2.71	1.172	0.986
30Leu	13.82	0.92	0.496	0.469
31Leu	12.15	0.87	0.424	0.367
32Leu	19.76	0.80	0.629	0.544
33Gln	7.52	0.74	0.435	0.397
34Thr	4.94	0.88	0.268	0.240
35Glu	3.37	1.17	0.239	0.198
36Phe	9.08	0.96	0.310	0.265
39Leu	7.03	0.70	0.395	0.376
40Leu	6.29	0.61	0.439	0.434
41Lys	3.23	0.70	0.372	0.329
46Leu	6.79	0.91	0.644	0.732
47Asp	13.60	0.61	0.712	0.861
48Glu	14.59	0.70	0.699	0.757

49Leu	30.90	0.88	1.168	1.301
50Phe	69.73	2.17	2.123	2.686
51Glu	127.53	7.06	1.750	2.232
68Val	99.18	7.51	1.156	0.942
69Leu	72.02	3.16	1.341	1.250
70Val	24.30	0.98	0.598	0.541
71Lys	11.99	0.84	0.452	0.409
72Lys	11.46	0.71	0.547	0.435
73Ile	9.19	0.65	0.411	0.402
74Ser	2.41	0.90	0.265	0.279
75Gln	2.38	0.52	0.191	0.173

Table S10: Data from experiment D (see Table S3)

	R2(PRE)	Error-R2(PRE)	PCS(H)	PCS(N)
1Lys	6.69	1.02	0.054	0.005
2Ser	6.80	0.92	-0.048	-0.029
4Glu	6.13	0.87	-0.184	-0.207
5Glu	12.05	0.88	-0.163	-0.199
6Leu	16.24	1.01	-0.167	-0.185
7Lys	18.15	1.17	-0.363	-0.405
8Gly	12.66	1.38	-0.373	-0.421
9Ile	20.37	1.37	-0.290	-0.325
11Glu	29.81	1.46	-0.762	-0.843
12Lys	19.79	1.16	-0.503	-0.536
13Tyr	35.64	1.44	-0.423	-0.415
14Ala	54.58	1.96	-0.806	-0.797
15Ala	30.81	1.73	-0.761	-0.797
16Lys	29.66	1.37	-0.488	-0.463
17Glu	30.88	1.16	-0.424	-0.342
18Gly	19.58	1.99	-0.253	-0.362
19Asp	116.43	33.32	-0.871	-0.935
21Asn	165.79	17.22	-2.184	-2.092
27Glu	92.73	5.02	0.592	0.528
28Leu	146.18	11.18	1.175	1.010
30Leu	27.60	1.17	0.497	0.488
31Leu	24.36	1.27	0.427	0.370
32Leu	24.22	1.24	0.636	0.564
33Gln	12.00	0.89	0.440	0.417
34Thr	7.21	0.95	0.267	0.257
35Glu	7.30	1.17	0.239	0.198
36Phe	15.21	1.27	0.311	0.275
39Leu	12.08	0.88	0.400	0.368
40Leu	9.91	0.97	0.442	0.433
41Lys	8.09	0.82	0.374	0.339
46Leu	12.54	1.15	0.648	0.771
47Asp	25.02	1.04	0.713	0.892
48Glu	26.06	1.24	0.703	0.774
49Leu	52.35	1.87	1.182	1.334
50Phe	126.16	10.79	2.145	2.742
51Glu	273.75	99.48	1.762	2.272

69Leu	141.06	13.28	1.387	1.312
70Val	46.59	2.02	0.622	0.570
71Lys	23.56	1.08	0.470	0.442
72Lys	18.57	0.99	0.564	0.482
73Ile	12.46	0.91	0.423	0.424
74Ser	7.00	1.06	0.271	0.258
75Gln	3.88	0.56	0.195	0.179

Table S11: Data from experiment E (see Table S3)

	R2 (PRE)	Error-R2 (PRE)	PCS (H)	PCS (N)
1Lys	22.66	1.09	0.011	0.088
4Glu	27.70	1.15	0.977	1.085
5Glu	67.94	1.99	0.826	0.881
6Leu	76.12	2.40	0.920	0.993
7Lys	47.47	1.78	1.677	1.926
8Gly	29.05	1.51	1.450	1.587
9Ile	69.37	2.57	1.017	1.015
11Glu	71.35	2.72	2.329	2.272
12Lys	60.55	2.28	1.227	1.171
13Tyr	95.24	2.95	0.578	0.398
16Lys	58.24	2.15	0.073	0.062
17Glu	78.08	2.42	-0.486	-0.434
18Gly	85.89	4.84	-0.726	-0.806
31Leu	48.64	2.47	-2.142	-1.771
32Leu	63.31	2.09	-2.447	-2.138
33Gln	73.08	2.14	-1.730	-1.647
34Thr	74.58	3.03	-1.140	-1.057
35Glu	35.42	1.54	-0.955	-0.790
36Phe	48.90	1.93	-1.130	-1.059
39Leu	60.49	1.64	-1.306	-1.247
40Leu	43.63	1.38	-1.477	-1.456
41Lys	43.46	1.18	-1.229	-1.147
42Gly	20.53	2.70	-1.036	-1.002
43Gly	14.68	7.54	-0.937	-0.939
46Leu	52.80	1.96	-1.984	-2.381
47Asp	75.98	2.65	-1.992	-2.595
48Glu	87.94	8.87	-1.988	-2.340
70Val	97.92	7.33	-1.049	-1.537
71Lys	46.34	1.66	-0.900	-0.774
72Lys	70.61	3.96	-1.466	-1.413
73Ile	70.92	2.24	-1.137	-1.213
74Ser	25.84	1.19	-0.639	-0.482
75Gln	18.42	0.62	-0.452	-0.380

Table S12: Data from experiment F (see Table S3)

	R2 (PRE)	Error-R2 (PRE)	PCS (H)	PCS (N)
1Lys	6.40	0.23	0.054	0.005
2Ser	6.91	0.19	-0.048	-0.029

4Glu	7.18	0.21	-0.184	-0.207
5Glu	15.08	0.23	-0.163	-0.199
6Leu	18.73	0.26	-0.167	-0.185
7Lys	19.78	0.32	-0.363	-0.405
8Gly	15.99	0.39	-0.373	-0.421
9Ile	20.64	0.42	-0.290	-0.325
11Glu	31.18	0.50	-0.762	-0.843
12Lys	21.43	0.35	-0.503	-0.536
13Tyr	37.83	0.57	-0.423	-0.415
14Ala	56.47	0.91	-0.806	-0.797
15Ala	31.26	0.54	-0.761	-0.797
16Lys	31.38	0.44	-0.488	-0.463
17Glu	32.51	0.39	-0.424	-0.342
18Gly	18.17	0.82	-0.253	-0.362
19Asp	140.05	24.54	-0.871	-0.935
21Asn	170.30	14.60	-2.184	-2.092
27Glu	96.65	3.21	0.592	0.528
28Leu	143.83	9.98	1.175	1.010
30Leu	28.26	0.37	0.497	0.488
31Leu	24.76	0.38	0.427	0.370
32Leu	24.83	0.39	0.636	0.564
33Gln	12.89	0.22	0.440	0.417
34Thr	8.48	0.26	0.267	0.257
35Glu	5.53	0.31	0.239	0.198
36Phe	18.12	0.36	0.311	0.275
39Leu	10.21	0.22	0.400	0.368
40Leu	11.07	0.24	0.442	0.433
41Lys	6.62	0.17	0.374	0.339
46Leu	12.32	0.33	0.648	0.771
47Asp	25.18	0.31	0.713	0.892
48Glu	25.14	0.40	0.703	0.774
49Leu	54.25	0.85	1.182	1.334
50Phe	129.36	8.12	2.145	2.742
51Glu	278.28	92.13	1.762	2.272
69Leu	137.07	11.21	1.387	1.312
70Val	48.99	0.81	0.622	0.570
71Lys	22.42	0.30	0.470	0.442
72Lys	16.11	0.27	0.564	0.482
73Ile	9.42	0.21	0.423	0.424
74Ser	6.18	0.22	0.271	0.258
75Gln	2.50	0.11	0.195	0.179

Table S13: Data from experiment G (see Table S3)

	R2(PRE)	Error-R2(PRE)	PCS(H)	PCS(N)
1Lys	29.43	0.32	0.011	0.088
4Glu	37.86	0.37	0.977	1.085
5Glu	87.93	1.12	0.826	0.881
6Leu	93.56	1.47	0.920	0.993
7Lys	61.20	0.83	1.677	1.926
8Gly	38.00	0.60	1.450	1.587

9Ile	82.49	1.54	1.017	1.015
11Glu	81.25	1.56	2.329	2.272
12Lys	79.50	1.27	1.227	1.171
13Tyr	110.93	2.38	0.578	0.398
16Lys	72.90	1.12	0.073	0.062
17Glu	94.40	1.34	-0.486	-0.434
18Gly	101.41	3.75	-0.726	-0.806
31Leu	61.96	1.14	-2.142	-1.771
32Leu	76.44	1.14	-2.447	-2.138
33Gln	92.23	1.18	-1.730	-1.647
34Thr	92.72	1.92	-1.140	-1.057
35Glu	46.00	0.58	-0.955	-0.790
36Phe	64.18	0.95	-1.130	-1.059
39Leu	77.17	0.95	-1.306	-1.247
40Leu	62.61	0.60	-1.477	-1.456
41Lys	52.32	0.46	-1.229	-1.147
42Gly	18.32	1.12	-1.036	-1.002
43Gly	17.89	4.86	-0.937	-0.939
46Leu	69.72	1.23	-1.984	-2.381
47Asp	87.93	1.63	-1.992	-2.595
48Glu	92.02	5.71	-1.988	-2.340
70Val	107.41	5.35	-1.049	-1.537
71Lys	52.40	0.69	-0.900	-0.774
72Lys	89.25	2.32	-1.466	-1.413
73Ile	94.90	1.41	-1.137	-1.213
74Ser	34.00	0.38	-0.639	-0.482
75Gln	21.51	0.15	-0.452	-0.380

Table S14: Data from experiment H (see Table S3)

	R2 (PRE)	Error-R2 (PRE)	PCS (H)	PCS (N)
1Lys	4.65	0.30	0.159	0.191
2Ser	4.53	0.30	0.297	0.289
4Glu	2.48	0.26	0.531	0.599
5Glu	14.37	0.29	0.512	0.553
6Leu	9.44	0.29	0.633	0.673
7Lys	11.73	0.30	1.012	1.142
8Gly	8.75	0.37	0.912	1.004
9Ile	14.45	0.39	0.809	0.806
10Phe	22.65	0.39	1.259	1.251
11Glu	22.48	0.43	1.601	1.656
12Lys	14.18	0.34	0.969	0.939
13Tyr	24.37	0.48	0.742	0.599
14Ala	38.26	0.56	1.237	1.015
15Ala	21.08	0.36	0.924	0.886
16Lys	23.52	0.32	0.221	0.181
17Glu	25.14	0.41	-0.275	-0.293
18Gly	19.02	0.62	-0.597	-0.647
19Asp	129.56	16.47	-0.793	-0.244
21Asn	157.68	14.35	2.823	2.992
28Leu	229.84	99.68	-2.724	-2.068

29Lys	31.89	1.06	-1.999	-1.698
30Leu	12.90	0.41	-1.071	-0.928
31Leu	19.46	0.32	-0.717	-0.548
32Leu	20.74	0.35	-0.780	-0.608
33Gln	12.50	0.28	-0.611	-0.531
34Thr	6.13	0.38	-0.339	-0.297
35Glu	4.45	0.39	-0.202	-0.139
36Phe	8.98	0.41	-0.266	-0.175
38Ser	7.80	0.53	-0.241	-0.246
39Leu	6.09	0.27	-0.351	-0.332
40Leu	5.38	0.29	-0.454	-0.456
41Lys	2.97	0.31	-0.397	-0.383
42Gly	5.69	0.44	-0.363	-0.343
43Met	6.02	1.94	-0.348	-0.338
44Ser	10.92	0.61	-0.427	-0.443
45Thr	14.14	0.36	-0.546	-0.581
46Leu	12.39	0.44	-0.892	-1.029
47Asp	13.15	0.31	-1.021	-1.247
48Glu	15.18	0.37	-0.812	-0.888
49Leu	33.64	0.60	-1.298	-1.428
50Phe	96.81	8.10	-2.570	-3.107
69Leu	100.55	7.85	-0.330	-0.497
70Val	32.93	0.58	0.181	0.046
71Lys	17.02	0.31	-0.052	-0.043
72Lys	12.52	0.31	-0.264	-0.254
73Ile	12.57	0.32	-0.151	-0.188
74Ser	4.04	0.32	-0.054	-0.027
75Gln	5.11	0.19	-0.048	-0.033

Table S15: Data from experiment I (see Table S3)

	R2 (PRE)	Error-R2 (PRE)	PCS (H)	PCS (N)
1Lys	3.19	0.81	0.205	0.250
2Ser	4.28	0.99	0.420	0.407
4Glu	6.49	0.89	0.828	0.930
5Glu	19.11	1.20	0.737	0.779
6Leu	18.56	1.34	0.883	0.923
7Lys	13.13	1.17	1.441	1.611
8Gly	8.53	1.32	1.213	1.311
9Ile	14.19	1.65	0.940	0.895
10Phe	21.97	2.01	1.425	1.317
11Glu	25.44	2.22	1.874	1.820
12Lys	20.56	1.94	0.941	0.854
13Tyr	25.32	2.36	0.388	0.177
14Ala	41.85	3.99	0.735	0.328
16Lys	29.99	2.93	-0.496	-0.429
17Glu	23.63	2.06	-1.247	-1.118
18Gly	17.44	3.36	-1.410	-1.619
21Asn	117.54	53.37	2.831	3.416
31Leu	18.04	3.58	-1.651	-1.319
32Leu	21.72	1.76	-1.598	-1.307

33Gln	11.31	1.25	-1.169	-1.031
34Thr	11.19	1.55	-0.761	-0.674
35Glu	5.88	1.18	-0.557	-0.435
36Phe	8.15	1.29	-0.600	-0.479
38Ser	5.62	1.56	-0.448	-0.433
39Leu	7.93	0.94	-0.618	-0.589
40Leu	10.65	0.94	-0.755	-0.753
41Lys	1.56	1.00	-0.622	-0.584
42Gly	2.98	1.06	-0.543	-0.507
43Met	5.89	5.78	-0.491	-0.506
44Ser	7.12	1.91	-0.578	-0.589
45Thr	12.17	1.45	-0.698	-0.752
46Leu	8.72	1.78	-1.207	-1.388
47Asp	20.28	2.13	-1.219	-1.520
48Glu	29.25	3.15	-0.888	-0.952
70Val	26.34	1.61	0.281	0.092
71Lys	23.61	1.88	0.031	0.034
72Lys	14.02	1.66	-0.372	-0.365
73Ile	11.88	1.29	-0.260	-0.324
74Ser	5.70	0.91	-0.091	-0.044
75Gln	3.18	0.53	-0.072	-0.046

Table S16: Data from experiment J (see Table S3)

	R2 (PRE)	Error-R2 (PRE)	PCS (H)	PCS (N)
1Lys	5.78	0.31	0.028	0.101
2Ser	5.77	0.28	0.391	0.381
4Glu	5.38	0.26	0.982	1.103
5Glu	30.42	0.33	0.825	0.895
6Leu	33.07	0.39	0.925	0.982
7Lys	21.57	0.34	1.678	1.893
8Gly	9.61	0.36	1.450	1.598
9Ile	15.56	0.40	1.036	1.027
10Phe	35.05	0.47	1.565	1.470
11Glu	31.35	0.52	2.351	2.324
12Lys	25.96	0.39	1.246	1.195
13Tyr	34.77	0.52	0.620	0.437
14Ala	41.94	0.67	1.322	0.954
15Ala	24.84	0.41	1.123	1.098
16Lys	23.70	0.37	0.091	0.096
17Glu	24.52	0.40	-0.476	-0.425
18Gly	17.51	0.71	-0.723	-0.775
19Asp	97.29	6.96	-1.204	-0.660
31Leu	22.96	1.08	-2.087	-1.732
32Leu	20.43	0.53	-2.391	-2.054
33Gln	17.28	0.32	-1.691	-1.573
34Thr	21.76	0.54	-1.104	-1.008
35Glu	9.81	0.42	-0.928	-0.770
36Phe	9.35	0.42	-1.096	-0.977
38Ser	6.35	0.49	-0.888	-0.873
39Leu	13.78	0.28	-1.243	-1.219

40Leu	19.47	0.46	-1.420	-1.445
41Lys	7.15	0.46	-1.201	-1.116
42Gly	5.78	0.42	-1.054	-0.986
43Met	8.70	1.91	-0.989	-1.011
44Ser	14.85	0.62	-1.220	-1.230
45Thr	12.73	0.40	-1.454	-1.500
46Leu	8.74	0.48	-1.982	-2.348
47Asp	22.08	1.00	-1.991	-2.528
48Glu	13.28	4.75	-1.983	-2.255
70Val	28.79	2.61	-1.014	-1.347
71Lys	14.23	0.35	-0.864	-0.741
72Lys	23.29	2.25	-1.451	-1.320
73Ile	12.71	0.55	-1.109	-1.146
74Ser	6.88	0.31	-0.624	-0.507
75Gln	2.62	0.19	-0.447	-0.379

Table S17: Data from experiment K (see Table S3)

	R2(PRE)	Error-R2(PRE)	PCS(H)	PCS(N)
1Lys	10.74	0.71	-0.023	-0.073
2Ser	4.67	0.48	-0.245	-0.229
5Glu	8.24	0.54	-0.531	-0.589
7Lys	4.24	0.48	-1.109	-1.278
8Gly	6.19	0.52	-1.091	-1.234
9Ile	8.49	0.64	-0.912	-0.948
10Phe	3.90	0.58	-1.424	-1.449
11Glu	7.58	0.66	-2.109	-2.248
12Lys	7.99	1.02	-1.512	-1.595
13Tyr	12.03	1.07	-1.115	-1.005
14Ala	1.31	0.56	-1.928	-1.734
16Lys	5.97	0.80	-1.010	-0.938
17Glu	8.34	0.51	-0.681	-0.555
18Gly	4.39	0.84	-0.305	-0.480
19Asp	38.79	2.44	-1.800	-1.975
24Ser	91.87	10.49	1.365	2.965
25Lys	104.08	13.49	7.570	7.235
26Glu	26.99	1.18	3.467	3.399
27Glu	29.35	1.52	2.518	2.160
28Leu	34.31	1.79	3.719	2.941
29Lys	15.66	0.91	3.003	2.681
30Leu	7.04	0.61	1.530	1.408
31Leu	8.18	0.58	1.150	0.943
32Leu	11.39	0.66	1.475	1.234
33Gln	4.43	0.54	1.095	0.996
34Thr	2.00	0.69	0.647	0.587
35Glu	0.36	0.78	0.482	0.395
36Phe	9.36	0.72	0.613	0.589
38Ser	7.31	1.02	0.544	0.536
39Leu	10.54	0.59	0.779	0.734
40Leu	11.97	0.62	0.935	0.959
41Lys	4.21	0.56	0.806	0.787

42Gly	8.71	0.81	0.722	0.667
43Met	9.38	3.33	0.685	0.710
44Ser	4.95	1.06	0.861	0.887
45Thr	8.84	0.63	1.103	1.169
46Leu	8.05	0.85	1.662	1.934
47Asp	8.37	0.53	1.934	2.405
48Glu	7.47	0.55	1.719	1.951
49Leu	17.66	0.91	2.656	2.949
50Phe	29.44	1.75	5.102	6.266
51Glu	54.91	3.45	4.103	5.129
52Glu	63.85	4.15	3.332	2.944
53Leu	191.74	66.02	8.584	8.378
67Gln	51.10	12.04	-1.658	-0.685
68Val	47.12	3.26	1.329	1.143
69Leu	35.42	1.71	1.854	1.805
70Val	9.85	0.69	0.646	0.787
71Lys	4.16	0.54	0.563	0.485
72Lys	2.34	0.51	0.819	0.744
73Ile	3.92	0.48	0.635	0.641
74Ser	0.38	0.59	0.366	0.300
75Gln	0.39	0.31	0.253	0.225

Table S18: Data from experiment L (see Table S3)

	R1(PRE)	Error-R1(PRE)	PCS(H)	PCS(N)
6Leu	0.120	0.020	0.916	0.967
7Lys	0.158	0.020	1.661	1.883
8Gly	0.127	0.019	1.439	1.582
9Ile	0.120	0.021	1.018	0.999
11Glu	0.310	0.024	2.323	2.271
12Lys	0.249	0.022	1.228	1.179
13Tyr	0.310	0.024	0.601	0.407
15Ala	0.264	0.023	1.120	1.112
16Lys	0.194	0.021	0.096	0.087
17Glu	0.445	0.020	-0.450	-0.410
19Asp	1.615	0.284	-1.095	-0.603
21Asn	1.253	0.238	4.129	4.930
31Leu	0.172	0.039	-2.094	-1.737
32Leu	0.178	0.025	-2.395	-2.063
33Gln	0.096	0.020	-1.699	-1.577
34Thr	0.078	0.028	-1.114	-1.018
35Glu	0.031	0.018	-0.930	-0.785
36Phe	0.047	0.019	-1.095	-1.022
39Leu	0.032	0.019	-1.274	-1.253
47Asp	0.147	0.048	-1.979	-2.529
48Glu	0.351	0.155	-1.959	-2.266
70Val	0.523	0.091	-0.999	-1.438
71Lys	0.175	0.033	-0.869	-0.724
72Lys	0.130	0.065	-1.416	-1.327
73Ile	0.131	0.032	-1.100	-1.132

Table S19: Data from experiment M (see Table S3)

	R1(PRE)	Error-R1(PRE)	PCS(H)	PCS(N)
6Leu	0.015	0.017	-0.169	-0.190
7Lys	0.098	0.017	-0.361	-0.405
8Gly	0.054	0.018	-0.368	-0.425
9Ile	0.059	0.018	-0.288	-0.319
11Glu	0.214	0.020	-0.753	-0.838
12Lys	0.123	0.017	-0.495	-0.531
13Tyr	0.202	0.019	-0.416	-0.413
14Ala	0.447	0.023	-0.793	-0.779
15Ala	0.216	0.040	-0.745	-0.793
16Lys	0.212	0.021	-0.476	-0.459
17Glu	0.210	0.018	-0.416	-0.340
19Asp	0.973	0.186	-0.865	-0.916
21Asn	1.209	0.073	-2.148	-2.072
27Glu	0.727	0.037	0.596	0.525
28Leu	1.195	0.054	1.168	0.985
30Leu	0.205	0.019	0.494	0.469
31Leu	0.236	0.017	0.422	0.364
32Leu	0.238	0.017	0.628	0.536
33Gln	0.066	0.016	0.435	0.395
34Thr	0.051	0.017	0.265	0.237
35Glu	0.031	0.017	0.235	0.194
36Phe	0.061	0.017	0.309	0.260
39Leu	0.037	0.018	0.393	0.371
47Asp	0.149	0.021	0.714	0.872
48Glu	0.279	0.026	0.699	0.763
49Leu	0.476	0.028	1.169	1.308
50Phe	1.258	0.065	2.123	2.690
51Glu	2.222	0.250	1.745	2.222
69Leu	1.053	0.065	1.335	1.243
70Val	0.375	0.026	0.597	0.533
71Lys	0.051	0.024	0.451	0.410
72Lys	0.136	0.019	0.545	0.448
73Ile	0.063	0.017	0.409	0.397

Table S20: Data from experiment N (see Table S3)

	R1(PRE)	Error-R1(PRE)	PCS(H)	PCS(N)
6Leu	0.085	0.038	-0.170	-0.189
7Lys	0.161	0.043	-0.362	-0.407
8Gly	0.053	0.045	-0.370	-0.420
9Ile	0.087	0.043	-0.290	-0.322
11Glu	0.157	0.052	-0.753	-0.830
12Lys	0.062	0.042	-0.496	-0.524
13Tyr	0.261	0.053	-0.428	-0.401
14Ala	0.459	0.051	-0.795	-0.775
15Ala	0.261	0.133	-0.758	-0.791
17Glu	0.185	0.042	-0.415	-0.333

19Asp	0.935	0.309	-0.859	-0.908
21Asn	0.947	0.136	-2.132	-2.031
24Ser	3.034	1.216	-0.109	0.169
27Glu	0.583	0.079	0.600	0.453
28Leu	1.227	0.128	1.168	0.993
29Lys	0.514	0.054	1.022	0.965
30Leu	0.174	0.044	0.496	0.479
31Leu	0.229	0.039	0.422	0.364
32Leu	0.398	0.043	0.625	0.544
35Glu	0.088	0.045	0.235	0.198
39Leu	0.064	0.043	0.392	0.364
49Leu	0.433	0.056	1.163	1.296
50Phe	1.310	0.145	2.118	2.693
51Glu	2.036	0.349	1.746	2.233
68Val	2.132	0.498	1.153	0.958
69Leu	1.077	0.158	1.342	1.235
70Val	0.357	0.062	0.599	0.546
71Lys	0.194	0.058	0.454	0.414
72Lys	0.318	0.047	0.545	0.455

Bruker pulse program of the $R_2(^1\text{H})$ pseudo-3D interleaved $[^{15}\text{N}-^1\text{H}]$ -HSQC relaxation experiment (see Figure S1)

```
;hsqcNwgt2ps3dr2.ho
;avance-version (02/05/31)
;15N-HSQC with watergate(using selective 90 deg. pulses) and R2 relaxation
;block
;32-step phase cycle, NS=8 is OK
;with decoupling during acquisition
;using f3 - channel
;water suppression using 90sel-180-90sel Watergate
;T2 relaxation measured via pseudo 3d interleaved acquisition

;Pulse sequence originally from
;Iwahara, J., Tang, C., & Clore, G. M. (2007).
;Practical Aspects of 1H Transverse Paramagnetic Relaxation Enhancement
;Measurements on Macromolecules. Journal of Magnetic Resonance
;(San Diego, Calif. : 1997), 184(2), 185–195

;Modifications by
;H. W. Orton and G. Otting 2018

;$DIM=3D

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>

prosol relations=<triple>

"d0=3u"
"in0=inf2/2"

"p2=p1*2"
"p22=p21*2"

"d0=in0*0.5*cnst10-p21*0.63-p2*0.5"
"d16=200u"
"d11=30m"
"d26=1s/(cnst4*4)"
"DELTA=d26-p16-d16-p11-16u"
"DELTA1=d26-p16-d16-p11-16u+p1*0.63"
"DELTA3=d26-p35*0.5"
"DELTA4=d26-p19-d16-4u"
"DELTA5=d26-4u-8u-4u-p10-4u-p19-d16"
"d10=3u"

aqseq 312

1 30m ze
   d11 st0
2 6m do:f3
3 3m
4 d1 p13:f3 p12:f2 p11:f1
   "TAU=vd*0.25+4u"
   4u UNBLKGRAD
   (p21 ph0):f3
   p16:gp7
   1m p10:f1
   (p11:sp1 ph6):f1
   4u
```



```

8u p11:f1
4u
(p1 ph5)
4u
p19:gp4
d16
DELTA4
TAU
(p22 ph8):f3
TAU
(p2 ph9):f1
4u
8u p10:f1
4u
(p10:sp2 ph10)
4u
p19:gp4
d16
DELTA5
4u p11:f1
TAU
(p22 ph8):f3
TAU
(p1 ph1)
4u
p19:gp4
d16
(p21 ph4):f3
if "d0>p8*0.5"{
    "d10=d0-p8*0.5"
    d10
    (center (p8:sp13 ph0):f2 (p2 ph1):f1)
    d10
}
else{
d0
(p2 ph1)
d0
}
(p21 ph0):f3
4u
p19:gp4
d16
(p1 ph1)
8u
p16:gp2
d16 p10:f1
DELTA
(p11:sp1 ph2:r):f1
4u
4u p11:f1
(center (p2 ph0) (p22 ph0):f3 )
4u p10:f1
(p11:sp1 ph2:r):f1
4u
p16:gp2
d16
DELTA1
4u BLKGRAD
4u p116:f3

goscnp ph31 cpd3:f3

```

```

3m do:f3
3m st ivd
lo to 3 times nbl

3m ipp4 ipp5 ipp6 ipp8 ipp9 ipp10 ipp31
lo to 4 times ns

d1 mc #0 to 4
  F1QF()
  F2PH(caliph(ph4, +90) & caliph(ph8, +90) & exec(rppall), caldel(d0, +in0))
exit

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

;td1: number of delays in vd-list
;td2: number of experiments in F2
;nbl: must be set to same values as tdl
;p11 : f1 channel - power level for pulse (default)
;p13 : f3 channel - power level for pulse (default)
;p116: f3 channel - power level for CPD/BB decoupling
;p1 : f1 channel - 90 degree high power pulse
;p8 : f2 channel - carbon decoupling pulse
;p11 : 1000u - 90 degree selective water
;p10 : 2000u - 180 degree selective water
;p15: 1500u - gaussian water refocusing pulse
;spnam1: Sincl.1000 - shaped pulse 90 degree Watergate
;sp1: 45.8 dB for 1.5 ms 90 deg. Sincl.1000 pulse
;p16: 0.9m gradient pulse of Watergate
;p19: 0.5m gradient pulse
;p20: pfg for initial spin echo and inept
;p21: f3 channel - 90 degree high power pulse
;p22: f3 channel - 180 degree high power pulse
;p24: (240us) f3 channel - 180 degree decoupling train
;p10: 120 dB
;vd : variable delay, taken from vd-list
;NBL: = tdl
;d0 : incremented delay (2D) [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d10: computed internally for C-decoupling
;d11: delay for disk I/O [30 msec]
;d16: delay for homospoil/gradient recovery
;cnst10: 1 for 90/-180, 2 for 180/-360 phase correction
;d26 : 1/(4J)NH (refocussing INEPT)
;in0: 1/(2 * SW(X)) = DW(X)
;nd0: 2
;NS: 4 * n
;DS: 16
;td1: number of experiments
;FnMODE: States-TPPI, TPPI, States or QSEC
;cpd3: decoupling according to sequence defined by cpdprg3
;pcpd3: f3 channel - 90 degree pulse for decoupling sequence

;for z-only gradients:

```

```

;gpz0: 15%
;gpz1: 10% (suppression of radiation damping)
;gpz2: 25% (Watergate)
;gpz3: 35%
;gpz4: 50%
;gpz7: 27% (kill initial N magnetisation)

;use gradient files:
;gpnam0: SINE.100
;gpnam1: SINE.100
;gpnam2: SINE.100
;gpnam3: SINE.100
;gpnam7: SINE.100

```

Bruker pulse program of the $R_1(^1\text{H})$ pseudo-3D interleaved $[^{15}\text{N}-^1\text{H}]$ -HSQC saturation–recovery relaxation experiment (see Figure 7 of main text)

```

;hsqcNwgtlps3d.ho
;avance-version (02/05/31)
;15N-HSQC with watergate(using selective 90 deg. pulses) with saturation
;recovery
;32-step phase cycle, NS=8 is OK
;with decoupling during acquisition
;using f3 - channel
;water suppression using 90sel-180-90sel Watergate
;t1 relaxation measured via pseudo 3d interleaved acquisition

;H. W. Orton and G. Otting

;$DIM=3D

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>

prosol relations=<triple>

;shaped pulse powers for 3.5 ms selective WET saturation
"p13=3500"
"spw2=plw1*pow((((0.25*(81.4/90.))/(p13*0.5889))/(0.25/p1)),2)" ;Sinc pulse power
"spw3=plw1*pow((((0.25*(101.4/90.))/(p13*0.5889))/(0.25/p1)),2)" ;Sinc pulse power
"spw4=plw1*pow((((0.25*(69.3/90.))/(p13*0.5889))/(0.25/p1)),2)" ;Sinc pulse power
"spw5=plw1*pow((((0.25*(161.1/90.))/(p13*0.5889))/(0.25/p1)),2)" ;Sinc pulse power

"spoal2=0.5"
"spoal3=0.5"
"spoal4=0.5"
"spoal5=0.5"

"spoffs2=0"
"spoffs3=0"
"spoffs4=0"
"spoffs5=0"

"d0=3u"

```

```

"in0=inf2/2"

"p2=p1*2"

"p22=p21*2"

"d0=in0*0.5*cnst10-p21*0.63-p2*0.5"
"d16=200u"
"d11=30m"
"d26=1s/(cnst4*4)"
"DELTA=d26-p16-d16-p11-16u"
"DELTA1=d26-p16-d16-p11-16u+p1*0.63"
"DELTA2=d26-p19-4u-d16"
"d10=3u"

```

aqseq 312

```

1 30m ze
   d11 st0
2 6m do:f3
3 3m
4 d1 pl3:f3 pl2:f2 pl1:f1
   "TAU=vd"
   (p21 ph0):f3
   8u
   4u UNBLKGRAD
   (p1*0.904 ph0)
   10u pl0:f1
   (p13:sp2 ph2):f1
   4u
   p16:gp21
   d16 pl1:f1
   (p1*1.127 ph1)
   10u pl0:f1
   (p13:sp3 ph3):f1
   4u
   p16:gp22
   d16 pl1:f1
   (p1*0.77 ph1)
   10u pl0:f1
   (p13:sp4 ph3):f1
   4u
   p16:gp23
   d16 pl1:f1
   (p1*1.789 ph1)
   10u pl0:f1
   (p13:sp5 ph3):f1
   4u
   p16:gp24
   d16 pl1:f1
   2u BLKGRAD
   TAU
   2u UNBLKGRAD
   (p1 ph5)
   4u
   p19:gp0
   d16
   DELTA2
   (center (p22 ph6):f3 (p2 ph1):f1)
   DELTA2
   4u
   p19:gp0
   d16

```

```

(p1 ph9)
4u
p19:gp4
d16
(p21 ph4):f3
if "d0>p8*0.5"{
  "d10=d0-p8*0.5"
  d10
  (center (p8:sp13 ph0):f2 (p2 ph8):f1)
  d10
}
else{
d0
(p2 ph8)
d0
}
(p21 ph11):f3
4u
p19:gp4
d16
(p1 ph10)
8u
p16:gp2
d16 p10:f1
DELTA
(p11:sp1 ph2:r):f1
4u
4u p11:f1
(center (p2 ph0) (p22 ph0):f3 )
4u p10:f1
(p11:sp1 ph2:r):f1
4u
p16:gp2
d16
DELTA1
4u BLKGRAD
4u p116:f3

goscnp ph31 cpd3:f3

3m do:f3
3m st ivd
lo to 3 times nbl

3m ipp4 ipp5 ipp8 ipp11 ipp9 ipp10 ipp31
lo to 4 times ns

d1 mc #0 to 4
  F1QF()
  F2PH(calph(ph4, +90) & calph(ph6, +90) & exec(rppall), caldel(d0, +in0))
exit

ph0=0
ph1=1
ph2=2
ph3=3

ph4=0 2
ph5=0 0 0 0 2 2 2 2
ph6=0
ph8=1 1 3 3
ph11=(4) {0}*16 {2}*16
ph9=(4) {1}*8 {3}*8

```

```

ph10=2 2 2 2 0 0 0 0
ph31=(4) {0 2 0 2 0 2 0 2 2 0 2 0 2 0 2 0}^2

;td1: number of delays in vd-list
;td2: number of experiments in F2

;p11 : f1 channel - power level for pulse (default)
;p13 : f3 channel - power level for pulse (default)
;p116: f3 channel - power level for CPD/BB decoupling
;p1 : f1 channel - 90 degree high power pulse
;p8 : f2 channel - carbon decoupling pulse
;p11 : 1500u - 90 degree
;spnam1: Sinc1.1000 - shaped pulse 90 degree Watergate
;sp1: Sinc1.1000 pulse
;p16: 0.9m gradient pulse of Watergate
;p19: 0.5m gradient pulse
;p21: f3 channel - 90 degree high power pulse
;p22: f3 channel - 180 degree high power pulse
;p24: (240us) f3 channel - 180 degree decoupling train
;p10: 120 dB

;vd : variable delay, taken from vd-list
;NBL: = td1

;d0 : incremented delay (2D) [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d10: computed internally for C-decoupling
;d11: delay for disk I/O [30 msec]
;d12: delay for power switching [20 usec]
;d16: delay for homospoil/gradient recovery
;cnst10: 1 for 90/-180, 2 for 180/-360 phase correction
;d26 : 1/(4J)NH (refocussing INEPT)
;in0: 1/(2 * SW(X)) = DW(X)
;nd0: 2
;NS: 4 * n
;DS: 16
;td1: number of experiments
;FnMODE: States-TPPI, TPPI, States or QSEC
;cpd3: decoupling according to sequence defined by cpdprg3
;pcpd3: f3 channel - 90 degree pulse for decoupling sequence

;for z-only gradients:
;gpz0: 15%
;gpz1: 8% (suppression of radiation damping)
;gpz2: 25% (Watergate)
;gpz3: 35% (strong gradient)
;gpz4: 50%
;gpz21: 80% (WET gradient 1)
;gpz22: 40% (WET gradient 2)
;gpz23: 20% (WET gradient 3)
;gpz24: 10% (WET gradient 4)

;use gradient files:
;gpnam0: SMSQ10.100
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
;gpnam3: SMSQ10.100
;gpnam4: SMSQ10.100
;gpnam21: SMSQ10.100
;gpnam22: SMSQ10.100
;gpnam23: SMSQ10.100
;gpnam24: SMSQ10.100

```

Bruker pulse program for $R_2(^1\text{H})$ pseudo-4D interleaved HNCO relaxation experiment (see Figure 6 of main text)

```
;hncogpwgps4dr2.ho
;avance-version (12/01/11)
;HNCO-R2(1H)
;3D sequence with psuedo R2 relaxation dimension
;  inverse correlation for triple resonance using multiple
;    inept transfer steps
;
;    F1(H, TAU(R2,t1)) -> F3(N) -> F2(C=O,t2) -> F3(N,t3) -> ; F1(H,t4)
;
;on/off resonance Ca and C=O pulses using shaped pulse
;phase sensitive (t1)
;using constant time in t2
;relaxation delays controlled by vdlist with pseudo-4D
;interleaved acquisition
;
;$DIM=4D

prosol relations=<triple>

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>

;;;;;;;;Gradient definitions;;;;;;;;;
"p16=600u"
"p19=900u"
"d16=200u"

;;;;;;;;High power pulse lengths ;;;;
"p2=p1*2"
"p4=p3*2"
"p22=p21*2"
"d11=30m"

;;;;;;;;;; J-coupling parameters ;;;;;;;;;;
;d21: 1/(2J(NH) [5.5 msec]
;d23: 1/(4J(NCO) [12 msec]
;d26: 1/(4J'(NH) [2.3 msec]
"d21=5.5m"
"d23=12m"
"d26=2.3m"

;;;;;;;;;;;;;Shaped pulse calculations;;;;;;;;;;;;;

;H2O Sinc Excitation/De-excitation
;spnam5 : Sinc.1000
"p5=1m"
"spw5= plw1*pow((90/(0.5889*p5))/(90/p1),2)"
"spoff5=0"

;H2O Sinc Inversion/Flipback
;spnam6 : Sinc.1000
"p6=1m"
"spw6= plw1*pow((180/(0.5889*p6))/(90/p1),2)"
"spoff6=0"
;H2O Square Excitation/Flipback
"p7=60u"
"plw19=plw1*pow((90/(1.0*p7))/(90/p1),2)"
```

```

;Carbon offsets
"cnst21=(sfo2-bf2)*1000000/bf2"
;cnst21 : Carbon carrier ppm
"cnst22= cnst21 + (173.7-cnst21) "
;cnst22 : CO frequency ppm
"cnst23= cnst21 + (53.7 -cnst21) "
;cnst23 : CA frequency ppm

;CO Q3 Inversion 15N INEPT
;spnam11 : Q3.1000
"p11 = 3.41/(60.0*bf2/1000000)"
"spw11= plw2*pow((180/(0.1515*p11))/(90/p3),2)"
"spoff11= (cnst22-cnst21)*bf2/1000000"
"spoal11=0.5"
;CO Q5 Excitation
;spnam12 : Q5.1000
"p12 = 6.18/(60.0*bf2/1000000)"
"spw12= plw2*pow((90/(0.0545*p12))/(90/p3),2)"
"spoff12= (cnst22-cnst21)*bf2/1000000"
"spoal12=1"
;CA inversion with Bloch-Seigert zero phase on CO
;spnam13 : Squal00.400
"p13 = (180/360)*pow(3,0.5)/(abs(cnst23-cnst21)*bf2/1000000)"
"spw13= plw2*pow((180/p13)/(90/p3),2)"
"spoff13= (cnst23-cnst21)*bf2/1000000"
"spoal13=0.5"
;CO Q5 Flipback
;spnam14 : Q5tr.1000
"p14 = 6.18/(60.0*bf2/1000000)"
"spw14= plw2*pow((90/(0.0545*p14))/(90/p3),2)"
"spoff14= (cnst22-cnst21)*bf2/1000000"
"spoal14=0"
;CA Q3 Inversion
;spnam15 : Q3.1000
"p15 = 3.41/(100.0*bf2/1000000)"
"spw15= plw2*pow((180/(0.1515*p15))/(90/p3),2)"
"spoff15= (cnst23-cnst21)*bf2/1000000"
"spoal15=0.5"

;;;;;;;;;; Evolution increments ;;;;;;;;;;
"in0=inf2/2"
"in10=inf1/4"
"in29=inf1/4"
"in30=inf1/4"

;;;;;;;;;; CO evolution delay ;;;;;;;;;;
"d0=in0*0.5*cnst10-0.5*larger(p13,p22)"
;cnst10 : if 1: phase correction 90/-180

;;;;;;;;;; N constant-time evolution delays ;;;;;;;;;;
"d10=d23/2-p11/2"
"d29=d23/2-p15/2-p7-d21-4u"
"d30=d23/2-p15/2"

;;;;;;;;;; Reverse INEPT delays ;;;;;;;;;;
"DELTA1=d23-d21-p7"
"DELTA2=d26-p16-d16-p6-12u"

;;;;;;;;;; INEPT+relaxation delays;;;;;;;;;
"DELTA4=d26-p16-d16-4u"
"DELTA5=d26-4u-8u-4u-p6-4u-p16-d16"

```



```

define list<delay> R2Delay = <$VDLIST>

1 d11 ze
  d11 pl16:f3
2 d11
3 d11 do:f3
4 d11
5 d1 pl1:f1 pl3:f3
  "TAU = R2Delay*0.25 + 2u"
  4u UNBLKGRAD
  (p21 ph0):f3
  p19:gp7 ;Kill1 nitrogen magnetisation
  1m pl0:f1
  (p5:sp5 ph4):f1 ;Water selective excitation
  8u
  16u pl1:f1
  8u
  ;;;;;;;;; Start Relaxation Block ;;;;;;;;;
  (p1 ph5):f1
  4u
  p16:gp4
  d16
  DELTA4
  TAU
  (p22 ph0):f3
  TAU
  (p2 ph0):f1
  4u
  8u pl0:f1
  4u
  (p6:sp6 ph2)
  4u
  p16:gp4
  d16
  DELTA5
  4u pl1:f1
  TAU
  (p22 ph0):f3
  TAU
  (p1 ph1):f1
  ;;;;;;;;; End Relaxation Block ;;;;;;;;;

  4u pl0:f1
  (p5:sp5 ph3:r):f1
  4u
  p16:gp1
  d16

  (p21 ph0):f3
  d21 pl19:f1
  (p7 ph1):f1
  DELTA1 cpds1:f1 ph0
  (center (pl1:sp11 ph0):f2 (p22 ph0):f3 )
  d23
  (p21 ph0):f3

  ;;;;;;;;; Start CO evolution ;;;;;;;;;
  (p12:sp12 ph6):f2;
  d0
  (center (p13:sp13 ph0):f2 (p22 ph0):f3 );
  d0

```

```

(p14:sp14 ph0):f2;
;;;;;;;;; End CO evolution ;;;;;;;;;;

4u do:f1
(p7 ph3):f1
4u
p16:gp2
d16
(p7 ph1):f1
20u cpds1:f1 ph0

;;;;;;;;;; Start N evolution ;;;;;;;;;;
(p21 ph7):f3
d30
(p15:sp15 ph0):f2;
d30
(center (p11:sp11 ph0):f2 (p22 ph0):f3 );
d10
(p15:sp15 ph0):f2;
d29
4u do:f1
(p7 ph3):f1
;;;;;;;;; End N evolution ;;;;;;;;;;
d21
(p21 ph0):f3

p16:gp3
d16 pl0:f1
(p5:sp5 ph2):f1;
4u
4u pl1:f1

(p1 ph0)
4u
p16:gp4
d16
DELTA2 pl0:f1
(p5:sp5 ph2):f1;
4u
4u pl1:f1
(center (p2 ph0) (p22 ph0):f3 )
4u pl0:f1
(p5:sp5 ph2):f1;
4u
DELTA2
p16:gp4
d16 pl16:f3
4u BLKGRAD

go=3 ph31 cpd3:f3
3m do:f3 zd

d11 st R2Delay.inc
lo to 4 times nbl

d11 st0 R2Delay.res
d11 mc #0 to 5
F3QF()
F2PH(calph(ph6, +90), caldel(d0, +in0))
F1PH(calph(ph7, +90), caldel(d10, +in10) & caldel(d29, +in29) &
caldel(d30, -in30))
exit

```

```

ph0=0
ph1=1
ph2=2
ph3=3
ph4=2 2 2 2 0 0 0 0
ph5=0 0 0 0 2 2 2 2
ph6=0 2
ph7=0 0 2 2
ph31=0 2 2 0 2 0 0 2

;p10 : 0W
;p11 : f1 channel - power level for pulse (default)
;p13 : f3 channel - power level for pulse (default)
;p116: f3 channel - power level for CPD/BB decoupling
;p119: f1 channel - power level for CPD/BB decoupling
;p1 : f1 channel - 90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p11: f1 channel - 90 degree shaped pulse [2 msec]
;p13: f2 channel - 90 degree shaped pulse
;p16: homospoil/gradient pulse [1 msec]
;p21: f3 channel - 90 degree high power pulse
;p22: f3 channel - 180 degree high power pulse
;d0 : incremented delay (F1 in 3D) [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d10: incremented delay (F2 in 3D) = d23/2-p14/2
;d11: delay for disk I/O [30 msec]
;d13: short delay [4 usec]
;d16: delay for homospoil/gradient recovery
;d29: incremented delay (F2 in 3D) = d23/2-p14/2-p26-d21-4u
;d30: decremented delay (F2 in 3D) = d23/2-p14/2
;inf1: 1/SW(CO) = 2 * DW(CO)
;inf2: 1/SW(N) = 2 * DW(N)
;in0: 1/(2 * SW(CO)) = DW(CO)
;nd0: 2
;in10: 1/(4 * SW(N)) = (1/2) DW(N)
;nd10: 4
;in29: = in10
;in30: = in10
;ns: 8 * n
;ds: >= 16
;td1: number of experiments in F1
;td2: number of experiments in F2 td2 max = 2 * d30 / in30
;FnMODE: States-TPPI (or TPPI) in F2
;FnMODE: States-TPPI (or TPPI) in F3
;cpds1: decoupling according to sequence defined by cpdprg1
;cpd3: decoupling according to sequence defined by cpdprg3
;pcpd1: f1 channel - 90 degree pulse for decoupling sequence
;pcpd3: f3 channel - 90 degree pulse for decoupling sequence

;use gradient files:
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
;gpnam3: SMSQ10.100
;gpnam4: SMSQ10.100
;gpnam5: SMSQ10.100

```

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