Solution Domain Dynamics of Monomeric SARS-CoV-2 Main Protease Revealed by Optimized NMR Residual Dipolar Coupling Measurements.

Supporting Information

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A. Experimental details

1. Expression and purification of $^{2}H/^{15}N/^{13}C$-enriched $MPro^{10-306,H41Q}$

A DNA insert encoding amino acids 10 through 306 of the SARS-CoV-2 main protease[1-2] with an active site H41Q substitution ($MPro^{10-306,H41Q}$, Figure S1A)[3], and a C-terminal GP-6H tag was cloned into the pJ414 vector (ATUM, Newark, CA). The construct was transformed into *E. coli* BL21-DE3 cells (Agilent, Santa Clara, CA). Cell culture (1 L) was grown at 37 °C in minimal medium, prepared in D_2O with $^{15}NH_4Cl$ and $^{13}C_6$-glucose as the sole nitrogen and carbon sources, to an optical density (600 nm) of 0.7-0.8. Next, expression was induced with 0.25 mM isopropyl β-d-1-thiogalactopyranoside for 16-18 h at 18 °C. Cells were suspended in 30 ml of 20 mM Tris-HCl, pH 8, 150 mM NaCl, 2 mM 2-mercaptoethanol and 20 mM imidazole, lysed by sonication, and centrifugated at 20,000g for 30 min at 4 °C. Soluble $MPro^{10–306,H41Q}$-GP-6H from the supernatant was purified by nickel-affinity chromatography (NAC, step 1). The bound fraction (~ 19 mg/2.5 ml) was subjected to isocratic fractionation on a Superose-12 column (1.6 cm x 60 cm, Cytiva Life Sciences) at a flow rate of 1.5 ml/min in 20 mM Tris-HCl, pH 8, 150 mM NaCl and 1 mM TCEP (step 2, Figure S1B). Peak fractions were pooled (~ 17 mg), and the GP-6H tag was removed by incubation with 68 µg of HRV-3C protease (Millipore-Sigma, Burlington, MA) overnight at 4 °C (step 3). $MPro^{10-306,H41Q}$ recovered in the unbound fraction following NAC (step 4) was concentrated using Amicon Ultra 10K centrifugal filtration (Millipore Corp, Bedford, MA) and another fractionation (step 5) on the Superose-12 column (1.6 cm x 60 cm) in NMR buffer (25 mM Hepes, pH 6.9, 20 mM NaCl and 1 mM TCEP). Purity was verified by SDS-PAGE (Figure S1C) on a 4-20% gradient mini-protean TGX precast gel (Bio-Rad, Herculus, CA), and HPLC with inline electrospray ionization mass spectrometry.[1] Peak fractions were pooled, concentrated to ~ 0.6 mM, and stored in aliquots at -80 °C until use.

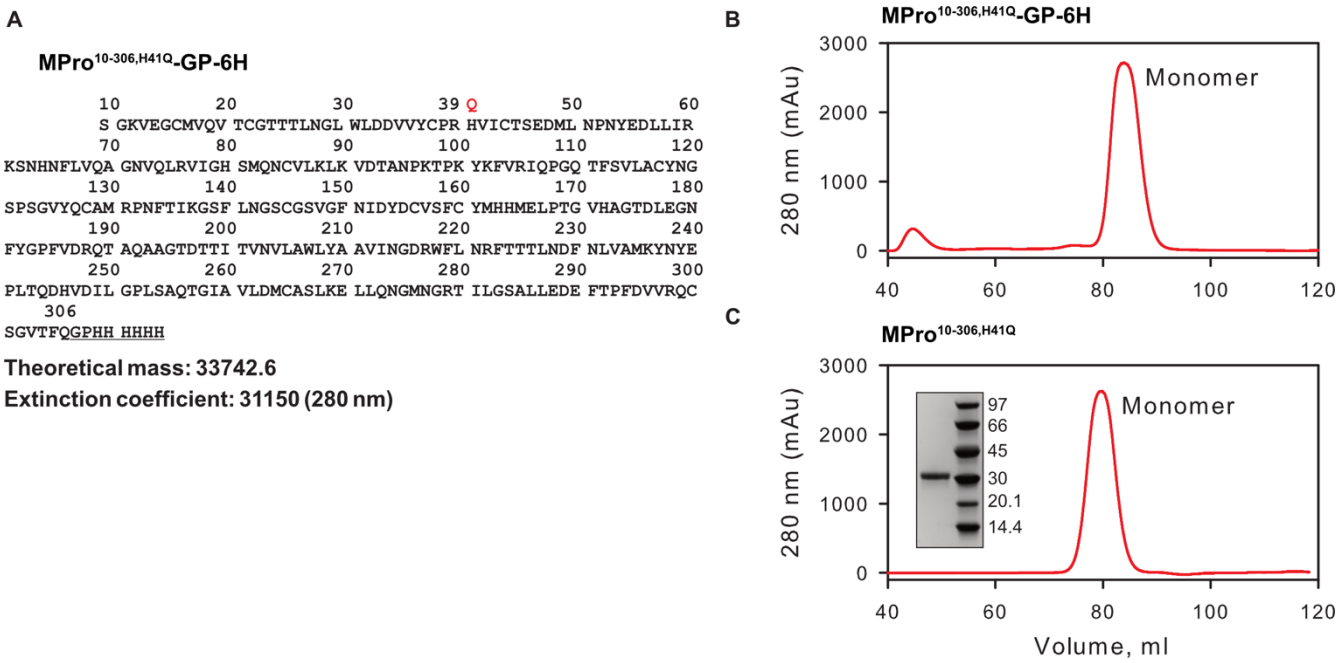


Figure S1. Amino acid sequence and purification of recombinant MPro$^{10\text{-}306,H41Q}$. (A) The H41Q mutation is indicated in red. Non-native GP-6H residues at the C-terminus are underlined. Calculated mass and extinction coefficient are indicated. The GP-6H-tag enables isolation by nickel-affinity chromatography followed by its removal with HRV-3C protease to yield the exact sequence of MPro$^{10\text{-}306,H41Q}$. (B) Elution profiles of MPro$^{10\text{-}306,H41Q}$ monomer on Superose-12 column before (step 2) and after (step 5) removal of the GP-6H tag. The inset shows the SDS-PAGE of purified MPro$^{10\text{-}306,H41Q}$ with molecular weight standards in kilodalton. The deconvoluted mass of MPro$^{10\text{-}306,H41Q}$-GP-6H and MPro$^{10\text{-}306,H41Q}$ determined by ESI-MS is 32741 and 32768 Da, consistent with the calculated mass of 33743 and 32766 Da, respectively. Based on the simple rule that when the following residue is Gly or Ser, the N-terminal methionine was fully excised by methionyl-aminopeptidase, as verified by mass spectrometry.[4-5] No dimer was detectable up to ~ 200 µM of MPro$^{10\text{-}306}$ by sedimentation-velocity analytical ultracentrifugation, even in the presence of inhibitor [1] or for up to ~ 600 µM of MPro$^{10\text{-}306,H41Q}$ by NMR.[3]

2. NMR experiments

All data were acquired on either a 600-MHz or 800-MHz Bruker Avance NEO spectrometer, running Topspin 4.1.4, with a 5-mm TXI or TCI cryoprobe, respectively, including a *z*-gradient coil with a maximum nominal gradient strength of 0.65 T m^{-1} and *x*- and *y*-gradients of 0.5 T m^{-1}. A 400-μM $MPro^{H41Q,10-306}$ sample with 25 mM HEPES buffer, 20 mM NaCl, 1 mM TCEP and 0.03% NaN3 was used for obtaining the scalar couplings; the RDC data used a similar sample with an additional 13 mg mL^{-1} Pf1.

The 2D ^{1}H-^{15}N ARTSY data was recorded at 800 MHz using full sampling. The data matrix consisted of 3072 complex points in the direct dimension (t_2, ^{1}H, 129 ms) and 512 complex points in the indirect dimension (t_1, ^{15}N, 98.8 ms). With an interscan delay of 1.8s and 8 scans per FID, the total acquisition time was 5 h.

The 2D ^{1}H-^{15}N mixed-time $QJ_{NC'}$ data were recorded at 800 MHz using full sampling. The data matrix consisted of 3072 complex points in the direct dimension (t_2, ^{1}H, 123 ms) and 400 complex points in the indirect dimension (t_1, ^{15}N, 77.2 ms). With an interscan delay of 1.8 s and 8 scans per FID, the total acquisition time was 4 h. The constant-time QJNC′ data used similar parameters but 170 points were acquired in the indirect dimension (32.8 ms) and the reference spectrum was acquired with 8 scans per FID, and the attenuated spectrum used 32 scans per FID, for a total acquisition time of 4 h.

The 3D ARTSY-HNCO data was recorded at 600 MHz using unweighted non-uniform sampling in the indirect dimensions (t_1 and t_2), with 22% of t_3 FIDs randomly selected from the full data matrix: 2048 points in t_3 (^{1}H, 118.7 ms), 260 points in t_2 (^{15}N, 65 ms), and 70 points in t_1 (^{13}C, 18.9 ms). With an interscan delay of 1.7 s and 4 scans per FID, the total acquisition time was 17.5 h.

The $QJ_{C\alpha C'}$ data were recorded at 600 MHz using unweighted non-uniform sampling in the indirect dimensions (t_1 and t_2), with 22% of t_3 FIDs randomly selected from the full data matrix: 2048 (t_3, ^{1}H, 118.7 ms) × 260 (t_2, ^{15}N, 65 ms) × 70 (t_1, ^{13}C, 19.2 ms) complex points. With an interscan delay of 1.7 s and 4 scans per FID, the total acquisition time was 17.5 h.

The TATER experiment was recorded at 600 MHz using unweighted non-uniform sampling in the indirect dimensions (t_1 and t_2), with 40% of t_3 FIDs randomly selected from the full data matrix: 2048 (t_3, ^{1}H, 118.7 ms) × 260 (t_2, ^{15}N, 65 ms) × 128 (t_1, ^{13}C, 35.2 ms) hypercomplex points. With an interscan delay of 1.7 s and 2 scans per FID, the total acquisition time was 28 h.

Schematic representations of the pulse sequences used for measuring $^{1}D_{NH}$, $^{1}D_{NC'}$, $^{1}D_{C'C\alpha}$ and $^{2}D_{C'H}$ are shown in Figures S2-S5. In all figures, black and grey narrow rectangles represent hard 90° and 180° pulses, respectively. The green open pulses represent ^{15}N composite 180° pulses (90_x-220_y-90_x) that compensate for both offset and RF inhomogeneity. The shaped, narrow, open ^{1}H pulses represent frequency-selective flip-back pulses for water suppression. Double-headed arrows represent fixed delays; single-headed arrows represent incremented delays. All gradients were either sine-bell or rectangular shaped as indicated in the pulse sequence diagrams.

2.1 ARTSY ^{1}H-^{15}N pulse scheme

Figure S2 shows the 2D ^{1}H-^{15}N ARTSY pulse sequence. The combination of the initial ^{15}N 90° pulse and the gradient G_1 destroyed the ^{15}N equilibrium magnetization. Two interleaved datasets were acquired that yielded a reference and attenuated spectrum, with the ^{15}N composite (green) pulses applied either at the midpoint between the ^{1}H 180° and 90° pulses or immediately after the ^{1}H 180° pulse. The period T was set to 10.75 ms, τ = 2.1 ms and τ' = 2.71 ms. Eight transients were acquired for the reference and attenuated datasets. The rectangular ^{13}C' and ^{13}C$^{\alpha}$ pulses were applied with an RF field strength of $\Delta f/\sqrt{3}$ where Δf is the frequency separation between the centers of the ^{13}C' and ^{13}C$^{\alpha}$ chemical shift regions. The weak rectangular gradient G_2 aids in dephasing the water signal and thereby prevents radiation damping; G_3 is a homospoil gradient; G_6 and G_7 serve for artifact suppression. Quadrature detection in F_1 is achieved using Echo-Antiecho encoding[6] by inverting the phases φ_3 and φ_5 together with the sign of G_4 and G_5. The duration of the delay τ was shortened to reduce anti-TROSY[7]artifacts.[7] ^{13}C decoupling was applied during acquisition of the FID.

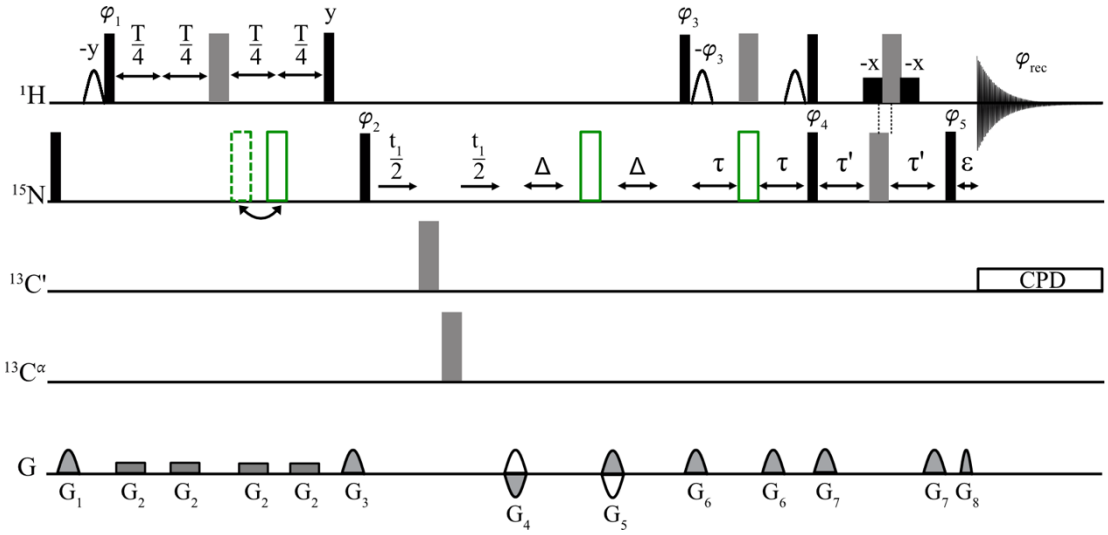


Figure S2. Schematic representation of the ARTSY pulse sequence. Black narrow and wide grey rectangles represent hard 90° and 180° pulses. The experiment is executed twice in an interleaved manner, with the first ^{15}N 180° composite pulses (denoted by the green open rectangles) applied at the midpoint between the ^{1}H 180° and 90° pulses corresponding to 5.38 ms $^{1}J_{NH}$+$^{1}D_{NH}$ dephasing, and immediately after the ^{1}H 180° pulse corresponding to a $^{1}J_{NH}$+$^{1}D_{NH}$ dephasing of 10.75 ms (dashed green pulse). The delays T, τ and τ' were set to 10.75 ms, 2.1 ms and 2.71 ms, respectively. Gradients $G_{1,3,4,5,6,7,8}$ are sine-bell shaped with durations of 1.0, 0.5, 0.5, 0.5, 0.15, 0.79 and 0.101 ms, respectively, and strengths of 33, 51, 51, -41, 15, 57 and 46 % of the maximum z-gradient strength. G_4, G_5 and G_8 were accompanied by transverse x,y gradients of 0.5,0.5,0.101 ms and gradient strengths 51, -41 and 46%, respectively. The rectangular gradients G_2 had durations of 2.53 ms and *z*-strengths of 3%. The phase cycling is shown below, with undefined pulses applied along x. Quadrature detection in F_1 is achieved by inverting the amplitudes of G_4 and G_5 together with φ_3 and φ_5. Chemical shift evolution during the decoding delay ε = 0.3 ms, and the resulting linear phase error, were compensated by offsetting the last pair of ^{1}H and ^{15}N 180° pulses by ε/2.

φ_1	x, -x
φ_2	y, y, -y, -y
φ_3	-y
φ_4	x_4, $-x_4$
φ_5	$-y_4$, y_4
φ_{rec}	y, -y, -y, y, -y, y, y, -y

2.2 ARTSY-HNCO pulse scheme

The schematic representation of the ARTSY-HNCO pulse sequence (Figure S3) is analogous to the 2D version except for the additional INEPT transfer and ^{13}C' chemical shift encoding periods. The ^{15}N chemical shift uses a mixed time approach where constant-time evolution is followed by real-time evolution with ζ_a=max(0,t_2/2) and ζ_b=max(T_b-t_2/2), with T_b=12.5 ms. The additional delays in the 3D pulse sequence are largely responsible for the substantial sensitivity difference between the 2D and 3D experiments. The open pulses on the ^{13}C' channel represent inversion pulses, shaped with the profile of the center lobe of a sin(*x*)/*x* function and typical durations of 200 μs at 150.9 MHz ^{13}C frequency centered at 176 ppm, while the open pulses on the $^{13}C^{\alpha}$ channel represent 300-μs 180° Q3 pulses.[8]

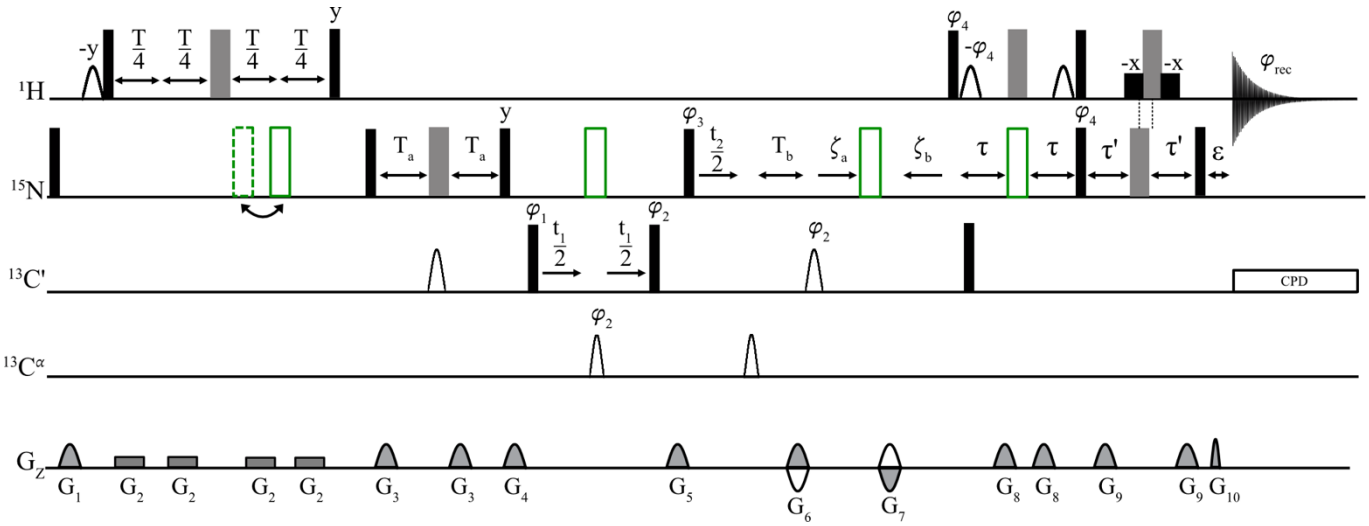


Figure S3. Schematic representation of the ARTSY-HNCO pulse sequence. Black narrow and wide grey rectangles represent hard 90° and 180° pulses, respectively, while green open rectangles represent composite 180° ^{15}N pulses. The experiment is executed twice in an interleaved fashion, with the first ^{15}N 180° composite pulse being applied at the midpoint between the ^{1}H 180° and 90° pulses, corresponding to 5.38 ms $^{1}J_{NH}$+$^{1}D_{NH}$ dephasing, or immediately after the ^{1}H 180° pulse (dashed green pulse), corresponding to $^{1}J_{NH}$+$^{1}D_{NH}$ dephasing for 10.75 ms. The delays T, τ and τ' were set to 10.75 ms, 2.1 ms and 2.71 ms, respectively. Initially a constant-time approach is used where t_2/2 is incremented and ζ_b is decremented (ζ_b =T_b-t_2/2), where T_b=12.5 ms. Once the constant-time evolution has been exhausted, a real time approach is implemented where t_2/2 and ζ_a are incremented. $G_{1,3,4,5,6,7,8,9,10}$ are sine-bell shaped pulsed field gradients with durations of 1.8, 3.0, 0.5, 0.5, 0.5, 0.5, 0.24, 0.7 and 0.101 ms, respectively, and strengths of 17, 17,

37, 41, 53, -43, 9, 53 and 48%. G_2 is a weak rectangular gradient with a duration of 2.53 ms and a gradient strength of 5.3%. The chemical shift evolution during the decoding delay $\varepsilon = 0.3$ ms, and the resulting linear phase error, are compensated by offsetting the last pair of ^{1}H and ^{15}N 180° pulses by $\varepsilon/2$. The phase cycling is as shown below, with undefined pulses being applied along x. Quadrature detection in F_1 was achieved by States-TPPI encoding, incrementing φ_1 by 90°. Quadrature F_2 detection was achieved by using Echo-AntiEcho encoding, inverting the amplitudes of G_6 and G_7 together with the phase of φ_4. Axial peaks were shifted to the edge of the spectrum by inverting φ_3 and the receiver phase in alternate increments.

φ_1	y
φ_2	y, -y
φ_3	y, y, -y, -y
φ_4	-y
φ_{rec}	y, -y, y, -y

2.3 Quantitative $J_{NC'}$ pulse scheme

The pulse sequence for the mixed-time quantitative J experiment for measuring $^1D_{NC'}$ values is shown in Figure S3. The initial INEPT uses a conventional $\tau=1/(4J_{NH})$ delay. Constant-time ^{15}N chemical shift evolution is used until the δ-$t_1/4$ delay becomes negative, with δ and ζ having initial values of $1/8J_{NC'}$ and 0 ms, respectively. Once the constant-time evolution has been exhausted, a real-time approach is used where ζ is incremented. The open shaped pulses on the ^{13}C' channel represent Q3 180° pulses with 350-μs durations at 201.2 MHz ^{13}C frequency. Two datasets are recorded in an interleaved manner with the ^{13}C' 180° Q3 pulses applied (A) immediately before and after the $t_1/4$ chemical shift evolution or (B) before and after an additional Δ delay of $1/8J_{NC'}$, corresponding to reference and attenuated spectra. The open shaped pulse on the $^{13}C^{\alpha}$ channel is a 200-μs Q3 pulse, applied at 56 ppm. Quadrature detection in F_1 is achieved by inverting the amplitudes of the G_3, G_4, G_5, and G_6 gradients together with φ_2.[6]

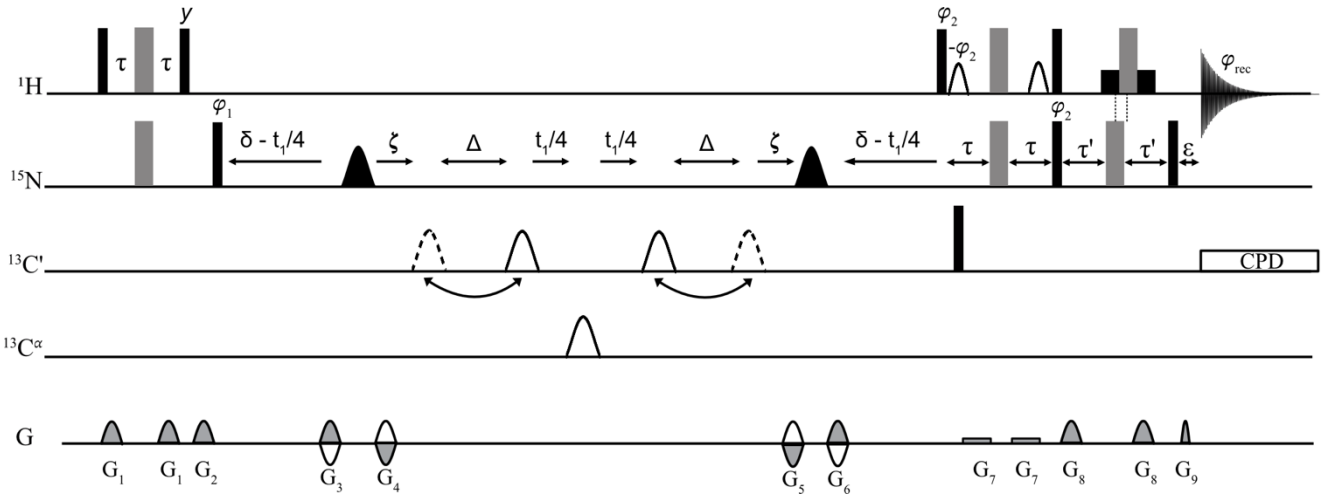


Figure S4. Schematic representation of the mixed-time quantitative-$J_{NC'}$ pulse sequence. Two datasets are acquired in an interleaved fashion with (A) the 350-μs ^{13}C' 180° Q3 pulses (denoted by open shapes)

applied immediately before and after the ^{15}N chemical shift encoding, or (B) before and after an additional delay, Δ, of $1/8^1J_{NC'}$ (dashed pulses). Narrow black and wide grey rectangles represent hard 90° and 180° pulses, respectively. The ^{15}N black shaped pulses are broadband 180° degree PIX pulses using the pix_10.800p1_bw1.49B1.mrf file available at (github.com/manuvsub/GENETICSAI/)[9] with a pulse duration of 432 μs. The open ^{13}C shapes denote Q3 inversion pulses of 200 μs and 350 μs durations at 800 MHz for $^{13}C^{\alpha}$ and ^{13}C' inversion, respectively. Gradients $G_{1,2,3,4,5,6,8,9}$ are sine-bell shaped with durations of 0.98, 1.2, 0.2, 0.3, 0.3, 0.2, 0.98 and 0.101 ms, respectively, and z-gradient strengths of 31, 41, 55, -55, -45, 45, 37 and 50%. $G_{3,4,5,6,9}$ also utilize *x*- and *y*- transverse gradients with the same durations and relative gradient strengths. The gradient G_7 is weak and rectangular with a duration of 1.37 ms and a relative gradient strength of 3 %. The periods δ and Δ are set the $1/(8^1J_{NC'})$ and $\zeta=\max(0, \Delta-t_1/4)$. The period τ was set to 2.1 ms and τ' was set to 2.71 ms. The chemical shift evolution during the decoding delay ε = 0.3 ms, and the resulting linear phase error, are compensated by offsetting the last pair of ^{1}H and ^{15}N 180° pulses by ε/2. Phase cycling was as shown below; undefined pulses were applied along x.

φ_1	y, -y, x, -x, -y, y, -x, x
φ_2	y
φ_{rec}	y, -y, -x, x, -y, y, x, -x

2.4 TROSY-anti-TROSY encoded RDC (TATER) pulse scheme

The schematic representation of the TATER RDC pulse sequence is shown in Figure S5. Two interleaved datasets are acquired, one without the ^{1}H tan/tanh inversion pulses [10] (depicted as orange shapes), and one where the ^{1}H spin state is inverted by the tan/tanh pulses, yielding a conventional HNCO and the ANTI-TROSY HNCO, respectively.

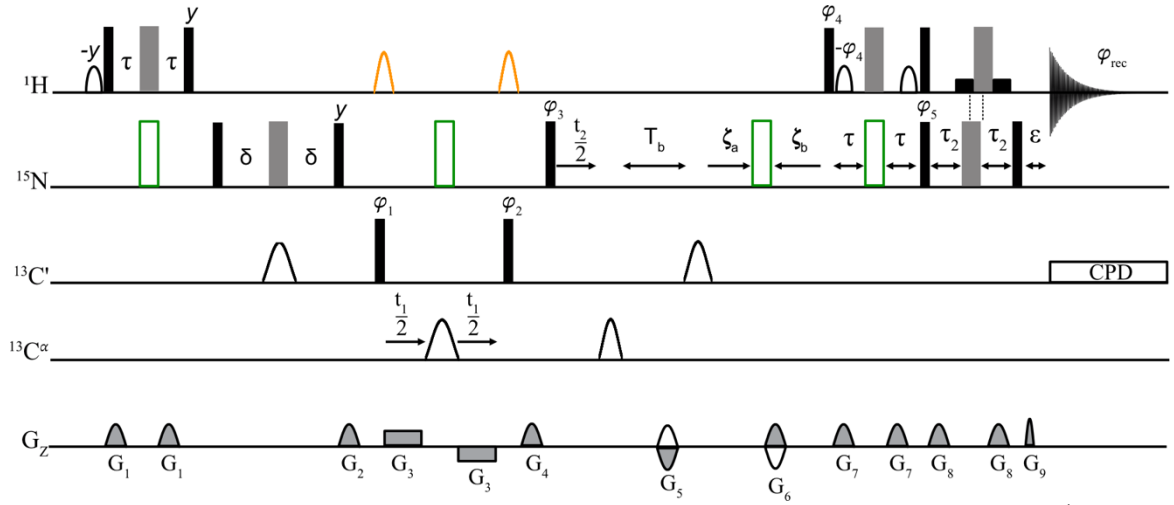


Figure S5. Schematic representation of the TATER pulse sequence for measuring $^{1}D_{C'H}$ RDCs in perdeuterated proteins. Two interleaved datasets are obtained: 1) a conventional TROSY-HNCO spectrum and 2) a dataset where 1-ms adiabatic tanh/tan inversion pulses (denoted by the shaped orange pulses) are employed to invert all ^{1}H nuclei immediately prior to and after the ^{13}C' chemical shift evolution. The ^{13}C' frequency difference between the two datasets corresponds to the sum of $^{2}J_{C'H}$ and $^{2}D_{C'H}$. Data for the isotropic and aligned samples were collected with the same parameters utilizing 40% NUS sampling with a total experiment time of 17 h. The open shaped ^{13}C' pulses denote 150-µs Sinc inversion pulses while the filled shaped $^{13}C^{\alpha}$ pulses are 180° Q3 decoupling pulses of 200-µs durations at 150.9 MHz ^{13}C frequency. Quadrature detection in F_1 was achieved by States-TPPI encoding, incrementing φ_1 by 90°. Quadrature F_2 detection was achieved by utilizing Echo-Antiecho encoding by inverting the amplitudes of G_6 and G_7, together with the phases φ_4 and φ_5, and changing φ_3 from y, y, -x, -x to y, y, x, x. The following delays were used $\tau = \tau_1 = 2.35$ ms, $\tau_2 = 2.7$ ms, $\delta = 12$ ms, $T_b = 12.5$ ms. This sequence also utilized mixed-time ^{15}N chemical shift evolution, where $\zeta_a = \max(0, t_2/2 - T_b)$ and $\zeta_b = \max(T_b - t_2/2, 0)$. Unless otherwise stated all pulses were applied along x. $G_{1,2,3,4,5,6,7,8,9}$ had relative z-gradient strengths of 11, 37, 1.7, 41, 53, -43, 9, 53 and 48% and durations of 1.8, 0.5, $t_1/2$, 0.5, 0.5, 0.5, 0.24, 0.7 and 0.1013 ms, respectively. The chemical shift evolution during the decoding delay $\varepsilon = 0.3$ ms, and the resulting linear phase error, are compensated by offsetting the last pair of ^{1}H and ^{15}N 180° pulses by $\varepsilon/2$. The phase cycling is as shown below with undefined pulses being applied along x:

φ_1	y
φ_2	y, -y
φ_3	y, y, -x, -x
$\varphi_{4,5}$	-y
φ_{rec}	y, -y, x, -x

2.5 Quantitative $J_{C'C\alpha}$ pulse scheme

The quantitative-$J_{C'C\alpha}$ pulse sequence is shown in Figure S6. The ^{13}C chemical shift utilizes constant-time evolution, whereas ^{15}N chemical shift utilizes mixed-time evolution, where ζ starts to increment once the constant-time evolution has completed. Two interleaved datasets are recorded with the 180° Q3 $^{13}C^{\alpha}$ inversion pulses (denoted by the open-shaped pulses) applied simultaneously with the composite ^{15}N 180° pulse (reference spectrum) or after a Δ delay of 14.2 ms (~3/4J_{CC}) corresponding to a total dephasing time of 28.4 ms (attenuated spectrum). The $^{13}C^{\alpha}$ 90° pulse applied with a RF field strength of $\Delta f/\sqrt{3}$ suppresses E. COSY distortions, where Δf is the frequency difference of ~120 ppm between $^{13}C^{\alpha}$ and $^{13}C'$ in units of Hz.

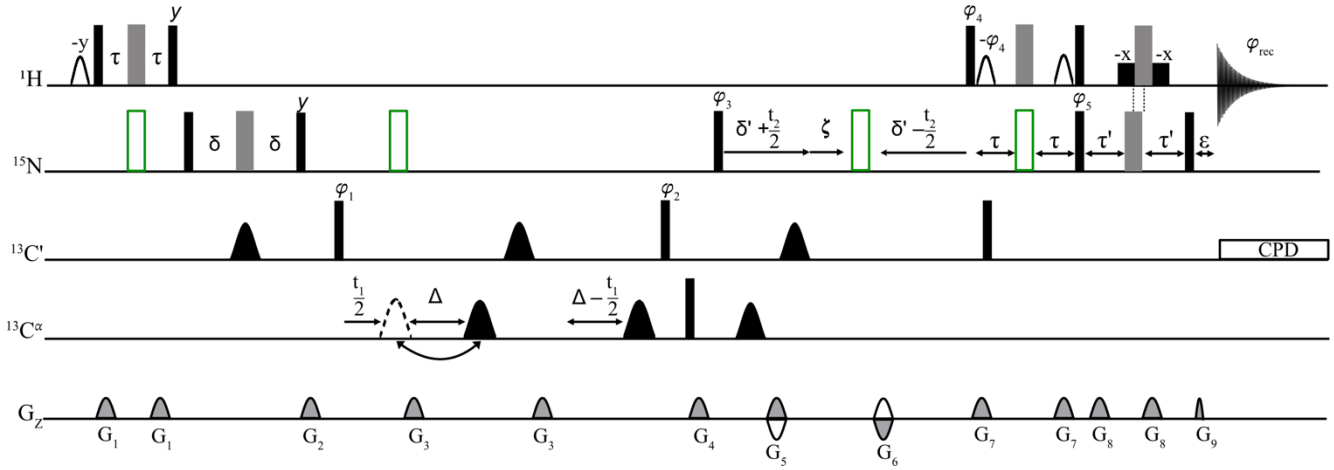


Figure S6. Schematic representation of the quantitative J-correlation pulse sequence for measuring $^{1}D_{C'C\alpha}$. Black and grey rectangles represent hard 90° and 180° pulses, respectively. Green open rectangles represent ^{15}N composite 180° pulses ($90°_x$-$220°_y$-$90°_x$). Black ^{13}C shaped pulses denote $^{13}C'$ and $^{13}C^{\alpha}$ Q3 inversion pulses of 600 μs and 500 μs duration at 600 MHz, respectively. Two datasets are acquired in an interleaved fashion with the Q3 pulse applied simultaneously with the ^{15}N composite pulse, or after an additional delay Δ of 14.2 ms, as shown by the curved double headed arrow. The gradients $G_{1,2,3,4,5,6,7,8,9}$ are sine-bell shaped with durations of 1.8, 0.5, 0.3, 1.0, 0.5, 0.5, 0.24, 0.7 and 0.1 ms, respectively, and relative gradient strengths of 11, 39, 36, 41, 53, -43, 9, 53, and 48%. Delays τ and τ' were set to 2.35 and 2.7 ms, respectively. Delays δ and δ' had durations of 12 and 12.5 ms. ζ initially has zero value until the δ-$t_2/2$ period becomes negative at which point ζ is incremented. Quadrature detection in F_1 was achieved by States-TPPI encoding, incrementing φ_1 by 90°. Quadrature F_2 detection was achieved by utilizing Echo-Antiecho encoding by inverting the amplitudes of G_5 and G_6, together with the phases φ_4 and φ_5. The phase cycling is shown below; undefined pulses are applied along x.

φ_1	y
φ_2	y, -y
φ_3	y, y, -y -y
φ_4	-y
φ_5	-y
φ_{rec}	y, -y, -y, y

3. Data Processing

3.1 Processing data in nmrPipe

All data were processed using nmrPipe, freely available at https://www.ibbr.umd.edu/nmrpipe/install.html. All Quantitative J-correlation methods used peak heights for the analysis. An example script for separating the interleaved datasets is shown below. Standard processing can then be performed with both the reference and attenuated spectra using the same parameters.

```
#!/bin/csh

bruk2pipe -verb -in ./ser \
  -bad 0.0 -ext -aswap -AMX -decim 1680 -dspfvs 20 -grpdly 68 -ws 8 -noi2f  \
  -xN              3072  -yN              1024  \
  -xT              1536  -yT              512  \
  -xMODE            DQD  -yMODE         Complex  \
  -xSW         11904.762  -ySW          2590.674  \
  -xOBS          800.134  -yOBS           81.086  \
  -xCAR            4.773  -yCAR         118.108  \
  -xLAB               HN  -yLAB             15N  \
  -ndim                3  -aq2D         Complex  \
 -out ./test.fid -ov
rm -fr fid
set idxExpmt  = (     1      2 )

set nExp=${#tauValues}
set n=1
set bits=()
while ( $n <= $nExp )
   set bits = ($bits 0)
   @ n++
end
foreach n (`seq 1 2`)
   set bits[$n] = 1
   echo $bits
   set outName = (`printf fid/test%03d.fid $idxExpmt[$n]`)
   echo "* output = $outName"
   rm -fr $outName
   nmrPipe -in test.fid \
   | nmrPipe -fn COADD -cList $bits -axis Y \
   | nmrPipe -fn MAC -macro $NMRTXT/bruk_ranceY.M -noRd -noWr \
           -out $outName -ov
   set bits[$n] = 0
end
rm -fr test.fid
```

All 3D experiments used non-uniform sampling (NUS), and the data was reconstructed as pseudo-4D datasets using the SMILE package distributed with nmrPipe, before splitting the two interleaved datasets. The example script below provides an example of this where "-yN" is set to the total number of acquired fids and the sample count is the number of points in the NUS list.

```
#!/bin/csh
bruk2pipe -verb -in ./ser \
  -bad 0.0 -ext -aswap -DMX -decim 2320 -dspfvs 20 -grpdly 68 -ws 8 -noi2f  \
```

```
  -xN            2048  -yN            8000  -zN              1  \
  -xT            1024  -yT             130  -zT             35  \
  -xMODE          DQD  -yMODE       Complex  -zMODE       Complex  \
  -xSW        8620.690  -ySW       2000.000  -zSW        1851.852  \
  -xOBS        600.433  -yOBS        60.848  -zOBS        151.004  \
  -xCAR          4.773  -yCAR       118.087  -zCAR        175.840  \
  -xLAB             HN  -yLAB           15N  -zLAB            13C  \
  -ndim              2  -aq2D        Complex                    \
-out ./test.fid -ov

rm -fr fid
set idxExpmt  = (     1      2)

set nExp=${#idxExpmnt}
set n=1
set bits=()

while ( $n <= $nExp )
   set bits = ($bits 0)
   @ n++
end

foreach n (`seq 1 2`)
      set bits[$n] = 1
   echo $bits
   set outName = (`printf fid/test%03d.fid $idxExpmt[$n]`)
   echo "* output = $outName"

   rm -fr $outName
   nmrPipe -in test.fid \
 | nmrPipe -fn COADD -cList $bits -axis Y \
 | nmrPipe -fn MAC -macro $NMRTXT/bruk_ranceY.M  -noRd -noWr \
 | nusExpand.tcl -mode pipe -in stdin -out stdout -qzSize 2  \
              -sample nuslist -sampleCount 1000 \
 | pipe2xyz -out $outName -x
   set bits[$n] = 0
end
rm -fr temp.fid test.fid


rm -fr ft
series3D.com fid/test*.fid
xyz2pipe -in fid/test%03d.fid                          \
| nmrPipe  -fn POLY -time                              \
| nmrPipe  -fn SP -off 0.4 -end 0.95 -pow 2 -c 0.5 \
| nmrPipe  -fn ZF -zf 2 -auto                          \
| nmrPipe  -fn FT                                  \
| nmrPipe  -fn PS -p0 0 -p1 0 -di                      \
| nmrPipe  -fn EXT -xn 5.4ppm -sw                        \
| nmrPipe  -fn FLATT -first -last                      \
| nmrPipe  -fn EXT -x1 11ppm -xn 6ppm -sw              \
| nmrPipe  -fn ZTP                                   \
| nmrPipe  -fn TP                                    \
| nmrPipe  -fn SMILE -sample nuslist -thresh 0.95       \
          -sampleCount 1000 -report 1 -pseudoND        \
          -xP0 0 -xP1 0 -xCT 47 -xT 130                \
          -yP0 0 -yP1 0 -yT 0 -yAlt                \
| nmrPipe  -fn SP -off 0.5 -end 0.98 -pow 1 -c 0.5      \
| nmrPipe  -fn ZF -zf 2 -auto                          \
| nmrPipe  -fn FT                                  \
| nmrPipe  -fn PS -p0 0 -p1 0 -di                      \
| nmrPipe  -fn TP                                    \
| nmrPipe  -fn SP -off 0.5 -end 0.98 -pow 1 -c 0.5      \
| nmrPipe  -fn ZF -zf 2 -auto                          \
```

```
| nmrPipe  -fn FT -alt                          \
| nmrPipe  -fn PS -p0  0 -p1 0 -di              \
| nmrPipe  -fn TP                               \
| nmrPipe  -fn ZTP                              \
| nmrPipe  -fn FLATT -first -last                \
| nmrPipe  -fn EXT -x1 11ppm -xn 6ppm -sw        \
| pipe2xyz -out ft/test%03d.ft3 -x

copyTau.com fid/test%03d.fid ft/test%03d.ft3 -noverb -all
/bin/rm -fr proj4Ref proj4Att

proj3D.tcl -in ft/test001.ft3 -outDir proj4Ref
proj3D.tcl -in ft/test002.ft3 -outDir proj4Att
```

3.2 Extracting RDCs using nmrPipe

Extracting the signal attenuation directly from nmrPipe is straightforward and follows the steps described below:

1) Peak pick the data in nmrDraw by navigating to Peak on the top toolbar and selecting peak detection
2) Ensure that the assigned signals are in nmrPipe format
3) From the terminal type the following command to transfer the assignments to the peak list generated in step 1, where ass.tab is the file containing the assignments and test.tab is the nmrPipe peak list.

```
ipap.tcl -specName1 ft/test001.ft2 -inName1 test.tab -outName1 test_ass.tab \
-xVar X_PPM -xAss X_PPM -assName ass.tab -yvar Y_PPM -yAss Y_PPM -single -xOff 0 -yOff 0 -ndim 2
```

4) The following command can then be executed to extract the signal attenuation, where spec.list contains the list of spectra. To avoid introducing bias, the peak intensity in the attenuated spectrum is determined using the exact peak position from the reference spectrum [11].

```
seriesTab -in test_ass.tab -out axt.tab -list spec.list -dX 0 -dY 0 -ndim 2
```

5) Use the appropriate equation and error analysis to extract the RDCs using a custom script.

3.3 Extracting RDCs from CCPN

The RDCs from the QJ-correlation and TATER methods can also be extracted using the CCPN v3 macro included in section C and at https://doi.org/10.5281/zenodo.16328473 (/CCPN macros/) as follows:

1) Load the spectra into CCPN.
2) Transfer the assignments from an assigned 2D TROSY or 3D HNCO spectrum to the reference spectrum
3) Ensure that the peaks were transferred correctly, fit the peaks using the “RP” or “RG” keyboard shortcuts
4) Copy the peaks to the attenuated spectrum once satisfied
5) Create a spectrum group containing the reference and attenuated spectra

6) Run the macro providing details such as the RDC type and output file path for the data and the noise level in the spectra. The output file is in a format directly compatible with the DC server that was used throughout this work.

B. Experimental data

1. Comparison of mixed-time and constant-time Quantitative J-correlation $^1D_{NC'}$ measurements

Figure S7 (A,B) shows the reference and attenuated spectra of a $QJ_{NC'}$ experiment utilizing constant-time evolution, while Figure S7(C,D) shows the corresponding spectra using mixed-time evolution. The mixed-time evolution provides higher resolution than the constant-time approach, albeit with a slight reduction in sensitivity. For resonances that are well resolved in both sets of spectra, the RDCs extracted from the $QJ_{NC'}$ experiment using mixed-time evolution are in excellent agreement with those extracted from the $QJ_{NC'}$ experiment using constant-time evolution, yielding a pairwise RMSD of 0.13 Hz (Figure S7E).

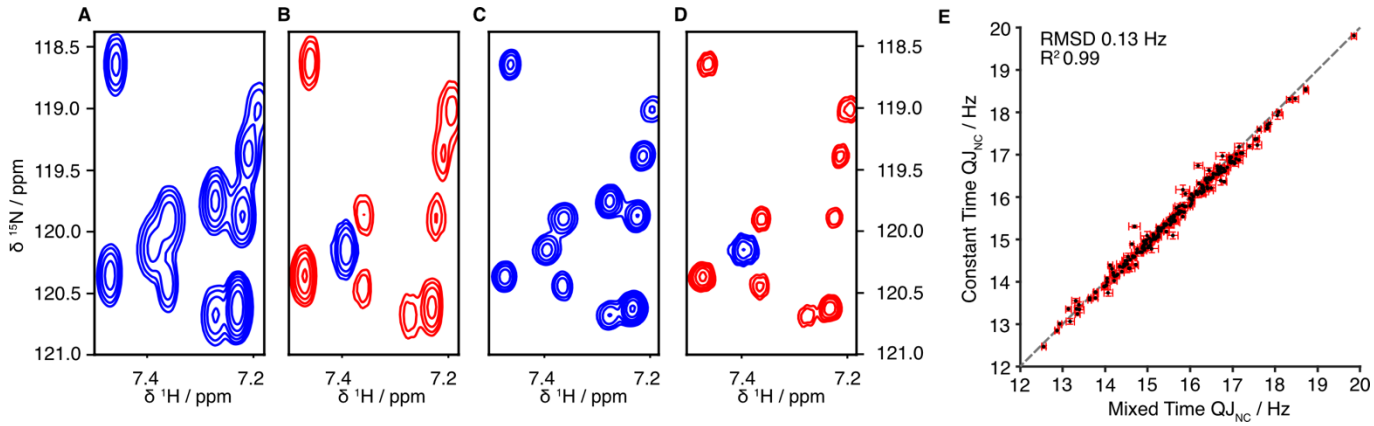


Figure S7. Comparison of constant-time and mixed-time $QJ_{NC'}$ experiments. (A,B) A small region of the 2D $QJ_{NC'}$ spectrum showing the reference and attenuated data, respectively, utilizing constant-time ^{15}N chemical shift evolution. (C,D) Analogous reference and attenuated spectra obtained with mixed-time chemical shift evolution. Attenuated spectra are contoured at five times lower levels than reference spectra. (E) Comparison of RDCs extracted from well-resolved signals in $QJ_{NC'}$ spectra utilizing constant-time and mixed-time ^{15}N chemical shift evolution. The uncertainties associated with the measured RDCs are marked by red error bars, with the CT approach consistently providing lower errors. The total measurement time to acquire the reference and attenuated spectra for the CT and MT experiments was 4 h. The attenuated spectrum in the CT measurement was acquired with 4x more scans than the reference spectrum.

2. 2D Quantitative J-correlation $^1D_{C'C\alpha}$ measurements

The $QJ_{C'C\alpha}$ experiment can also be acquired in a 2D manner by fixing t_1=0 in Figure S6, resulting in a 2D ^1H-^{15}N TROSY-HSQC readout. Although such measurements then are much faster than the 3D experiment, the lower resolution reduces the number of obtainable RDCs. Figure S8 shows that with a pairwise RMSD of 0.29 Hz, the RDCs extracted from the 2D $QJ_{C'C\alpha}$ experiment are in excellent agreement with the 3D $QJ_{C'C\alpha}$ RDCs.

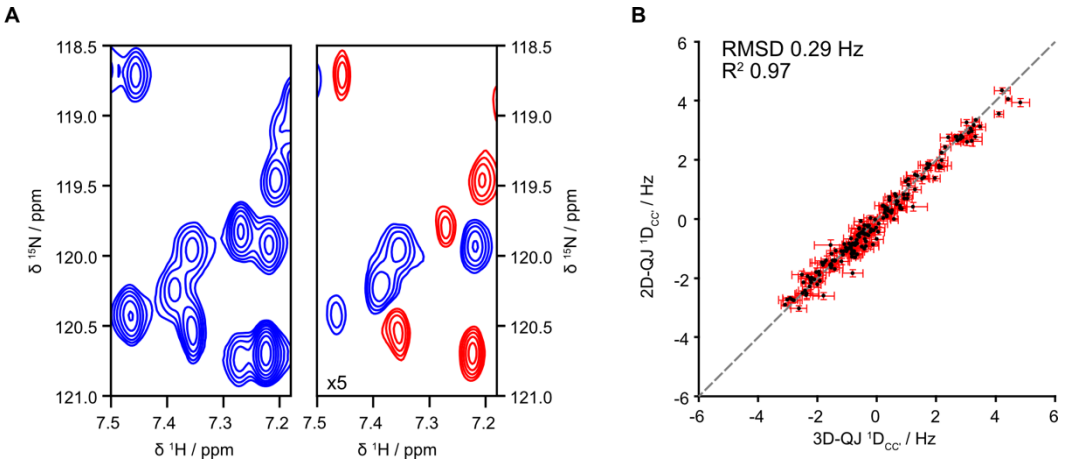


Figure S8. Comparison of 2D and 3D $QJ_{C'C\alpha}$ experiments. (A) A small region of the 2D $QJ_{C'C\alpha}$ spectrum showing the reference and attenuated data. (B) Correlation of the RDCs extracted from the 3D HNCO and 2D ^{1}H-^{15}N TROSY-based experiments, along with the associated errors as red error bars.

3. Reducing experimental errors by performing differential number of scans

Figure S9 shows the error improvement in the measured RDCs per unit time obtained by acquiring the attenuated spectrum with more transients than the reference spectrum. When the intensity ratio (Q) between the reference and attenuated spectrum is close to zero, an improvement factor that approaches √2 can be obtained. Smaller improvement factors are observed when Q deviates from 0. In practice, acquiring the attenuated spectrum with up to 4 times more scans than the reference spectrum offers a good compromise.

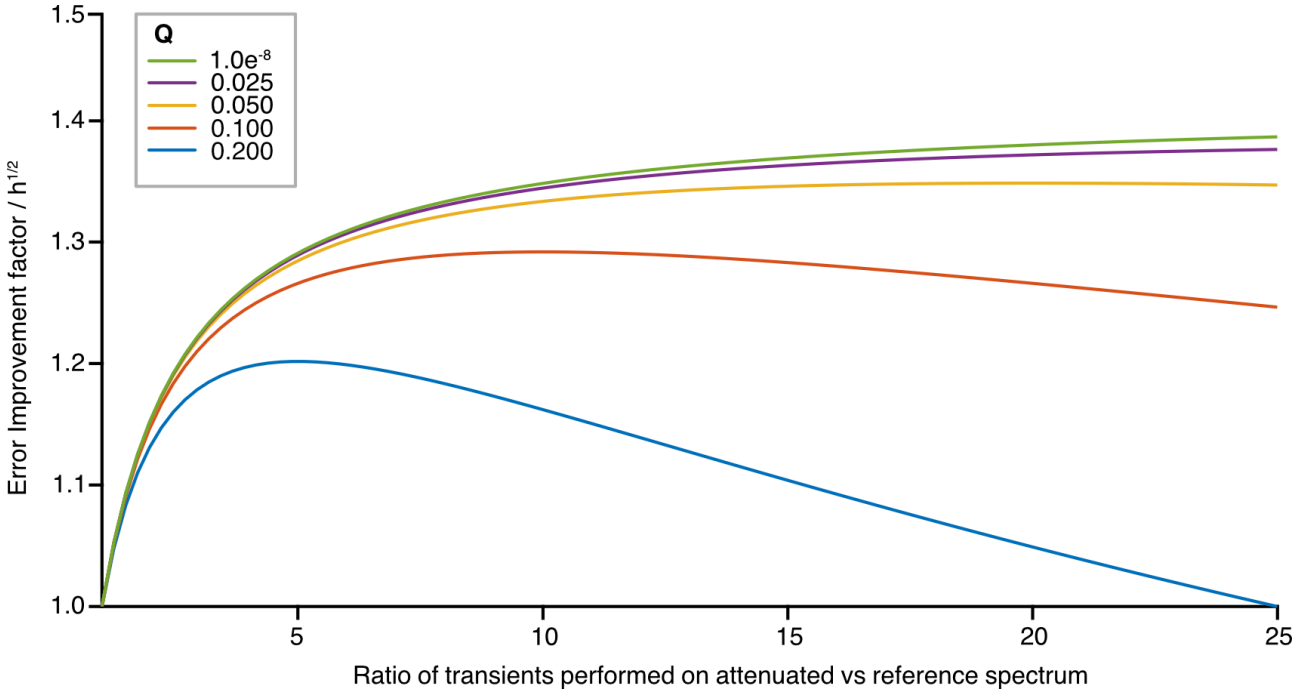


Figure S9. Improvement factor of the errors in RDC measurements as a function of the ratio of the number of scans performed on the attenuated vs the reference spectrum for a constant total number of transients acquired for the reference and attenuated spectra, i.e. a fixed total measurement time. Improvement factors are shown for the signal attenuation (Q) ratios ranging from $1.0e^{-8}$ to 0.2.

4. Structural insights from fitting the RDC datasets

Fitting the RDC data to the average vector orientation of 350 PDB MPro structures results in lower Q_{JK} factors for domains 1 and 3 (Figure S10), indicating that structural noise limits the quality of the fit. The Q_{JK} value remains high for domain 2 when fitting to 7K3T alone or to the ensemble of 350 X-ray structures.

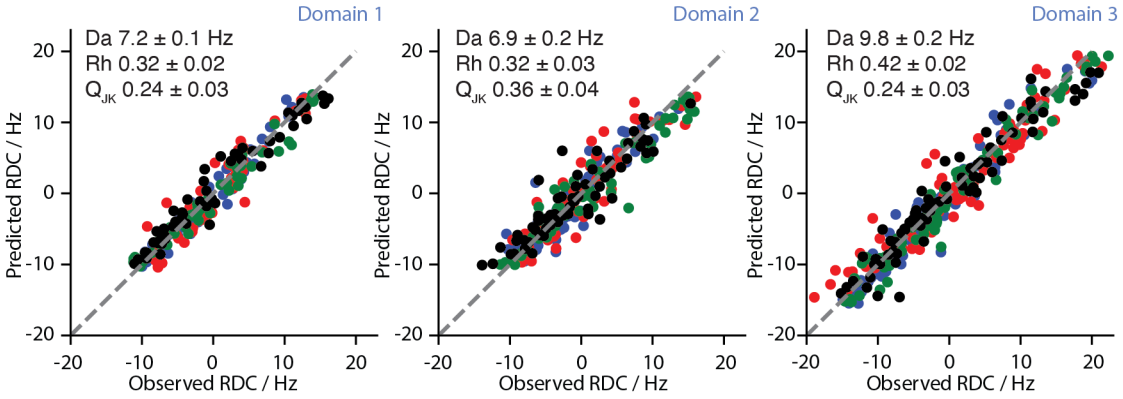


Figure S10. Simultaneous SVD fits of $^{1}D_{NH}$ (blue), $^{1}D_{NC'}$ (red), $^{1}D_{C'C\alpha}$ (green) and $^{2}D_{C'H}$ (black) to an ensemble of 350 PDB structures for Domains 1, 2 and 3. RDCs corresponding to residues within the active site oxyanion loop (G138-C145) have been removed from this analysis.

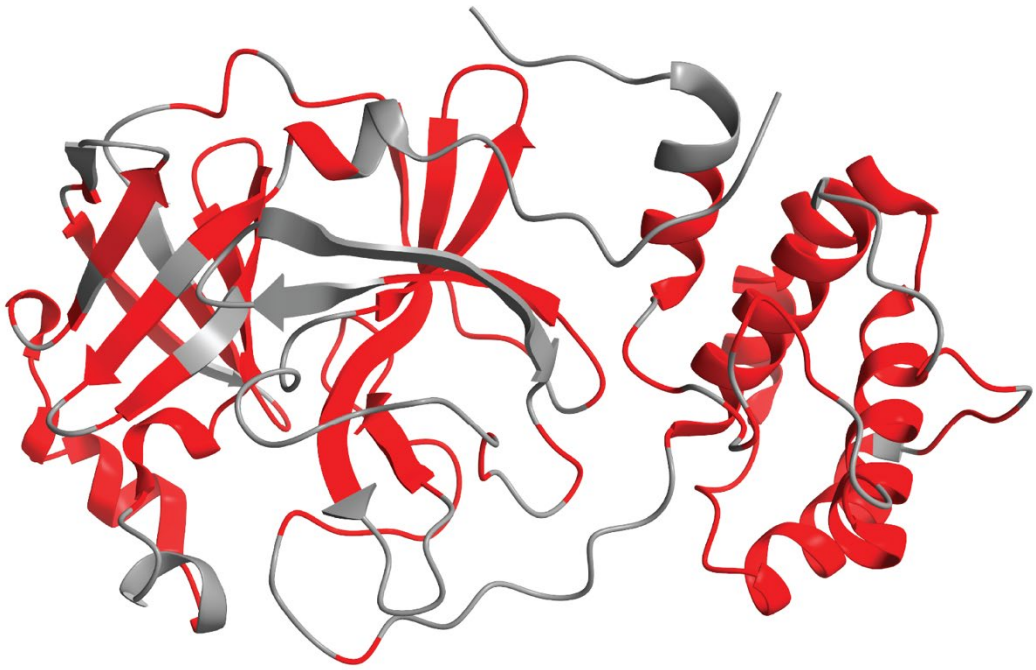


Figure S11. Crystal structure of MPro (PDB 7K3T) with red highlighted residues corresponding to those that have less than 0.6 Å difference in backbone coordinates between monomeric (2QCY) and homodimeric (7K3T) structures, and an average orientation difference between the of N-H, N-C', and C'-C^{α} vectors that is less than 4°. The invariant residue numbers are: 14 15 17 18 20 21 22 25 26 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 51 52 53 54 55 56 57 58 59 60 61 62 63 64 66 68 69 70 75 77 78 79 80 81 84 85 86 87 88 94 95 96 98 99 100 100 101 102 103 104 105 106 109 110 111 112 113 114 115 131 132 135 136 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 172 173 174 175 176 177 178 179 180 181 182 183 184

185 186 187 191 200 201 202 203 204 205 206 207 208 209 210 211 212 213 219 220 221 223 224 225 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 280 281 282 283 285 287 289 290 292 293 294 295 296 297.

Comparing the fit of the $^{1}D_{NH}$ RDC data for domain 2 of MPro (residues 101-184) to various X-ray structures (Figure S12) shows the best fit to the SARS-CoV MPro structure (Fig S12A, PDB 2QCY), followed closely by the SARS-CoV-2 domain 3 deletion mutant, MPro$^{1\text{-}199}$ (Fig S12B, PDB 7UJ9). These two structures are very similar with a heavy atom RMSD of 0.64 Å, and both feature a 3_{10}-helix conformation in the oxyanion loop, highlighted in red, precluding substrate access to the sidechains of the catalytic dyad (H41/C145). However, the higher Q_{JK} value for 7UJ9 highlights the sensitivity of RDCs to even small structural differences. In contrast, the fit to the N-terminally extended mutant (PDB 7KFI, Figure S10C), which is unable to dimerize, is comparable to that of the full-length homodimer (Figure S12D), indicating that these two structures adopt similar active site conformations.

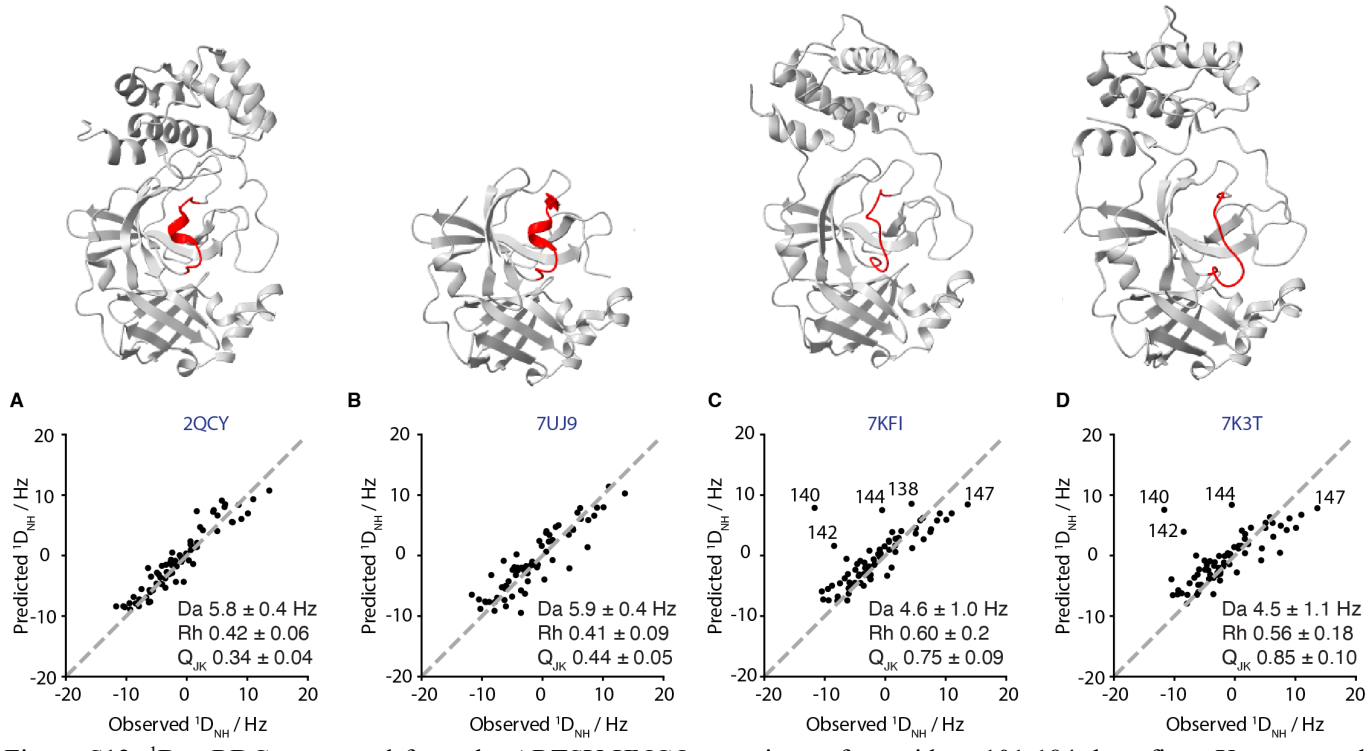


Figure S12. $^{1}D_{NH}$ RDCs extracted from the ARTSY-HNCO experiment for residues 101-184, best fit to X-ray crystal structures of (A) the monomeric MPro^{R298A} mutant of SARS-CoV (PDB 2QCY), (B) a monomeric SARS-CoV-2 MPro mutant lacking domain 3 (MPro$^{1\text{-}199}$, PDB 7UJ9), (C) an N-terminus extended MPro mutant (PDB 7KFI, [12]), and (D) a monomeric subunit of the homodimeric X-ray crystal structure (PDB 7K3T). The corresponding PDB codes and structures are provided above each of the correlation plots. The red highlighted regions in the structures correspond to the oxyanion loop residues (G138-C145)

Figure S13 shows the per-residue angular and distance RMSDs between the monomeric 2QCY structure and the dimeric 7K3T structure. These analyses reveal substantial conformational changes in the catalytic domain, whereas the helical domain remains structurally well conserved. The major differences in the catalytic domain involve rearrangements of the oxyanion loop (residues G138–C145) and the β-strand spanning residues S121–A129, which align with the key structural changes identified in the XPLOR refinement protocol used by us.

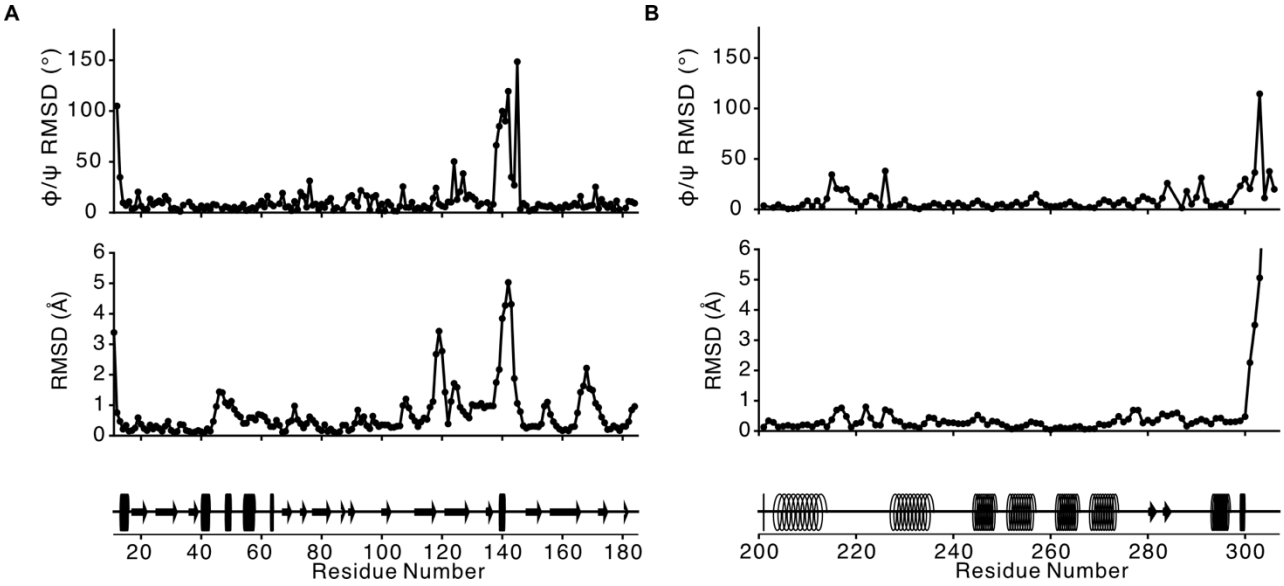


Figure S13. Per residue RMSD in φ/ϕ backbone torsion angles and backbone heavy atom co-ordinates between the 2QCY monomeric structure and the 7K3T dimeric structure for (A) the catalytic domain and (B) the helical domain of MPro. The secondary structure elements are indicated along the bottom with arrows representing β-sheets and coils representing α-helices.

Table S1. Jackknifed Q_{JK} values for fits of $^1D_{NC'}$, $^1D_{C'C\alpha}$, $^2D_{C'H}$ and $^1D_{NH}$ RDCs to (1) the X-ray structure of monomeric MPro (PDB 2QCY); (2) a monomer subunit of the active homodimer (PDB 7K3T); (3) AlphaFold-predicted structure with homologs excluded from training (AF^{HE}); (4) AlphaFold-predicted structure without homolog exclusion (AF^{HI}); (5) A 3-structure ensemble of PDB entries 2QCY, 7K3T and AF^{HE}; and (6) the average vector orientation of the three models. This analysis was performed using the well-defined peptide planes shown in Figure S11.

	Frequency based			**Quantitative-J**		
$^1D_{NC'}$	**Domain 1**	**Domain 2**	**Domain 3**	**Domain 1**	**Domain 2**	**Domain 3**
2QCY	0.31	0.31	0.24	0.31	0.33	0.29
7K3T	0.36	0.34	0.24	0.30	0.39	0.25
AF^{HE}	0.30	0.32	0.21	0.27	0.33	0.24
AF^{HI}	0.30	0.32	0.21	0.27	0.33	0.24
7K3T+2QCY+AF^{HE}	0.29	0.32	0.21	0.27	0.33	0.24
<7K3T+2QCY+AF^{HE}>	0.30	0.32	0.21	0.27	0.33	0.24
$^1D_{C'C\alpha}$						
2QCY	0.24	0.28	0.20	0.21	0.25	0.23
7K3T	0.22	0.31	0.23	0.19	0.25	0.23
AF^{HE}	0.22	0.27	0.18	0.18	0.22	0.21
AF^{HI}	0.22	0.27	0.18	0.18	0.22	0.21
7K3T+2QCY+AF^{HE}	0.22	0.26	0.19	0.18	0.22	0.21
<7K3T+2QCY+AF^{HE}>	0.22	0.27	0.18	0.18	0.22	0.21
$^2D_{C'H}$						
2QCY	0.15	0.33	0.26			
7K3T	0.17	0.36	0.22			
AF^{HE}	0.13	0.35	0.21			
AF^{HI}	0.13	0.35	0.21			
7K3T+2QCY+AF^{HE}	0.13	0.35	0.21			
<7K3T+2QCY+AF^{HE}>	0.13	0.35	0.21			
$^1D_{NH}$						
2QCY				0.22	0.27	0.21
7K3T				0.25	0.36	0.23
AF^{HE}				0.21	0.30	0.20
AF^{HI}				0.21	0.30	0.20
7K3T+2QCY+AF^{HE}				0.21	0.30	0.20
<7K3T+2QCY+AF^{HE}>				0.21	0.30	0.20

Table S2. Jackknifed quality factors as for Table S1 but with all residues included in the fit.

	Frequency based			Quantitative-J		
$^1D_{NC'}$	**Domain 1**	**Domain 2**	**Domain 3**	**Domain 1**	**Domain 2**	**Domain 3**
2QCY	0.36	0.39	0.31	0.31	0.40	0.34
7K3T	0.37	0.48	0.29	0.31	0.49	0.28
AF^{HE}	0.33	0.52	0.25	0.27	0.53	0.24
AF^{HI}	0.31	0.52	0.25	0.28	0.52	0.24
7K3T+2QCY+AF^{HE}	0.31	0.39	0.22	0.25	0.38	0.22
<7K3T+2QCY+AF^{HE}>	0.32	0.49	0.25	0.25	0.48	0.24
$^1D_{C'C\alpha}$						
2QCY	0.24	0.40	0.28	0.20	0.34	0.27
7K3T	0.24	0.64	0.22	0.23	0.56	0.22
AF^{HE}	0.21	0.56	0.19	0.19	0.48	0.20
AF^{HI}	0.20	0.57	0.20	0.18	0.50	0.20
7K3T+2QCY+AF^{HE}	0.20	0.46	0.18	0.18	0.37	0.18
<7K3T+2QCY+AF^{HE}>	0.20	0.48	0.21	0.18	0.40	0.20
$^2D_{C'H}$						
2QCY	0.24	0.55	0.38			
7K3T	0.26	0.78	0.26			
AF^{HE}	0.19	0.63	0.25			
AF^{HI}	0.21	0.56	0.29			
7K3T+2QCY+AF^{HE}	0.14	0.54	0.21			
<7K3T+2QCY+AF^{HE}>	0.18	0.71	0.24			
$^1D_{NH}$						
2QCY				0.28	0.37	0.33
7K3T				0.28	0.92	0.26
AF^{HE}				0.19	0.72	0.27
AF^{HI}				0.20	0.69	0.28
7K3T+2QCY+AF^{HE}				0.18	0.50	0.21
<7K3T+2QCY+AF^{HE}>				0.19	0.60	0.22

5. $^{1}D_{NH}$, $^{1}D_{C'C\alpha}$, $^{1}D_{NC'}$ and $^{2}D_{C'H}$ MPro$^{10\text{-}306,H41Q}$ RDCs

Table S3. 3D ARTSY-HNCO derived $^{1}J_{NH}$ and $^{1}D_{NH}$ RDCs for MPro$^{10\text{-}306,\ H41Q}$

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
13	Val	N	13	Val	HN	92.76	0.27	-6.97	0.59
14	Glu	N	14	Glu	HN	93.61	0.72	-9.54	0.92
15	Gly	N	15	Gly	HN	94.83	0.17	-6.34	0.36
16	Cys	N	16	Cys	HN	91.13	0.27	-4.71	0.55
17	Met	N	17	Met	HN	93.90	0.16	-2.36	0.32
19	Gln	N	19	Gln	HN	92.30	0.18	5.19	0.38
21	Thr	N	21	Thr	HN	93.06	0.08	7.99	0.16
22	Cys	N	22	Cys	HN	93.59	0.10	4.68	0.19
24	Thr	N	24	Thr	HN	93.10	0.17	1.87	0.50
25	Thr	N	25	Thr	HN	93.45	0.10	-2.12	0.24
40	Arg	N	40	Arg	HN	93.52	0.16	-7.67	0.44
41	Gln	N	41	Gln	HN	93.23	0.27	-9.99	0.65
42	Val	N	42	Val	HN	94.24	0.17	0.78	0.33
43	Ile	N	43	Ile	HN	93.18	0.22	-8.52	0.60
44	Cys	N	44	Cys	HN	93.40	0.13	-6.95	0.30
45	Thr	N	45	Thr	HN	92.35	0.09	-3.10	0.19
46	Ser	N	46	Ser	HN	93.36	0.25	-4.33	1.02
48	Asp	N	48	Asp	HN	93.49	0.15	2.46	0.36
49	Met	N	49	Met	HN	93.42	0.16	-4.37	0.42
50	Leu	N	50	Leu	HN	93.54	0.14	-9.23	0.32
51	Asn	N	51	Asn	HN	93.64	0.13	-3.19	0.25
53	Asn	N	53	Asn	HN	93.54	0.09	-7.50	0.18
54	Tyr	N	54	Tyr	HN	93.58	0.13	-4.96	0.28
55	Glu	N	55	Glu	HN	92.91	0.18	3.91	0.39
56	Asp	N	56	Asp	HN	93.09	0.23	4.29	0.49
57	Leu	N	57	Leu	HN	93.53	0.20	-3.34	0.65
59	Ile	N	59	Ile	HN	92.96	0.36	6.85	0.65
60	Arg	N	60	Arg	HN	92.32	0.30	-0.58	0.61
61	Lys	N	61	Lys	HN	93.45	0.12	-6.27	0.24
65	Asn	N	65	Asn	HN	93.53	0.19	-3.94	0.44
66	Phe	N	66	Phe	HN	93.39	0.12	5.76	0.25
67	Leu	N	67	Leu	HN	93.08	0.12	10.25	0.24
68	Val	N	68	Val	HN	92.39	0.12	2.73	0.21
69	Gln	N	69	Gln	HN	93.11	0.18	5.61	0.36
70	Ala	N	70	Ala	HN	92.56	0.11	-1.94	0.18
71	Gly	N	71	Gly	HN	95.57	0.07	7.77	0.15

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
73	Val	N	73	Val	HN	93.11	0.08	-7.63	0.16
74	Gln	N	74	Gln	HN	92.99	0.08	1.98	0.14
75	Leu	N	75	Leu	HN	93.43	0.12	11.04	0.22
76	Arg	N	76	Arg	HN	93.64	0.08	10.57	0.15
77	Val	N	77	Val	HN	93.54	0.10	-0.11	0.15
78	Ile	N	78	Ile	HN	91.35	0.58	-9.34	1.09
79	Gly	N	79	Gly	HN	93.91	0.19	-4.67	0.38
81	Ser	N	81	Ser	HN	93.89	0.17	-6.40	0.25
82	Met	N	82	Met	HN	92.95	0.08	-5.88	0.15
83	Gln	N	83	Gln	HN	93.23	0.10	-2.59	0.19
84	Asn	N	84	Asn	HN	94.96	0.08	-5.43	0.15
85	Cys	N	85	Cys	HN	92.91	0.18	-9.17	0.44
86	Val	N	86	Val	HN	93.39	0.17	-2.39	0.29
92	Asp	N	92	Asp	HN	93.55	0.15	1.38	0.33
93	Thr	N	93	Thr	HN	92.27	0.10	12.67	0.23
94	Ala	N	94	Ala	HN	93.75	0.08	10.60	0.14
95	Asn	N	95	Asn	HN	93.19	0.21	3.91	0.38
100	Lys	N	100	Lys	HN	91.93	0.07	-3.78	0.15
101	Tyr	N	101	Tyr	HN	93.45	0.11	-1.18	0.20
102	Lys	N	102	Lys	HN	92.35	0.13	0.60	0.25
103	Phe	N	103	Phe	HN	92.51	0.08	0.61	0.14
104	Val	N	104	Val	HN	93.56	0.11	-2.62	0.21
105	Arg	N	105	Arg	HN	92.97	0.08	-0.92	0.14
106	Ile	N	106	Ile	HN	92.99	0.24	-3.54	0.44
107	Gln	N	107	Gln	HN	92.84	0.11	1.04	0.21
109	Gly	N	109	Gly	HN	93.87	0.08	-2.89	0.26
110	Gln	N	110	Gln	HN	93.62	0.16	-5.92	0.38
111	Thr	N	111	Thr	HN	94.54	0.11	-6.41	0.17
112	Phe	N	112	Phe	HN	94.11	0.16	10.10	0.33
113	Ser	N	113	Ser	HN	92.70	0.17	6.23	0.35
114	Val	N	114	Val	HN	92.93	0.18	-2.88	0.32
115	Leu	N	115	Leu	HN	92.57	0.14	-4.94	0.29
116	Ala	N	116	Ala	HN	94.16	0.14	-6.28	0.27
117	Cys	N	117	Cys	HN	93.46	0.16	1.64	0.33
118	Tyr	N	118	Tyr	HN	93.08	0.13	5.79	0.26
119	Asn	N	119	Asn	HN	94.70	0.21	5.07	0.59
120	Gly	N	120	Gly	HN	93.56	0.19	1.15	0.50
121	Ser	N	121	Ser	HN	93.44	0.09	10.98	0.19
123	Ser	N	123	Ser	HN	92.28	0.17	-0.77	0.36
124	Gly	N	124	Gly	HN	94.36	0.11	0.32	0.21

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
125	Val	N	125	Val	HN	92.36	0.09	-2.42	0.15
126	Tyr	N	126	Tyr	HN	93.20	0.19	-6.70	0.37
127	Gln	N	127	Gln	HN	93.27	0.09	-1.81	0.16
128	Cys	N	128	Cys	HN	94.45	0.15	8.56	0.30
129	Ala	N	129	Ala	HN	92.92	0.09	9.02	0.16
130	Met	N	130	Met	HN	93.48	0.20	6.34	0.45
131	Arg	N	131	Arg	HN	93.65	0.16	-9.48	0.30
133	Asn	N	133	Asn	HN	92.55	0.19	-3.33	0.40
134	Phe	N	134	Phe	HN	93.60	0.19	-9.36	0.42
135	Thr	N	135	Thr	HN	93.18	0.31	-3.56	0.79
136	Ile	N	136	Ile	HN	93.01	0.17	-10.50	0.48
137	Lys	N	137	Lys	HN	93.45	0.11	-5.02	0.22
138	Gly	N	138	Gly	HN	94.29	0.11	4.34	0.21
140	Phe	N	140	Phe	HN	93.15	0.24	-11.68	0.67
142	Asn	N	142	Asn	HN	94.54	0.25	-8.46	0.50
143	Gly	N	143	Gly	HN	93.69	0.21	4.45	0.80
144	Ser	N	144	Ser	HN	92.35	0.20	-0.52	0.51
145	Cys	N	145	Cys	HN	92.74	0.46	7.46	0.79
146	Gly	N	146	Gly	HN	93.92	0.39	1.67	0.95
147	Ser	N	147	Ser	HN	93.01	0.51	13.63	1.30
148	Val	N	148	Val	HN	92.79	0.17	1.69	0.34
149	Gly	N	149	Gly	HN	92.19	0.28	-4.39	0.54
150	Phe	N	150	Phe	HN	92.94	0.19	-3.44	0.40
151	Asn	N	151	Asn	HN	91.84	0.15	-2.70	0.31
152	Ile	N	152	Ile	HN	92.84	0.10	-2.46	0.23
153	Asp	N	153	Asp	HN	93.28	0.10	-7.86	0.18
155	Asp	N	155	Asp	HN	93.35	0.18	-0.12	0.48
156	Cys	N	156	Cys	HN	93.18	0.10	-7.93	0.25
157	Val	N	157	Val	HN	92.98	0.13	-1.93	0.26
158	Ser	N	158	Ser	HN	93.03	0.16	-5.61	0.35
159	Phe	N	159	Phe	HN	93.63	0.16	2.20	0.33
160	Cys	N	160	Cys	HN	91.06	0.47	-4.12	1.10
161	Tyr	N	161	Tyr	HN	92.64	0.37	7.58	0.98
162	Met	N	162	Met	HN	93.87	0.16	2.65	0.31
163	His	N	163	His	HN	94.36	0.19	5.03	0.39
165	Met	N	165	Met	HN	93.21	0.37	-6.05	1.26
166	Glu	N	166	Glu	HN	92.20	0.15	-10.27	0.26
167	Leu	N	167	Leu	HN	93.97	0.34	-4.29	0.68
172	His	N	172	His	HN	91.19	0.27	-8.80	0.55
174	Gly	N	174	Gly	HN	95.02	0.27	-7.49	0.62

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
175	Thr	N	175	Thr	HN	93.29	0.19	-0.90	0.40
176	Asp	N	176	Asp	HN	92.29	0.16	-1.69	0.34
177	Leu	N	177	Leu	HN	93.07	0.25	-6.06	0.53
178	Glu	N	178	Glu	HN	90.29	0.33	-2.41	0.83
179	Gly	N	179	Gly	HN	94.87	0.31	-6.18	0.69
180	Asn	N	180	Asn	HN	94.04	0.16	5.56	0.39
181	Phe	N	181	Phe	HN	94.91	0.12	-4.08	0.24
182	Tyr	N	182	Tyr	HN	91.79	0.12	-4.64	0.25
183	Gly	N	183	Gly	HN	95.50	0.08	0.66	0.17
185	Phe	N	185	Phe	HN	93.25	0.10	3.50	0.18
186	Val	N	186	Val	HN	92.09	0.24	8.27	0.52
187	Asp	N	187	Asp	HN	93.52	0.14	0.07	0.27
188	Arg	N	188	Arg	HN	91.79	0.23	-8.36	0.47
189	Gln	N	189	Gln	HN	93.24	0.14	-10.09	0.26
190	Thr	N	190	Thr	HN	93.40	0.10	-0.83	0.21
191	Ala	N	191	Ala	HN	92.84	0.47	-1.16	3.24
193	Ala	N	193	Ala	HN	93.46	0.19	2.48	0.48
194	Ala	N	194	Ala	HN	93.63	0.09	-1.60	0.22
195	Gly	N	195	Gly	HN	94.35	0.15	4.31	0.38
196	Thr	N	196	Thr	HN	93.41	0.14	-2.35	0.32
197	Asp	N	197	Asp	HN	92.99	0.19	4.18	0.37
198	Thr	N	198	Thr	HN	93.31	0.12	9.27	0.25
199	Thr	N	199	Thr	HN	93.41	0.14	19.38	0.38
200	Ile	N	200	Ile	HN	93.38	0.10	4.11	0.26
201	Thr	N	201	Thr	HN	91.85	0.41	-7.22	0.92
202	Val	N	202	Val	HN	93.38	0.21	-6.58	0.48
203	Asn	N	203	Asn	HN	92.01	0.24	-13.18	0.83
204	Val	N	204	Val	HN	93.31	0.32	-6.62	0.63
214	Asn	N	214	Asn	HN	93.58	0.23	-1.17	0.40
215	Gly	N	215	Gly	HN	93.57	0.16	-4.84	0.24
216	Asp	N	216	Asp	HN	93.54	0.12	8.42	0.20
217	Arg	N	217	Arg	HN	90.32	0.15	-4.05	0.21
218	Trp	N	218	Trp	HN	92.21	0.22	7.23	0.46
220	Leu	N	220	Leu	HN	94.14	0.10	-2.26	0.14
221	Asn	N	221	Asn	HN	93.61	0.09	3.20	0.15
222	Arg	N	222	Arg	HN	91.87	0.20	3.55	0.52
223	Phe	N	223	Phe	HN	93.03	0.05	-0.71	0.08
224	Thr	N	224	Thr	HN	92.83	0.05	11.45	0.09
225	Thr	N	225	Thr	HN	93.55	0.06	4.34	0.10
226	Thr	N	226	Thr	HN	92.98	0.09	-6.39	0.15

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
227	Leu	N	227	Leu	HN	93.52	0.09	-12.38	0.17
228	Asn	N	228	Asn	HN	93.82	0.11	-10.22	0.18
229	Asp	N	229	Asp	HN	94.08	0.16	-12.75	0.22
230	Phe	N	230	Phe	HN	94.71	0.15	-12.53	0.22
231	Asn	N	231	Asn	HN	93.59	0.06	-10.96	0.18
232	Leu	N	232	Leu	HN	94.06	0.17	-8.88	0.26
233	Val	N	233	Val	HN	92.92	0.23	-14.06	0.65
234	Ala	N	234	Ala	HN	94.35	0.25	-13.40	0.54
235	Met	N	235	Met	HN	93.66	0.21	-3.67	0.29
236	Lys	N	236	Lys	HN	93.33	0.25	-7.16	0.37
237	Tyr	N	237	Tyr	HN	92.66	0.31	-12.29	0.48
238	Asn	N	238	Asn	HN	93.98	0.26	7.99	0.43
239	Tyr	N	239	Tyr	HN	92.26	0.21	19.64	0.68
240	Glu	N	240	Glu	HN	94.06	0.21	3.73	0.52
243	Thr	N	243	Thr	HN	92.51	0.14	12.88	0.26
244	Gln	N	244	Gln	HN	93.16	0.12	-2.50	0.31
245	Asp	N	245	Asp	HN	93.75	0.12	-9.26	0.21
246	His	N	246	His	HN	94.05	0.17	0.65	0.25
247	Val	N	247	Val	HN	93.71	0.16	-3.58	0.23
248	Asp	N	248	Asp	HN	94.13	0.15	-6.84	0.24
249	Ile	N	249	Ile	HN	93.88	0.23	-7.35	0.56
250	Leu	N	250	Leu	HN	93.27	0.30	6.42	0.91
254	Ser	N	254	Ser	HN	93.66	0.25	-1.05	0.66
255	Ala	N	255	Ala	HN	93.54	0.19	4.41	0.32
256	Gln	N	256	Gln	HN	92.84	0.11	1.04	0.21
257	Thr	N	257	Thr	HN	92.21	0.64	-5.21	1.05
258	Gly	N	258	Gly	HN	95.04	0.30	6.77	0.52
259	Ile	N	259	Ile	HN	93.46	0.19	16.54	0.37
260	Ala	N	260	Ala	HN	94.26	0.09	11.73	0.17
261	Val	N	261	Val	HN	93.51	0.10	-5.67	0.15
262	Leu	N	262	Leu	HN	93.39	0.15	-11.34	0.24
263	Asp	N	263	Asp	HN	91.74	0.21	0.87	0.31
264	Met	N	264	Met	HN	94.64	0.31	-4.29	0.46
265	Cys	N	265	Cys	HN	93.27	0.31	-9.48	0.55
266	Ala	N	266	Ala	HN	94.40	0.19	-13.34	0.36
267	Ser	N	267	Ser	HN	93.87	0.21	-4.54	0.36
269	Lys	N	269	Lys	HN	93.72	0.20	-13.54	0.31
270	Glu	N	270	Glu	HN	93.30	0.20	-11.87	0.36
271	Leu	N	271	Leu	HN	93.64	0.22	-7.02	0.32
273	Gln	N	273	Gln	HN	93.99	0.18	-14.92	0.31

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
274	Asn	N	274	Asn	HN	92.39	0.22	-7.50	0.31
275	Gly	N	275	Gly	HN	95.19	0.15	-4.21	0.20
276	Met	N	276	Met	HN	91.84	0.09	-6.74	0.13
277	Asn	N	277	Asn	HN	93.96	0.13	-7.58	0.30
278	Gly	N	278	Gly	HN	94.38	0.10	-3.46	0.19
279	Arg	N	279	Arg	HN	92.69	0.06	2.02	0.09
280	Thr	N	280	Thr	HN	93.34	0.07	-8.44	0.13
281	Ile	N	281	Ile	HN	92.49	0.14	-6.51	0.27
282	Leu	N	282	Leu	HN	93.68	0.25	-1.30	0.46
283	Gly	N	283	Gly	HN	93.67	0.11	10.53	0.22
284	Ser	N	284	Ser	HN	93.00	0.21	-9.99	0.49
285	Ala	N	285	Ala	HN	92.16	0.12	6.42	0.29
286	Leu	N	286	Leu	HN	92.02	0.11	6.27	0.20
287	Leu	N	287	Leu	HN	93.39	0.20	-2.25	0.35
290	Glu	N	290	Glu	HN	95.03	5.31	-1.30	5.35
291	Phe	N	291	Phe	HN	93.34	0.21	20.01	0.76
292	Thr	N	292	Thr	HN	93.53	0.12	19.07	0.31
294	Phe	N	294	Phe	HN	92.93	0.13	-9.82	0.23
295	Asp	N	295	Asp	HN	93.71	0.17	-4.64	0.29
296	Val	N	296	Val	HN	93.53	0.15	-4.31	0.25
298	Arg	N	298	Arg	HN	94.10	0.13	-10.72	0.22
299	Gln	N	299	Gln	HN	93.40	0.10	-3.38	0.15
300	Cys	N	300	Cys	HN	92.66	0.12	-4.40	0.19
301	Ser	N	301	Ser	HN	93.36	0.08	-3.79	0.14
302	Gly	N	302	Gly	HN	93.95	0.06	-2.94	0.15
303	Val	N	303	Val	HN	93.34	0.03	-1.43	0.04
304	Thr	N	304	Thr	HN	93.31	0.03	0.86	0.06
305	Phe	N	305	Phe	HN	93.12	0.03	0.18	0.05
306	Gln	N	306	Gln	HN	92.67	0.02	-0.13	0.02

Table S4. 2D MT-QJ$_{NC}$, derived $^{1}J_{NC}$, and $^{1}D_{NC}$, couplings for MPro$^{10\text{-}306,\ H41Q}$

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
13	Val	N	12	Lys	C	15.99	0.08	1.44	0.11
14	Glu	N	13	Val	C	13.80	0.08	-0.23	0.11
15	Gly	N	14	Glu	C	16.26	0.04	1.59	0.07
16	Cys	N	15	Gly	C	16.16	0.06	0.47	0.1
17	Met	N	16	Cys	C	15.92	0.04	-0.22	0.06
19	Gln	N	18	Val	C	13.96	0.04	-1.14	0.07
21	Thr	N	20	Val	C	14.30	0.02	-0.95	0.03
22	Cys	N	21	Thr	C	15.39	0.02	1.40	0.04
23	Gly	N	22	Cys	C	14.35	0.12	-0.88	0.32
24	Thr	N	23	Gly	C	15.98	0.04	1.06	0.07
25	Thr	N	24	Thr	C	15.95	0.03	-0.53	0.05
40	Arg	N	39	Pro	C	14.22	0.06	-0.06	0.11
41	Gln	N	40	Arg	C	14.39	0.07	0.31	0.1
42	Val	N	41	Gln	C	16.14	0.04	1.67	0.06
43	Ile	N	42	Val	C	13.82	0.05	-0.12	0.08
45	Thr	N	44	Cys	C	15.04	0.02	0.27	0.04
46	Ser	N	45	Thr	C	14.43	0.06	0.30	0.12
47	Glu	N	46	Ser	C	14.12	0.03	-1.12	0.05
48	Asp	N	47	Glu	C	15.97	0.04	1.42	0.06
49	Met	N	48	Asp	C	13.87	0.04	-0.69	0.06
50	Leu	N	49	Met	C	15.70	0.04	0.52	0.06
51	Asn	N	50	Leu	C	14.55	0.04	-0.02	0.05
53	Asn	N	52	Pro	C	13.80	0.02	-0.50	0.04
54	Tyr	N	53	Asn	C	14.05	0.03	-0.27	0.05
55	Glu	N	54	Tyr	C	14.25	0.04	-0.15	0.07
56	Asp	N	55	Glu	C	16.09	0.05	1.49	0.08
57	Leu	N	56	Asp	C	14.17	0.07	-0.60	0.1
59	Ile	N	58	Leu	C	17.10	0.07	-1.00	0.1
60	Arg	N	59	Ile	C	15.63	0.08	0.51	0.12
61	Lys	N	60	Arg	C	15.72	0.04	-0.40	0.06
65	Asn	N	64	His	C	15.42	0.05	0.43	0.07
66	Phe	N	65	Asn	C	14.08	0.03	0.54	0.05
67	Leu	N	66	Phe	C	14.34	0.03	-0.24	0.04
68	Val	N	67	Leu	C	15.74	0.03	1.52	0.05
69	Gln	N	68	Val	C	13.65	0.04	-0.36	0.06
70	Ala	N	69	Gln	C	15.62	0.02	1.51	0.04
71	Gly	N	70	Ala	C	14.89	0.02	0.35	0.03
73	Val	N	72	Asn	C	16.00	0.02	-0.80	0.04
74	Gln	N	73	Val	C	13.46	0.02	-0.99	0.03
75	Leu	N	74	Gln	C	14.34	0.03	-0.10	0.04

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
76	Arg	N	75	Leu	C	14.50	0.02	0.34	0.03
79	Gly	N	78	Ile	C	16.84	0.06	0.03	0.08
81	Ser	N	80	His	C	14.91	0.04	-0.97	0.05
82	Met	N	81	Ser	C	15.91	0.02	0.50	0.03
83	Gln	N	82	Met	C	13.65	0.03	-1.00	0.04
84	Asn	N	83	Gln	C	14.78	0.02	0.86	0.03
85	Cys	N	84	Asn	C	14.79	0.04	-0.63	0.07
86	Val	N	85	Cys	C	16.61	0.04	1.58	0.07
92	Asp	N	91	Val	C	12.98	0.03	-0.15	0.05
93	Thr	N	92	Asp	C	15.76	0.03	-1.14	0.05
94	Ala	N	93	Thr	C	15.09	0.02	0.50	0.03
95	Asn	N	94	Ala	C	14.38	0.05	0.39	0.07
100	Lys	N	99	Pro	C	13.54	0.02	-0.35	0.03
101	Tyr	N	100	Lys	C	14.73	0.03	-0.13	0.04
102	Lys	N	101	Tyr	C	16.65	0.03	0.97	0.05
104	Val	N	103	Phe	C	15.45	0.03	0.43	0.04
105	Arg	N	104	Val	C	14.79	0.02	-0.09	0.03
106	Ile	N	105	Arg	C	14.95	0.06	0.52	0.1
109	Gly	N	108	Pro	C	14.64	0.03	-0.50	0.05
110	Gln	N	109	Gly	C	16.04	0.05	-0.14	0.07
111	Thr	N	110	Gln	C	15.17	0.03	0.00	0.04
112	Phe	N	111	Thr	C	15.41	0.05	-1.18	0.07
113	Ser	N	112	Phe	C	16.12	0.04	0.26	0.07
114	Val	N	113	Ser	C	14.93	0.04	0.26	0.06
115	Leu	N	114	Val	C	13.31	0.04	0.04	0.06
116	Ala	N	115	Leu	C	14.59	0.04	0.63	0.05
117	Cys	N	116	Ala	C	13.97	0.04	-0.92	0.06
118	Tyr	N	117	Cys	C	14.45	0.04	-0.47	0.06
119	Asn	N	118	Tyr	C	13.84	0.07	-0.43	0.11
120	Gly	N	119	Asn	C	15.19	0.06	-0.57	0.1
121	Ser	N	120	Gly	C	15.56	0.03	-0.32	0.04
123	Ser	N	122	Pro	C	15.21	0.05	0.39	0.08
124	Gly	N	123	Ser	C	16.12	0.05	-0.44	0.07
125	Val	N	124	Gly	C	15.19	0.02	-1.02	0.04
126	Tyr	N	125	Val	C	15.05	0.04	0.25	0.07
127	Gln	N	126	Tyr	C	13.84	0.02	-0.90	0.04
128	Cys	N	127	Gln	C	14.72	0.03	-0.54	0.05
129	Ala	N	128	Cys	C	16.87	0.02	0.96	0.04
130	Met	N	129	Ala	C	13.98	0.05	-0.27	0.08
131	Arg	N	130	Met	C	16.80	0.04	1.52	0.06
133	Asn	N	132	Pro	C	15.39	0.05	-0.21	0.07

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
134	Phe	N	133	Asn	C	17.32	0.05	0.92	0.08
135	Thr	N	134	Phe	C	16.27	0.08	0.02	0.13
136	Ile	N	135	Thr	C	14.51	0.06	-0.51	0.09
137	Lys	N	136	Ile	C	13.53	0.03	-0.71	0.05
138	Gly	N	137	Lys	C	13.98	0.03	-0.60	0.04
140	Phe	N	139	Ser	C	15.96	0.07	0.88	0.11
142	Asn	N	141	Leu	C	16.03	0.07	0.65	0.1
143	Gly	N	142	Asn	C	14.17	0.15	-1.08	0.19
144	Ser	N	143	Gly	C	15.31	0.06	-0.94	0.09
145	Cys	N	144	Ser	C	15.99	0.14	0.03	0.18
146	Gly	N	145	Cys	C	15.29	0.16	-0.21	0.22
147	Ser	N	146	Gly	C	14.61	0.16	-0.76	0.2
148	Val	N	147	Ser	C	16.48	0.06	0.74	0.08
149	Gly	N	148	Val	C	13.31	0.07	-0.63	0.1
150	Phe	N	149	Gly	C	14.40	0.05	-0.69	0.07
151	Asn	N	150	Phe	C	15.86	0.04	0.53	0.06
152	Ile	N	151	Asn	C	15.25	0.02	-0.20	0.04
153	Asp	N	152	Ile	C	15.06	0.02	0.66	0.04
155	Asp	N	154	Tyr	C	15.17	0.05	-0.07	0.09
156	Cys	N	155	Asp	C	16.15	0.03	0.47	0.05
157	Val	N	156	Cys	C	14.29	0.03	-0.91	0.05
158	Ser	N	157	Val	C	14.33	0.04	0.62	0.06
159	Phe	N	158	Ser	C	12.93	0.03	-0.85	0.06
160	Cys	N	159	Phe	C	15.08	0.09	1.78	0.14
161	Tyr	N	160	Cys	C	15.30	0.1	-0.97	0.14
162	Met	N	161	Tyr	C	16.31	0.04	-0.03	0.06
163	His	N	162	Met	C	14.57	0.05	-0.29	0.07
164	His	N	163	His	C	15.69	0.17	1.18	0.25
165	Met	N	164	His	C	14.66	0.08	0.39	0.15
166	Glu	N	165	Met	C	14.82	0.04	0.18	0.06
167	Leu	N	166	Glu	C	14.36	0.12	-0.09	0.16
172	His	N	171	Val	C	15.79	0.07	1.41	0.11
174	Gly	N	173	Ala	C	15.74	0.08	0.16	0.12
175	Thr	N	174	Gly	C	17.19	0.05	0.93	0.08
176	Asp	N	175	Thr	C	14.97	0.03	-0.09	0.05
177	Leu	N	176	Asp	C	16.04	0.06	1.97	0.09
179	Gly	N	178	Glu	C	15.91	0.07	0.37	0.11
181	Phe	N	180	Asn	C	15.72	0.03	1.73	0.05
182	Tyr	N	181	Phe	C	14.55	0.03	-0.75	0.05
183	Gly	N	182	Tyr	C	14.82	0.02	-0.25	0.03
185	Phe	N	184	Pro	C	15.70	0.04	0.09	0.06

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
186	Val	N	185	Phe	C	15.12	0.08	-0.56	0.11
187	Asp	N	186	Val	C	14.80	0.04	0.20	0.06
188	Arg	N	187	Asp	C	16.24	0.06	1.58	0.1
189	Gln	N	188	Arg	C	14.54	0.03	0.00	0.05
190	Thr	N	189	Gln	C	15.01	0.02	0.41	0.05
193	Ala	N	192	Gln	C	15.06	0.06	0.20	0.1
194	Ala	N	193	Ala	C	15.08	0.03	0.02	0.05
195	Gly	N	194	Ala	C	15.03	0.06	-0.18	0.09
196	Thr	N	195	Gly	C	15.43	0.04	-0.08	0.07
197	Asp	N	196	Thr	C	15.84	0.09	0.45	0.11
199	Thr	N	198	Thr	C	15.23	0.04	0.07	0.06
200	Ile	N	199	Thr	C	15.51	0.05	1.29	0.06
201	Thr	N	200	Ile	C	14.47	0.09	1.81	0.14
202	Val	N	201	Thr	C	14.94	0.05	1.03	0.07
203	Asn	N	202	Val	C	13.94	0.07	-1.19	0.11
204	Val	N	203	Asn	C	15.96	0.08	1.60	0.13
215	Gly	N	214	Asn	C	17.47	0.05	1.34	0.07
216	Asp	N	215	Gly	C	14.37	0.03	-1.66	0.05
217	Arg	N	216	Asp	C	14.18	0.03	1.98	0.04
218	Trp	N	217	Arg	C	13.13	0.04	-1.04	0.07
220	Leu	N	219	Phe	C	14.30	0.03	-2.32	0.04
221	Asn	N	220	Leu	C	15.09	0.03	0.07	0.04
222	Arg	N	221	Asn	C	16.40	0.05	1.51	0.09
223	Phe	N	222	Arg	C	14.74	0.02	-1.53	0.03
224	Thr	N	223	Phe	C	14.87	0.01	-0.18	0.02
225	Thr	N	224	Thr	C	15.63	0.02	0.77	0.03
226	Thr	N	225	Thr	C	14.10	0.02	-0.81	0.04
227	Leu	N	226	Thr	C	15.61	0.02	1.10	0.04
229	Asp	N	228	Asn	C	15.11	0.03	0.10	0.05
230	Phe	N	229	Asp	C	15.74	0.03	0.94	0.05
231	Asn	N	230	Phe	C	14.78	0.03	0.12	0.05
232	Leu	N	231	Asn	C	17.00	0.04	2.33	0.06
233	Val	N	232	Leu	C	14.25	0.05	-0.90	0.11
234	Ala	N	233	Val	C	15.10	0.06	1.20	0.11
235	Met	N	234	Ala	C	14.85	0.05	0.23	0.08
236	Lys	N	235	Met	C	16.34	0.06	1.63	0.1
237	Tyr	N	236	Lys	C	15.55	0.06	0.17	0.1
238	Asn	N	237	Tyr	C	15.19	0.07	-0.37	0.1
239	Tyr	N	238	Asn	C	14.65	0.06	-1.73	0.09
240	Glu	N	239	Tyr	C	15.80	0.05	1.47	0.08
243	Thr	N	242	Leu	C	15.77	0.04	1.31	0.06

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
244	Gln	N	243	Thr	C	13.27	0.03	-0.64	0.05
245	Asp	N	244	Gln	C	14.31	0.03	-0.39	0.05
246	His	N	245	Asp	C	14.81	0.04	-0.33	0.05
247	Val	N	246	His	C	15.53	0.04	1.37	0.06
248	Asp	N	247	Val	C	15.06	0.04	0.60	0.06
249	Ile	N	248	Asp	C	13.21	0.06	-1.95	0.1
250	Leu	N	249	Ile	C	17.03	0.07	2.62	0.12
251	Gly	N	250	Leu	C	15.14	0.05	-1.08	0.08
255	Ala	N	254	Ser	C	15.68	0.05	1.11	0.08
257	Thr	N	256	Gln	C	15.00	0.15	0.38	0.22
258	Gly	N	257	Thr	C	15.65	0.08	-0.67	0.12
259	Ile	N	258	Gly	C	14.75	0.06	-2.04	0.08
260	Ala	N	259	Ile	C	15.69	0.03	1.26	0.04
261	Val	N	260	Ala	C	14.94	0.03	0.37	0.04
262	Leu	N	261	Val	C	14.67	0.04	1.23	0.06
263	Asp	N	262	Leu	C	13.61	0.04	-1.28	0.07
264	Met	N	263	Asp	C	16.30	0.08	2.20	0.12
265	Cys	N	264	Met	C	14.23	0.08	0.07	0.12
266	Ala	N	265	Cys	C	14.61	0.05	-0.17	0.08
267	Ser	N	266	Ala	C	14.69	0.03	-0.25	0.04
269	Lys	N	268	Leu	C	14.95	0.05	0.81	0.08
270	Glu	N	269	Lys	C	13.89	0.05	-0.51	0.07
271	Leu	N	270	Glu	C	16.63	0.07	2.27	0.11
273	Gln	N	272	Leu	C	15.43	0.05	0.95	0.07
274	Asn	N	273	Gln	C	14.03	0.05	-0.68	0.07
275	Gly	N	274	Asn	C	18.30	0.05	1.67	0.07
277	Asn	N	276	Met	C	14.70	0.03	-0.10	0.06
278	Gly	N	277	Asn	C	16.64	0.03	1.04	0.05
279	Arg	N	278	Gly	C	15.62	0.02	-1.43	0.02
280	Thr	N	279	Arg	C	15.63	0.02	0.42	0.03
281	Ile	N	280	Thr	C	15.91	0.04	1.03	0.06
282	Leu	N	281	Ile	C	13.27	0.08	-0.63	0.12
283	Gly	N	282	Leu	C	15.58	0.04	0.46	0.06
284	Ser	N	283	Gly	C	16.03	0.09	-0.13	0.16
285	Ala	N	284	Ser	C	12.65	0.03	-1.74	0.05
286	Leu	N	285	Ala	C	17.50	0.04	0.99	0.05
287	Leu	N	286	Leu	C	15.03	0.07	0.51	0.1
289	Asp	N	288	Glu	C	13.14	0.08	-0.33	0.12
290	Glu	N	289	Asp	C	15.56	0.09	0.69	0.12
291	Phe	N	290	Glu	C	14.72	0.07	-1.32	0.1
292	Thr	N	291	Phe	C	15.31	0.03	1.15	0.05

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
294	Phe	N	293	Pro	C	16.17	0.04	1.31	0.06
296	Val	N	295	Asp	C	16.88	0.04	2.33	0.07
297	Val	N	296	Val	C	14.19	0.04	-0.30	0.07
299	Gln	N	298	Arg	C	15.00	0.03	0.18	0.04
300	Cys	N	299	Gln	C	15.29	0.03	0.50	0.05
301	Ser	N	300	Cys	C	16.15	0.02	0.93	0.04
302	Gly	N	301	Ser	C	15.29	0.02	-0.51	0.04
303	Val	N	302	Gly	C	16.20	0.01	0.25	0.02
304	Thr	N	303	Val	C	14.53	0.01	-0.27	0.02
305	Phe	N	304	Thr	C	15.14	0.01	0.11	0.02
306	Gln	N	305	Phe	C	15.78	0.01	-0.17	0.01

Table S5. 3D QJ derived $^{1}J_{C'C\alpha}$ and $^{1}D_{C'C\alpha}$ couplings for MPro$^{10\text{-}306,\ H41Q}$

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
12	Lys	C	12	Lys	CA	53.76	0.15	0.62	0.25
13	Val	C	13	Val	CA	53.62	0.16	-1.54	0.26
14	Glu	C	14	Glu	CA	53.93	0.12	-0.43	0.20
15	Gly	C	15	Gly	CA	53.34	0.15	-0.77	0.23
16	Cys	C	16	Cys	CA	52.21	0.09	2.11	0.14
18	Val	C	18	Val	CA	52.67	0.11	2.43	0.19
20	Val	C	20	Val	CA	53.87	0.05	2.18	0.08
21	Thr	C	21	Thr	CA	52.53	0.07	-1.47	0.10
23	Gly	C	23	Gly	CA	53.76	0.15	-0.41	0.30
24	Thr	C	24	Thr	CA	53.45	0.07	-2.03	0.12
39	Pro	C	39	Pro	CA	55.58	0.16	-2.21	0.28
40	Arg	C	40	Arg	CA	54.03	0.16	0.80	0.27
41	Gln	C	41	Gln	CA	53.18	0.11	-0.93	0.17
42	Val	C	42	Val	CA	53.78	0.14	-2.18	0.26
43	Ile	C	43	Ile	CA	53.25	0.08	2.78	0.14
44	Cys	C	44	Cys	CA	53.24	0.06	-0.52	0.10
45	Thr	C	45	Thr	CA	51.19	0.25	0.66	0.69
47	Glu	C	47	Glu	CA	53.24	0.10	-0.09	0.16
48	Asp	C	48	Asp	CA	53.66	0.13	-2.22	0.24
49	Met	C	49	Met	CA	54.06	0.09	1.82	0.15
50	Leu	C	50	Leu	CA	53.67	0.09	-0.62	0.13
52	Pro	C	52	Pro	CA	54.47	0.06	-0.31	0.10
53	Asn	C	53	Asn	CA	55.23	0.09	2.59	0.16

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
54	Tyr	C	54	Tyr	CA	53.66	0.12	0.70	0.18
55	Glu	C	55	Glu	CA	53.46	0.14	-2.11	0.21
56	Asp	C	56	Asp	CA	53.54	0.15	-0.43	0.26
57	Leu	C	57	Leu	CA	53.45	0.16	2.76	0.27
58	Leu	C	58	Leu	CA	54.00	0.22	-0.69	0.31
59	Ile	C	59	Ile	CA	53.23	0.18	-1.49	0.26
60	Arg	C	60	Arg	CA	53.44	0.08	0.48	0.12
64	His	C	64	His	CA	54.31	0.13	1.71	0.23
65	Asn	C	65	Asn	CA	53.17	0.08	-1.96	0.13
66	Phe	C	66	Phe	CA	53.87	0.08	0.76	0.12
67	Leu	C	67	Leu	CA	53.60	0.07	-1.92	0.11
68	Val	C	68	Val	CA	54.01	0.11	0.66	0.16
69	Gln	C	69	Gln	CA	54.24	0.07	-1.90	0.10
70	Ala	C	70	Ala	CA	53.21	0.05	-0.35	0.08
72	Asn	C	72	Asn	CA	53.92	0.05	-0.15	0.08
73	Val	C	73	Val	CA	54.00	0.05	0.79	0.07
74	Gln	C	74	Gln	CA	53.46	0.07	0.38	0.10
75	Leu	C	75	Leu	CA	53.43	0.06	-1.08	0.08
76	Arg	C	76	Arg	CA	53.70	0.07	-0.66	0.10
77	Val	C	77	Val	CA	54.01	0.31	-0.93	0.54
78	Ile	C	78	Ile	CA	53.32	0.13	-0.13	0.17
80	His	C	80	His	CA	54.98	0.25	-0.44	0.27
81	Ser	C	81	Ser	CA	53.31	0.07	0.21	0.10
82	Met	C	82	Met	CA	53.74	0.07	-1.28	0.11
83	Gln	C	83	Gln	CA	53.47	0.06	1.36	0.09
84	Asn	C	84	Asn	CA	54.93	0.13	-0.17	0.22
85	Cys	C	85	Cys	CA	52.64	0.12	1.07	0.16
91	Val	C	91	Val	CA	52.69	0.11	2.83	0.18
92	Asp	C	92	Asp	CA	55.16	0.07	0.44	0.10
93	Thr	C	93	Thr	CA	53.37	0.06	-1.71	0.08
94	Ala	C	94	Ala	CA	52.36	0.13	-1.19	0.19
99	Pro	C	99	Pro	CA	51.62	0.05	-0.78	0.09
100	Lys	C	100	Lys	CA	52.83	0.07	2.73	0.11
101	Tyr	C	101	Tyr	CA	54.75	0.08	-0.62	0.13
102	Lys	C	102	Lys	CA	52.25	0.05	3.03	0.08
103	Phe	C	103	Phe	CA	54.45	0.08	-0.94	0.12
104	Val	C	104	Val	CA	53.38	0.06	3.01	0.09
105	Arg	C	105	Arg	CA	53.41	0.15	-1.77	0.23
106	Ile	C	106	Ile	CA	52.84	0.07	2.34	0.11
108	Pro	C	108	Pro	CA	54.76	0.08	-1.53	0.14
109	Gly	C	109	Gly	CA	53.23	0.10	2.95	0.18

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
110	Gln	C	110	Gln	CA	53.40	0.07	-1.00	0.10
111	Thr	C	111	Thr	CA	52.89	0.10	0.49	0.16
112	Phe	C	112	Phe	CA	53.30	0.11	-0.98	0.17
113	Ser	C	113	Ser	CA	52.20	0.10	-1.92	0.16
114	Val	C	114	Val	CA	53.45	0.09	-0.60	0.15
115	Leu	C	115	Leu	CA	52.57	0.09	0.31	0.14
116	Ala	C	116	Ala	CA	53.74	0.11	-1.18	0.17
117	Cys	C	117	Cys	CA	53.60	0.09	0.83	0.14
118	Tyr	C	118	Tyr	CA	53.83	0.15	-0.74	0.28
119	Asn	C	119	Asn	CA	54.19	0.14	3.15	0.29
120	Gly	C	120	Gly	CA	53.98	0.06	-0.45	0.09
122	Pro	C	122	Pro	CA	54.93	0.11	-0.46	0.17
123	Ser	C	123	Ser	CA	54.05	0.07	-1.85	0.11
124	Gly	C	124	Gly	CA	52.62	0.06	-0.72	0.09
125	Val	C	125	Val	CA	53.75	0.12	0.33	0.18
126	Tyr	C	126	Tyr	CA	53.60	0.06	-0.83	0.09
127	Gln	C	127	Gln	CA	53.75	0.10	2.35	0.15
128	Cys	C	128	Cys	CA	53.51	0.06	-0.84	0.09
129	Ala	C	129	Ala	CA	52.73	0.14	1.32	0.22
130	Met	C	130	Met	CA	52.47	0.11	0.27	0.17
132	Pro	C	132	Pro	CA	55.85	0.13	-1.19	0.20
133	Asn	C	133	Asn	CA	53.31	0.12	-0.05	0.20
134	Phe	C	134	Phe	CA	53.53	0.17	-1.35	0.31
135	Thr	C	135	Thr	CA	54.07	0.13	-0.20	0.23
136	Ile	C	136	Ile	CA	53.42	0.08	0.84	0.12
137	Lys	C	137	Lys	CA	53.09	0.07	0.85	0.12
139	Ser	C	139	Ser	CA	53.29	0.18	1.54	0.33
141	Leu	C	141	Leu	CA	53.54	0.13	-0.15	0.20
142	Asn	C	142	Asn	CA	54.04	0.25	-0.25	0.39
143	Gly	C	143	Gly	CA	53.53	0.13	2.61	0.24
144	Ser	C	144	Ser	CA	53.29	0.45	0.48	0.59
145	Cys	C	145	Cys	CA	55.21	0.24	2.25	0.41
146	Gly	C	146	Gly	CA	53.70	0.29	-0.43	0.46
147	Ser	C	147	Ser	CA	51.02	0.11	-0.96	0.18
148	Val	C	148	Val	CA	54.20	0.16	-2.11	0.27
149	Gly	C	149	Gly	CA	52.10	0.12	1.58	0.20
150	Phe	C	150	Phe	CA	54.33	0.09	-1.92	0.15
151	Asn	C	151	Asn	CA	53.33	0.08	2.84	0.16
152	Ile	C	152	Ile	CA	54.48	0.06	-1.17	0.09
154	Tyr	C	154	Tyr	CA	53.68	0.13	-1.67	0.26
155	Asp	C	155	Asp	CA	54.43	0.06	-0.67	0.10

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
156	Cys	C	156	Cys	CA	55.17	0.08	2.59	0.13
157	Val	C	157	Val	CA	53.86	0.09	0.23	0.15
158	Ser	C	158	Ser	CA	52.34	0.09	1.94	0.15
159	Phe	C	159	Phe	CA	53.45	0.29	1.80	0.45
160	Cys	C	160	Cys	CA	56.22	0.23	1.23	0.39
161	Tyr	C	161	Tyr	CA	54.63	0.11	-1.67	0.17
162	Met	C	162	Met	CA	53.17	0.13	2.97	0.21
164	His	C	164	His	CA	55.15	0.25	2.56	0.53
165	Met	C	165	Met	CA	55.02	0.11	-0.76	0.16
166	Glu	C	166	Glu	CA	53.15	0.23	2.18	0.37
171	Val	C	171	Val	CA	52.48	0.18	-0.66	0.28
172	His	C	172	His	CA	54.28	0.07	-1.18	0.09
173	Ala	C	173	Ala	CA	53.89	0.21	1.88	0.35
174	Gly	C	174	Gly	CA	52.94	0.13	0.04	0.20
175	Thr	C	175	Thr	CA	52.72	0.10	1.46	0.17
176	Asp	C	176	Asp	CA	51.68	0.17	-0.76	0.27
177	Leu	C	177	Leu	CA	53.69	0.19	-0.88	0.32
178	Glu	C	178	Glu	CA	52.69	0.18	1.68	0.30
180	Asn	C	180	Asn	CA	53.86	0.10	0.18	0.16
181	Phe	C	181	Phe	CA	52.60	0.08	-2.25	0.13
182	Tyr	C	182	Tyr	CA	52.66	0.06	2.97	0.10
184	Pro	C	184	Pro	CA	52.98	0.07	-1.50	0.10
185	Phe	C	185	Phe	CA	54.00	0.17	-0.60	0.27
186	Val	C	186	Val	CA	53.52	0.09	-1.18	0.14
187	Asp	C	187	Asp	CA	54.15	0.14	-0.38	0.21
188	Arg	C	188	Arg	CA	52.10	0.10	0.97	0.15
189	Gln	C	189	Gln	CA	54.09	0.08	1.42	0.12
190	Thr	C	190	Thr	CA	53.00	0.48	-5.51	2.45
192	Gln	C	192	Gln	CA	53.17	0.15	-1.36	0.27
193	Ala	C	193	Ala	CA	53.35	0.07	-0.52	0.12
194	Ala	C	194	Ala	CA	53.51	0.10	-0.75	0.18
195	Gly	C	195	Gly	CA	53.04	0.11	-0.44	0.18
196	Thr	C	196	Thr	CA	53.73	0.11	-0.70	0.16
197	Asp	C	197	Asp	CA	53.70	0.07	-1.13	0.12
198	Thr	C	198	Thr	CA	52.69	0.09	-1.32	0.15
199	Thr	C	199	Thr	CA	52.35	0.10	-2.54	0.15
200	Ile	C	200	Ile	CA	54.40	0.26	-1.18	0.39
201	Thr	C	201	Thr	CA	54.60	0.18	-1.67	0.29
202	Val	C	202	Val	CA	53.43	0.16	1.30	0.28
203	Asn	C	203	Asn	CA	53.59	0.19	1.35	0.29
213	Ile	C	213	Ile	CA	53.44	0.12	-0.57	0.17

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
214	Asn	C	214	Asn	CA	54.62	0.11	2.21	0.14
215	Gly	C	215	Gly	CA	53.66	0.07	0.40	0.10
216	Asp	C	216	Asp	CA	55.00	0.11	0.93	0.14
217	Arg	C	217	Arg	CA	53.69	0.14	2.63	0.22
219	Phe	C	219	Phe	CA	53.93	0.06	-1.33	0.08
220	Leu	C	220	Leu	CA	53.87	0.06	3.98	0.08
221	Asn	C	221	Asn	CA	54.37	0.17	-1.76	0.29
222	Arg	C	222	Arg	CA	53.58	0.03	-0.35	0.04
223	Phe	C	223	Phe	CA	53.29	0.03	1.65	0.04
224	Thr	C	224	Thr	CA	52.71	0.04	-2.21	0.06
225	Thr	C	225	Thr	CA	51.94	0.06	-0.14	0.10
226	Thr	C	226	Thr	CA	51.98	0.07	0.52	0.10
227	Leu	C	227	Leu	CA	54.24	0.07	-1.75	0.10
228	Asn	C	228	Asn	CA	54.39	0.09	-0.52	0.12
229	Asp	C	229	Asp	CA	53.25	0.10	0.63	0.13
230	Phe	C	230	Phe	CA	54.39	0.10	-1.33	0.12
231	Asn	C	231	Asn	CA	54.99	0.10	-0.86	0.13
232	Leu	C	232	Leu	CA	53.30	0.13	-0.70	0.24
233	Val	C	233	Val	CA	53.19	0.15	3.09	0.23
234	Ala	C	234	Ala	CA	53.66	1.54	-1.93	1.54
235	Met	C	235	Met	CA	53.46	0.13	-1.46	0.18
236	Lys	C	236	Lys	CA	53.40	0.16	0.19	0.21
237	Tyr	C	237	Tyr	CA	52.60	0.17	3.80	0.22
238	Asn	C	238	Asn	CA	53.89	0.12	-0.59	0.21
239	Tyr	C	239	Tyr	CA	52.27	0.13	-0.33	0.20
242	Leu	C	242	Leu	CA	52.96	0.08	-0.93	0.11
243	Thr	C	243	Thr	CA	52.97	0.09	-2.72	0.18
244	Gln	C	244	Gln	CA	53.96	0.07	3.27	0.11
245	Asp	C	245	Asp	CA	53.31	0.11	-0.82	0.15
246	His	C	246	His	CA	53.67	0.10	-0.23	0.12
247	Val	C	247	Val	CA	53.18	0.10	-0.09	0.13
248	Asp	C	248	Asp	CA	53.69	0.15	2.47	0.29
249	Ile	C	249	Ile	CA	53.02	0.17	-0.61	0.32
250	Leu	C	250	Leu	CA	53.38	0.11	-2.50	0.16
253	Leu	C	253	Leu	CA	53.96	0.15	3.19	0.25
254	Ser	C	254	Ser	CA	53.14	0.12	-2.90	0.16
255	Ala	C	255	Ala	CA	53.63	0.11	-0.64	0.16
256	Gln	C	256	Gln	CA	53.41	0.34	2.86	0.46
257	Thr	C	257	Thr	CA	54.91	0.19	2.34	0.25
258	Gly	C	258	Gly	CA	52.90	0.10	0.56	0.14
259	Ile	C	259	Ile	CA	54.08	0.06	-0.83	0.08

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
260	Ala	C	260	Ala	CA	52.51	0.07	-2.76	0.09
261	Val	C	261	Val	CA	53.74	0.09	1.85	0.12
262	Leu	C	262	Leu	CA	54.50	0.13	0.44	0.17
263	Asp	C	263	Asp	CA	52.94	0.22	-0.40	0.27
264	Met	C	264	Met	CA	53.06	0.17	-1.72	0.24
265	Cys	C	265	Cys	CA	51.85	0.11	3.06	0.16
266	Ala	C	266	Ala	CA	53.21	0.13	-1.03	0.17
268	Leu	C	268	Leu	CA	53.37	0.11	0.62	0.15
269	Lys	C	269	Lys	CA	53.92	0.12	0.24	0.17
270	Glu	C	270	Glu	CA	53.44	0.11	-0.30	0.15
272	Leu	C	272	Leu	CA	54.04	0.10	2.04	0.14
273	Gln	C	273	Gln	CA	54.15	0.13	-0.30	0.16
274	Asn	C	274	Asn	CA	55.58	0.11	1.06	0.13
275	Gly	C	275	Gly	CA	52.32	0.06	-1.74	0.08
276	Met	C	276	Met	CA	53.99	0.09	0.26	0.16
277	Asn	C	277	Asn	CA	54.45	0.07	0.36	0.11
278	Gly	C	278	Gly	CA	53.51	0.03	-0.78	0.05
279	Arg	C	279	Arg	CA	53.01	0.05	2.96	0.07
280	Thr	C	280	Thr	CA	53.25	0.08	-2.64	0.13
281	Ile	C	281	Ile	CA	53.56	0.16	4.43	0.27
282	Leu	C	282	Leu	CA	53.02	0.10	-1.81	0.14
283	Gly	C	283	Gly	CA	53.48	0.11	-2.57	0.20
284	Ser	C	284	Ser	CA	54.09	0.07	3.82	0.14
285	Ala	C	285	Ala	CA	52.89	0.07	-0.57	0.10
286	Leu	C	286	Leu	CA	52.84	0.13	-2.31	0.21
288	Glu	C	288	Glu	CA	55.62	0.17	-0.34	0.32
289	Asp	C	289	Asp	CA	55.91	0.17	4.07	0.33
290	Glu	C	290	Glu	CA	54.48	0.13	-2.42	0.22
291	Phe	C	291	Phe	CA	54.07	0.07	-0.36	0.11
293	Pro	C	293	Pro	CA	56.10	0.08	1.58	0.12
294	Phe	C	294	Phe	CA	53.57	0.10	-0.25	0.15
295	Asp	C	295	Asp	CA	53.61	0.10	-0.12	0.14
297	Val	C	297	Val	CA	53.17	0.07	2.30	0.10
298	Arg	C	298	Arg	CA	53.36	0.07	-0.73	0.08
299	Gln	C	299	Gln	CA	53.81	0.08	-0.65	0.10
300	Cys	C	300	Cys	CA	53.02	0.05	-1.92	0.08
301	Ser	C	301	Ser	CA	53.59	0.04	-0.34	0.08
302	Gly	C	302	Gly	CA	53.14	0.02	0.41	0.02
303	Val	C	303	Val	CA	53.61	0.02	-0.48	0.03
304	Thr	C	304	Thr	CA	53.41	0.02	0.63	0.03
305	Phe	C	305	Phe	CA	53.81	0.01	0.00	0.15

Table S6. 3D TATER derived $^{2}J_{C'H}$ and $^{2}D_{C'H}$ couplings for MPro$^{10\text{-}306,\ H41Q}$

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
12	Lys	C	13	Val	HN	4.55	0.18	1.28	0.41
13	Val	C	14	Glu	HN	5.02	0.19	-2.20	0.43
14	Glu	C	15	Gly	HN	5.19	0.13	2.07	0.30
15	Gly	C	16	Cys	HN	4.61	0.17	0.32	0.37
16	Cys	C	17	Met	HN	4.58	0.11	-1.66	0.24
18	Val	C	19	Gln	HN	4.51	0.12	-2.44	0.29
20	Val	C	21	Thr	HN	4.66	0.06	-1.17	0.12
21	Thr	C	22	Cys	HN	4.66	0.07	4.98	0.13
23	Gly	C	24	Thr	HN	4.60	0.14	3.43	0.43
24	Thr	C	25	Thr	HN	4.80	0.07	0.07	0.16
39	Pro	C	40	Arg	HN	4.53	0.23	-1.32	0.45
40	Arg	C	41	Gln	HN	4.03	0.18	-2.08	0.47
41	Gln	C	42	Val	HN	4.92	0.12	3.83	0.25
42	Val	C	43	Ile	HN	4.33	0.14	-1.63	0.41
43	Ile	C	44	Cys	HN	4.47	0.09	-2.87	0.22
44	Cys	C	45	Thr	HN	4.17	0.07	-0.37	0.16
45	Thr	C	46	Ser	HN	4.87	0.22	-1.37	0.96
47	Glu	C	48	Asp	HN	4.85	0.12	4.04	0.30
48	Asp	C	49	Met	HN	4.43	0.12	-1.34	0.33
49	Met	C	50	Leu	HN	5.07	0.10	-1.71	0.23
50	Leu	C	51	Asn	HN	4.67	0.10	-0.16	0.22
52	Pro	C	53	Asn	HN	4.95	0.06	-3.26	0.15
53	Asn	C	54	Tyr	HN	4.47	0.10	-3.41	0.22
54	Tyr	C	55	Glu	HN	5.07	0.12	-0.29	0.27
55	Glu	C	56	Asp	HN	4.83	0.16	4.75	0.35
56	Asp	C	57	Leu	HN	4.85	0.17	-2.58	0.45
57	Leu	C	58	Leu	HN	4.74	0.17	-2.34	0.43
58	Leu	C	59	Ile	HN	4.86	0.08	0.94	0.43
59	Ile	C	60	Arg	HN	4.66	0.19	1.27	0.41
60	Arg	C	61	Lys	HN	4.68	0.09	-2.23	0.18
64	His	C	65	Asn	HN	4.90	0.13	-1.11	0.33
65	Asn	C	66	Phe	HN	4.90	0.25	3.48	0.29
66	Phe	C	67	Leu	HN	4.78	0.08	1.08	0.16

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
67	Leu	C	68	Val	HN	4.57	0.08	4.81	0.15
68	Val	C	69	Gln	HN	4.62	0.11	-0.57	0.24
69	Gln	C	70	Ala	HN	4.75	0.07	3.69	0.13
70	Ala	C	71	Gly	HN	5.19	0.05	2.39	0.11
72	Asn	C	73	Val	HN	4.72	0.05	-2.45	0.11
73	Val	C	74	Gln	HN	4.57	0.05	-2.29	0.11
74	Gln	C	75	Leu	HN	4.37	0.08	1.70	0.15
75	Leu	C	76	Arg	HN	4.65	0.06	3.25	0.11
76	Arg	C	77	Val	HN	5.04	0.07	2.88	0.12
77	Val	C	78	Ile	HN	4.19	0.52	-1.73	0.87
78	Ile	C	79	Gly	HN	4.98	0.12	-0.41	0.27
80	His	C	81	Ser	HN	4.58	0.15	-2.95	0.22
81	Ser	C	82	Met	HN	4.59	0.05	-0.30	0.12
82	Met	C	83	Gln	HN	5.01	0.07	-2.08	0.15
83	Gln	C	84	Asn	HN	4.93	0.06	-0.07	0.12
84	Asn	C	85	Cys	HN	5.14	0.13	-2.89	0.33
85	Cys	C	86	Val	HN	4.67	0.13	2.45	0.24
91	Val	C	92	Asp	HN	4.48	0.11	-2.23	0.26
92	Asp	C	93	Thr	HN	3.90	0.08	0.77	0.17
93	Thr	C	94	Ala	HN	4.56	0.05	3.98	0.09
94	Ala	C	95	Asn	HN	4.36	0.15	1.22	0.29
99	Pro	C	100	Lys	HN	4.03	0.06	-2.13	0.12
100	Lys	C	101	Tyr	HN	4.61	0.08	-1.52	0.16
101	Tyr	C	102	Lys	HN	4.27	0.10	2.80	0.19
102	Lys	C	103	Phe	HN	4.57	0.05	-1.85	0.12
103	Phe	C	104	Val	HN	4.50	0.08	1.25	0.17
104	Val	C	105	Arg	HN	4.43	0.06	-2.10	0.11
105	Arg	C	106	Ile	HN	3.97	0.17	1.33	0.34
106	Ile	C	107	Gln	HN	4.67	0.08	-2.91	0.17
108	Pro	C	109	Gly	HN	4.67	0.09	-0.56	0.22
109	Gly	C	110	Gln	HN	4.49	0.11	-2.76	0.28
110	Gln	C	111	Thr	HN	4.76	0.08	-0.82	0.14
111	Thr	C	112	Phe	HN	4.85	0.11	-0.29	0.23
112	Phe	C	113	Ser	HN	5.03	0.12	2.71	0.28
113	Ser	C	114	Val	HN	4.54	0.12	1.03	0.23
114	Val	C	115	Leu	HN	4.53	0.11	-1.33	0.24
115	Leu	C	116	Ala	HN	4.33	0.10	-0.85	0.21
116	Ala	C	117	Cys	HN	4.75	0.11	-1.01	0.27
117	Cys	C	118	Tyr	HN	4.49	0.09	-0.24	0.20
118	Tyr	C	119	Asn	HN	4.95	0.15	0.30	0.44
119	Asn	C	120	Gly	HN	4.99	0.14	-1.98	0.40

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
120	Gly	C	121	Ser	HN	4.67	0.06	2.28	0.12
122	Pro	C	123	Ser	HN	4.21	0.12	1.22	0.27
123	Ser	C	124	Gly	HN	4.91	0.07	0.81	0.16
124	Gly	C	125	Val	HN	4.49	0.07	-2.12	0.12
125	Val	C	126	Tyr	HN	4.31	0.13	-1.15	0.25
126	Tyr	C	127	Gln	HN	4.53	0.07	-1.62	0.13
127	Gln	C	128	Cys	HN	4.76	0.10	-0.38	0.22
128	Cys	C	129	Ala	HN	4.67	0.07	4.73	0.12
129	Ala	C	130	Met	HN	4.87	0.15	-0.16	0.35
130	Met	C	131	Arg	HN	4.23	0.11	0.80	0.24
132	Pro	C	133	Asn	HN	4.44	0.13	-0.50	0.30
133	Asn	C	134	Phe	HN	4.52	0.12	0.68	0.29
134	Phe	C	135	Thr	HN	4.52	0.20	0.30	0.52
135	Thr	C	136	Ile	HN	4.63	0.15	-3.50	0.36
136	Ile	C	137	Lys	HN	4.39	0.09	-3.42	0.18
137	Lys	C	138	Gly	HN	4.60	0.08	-0.72	0.17
139	Ser	C	140	Phe	HN	4.17	0.18	-1.48	0.48
140	Phe	C	141	Leu	HN	4.63	0.55	-7.21	9.73
141	Leu	C	142	Asn	HN	4.56	0.17	0.26	0.35
142	Asn	C	143	Gly	HN	4.33	0.22	-0.09	0.61
143	Gly	C	144	Ser	HN	4.30	0.13	-2.81	0.38
144	Ser	C	145	Cys	HN	3.90	0.36	3.22	0.67
145	Cys	C	146	Gly	HN	5.12	0.25	-2.48	0.61
146	Gly	C	147	Ser	HN	3.91	0.31	2.04	0.82
147	Ser	C	148	Val	HN	4.64	0.13	2.89	0.25
148	Val	C	149	Gly	HN	4.09	0.18	-1.52	0.39
149	Gly	C	150	Phe	HN	3.88	0.14	-2.27	0.31
150	Phe	C	151	Asn	HN	4.08	0.10	1.98	0.24
151	Asn	C	152	Ile	HN	4.72	0.07	-2.44	0.21
152	Ile	C	153	Asp	HN	4.66	0.06	0.18	0.13
154	Tyr	C	155	Asp	HN	4.92	0.14	0.61	0.43
155	Asp	C	156	Cys	HN	4.63	0.07	-3.92	0.16
156	Cys	C	157	Val	HN	4.51	0.08	-3.83	0.19
157	Val	C	158	Ser	HN	4.82	0.11	-1.01	0.26
158	Ser	C	159	Phe	HN	3.86	0.11	-2.81	0.25
159	Phe	C	160	Cys	HN	4.44	0.33	0.49	0.76
160	Cys	C	161	Tyr	HN	5.31	0.27	-0.79	0.63
161	Tyr	C	162	Met	HN	4.35	0.12	3.12	1.68
162	Met	C	163	His	HN	4.67	0.14	-1.86	0.31
163	His	C	164	His	HN	4.56	0.38	3.33	1.12
164	His	C	165	Met	HN	5.55	0.25	-3.05	0.84

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
165	Met	C	166	Glu	HN	4.70	0.10	-1.84	0.20
166	Glu	C	167	Leu	HN	4.95	0.23	-4.29	0.51
171	Val	C	172	His	HN	3.57	0.17	1.72	0.38
173	Ala	C	174	Gly	HN	4.53	0.20	-1.86	0.48
174	Gly	C	175	Thr	HN	4.11	0.14	2.83	0.32
175	Thr	C	176	Asp	HN	4.06	0.12	-1.10	0.28
176	Asp	C	177	Leu	HN	5.93	0.17	2.68	0.39
177	Leu	C	178	Glu	HN	4.57	0.21	-0.13	0.56
178	Glu	C	179	Gly	HN	5.13	0.22	-1.33	0.52
180	Asn	C	181	Phe	HN	4.56	0.09	2.57	0.22
181	Phe	C	182	Tyr	HN	4.13	0.09	-1.35	0.21
182	Tyr	C	183	Gly	HN	5.20	0.06	-1.79	0.14
184	Pro	C	185	Phe	HN	3.59	0.07	2.34	0.13
185	Phe	C	186	Val	HN	4.51	0.16	0.51	0.36
186	Val	C	187	Asp	HN	5.89	0.10	0.62	0.20
187	Asp	C	188	Arg	HN	3.91	0.16	1.85	0.33
188	Arg	C	189	Gln	HN	4.62	0.09	-2.95	0.19
189	Gln	C	190	Thr	HN	4.37	0.07	-2.81	0.17
190	Thr	C	191	Ala	HN	4.73	0.41	3.42	2.49
192	Gln	C	193	Ala	HN	4.83	0.13	1.65	0.38
193	Ala	C	194	Ala	HN	4.68	0.07	0.42	0.17
194	Ala	C	195	Gly	HN	5.13	0.10	1.29	0.27
195	Gly	C	196	Thr	HN	4.49	0.11	0.02	0.26
196	Thr	C	197	Asp	HN	5.06	0.12	1.94	0.27
197	Asp	C	198	Thr	HN	4.35	0.07	0.29	0.17
198	Thr	C	199	Thr	HN	4.06	0.10	-6.69	0.31
199	Thr	C	200	Ile	HN	4.55	0.11	5.04	0.23
200	Ile	C	201	Thr	HN	3.95	0.26	2.27	0.66
201	Thr	C	202	Val	HN	4.87	0.18	0.92	0.45
202	Val	C	203	Asn	HN	4.30	0.15	-6.51	0.50
203	Asn	C	204	Val	HN	4.57	0.28	1.04	0.50
213	Ile	C	214	Asn	HN	4.49	0.13	-4.97	0.25
214	Asn	C	215	Gly	HN	4.58	0.11	1.32	0.17
215	Gly	C	216	Asp	HN	4.76	0.08	-0.90	0.14
216	Asp	C	217	Arg	HN	3.81	0.10	2.26	0.16
217	Arg	C	218	Trp	HN	4.05	0.16	-2.51	0.40
219	Phe	C	220	Leu	HN	4.65	0.07	-3.74	0.11
220	Leu	C	221	Asn	HN	4.53	0.06	-1.71	0.11
221	Asn	C	222	Arg	HN	4.63	0.17	5.93	0.46
222	Arg	C	223	Phe	HN	4.47	0.04	-2.37	0.06
223	Phe	C	224	Thr	HN	4.47	0.03	1.36	0.07

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
224	Thr	C	225	Thr	HN	4.19	0.04	4.06	0.07
225	Thr	C	226	Thr	HN	3.61	0.06	-3.18	0.12
226	Thr	C	227	Leu	HN	5.12	0.07	-0.60	0.13
227	Leu	C	228	Asn	HN	4.73	0.09	0.55	0.15
228	Asn	C	229	Asp	HN	5.16	0.12	-2.26	0.18
229	Asp	C	230	Phe	HN	5.13	0.11	-1.56	0.19
230	Phe	C	231	Asn	HN	4.56	0.10	-1.93	0.17
231	Asn	C	232	Leu	HN	4.72	0.12	3.62	0.20
232	Leu	C	233	Val	HN	4.52	0.16	-3.57	0.49
233	Val	C	234	Ala	HN	4.50	0.16	-2.34	0.36
234	Ala	C	235	Met	HN	4.68	0.15	-0.14	0.23
235	Met	C	236	Lys	HN	4.83	0.18	1.78	0.29
236	Lys	C	237	Tyr	HN	4.78	0.20	-3.25	0.34
237	Tyr	C	238	Asn	HN	4.83	0.18	-1.48	0.31
238	Asn	C	239	Tyr	HN	4.63	0.14	1.06	0.36
239	Tyr	C	240	Glu	HN	4.58	0.14	5.45	0.38
242	Leu	C	243	Thr	HN	4.29	0.10	6.11	0.20
243	Thr	C	244	Gln	HN	4.82	0.09	-1.22	0.27
244	Gln	C	245	Asp	HN	4.74	0.09	-2.43	0.17
245	Asp	C	246	His	HN	4.96	0.12	-0.82	0.20
246	His	C	247	Val	HN	4.23	0.11	1.60	0.18
247	Val	C	248	Asp	HN	4.41	0.11	-1.01	0.18
249	Ile	C	250	Leu	HN	4.59	0.21	3.54	0.53
250	Leu	C	251	Gly	HN	4.76	0.12	-1.43	0.23
253	Leu	C	254	Ser	HN	4.55	0.17	-0.97	0.46
254	Ser	C	255	Ala	HN	4.71	0.13	3.56	0.24
255	Ala	C	256	Gln	HN	4.75	0.16	-2.15	0.35
256	Gln	C	257	Thr	HN	4.05	0.37	-2.63	0.72
257	Thr	C	258	Gly	HN	5.00	0.20	-0.79	0.36
258	Gly	C	259	Ile	HN	4.40	0.13	0.87	0.22
259	Ile	C	260	Ala	HN	4.32	0.07	5.80	0.13
260	Ala	C	261	Val	HN	4.58	0.07	1.24	0.12
261	Val	C	262	Leu	HN	4.70	0.11	-1.25	0.19
262	Leu	C	263	Asp	HN	4.47	0.14	-3.00	0.22
263	Asp	C	264	Met	HN	4.63	0.25	4.17	0.38
264	Met	C	265	Cys	HN	4.01	0.23	-1.91	0.41
265	Cys	C	266	Ala	HN	4.69	0.13	-3.42	0.27
266	Ala	C	267	Ser	HN	4.42	0.14	-0.18	0.29
268	Leu	C	269	Lys	HN	4.59	0.14	-2.07	0.23
269	Lys	C	270	Glu	HN	4.80	0.13	-4.68	0.26
270	Glu	C	271	Leu	HN	4.59	0.21	3.13	0.28

Residue Number	Residue Name	Atom	Residue Number	Residue Name	Atom	J [Hz]	Error [Hz]	D [Hz]	Error [Hz]
272	Leu	C	273	Gln	HN	4.45	0.12	-1.10	0.23
273	Gln	C	274	Asn	HN	4.39	0.14	-2.88	0.23
274	Asn	C	275	Gly	HN	5.14	0.11	2.75	0.16
275	Gly	C	276	Met	HN	4.42	0.06	0.57	0.11
276	Met	C	277	Asn	HN	5.16	0.10	-2.54	0.25
277	Asn	C	278	Gly	HN	5.23	0.08	1.86	0.16
278	Gly	C	279	Arg	HN	4.24	0.04	-1.61	0.07
279	Arg	C	280	Thr	HN	4.76	0.05	-3.03	0.10
280	Thr	C	281	Ile	HN	4.59	0.09	1.64	0.21
281	Ile	C	282	Leu	HN	4.69	0.16	-4.43	0.40
282	Leu	C	283	Gly	HN	4.80	0.12	3.64	0.18
283	Gly	C	284	Ser	HN	4.28	0.13	-1.19	0.35
284	Ser	C	285	Ala	HN	4.82	0.08	-3.10	0.24
285	Ala	C	286	Leu	HN	4.45	0.08	5.45	0.16
286	Leu	C	287	Leu	HN	4.51	0.15	1.17	0.28
289	Asp	C	290	Glu	HN	4.03	0.17	-0.82	0.52
290	Glu	C	291	Phe	HN	4.97	0.15	2.31	0.41
291	Phe	C	292	Thr	HN	4.46	0.08	6.47	0.18
293	Pro	C	294	Phe	HN	4.50	0.10	-0.43	0.18
294	Phe	C	295	Asp	HN	4.97	0.12	-4.15	0.26
295	Asp	C	296	Val	HN	4.44	0.11	3.52	0.20
297	Val	C	298	Arg	HN	4.95	0.09	-2.31	0.17
298	Arg	C	299	Gln	HN	4.64	0.08	-0.10	0.12
299	Gln	C	300	Cys	HN	4.33	0.08	0.45	0.15
300	Cys	C	301	Ser	HN	4.43	0.05	2.38	0.11
301	Ser	C	302	Gly	HN	5.18	0.04	-1.72	0.12
302	Gly	C	303	Val	HN	4.32	0.02	0.27	0.03
303	Val	C	304	Thr	HN	4.56	0.02	0.03	0.04
304	Thr	C	305	Phe	HN	4.53	0.02	0.08	0.04
305	Phe	C	306	Gln	HN	4.45	0.01	0.17	0.32

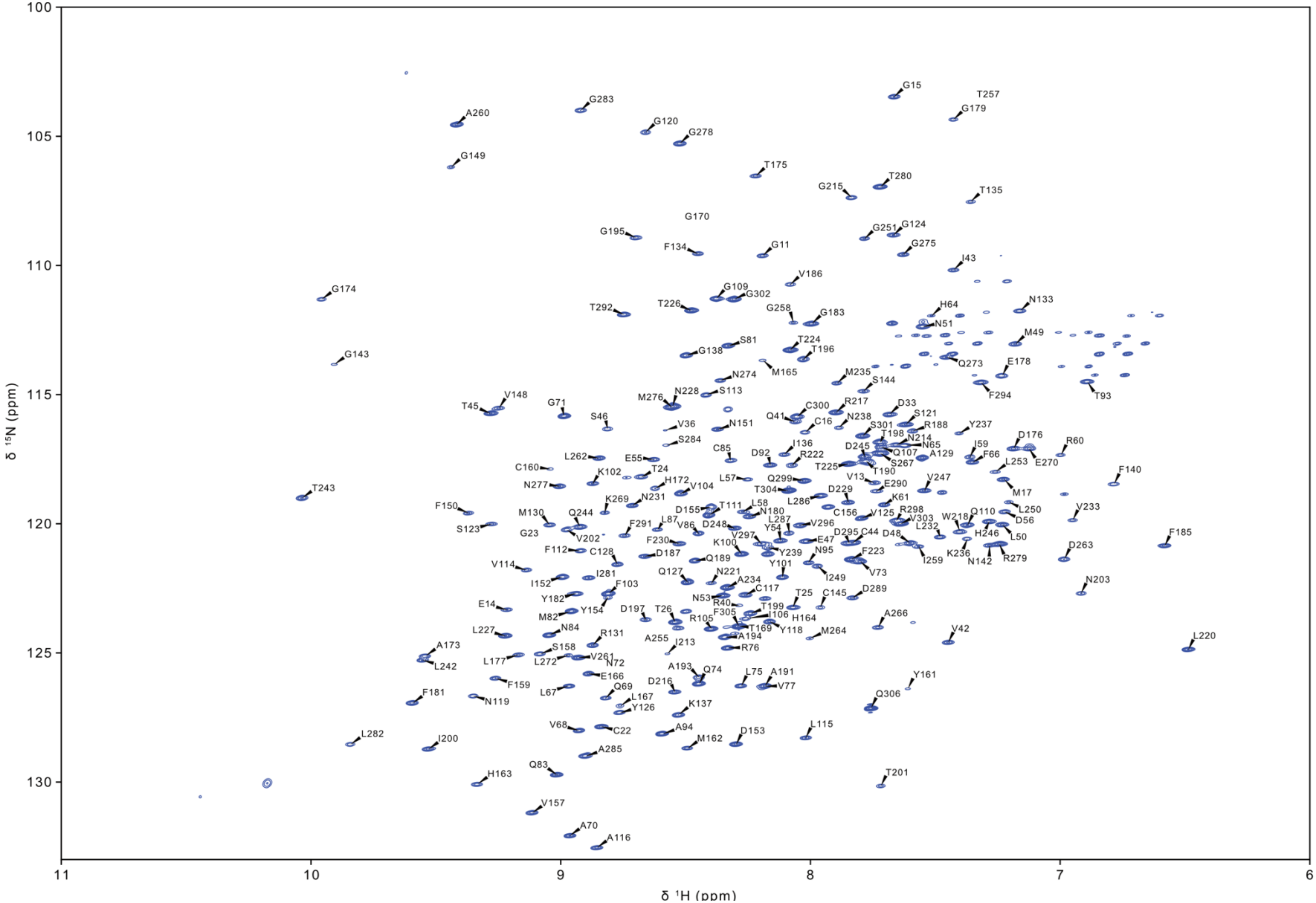
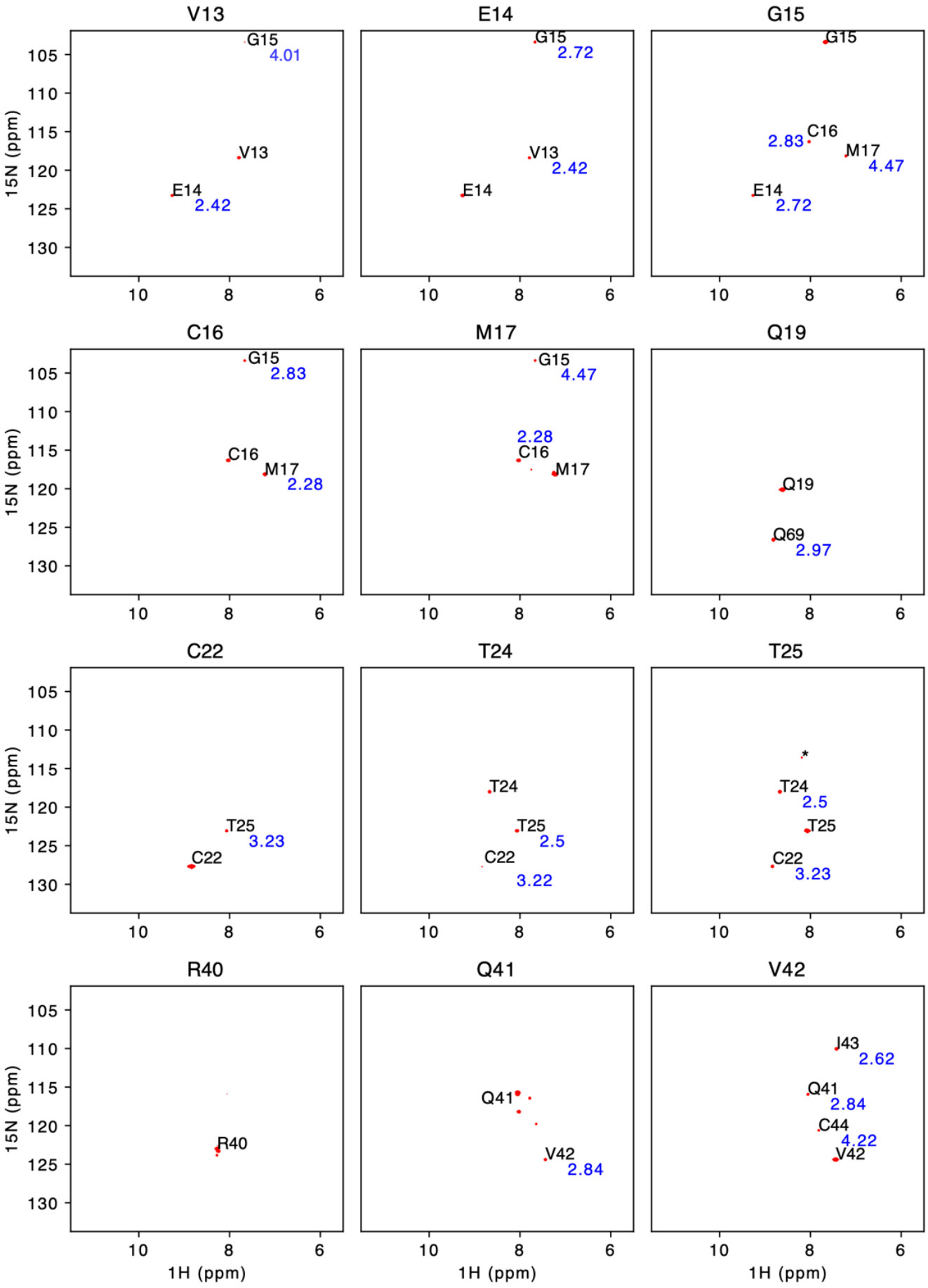
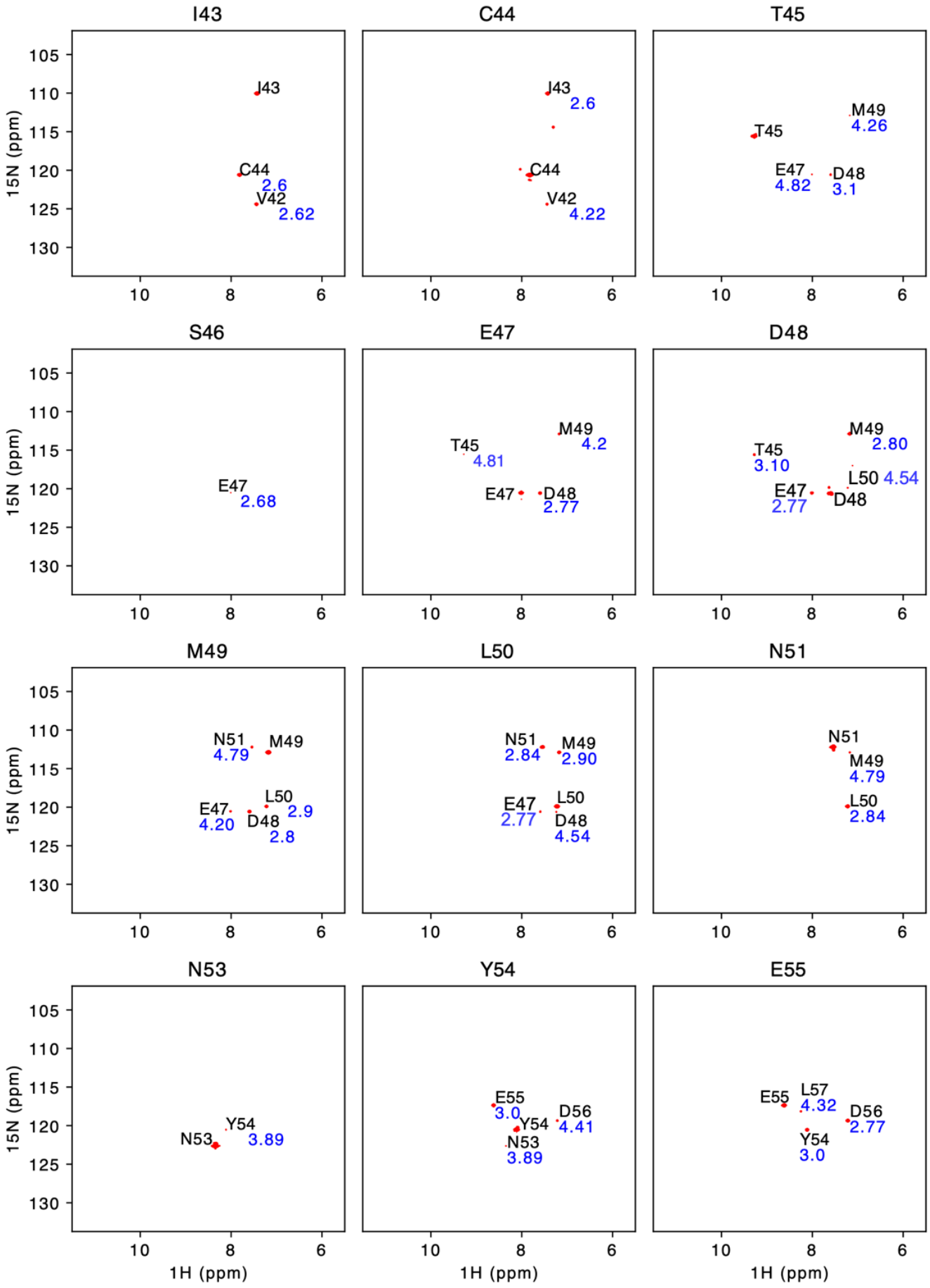
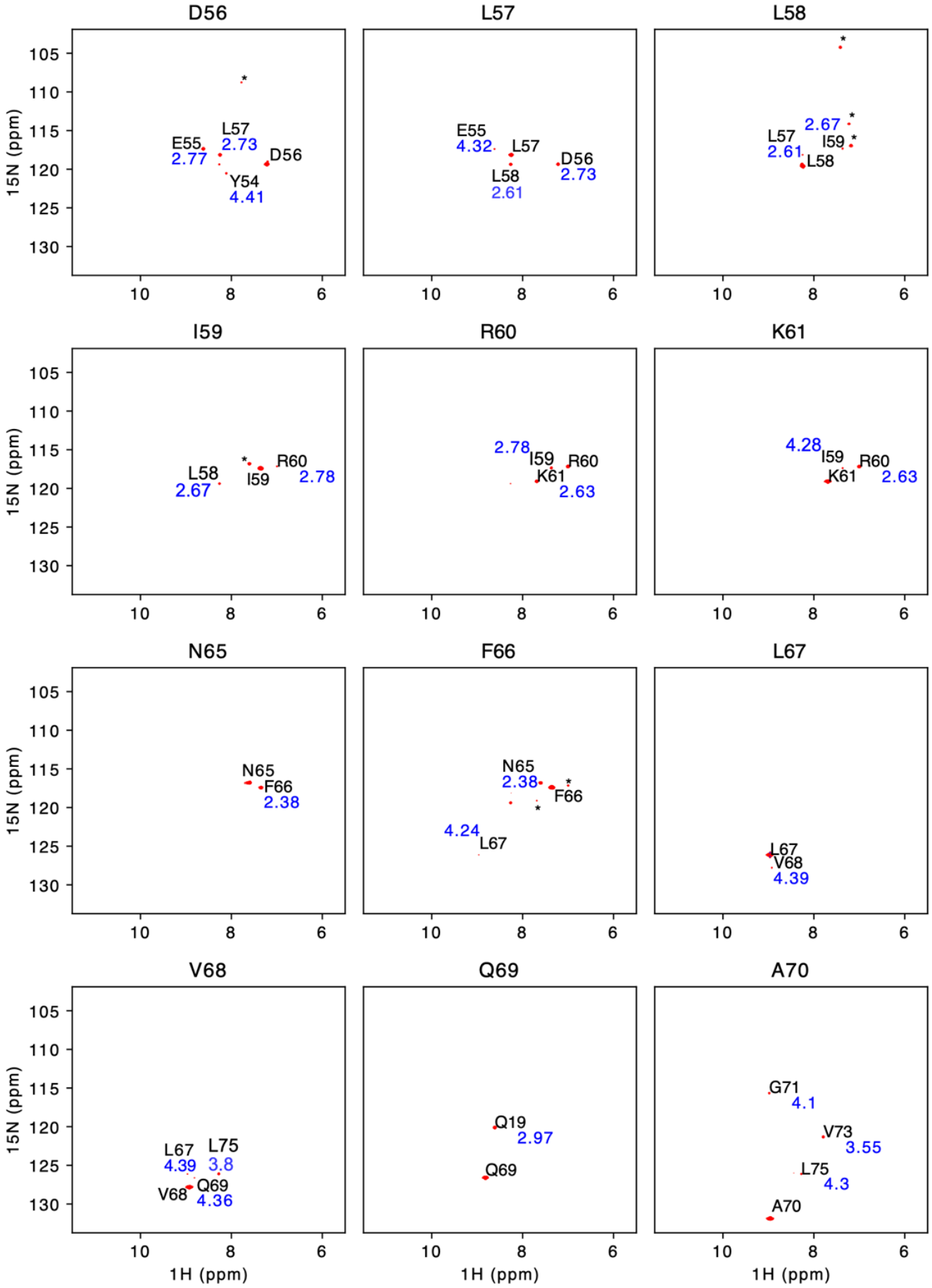


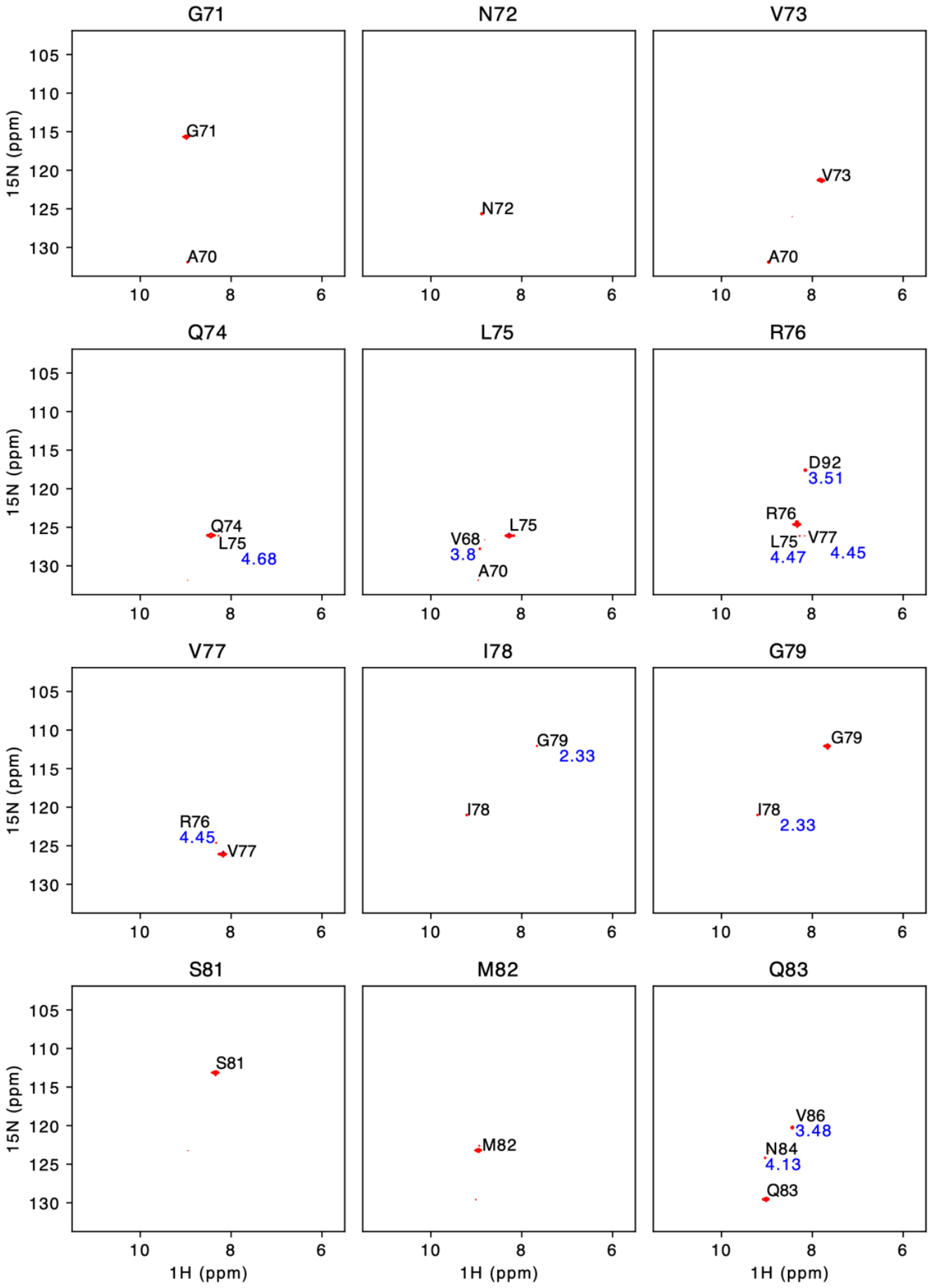
Figure S14. ^{1}H-^{15}N TROSY-HSQC spectrum of MPro$^{10\text{-}306,H41Q}$. The assignment numbering corresponds to the full residue sequence of MPro.

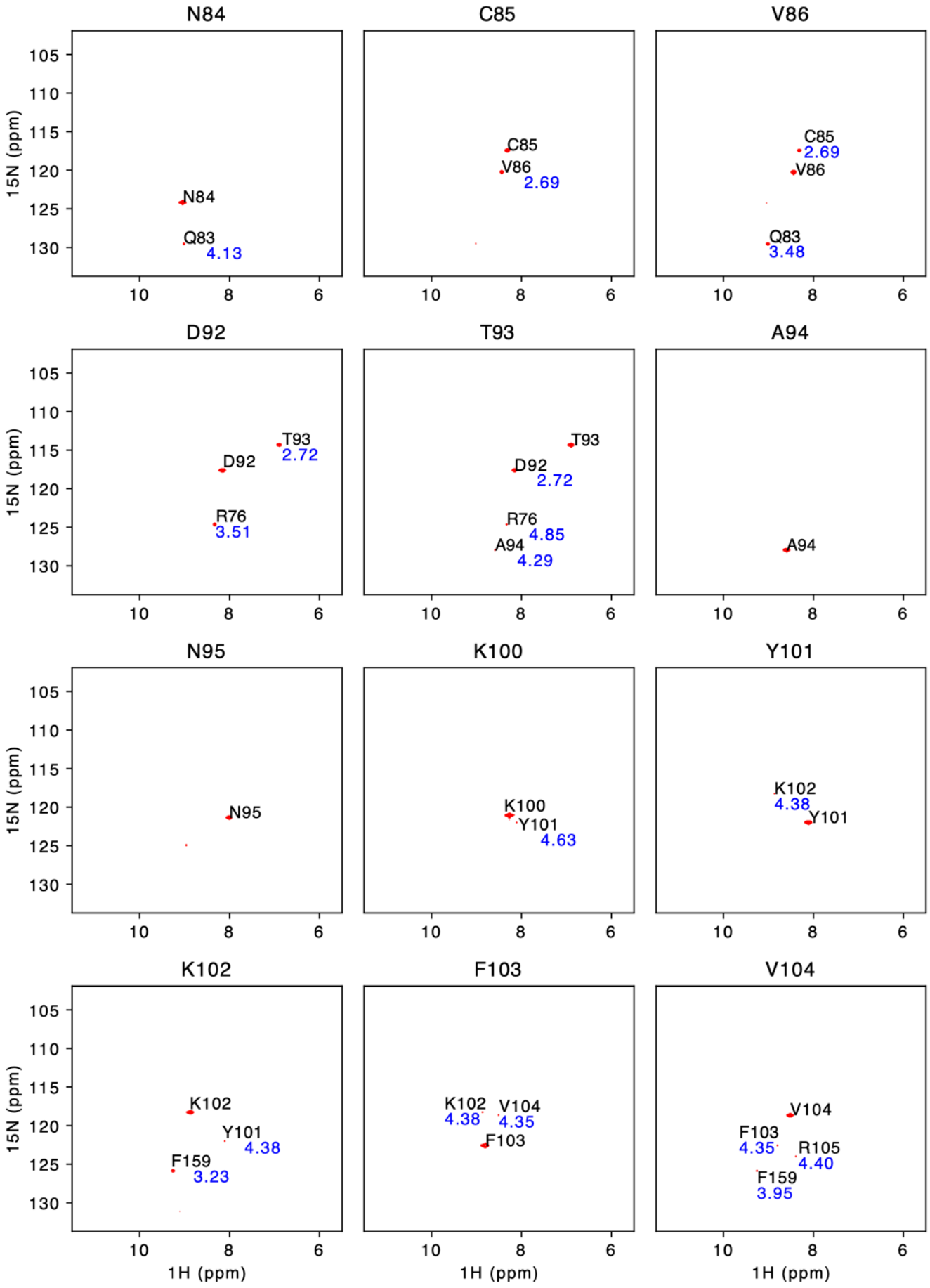
Figure S15. (overleaf) (F_1,F_2) cross-sections through the 4D ^{1}H-^{15}N TROSY-NOESY-TROSY spectrum taken orthogonal to the F_3/F_4 frequencies of the amides marked in each panel. Peak assignments are provided in black and the amide ^{1}H internuclear distance (Å) based on the 7K3T crystal structure are shown in blue. Signals marked with an asterisk are correlations from amides that are (partially) overlapped in the F_3/F_4 dimensions with the selected amide.

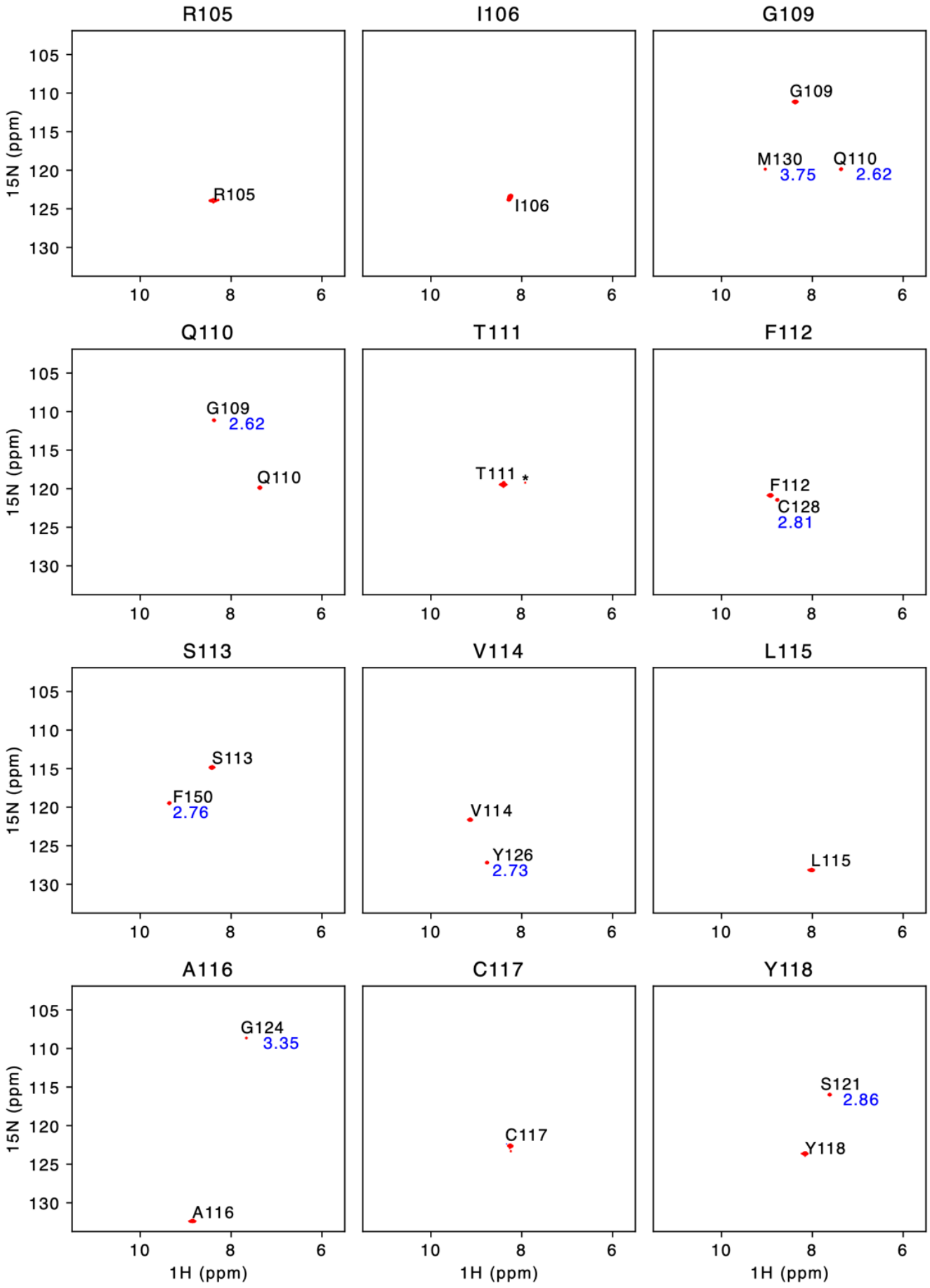

V13
G15
4.01
V13
E14
2.42
E14
G15
2.72
V13
2.42
E14
G15
C16
2.83
M17
4.47
E14
2.72
C16
G15
2.83
C16
M17
2.28
M17
G15
4.47
2.28
C16
M17
Q19
Q19
Q69
2.97
C22
T25
3.23
C22
T24
T24
T25
2.5
C22
3.22
T25
*
T24
2.5
T25
C22
3.23
R40
R40
Q41
Q41
V42
2.84
I43
2.62
Q41
2.84
C44
4.22
V42
15N (ppm)
1H (ppm)
105
110
115
120
125
130
10
8
6

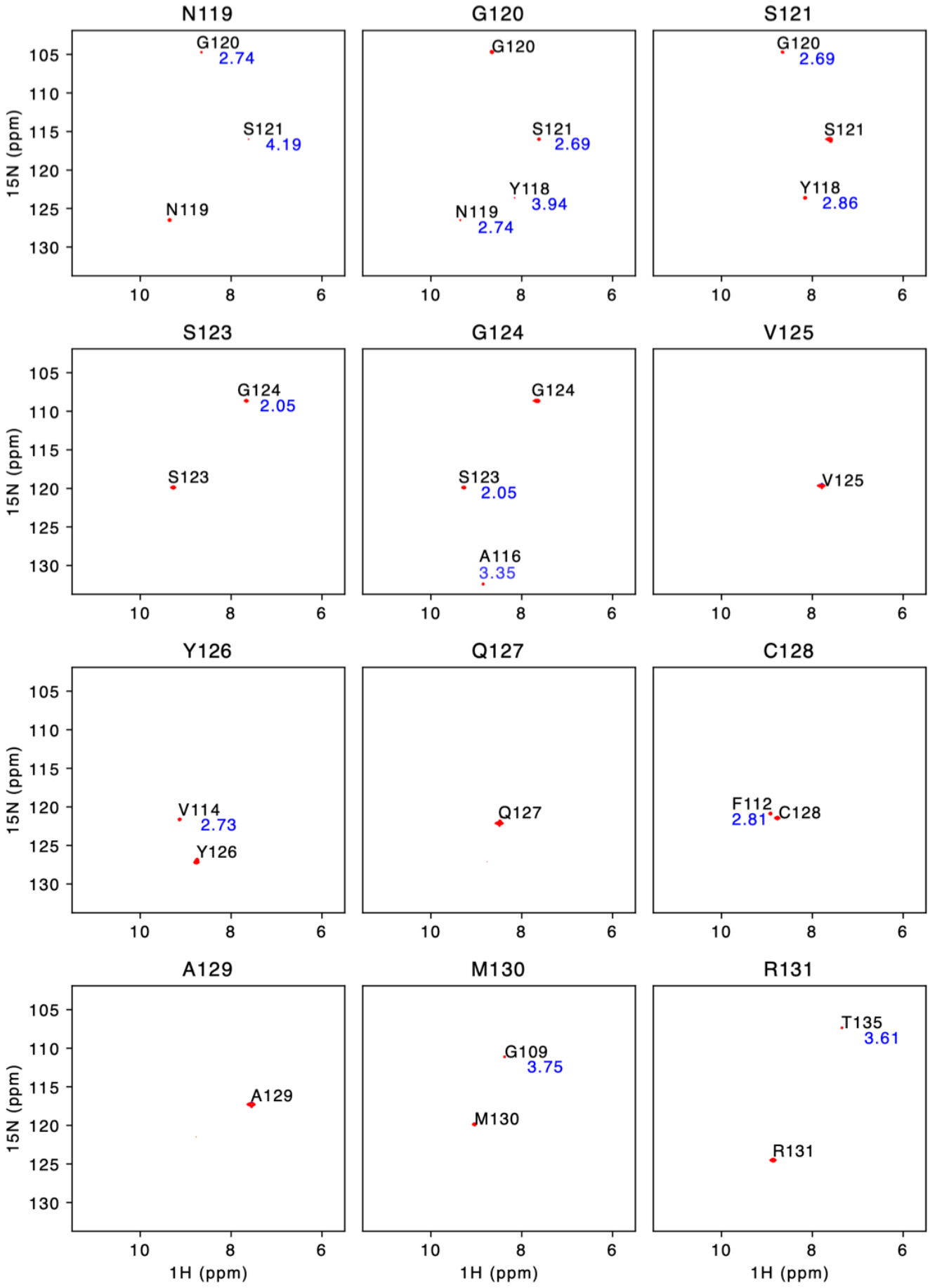

I43
C44
T45
S46
E47
D48
M49
L50
N51
N53
Y54
E55
15N (ppm)
1H (ppm)
105
110
115
120
125
130
10
8
6
I43
C44
2.6
V42
2.62
I43
2.6
C44
V42
4.22
M49
4.26
T45
E47
4.82
D48
3.1
E47
2.68
M49
4.2
T45
4.81
E47
D48
2.77
M49
2.80
T45
3.10
L50 4.54
E47
2.77
D48
N51
4.79
M49
L50
E47
4.20
D48
2.9
2.8
N51
2.84
M49
2.90
E47
2.77
L50
D48
4.54
N51
M49
4.79
L50
2.84
N53
Y54
3.89
E55
3.0
D56
4.41
Y54
N53
3.89
E55
L57
4.32
D56
2.77
Y54
3.0

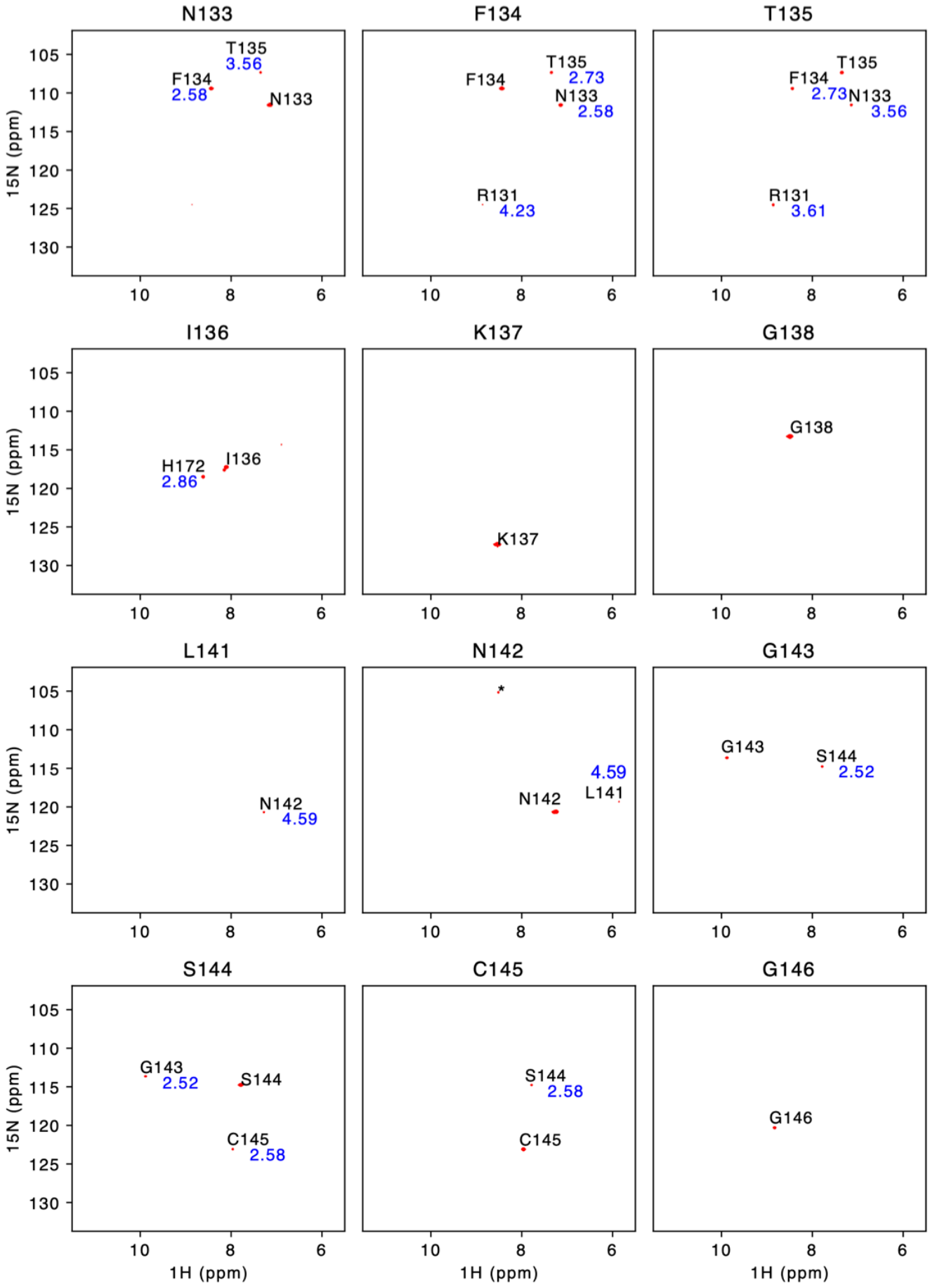

D56
L57
L58
I59
R60
K61
N65
F66
L67
V68
Q69
A70
15N (ppm)
1H (ppm)
E55 2.77
L57 2.73
D56
Y54 4.41
E55 4.32
L57
D56 2.73
L58 2.61
L57 2.61
2.67
I59
L58
L58 2.67
I59
R60 2.78
2.78
I59
R60
K61
2.63
4.28
I59
R60
K61
2.63
N65
F66 2.38
N65 2.38
F66
4.24
L67
L67
V68
4.39
L67 4.39
L75 3.8
V68
Q69 4.36
Q19 2.97
Q69
G71 4.1
V73 3.55
L75 4.3
A70

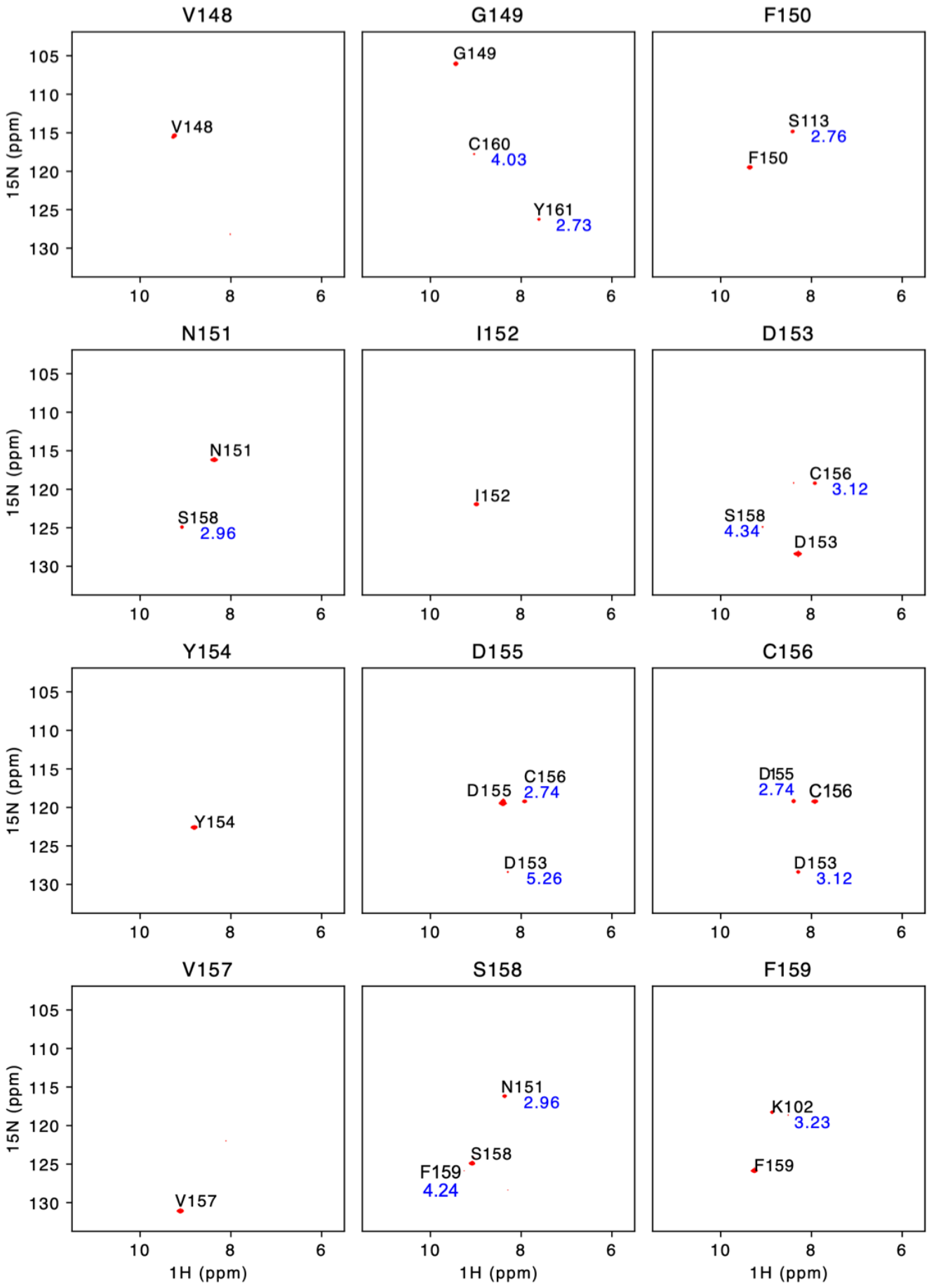

G71
N72
V73
Q74
L75
R76
V77
I78
G79
S81
M82
Q83
15N (ppm)
1H (ppm)
G71
A70
N72
V73
A70
Q74
L75
4.68
V68
3.8
L75
A70
D92
3.51
R76
L75
4.47
V77
4.45
R76
4.45
V77
G79
2.33
I78
G79
I78
2.33
S81
M82
V86
3.48
N84
4.13
Q83

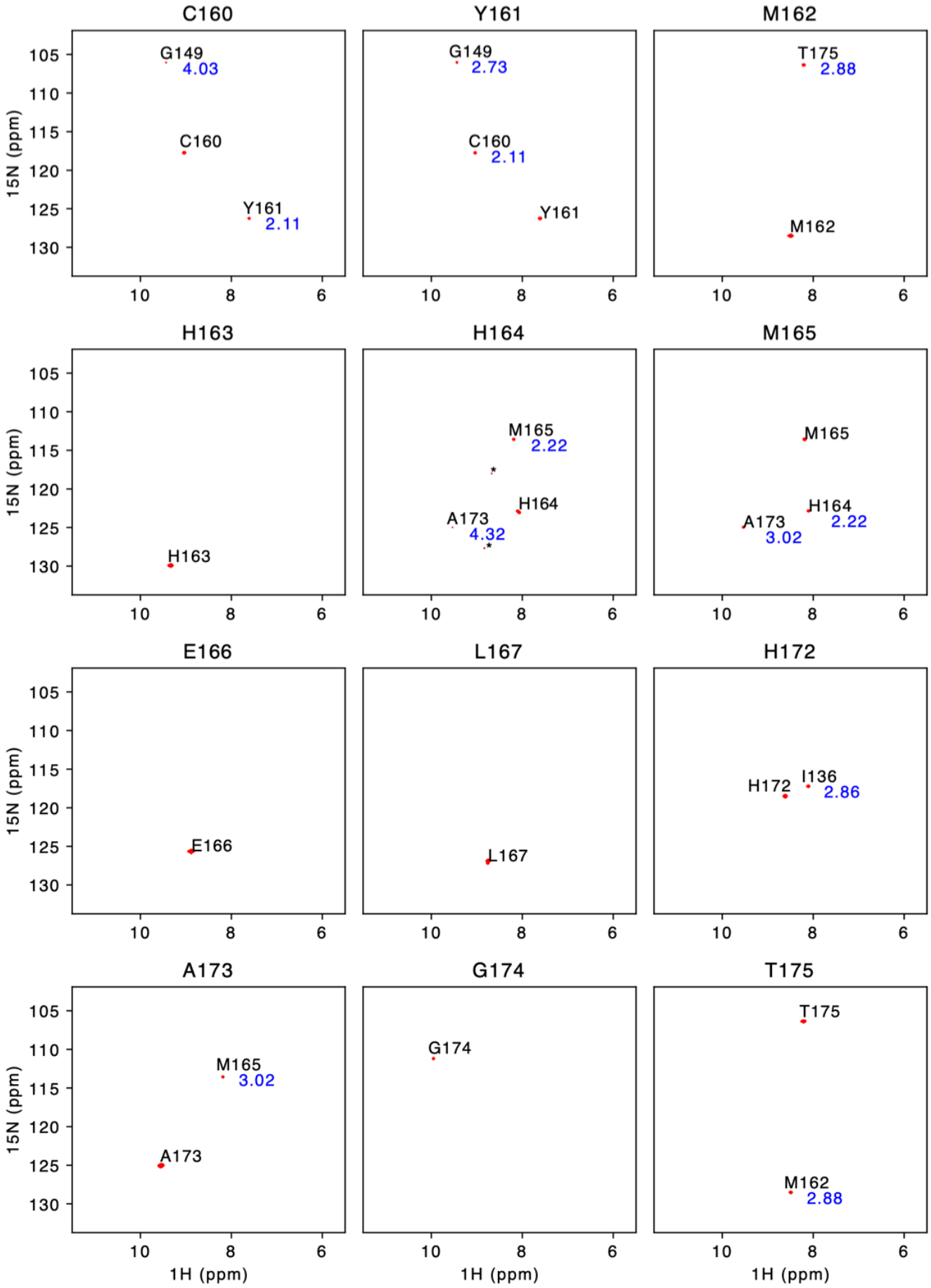

N84
C85
V86
D92
T93
A94
N95
K100
Y101
K102
F103
V104
15N (ppm)
1H (ppm)
N84
Q83
4.13
C85
V86
2.69
C85
2.69
V86
Q83
3.48
D92
T93
2.72
R76
3.51
T93
D92
2.72
R76
4.85
A94
4.29
A94
N95
K100
Y101
4.63
K102
4.38
Y101
K102
Y101
4.38
F159
3.23
K102
4.38
V104
4.35
F103
V104
F103
4.35
R105
4.40
F159
3.95

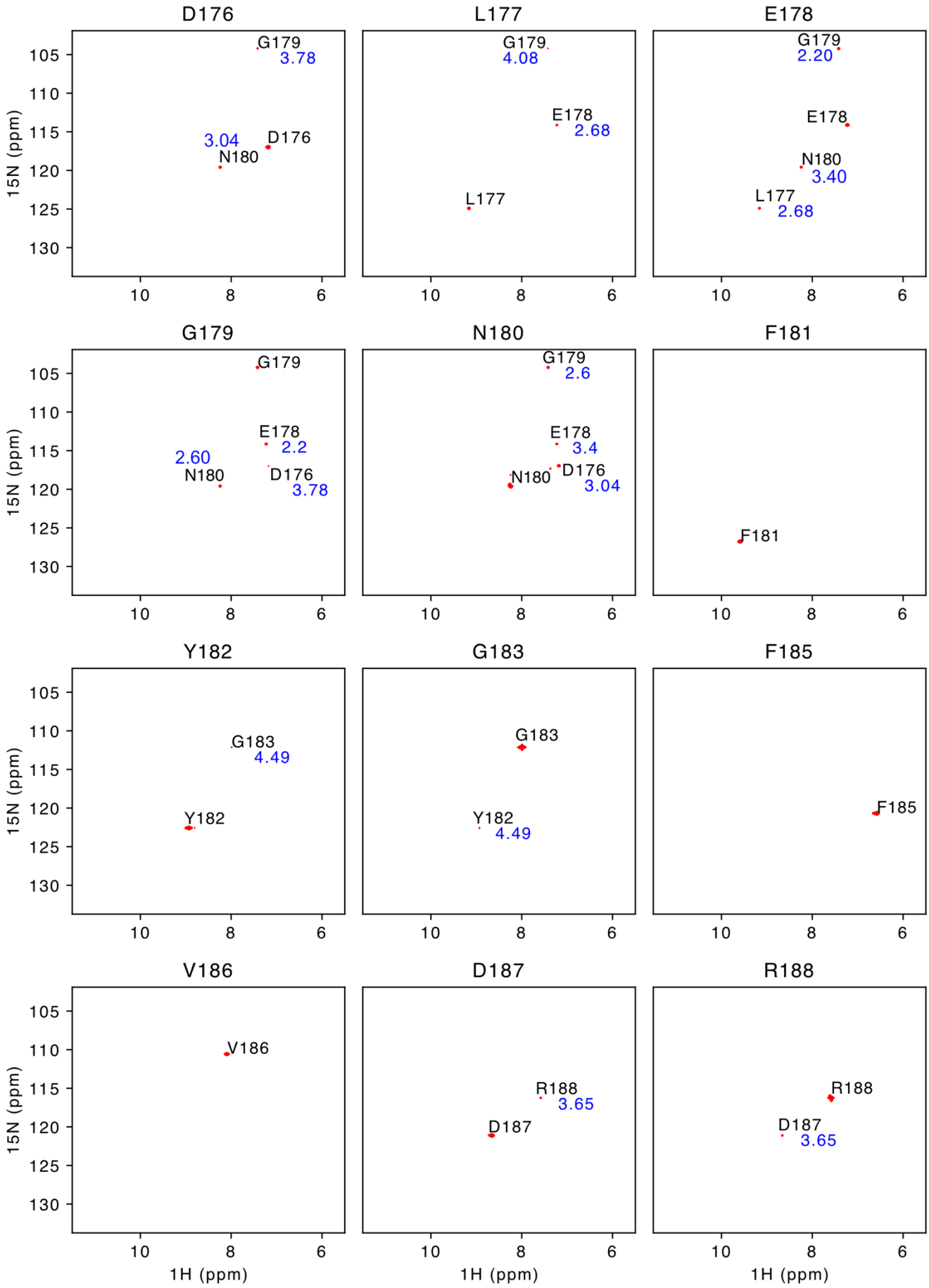

R105
I106
G109
Q110
T111
F112
S113
V114
L115
A116
C117
Y118
15N (ppm)
1H (ppm)
R105
I106
G109
M130
3.75
Q110
2.62
G109
2.62
Q110
T111
F112
C128
2.81
S113
F150
2.76
V114
Y126
2.73
L115
G124
3.35
A116
C117
S121
2.86
Y118


N119
G120 2.74
S121 4.19
N119
G120
G120
S121 2.69
Y118 3.94
N119 2.74
S121
G120 2.69
S121
Y118 2.86
S123
G124 2.05
S123
G124
G124
S123 2.05
A116 3.35
V125
V125
Y126
V114 2.73
Y126
Q127
Q127
C128
F112 2.81
C128
A129
A129
M130
G109 3.75
M130
R131
T135 3.61
R131
15N (ppm)
1H (ppm)


N133
T135
3.56
F134
2.58
N133
F134
T135
2.73
N133
2.58
R131
4.23
T135
F134
2.73
N133
3.56
R131
3.61
I136
H172
2.86
I136
K137
K137
G138
G138
L141
N142
4.59
N142
*
4.59
L141
G143
S144
2.52
S144
G143
2.52
C145
2.58
S144
2.58
C145
G146
G146
15N (ppm)
1H (ppm)


V148
V148
G149
G149
C160
4.03
Y161
2.73
F150
S113
2.76
F150
N151
N151
S158
2.96
I152
I152
D153
C156
3.12
S158
4.34
D153
Y154
Y154
D155
C156
D155
2.74
D153
5.26
C156
D155
2.74
C156
D153
3.12
V157
V157
S158
N151
2.96
S158
F159
4.24
F159
K102
3.23
F159
15N (ppm)
1H (ppm)


C160
G149
4.03
C160
Y161
2.11
Y161
G149
2.73
C160
2.11
Y161
M162
T175
2.88
M162
H163
H163
H164
M165
2.22
*
H164
A173
4.32
*
M165
M165
H164
A173
2.22
3.02
E166
E166
L167
L167
H172
H172
I136
2.86
A173
M165
3.02
A173
G174
G174
T175
T175
M162
2.88
15N (ppm)
1H (ppm)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G179
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D176
N180
L177
G179
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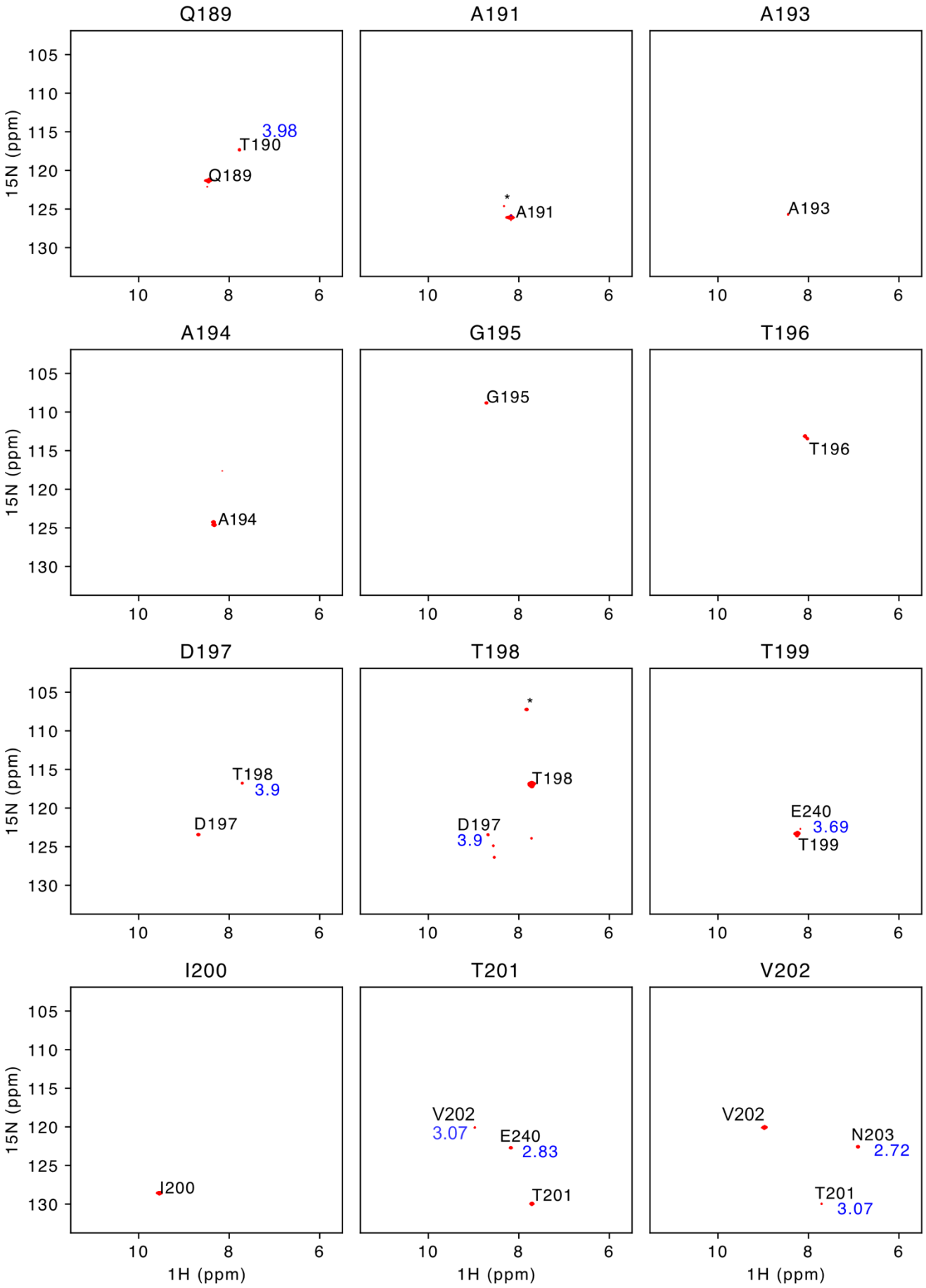
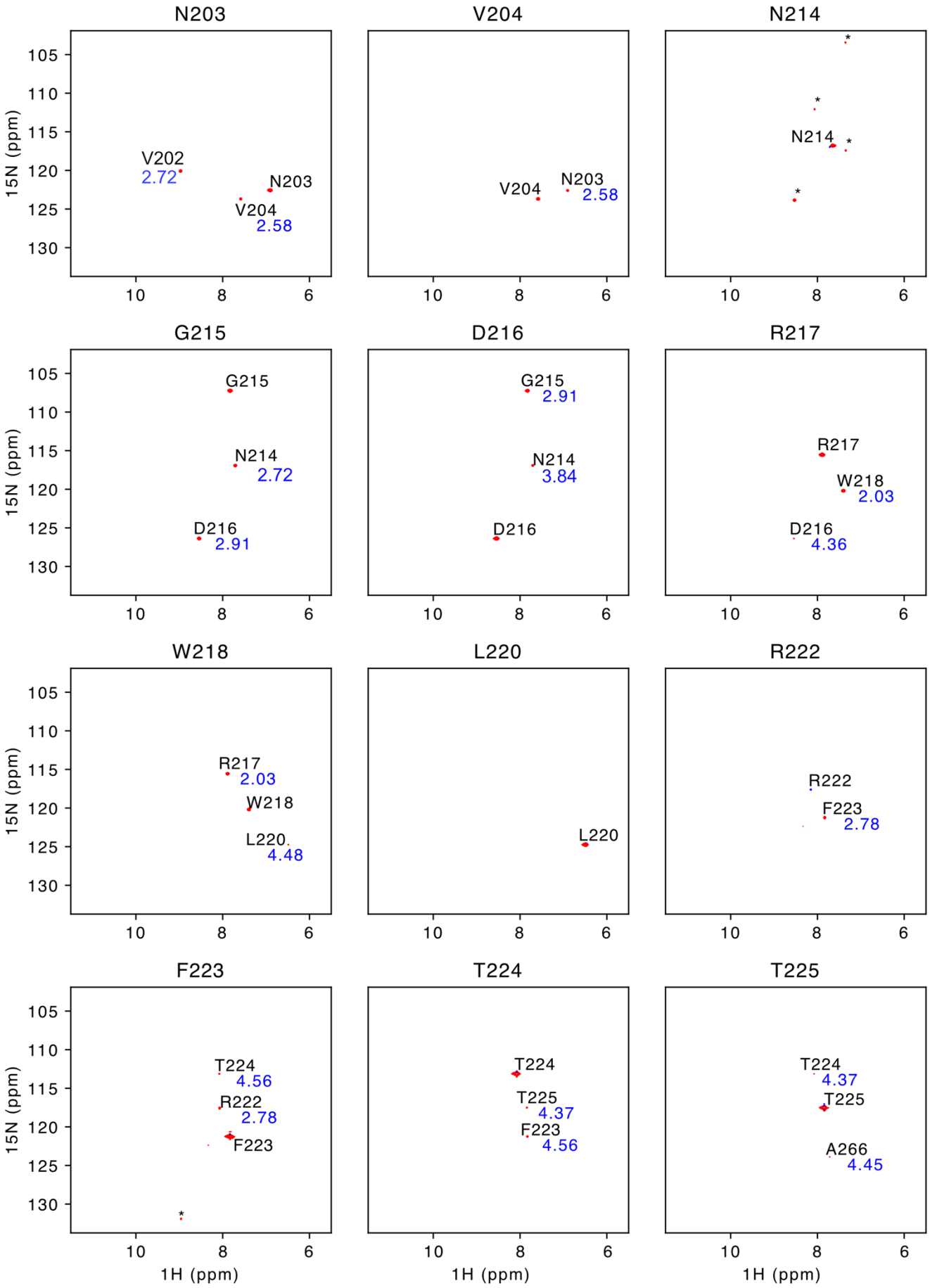
E178
2.68
L177
E178
G179
2.20
E178
N180
3.40
L177
2.68
G179
G179
E178
2.2
2.60
N180
D176
3.78
N180
G179
2.6
E178
3.4
N180
D176
3.04
F181
F181
Y182
G183
4.49
Y182
G183
G183
Y182
4.49
F185
F185
V186
V186
D187
R188
3.65
D187
R188
R188
D187
3.65
15N (ppm)
1H (ppm)
105
110
115
120
125
130
10
8
6


Q189
A191
A193
A194
G195
T196
D197
T198
T199
I200
T201
V202
15N (ppm)
1H (ppm)
105
110
115
120
125
130
10
8
6
3.98
T190
Q189
A191
A193
A194
G195
T196
T198
3.9
D197
T198
D197
3.9
E240
3.69
T199
I200
V202
3.07
E240
2.83
T201
V202
N203
2.72
T201
3.07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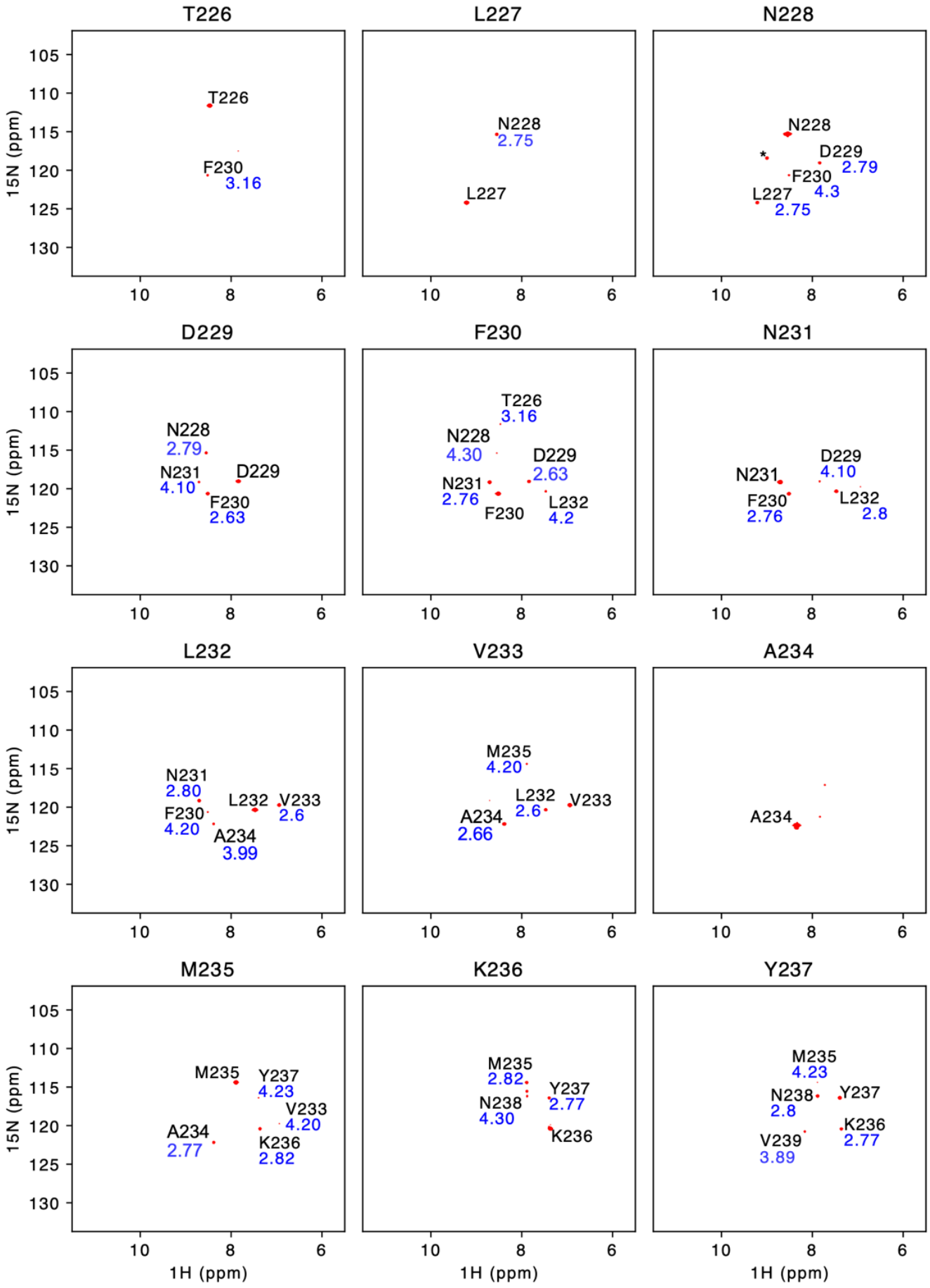
N203
V204
2.58
V204
N203
2.58
N214
N214
G215
G215
N214
2.72
D216
2.91
D216
G215
2.91
N214
3.84
D216
R217
R217
W218
2.03
D216
4.36
W218
R217
2.03
W218
L220
4.48
L220
L220
R222
R222
F223
2.78
F223
T224
4.56
R222
2.78
F223
T224
T224
T225
4.37
F223
4.56
T225
T224
4.37
T225
A266
4.45
15N (ppm)
1H (ppm)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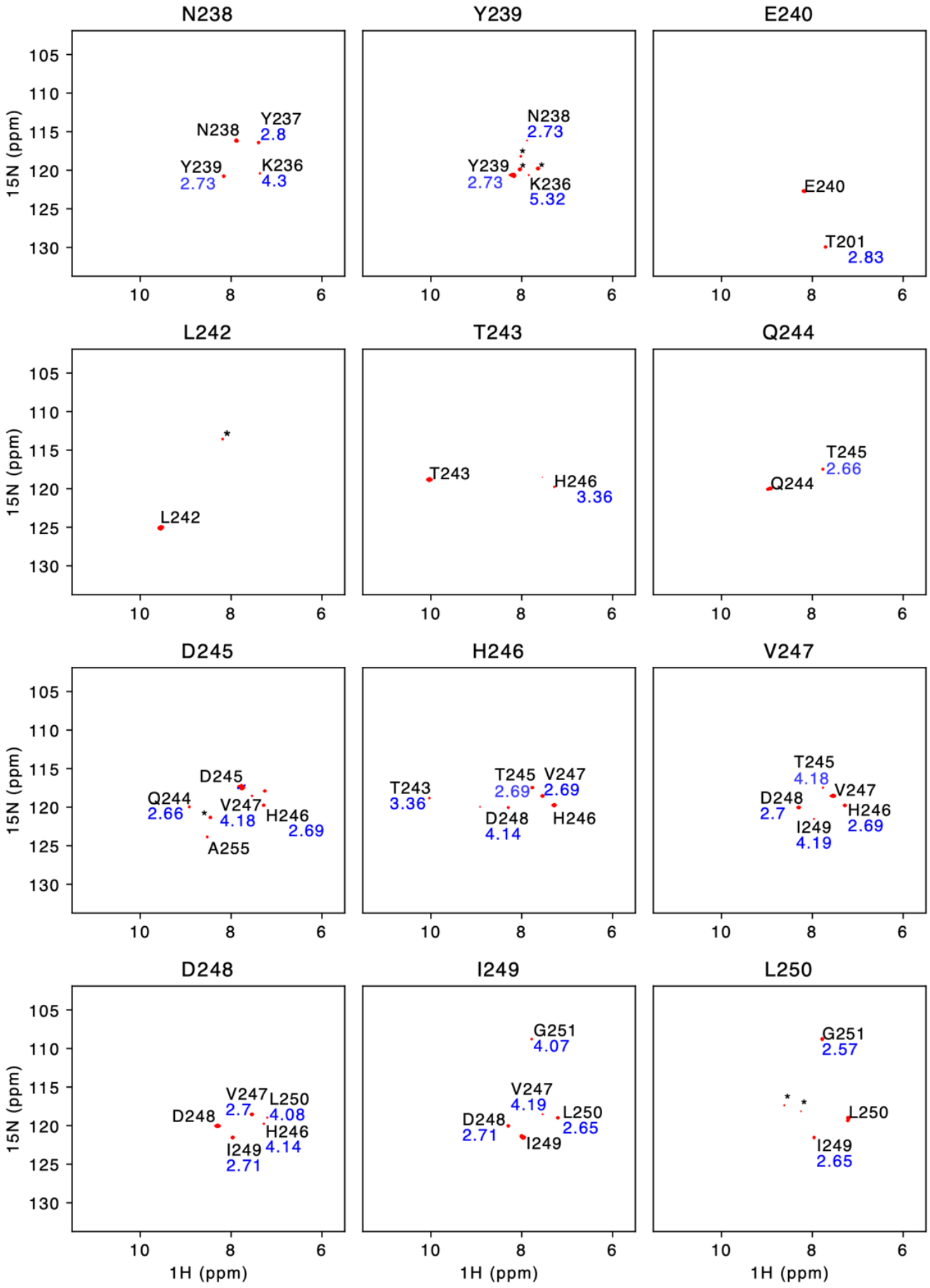
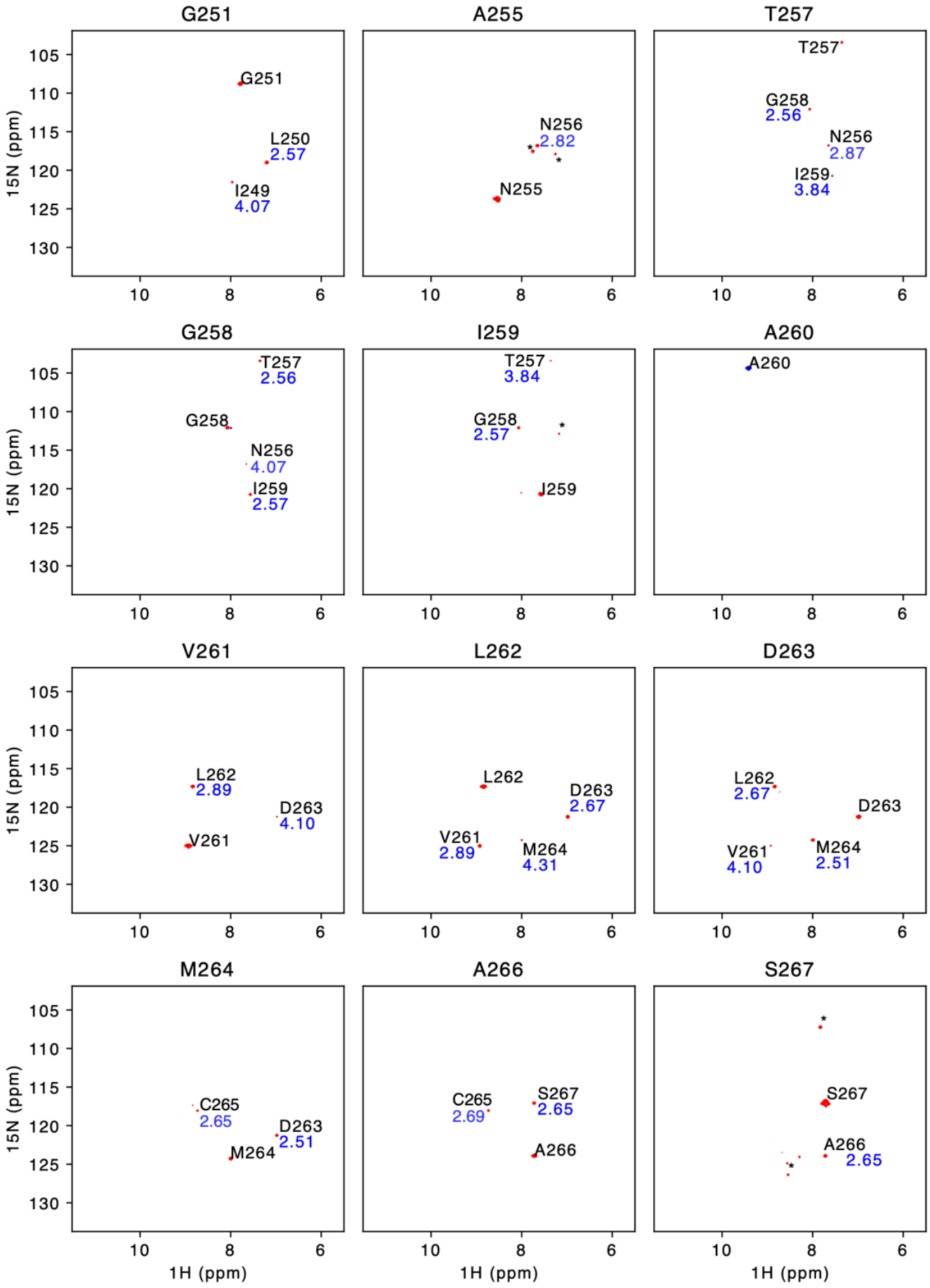
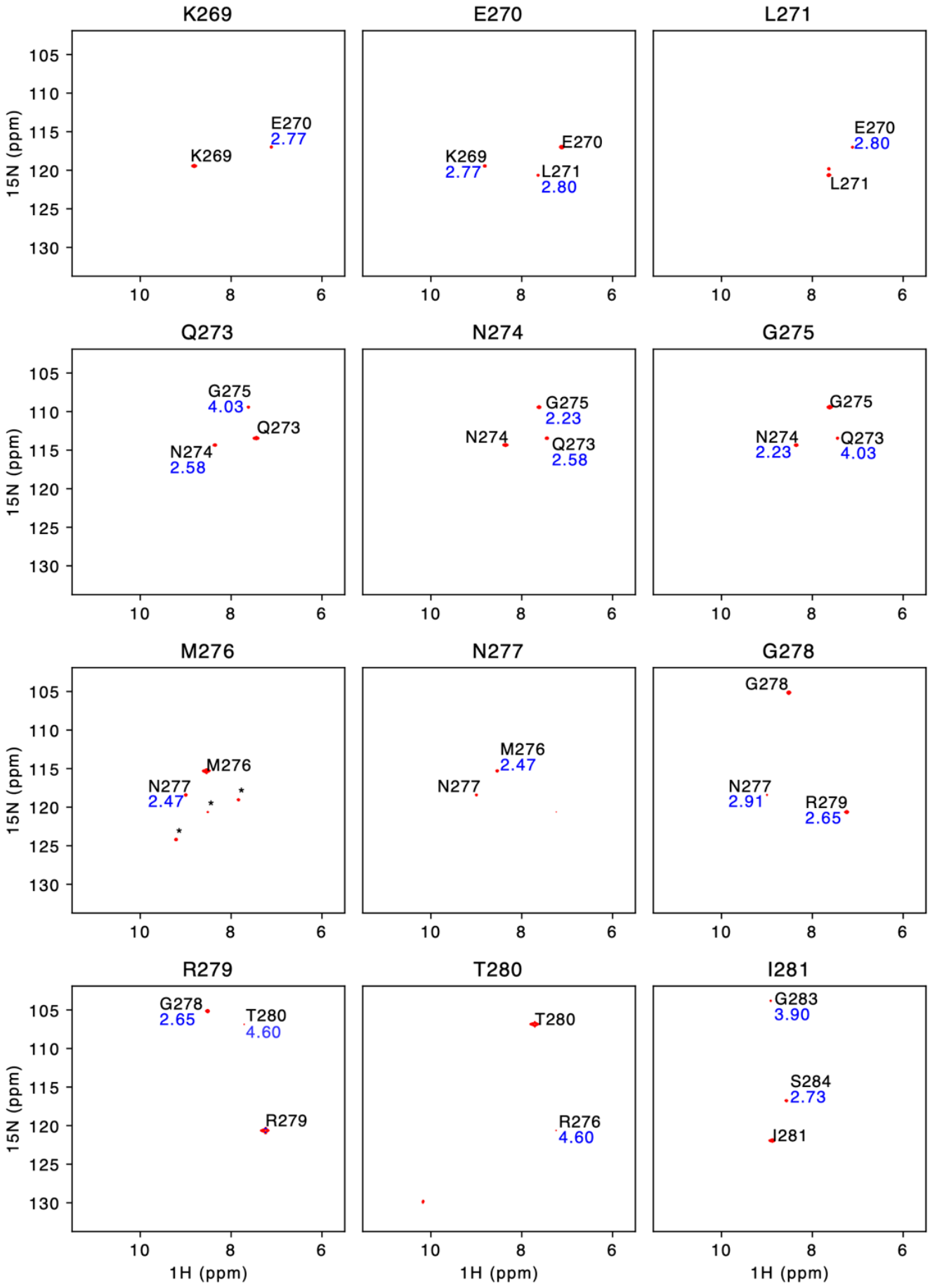
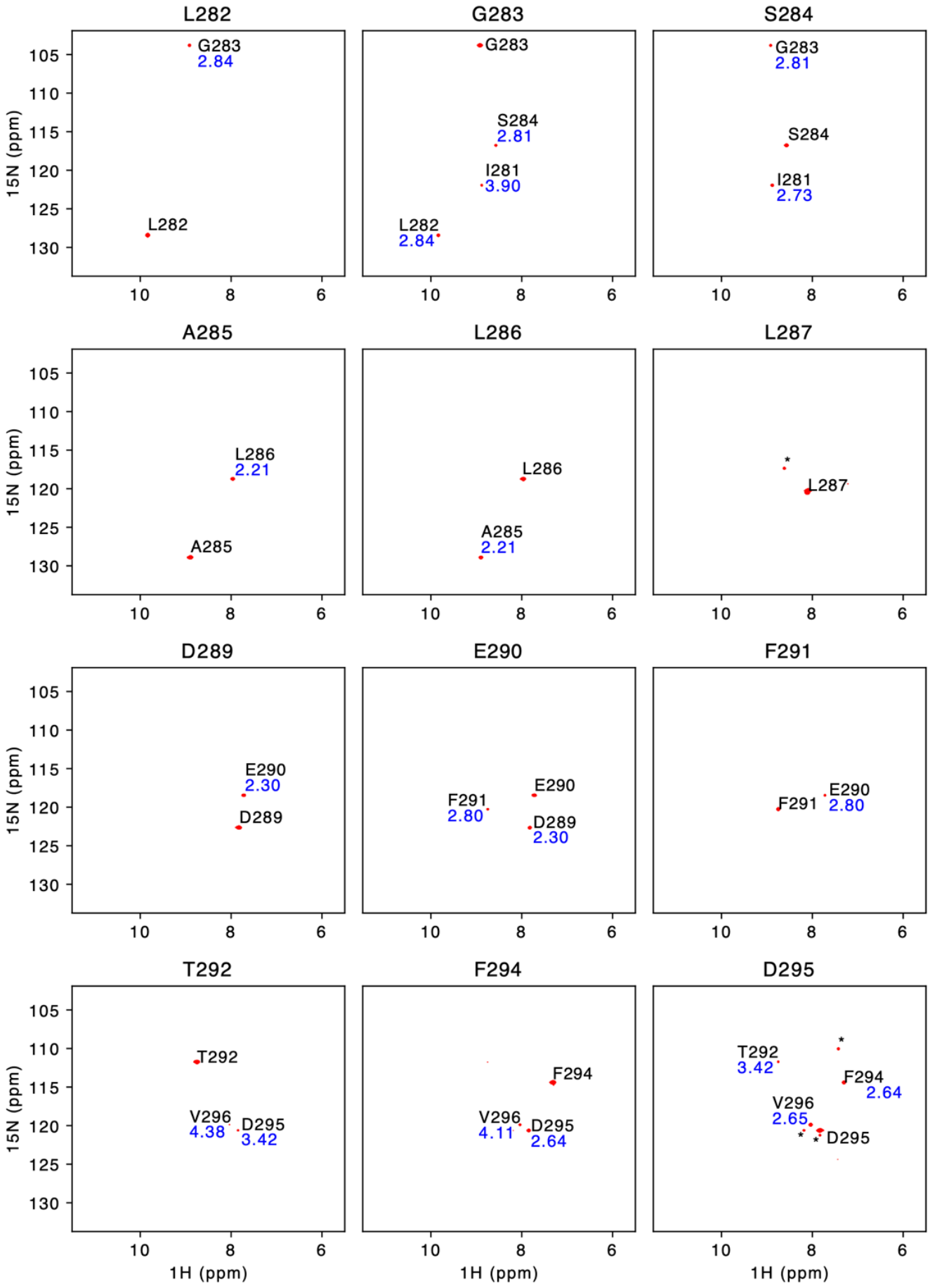
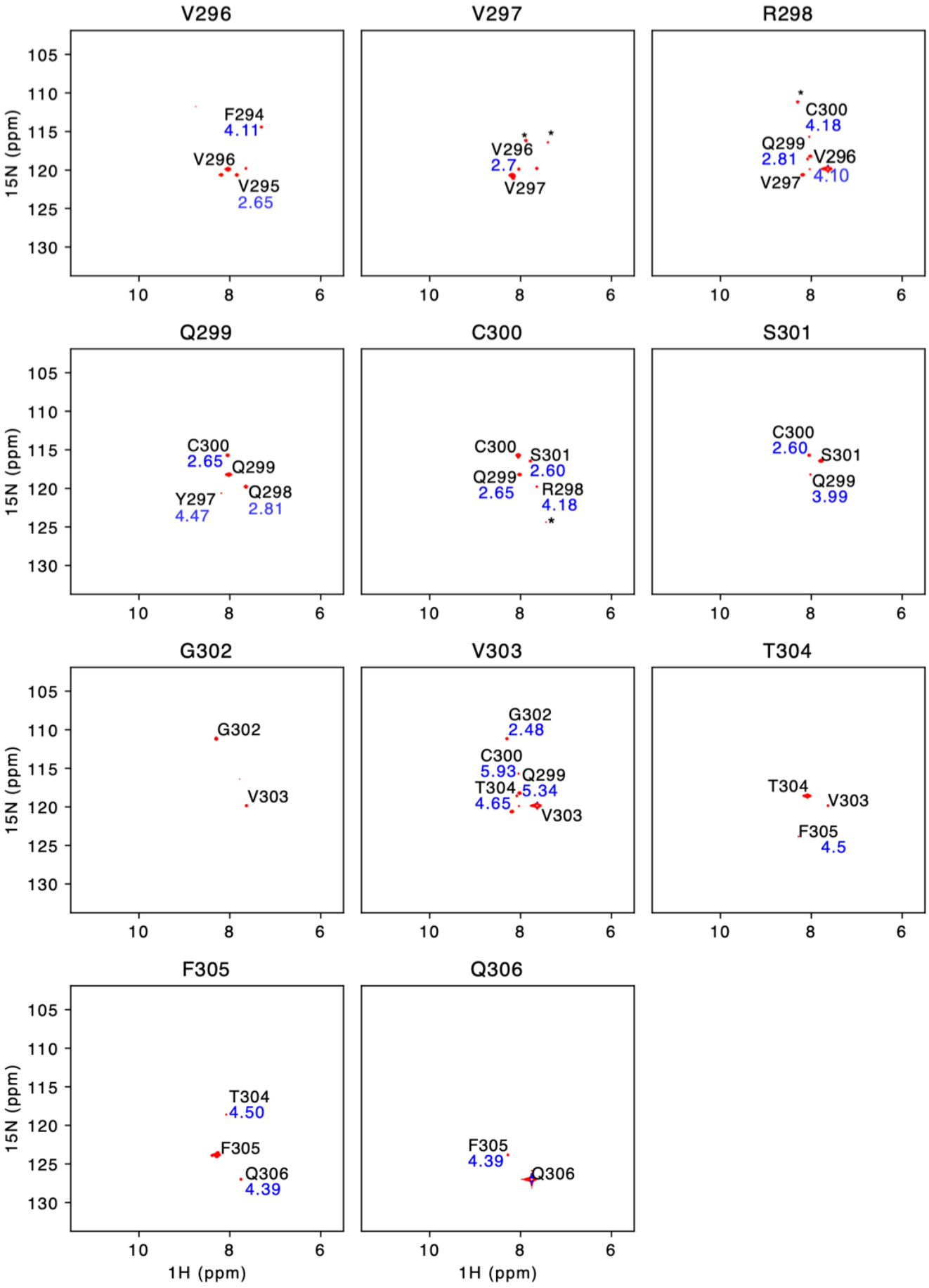
L227
N228
N228
D229
2.79
F230
L227
4.3
2.75
D229
N228
2.79
N231
4.10
D229
F230
2.63
F230
T226
3.16
N228
4.30
D229
2.63
N231
2.76
F230
L232
4.2
N231
D229
4.10
N231
F230
2.76
L232
2.8
L232
N231
2.80
L232
V233
2.6
F230
4.20
A234
3.99
V233
M235
4.20
L232
2.6
V233
A234
2.66
A234
A234
M235
M235
Y237
4.23
V233
4.20
A234
2.77
K236
2.82
K236
M235
2.82
Y237
2.77
N238
4.30
K236
Y237
M235
4.23
N238
2.8
Y237
K236
2.77
V239
3.89
15N (ppm)
1H (ppm)
105
110
115
120
125
130
10
8
6


N238
Y237 2.8
N238
Y239 2.73
K236 4.3
Y239
N238 2.73
Y239 2.73
K236 5.32
E240
E240
T201 2.83
L242
L242
T243
T243
H246 3.36
Q244
T245 2.66
Q244
D245
D245
Q244 2.66
V247 4.18
H246 2.69
A255
H246
T243 3.36
T245 V247 2.69 2.69
D248 4.14
H246
V247
T245 4.18
D248 2.7
V247
H246 2.69
I249 4.19
D248
V247 2.7
L250 4.08
D248
H246 4.14
I249 2.71
I249
G251 4.07
V247 4.19
D248 2.71
L250 2.65
I249
L250
G251 2.57
L250
I249 2.65
15N (ppm)
1H (ppm)
105
110
115
120
125
130
10
8
6


G251
G251
L250
2.57
I249
4.07
A255
N256
2.82
N255
T257
T257
G258
2.56
N256
2.87
I259
3.84
G258
T257
2.56
G258
N256
4.07
I259
2.57
I259
T257
3.84
G258
2.57
I259
A260
A260
V261
L262
2.89
D263
4.10
V261
L262
L262
D263
2.67
V261
2.89
M264
4.31
D263
L262
2.67
D263
V261
4.10
M264
2.51
M264
C265
2.65
D263
2.51
M264
A266
C265
2.69
S267
2.65
A266
S267
S267
A266
2.65
15N (ppm)
1H (ppm)
105
110
115
120
125
130
10
8
6


K269
E270
L271
Q273
N274
G275
M276
N277
G278
R279
T280
I281
15N (ppm)
1H (ppm)
E270 2.77
K269
K269 2.77
E270
L271 2.80
E270 2.80
L271
G275 4.03
Q273
N274 2.58
G275 2.23
N274
Q273 2.58
G275
N274 2.23
Q273 4.03
M276
N277 2.47
M276 2.47
N277
G278
N277 2.91
R279 2.65
G278 2.65
T280 4.60
R279
T280
R276 4.60
G283 3.90
S284 2.73
I281


L282
G283
2.84
L282
G283
G283
S284
2.81
I281
3.90
L282
2.84
S284
G283
2.81
S284
I281
2.73
A285
L286
2.21
A285
L286
L286
A285
2.21
L287
*
L287
D289
E290
2.30
D289
E290
F291
2.80
E290
D289
2.30
F291
F291
E290
2.80
T292
T292
V296
4.38
D295
3.42
F294
F294
V296
4.11
D295
2.64
D295
T292
3.42
F294
2.64
V296
2.65
D295
15N (ppm)
1H (ppm)


V296
F294
4.11
V296
V295
2.65
V297
V296
2.7
V297
R298
C300
4.18
Q299
2.81
V296
4.10
V297
Q299
C300
2.65
Q299
Y297
4.47
Q298
2.81
C300
C300
S301
Q299
2.65
2.60
R298
4.18
S301
C300
2.60
S301
Q299
3.99
G302
G302
V303
V303
G302
2.48
C300
5.93
Q299
T304
5.34
4.65
V303
T304
T304
V303
F305
4.5
F305
T304
4.50
F305
Q306
4.39
F305
4.39
Q306
15N (ppm)
1H (ppm)

C. Bruker pulse sequence programs and CCPN Macros

All pulse sequence code used to acquire the RDCs in this work and the CCPN macro are also available from https://doi.org/10.5281/zenodo.16328473.

1. CCPN Macro for extracting RDCs from QJ $^1D_{NH}$, $^1D_{NC'}$, $^1D_{CC'}$ and TATER experiments

```
import pandas as pd
import numpy as np
from ccpn.core.lib.ContextManagers import undoBlock, undoBlockWithoutSideBar, notificationEchoBlocking
from ccpn.core.lib.peakUtils import estimateVolumes, updateHeight
from ccpn.ui.gui.widgets import MessageDialog
from ccpn.ui.gui.popups.SpectrumGroupEditor import SpectrumGroupEditor
from ccpn.core.lib.peakUtils import _getSpectralPeakPropertyAsDataFrame, NR_ID, HEIGHT , POSITIONS, VOLUME
from ccpn.util.Common import makeIterableList, percentage
from pandas import MultiIndex as m_ix
import re
import builtins

#####################################     User input      ###################################

exportPath = "/u/smithmarsj/Documents/MPRO_RDC/13pf1_RDCs/ccpn_couplings/" #Export path for RDCs in csv/xlsx
format
outpath="/u/smithmarsj/Documents/MPRO_RDC/13pf1_RDCs/ccpn_couplings/2D_MT_QJNC_13pf1.tab"    #Output for the
RDC in DC server fomat

exportFileName = 'TATER.csv' # Name for csv export table
Export          = True  # If you want export to other formats, change the file name and export command below. Flag for csv
export
Export_to_DC = False # Flag to export RDCs in DC server format

recalculateHeight = False
recalculateVolume = False #this will also refit lineWidth maintaining the original position

spectrumGroupName = 'tater_iso'#isotropic
spectrumGroupName2='tater_13pf1' #aligned
chainName='MC:A' #Chain name from "Chains" drop down menu

sortByDataByNR_ID = True # Sort table by NmrResidue ID.
PeakProperty      = 'height' # one of: 'volume', 'height', 'positions' (lower case)

offset=0 # Chain numbering offset
isotope1='HN' # Atom type for meausured RDC. Valid options are HN, N, C, CA
isotope2='C' #Second atom type for measured RDC. Valid options are HN, N, C, CA


noise = [2.84442e+07, 2.84442e+07] #Noise in reference and attenuated spectrum - isotropic
noise2=[2.83338e+07, 2.83338e+07] #Noise in reference and attenuated spectrum - aligned

#####################################   Start of the code    ###################################


def getDFforPeakProperty(spectra, peakProperty, peakListIndexes:list=None) -> pd.DataFrame:
    df = _getSpectralPeakPropertyAsDataFrame(spectra, peakProperty=HEIGHT, peakListIndexes=peakListIndexes)
    newDf = df[df[NR_ID] != ''] # remove rows if NR_ID is not defined
    newDf = newDf.reset_index(drop=True).groupby(NR_ID).max()
    return newDf

def getPeakPositions(spectra,dimension_label,peakListIndexes:list=None):
```

```
    dfs = []
    if peakListIndexes is None: peakListIndexes = [-1] * len(spectra)
    for spectrum, ix in zip(spectra, peakListIndexes):
        positions = []
        values=[]
        values2 = []
        values3=[]
        nmrResidues = []
        serieValue = spectrum.name  # use spectrumName as default. if series defined use that instead.
        if len(spectrum.spectrumGroups) > 0:
            sGserieValue = spectrum._getSeriesItem(spectrum.spectrumGroups[-1])
            if sGserieValue is not None:
                serieValue = sGserieValue
        peaks = spectrum.peakLists[ix].peaks
        peaks.sort(key=lambda x: x.position, reverse=True)
        for peak in peaks:
            print(dimension_label)
            dimension= spectrum.axisCodes.index(dimension_label) if dimension_label in spectrum.axisCodes else None

            position_array = np.array(peak.position)
            frequencies = np.array(peak.peakList.spectrum.spectrometerFrequencies)
            scaled_position = position_array * frequencies
            positions.append(scaled_position)
            positions_array = np.array(positions)
            values.append(peak.position[dimension]*peak.peakList.spectrum.spectrometerFrequencies[dimension])

            values3.append(peak.lineWidths[dimension])
            values2.append(getattr(peak, HEIGHT, None))
            assignedResidues = list(set(filter(None, map(lambda x: x.nmrResidue.id,
                                                         makeIterableList(peak.assignments)))))
            nmrResidues.append(", ".join(assignedResidues))
        _df = pd.DataFrame({f"{serieValue}_positions": values, f"{serieValue}_height": values2, f"{serieValue}_LW": values3},
index=pd.MultiIndex.from_tuples(positions, names=spectrum.axisCodes))

        _df[NR_ID] = nmrResidues
        _df = _df[~_df.index.duplicated()]
        dfs.append(_df)

    df = pd.concat(dfs, axis=1)
    df[NR_ID] = df.T[df.columns.values == NR_ID].apply(lambda x: ' '.join(set([item for item in x[x.notnull()]])))
    df = df.loc[:, ~df.columns.duplicated()]

    cols = list(df.columns)
    resColumn = cols.pop(cols.index(NR_ID))
    sortedCols = sorted(cols, reverse=False)
    sortedCols.insert(0, resColumn)
    df=df[sortedCols]
    newDf = df[df[NR_ID] != ''] # remove rows if NR_ID is not defined
    newDf = newDf.reset_index(drop=True).groupby(NR_ID).max()

    return newDf

    #return tuple([peak.position[i]*peak.peakList.spectrum.spectrometerFrequencies[i] for i,v in enumerate(peak.position)])


spectrumGroup = get('SG:'+spectrumGroupName)
spectrumGroup2 = get('SG:'+spectrumGroupName2)

def _queryForNewSpectrumGroup(spectrumGroup=None):

    msg = 'This macro requires a SpectrumGroup containing spectra and defined series'
    if not spectrumGroup:
        needCreateNewSG = MessageDialog.showYesNo('SpectrumGroup not found','%s. Create new?'% msg)
        if needCreateNewSG:
```

```
            sge = SpectrumGroupEditor(parent=mainWindow, mainWindow=mainWindow, editMode=False)
            sge.exec_()
            spectrumGroup = sge.obj
            if spectrumGroup.name != spectrumGroupName:
                warning('The spectrumGroupName variable in this macro is different from the new spectrumGroup name.'
                        'If you run this macro again it will prompt another Popup.')
        else:
            raise RuntimeError('SpectrumGroup not found. %s'% msg)

    return spectrumGroup

while not spectrumGroup:
    spectrumGroup = _queryForNewSpectrumGroup(spectrumGroup)

def one_to_three(aa):
    # Dictionary mapping one-letter codes to three-letter codes
        aa_map = {
            'A': 'Ala', 'R': 'Arg', 'N': 'Asn', 'D': 'Asp',
            'C': 'Cys', 'E': 'Glu', 'Q': 'Gln', 'G': 'Gly',
            'H': 'His', 'I': 'Ile', 'L': 'Leu', 'K': 'Lys',
            'M': 'Met', 'F': 'Phe', 'P': 'Pro', 'S': 'Ser',
            'T': 'Thr', 'W': 'Trp', 'Y': 'Tyr', 'V': 'Val',
            'B': 'Asx', 'Z': 'Glx', 'X': 'Xaa'
        }

    # Convert input amino acid
        three_letter = aa_map.get(aa.upper())
        if three_letter:
            return three_letter
        else:
            return "Unknown amino acid"
def natural_key(key):
    return [int(text) if text.isdigit() else text for text in re.split(r'(\d+)', key)]

######## Get the spectra from the spectrumGroup and their peaks from the last added peakList
spectra = [sp for sp in spectrumGroup.spectra]
peaks = [pk for sp in spectra for pk in sp.peakLists[-1].peaks]
info('Using peaks contained in the last peakList for spectra %s' % ', '.join(map(str, spectra)))

spectra2 = [sp2 for sp2 in spectrumGroup2.spectra]
peaks2 = [pk2 for sp2 in spectra2 for pk2 in sp2.peakLists[-1].peaks]
info('Using peaks contained in the last peakList for spectra %s' % ', '.join(map(str, spectra2)))

########  Recalculate peak properties
info('Recalculating peak properties...')
with undoBlockWithoutSideBar():
    with notificationEchoBlocking():
        if recalculateHeight:
            list(map(lambda x: updateHeight(x), peaks))
        if recalculateVolume:
            list(map(lambda x: x.fit(keepPosition=True), peaks))
            list(map(lambda x: x.estimateVolume(), peaks))
info('Recalculating peak properties completed.')

info('Recalculating peak properties...')
with undoBlockWithoutSideBar():
    with notificationEchoBlocking():
        if recalculateHeight:
            list(map(lambda x: updateHeight(x), peaks2))
        if recalculateVolume:
            list(map(lambda x: x.fit(keepPosition=True), peaks2))
            list(map(lambda x: x.estimateVolume(), peaks2))
info('Recalculating peak properties completed.')
```

```
######## Get dataframes for the spectrumGroup

if (isotope1=='C' and isotope2=='HN') or (isotope1=='HN' and isotope2=='C') :
    dimension_label="C"
    df = getPeakPositions(spectra,dimension_label)
    df2= getPeakPositions(spectra2,dimension_label)
    print(df.iloc[:,[2,5]])
    print(df2.iloc[:,[2,5]])
else:
    df = getDFforPeakProperty(spectra, PeakProperty)
    df2 = getDFforPeakProperty(spectra2, PeakProperty)

def naturalSort(df, col):
    df['_int'] = df[col].str.extract('(\d+)')
    _nonIntDF = df[df['_int'].isnull()]
    df = df[~df['_int'].isnull()]
    df['_int'] = df['_int'].astype(int)
    df = df.sort_values(by=['_int'])
    df = pd.concat([df, _nonIntDF])
    df.drop(['_int'], axis=1, inplace = True)

    return df

if sortByDataByNR_ID:
    df = df.reindex(sorted(df.index, key=natural_key))
    df2 = df2.reindex(sorted(df2.index, key=natural_key))

if not ((isotope1 == 'C' and isotope2 == 'HN') or (isotope1 == 'HN' and isotope2 == 'C')):
    df['axt'] = df.iloc[:, 1] / df.iloc[:, 0]
    df2['axt'] = df2.iloc[:, 1] / df2.iloc[:, 0]

if (isotope1=='N' and isotope2=='HN') or (isotope1=='HN' and isotope2=='N') :
    df['j']=2 / np.pi / 0.0107 * np.arccos(df.axt / 2)
    err_ratio = (1 + df['axt']**2) / (4 - df['axt']**2)
    fnoise = noise[1] / df.iloc[:, 0];
    df['err']=2*fnoise/(np.pi*0.0107)*np.sqrt(err_ratio);

    df2['j']=2 / np.pi / 0.0107 * np.arccos(df2.axt / 2)
    err_ratio = (1 + df2['axt']**2) / (4 - df2['axt']**2)
    fnoise2 = noise2[1] / df2.iloc[:, 0];
    df2['err']=2*fnoise2/(np.pi*0.0107)*np.sqrt(err_ratio);

    weight=1
    nominal_error=1

elif (isotope1=='N' and isotope2=='C') or (isotope1=='N' and isotope2=='C') :
        df['j']=np.arccos(df.axt/0.95)/(2*np.pi*0.0333/2);

        sigma_err=df.axt*np.sqrt((noise[0]/df.iloc[:,0])**2+(noise[1]/df.iloc[:,1])**2)
        df['err']=1/(2*np.pi*0.0333/2*np.sqrt(1-df.axt**2))*sigma_err

        df2['j']=np.arccos(df2.axt/0.95)/(2*np.pi*0.0333/2);

        sigma_err=df2.axt*np.sqrt((noise2[0]/df2.iloc[:,0])**2+(noise2[1]/df2.iloc[:,1])**2)
        df2['err']=1/(2*np.pi*0.0333/2*np.sqrt(1-df2.axt**2))*sigma_err

        weight=8
        nominal_error=0.125

elif (isotope1=='C' and isotope2=='CA') or (isotope1=='CA' and isotope2=='C') :
        f=0.96 #Incomplete labelling correction factor
        df['j']=(np.arccos(-1*(1/f)*(df.axt-(1-f)))/(2*np.pi*0.0139)+(1/(2*0.0139)))
        sigma_err=df.axt*np.sqrt((noise[0]/df.iloc[:,0])**2+(noise[1]/df.iloc[:,1])**2)
        df['err']=1/(2*np.pi*0.0139*np.sqrt(1-df.axt**2))*sigma_err
```

```
        df2['j']=(np.arccos(-1*(1/f)*(df2.axt-(1-f)))/(2*np.pi*0.0139)+(1/(2*0.0139)))
        sigma_err=df2.axt*np.sqrt((noise2[0]/df2.iloc[:,1])**2+(noise2[1]/df2.iloc[:,0])**2)
        df2['err']=1/(2*np.pi*0.0139*np.sqrt(1-df2.axt**2))*sigma_err

        weight=5
        nominal_error=0.2
elif (isotope1=='C' and isotope2=='HN') or (isotope1=='HN' and isotope2=='C') :
        # We have assumed that the TATER method has been used for CH couplings
        # this will use peak positions to calculate J and D
        df['j']=df.iloc[:, 2] - df.iloc[:, 5]
        df['err']=np.sqrt((df.iloc[:, 0]/(df.iloc[:, 1]/noise[1])**2)+(df.iloc[:, 3]/(df.iloc[:, 4]/noise[1]))**2)
        print(df)
        df2['j']=df2.iloc[:, 2] - df2.iloc[:, 5]
        df2['err']=np.sqrt((df2.iloc[:, 0]/(df2.iloc[:, 1]/noise2[1]))**2+(df2.iloc[:, 3]/(df2.iloc[:, 4]/noise2[1]))**2)
        print(df2.iloc[:,[2,5,6]])
        weight=3
        nominal_error=0.33

else:
    raise RuntimeError('Unknown coupling type check the isotopes')

#Calculate the RDC and the error
result = [df2.loc[df2.index.isin(df.index), 'j']-df.loc[df.index.isin(df2.index), 'j'], np.sqrt((df2.loc[df2.index.isin(df.index),
'err'])**2+(df.loc[df.index.isin(df2.index), 'err'])**2)]
result=pd.DataFrame(result).T

n=1
if Export:
    result.to_csv(exportPath+exportFileName) # or use df.to_csv() for "file.csv" fileNames. etc
    info('DataFrame exported in: %s' %exportPath+exportFileName)

    ## Make export to DC server
if Export_to_DC:
    # Export to DC server format
    mc=get(chainName)
    aa_sequence=mc.sequence

    with builtins.open(outpath,'w') as file:
        string='FORMAT %5d %6s %6s %5d %6s %6s %9.3f %9.3f %.2f'
        print('DATA SEQUENCE %s' % aa_sequence,file=file)
        print('VARS RESID_I RESNAME_I ATOMNAME_I RESID_J RESNAME_J ATOMNAME_J D DD W',file=file)
        print(string,file=file)
        for index, row in result.iterrows():
            if '@' in index:
                continue
            if result.loc[index].isna().any(): #remove any values that are nan
                continue
            num=int(re.findall(r'\d+', index)[0])
            n=n+1
            if isotope1=='C' or isotope1=='CA':
                aa=one_to_three(aa_sequence[num-2])
                num1=num-1
            else:
                aa=one_to_three(aa_sequence[num-1])
                num1=num

            if isotope2=='C' or isotope2=='CA':
                aa2=one_to_three(aa_sequence[num-2])
                num2=num-1
            else:
                aa2=one_to_three(aa_sequence[num-1])
                num2=num
```

```
        num2=num2+offset
        num1=num1+offset
        if result.loc[index,'err'] < nominal_error:
            print('%5d %6s %6s %5d %6s %6s %9.3f %9.3f
%.2f'%(num1,aa,isotope1,num2,aa2,isotope2,result.loc[index,'j'],nominal_error,weight),file=file)
        else:
            print('%5d %6s %6s %5d %6s %6s %9.3f %9.3f
%.2f'%(num1,aa,isotope1,num2,aa2,isotope2,result.loc[index,'j'],result.loc[index,'err'],weight),file=file)
```

2. 2D ^{1}H-^{15}N Amide RDC by TROSY (ARTSY) pulse program

```
; title:   artsy
; summary: ARTSY-2D TROSY for measuring JNH and DNH
; author:  Nick Fitzkee
;         Jinfa Ying
;         Marshall Smith
;         Ad Bax
; date:    April 14, 2025
;
;
;
;
#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>
;SWITCH
#define CARBON_LABEL
#define INTERLEAVE

#define H f1
#define N f3
#define C1 f2    ; 13C centered at 76 ppm (if available)
#define C2 f2    ; 13C centered at 156 ppm

define pulse PG1
define pulse PG3
define pulse PG4
define pulse PG5
define pulse PG6
define pulse PG7



;Gradient Pulses
"PG1=1m"       ;half-encoding
"PG3=500u"
"PG4=500u"     ;half-encoding
"PG5=150u"
"PG6=799u"
"PG7=101.3u"   ;decoding

"l3=td1/4"
"l5=1"
"l6=1"

"in0=inf1*0.5"

"d0=3u"                      ;use if set up for (0,0) 15N phase

#ifdef CARBON_LABEL
"DELTA4=p14*2+4u+d0*2+p21*1.274-p1"   ;phc0=0 phc1=0 aliased peaks same sign
;"d0=in0*0.5-p21*0.637-2u-p14+p1*0.5" ;ph0=90deg ph1=-180deg
#else
"DELTA4=d0*2+2u+p21*1.274-p1"    ;phc0=0 phc1=0  aliased peaks same sign
;"d0=in0*0.5-p21*0.637-1u"       ;ph0=90deg ph1=-180deg
#endif
"p2=p1*2"
"p22=p21*2"

"d11=30m"
"d12=100u"
"d16=190u"                   ;Gradient recovery
```

```
"d28=p6+5u+p1-p21"

; Delays to compensate composite 15N 180
"d29=p21*4.34"

; Adjust for Clean TROSY (with phcor14) - total time for 1st S3E element
"d25=2.1m"

; Total time of 1st transfer step (1/JNH)
; Adjust this so 2nd FID is a null for isotropic sample
; In the attenuated spectrum, the coupling will evolve for d27, and in
; the reference spectrum, it will evolve for d27*0.5 + 0.637*p1.
"d27=10.75m"

"DELTA1=d27-d29*3-p1*1.237-80u"
"DELTA2=d25-PG5-p29"
"DELTA3=2.71m-PG6-p6-205u"
"DELTA5=300u-PG7-20u-p1-3u"

; Comment out when multiple SGUs in use for C1/C2
; You will need to set the offset of sp3 manually (to 56 ppm)
"cnst21=176"
"cnst22=56"
"spoff3=bf2*((cnst22-cnst21)/1000000)"
"spoff5=0"

;mjs adding wavemaker compatibilty to the pulse sequence

;sp12(p29):wvm:mjs_sinc90_fb:f1 sinc90_fb(p29 us; PA=0.5)
;sp2(p29):wvm:mjs_sinc90_fb:f1 sinc90_fb(p29 us; PA=0.5)
;sp1(p29):wvm:mjs_sinc90_fb:f1 sinc90_fb(p29 us; PA=0.5)
;sp3(p14):wvm:mjs_rect_CA:f2 square180(120 ppm;NPOINTS=1000)
;sp5(p14):wvm:mjs_rect_CO:f2 square180(120 ppm; NPOINTS=1000)
;sp31(pcpd2,pl12):wvm:mjs_garp:f2 garp1(260 us)

1       ze
        1m
2       10u do:C2
        1m BLKGRAD
        d11
        d12*6
3       d12*6
4       d12*6
5       d1
        1m UNBLKGRAD
        10u pl0:H
        10u pl3:N
        10u pl0:C1
        10u pl0:C2

; start 90-degree on hn
        (p21 ph0):N             ; along with cycling ph5,
        3u                      ; this thoroughly eliminates
        PG1:gp1                 ; 15N Boltzmann magnetization
        500u

        (p29:sp12 ph11:r):H
        3u
        3u pl1:H
10      (p1 ph5):H
        5u
        5u gron2
        DELTA1*0.25
        10u groff
```

```
        d29*2
        5u
        5u gron2
        DELTA1*0.25
        10u groff

        if "l6==1" goto 20
       (p2 ph0):H
       (p21 ph0 p21*2.44 ph1 p21 ph0):N
        5u
        5u gron2
        DELTA1*0.25
        10u groff
        d29
        goto 25

20      (p2 ph0):H
        d29*0.75
        5u
        5u gron2
        DELTA1*0.25
        10u groff
       (p21 ph0 p21*2.44 ph1 p21 ph0):N
        d29*0.25

25      5u
        5u gron2
        DELTA1*0.25
        10u groff
       (p1 ph1):H
30      3u

;goto 100              ;WARNING - make sure pl12 is set properly for C' dec
;                          or comment out C' AQ cpd before using this
;                          statement.  Ditto for all 'goto 100's'.
        PG3:gp3
        d16

; quantitative-J finished, start 15N evolution

       if "l5==1" {
       (p21 ph17):N
        } else {
       (p21 ph7):N
        }

        d0

#ifdef CARBON_LABEL
       (p14:sp3 ph0):C1
        4u
       (p14:sp5 ph0):C2
#else
        2u
#endif

        d0

        PG4:gp3*EA        ;half-encoding pulse
        200u
       if "l5==1" {
       (p21 ph0 p21*2.44 ph1 p21 ph0):N ;(p22 ph17):N
        } else {
       (p21 ph0 p21*2.44 ph1 p21 ph0):N ;(p22 ph7):N
```

```
        }
        PG4:gp4*EA     ;half-encoding pulse
        200u
        DELTA4         ;comment out if 90,180 delay selected

;;;;;;;;; start final TROSY back-transfer ;;;;;;;;;;

       (p1 ph12):H
        3u
        2u pl0:H
       (p29:sp1 ph21:r):H
;goto 100
        5u
        PG5:gp5
        DELTA2 pl1:H
       (center (p2 ph0):H (p21 ph0 p21*2.44 ph1 p21 ph0):N) ;(p22 ph0):N)
        5u
        PG5:gp5
        DELTA2 pl0:H
       (p29:sp2 ph22:r):H
        3u
        2u pl1:H
       (p1 ph0):H (p21 ph15):N
;goto 100
        5u
        PG6:gp6
        195u pl7:H
        DELTA3
       (150u p6 ph10:r 3u 2u pl1 p2 ph0 3u 2u pl7 p6 ph10:r):H (d28 p22 ph15):N
        3u
        2u pl1:H
;goto 100
        PG6:gp6
        DELTA3
        45u
       (p21 ph14:r):N
        3u
        PG7:gp7        ;decoding gradient
        DELTA5
100     5u
        5u pl12:C2
        10u BLKGRAD
#ifdef CARBON_LABEL
        go=2 ph31 cpds2:C1
#else
        go=2 ph31
#endif
        10u do:C2
        1m BLKGRAD
        d11 wr #0 if #0 zd
#ifdef INTERLEAVE
        d12*6 iu6
       lo to 3 times 2
#else
        d12*6
#endif
        d12 igrad EA
        d12 ip12*2
        d12 ip21*2
        d12 ip14*2
        d12 iu5
        d12 ru6
       lo to 4 times 2
        d12 ip7*2
```

```
      d12 ip17*2
      d12 ip31*2
      d12 id0
      d12 ru5
      d12
     lo to 5 times l3

1m do:C1
1m do:N
1m BLKGRAD
exit

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

ph5=0 2
ph7=1 1 3 3
ph10=2
ph11=3
ph12=3
ph14=3 3 3 3 1 1 1 1
ph15=0 0 0 0 2 2 2 2
ph17=1 1 3 3
ph21=1
ph22=0
ph31=1 3 3 1 3 1 1 3


;;;;;;;;;; NOTES ;;;;;;;

; tested on Bruker AVIII TS2-PL3 consoles
; Note: when used on older dmx/drx consoles, add 3u between pulses
; in the 15N composite pulse (and adjust d29, d30)

; All "goto 100" statements are at points where water flip-backs can
; be tuned.  See note above at first statement.

;1H pulses (H=f1, center on H2O):
;p1 = 90 deg (10us) hard 1H pulse pl1
;p6 = 1.2ms soft 90 deg pulse pl7
;p29 = 1.9m sp1, sp2, and sp12  (spnam1 = sinc1.0)

;15N pulses (N=f3, carrier=119ppm):
;p21 = 90 deg (~50us) 15N pulse pl3

;13C pulses (C1=C2=f2, carrier=176ppm; must modify for two channels):
;p14 = selective 180 deg (23.7*2us 600MHz)
;spnam3 and spnam5 = RECT.1000  (~1000 points req'd for spoffs)
;cpdprg2 = garp  (aq C' dec program for 13C samples)
;pcpd2   = pw for C' aq dec (140u) pl12

;N  SW = 1/(2*in0)
;echo-antiecho in N15

;RECT pulse pairs do not work well on an AVIII
;gpz1 = +33%  sine.100   Boltzmann killer
;gpz2 = +0.5  (gron2)
;gpz3 = +17%  sine.100   zz crusher gradient
;gpz4 = +70%  sine.20    encoding gradient
;gpz5 = +15%  sine.50
;gpz6 = +23%  sine.50
;gpz7 = +70%  sine.20    decoding gradient
```

3. 3D ARTSY-HNCO pulse program

```
;   Pulse sequence for interleaved 3D ARTSY-HNCO for measurement
;of 1JHN couplings

;   The N15 dimension uses the so-called mixed time evolution,
;see JBNMR 2007, 37, 195-204
;   It starts with a constant-time evolution by incrementing d10
;and decrementing d0 (d18=d0)
;   When d0 becomes negative, d18 stops decrementing as d0 and
;the N15 evolution in F2 switches from constant-time to real-time
;by incrementing both d10 and d9 (d19 calculated from d9)
;   By mixed-time evolution, the N15 acquisition time is no longer
;limited by the JNC' rephasing delay (25m in this experiment),
;thereby improving the resolution in the N15 dimension.

#include "bits.jlb"

"d29=p21*4.44"          ;duration for the N15 composite 180
#define N180Comp p21 ph0 p21*2.44 ph1 p21 ph0

#define H180Comp p1 ph0 3u p1*2.44 ph1 3u p1 ph0

"in0=inf2*0.5"
"in10=in0"
"in9=in0"

"in20=inf1*0.5"

;Gradient Pulses
"PG1=1.8m"
"PG2=240u"
"PG3=700u"
"PG4=101.3u"
"PG5=500u"
"PG8=3m"
"PG20=500u"
"PG22=500u"

"l5=1"

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


"d11=30m"
"d16=190u"  ;Grad Recovery
"d26=2.7m"
"d6=2.35m"
"d27=12m-PG8-10u"

"d0=12.5m-PG5-200u"
"d10=10u"
"d9=3u"

"d20=in20*0.5-p13*0.636-5u-larger(d29*0.5, p24*0.5)"

"l9=trunc(d0/in0)"  ;number of increments for CT

define delay d0stop
"d0stop=d0-l9*in0"  ;the d0 before it gets negative

;ARTSY block
```

```
;d29 defined on the top for p21*4.44, duration of the 15N composite pulse
"d23=10.75m"              ;the delay T for the J evolution in the attenuated
"d39=d29*0.75+p1*0.3183"  ;delay to make the J evolution exactly T/2 in the reference
"d38=d29*0.25-p1*0.3183"  ;delay to make the J evolution exactly T/2 in the reference
"DELTA1=d23-d29*3-p1*1.2732-80u"
"l6=1"

"DELTA2=2.35m-PG2-p29-d16"
"DELTA3=d26-PG3-p27-50u"
"DELTA4=d0-d10-p24-p14-d9"
"DELTA6=p21*1.274"
"DELTA8=300u-p1*0.363-PG4-20u-de" ;if PG4 is increased, make sure this Grad. Rec. is adequate.

;  set C13 frequencies, set o2p=176ppm from command line
"cnst21=o2/bf2"
"cnst22=54"

"spoff2=bf2*((cnst22-cnst21)/1000000)"
"spoff4=0"
"spoff5=0"
"spoff8=0"
"spoff9=bf2*((cnst22-cnst21)/1000000)"

; FLIP BACK PULSES
;sp1(p29):wvm:mjs_sinc90_fb:f1 sinc90_fb(p29 us; PA=0.5)
;sp11(p29):wvm:mjs_sinc90_fb:f1 sinc90_fb(p29 us; PA=0.5)
;sp21(p29):wvm:mjs_sinc90_fb:f1 sinc90_fb(p29 us; PA=0.5)

;sp4(p13):wvm:mjs_rect:f2 square90(p13 us; NPOINTS=1000)
;sp5(p14):wvm:mjs_sinc180_CO:f2 sinc180nr(150 us; NPOINTS=1000)
;sp9(p24):wvm:mjs_q3_CA:f2 Q3(200 us; NPOINTS=1000)
;sp31(pcpd2,pl12):wvm:mjs_garp:f2 garp1(260 us)


aqseq 321

1       d11 ze
        1m
2       d11 do:C2
        50u LOCKH_OFF
        d1
        1m UNBLKGRAD
        10u pl0:H
        10u pl3:N
        10u pl0:C1
        10u pl0:C2
;First remove 15N Boltzmann
       (p21 ph0):N
        3u
        PG1:gp0
        100u
       (p21 ph1):N
        5u
        PG1:gp1
        500u

;start 90-degree on hn
        (p29:sp21 ph11:r):H
        3u
        2u pl1:H

        (p1 ph0):H
        5u
        5u gron7
```

```
        DELTA1*0.25
        10u groff
        d29*2

        5u
        5u gron7
        DELTA1*0.25
        10u groff

        (p2 ph0):H

if "l6%l7==1"
  {
        d39 ;=d29*0.75+p1*0.3183
        5u
        5u gron7
        DELTA1*0.25
        10u groff
        (N180Comp):N
        d38 ;=d29*0.25-p1*0.3183
  }
else
  {
        (N180Comp):N
        5u
        5u gron7
        DELTA1*0.25
        10u groff
        d29
  }

        5u
        5u gron7
        DELTA1*0.25
        10u groff
        (p1 ph8):H
        5u
;goto 100
        (p21 ph0):N
        10u
        PG8:gp8
        d27
        (center (p22 ph0):N (p14:sp5 ph0):C2)
        10u
        PG8:gp8
        d27
        (p21 ph1):N
        3u
        PG20:gp20
        d16 pl0:H

        (p13:sp4 ph5):C2
        d20
        5u
        (center (N180Comp):N (p24:sp9 ph6):C1)
        d20
        5u
        (p13:sp4 ph6):C2
        3u
        PG22:gp22
        d16 pl1:H
        10u

;since Topspin's macros F1PH() and F2EA() etc do not support conditional statement,
```

```
;we place the condition within the pulse program to do the mixed time evolution.
;specifically:
; 1) d10 and d9 always increment while d0 always keeps decrementing.
; 2) When d0 is positive, d18=d0, CT evolution realized by incrementing d10 and
;    decrementing d0 (so d18 being decremented too).
; 3) When d0 is negative, d18 stops decrementing with d0 but is fixed at
;    the residual d0 delay before it turns negative.
; 4) Instead, both d10 and d19 increment, with d19 calculated from incrementing d9,
;    therefore turning the N15 evolution from constant-time to real-time.
; 5) Note that l9 is the number of constant-time loops before d0 turns negative.

;condition for doing CT or RT:
if "d9 > l9*in9"  ;d0<0
  {
   "d19=d9-l9*in9"
   "d18=d0stop"
  }
else
  {
   "d18=d0"
   "d19=3u"   ;set to original d9
  }


 ;****************************************
       (p21 ph7):N
       d10
       (p24:sp9 ph0):C1
       DELTA4

       PG5:gp6*EA
       200u
       (p14:sp5 ph6):C2
       d19
       (N180Comp):N
       5u
       PG5:gp5*EA

       190u
       DELTA6
       d18
       5u pl1:H
       (p1 ph12):H
       3u
       2u pl0:H
       (p29:sp1 ph21:r):H (p13:sp4 ph0):C2
       5u
;goto 100
       PG2:gp2
       DELTA2
       d16 pl1:H
       (center (p2 ph0):H (N180Comp):N)
       5u
       DELTA2
       PG2:gp2
       d16 pl0:H
       (p29:sp11 ph22:r):H
       3u
       2u pl1:H
       (p1 ph0):H
       (p21 ph14):N
       5u
;goto 100
       PG3:gp3
```

```
      45u pl18:H
      DELTA3
     (center (300u p27 ph10:r 3u 2u pl1 p2 ph0 3u 2u pl18 p27 ph10:r):H (p22 ph0):N)
;goto 100
     3u
      2u pl1:H
      PG3:gp3
      DELTA3
      45u
      (p21 ph0):N
      PG4:gp4              ;decoding gradient
      DELTA8
100    5u
      5u pl12:C2
      10u BLKGRAMP
     go=2 ph31 cpd2:C2
      d11 do:C2 mc #0 to 2
      F1PH(calph(ph5, +90), caldel(d20, +in20))
      F2I(iu6, I7)  ;ARTSY interleaving loop, i.e. ref vs attenuated
                ;use F2I() for aqseq 321 and F1I() for 312, but always use F2I() for NUS
                ;because topspin always uses 321 for NUS, even if it is explicitly defined as 312
      F2EA(calclc(I5, +1) & calgrad(EA) & calph(ph12, +180) & calph(ph21, +180) & calph(ph14, +180), caldel(d10, +in10) &
caldel(d9, +in9) & caldel(d0, -in0) & calph(ph7, +180) & calph(ph31, +180))


1m do:C2
1m LOCKH_OFF
1m
exit

ph0=0
ph1=1
ph5=1
ph6=1 3
ph7=1 1 3 3
ph8=1
ph10=2
ph11=3
ph12=3
ph14=3
ph21=1
ph22=0
ph31=1 3 3 1

;pl1 : f1 channel - power level for 1H pulses
;pl2 : f2 channel - power level for pulse (default, not used)
;pl3 : f3 channel - power level for n15 pulses
;pl12: f2 channel - power level for C' CPD decoupling
;pl18: f1 channel - power level for soft 90 degree rectangular pulses p27 in WATERGATE
;sp0 : f1 channel - tanh/tan adiabatic 1H inversion pulse p10
;sp1 : f1 channel -  90 degree Sinc1.1000 pulse p29 on water during TROSY back transfer
;sp4 : f2 channel -  90 degree rectangular pulse p13 on 13C (C' on resonance)
;sp5 : f2 channel - 180 degree Sinc1.1000 pulse p14 on 13C (C' on resonance)
;sp9 : f2 channel - 180 degree Q3.1000 pulse p24 on 13C (Ca on resonance)
;sp11: f1 channel -  90 degree Sinc1.1000 pulse p29 on water during TROSY back transfer
;sp21: f1 channel -  90 degree Sinc1.1000 pulse p29 on water before the initial INEPT

;p1 : f1 channel -  90 degree high power pulse (pl1)
;p2 : f1 channel - 180 degree high power pulse (pl1)
;p10: f1 channel - 180 degree tanh/tan adiabatic pulse (sp0)
;p13: f2 channel -  90 degree high power pulse (sp4)
;p14: f2 channel - 180 degree Sinc1.1000 pulse (sp5)
;p21: f3 channel -  90 degree high power pulse (pl3)
;p22: f3 channel - 180 degree high power pulse (pl3)
```

```
;p24: f2 channel - 180 degree Q3.1000 pulse (sp9)
;p27: f1 channel -  90 degree rectangular soft pulse for WATERGATE
;p29: f1 channel -  90 degree water flipback sinc pulses (sp1, sp11, & sp21)

;d0 : decremented delay (F2 in 3d) = 12.5m-PG5-200u
;d0stop: shortest positive d0 before it is decremented to a negative value
;d1 : relaxation delay                    [1.7 sec ]
;d6 : delay for initial INEPT transfer          [2.35 msec]
;d9 : incremented delay (F2 in 3d)          [3 usec]
;d10: incremented delay (F2 in 3d)          [10 usec]
;d11: delay for disk I/O                  [30 msec]
;d16: delay for gradient recovery          [190 usec]
;d18: delay for mixed time N15 evolution (= d0 or d0stop if d0 becomes negative)
;d19: delay for mixed time N15 evolution (= 3u or d9-l9*in9 if d0 becomes negative)
;d20: incremented delay (F1 in 3d) = in20*0.5-p13*0.635-5u-larger(p21*2.22, p24*0.5)
;d26: delay for half of the second INEPT transfer during the trosy back transfer [2.7msec, 1/(4JNH)]
;d27: delay for half of the N15->C' INEPT transfer [12msec, ~1/(4JNC')]
;DELTA : delay used in the initial INEPT transfer = d6-PG1-3u-d16
;DELTA2: delay used in the first INEPT transfer of the trosy back transfer = 2.35m-PG2-p29-d16
;DELTA3: delay used in the second INEPT transfer of the trosy back transfer = d26-PG3-p27-50u
;DELTA4: delay used to refocus N15 chemical shift evolution = d0-d10-p24-p14-d9
;DELTA6: delay for compensating the N15 chemical shift evolution during pulses = p21*1.274-p1
;DELTA8: delay for decoding gradient recovery = 300u-p1*0.363-PG4-20u-de

;cnst21: C' chemical shift (offset, in ppm)
;cnst22: Ca chemical shift (offset, in ppm)
;o2p:    C' chemical shift (cnst21, 175.6ppm)

;inf1: 1/SW(C') = 2 * DW(C')
;inf2: 1/SW(N15) = 2 * DW(N15)
;in0 : 1/(2 * SW(N15)) = DW(N15)
;in9 : 1/(2 * SW(N15)) = DW(N15)
;in10: 1/(2 * SW(N15)) = DW(N15)
;in20: 1/(2 * SW(C')) = DW(C')
;ns:   2 * n
;ds:   >= 2
;td1: number of experiments in F1
;td2: number of experiments in F2
;FnMODE: States-TPPI in F1
;FnMODE: Echo-Antiecho in F2
;cpd2:  C' decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence

;l5: loopcounter used to control N15 90 pulse phase rotation sense
;l6: loopcounter used to control ref/attenuated interleave
;l9: Number of N15 increments in constant time before being switched to real time

;PG1 : gradient pulse 1.8m
;PG2 : gradient pulse 240u
;PG3 : gradient pulse 700u
;PG4 : gradient pulse 101.3u, for coherence decoding
;PG5 : gradient pulse 500u, for coherence encoding
;PG20: gradient pulse 500u
;PG22: gradient pulse 500u

;for z-only gradients:
;gpz0 :   1.7% (d20)
;gpz1 :  11.0% (PG1)
;gpz2 :   9.0% (PG2)
;gpz3 :  53.0% (PG3)
;gpz4 :  48.0% (PG4, E/A decoding gradient)
;gpz5 : -43.0% (PG5, E/A encoding gradient)
;gpz6 :  53.0% (PG5, E/A encoding gradient)
;gpz20:  37.0% (PG20)
```

;gpz22: 41.0% (PG22)

;use gradient files:
;gpnam1 : SINE.20
;gpnam2 : SINE.20
;gpnam3 : SINE.50
;gpnam4 : SINE.20
;gpnam5 : SINE.20
;gpnam6 : SINE.20
;gpnam20: SINE.50
;gpnam22: SINE.20

4. 2D ^{1}H-^{15}N Mixed time quantitative $J_{NC'}$ pulse program

```
;trosy_qJNC_MT.mjs
;NEO-version

; Quantitative J-Correlation for measuring NC' couplings
; 2D experiment using Mixed Time evolution for improved resolution
; Experiment can be performed with increased number of scans for the
; reference vs. the attenuated spectrum
;
; Automatic setup can be achieved by running the "mjs_QJNC" macro from the command line


;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=



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

#define H f1
#define N f3
#define D f4
#define C1 f2
#define C2 f2

;written by abx 7/28/98
;taken from trosy-ge.dw by Jordan 10/11/04
;HN(CA)CB trosy for full transfer to Cb


"in0=inf1*0.25"
"in10=in0"

;total JNC'+DJNC' evolution in the attenuated, ~1/JNC'
;but the coupling cannnot be fully rephased, with a net evolution of p15*2+p14*2+d0*4
"d4=33.3m"

"d25=p22+100u+de+p1*0.363"
"d26=p7-p1"
"d0=3u"

;delays below give a net J evolution of:
;1) p15*2+p14*2+d0*4 as approximation to fully refocusing
;2) d4 (i.e. 1/2JNC') when is fully dephased
;15N C.S. evolution is always 100% refocused at t1=0
"d10=d4*0.25+p15*0.5-p23-45u-p1*0.5"
"d17=d4*0.25-p24-45u-d0-p14*0.5-p15*0.5"

"l9=trunc(d10/in0)" ; number of CT steps
"in9=in10"

define delay d10stop

"d10stop=d10-l9*in9" ; final time step before turning negative

"d11=50m"
"d21=2.1m-p21"
```

```
"d22=2.7m-p21-p2-5u"
"d23=2.4m-p2-30u"
"d18=0u"
"d19=0u"
"d9=3u"


"l1=1"
"l2=1"
;;;;;;;;;;;;;;;; Wavemaker compatibility;;;;;;;;;;;;;

;sp0(p2):wvm:mjs_sinc90_fb:f1 sinc90_fb(p2 us; PA=0.5)
;sp1(p2):wvm:mjs_sinc90_fb:f1 sinc90_fb(p2 us; PA=0.5)
;sp4(p14):wvm:mjs_q3_CA:f2 Q3(89 ppm; NPOINTS=1000)
;sp5(p15):wvm:mjs_q3_CO:f2 Q3(51 ppm; NPOINTS=1000)
;sp31(pcpd2,pl30):wvm:mjs_garp:f2 garp1(260 us)

1       ze
        1m ;RESET
2       d11 do:C2 do:N LOCKH_OFF
        1m do:N
        d1 BLKGRAD  ;LOCK_ON
        1m UNBLKGRAD ;LOCK_OFF
        10u pl1:H
        10u pl7:N
;start 90-degree on hn
       (p1 ph0):H
        3u
        p21:gp1
        d21
        300u
       (d26 p1*2 ph0):H (p7*2 ph0):N
        d21
        p21:gp1
        303u
       (p1 ph1):H               ;INEPT to 15N
        3u
        3u pl12:H
       (p2 ph11:r):H
;goto 999 ;pldb12 phcor11
        6u
        4u pl1:H
        p20:gp0
        200u
        10u pl0:C1
   if "l1%2==1"
     {
      (p7 ph7):N
     }
   else
     {
      (p7 ph17):N
     }

; condition for switch between real time and constant time
; d9 and d0 increase while d10 decreases
; once d10 is negative d18 begins to increase
if "d9 > l9*in9" ; once d9 increases passed CT points switch to RT
      {
      "d19=d10stop" ;d10 before turns negative
      "d18=d9-l9*in9"
      }
      else
      {
```

```
    "d19=d10" ; decrement till negative
    "d18=0u"
    }

     d19 ;d10 in the original pulse sequence
     p23:gp6*EA
     45u pl0:N
    (p17:sp7 ph0):N


;interleave for minimal JNC' volution or 1/2JNC
  if "l2%l7==1"
    {
     5u
     p24:gp6*EA*-1
     40u
     d17
                                        d18 ; mixed time evolution increment once d10 negative
    (p15:sp5 ph0):C2
     d0
    (p14:sp4 ph0):C1
     d0
    (p15:sp5 ph0):C2
                                        d18 ; mixed time evolution
     d17
     p24:gp5*EA*-1
     45u
    }
  else
    {
                                        d18 ;mixed time evolution
    (p15:sp5 ph0):C2
     5u
     p24:gp6*EA*-1
     40u
     d17
     d0
    (p14:sp4 ph0):C1
     d0
     d17
     p24:gp5*EA*-1
     45u
    (p15:sp5 ph0):C2
                              d18 ; mixed time evolution
    }
    (p17:sp7 ph0):N
     5u
     p23:gp5*EA
     40u
     d19 pl7:N

  if "l1%2==1"
    {
    (p1 ph12):H
     3u pl4:C2
     3u pl0:H
    (p2:sp0 ph2:r):H (p3 ph0):C2
    }
  else
    {
    (p1 ph2):H
     3u pl4:C2
     3u pl0:H
    (p2:sp0 ph12:r):H (p3 ph0):C2
```

```
    }
;goto 999     ;spdb0 phcor12
       6u gron2
       d23 pl1:H
       18u groff
      (center (p1*2 ph0):H (p7*2 ph0):N)
       6u gron2
       d23 pl0:H
       18u groff
      (p2:sp1 ph0:r):H
       3u
       3u pl1:H
  if "l1%2==1"
    {
     (p1 ph0):H (p7 ph14):N
    }
  else
    {
     (p1 ph0):H (p7 ph4):N
    }
       5u
;goto 999 ;spdb1 phcor0
       p21:gp3
       d22 pl22:H
      (center (d25 p2 ph10:r 3u 2u pl1 p1*2 ph0 3u 2u pl22 p2 ph10:r):H (p7*2 ph0):N)
;goto 999 ;pldb22 phcor10
       5u pl1:H
       p21:gp3
       d22
      (p7 ph0):N
       p22:gp4
999    95u pl30:C2
       5u BLKGRAMP
      go=2 ph31 cpd2:C2
      d11 do:N do:C2 LOCKH_OFF mc #0 to 2
      F1I(iu2, l7)
                                          F1EA(calclc(l1, 1) & calgrad(EA), caldel(d0, +in0) & caldel(d10, -in10) & caldel(d9, +in9) &
calph(ph7, +180) & calph(ph17, +180) & calph(ph31, +180))
1m do:C1
1m do:N
1m
1m BLKGRAD
exit

ph0=0
ph1=1
ph2=3
ph4=3
ph5=0
ph10=2
ph11=0
ph12=1
ph7=1 3 0 2 3 1 2 0
ph17=1 3 2 0 3 1 0 2
ph14=1
ph31=1 3 2 0 3 1 0 2

;Power level
;pl0 : zero power (0 W)
;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default) not used in PP
;pl3 : f3 channel - power level for pulse (default)
;pl7 : f3 channel - power level for pulse (default)
;sp7 : f3 channel - power level for 180 degree shaped pulse (default)
```

;pl4 : f2 channel - power level for default pulse (=PLW2)
;pl12 : f1 channel - power level for soft rectangular pulse (P2)
;pl22 : f1 channel - power level for soft rectangular pulse (P2)
;pl30 : f2 channel - power for 13C CPD/BB decoupling
;sp0 : f1 channel - power level for Sinc flipback pulse (P2)
;sp1 : f1 channel - power level for Sinc flipback pulse (P2)
;sp4 : f2 channel - power level for Q3 180 degree shaped pulse (P14)
;sp5 : f2 channel - power level for Q3 180 degree shaped pulse (P15)

;PULSE DURATION

;p1 : f1 channel - 90 degree high powered pulse
;p2 : f1 channel - soft rectangular pulse [1 ms]
;p3 : f2 channel - 90 degree high powered pulse
;p7 : f3 channel -f 90 degree high powered pulse
;p14 : f2 channel - 180 degree shaped inversion pulse [200 us]
;p15 : f2 channel - 180 degree shaped inversion pulse [350 us]
;p17 : f3 channel - 180 degree broadband inversion pulse [10.8*p7]

;GRADIENT DURATION
;p20 : duration of homospoil gradient [1.2 ms]
;p21 : duration of CTP gradients [977 us]
;p22 : duration of deconing gradient [101 us]
;p24 : duration of encoding gradient [200 us]
;p24 : duration of encoding gradient [300 us]

;DELAYS
;d1 : relaxation delay 1-5 * T1
;d4 : 1/(2 * JNC)
;d9 : incremented delay in the constant time evolution
;d10 : decremented delay in the constant time evolution 1/(8 * JNC)
;d18 : incremented delay once constant time has been exhausted
;d19 : decremented delay during the chemical shift evolution

;PULSE SHAPE

;spnam0 : f1 channel - file name for the flip back pulse [Sinc]
;spnam1 : f1 channel - file name for the flip back pulse [Sinc]
;spnam4 : f2 channel - file name for the shaped CA 180 degree pulse [Q3]
;spnam5 : f2 channel - file name for the shaped CO 180 degree pulse [Q3]
;spnam7 : f3 channel - file name for BB 180 degree pulse [pix_10.800p1_bw1.49B1.mf]

;GRADIENT STRENGTH

;gpz0 : homospoil gradient 41%
;gpz1 : CTP gradient 31%
;gpz2 : long CTP selection gradient 0.5%
;gpz3 : CTP gradient 37%
;gpz4 : encoding gradient 50%
;gpx4 : encoding gradient 50%
;gpy4 : encoding gradient 50%
;gpz5 : encoding gradient 45%
;gpy5 : encoding gradient 45%
;gpx5 : encoding gradient 45%
;gpz6 : decoding gradient 55%
;gpy6 : decoding gradient 55%
;gpx6 : decoding gradient 55%

;GRADIENT SHAPE

;gpnam0 : SINE.20
;gpnam1 : SINE.20
;gpnam3 : SINE.20
;gpnam4 : SINE.20

;gpnam5 : SINE.20
;gpnam6 : SINE.20

; OTHER
; l7 : scan factor for the attenuated spectrum
; l9 : number of constant time points
; d10stop : last value before switching from constant time to real time
; NS : number of scans for the reference spectrum
; o2 : centred on the 13C' chemical shift window [176 ppm]

5. 3D $QJ_{C'C\alpha}$ based HNCO experiment pulse program

```
;   Pulse sequence for interleaved QJCC 3D TROSY-HNCO for measurement
;of 1J(C'-CA) couplings

;   The N15 dimension uses the so-called mixed time evolution,
;see JBNMR 2007, 37, 195-204
;   It starts with a constant-time evolution by incrementing d10
;and decrementing d0 (d18=d0)
;   When d0 becomes negative, d18 stops decrementing as d0 and
;the N15 evolution in F2 switches from constant-time to real-time
;by incrementing both d10 and d9 (d19 calculated from d9)
;   By mixed-time evolution, the N15 acquisition time is no longer
;limited by the JNC' rephasing delay (25m in this experiment),
;thereby improving the resolution in the N15 dimension.


#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>
#include <De.incl>
;SWITCH
#define CARBON_LABEL
#define INTERLEAVE

#define H f1
#define N f3
#define C1 f2    ; 13C centered at 76 ppm (if available)
#define C2 f2    ; 13C centered at 156 ppm
define pulse PG1
define pulse PG2
define pulse PG3
define pulse PG4
define pulse PG5
define pulse PG6
define pulse PG7
define pulse PG20
define pulse PG21
define pulse PG22

"in0=inf2*0.5"
"in10=in0"
"in9=in0"

"in20=inf1*0.5"
"in30=in20"

;Gradient Pulses
"PG1=1.8m"
"PG2=240u"
"PG3=700u"
"PG4=101.3u"
"PG5=500u"
"PG20=500u"
"PG21=300u"
"PG22=1m"

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


"d11=30m"
"d16=190u"  ;Grad Recovery
```

```
"d26=2.7m"
"d6=2.35m"
"d27=12m"

"d0=12.5m-PG5-200u"
"d10=10u"
"d9=3u"

"d20=3u"
"d30=14.2m-PG21"                    ;for total J evolution of 14.2m*2=28.4m in the attenuated
"d21=d30-d20-larger(p21*4.44, p24)"

"l9=trunc(d0/in0)"  ;number of increments for CT

define delay d0stop
"d0stop=d0-l9*in0"  ;the d0 before it gets negative

"DELTA=d6-PG1-3u-d16"
"DELTA2=2.35m-PG2-p29-d16"
"DELTA3=d26-PG3-p27-50u"
"DELTA4=d0-d10-p25-p14-d9"
"DELTA6=p21*1.274"
"DELTA8=300u-p1*0.363-PG4-20u-de" ;if PG4 is increased, make sure this Grad. Rec. is adequate.

; set C13 frequencies, set o2p=176ppm from command line
"cnst20=180"
"cnst21=o2/bf2"
"cnst22=55"

"spoffs2=0"
"spoffs3=bf2*((cnst22-cnst21)/1000000)"
"spoffs4=0"
"spoffs5=bf2*((cnst20-cnst21)/1000000)"
"spoffs9=bf2*((cnst22-cnst21)/1000000)"
"spoffs19=bf2*((cnst22-cnst21)/1000000)"
"spw19=0"

#define N180Comp p21 ph0 p21*2.44 ph1 p21 ph0
#define H180Comp p1 ph0 3u p1*2.44 ph1 3u p1 ph0
"cnst2=p11/2"
"l2=1"
;sp1(p29):wvm:mjs_sinc90_fb:f1 sinc90_fb(p29 us; PA=0.5)
;sp11(p29):wvm:mjs_sinc90_fb:f1 sinc90_fb(p29 us; PA=0.5)
;sp21(p29):wvm:mjs_sinc90_fb:f1 sinc90_fb(p29 us; PA=0.5)

;sp2(p11):wvm:mjs_rect_CO:f2 square90(p11 us; NPOINTS=1000)
;sp3(p11):wvm:mjs_rect_CA:f2 square90(p11 us; NPOINTS=1000)
;sp4(p13):wvm:mjs_rect_CO_90:f2 square90(p13 us; NPOINTS=1000)
;sp5(p14):wvm:mjs_q3_CO:f2 Q3(39.7 ppm; NPOINTS=1000)
;sp9(p24):wvm:mjs_hsec:f2 sech(2 ms; Q=6, NPOINTS=1000)
;sp10(p25):wvm:mjs_q3_CA:f2 Q3(47.6 ppm; NPOINTS=1000)
;sp19(p24):wvm:mjs_hsec:f2 sech(2 ms; Q=6, NPOINTS=1000)
;sp31(pcpd2,pl12):wvm:mjs_garp:f2 garp1(260 us)



aqseq 321

1      d11 ze
       1m
2      d11 do:C2
       50u LOCKH_OFF
       d1
```

```
        1m UNBLKGRAD
        10u pl0:H
        10u pl3:N
        10u pl0:C1
        10u pl0:C2
;start 90-degree on hn
        (p29:sp21 ph11:r):H
        3u
        2u pl1:H
        (p1 ph0):H
        3u
        PG1:gp1
        DELTA
        d16
        (center (p2 ph0):H (N180Comp):N)
        3u
        DELTA
        PG1:gp1
        d16
        (p1 ph8):H
        5u
;goto 100

        (p21 ph0):f3
        d27
        (center (p22 ph0):f3 (p14:sp5 ph0):f2)
        d27
        (p21 ph1):N
        3u
        PG20:gp20
        d16

        (p13:sp4 ph5):C2
        d20
if "l2%l7==1"
  {
        (N180Comp):N (p24:sp9 ph0):C1  ;reference
        3u
        PG21:gp21
        d21
        (p24:sp19 ph0):C1               ;dummy pulse as space holder, always off
  }
else
  {
        (N180Comp):N (p24:sp19 ph0):C1  ;dummy pulse as space holder, always off
        3u
        PG21:gp21
        d21                         ;d21=d30-d20-larger(p21*4.44, p24)
        (p24:sp9 ph0):C1                ;attenuated
  }
        3u
        (p14:sp5 ph0):C2   ;C' refocusing
        3u
        PG21:gp21
        d30               ;CT/2, d30=14.2m-PG21
        (p24:sp9 ph0):C1   ;BSP
        3u
        (p13:sp4 ph6):C2
        3u
        PG22:gp22
        d16
        (p11:sp3 ph0):C1   ;CA purge pulse
        5u
;since Topspin's macros F1PH() and F2EA() etc do not support conditional statement,
```

```
;we place the condition within the pulse program to do the mixed time evolution.
;specifically:
; 1) d10 and d9 always increment while d0 always keeps decrementing.
; 2) When d0 is positive, d18=d0, CT evolution realized by incrementing d10 and
;    decrementing d0 (so d18 being decremented too).
; 3) When d0 is negative, d18 stops decrementing with d0 but is fixed at
;    the residual d0 delay before it turns negative.
; 4) Instead, both d10 and d19 increment, with d19 calculated from incrementing d9,
;    therefore turning the N15 evolution from constant-time to real-time.
; 5) Note that l9 is the number of constant-time loops before d0 turns negative.

;condition for doing CT or RT:
if "d9 > l9*in9"  ;d0<0
  {
   "d19=d9-l9*in9"
   "d18=d0stop"
  }
else
  {
   "d18=d0"
   "d19=3u"   ;set to original d9
  }



       (p21 ph7):N
       d10
       (p25:sp10 ph0):C1
       DELTA4

       PG5:gp6*EA
       200u
       (p14:sp5 ph0):C2
       d19
       (N180Comp):N
       5u
       PG5:gp5*EA

       190u
       DELTA6
       d18
       5u pl1:H
       (p1 ph12):H
       3u
       2u pl0:H
       (lalign (p29:sp1 ph21:r):f1 (p11:sp2 ph0):f2)   ;sp2 C' purge pulse
       5u
;goto 100
       PG2:gp2
       DELTA2
       d16 pl1:H
       (center (p2 ph0):H (N180Comp):N)
       5u
       DELTA2
       PG2:gp2
       d16 pl0:H
       (p29:sp11 ph22:r):H
       3u
       2u pl1:H
       (p1 ph0):H
       (p21 ph14):N
       5u
;goto 100
       PG3:gp3
```

```
      45u pl18:H
      DELTA3
     (center (300u p27 ph10:r 3u 2u pl1 p2 ph0 3u 2u pl18 p27 ph10:r):H (p22 ph0):N)
;goto 100
     3u
      2u pl1:H
      PG3:gp3
      DELTA3
      45u
      (p21 ph0):N
      PG4:gp4              ;decoding gradient
      DELTA8
100    5u
      5u pl12:C2
      10u BLKGRAMP
     go=2 ph31 cpd2:C2
     5u do:f2
      d11 mc #0 to 2
      F1PH(calph(ph5, +90), caldel(d20, +in20) & caldel(d30, -in30))
      F2I(iu2, I7)
      F2EA(calgrad(EA) & calph(ph12, +180) & calph(ph21, +180) & calph(ph14, +180), caldel(d10, +in10) & caldel(d9, +in9) &
caldel(d0, -in0) & calph(ph7, +180) & calph(ph31, +180))


1m do:C2
1m LOCKH_OFF
1m
exit

ph0=0
ph1=1
ph5=1
ph6=1 3
ph7=1 1 3 3
ph8=1
ph10=2
ph11=3
ph12=3
ph14=3
;ph17=0 0 2 2
ph21=1
ph22=0
ph31=1 3 3 1

;pl1 : f1 channel - power level for 1H pulses
;pl2 : f2 channel - power level for pulse (default, not used)
;pl3 : f3 channel - power level for n15 pulses
;pl12: f2 channel - power level for C' CPD decoupling
;pl18: f1 channel - power level for soft 90 degree rectangular pulses p27 in WATERGATE
;sp0 : f1 channel - tanh/tan adiabatic 1H inversion pulse p10
;sp1 : f1 channel -  90 degree Sinc1.1000 pulse p29 on water during TROSY back transfer
;sp4 : f2 channel -  90 degree rectangular pulse p13 on 13C (C' on resonance)
;sp5 : f2 channel - 180 degree Sinc1.1000 pulse p14 on 13C (C' on resonance)
;sp9 : f2 channel - 180 degree Q3.1000 pulse p24 on 13C (Ca on resonance)
;sp11: f1 channel -  90 degree Sinc1.1000 pulse p29 on water during TROSY back transfer
;sp21: f1 channel -  90 degree Sinc1.1000 pulse p29 on water before the initial INEPT

;p1 : f1 channel -  90 degree high power pulse (pl1)
;p2 : f1 channel - 180 degree high power pulse (pl1)
;p10: f1 channel - 180 degree tanh/tan adiabatic pulse (sp0)
;p13: f2 channel -  90 degree high power pulse (sp4)
;p14: f2 channel - 180 degree Sinc1.1000 pulse (sp5)
;p21: f3 channel -  90 degree high power pulse (pl3)
;p22: f3 channel - 180 degree high power pulse (pl3)
```

```
;p24: f2 channel - 180 degree Q3.1000 pulse (sp9)
;p27: f1 channel -  90 degree rectangular soft pulse for WATERGATE
;p29: f1 channel -  90 degree water flipback sinc pulses (sp1, sp11, & sp21)

;d0 : decremented delay (F2 in 3d) = 12.5m-PG5-200u
;d0stop: shortest positive d0 before it is decremented to a negative value
;d1 : relaxation delay                    [1.7 sec ]
;d6 : delay for initial INEPT transfer    [2.35 msec]
;d9 : incremented delay (F2 in 3d)        [3 usec]
;d10: incremented delay (F2 in 3d)        [10 usec]
;d11: delay for disk I/O                  [30 msec]
;d16: delay for gradient recovery         [190 usec]
;d18: delay for mixed time N15 evolution (= d0 or d0stop if d0 becomes negative)
;d19: delay for mixed time N15 evolution (= 3u or d9-l9*in9 if d0 becomes negative)
;d20: incremented delay (F1 in 3d) = in20*0.5-p13*0.635-5u-larger(p21*2.22, p24*0.5)
;d26: delay for half of the second INEPT transfer during the trosy back transfer [2.7msec, 1/(4JNH)]
;d27: delay for half of the N15->C' INEPT transfer [12msec, ~1/(4JNC')]
;DELTA : delay used in the initial INEPT transfer = d6-PG1-3u-d16
;DELTA2: delay used in the first INEPT transfer of the trosy back transfer = 2.35m-PG2-p29-d16
;DELTA3: delay used in the second INEPT transfer of the trosy back transfer = d26-PG3-p27-50u
;DELTA4: delay used to refocus N15 chemical shift evolution = d0-d10-p24-p14-d9
;DELTA6: delay for compensating the N15 chemical shift evolution during pulses = p21*1.274-p1
;DELTA8: delay for decoding gradient recovery = 300u-p1*0.363-PG4-20u-de

;cnst21: C' chemical shift (offset, in ppm)
;cnst22: Ca chemical shift (offset, in ppm)
;o2p:    C' chemical shift (cnst21, 175.6ppm)

;inf1: 1/SW(C') = 2 * DW(C')
;inf2: 1/SW(N15) = 2 * DW(N15)
;in0 : 1/(2 * SW(N15)) = DW(N15)
;in9 : 1/(2 * SW(N15)) = DW(N15)
;in10: 1/(2 * SW(N15)) = DW(N15)
;in20: 1/(2 * SW(C')) = DW(C')
;ns:   2 * n
;ds:   >= 2
;td1: number of experiments in F1
;td2: number of experiments in F2
;FnMODE: States-TPPI in F1
;FnMODE: Echo-Antiecho in F2
;cpd2:  C' decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence

;l9: Number of N15 increments in constant time before being switched to real time

;PG1 : gradient pulse 1.8m
;PG2 : gradient pulse 240u
;PG3 : gradient pulse 700u
;PG4 : gradient pulse 101.3u, for coherence decoding
;PG5 : gradient pulse 500u, for coherence encoding
;PG20: gradient pulse 500u
;PG22: gradient pulse 500u

;for z-only gradients:
;gpz0 :   1.7% (d20)
;gpz1 :  11.0% (PG1)
;gpz2 :   9.0% (PG2)
;gpz3 :  53.0% (PG3)
;gpz4 :  48.0% (PG4, E/A decoding gradient)
;gpz5 : -43.0% (PG5, E/A encoding gradient)
;gpz6 :  53.0% (PG5, E/A encoding gradient)
;gpz20:  37.0% (PG20)
;gpz22:  41.0% (PG22)
```

;use gradient files:
;gpnam1 : SINE.20
;gpnam2 : SINE.20
;gpnam3 : SINE.50
;gpnam4 : SINE.20
;gpnam5 : SINE.20
;gpnam6 : SINE.20
;gpnam20: SINE.50
;gpnam22: SINE.20

6. TROSY/ANTI-TROSY encoded RDC pulse program

```
;   Pulse sequence for interleaved TATER 3d TROSY-HNCO for measurement
;of 2J(C'-HN) couplings

;   The N15 dimension uses the so-called mixed time evolution,
;see JBNMR 2007, 37, 195-204
;   It starts with a constant-time evolution by incrementing d10
;and decrementing d0 (d18=d0)
;   When d0 becomes negative, d18 stops decrementing as d0 and
;the N15 evolution in F2 switches from constant-time to real-time
;by incrementing both d10 and d9 (d19 calculated from d9)
;   By mixed-time evolution, the N15 acquisition time is no longer
;limited by the JNC' rephasing delay (25m in this experiment),
;thereby improving the resolution in the N15 dimension.


#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>
#include <De.incl>
;SWITCH
#define CARBON_LABEL
#define INTERLEAVE

#define H f1
#define N f3
#define C1 f2    ; 13C centered at 76 ppm (if available)
#define C2 f2    ; 13C centered at 156 ppm
define pulse PG1
define pulse PG2
define pulse PG3
define pulse PG4
define pulse PG5
define pulse PG6
define pulse PG7
define pulse PG20
define pulse PG21
define pulse PG22

"in0=inf2*0.5"
"in10=in0"
"in9=in0"

"in20=inf1*0.5"
"in30=in20"

;Gradient Pulses
"PG1=1.8m"
"PG2=240u"
"PG3=700u"
"PG4=101.3u"
"PG5=500u"
"PG20=500u"
"PG21=300u"
"PG22=1m"

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


"d11=30m"
```

```
"d16=190u"  ;Grad Recovery
"d26=2.7m"
"d6=2.35m"
"d27=12m"

"d0=12.5m-PG5-200u"
"d10=10u"
"d9=3u"

"d20=3u"
"d30=14.2m-PG21"                    ;for total J evolution of 14.2m*2=28.4m in the attenuated
"d21=d30-d20-larger(p21*4.44, p24)"

"l9=trunc(d0/in0)"  ;number of increments for CT

define delay d0stop
"d0stop=d0-l9*in0"  ;the d0 before it gets negative

"DELTA=d6-PG1-3u-d16"
"DELTA2=2.35m-PG2-p29-d16"
"DELTA3=d26-PG3-p27-50u"
"DELTA4=d0-d10-p24-p14-d9"
"DELTA6=p21*1.274"
"DELTA8=300u-p1*0.363-PG4-20u-de" ;if PG4 is increased, make sure this Grad. Rec. is adequate.

; set C13 frequencies, set o2p=176ppm from command line
"cnst20=180"
"cnst21=o2/bf2"
"cnst22=55"

"spoffs2=0"
"spoffs3=bf2*((cnst22-cnst21)/1000000)"
"spoffs4=0"
"spoffs5=bf2*((cnst20-cnst21)/1000000)"
"spoffs9=bf2*((cnst22-cnst21)/1000000)"
"spoffs19=bf2*((cnst22-cnst21)/1000000)"
"spw19=0"

#define N180Comp p21 ph0 p21*2.44 ph1 p21 ph0
#define H180Comp p1 ph0 3u p1*2.44 ph1 3u p1 ph0
"cnst2=p11/2"
"l2=1"
;sp1(p29):wvm:mjs_sinc90_fb:f1 sinc90_fb(p29 us; PA=0.5)
;sp11(p29):wvm:mjs_sinc90_fb:f1 sinc90_fb(p29 us; PA=0.5)
;sp21(p29):wvm:mjs_sinc90_fb:f1 sinc90_fb(p29 us; PA=0.5)

;sp2(p11):wvm:mjs_rect_CO:f2 square90(p11 us; NPOINTS=1000)
;sp3(p11):wvm:mjs_rect_CA:f2 square90(p11 us; NPOINTS=1000)
;sp4(p13):wvm:mjs_rect_CO_90:f2 square90(p13 us; NPOINTS=1000)
;sp5(p14):wvm:mjs_q3_CO:f2 Q3(39.7 ppm; NPOINTS=1000)
;sp9(p24):wvm:mjs_q3_CA:f2 Q3(47.6 ppm; NPOINTS=1000)
;sp19(p24):wvm:mjs_q3_CA:f2 Q3(47.6 ppm; NPOINTS=1000)
;sp31(pcpd2,pl12):wvm:mjs_garp:f2 garp1(260 us)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1      d11 ze
       1m
2      d11 do:C2
       50u LOCKH_OFF
       d1
       1m UNBLKGRAD
       10u pl0:H
```

```
        10u pl3:N
        10u pl0:C1
        10u pl0:C2
;start 90-degree on hn
        (p29:sp21 ph11:r):H
        3u
        2u pl1:H
        (p1 ph0):H
        3u
        PG1:gp1
        DELTA
        d16
        (center (p2 ph0):H (N180Comp):N)
        3u
        DELTA
        PG1:gp1
        d16
        (p1 ph8):H
        5u
;goto 100

        (p21 ph0):f3
        d27
        (center (p22 ph0):f3 (p14:sp5 ph0):f2)
        d27
        (p21 ph1):N
        3u
        PG20:gp20
        d16

        (p13:sp4 ph5):C2
        d20
if "l2%l7==1"
  {
        (N180Comp):N (p24:sp9 ph0):C1  ;reference
        3u
        PG21:gp21
        d21
        (p24:sp19 ph0):C1               ;dummy pulse as space holder, always off
  }
else
  {
        (N180Comp):N (p24:sp19 ph0):C1  ;dummy pulse as space holder, always off
        3u
        PG21:gp21
        d21                         ;d21=d30-d20-larger(p21*4.44, p24)
        (p24:sp9 ph0):C1                ;attenuated
  }
        3u
        (p14:sp5 ph0):C2   ;C' refocusing
        3u
        PG21:gp21
        d30              ;CT/2, d30=14.2m-PG21
        (p24:sp9 ph0):C1   ;BSP
        3u
        (p13:sp4 ph6):C2
        3u
        PG22:gp22
        d16
        (p11:sp3 ph0):C1   ;CA purge pulse
        5u
;since Topspin's macros F1PH() and F2EA() etc do not support conditional statement,
;we place the condition within the pulse program to do the mixed time evolution.
;specifically:
```

```
; 1) d10 and d9 always increment while d0 always keeps decrementing.
; 2) When d0 is positive, d18=d0, CT evolution realized by incrementing d10 and
;    decrementing d0 (so d18 being decremented too).
; 3) When d0 is negative, d18 stops decrementing with d0 but is fixed at
;    the residual d0 delay before it turns negative.
; 4) Instead, both d10 and d19 increment, with d19 calculated from incrementing d9,
;    therefore turning the N15 evolution from constant-time to real-time.
; 5) Note that l9 is the number of constant-time loops before d0 turns negative.

;condition for doing CT or RT:
if "d9 > l9*in9"  ;d0<0
  {
   "d19=d9-l9*in9"
   "d18=d0stop"
  }
else
  {
   "d18=d0"
   "d19=3u"   ;set to original d9
  }



       (p21 ph7):N
       d10
       (p24:sp9 ph0):C1
       DELTA4

       PG5:gp6*EA
       200u
       (p14:sp5 ph0):C2
       d19
       (N180Comp):N
       5u
       PG5:gp5*EA

       190u
       DELTA6
       d18
       5u pl1:H
       (p1 ph12):H
       3u
       2u pl0:H
       (lalign (p29:sp1 ph21:r):f1 (p11:sp2 ph0):f2)   ;sp2 C' purge pulse
       5u
;goto 100
       PG2:gp2
       DELTA2
       d16 pl1:H
       (center (p2 ph0):H (N180Comp):N)
       5u
       DELTA2
       PG2:gp2
       d16 pl0:H
       (p29:sp11 ph22:r):H
       3u
       2u pl1:H
       (p1 ph0):H
       (p21 ph14):N
       5u
;goto 100
       PG3:gp3
       45u pl18:H
       DELTA3
```

```
      (center (300u p27 ph10:r 3u 2u pl1 p2 ph0 3u 2u pl18 p27 ph10:r):H (p22 ph0):N)
;goto 100
      3u
       2u pl1:H
       PG3:gp3
       DELTA3
       45u
       (p21 ph0):N
       PG4:gp4              ;decoding gradient
       DELTA8
100    5u
       5u pl12:C2
       10u BLKGRAMP
      go=2 ph31 cpd2:C2
      5u do:f2
       d11 mc #0 to 2
       F1PH(calph(ph5, +90), caldel(d20, +in20) & caldel(d30, -in30))
       F2I(iu2, I7)
       F2EA(calgrad(EA) & calph(ph12, +180) & calph(ph21, +180) & calph(ph14, +180), caldel(d10, +in10) & caldel(d9, +in9) &
caldel(d0, -in0) & calph(ph7, +180) & calph(ph31, +180))


1m do:C2
1m LOCKH_OFF
1m
exit

ph0=0
ph1=1
ph5=1
ph6=1 3
ph7=1 1 3 3
ph8=1
ph10=2
ph11=3
ph12=3
ph14=3
;ph17=0 0 2 2
ph21=1
ph22=0
ph31=1 3 3 1

;pl1 : f1 channel - power level for 1H pulses
;pl2 : f2 channel - power level for pulse (default, not used)
;pl3 : f3 channel - power level for n15 pulses
;pl12: f2 channel - power level for C' CPD decoupling
;pl18: f1 channel - power level for soft 90 degree rectangular pulses p27 in WATERGATE
;sp0 : f1 channel - tanh/tan adiabatic 1H inversion pulse p10
;sp1 : f1 channel -  90 degree Sinc1.1000 pulse p29 on water during TROSY back transfer
;sp4 : f2 channel -  90 degree rectangular pulse p13 on 13C (C' on resonance)
;sp5 : f2 channel - 180 degree Sinc1.1000 pulse p14 on 13C (C' on resonance)
;sp9 : f2 channel - 180 degree Q3.1000 pulse p24 on 13C (Ca on resonance)
;sp11: f1 channel -  90 degree Sinc1.1000 pulse p29 on water during TROSY back transfer
;sp21: f1 channel -  90 degree Sinc1.1000 pulse p29 on water before the initial INEPT

;p1 : f1 channel -  90 degree high power pulse (pl1)
;p2 : f1 channel - 180 degree high power pulse (pl1)
;p10: f1 channel - 180 degree tanh/tan adiabatic pulse (sp0)
;p13: f2 channel -  90 degree high power pulse (sp4)
;p14: f2 channel - 180 degree Sinc1.1000 pulse (sp5)
;p21: f3 channel -  90 degree high power pulse (pl3)
;p22: f3 channel - 180 degree high power pulse (pl3)
;p24: f2 channel - 180 degree Q3.1000 pulse (sp9)
;p27: f1 channel -  90 degree rectangular soft pulse for WATERGATE
```

```
;p29: f1 channel -  90 degree water flipback sinc pulses (sp1, sp11, & sp21)

;d0 : decremented delay (F2 in 3d) = 12.5m-PG5-200u
;d0stop: shortest positive d0 before it is decremented to a negative value
;d1 : relaxation delay                    [1.7 sec ]
;d6 : delay for initial INEPT transfer          [2.35 msec]
;d9 : incremented delay (F2 in 3d)           [3 usec]
;d10: incremented delay (F2 in 3d)           [10 usec]
;d11: delay for disk I/O                  [30 msec]
;d16: delay for gradient recovery           [190 usec]
;d18: delay for mixed time N15 evolution (= d0 or d0stop if d0 becomes negative)
;d19: delay for mixed time N15 evolution (= 3u or d9-l9*in9 if d0 becomes negative)
;d20: incremented delay (F1 in 3d) = in20*0.5-p13*0.635-5u-larger(p21*2.22, p24*0.5)
;d26: delay for half of the second INEPT transfer during the trosy back transfer [2.7msec, 1/(4JNH)]
;d27: delay for half of the N15->C' INEPT transfer [12msec, ~1/(4JNC')]
;DELTA : delay used in the initial INEPT transfer = d6-PG1-3u-d16
;DELTA2: delay used in the first INEPT transfer of the trosy back transfer = 2.35m-PG2-p29-d16
;DELTA3: delay used in the second INEPT transfer of the trosy back transfer = d26-PG3-p27-50u
;DELTA4: delay used to refocus N15 chemical shift evolution = d0-d10-p24-p14-d9
;DELTA6: delay for compensating the N15 chemical shift evolution during pulses = p21*1.274-p1
;DELTA8: delay for decoding gradient recovery = 300u-p1*0.363-PG4-20u-de

;cnst21: C' chemical shift (offset, in ppm)
;cnst22: Ca chemical shift (offset, in ppm)
;o2p:    C' chemical shift (cnst21, 175.6ppm)

;inf1: 1/SW(C') = 2 * DW(C')
;inf2: 1/SW(N15) = 2 * DW(N15)
;in0 : 1/(2 * SW(N15)) = DW(N15)
;in9 : 1/(2 * SW(N15)) = DW(N15)
;in10: 1/(2 * SW(N15)) = DW(N15)
;in20: 1/(2 * SW(C')) = DW(C')
;ns:   2 * n
;ds:   >= 2
;td1: number of experiments in F1
;td2: number of experiments in F2
;FnMODE: States-TPPI in F1
;FnMODE: Echo-Antiecho in F2
;cpd2:  C' decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence

;l9: Number of N15 increments in constant time before being switched to real time

;PG1 : gradient pulse 1.8m
;PG2 : gradient pulse 240u
;PG3 : gradient pulse 700u
;PG4 : gradient pulse 101.3u, for coherence decoding
;PG5 : gradient pulse 500u, for coherence encoding
;PG20: gradient pulse 500u
;PG22: gradient pulse 500u

;for z-only gradients:
;gpz0 :   1.7% (d20)
;gpz1 :  11.0% (PG1)
;gpz2 :   9.0% (PG2)
;gpz3 :  53.0% (PG3)
;gpz4 :  48.0% (PG4, E/A decoding gradient)
;gpz5 : -43.0% (PG5, E/A encoding gradient)
;gpz6 :  53.0% (PG5, E/A encoding gradient)
;gpz20:  37.0% (PG20)
;gpz22:  41.0% (PG22)


;use gradient files:
```

```
;gpnam1 : SINE.20
;gpnam2 : SINE.20
;gpnam3 : SINE.50
;gpnam4 : SINE.20
;gpnam5 : SINE.20
;gpnam6 : SINE.20
;gpnam20: SINE.50
;gpnam22: SINE.20
```

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