Supporting Information

Revisiting $^{1}H^{N}$ CPMG relaxation dispersion experiments: a simple modification can eliminate large artifacts

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Supplementary Figures

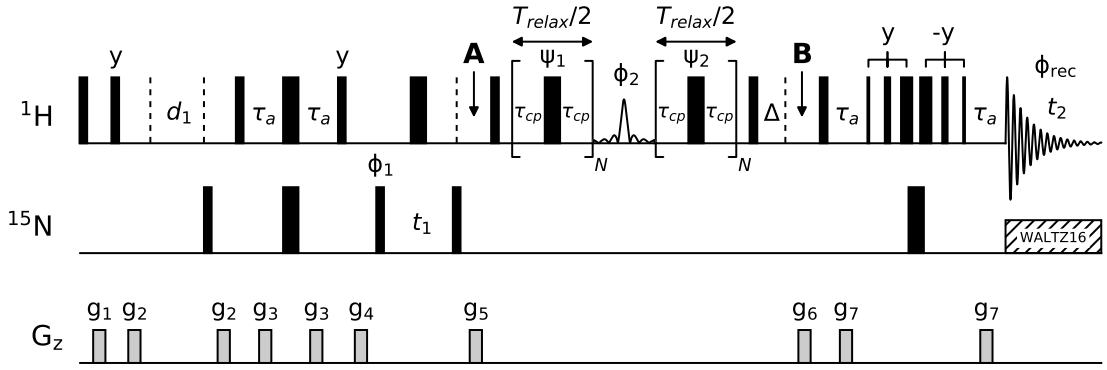


Fig. S1 Previous $^1H^N$ CPMG pulse scheme used by our laboratory for studies of fully protonated proteins with a central amide proton selective REBURP pulse. Details are as described in the legend to Figure 3, with the exception of the REBURP pulse that is typically of duration 1.5 ms for applications at 800 MHz.

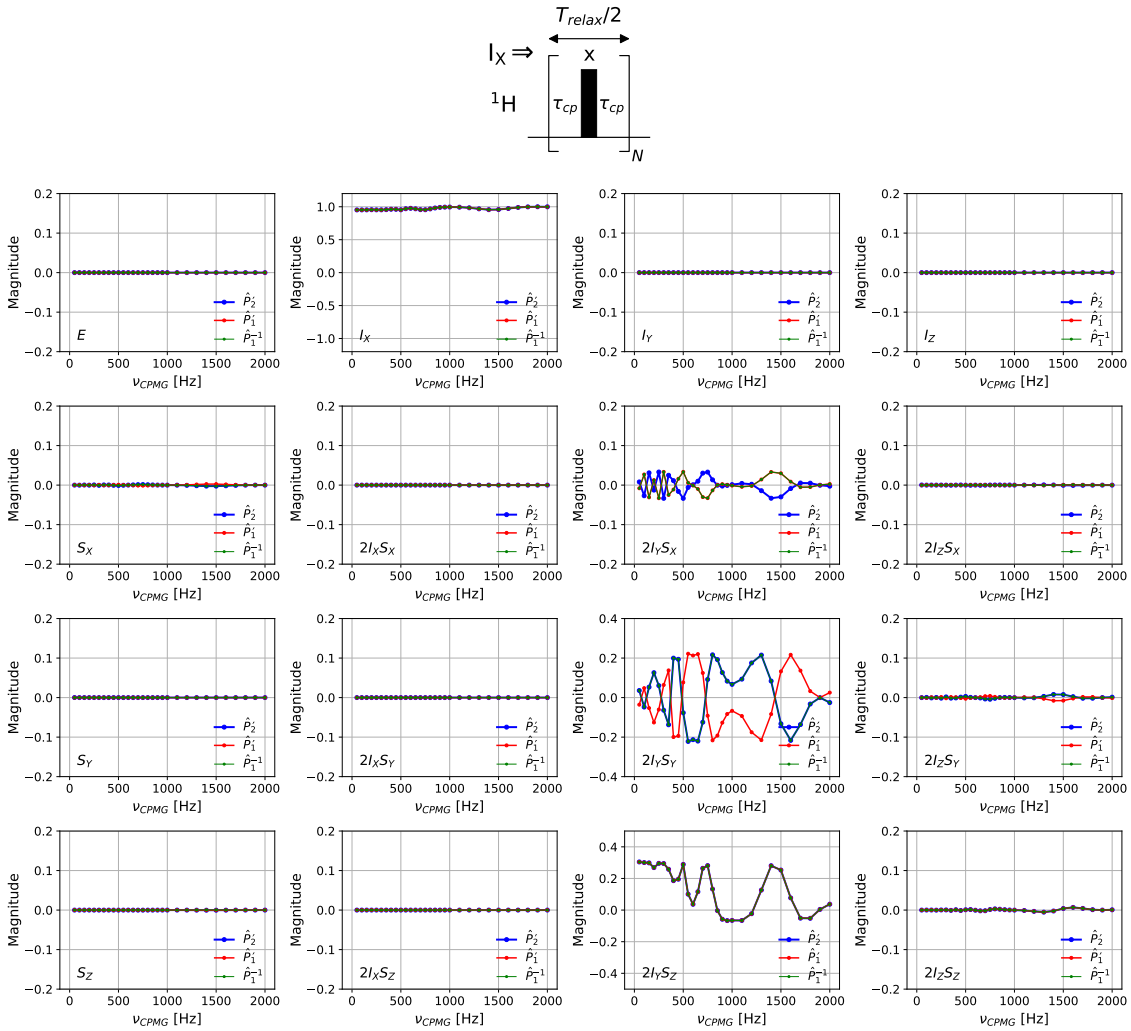


Fig. S2 (Top) Pulse scheme used for numerical simulations of the evolution of each of the 16 density operators in a two-spin I–S spin system where I = ^{1}H$^{\mathrm{N}}$ and S = ^{1}H$^{\alpha}$, starting with I_X (Bottom). Each of the operators is evaluated at the end of N cycles as $\hat{P}_2'\hat{O}(\hat{P}_2')^{-1}$, $\hat{P}_1'\hat{O}(\hat{P}_1')^{-1}$ and $(\hat{P}_1)^{-1}\hat{O}\hat{P}_1$, denoted in the figure as $\hat{P}_2'$, $\hat{P}_1'$ and $\hat{P}_1^{-1}$, respectively (see Eqs. (3) and (4)). Values of T_{relax} = 20 ms, $^3J_{\mathrm{HNH}\alpha}$ = 10 Hz, ν_1 = 25 kHz, ^{1}H$^{\mathrm{N}}$ and ^{1}H$^{\alpha}$ chemical shifts of 8.2 ppm and 4.5 ppm, respectively, were used for all simulations, that assumed a ^{1}H resonance frequency of 800 MHz. The full ^{1}H$^{\mathrm{N}}$–^{1}H$^{\alpha}$ scalar-coupled Hamiltonian was considered in each simulation (although this makes little difference relative to using the expression for weak coupling due to the large separation of ^{1}H$^{\mathrm{N}}$ and ^{1}H$^{\alpha}$ chemical shifts) and the ^{1}H RF carrier was positioned at 8.2 ppm. Note, ν_{CPMG} = $1/(4\tau_{cp})$, $T_{relax} = 4N\tau_{cp}$.

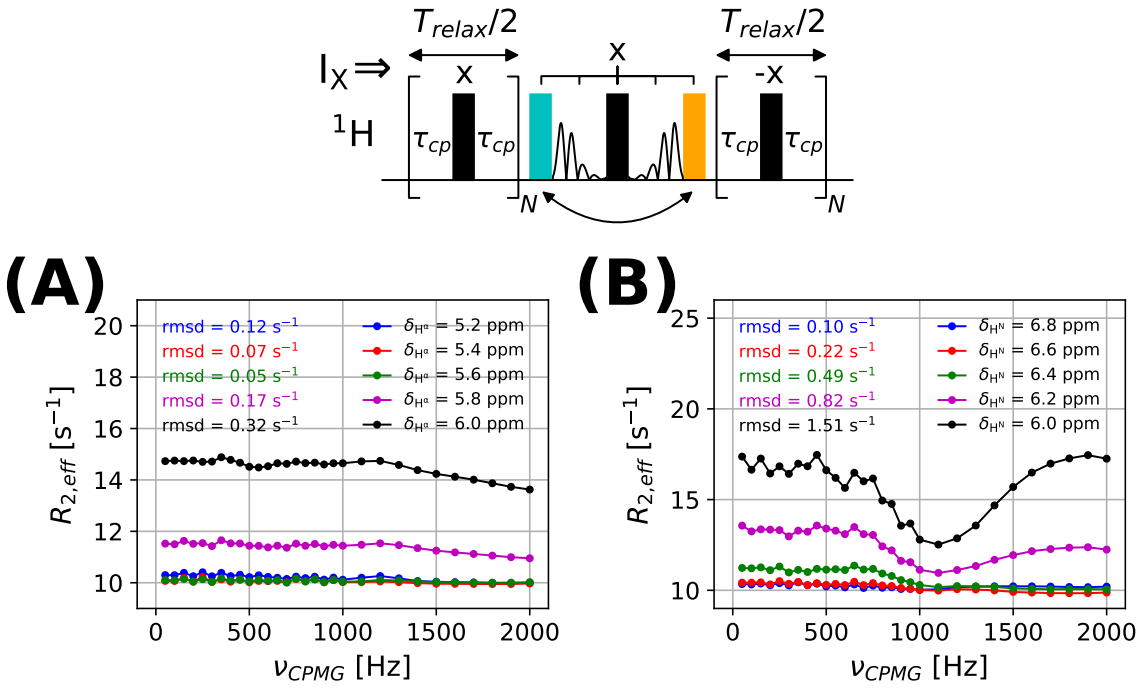
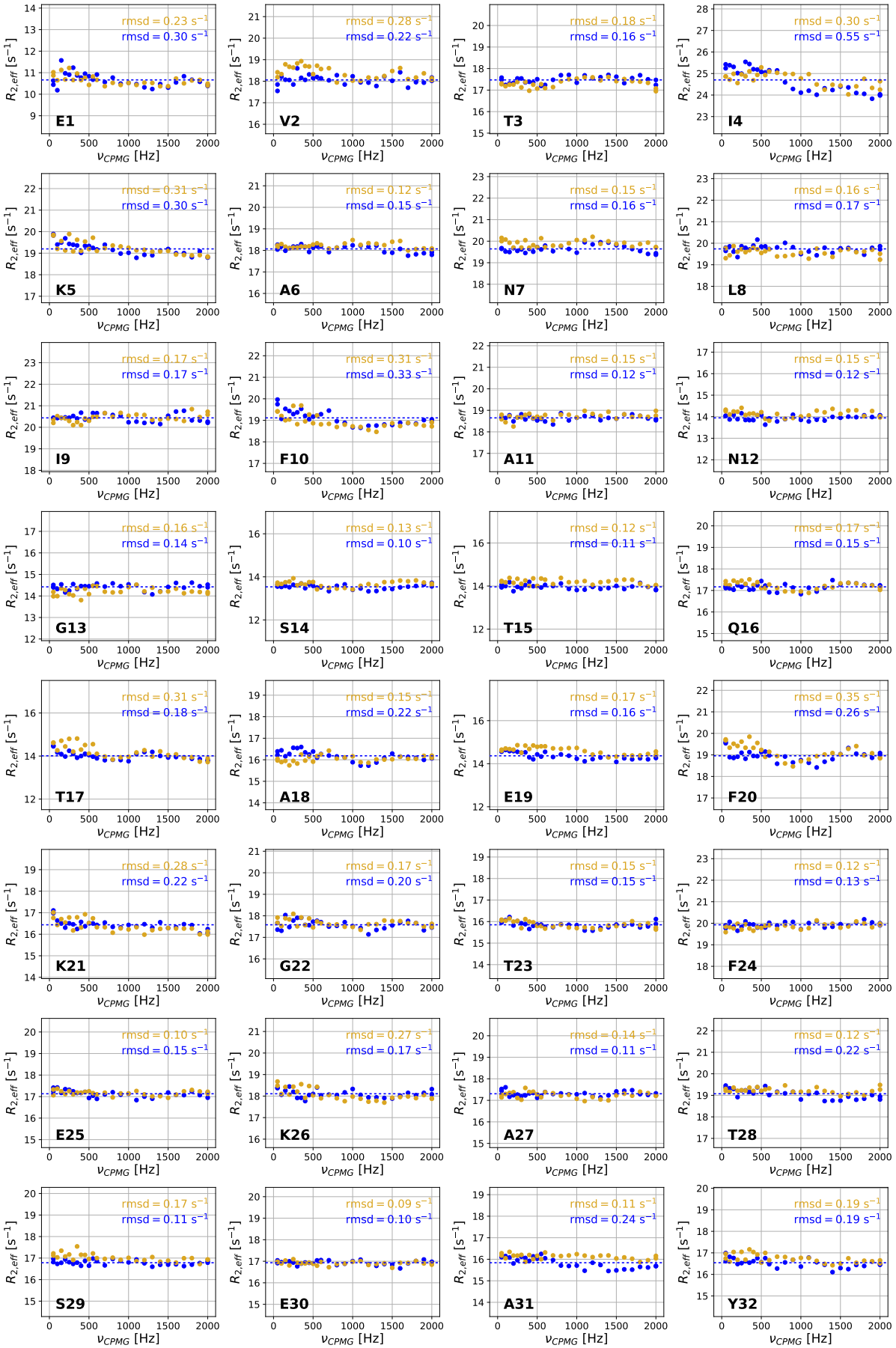


Fig. S3 Numerical simulations of $^1H^N$ dispersion profiles, considering a three-spin {$^1H^N$, $^1H^\alpha$, $^1H^\beta$}, spin-system as a function of $^1H^\alpha$ (A) or $^1H^N$ (B) chemical shifts. The pulse scheme used is shown above the profiles. Values of T_{relax} = 20 ms and ν_1 = 25 kHz were used for all simulations, that assumed a ^1H resonance frequency of 800 MHz. Scalar couplings were $^3J_{HNH\alpha}$ = 10 Hz, $^4J_{HNH\beta}$ = 2 Hz and $^3J_{H\alpha H\beta}$ = 10 Hz and a full $^1H^N$–$^1H^\alpha$ scalar-coupled Hamiltonian was considered. The ^1H RF carrier was positioned at 8.2 ppm. In panel A the $^1H^N$ chemical shift is 8.2 ppm and a number of far downfield $^1H^\alpha$ chemical shifts are considered while in panel B the $^1H^\alpha$ resonance position is 4.5 ppm and several far upfield $^1H^N$ chemical shifts are used. The RF carrier is placed at 8.2 ppm and the EBURP excitation bandwidth is 4.0 ppm (B_1 field of 2.8 kHz). In a few pathological cases where the EBURP excitation profile leads to incomplete excitation of the amides or partial excitation of α-protons artifacts are observed. Nearly identical profiles were obtained when $^3J_{H\alpha H\beta}$ = 0 Hz, indicating effectively no transfer of magnetization past $^1H^\alpha$.


E1 rmsd = 0.23 s⁻¹ rmsd = 0.30 s⁻¹
V2 rmsd = 0.28 s⁻¹ rmsd = 0.22 s⁻¹
T3 rmsd = 0.18 s⁻¹ rmsd = 0.16 s⁻¹
I4 rmsd = 0.30 s⁻¹ rmsd = 0.55 s⁻¹
K5 rmsd = 0.31 s⁻¹ rmsd = 0.30 s⁻¹
A6 rmsd = 0.12 s⁻¹ rmsd = 0.15 s⁻¹
N7 rmsd = 0.15 s⁻¹ rmsd = 0.16 s⁻¹
L8 rmsd = 0.16 s⁻¹ rmsd = 0.17 s⁻¹
I9 rmsd = 0.17 s⁻¹ rmsd = 0.17 s⁻¹
F10 rmsd = 0.31 s⁻¹ rmsd = 0.33 s⁻¹
A11 rmsd = 0.15 s⁻¹ rmsd = 0.12 s⁻¹
N12 rmsd = 0.15 s⁻¹ rmsd = 0.12 s⁻¹
G13 rmsd = 0.16 s⁻¹ rmsd = 0.14 s⁻¹
S14 rmsd = 0.13 s⁻¹ rmsd = 0.10 s⁻¹
T15 rmsd = 0.12 s⁻¹ rmsd = 0.11 s⁻¹
Q16 rmsd = 0.17 s⁻¹ rmsd = 0.15 s⁻¹
T17 rmsd = 0.31 s⁻¹ rmsd = 0.18 s⁻¹
A18 rmsd = 0.15 s⁻¹ rmsd = 0.22 s⁻¹
E19 rmsd = 0.17 s⁻¹ rmsd = 0.16 s⁻¹
F20 rmsd = 0.35 s⁻¹ rmsd = 0.26 s⁻¹
K21 rmsd = 0.28 s⁻¹ rmsd = 0.22 s⁻¹
G22 rmsd = 0.17 s⁻¹ rmsd = 0.20 s⁻¹
T23 rmsd = 0.15 s⁻¹ rmsd = 0.15 s⁻¹
F24 rmsd = 0.12 s⁻¹ rmsd = 0.13 s⁻¹
E25 rmsd = 0.10 s⁻¹ rmsd = 0.15 s⁻¹
K26 rmsd = 0.27 s⁻¹ rmsd = 0.17 s⁻¹
A27 rmsd = 0.14 s⁻¹ rmsd = 0.11 s⁻¹
T28 rmsd = 0.12 s⁻¹ rmsd = 0.22 s⁻¹
S29 rmsd = 0.17 s⁻¹ rmsd = 0.11 s⁻¹
E30 rmsd = 0.09 s⁻¹ rmsd = 0.10 s⁻¹
A31 rmsd = 0.11 s⁻¹ rmsd = 0.24 s⁻¹
Y32 rmsd = 0.19 s⁻¹ rmsd = 0.19 s⁻¹
$R_{2,eff}$ [s^{-1}]
ν_{CPMG} [Hz]

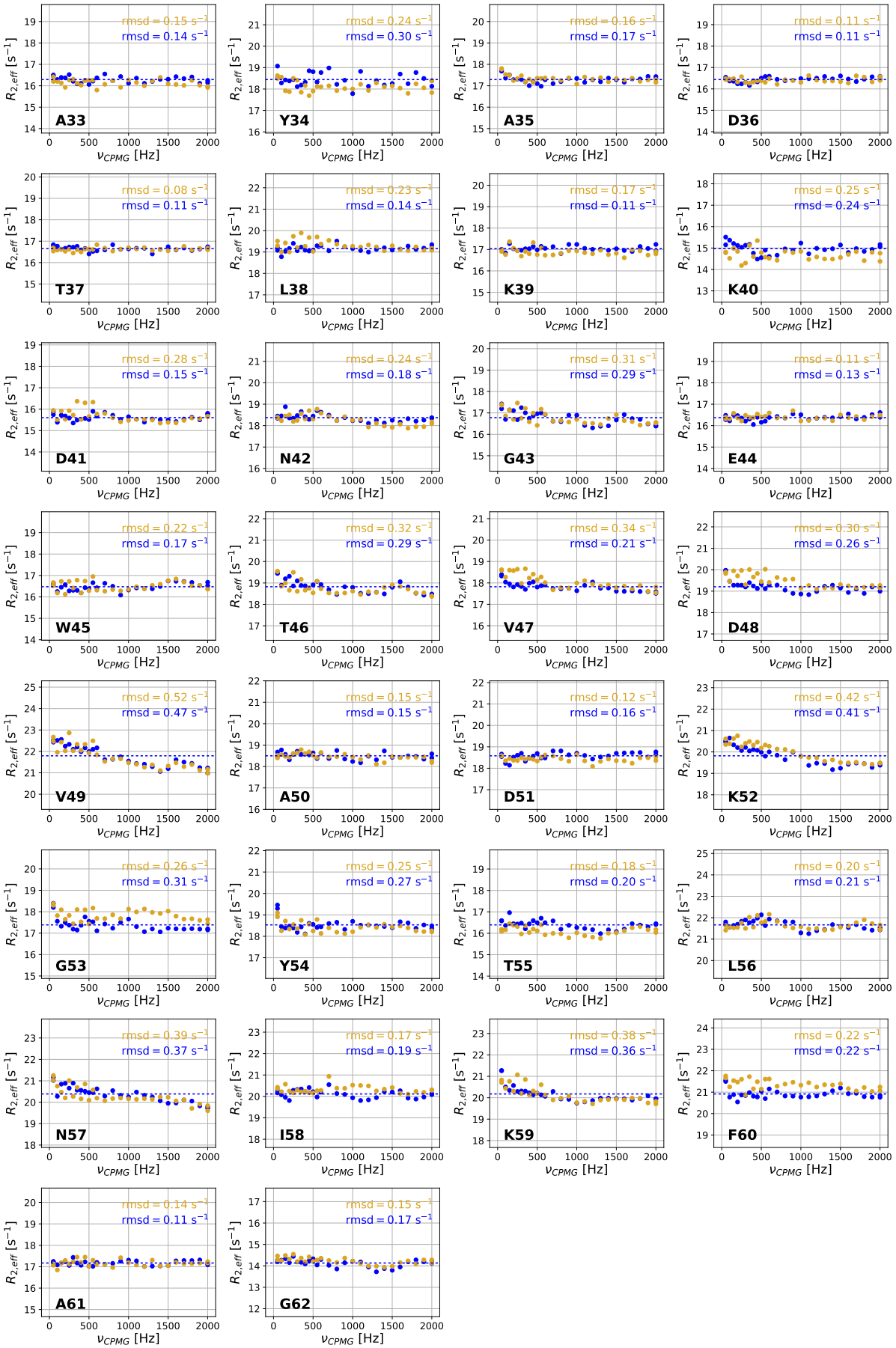

A33
rmsd = 0.15 s⁻¹
rmsd = 0.14 s⁻¹
Y34
rmsd = 0.24 s⁻¹
rmsd = 0.30 s⁻¹
A35
rmsd = 0.16 s⁻¹
rmsd = 0.17 s⁻¹
D36
rmsd = 0.11 s⁻¹
rmsd = 0.11 s⁻¹
T37
rmsd = 0.08 s⁻¹
rmsd = 0.11 s⁻¹
L38
rmsd = 0.23 s⁻¹
rmsd = 0.14 s⁻¹
K39
rmsd = 0.17 s⁻¹
rmsd = 0.11 s⁻¹
K40
rmsd = 0.25 s⁻¹
rmsd = 0.24 s⁻¹
D41
rmsd = 0.28 s⁻¹
rmsd = 0.15 s⁻¹
N42
rmsd = 0.24 s⁻¹
rmsd = 0.18 s⁻¹
G43
rmsd = 0.31 s⁻¹
rmsd = 0.29 s⁻¹
E44
rmsd = 0.11 s⁻¹
rmsd = 0.13 s⁻¹
W45
rmsd = 0.22 s⁻¹
rmsd = 0.17 s⁻¹
T46
rmsd = 0.32 s⁻¹
rmsd = 0.29 s⁻¹
V47
rmsd = 0.34 s⁻¹
rmsd = 0.21 s⁻¹
D48
rmsd = 0.30 s⁻¹
rmsd = 0.26 s⁻¹
V49
rmsd = 0.52 s⁻¹
rmsd = 0.47 s⁻¹
A50
rmsd = 0.15 s⁻¹
rmsd = 0.15 s⁻¹
D51
rmsd = 0.12 s⁻¹
rmsd = 0.16 s⁻¹
K52
rmsd = 0.42 s⁻¹
rmsd = 0.41 s⁻¹
G53
rmsd = 0.26 s⁻¹
rmsd = 0.31 s⁻¹
Y54
rmsd = 0.25 s⁻¹
rmsd = 0.27 s⁻¹
T55
rmsd = 0.18 s⁻¹
rmsd = 0.20 s⁻¹
L56
rmsd = 0.20 s⁻¹
rmsd = 0.21 s⁻¹
N57
rmsd = 0.39 s⁻¹
rmsd = 0.37 s⁻¹
I58
rmsd = 0.17 s⁻¹
rmsd = 0.19 s⁻¹
K59
rmsd = 0.38 s⁻¹
rmsd = 0.36 s⁻¹
F60
rmsd = 0.22 s⁻¹
rmsd = 0.22 s⁻¹
A61
rmsd = 0.14 s⁻¹
rmsd = 0.11 s⁻¹
G62
rmsd = 0.15 s⁻¹
rmsd = 0.17 s⁻¹
$R_{2,eff}$ [s^{-1}]
ν_{CPMG} [Hz]

Fig. S4 ***(previous pages)*** $^{1}H^{N}$ CPMG dispersion profiles recorded on a fully protonated protein L sample, 800 MHz, 25 °C, using the pulse scheme of Figure 3 with either [0013] (blue) or [0000] (gold) phase cycling of the CPMG pulses. Residues V49 and K52 have small CPMG relaxation dispersions, suggesting millisecond timescale dynamics at these positions. The rmsd for each dispersion profile is shown in each panel.

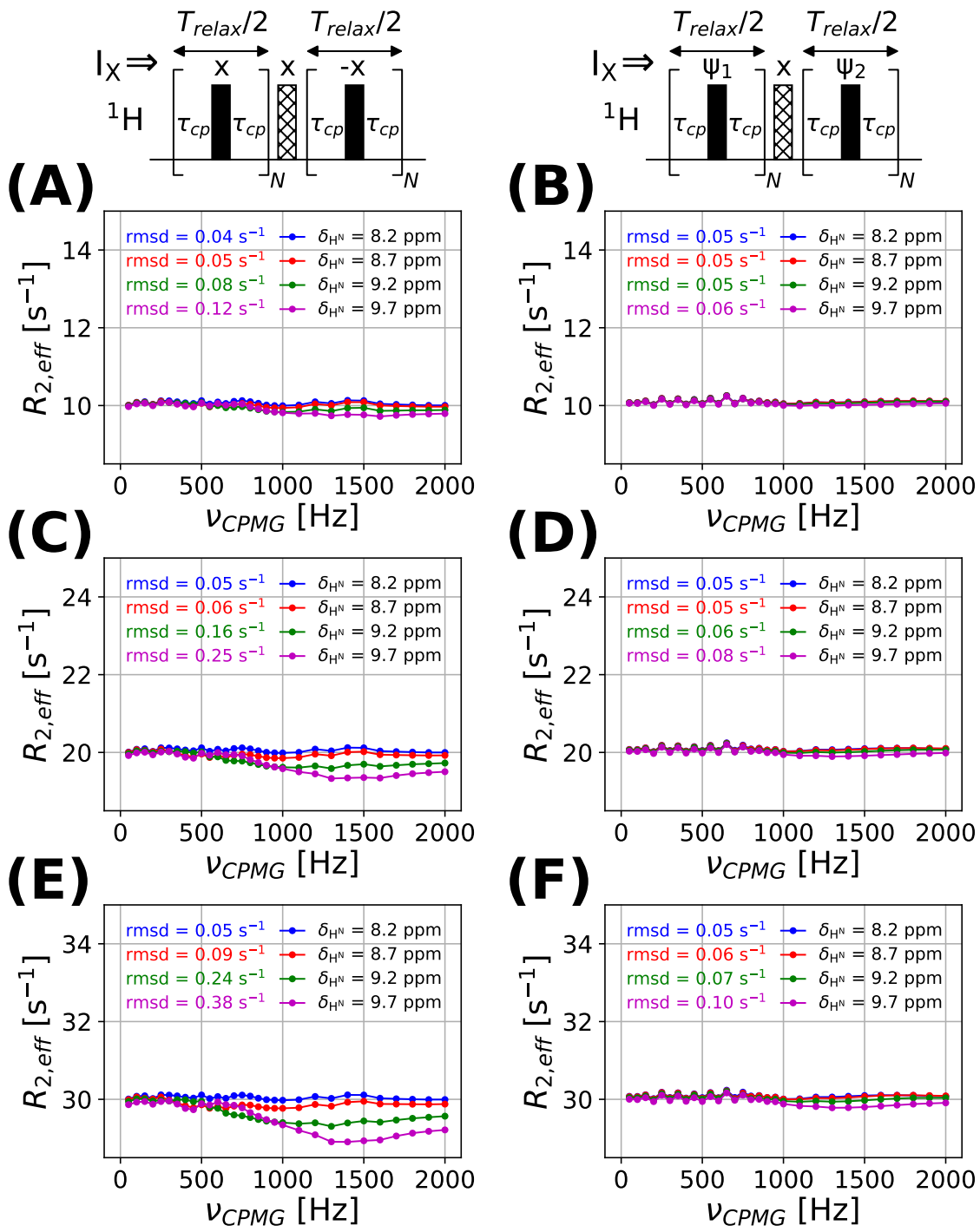

(A)
(B)
(C)
(D)
(E)
(F)
$T_{relax}/2$
$T_{relax}/2$
$I_X \Rightarrow$
^1H
x
-x
ψ_1
ψ_2
τ_{cp}
N
$R_{2,eff}$ [s^{-1}]
ν_{CPMG} [Hz]
rmsd = 0.04 s^{-1}
rmsd = 0.05 s^{-1}
rmsd = 0.08 s^{-1}
rmsd = 0.12 s^{-1}
rmsd = 0.05 s^{-1}
rmsd = 0.05 s^{-1}
rmsd = 0.05 s^{-1}
rmsd = 0.06 s^{-1}
rmsd = 0.05 s^{-1}
rmsd = 0.06 s^{-1}
rmsd = 0.16 s^{-1}
rmsd = 0.25 s^{-1}
rmsd = 0.05 s^{-1}
rmsd = 0.05 s^{-1}
rmsd = 0.06 s^{-1}
rmsd = 0.08 s^{-1}
rmsd = 0.05 s^{-1}
rmsd = 0.09 s^{-1}
rmsd = 0.24 s^{-1}
rmsd = 0.38 s^{-1}
rmsd = 0.05 s^{-1}
rmsd = 0.06 s^{-1}
rmsd = 0.07 s^{-1}
rmsd = 0.10 s^{-1}
δ_{H^N} = 8.2 ppm
δ_{H^N} = 8.7 ppm
δ_{H^N} = 9.2 ppm
δ_{H^N} = 9.7 ppm

Fig. S5 ***(previous page)*** Numerical simulations of $^1H^N$ dispersion profiles for a $\{^1H^N, ^1H^\alpha\}$ two-spin, non-exchanging, spin-system using either [0000] (panels A, C, E) or [0013] (panels B, D, F) phase cycling schemes for CPMG pulses. The pulse schemes used are shown above each set of profiles and the central pulse (infinitely short, represented by a hatched bar) is assumed to generate an ideal, selective 180° rotation on $^1H^N$ magnetization. Values of T_{relax} = 20 ms and ν_1 = 25 kHz were used for all simulations, that assumed a ^1H resonance frequency of 800 MHz. $^3J_{HNH\alpha}$ = 10 Hz and a full $^1H^N$–$^1H^\alpha$ scalar-coupled Hamiltonian was considered. The ^1H RF carrier was positioned at 8.2 ppm, with $^1H^N$ chemical shifts indicated in each panel, and the $^1H^\alpha$ chemical shift was set to 4.5 ppm. The intrinsic $^1H^N$ relaxation rates were set to R_1 = 2 s^{-1}, and R_2 = 10 s^{-1} (panels A, B), 20 s^{-1} (panels C, D) or 30 s^{-1} (panels E, F). Note that as the difference in R_1 and R_2 rates increases the profiles simulated with the [0000] scheme become increasingly less flat for off-resonance $^1H^N$ spins. This is because imperfections in the CPMG pulses that are not corrected during the course of the pulse train place magnetization components along the z-axis in addition to the transverse plane so that the relaxation during the τ_{cp} intervals varies in a manner that reflects the amount of X, Y and Z-magnetization present, that in turn depends on ν_{CPMG}. In contrast, the [0013] scheme places magnetization completely in the transverse plane after every 4 pulses, decreasing the effects of differential relaxation. This effect can be taken into account by fitting routines (in the case of the [0000] scheme), however, it is preferable to avoid it altogether.

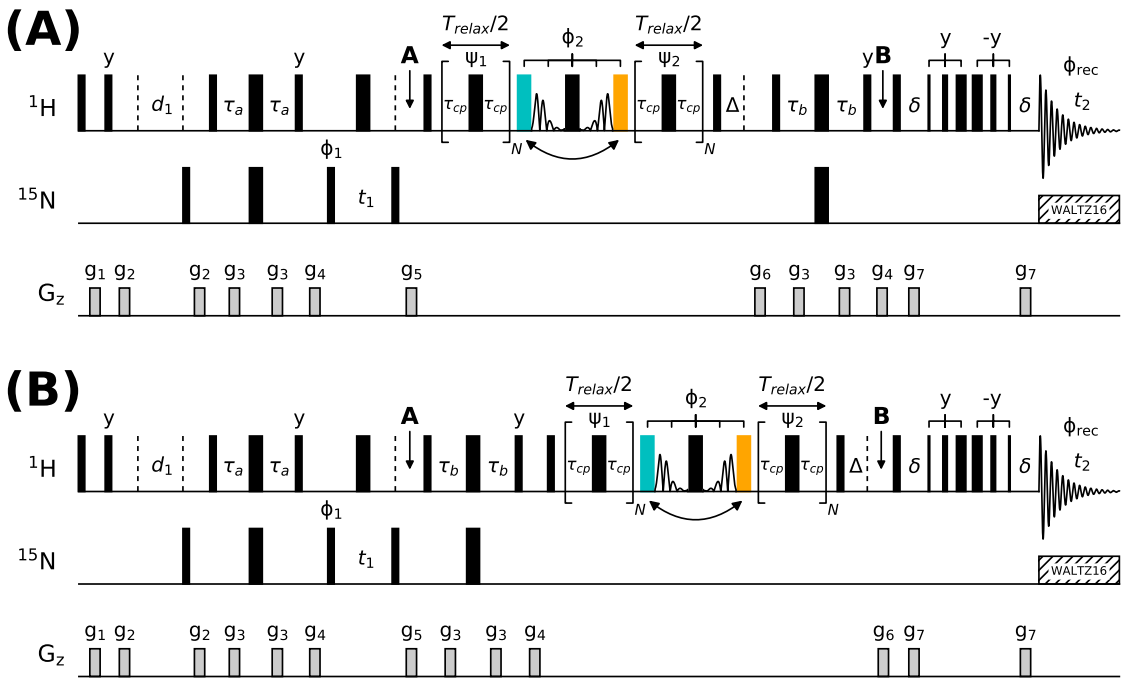


Fig. S6 Modified $^1H^N$ CPMG pulse schemes starting from $^1H^N$ anti-phase (A) or in-phase (B) magnetization. Pulse sequence details are as described in the legend to Figure 3 with τ_b = 2.68 ms. The differential relaxation rates of in-phase and anti-phase coherences can be obtained by recording a series of data sets with different T_{relax} values using a large ν_{CPMG} rate (1–2 kHz) to suppress potential chemical exchange effects. In cases where dispersion profiles are desired that are free of contributions from differential relaxation the resulting curves obtained from sequences (A) and (B) can be added. As described in the text, this effect is small, however.

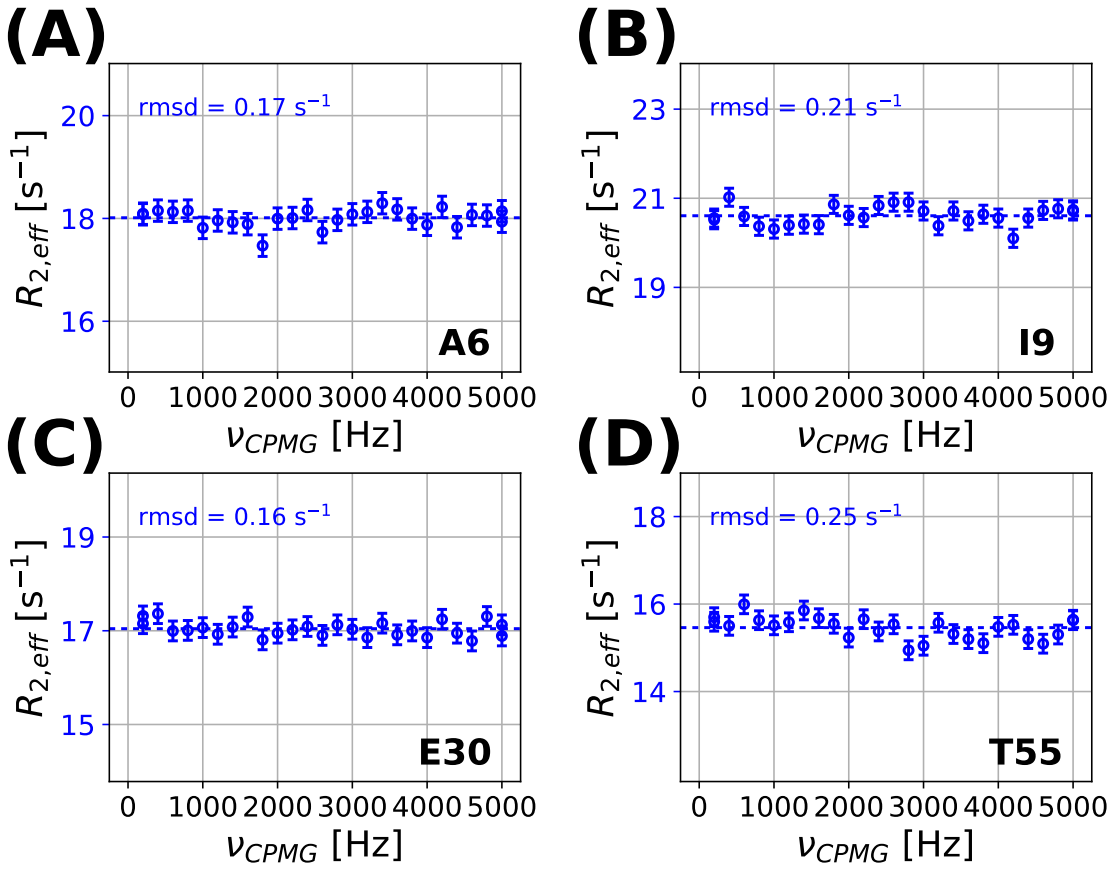


Fig. S7 Experimental $^{1}H^{N}$ CPMG dispersion profiles measured on a fully protonated protein L sample using the pulse scheme of Figure 3, 800 MHz, 25 °C. A value of T_{relax} = 10 ms was used. Profiles from the same residues as in Figure 4 are shown.

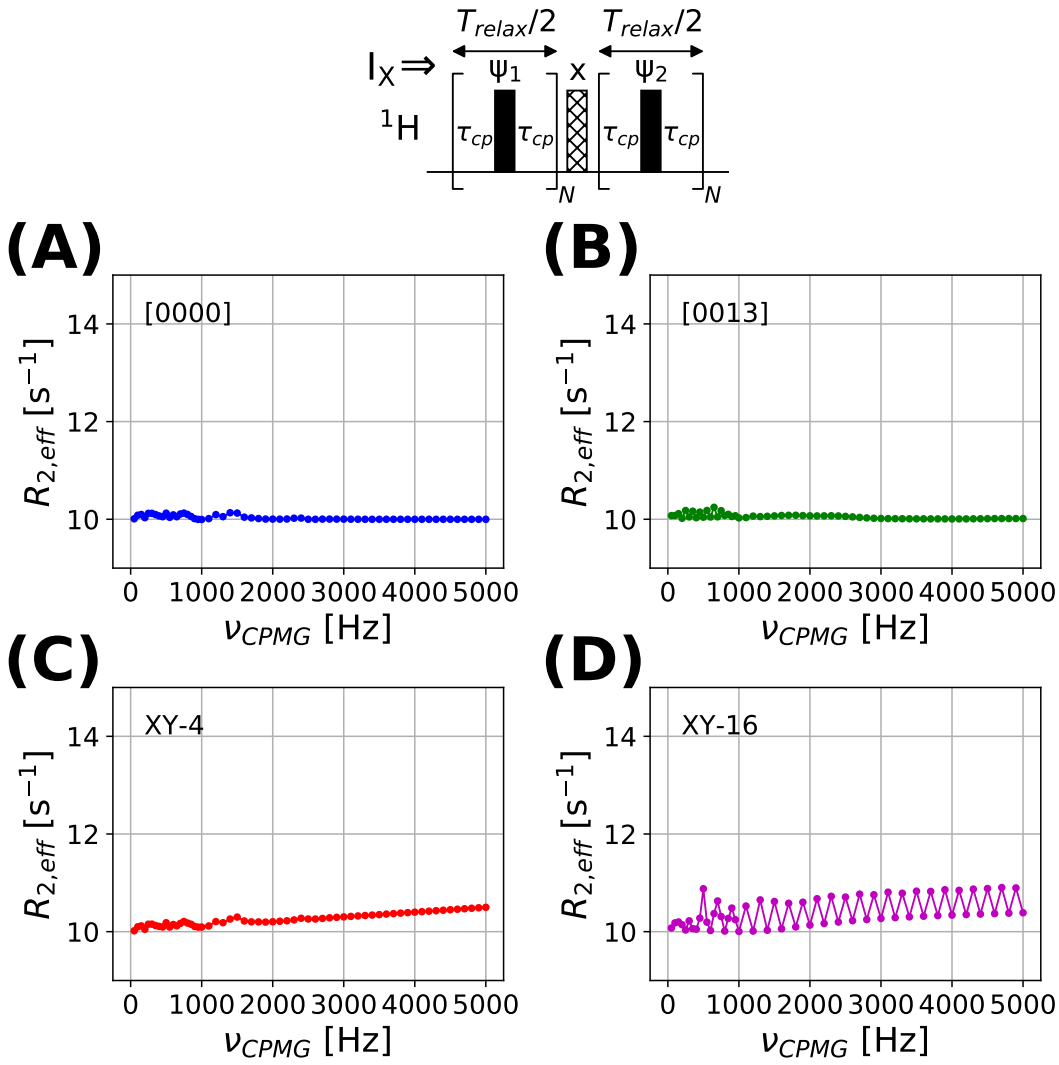


Fig. S8 Numerical simulations of $^1H^N$ dispersion profiles, considering a three-spin {$^1H^N$, $^1H^\alpha$, $^1H^\beta$}, spin-system and different phase schemes ψ_1/ψ_2 for CPMG pulses. The pulse scheme used is shown above the profiles, the central pulse (infinitely short, represented by a hatched bar) is assumed to generate an ideal, selective 180° rotation on $^1H^N$ magnetization. Values of T_{relax} = 20 ms and ν_1 = 25 kHz were used for all simulations, that assumed a ^1H resonance frequency of 800 MHz. Scalar couplings were $^3J_{HNH\alpha}$ = 10 Hz, $^4J_{HNH\beta}$ = 2 Hz and $^3J_{H\alpha H\beta}$ = 10 Hz and a full $^1H^N$–$^1H^\alpha$ scalar-coupled Hamiltonian was considered. The ^1H RF carrier was positioned at 8.2 ppm, with $^1H^N$, $^1H^\alpha$ and $^1H^\beta$ chemical shifts set to 8.2 ppm, 4.5 ppm and 2.0 ppm respectively. The profile in panel A is simulated with constant phase for all CPMG pulses, ψ_1 = x, ψ_2 = −x, while profiles in panels B, C and D are simulated with [0013] (current scheme), XY-4, and XY-16 phase cycles, respectively.

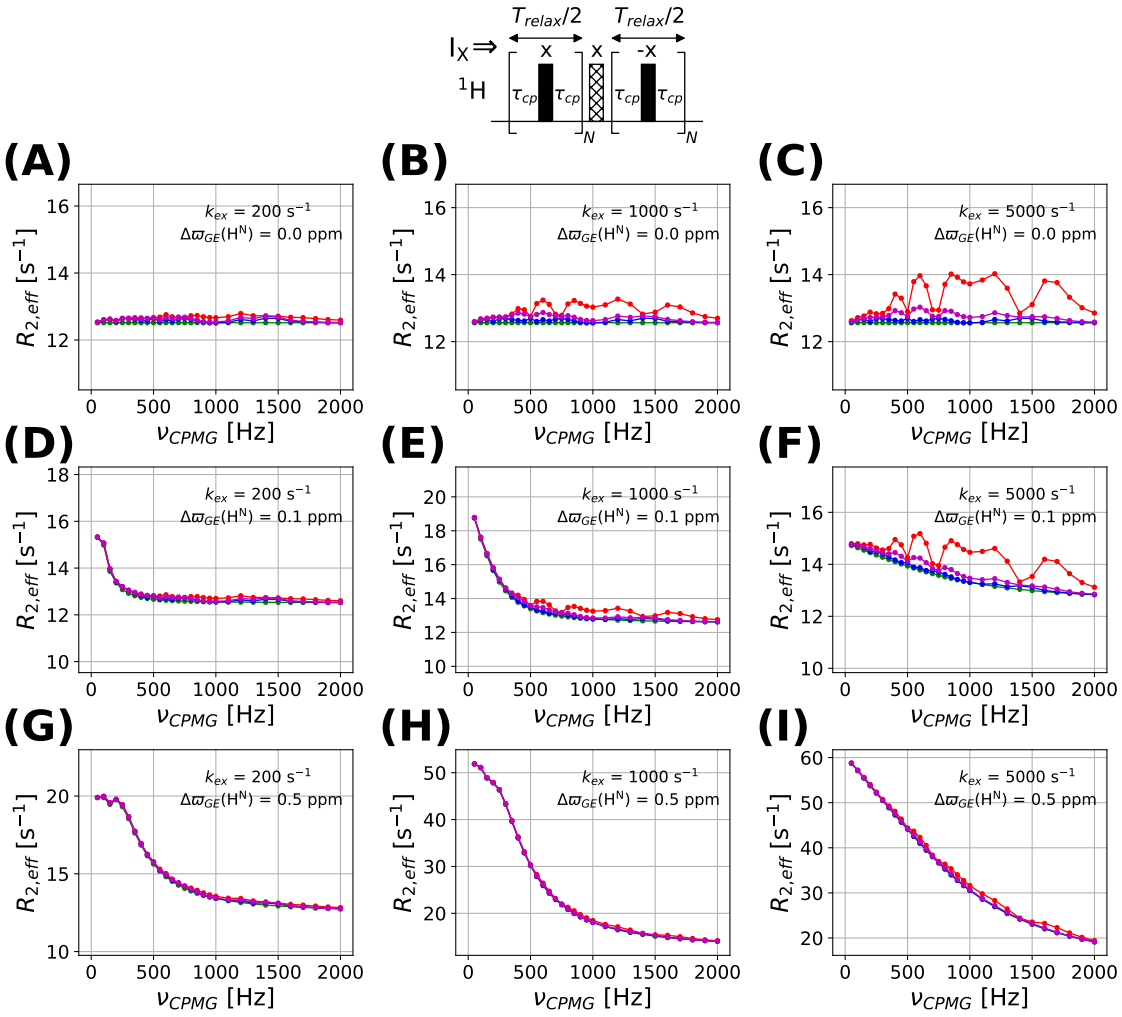


Fig. S9 Simulated $^1H^N$ CPMG dispersion profiles using the pulse scheme shown above the plots for $\Delta\varpi_{GE}(^1H^\alpha)$ = 0 ppm (blue), $\Delta\varpi_{GE}(^1H^\alpha)$ = 0.5 ppm (magenta), $\Delta\varpi_{GE}(^1H^\alpha)$ = 2.5 ppm (red), $\Delta\varpi_{GE}(^1H^N)$ = 0 ppm, 0.1 ppm and 0.5 ppm (shown in each panel), p_E = 0.10, k_{ex} = 200 s^{-1}, 1000 s^{-1} and 5000 s^{-1} (shown in each panel). $^3J_{HNH\alpha}$ = 10 Hz for all curves with the exception of those in green for which $^3J_{HNH\alpha}$ = 0 Hz. Values of T_{relax} = 20 ms and ν_1 = 25 kHz were used for all simulations, that assumed a ^1H resonance frequency of 800 MHz. The ^1H RF carrier was positioned at 8.2 ppm, on resonance with the amide proton (ground state), while the chemical shift of the $^1H^\alpha$ spin (ground state) is 4.5 ppm. The central, hatched pulse inverts the $^1H^N$ spins selectively and is of infinitely short duration.

```
/* NH_H1CPMG_unenh_lek_800_cp

   This pulse sequence will allow one to perform the following experiment:
   1HN relaxation dispersion, detected via 1H-15N unenhanced HSQC

    2D       F1       15N
             F2(acq)  1H (NH)

    Experiment setup-
    Uses three channels:
        f1)  1H        - SFO1 (tof)  @  4.73ppm [H2O]
        f2) 13C        - SFO2 (dof)  @117.00ppm [middle between Calpha and C']
        f3) 15N        - SFO3 (dof2) @119.00ppm [centre of amide 15N]

    FnMODE: F1-States, F2-QF, F3-QF

    Flags (zgotns)
        fsat            'y' for presaturation of H2O
        fscuba          'y' to apply scuba pulse after presaturation of H2O
        mess_flg        'y' for Messerle type purging pulse
        f1180           'y' for 180 deg linear phase correction in F1
                            otherwise 0 deg linear phase correction
        c_flg           'y' for 15N and 13C labelled samples
        H_heat          'y' to get same heating effects as 15N CW CPMG expt
                            due to 1H CW

        Standard Settings
        fsat='n',fscuba='n',mess_flg='n',f1180='y',c_flg='n' H_heat='n'
        - i.e. zgoptns -Df1180

   ncyc_cp can be set as odd or even, ncyc_cp_max should be set properly

   For REBURP/EBURP pulses also need to check PHCOR2

   For fully protonated samples -Deburp_flg option shows better performance than
     -Dreburp_flg (i.e. less artifact caused by 1H-1H J-couplings) while overall
     pulse duration becomes longer. For deuterated samples it is not necessary to
     use -Deburp_flg or -Dreburp_flg

   Modified on Jan 31, 2019 to add 3-9-19 watergate option by using -Dwgate_flg

   Modified on May 1, 2019 to add -Dipap_flg option for measuring IP/AP differential
     relaxation, this option can also suppress IP/AP differential relaxation by
     measuring two separate datasets starting from AP (l3=0) and IP (l3=1)
     respectively (F3-axis dimension should be set as 2 for such purpose). When
     -Dipap_flg is not used it always starts from AP for CPMG
*/

prosol relations=<triple>

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

/***********************/
/*   Define phases     */
/***********************/
#define zero ph=0.0
#define one ph=90.0
#define two ph=180.0
#define three ph=270.0

/*****************************************/
/* Calculate parameters for shaped pulses */
/*****************************************/
#ifdef eburp_flg
  "p9=4.6/(cnst2*bf1/1e6)"    /*  EBURP pulse length   */
  "spw9=plw17*(pow((p17/p9)/0.06103,2))"    /* EBURP power level  */
  "spw19=spw9"
```

```
  "spoal9=1"
  "spoal19=0"
  "spoff9=0"
  "spoff19=0"
#endif

#ifdef reburp_flg
  "p16=4.875/(cnst2*bf1/1e6)"   /* REBURP pulse length  */
  "spw16=plw17*(pow((p17*2.0/p16)/0.07981,2))"   /* REBURP power level  */

  "spoff16=0"
  "spoal16=0.5"
#endif

/*************************/
/*    Define delays       */
/*************************/
define delay hscuba      /* length of 1/2 scuba delay */
  "hscuba=30m"

define delay taua        /* 1 / 4J(XH)                */
  "taua=d3"              /* 2.25m use JNH=105 to decreas the tauxh duration */

define delay time_T2
  "time_T2=d17"

#ifdef H_heat
  define delay time_T2_N /* used only when heating is to be the same as 15N CW expt du
e to 1H CW */
    "time_T2_N=d18"
#endif

define delay tauCPMG
define delay tauCPMG1

"d11=30m"
"in0=inf1/2"             /* t1/2 increment */
"TAU2=0.2u"

#ifdef wgate_flg
  define delay tau_wgate
    "tau_wgate = abs(1.0/(4.0*cnst1))*2.0"
#endif

/************************/
/*    Define pulses      */
/************************/
define pulse dly_pg1     /* Messerle purge pulse */
  "dly_pg1=5m"

define pulse dly_pg2     /* Messerle purge pulse */
  "dly_pg2=dly_pg1/1.62"

define pulse pwh
  "pwh=p1"               /* 1H hard pulse at power level pl1 (tpwr) */

define pulse pwn
  "pwn=p3"               /* PW90 for N pulse at power level pl3 (dhpwr2)  */

#ifndef wgate_flg
  define pulse pw_sl
    "pw_sl=p13"

  define pulse pw_sl1
    "pw_sl1=p14"
#endif

define pulse pwh_cp
```

```
  "pwh_cp=p15"           /* 1H 90 degree pulse for 1H CPMG at tpwr_cp, pl15 */

#ifdef eburp_flg
  define pulse pwh_eburp
    "pwh_eburp=p9"
  define pulse pwh_eburptr
    "pwh_eburptr=p9"
#endif

#ifdef reburp_flg
  define pulse pwh_reb
    "pwh_reb=p16"        /* 1H reburp 180 pulse at tpwr_reb, pl16 */
#endif

#ifdef c_flg
  define pulse pwc_ad
    "pwc_ad=p22"         /* PW180 for C adiabatic inversion pulse at power level spw22
 (dpwr_ad)   */
#endif

#ifdef N_sel
  define pulse pwn_sl
    "pwn_sl=p32"
#endif

/*********************************/
/*    Define other parameters     */
/*********************************/
define list<loopcounter> ncyc_cp=<$VCLIST>

define loopcounter ncyc_cp_max /* max value of ncyc used */
  "ncyc_cp_max=l8"

/*****************************************************************/
/* f1180: Start t1 at half dwell to get -90/180 phase correction */
/*        in F1 (15N) dim - set zgoptns -Df1180                  */
/*****************************************************************/
#ifdef f1180
  "d0=(in0/2)"
#else
  "d0=(0.2u/2)"
#endif

/*******************************************************/
/* Assign cnsts to check validity of parameter range */
/*******************************************************/
#ifdef fsat
  "cnst10=plw10"         /* tsatpwr - set max at 0.00005W */
#endif

#ifdef H_heat
  "cnst12=plw12"         /* tpwrml - set max at 9W */
#endif

#ifndef wgate_flg
  "cnst13=spw13"
  "cnst14=spw14"

  "spoal13=0"
  "spoal14=1"
  "spoff13=0"
  "spoff14=0"
#endif

  "cnst15=plw15"         /* tpwrcp - set max at 15W */

#ifdef c_flg
  "cnst22=spw22"         /* dpwr_ad - set max at 50W */
  "spoff22=0"
```

```
  "spoal22=0.5"

  "plw2=0"
#endif

#ifdef N_sel
  "cnst32=spw32"
  "spoal32=0.5"
#endif

  "cnst3=plw3"           /* dhpwr2 - set max at 150W */
  "cnst31=plw31"         /* dpwr2 - set max at 8W */

/**********************/
/* Define CPMG pulses */
/**********************/
#define cpmg_F if "nsdone%2 == 0" {\n (pwh_cp*2.0 ph11):f1 \n}\n else {\n (pwh_cp*2.0
ph12):f1 \n}
#define cpmg_R if "nsdone%2 == 0" {\n (pwh_cp*2.0 ph21):f1 \n}\n else {\n (pwh_cp*2.0
ph22):f1 \n}

/************************/
/* Initialize variables */
/************************/
"l2=0"
"l4=0"
"l5=0"

#ifndef ipap_flg
  "l3=0"
#endif

"acqt0=0"
baseopt_echo

/*******************************/
/* BEGIN ACTUAL PULSE SEQUENCE */
/*******************************/
1 ze
/*****************************************************************/
/* Check validity of parameters and assign values to some of them */
/*****************************************************************/
#ifdef fsat
  if "cnst10 > 0.00005" {
    2u
    print "error: tsatpwr pl10 too large !!!  "
    goto HaltAcqu
  }
#endif

#ifdef H_heat
  if "cnst12 > 9" {
    2u
    print "error: tpwrml pl12 too large !!!  "
    goto HaltAcqu
  }
#endif

#ifndef wgate_flg
  if "cnst13 > 0.001" {
    2u
    print "error: tpwrsl spw13 too large !!!  "
    goto HaltAcqu
  }

  if "cnst14 > 0.001" {
    2u
    print "error: tpwrsl1 spw14 too large !!!  "
    goto HaltAcqu
```

```
  }
#endif

  if "cnst15 > 15" {
    2u
    print "error: tpwr_cp pl15 too large !!!  "
    goto HaltAcqu
  }

#ifdef c_flg
  if "cnst22 > 50" {
    2u
    print "error: dpwr_ad spw22 too large !!!  "
    goto HaltAcqu
  }
#endif

#ifdef N_sel
  if "cnst32 > 120" {
    2u
    print "error: power level for selective 15N pulse spw32 too large < 120W !!!  "
    goto HaltAcqu
  }
#endif

  if "cnst3 > 150" {
    2u
    print "error: dhpwr2 pl3 too large !!!  "
    goto HaltAcqu
  }

  if "cnst31 > 8" {
    2u
    print "error: dpwr2 pl31 too large !!!  "
    goto HaltAcqu
  }

  if "pwh_cp < 8u" {
    2u
    print "error: pwh_cp p15 too short!!!  "
    goto HaltAcqu
  }

  if "pwh_cp > 20u" {
    2u
    print "error: pwh_cp p15 too long !!!  "
    goto HaltAcqu
  }

  if "time_T2 > 40m" {
    2u
    print "error:time_T2 too long"
    goto HaltAcqu
  }

  if "aq > 80m" {
    2u
    print "error: aq is too long"
    goto HaltAcqu
  }

#ifdef H_heat
  if "time_T2_N > 40m" {
    2u
    print "error:time_T2_N too long"
    goto HaltAcqu
  }
#endif
```

```
#if defined(eburp_flg) && defined(reburp_flg)
  2u
  print "error:eburp_flg and reburp_flg should not be used together"
  goto HaltAcqu
#endif

2 d11 do:f3
/************************/
/* Update list pointers */
/************************/
  2u
  "ncyc_cp.idx=l2"
  2u rpp11 rpp12 rpp21 rpp22

/****************************************/
/* Continue to check real time variables  */
/****************************************/
  "l4 = (trunc(ncyc_cp + 0.3))"
  "l5 = (trunc(ncyc_cp + 0.3 - 1))"
  2u

  if "ncyc_cp > 0" {
    "tauCPMG = time_T2*0.25/ncyc_cp - pwh_cp*0.75"
    "tauCPMG1 = tauCPMG - pwh_cp*2.0/PI"

    if "tauCPMG + pwh_cp < 30.0u" {
      2u
      print "error: tauCPMG < 30u too short for CPMG"
      goto HaltAcqu
    }
  }

  if "ncyc_cp_max > 80" {
    2u
    print "error: tauCPMG too short for CPMG"
    goto HaltAcqu
  }

  if "ncyc_cp > ncyc_cp_max" {
    2u
    print "error: ncyc_cp_max must be greater than or equal to ncyc_cp !!!"
    goto HaltAcqu
  }

/*********************/
/* 1H Heating period */
/*********************/
  4u pl17:f1

#ifdef H_heat
  4u pl12:f1
  time_T2_N cw:f1 ph26
  2u do:f1
#endif

/************************************************/
/* Destroy residual 1H magnetization prior to d1 */
/************************************************/
  4u pl1:f1              /* power pl1 for 1H pulses */
  20u UNBLKGRAD

  (pwh ph26):f1

  2u
  p51:gp1*0.5
  d16

  (pwh ph27):f1
```

```
  2u
  p51:gp1
  d16

  4u BLKGRAD

/*********************************************/
/* Presaturation Period                      */
/* option for Messerle purge zgoptn -Dmess_flg */
/*********************************************/
#ifdef mess_flg
  4u pl11:f1
  (dly_pg1 ph26):f1
  20u
  (dly_pg2 ph27):f1
  4u
#endif

#ifdef fsat
  4u pl10:f1
  d1 cw:f1 ph26
  2u do:f1
  4u pl1:f1
#ifdef fscuba
  hscuba
  (pwh ph26 pwh*2.0 ph27 pwh ph26):f1
  hscuba
#endif

#else
  4u pl1:f1
  d1
#endif

/*********************************************/
/* Eliminate equilibrium magnetization on 15N */
/*********************************************/
  20u UNBLKGRAD

  4u pl3:f3
  (pwn ph26):f3

  2u
  p51:gp1
  d16

/*************************/
/* This is the real start */
/*************************/
  (pwh ph26):f1

  2u
  p52:gp2
  d16

#ifdef N_sel
  "DELTA = taua - 2u - p52 - d16 - pwn_sl*0.5"
  DELTA

  (center (pwh*2.0 ph26):f1 (pwn_sl:sp32 ph26):f3)

  DELTA pl3:f3
#else
  "DELTA = taua - 2u - p52 - d16"
  DELTA

  (center (pwh*2.0 ph26):f1 (pwn*2.0 ph26):f3)

  DELTA
```

```
#endif

  2u
  p52:gp2
  d16

  (pwh ph27):f1

  2u
  p53:gp3
  d16

  (pwn ph1):f3

  "TAU1=larger(d0-pwn*2.0/PI-pwh*2.0, TAU2)"

#ifdef c_flg
  if "d0 - pwn*2.0/PI - pwc_ad*0.5 > 0.2u" {
    "DELTA = d0 - pwn*2.0/PI - pwc_ad*0.5"
    DELTA
    (center (pwh ph26 pwh*2.0 ph27 pwh ph26):f1 (pwc_ad:sp22 ph26):f2)
    DELTA
  }
  else {
    TAU1
    (pwh ph27 pwh*2.0 ph26 pwh ph27):f1
    TAU1
  }
#else
  TAU1
  (pwh ph27 pwh*2.0 ph26 pwh ph27):f1
  TAU1
#endif

  (pwn ph26):f3

  2u
  p54:gp4
  d16

  10u fq=cnst1(sfo hz):f1

#ifdef ipap_flg
  if "l3%2 == 1" {
    (pwh ph26):f1

    2u
    p52:gp2
    d16

    "DELTA = taua - 2u - p52 - d16"
    DELTA

    (center (pwh*2.0 ph26):f1 (pwn*2.0 ph26):f3)

    DELTA

    2u
    p52:gp2
    d16

    (pwh ph27):f1

    2u
    p53:gp3
    d16
  }
#endif
```

```
/* First half of CPMG relaxation period */
  4u pl15:f1
  (pwh_cp ph26):f1

  if "l4 > 0" {
    tauCPMG1
    cpmg_F
    tauCPMG ipp11 ipp12 ipp21 ipp22

    if "l5 > 0" {
5     tauCPMG
      cpmg_F
      tauCPMG ipp11 ipp12 ipp21 ipp22
      lo to 5 times l5
    }
  }

/* Different options for center 180 pulse, EBURP or REBURP */
#ifdef eburp_flg
  if "nsdone%4 < 2" {
    (pwh_cp*2.0 ph2):f1
  }

  4u
  (pwh_eburptr:sp19 ph2:r):f1
  4u pl15:f1

  (pwh_cp*2.0 ph2):f1

  4u
  (pwh_eburp:sp9 ph2:r):f1
  4u pl15:f1

  if "nsdone%4 >= 2" {
    (pwh_cp*2.0 ph2):f1
  }
#else /*eburp_flg*/
#ifdef reburp_flg
  4u
  (pwh_reb:sp16 ph2:r):f1
  4u pl15:f1
#else /*reburp_flg*/
  (pwh_cp*2.0 ph2):f1
#endif /*reburp_flg*/
#endif /*eburp_flg*/

/* Second half of CPMG relaxation period */
  if "l4 > 0" {
    if "l5 > 0" {
6     tauCPMG dpp11 dpp12 dpp21 dpp22
      cpmg_R
      tauCPMG
      lo to 6 times l5
    }

    tauCPMG dpp11 dpp12 dpp21 dpp22
    cpmg_R
    tauCPMG1
  }

  (pwh_cp ph26):f1

  /* Compensation for R1 relaxation during CPMG pulses */
  "DELTA = (ncyc_cp_max - ncyc_cp) * pwh_cp + 2u"
  DELTA

  2u
  p55:gp5
  d16
```

```
  4u pl1:f1

#ifdef ipap_flg
  if "l3%2 == 0" {
    (pwh ph26):f1

    2u
    p52:gp2
    d16

    "DELTA = taua - 2u - p52 - d16"
    DELTA

    (center (pwh*2.0 ph26):f1 (pwn*2.0 ph26):f3)

    DELTA

    2u
    p52:gp2
    d16

    (pwh ph27):f1

    2u
    p53:gp3
    d16
  }
#endif

  10u fq=0(sfo hz):f1

#ifdef ipap_flg
  (pwh ph29):f1
#else
  (pwh ph26):f1
#endif

  2u
  p56:gp6
  d16

#ifdef wgate_flg
#ifdef ipap_flg
  "DELTA = 4u + 4u + de"
  DELTA

  (pwh*0.231 ph27):f1
  tau_wgate
  (pwh*0.692 ph27):f1
  tau_wgate
  (pwh*1.462 ph27):f1
  tau_wgate
  (pwh*1.462 ph29):f1
  tau_wgate
  (pwh*0.692 ph29):f1
  tau_wgate
  (pwh*0.231 ph29):f1

  "DELTA = pwh*2.0/PI"
  DELTA
#else /*ipap_flg*/
  "DELTA = taua - 2u - p56 - d16 - tau_wgate*3.367*0.5"
  DELTA

  (center
  (pwh*0.231 ph27 tau_wgate pwh*0.692 ph27 tau_wgate pwh*1.462 ph27 tau_wgate pwh*1.46
2 ph29 tau_wgate pwh*0.692 ph29 tau_wgate pwh*0.231 ph29):f1
  (pwn*2.0 ph26):f3)
```

```
  "DELTA = taua - tau_wgate*3.367*0.5 - 2u - p56 - d16 - 4u - 4u - de + pwh*2.0/PI"
  DELTA
#endif /*ipap_flg*/
#else /*wgate_flg*/
#ifdef ipap_flg
  "DELTA = larger(pw_sl1, pw_sl) - pw_sl1 + 4u + 4u + de"
  DELTA

  /* shaped pulse */
  4u
  (pw_sl1:sp14 ph28):f1
  4u pl1:f1
  /* shaped pulse */

  (pwh*2.0 ph26):f1

  /* shaped pulse */
  4u
  (pw_sl:sp13 ph28):f1
  4u
  /* shaped pulse */

  "DELTA = larger(pw_sl1, pw_sl) - pw_sl + pwh*2.0/PI"
  DELTA
#else /*ipap_flg*/
  "DELTA = taua - 2u - p56 - d16 - 4u - pw_sl1 - 4u"
  DELTA

  /* shaped pulse */
  4u
  (pw_sl1:sp14 ph28):f1
  4u pl1:f1
  /* shaped pulse */

  (center (pwh*2.0 ph26):f1 (pwn*2.0 ph26):f3)

  /* shaped pulse */
  4u
  (pw_sl:sp13 ph28):f1
  4u
  /* shaped pulse */

  "DELTA = taua - 4u - pw_sl - 4u - 2u - p56 - d16 - 4u - 4u - de + pwh*2.0/PI"
  DELTA
#endif /*ipap_flg*/
#endif /*wgate_flg*/

  2u
  p56:gp6
  d16

  4u pl31:f3
  4u BLKGRAD

/***************************************/
/*   Signal detection and looping      */
/***************************************/
  go=2 ph31 cpds3:f3
  d11 do:f3 mc #0 to 2
    F3QF(calclc(l3, 1))
    F2QF(calclc(l2, 1))
    F1PH(calph(ph1, +90), caldel(d0, +in0) & calph(ph1, +180) & calph(ph31, +180))

HaltAcqu, 1m
exit

ph1=0 2 2 0 2 0 0 2
ph2=1 1 1 1 3 3 3 3 3 3 3 3 1 1 1 1
```

```
ph11=1 1 2 0 1 1 0 2 1 1 0 2 1 1 2 0
ph12=2 0 3 3 0 2 3 3 0 2 3 3 2 0 3 3
ph21=3 3 2 0 3 3 0 2 3 3 0 2 3 3 2 0
ph22=2 0 1 1 0 2 1 1 0 2 1 1 2 0 1 1
ph26=0
ph27=1
ph28=2
ph29=3
ph31=0 2 2 0 2 0 0 2

;d1: Repetition delay d1
;d3: taua (~ 2.4ms < 1/4JNH)
;d11: delay for disk i/o, 30ms
;d13: delay time prior to tap reading pulse
;d16: gradient recovery delay, 200us
;d17: time_T2
;d18: time_T2_N Used only when heating is to be the same as for 15N CW CPMG
;pl1: tpwr - power level for pwh
;pl3: dhpwr2 - power level for 15N pulse pwn
;pl10: tsatpwr - power level for water presat
;pl11: tpwrmess - power level for Messerle purge
;pl12: tpwrml - power level for 1H CW in 15N CPMG - used to preserve heating
;pl15: tpwr_cp - power level for 1H CPMG
;pl17: tpwr_ref - power level for 1H pulse calibration
;pl31: dpwr2 - power level for 15N decoupling
;sp13: tpwrsl - power level for water selective pulse pw_sl
;sp14: tpwrsl1 - power level for water selective pulse pw_sl1
;sp22: dpwr_ad - power level for 13C adiabatic inversion pulse pwc_ad (p22)
;sp32: power level for 15N selective reburp
;spnam9: Eburp2.1000
;spnam13: shape for pw_sl
;spnam14: shape for pw_sl1
;spnam16: Reburp.1000
;spnam19: Eburp2tr.1000
;spnam22: shape for 13C adiabatic pulse
;spnam32: shape for 15N selective pulse to remove Arg
;p1: pwh - 1H 90 degree pulse
;p3: pwn - 15N 90 degree pulse
;p13: pw_sl
;p14: pw_sl1
;p15: pwh_cp
;p16: pwh_reb
;p17: pwh_ref at pl17
;p22: pwc_ad - 13C adiabatic inversion pulse
;p32: pwn_sl - 15N 180 degree selective pulse
;p51: gradient pulse 51                              [1000 usec]
;p52: gradient pulse 52                              [500 usec]
;p53: gradient pulse 53                              [1000 usec]
;p54: gradient pulse 54                              [800 usec]
;p55: gradient pulse 55                              [1000 usec]
;p56: gradient pulse 56                              [800 usec]
;pcpd3 : pwndec - 15N 90 degree pulse at power pl31 for 15N decouling during acqt
;cnst1: set to center of NHs - o1 (Hz)
;cnst2: bandwidth (ppm) of NHs region
;l3: 0 for AP, 1 for IP
;l8: ncyc_cp_max (MUST BE SET PROPERLY!)
;vclist: variable counter list for ncyc_cp
;inf1: 1/SW(X) = 2*DW(X)
;in0: 1/(2*SW(x))=DW(X)
;nd0: 2
;ns: 4*n
;FnMODE: States in F1
;FnMODE: QF in F2
;FnMODE: QF in F3

;for z-only gradients:
;gpz1: 15%
;gpz2: 20%
;gpz3: 30%
```

```
;gpz4: -50%
;gpz5: 40%
;gpy6: 0% (Z-gradient) or 80% (XYZ-gradient)
;gpz6: 80% (Z-gradient) or 0% (XYZ-gradient)

;use gradient files:
;gpnam1: SMSQ10.32
;gpnam2: SMSQ10.32
;gpnam3: SMSQ10.32
;gpnam4: SMSQ10.32
;gpnam5: SMSQ10.32
;gpnam6: SMSQ10.32

;zgoptns: Df1180, Dc_flg, DH_heat, DN_sel, Dwgate_flg, Deburp_flg, Dreburp_flg, Dipap_
flg
```