

## **Supporting Material for**

# **Multiquantum Chemical Exchange Saturation Transfer NMR to Quantify Symmetrical Exchange: Application to Rotational Dynamics of the Guanidinium Group in Arginine Side Chains**

Gogulan Karunanithy<sup>1</sup>, Jochen Reinstein<sup>2</sup>, and D. Flemming Hansen<sup>1,\*</sup>

1) Institute of Structural and Molecular Biology, Division of Biosciences, University College London, London, UK, WC1E 6BT

2) Department of Biomolecular Mechanisms, Max Planck Institute for Medical Research, Jahnstrasse 29, D-69120 Heidelberg, Germany.

\*) To whom correspondence should be addressed, E-mail: [d.hansen@ucl.ac.uk](mailto:d.hansen@ucl.ac.uk)

## Detailed Methods

### *Sample Preparation.*

A sample of L99A T4 Lysozyme was prepared according to previously published protocols<sup>1</sup>. The concentration of D<sub>2</sub>O was kept to a minimum ( $\approx 1\%$ ) to minimise the presence of isotopically shifted <sup>15</sup>N resonances<sup>2</sup>. [<sup>13</sup>C<sub>6</sub>-<sup>15</sup>N<sub>4</sub>]-L-arginine hydrochloride was purchased from Sigma-Aldrich.

### *NMR Spectroscopy.*

For all experiments, the spectrometer temperature was calibrated using a sample of d<sub>4</sub>-methanol and following standard protocols.

Longitudinal EXSY<sup>3</sup> zz-exchange experiments on free [<sup>13</sup>C<sub>6</sub>,<sup>15</sup>N<sub>4</sub>]-L-arginine were carried out and analysed as described previously<sup>1</sup>. All zz-exchange NMR spectra were recorded at a static magnetic field of 14.1 T on a Bruker Avance II spectrometer and using a room-temperature TXI HCN inverse probe. In all of these experiments, 20 mixing times between 2 ms and 300 ms were used, including two repeats for error estimation. The carrier frequencies were set to 4.7 ppm (<sup>1</sup>H) and 70.3 ppm (<sup>15</sup>N). The sweep width in the indirect dimension was set to 16.5 ppm with 40 complex points recorded. Four scans were recorded per transient and the recycle recovery delay (d1) was 1 s, giving a total recording time of  $\sim 2$  hours per experiment.

The presented MQ-CEST profiles were all recorded using the pulse sequence in Fig 1D (with <sup>13</sup>C detection). The parameters employed in the MQ-CEST experiments are detailed in Table S1. The  $B_1$  field strengths and inhomogeneity were calibrated for each radio-frequency probe using the approach of Guenneugues *et al*<sup>4</sup>.

### *Data analysis.*

**Simulation and Fitting of MQ-CEST Data.** For the simulation and fitting of MQ-CEST data in arginine guanidinium groups the three-spin system,  $^{13}\text{C}^{\zeta}{}^{15}\text{N}^{\eta_1}{}^{15}\text{N}^{\eta_2}$ , is considered. The composite decoupling scheme ensures that the <sup>1</sup>H <sup>$\eta$</sup>  nuclei have no significant effect on this spin system during the CEST period, although these protons are an important source of auto-relaxation. All combinations of direct products of the individual spin product operators in  $^{13}\text{C}^{\xi}{}^{15}\text{N}^{\eta_1}{}^{15}\text{N}^{\eta_2}$  and the identity operator give rise to 64 product operators<sup>5</sup> required to fully describe this spin system. To account for the rotational exchange about the C <sup>$\zeta$</sup> -N <sup>$\epsilon$</sup>  bond

(symmetrical exchange of  $N^{\eta_1}$  and  $N^{\eta_2}$ ) a further 63 product operators are required: for convenience (in common with literature conventions) we can refer to the two states as the ground (G) and excited (E) state although the symmetric exchange here means that these states have equal energy and population. This gives a total of 127 product operators, describing the full spin system. In order to fit or simulate MQ-CEST data the Bloch-McConnell equation for the system<sup>6,7</sup> needs to be solved,

$$\frac{d}{dt} \mathbf{m}(t) = -\mathbf{L} \cdot \mathbf{m}(t) \quad (1)$$

Here,  $\mathbf{m}(t)$  is an  $(n \times 1)$  column vector containing the  $n$  product operator terms for the spin system and  $\mathbf{L}$  is the  $(n \times n)$  Liouvillian matrix that describes its dynamics and time-dependence. To calculate the Liouvillian matrix we first write down the Hamiltonian for each spin interaction: in the present case the chemical shift of the two  $^{15}\text{N}^{\eta}$  nuclei, the CEST pulse and  $J$ -couplings between the  $^{15}\text{N}^{\eta}$  nuclei and the  $^{13}\text{C}^{\zeta}$  nucleus are included. The small scalar-coupling between the two  $^{15}\text{N}^{\eta}$  is ignored<sup>8</sup>. For each Hamiltonian,  $\hat{H}$ , the commutator superoperator,  $\hat{\hat{H}}$ , is calculated as:

$$\hat{\hat{H}} = \hat{H} \otimes \hat{E} - \hat{E} \otimes \tilde{\hat{H}} \quad (2)$$

In which  $\hat{E}$  is the identity matrix and the tilde denotes the complex conjugate. The elements of  $\mathbf{L}$  can then be found by transforming  $\hat{\hat{H}}$  to the product operator basis<sup>9</sup>.

Having added these interactions to the Liouvillian, relaxation terms are added to the matrix phenomenologically, following the approach of Helgstrand *et al*<sup>10</sup>. Due to the small gyromagnetic ratios of  $^{13}\text{C}$  and  $^{15}\text{N}$  dipolar interactions between them will be a relatively small source of relaxation<sup>11</sup>, which are ignored. Instead, the auto-relaxations will be dominated by dipolar relaxation to proximal  $^1\text{H}$  nuclei and the chemical shift anisotropy of the individual spins. Consequently, to a good approximation, we can write the spin relaxation of higher order product operators as the sum of the individual longitudinal and transverse single order spin operators:

$$R_{C_j^{\zeta} N_k^{\eta_1} N_l^{\eta_2}} = R_{C_j^{\zeta}} + R_{N_k^{\eta_1}} + R_{N_l^{\eta_2}} \quad (3)$$

Where  $j, k, l \in (x, y, z)$ . It is also assumed that  $R_{N_k^{\eta_1}} = R_{N_k^{\eta_2}}$ . This approach considerably reduces the number of parameters to be optimised in the model and, as shown below, the derived  $k_{\text{ex}}$  and  $\Delta\omega$  values are relatively uncorrelated from the transverse relaxation rates (Fig S8). It should however be noted that the MQ-CEST approach should not be treated as a method of attaining relaxation rates of the various product operator terms. It should also be noted that the  $^{15}\text{N}^\eta$  nuclei will undergo multiexponential relaxation, which we approximate here with a single exponential, as a result of cross-correlated dipolar relaxation with each of the two directly bonded  $^1\text{H}^\eta$  nuclei<sup>12</sup>.

To complete the Liouvillian matrix,  $\mathbf{L}$ , chemical exchange between the two  $^{15}\text{N}^\eta$  nuclei needs to be added. The  $127 \times 127$  exchange matrix  $\mathbf{k}$  is defined as:

$$\mathbf{k} = \begin{pmatrix} 0 & 0 \dots 0 \\ 0 & \begin{pmatrix} k_{GE} & -k_{EG} \\ -k_{GE} & k_{EG} \end{pmatrix} \otimes \mathbf{I}_{63} \\ \vdots & \\ 0 & \end{pmatrix}, \quad (4)$$

where  $k_{GE}$  and  $k_{EG}$  are the forward and backwards rates of rotation, respectively,  $k_{\text{ex}} = k_{GE} + k_{EG}$  and  $\mathbf{I}_{63}$  is the  $63 \times 63$  identity matrix. Since the exchange between  $^{15}\text{N}^{\eta_1}$  and  $^{15}\text{N}^{\eta_2}$  is symmetric,  $k_{EG} = k_{GE}$ . Finally, to obtain the full Liouvillian,  $\mathbf{L}$ ,  $\mathbf{k}$  is added to Eq 2.

To reduce the size of the Liouvillian matrix used to propagate the spin-system, it is noted that, in the MQ-CEST experiment, transverse  $^{13}\text{C}^\zeta$  magnetisation cannot be created and so any terms containing these are ignored. Removing these terms, we arrive at a  $63 \times 63$  matrix,  $\mathbf{L}$ , that describes the evolution of the three-spin  $C^\zeta N^{\eta_1} N^{\eta_2}$  spin system during the MQ-CEST experiment.

Once  $\mathbf{L}$  is known and since  $\mathbf{L}$  is time-independent, Eq. 1 can be solved according to,

$$\mathbf{m}(t) = \exp(-\mathbf{L}t) \cdot \mathbf{m}(0), \quad (5)$$

where Eq. 5 in turn can be solved by directly taking the matrix exponential of  $\mathbf{L}$ , using the Padé approximation (as implemented in the python module *scipy*<sup>13</sup>) or similar. For analysis of the MQ-CEST data, the matrix exponential can be calculated more rapidly through a Taylor expansion with scaling and squaring<sup>14</sup>. The initial condition of the column vector,  $\mathbf{m}(0)$ , is set to  $4C_z^\zeta N_z^{\eta_1} N_z^{\eta_2}(G) = p_G$  and  $4C_z^\zeta N_z^{\eta_1} N_z^{\eta_2}(E) = p_E$  with  $p_G = p_E = 0.5$  for the symmetrical exchange.

When simulating MQ-CEST data it is important to take  $B_1$  field inhomogeneity into account. To do this, for each CEST offset frequency, the CEST intensity is calculated for ten  $B_1$  fields evenly spaced between  $\pm 2\sigma$  of the average CEST pulse strength<sup>15</sup> (in simulations  $\sigma$  is set to 10% of the CEST pulse strength while for fitting experimentally determined values are used) and the final intensity result is calculated as the sum of the results weighted by Gaussian coefficients.

For fitting experimental data, the results of Eq. 5 need to be passed to an optimiser to minimise the standard  $\chi^2$  equation<sup>16</sup>:

$$\chi(x)^2 = \sum_{i=1}^n \frac{(I_{\text{exp},i} - I_{\text{calc},i}(x))^2}{\sigma_{\text{exp},i}^2} \quad (6)$$

Here the sum extends over all experimental data points per residue, including data at multiple CEST field strengths and/or multiple static magnetic field strengths, and  $x$  refers to the parameters to be optimised, including  $k_{\text{ex}}$ ,  $\Delta\omega$ , average  $^{15}\text{N}^n$  chemical shifts and relaxation rates. The uncertainties,  $\sigma_{\text{exp},i}$  are estimated from the scatter in the CEST profiles<sup>17</sup>. A python script, which makes extensive use of the *numpy* and *scipy* modules<sup>13</sup> has been written for the simulation and fitting of MQ-CEST data. The least-squares optimiser is implemented using the LMFIT module<sup>18</sup>. This script is available upon request.

**Analysis of longitudinal zz-exchange spectra.** For each temperature the intensities of the cross and diagonal peaks for the  $\text{N}^n$  nuclei are quantified using FuDA<sup>19</sup>. They are then fit to the following equation with  $k_{\text{ex}}$  as a fitting parameter<sup>1</sup>:

$$\frac{I_{\text{ab}} + I_{\text{ba}}}{I_{\text{aa}} + I_{\text{bb}}} = \frac{1 - \exp(-k_{\text{ex}}t)}{1 + \exp(-k_{\text{ex}}t)} \quad (7)$$

Where  $I_{\text{ab}}$  and  $I_{\text{ba}}$  are the cross-peak intensities and  $I_{\text{aa}}$  and  $I_{\text{bb}}$  are the intensities of the two diagonal peaks.

## Supporting Table

**Table S1:** Acquisition parameters used in MQ-CEST NMR Experiments (all experiments were carried out using the  $^{13}\text{C}$  detect pulse sequence in Fig 1D)

Temp (K)	B <sub>0</sub> Field (T), probe <sup>a</sup>	B <sub>1</sub> Field <sup>b</sup> (Hz)	CEST offsets	$^{15}\text{N}$ SW (ppm), $t_1$ complex points	d1 <sup>c</sup> (sec)	NS <sup>d</sup>	Time <sup>e</sup> (hours)
<b>50 mM [<math>^{13}\text{C}_6</math>, <math>^{15}\text{N}_4</math>] arginine in 50% H<sub>2</sub>O/50% MeOH. MeOD insert provides lock signal</b>							
258.1	14.1, TXI	10.0	24 evenly spaced offsets between 66.9-74.1 ppm (20 Hz gap)	9.7 ppm, 14 complex points	1.5	4	1.5
258.1	14.1, TXI	20.0					
264.0	14.1, TXI	10.0					
264.0	14.1, TXI	20.0					
269.6	14.1, TXI	10.0					
269.6	14.1, TXI	20.0					
275.5	14.1, TXI	10.0					
275.5	14.1, TXI	14.1					
<b>1.1 mM [U-<math>^{13}\text{C}</math>, <math>^{15}\text{N}</math>]-labelled L99A mutant of T4 Lysozyme</b>							
293.0	14.1, TXO	10.9	39 evenly spaced offsets between 63.9-76.4ppm (20 Hz gap)	8.5 ppm, 12 complex points	2.0	48	32
293.0	14.1, TXO	18.9	39 evenly spaced offsets between 63.9-76.4ppm (20 Hz gap)	8.5 ppm, 12 complex points	2.0	40	26.5
293.0	16.4, TCI	10.6	42 evenly spaced offsets between 65-76.5 ppm (20 Hz gap)	8.5 ppm, 14 complex points	2.0	72	58.5
293.0	18.8, TCI	10.7	46 evenly spaced offsets between 65.5-76.6 ppm (20 Hz gap)	8.5 ppm, 16 complex points	2.0	80	83
293.0	22.3, TCI	11.2	55 evenly spaced offsets between 65.3-76.5 ppm (20 Hz gap)	8.7 ppm, 19 complex points	2.0	40	58

a) TXI: HCN inverse room-temperature probe. TCI: Cryogenic HNC inverse probe with cooled  $^1\text{H}$  and  $^{13}\text{C}$  preamplifiers,  $^1\text{H}$  inner coil and  $^{13}\text{C}$  outer coil. TXO:  $^{13}\text{C}$ -optimised cryogenic CNH probe with cooled  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$  preamplifiers,  $^{13}\text{C}$  inner coil and  $^1\text{H}$  outer coil.

b) Shown are values calibrated as described above.

c) Recovery delay.

d) Number of transients (scans) per FID.

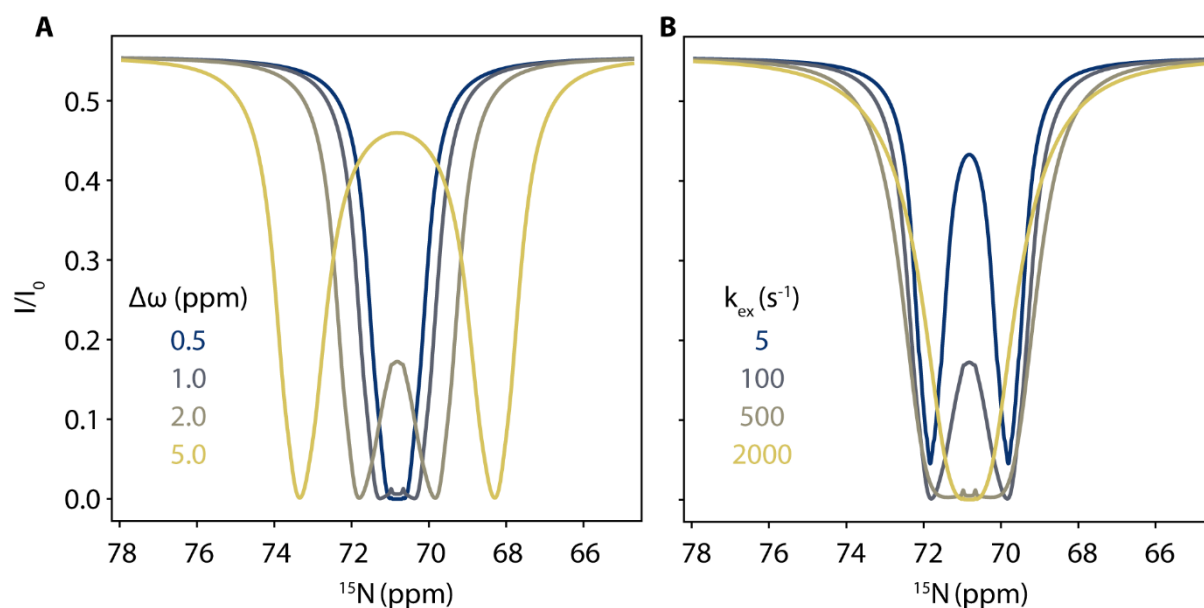
e) Total acquisition time of the experiment.

**Table S2:** Parameters obtained from MQ-CEST for L99A T4 Lysozyme at 293 K

Residue	$k_{\text{ex}}$ (s <sup>-1</sup> ) <sup>a</sup>	$\Delta\omega$ (ppm) <sup>a</sup>
R8	814±65	1.54±0.04
R14	1722±61	2.76±0.04
R52	19±2	1.19±0.01
R76	892±18	1.92±0.01
R80	1184±75	1.43±0.04
R95	<2.7	5.41±0.02
R96	156±4	1.98±0.01
R119	788±26	1.61±0.02
R125	558±32	1.79±0.02
R137	1106±21	1.98±0.02
R154	518±14	1.64±0.01

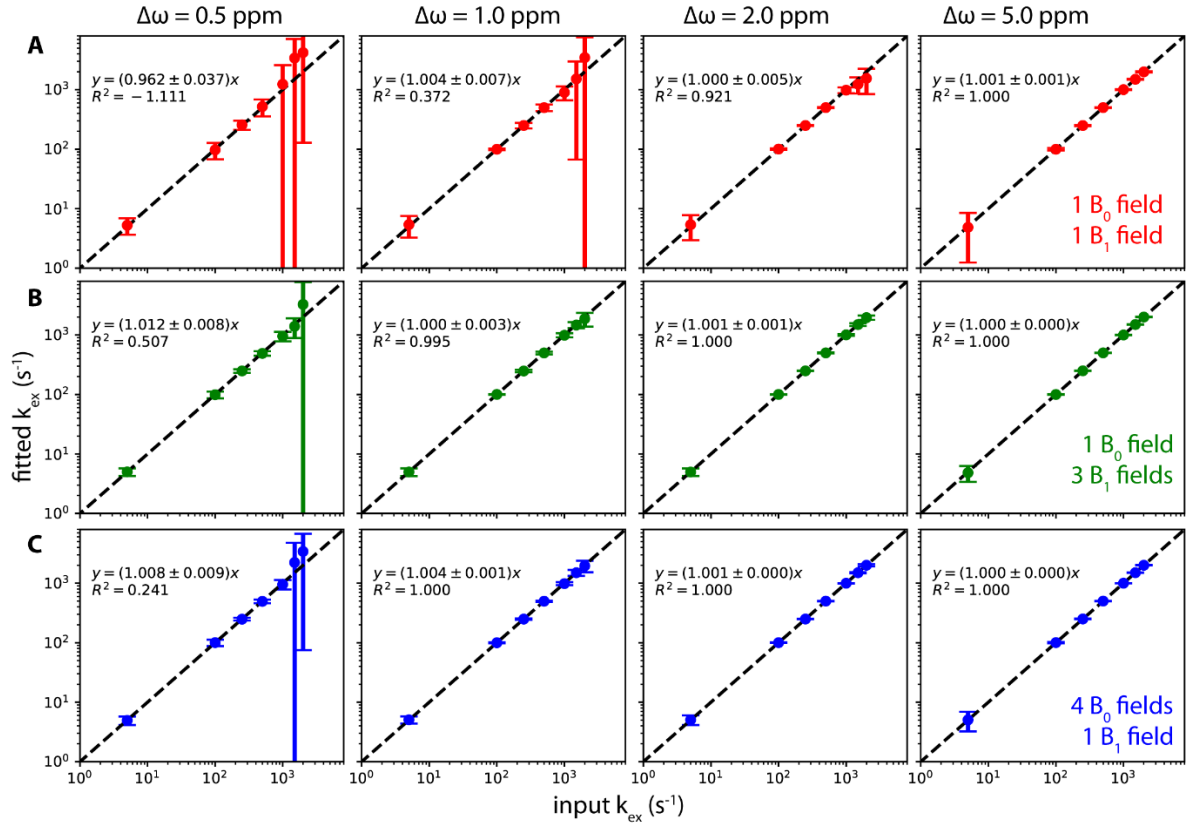
a) In all cases errors are taken from the covariance matrix of the fits<sup>16</sup>. These errors are cross validated by performing fits using grid searched  $k_{\text{ex}}$  values (see Fig S8).

## Supporting Figures

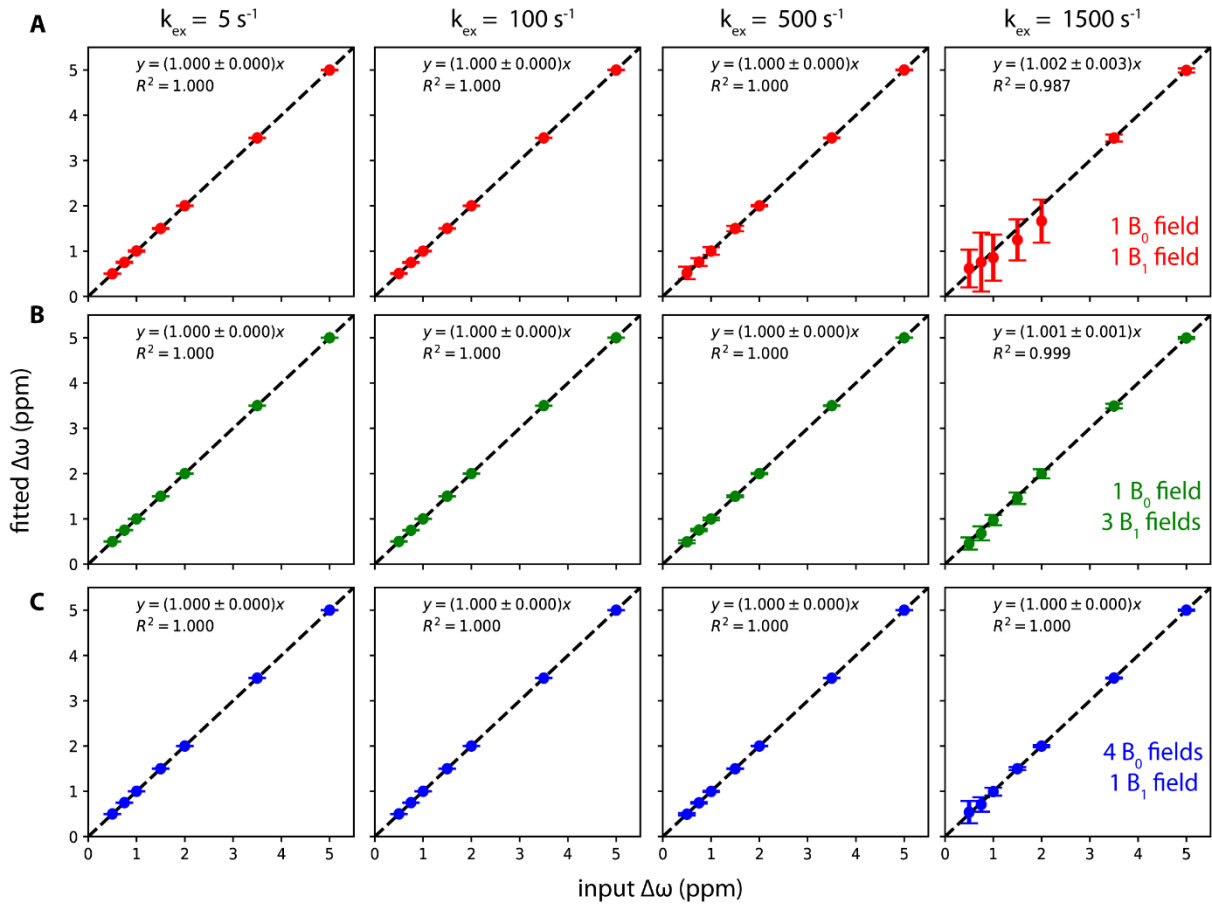


**Fig S1.** Simulation of MQ-CEST profiles with varying  $\Delta\omega$  and  $k_{\text{ex}}$ . In both cases the static magnetic field strength ( $B_0$ ) is 16.4 T, the CEST  $B_1$  strength is 10 Hz (inhomogeneity of 1 Hz) and is applied for  $T_{\text{CEST}} = 250 \text{ ms}$ . The average of the two  $^{15}\text{N}^{\text{H}}$  chemical shifts is set to 71 ppm. (A) The exchange rate is constant,  $k_{\text{ex}} = 100 \text{ s}^{-1}$ , and  $\Delta\omega$  is varied (B) The chemical shift difference is kept constant,  $\Delta\omega = 2 \text{ ppm}$ , and the exchange rate  $k_{\text{ex}}$  is varied.

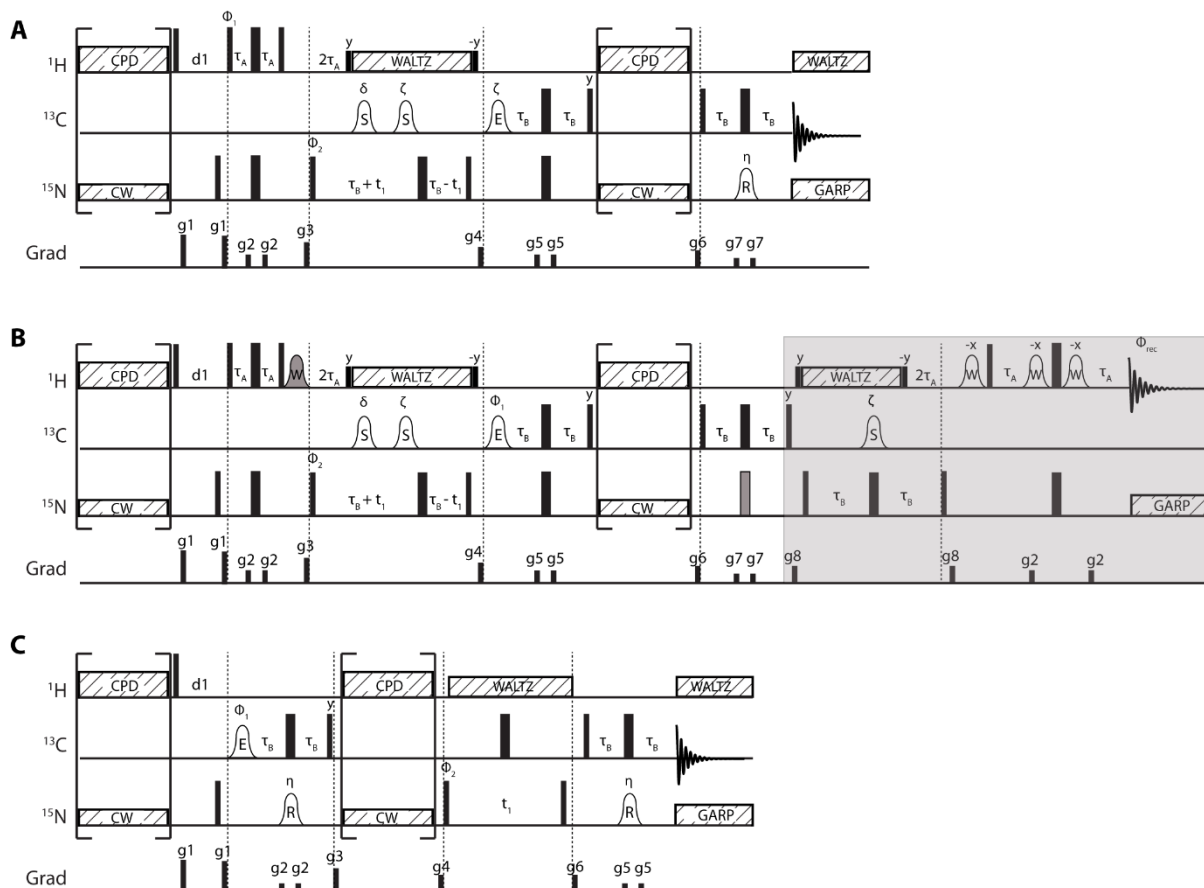




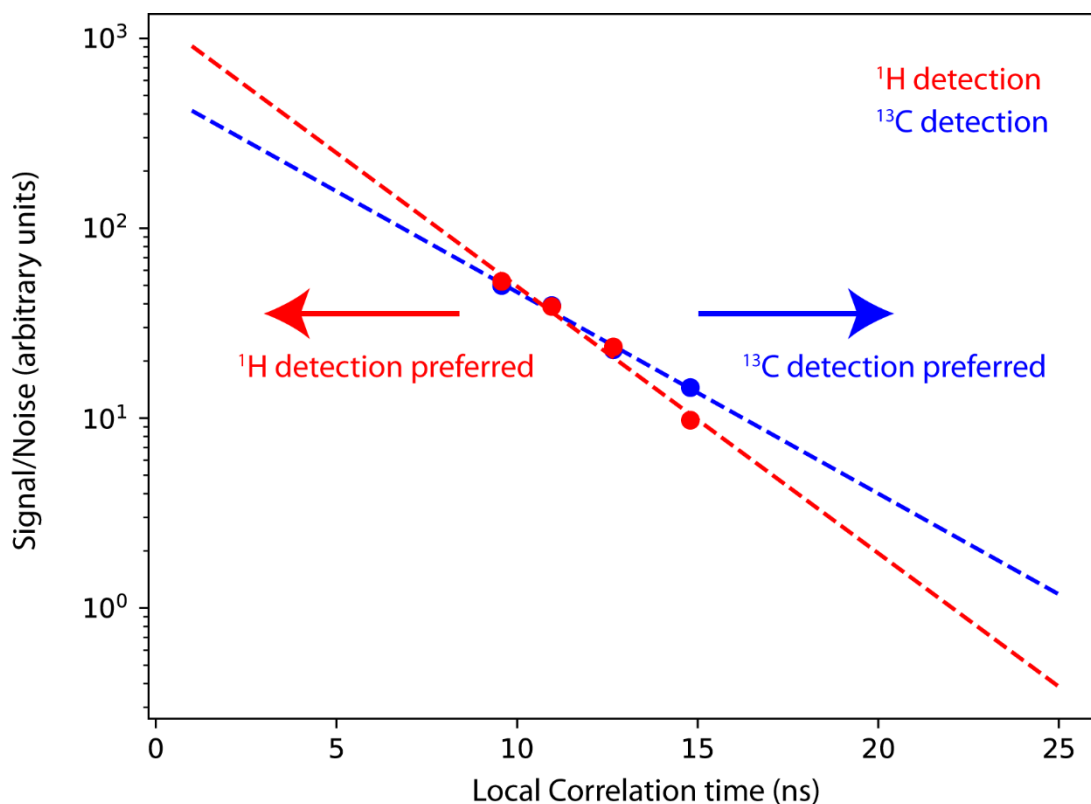
**Fig S2.** Correlation plot of fitted  $k_{ex}$  versus  $k_{ex}$  used as input for the simulated data under different conditions. In each of the 12 plots, each datapoint represents the average (circle) and standard deviation (vertical line) of the  $k_{ex}$  values obtained from 100 simulated datasets under different conditions ( $k_{ex}$ ,  $\Delta\omega$ ,  $B_0$  and  $B_1$ ). Random Gaussian noise with standard deviation equal to 2% of the signal intensity at each point was added to each simulation prior to the fit. (A) A  $B_0$  field strength of 16.4 T and a  $B_1$  strength of 10 Hz (red) were used to simulate the data. (B) A  $B_0$  field strength of 16.4 T and  $B_1$  field strengths of 10, 15 and 20 Hz were used (green). (C)  $B_0$  field strengths of 14.1 T, 16.4 T, 18.8 T, and 22.3 T and a  $B_1$  field strength of 10 Hz were used. For all simulations a saturation pulse length of  $T_{CEST} = 250$  ms was used and assuming a  $B_1$  field inhomogeneity of 10%. For each calculation 10  $B_1$  fields are evenly spaced between the mean  $B_1$  field strength  $\pm 2\sigma$  and weighted according to a Gaussian distribution.



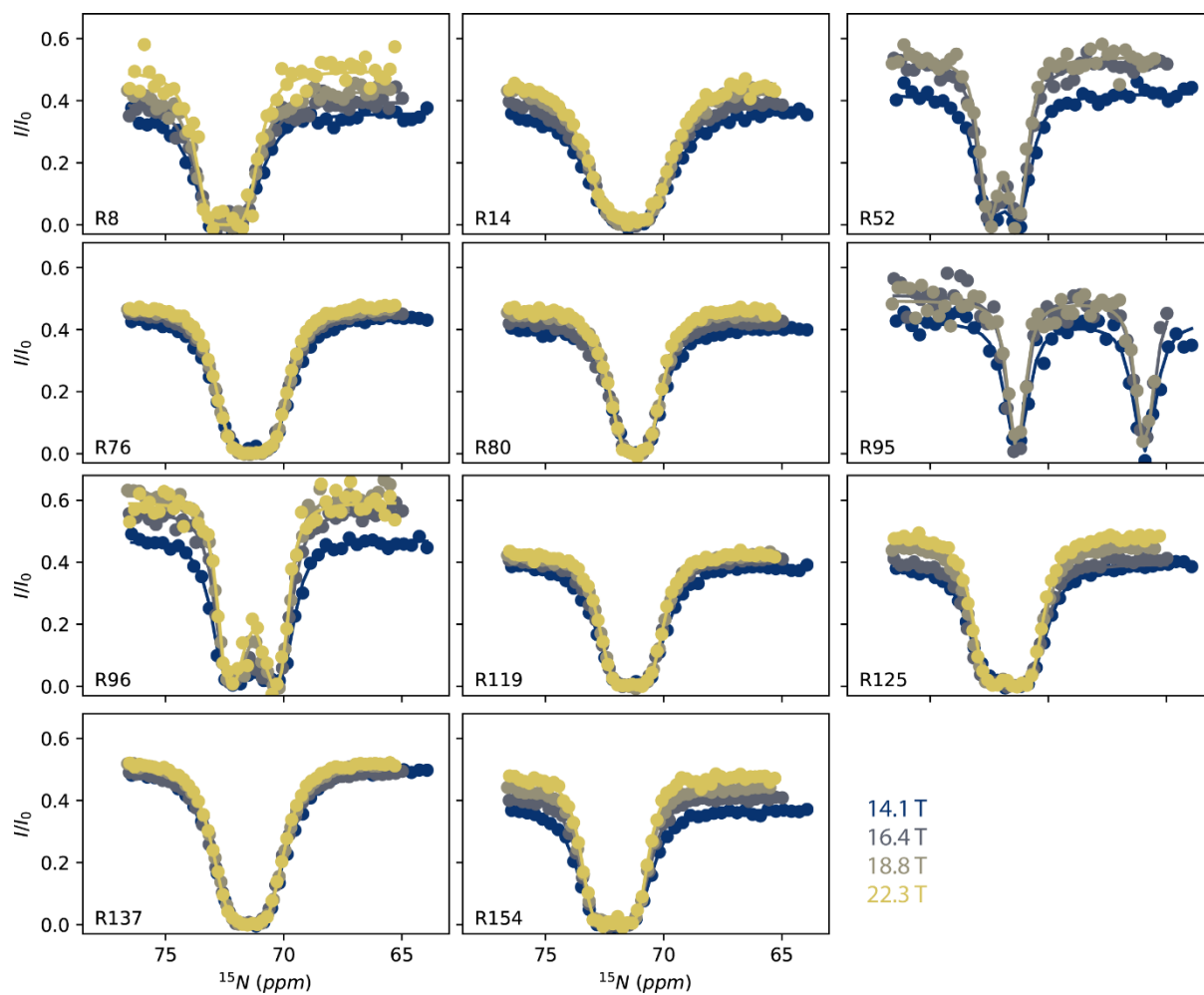
**Fig S3.** Correlation plot of fitted  $\Delta\omega$  versus  $\Delta\omega$  used as input for the simulated data under different conditions. In each of the 12 plots, each datapoint represents the average (circle) and standard deviation (vertical line) of the  $\Delta\omega$  values obtained from 100 simulated datasets under different conditions ( $k_{ex}$ ,  $\Delta\omega$ ,  $B_0$  and  $B_1$ ). Random Gaussian noise with standard deviation equal to 2% of the signal intensity at each point was added to each simulation prior to the fit. **(A)** A  $B_0$  field strength of 16.4 T and a  $B_1$  strength of 10 Hz (red) were used to simulate the data. **(B)** A  $B_0$  field strength of 16.4 T and  $B_1$  field strengths of 10, 15 and 20 Hz were used (green). **(C)**  $B_0$  field strengths of 14.1 T, 16.4 T, 18.8 T, and 22.3 T and a  $B_1$  field strength of 10 Hz were used. For all simulations a saturation pulse length of  $T_{CEST} = 250$  ms was used and assuming a  $B_1$  field inhomogeneity of 10%. For each calculation 10  $B_1$  fields are evenly spaced between the mean  $B_1$  field strength  $\pm 2\sigma$  and weighted according to a Gaussian distribution.



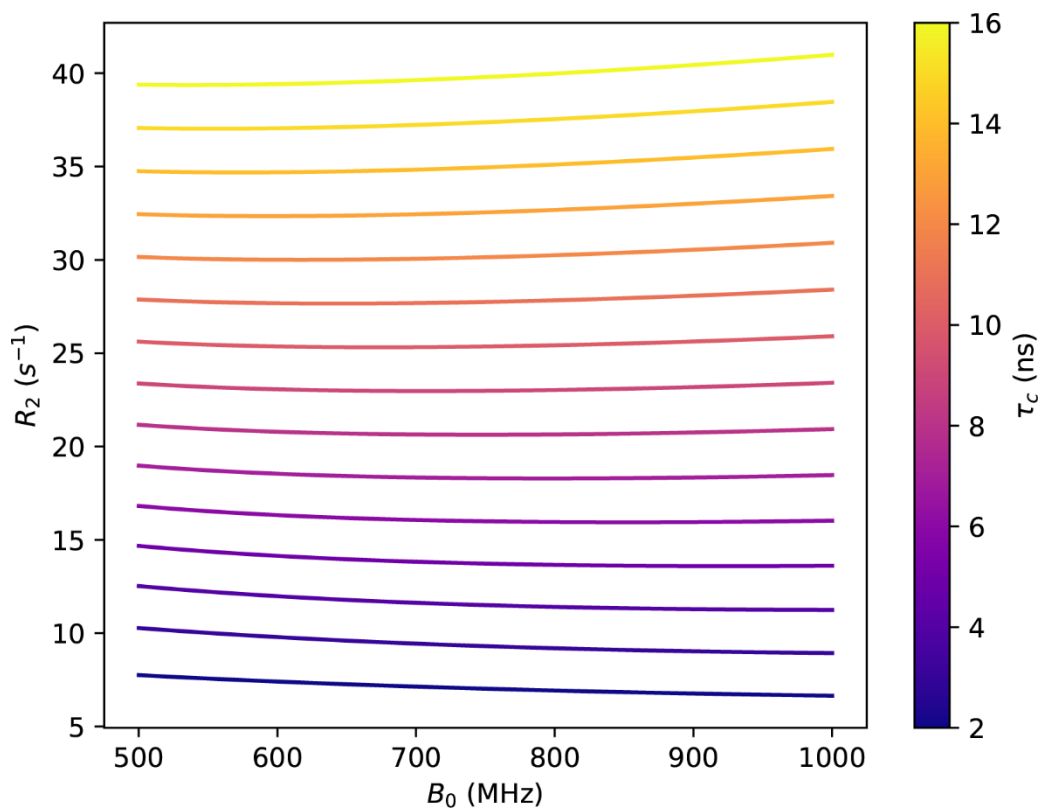
**Fig S4.** Pulse sequences for performing the MQ-CEST experiment with (A) CEST profiles encoded onto  $^{13}\text{C}^{\zeta}\text{-}^{15}\text{N}^{\text{c}}$  coherences, (B) CEST profiles encoded onto  $^1\text{H}^{\text{e}}\text{-}^{15}\text{N}^{\text{c}}$  resonances and (C) CEST profiles encoded onto  $^{13}\text{C}^{\zeta}\text{-}^{15}\text{N}^{\text{n}}$  double quantum coherences<sup>2</sup>. The carrier frequencies are set to 157 ppm ( $^{13}\text{C}$ ) in all cases, (A) and (B) 84 ppm ( $^{15}\text{N}$ ), (C) 71 ppm ( $^{15}\text{N}$ ), and (A) and (C) 7.15 ppm ( $^1\text{H}$ ), (B) 4.77 ppm ( $^1\text{H}$ ) except for during the CEST period when it is moved to 8 ppm ( $^1\text{H}$ ) for this sequence. Narrow and wide bars denote  $90^\circ$  and  $180^\circ$  pulses, respectively with all pulses applied along  $x$  unless otherwise indicated. The bell shapes denote shaped and frequency-selective pulses with the letter indicating the type: S refers to a Seduce pulse<sup>20</sup>, E to an EBURP pulse<sup>21</sup>, R to a REBURP pulse and W to a sinc pulse. Shaped pulses are applied to the nuclei indicated above the pulse and all sinc pulses are applied to the water resonance. The shaped pulses are applied for durations of 300  $\mu\text{s}$  (S), 1.5 ms (E), 4.5 ms (R) and 1 ms (W). Striped boxes indicate decoupling or CEST periods with the text indicating the scheme used: CPD (composite decoupling scheme of  $90_x 240_y 90_x$  applied at 6.25 kHz), CW (continuous wave, power set as desired for the CEST experiment typically 10-20 Hz and applied at CEST offset frequency for 250 ms), WALTZ<sup>22</sup> (3.125 KHz), GARP<sup>23</sup> (0.7 KHz). The delays are set to:  $d1 = 1.5$  s,  $\tau_a = 1/(4J_{\text{HN}}) = 2.7$  ms and  $\tau_b = 1/(4J_{\text{NC}}) = 12.5$  ms. The phase cycle used is  $\phi_1 = x, -x$ ;  $\phi_2 = 2(x), 2(-x)$ ; and  $\phi_{\text{rec}} = x, -x, -x, x$ . Quadrature in  $t_1$  is achieved by States-TPPI of  $\phi_2$ <sup>24</sup>. All gradients are 1 ms in length and applied with the following strengths:  $g1 = 12.3$  G/cm,  $g2 = 5.9$  G/cm,  $g3 = 16.6$  G/cm,  $g4 = 10.2$  G/cm,  $g5 = 7.0$  G/cm,  $g6 = 15.5$  G/cm,  $g7 = 9.1$  G/cm and  $g8 = 19.8$  G/cm.



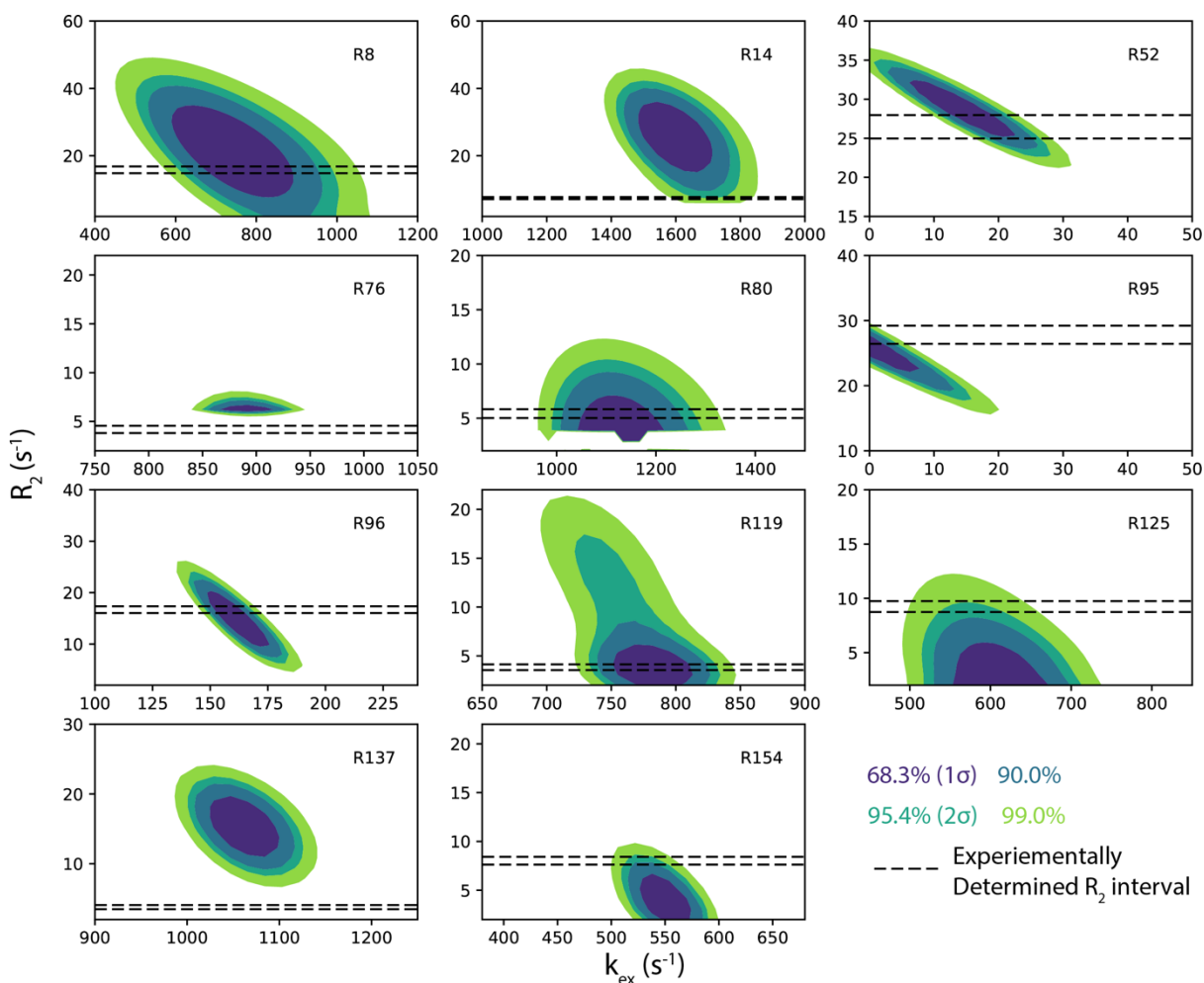
**Fig S5.** Comparison of signal/noise ratios for  $^{13}\text{C}$  (Fig S4A) and  $^1\text{H}$ -detected (Fig S4B) reference planes of the MQ-CEST experiment. The signal/noise are calculated for R52 in L99A T4 Lysozyme. The comparison here ignores the role of hydrogen-exchange with the solvent (very slow under the conditions employed here for R52<sup>25</sup>). The spectra were acquired with the same number of scans, complex points (and acquisition time) in  $t_1$  and recycle delay on a 700 MHz spectrometer equipped with a TCI cryogenic probe and processed identically. The temperature of the sample was altered in steps of 5 K between 283 K and 298 K to change the correlation time. The global correlation time,  $\tau_c$ , is estimated at each temperature using an empirical relationship<sup>26</sup>. The local correlation time is then estimated from this as  $S^2\tau_c$  with the value for the  $^{15}\text{N}$ - $^1\text{H}$  order parameters  $S^2$  taken from Werbeck *et al*<sup>27</sup>. The measured signal to noise ratios were plotted against the estimated local correlation times above. The dotted lines indicate fits to a decaying exponential function. This fit is extrapolated between 1 and 25 ns. Overall,  $^{13}\text{C}$  detection is preferable when the local correlation time of a residue exceeds approximately 10 ns. On a  $^{13}\text{C}$  optimised TXO cryogenic probe,  $^{13}\text{C}$  detection is preferable for residues with even smaller local correlation times. Of note is that flexible arginine side chains, with small local correlation times, are often severely affected by hydrogen-exchange.



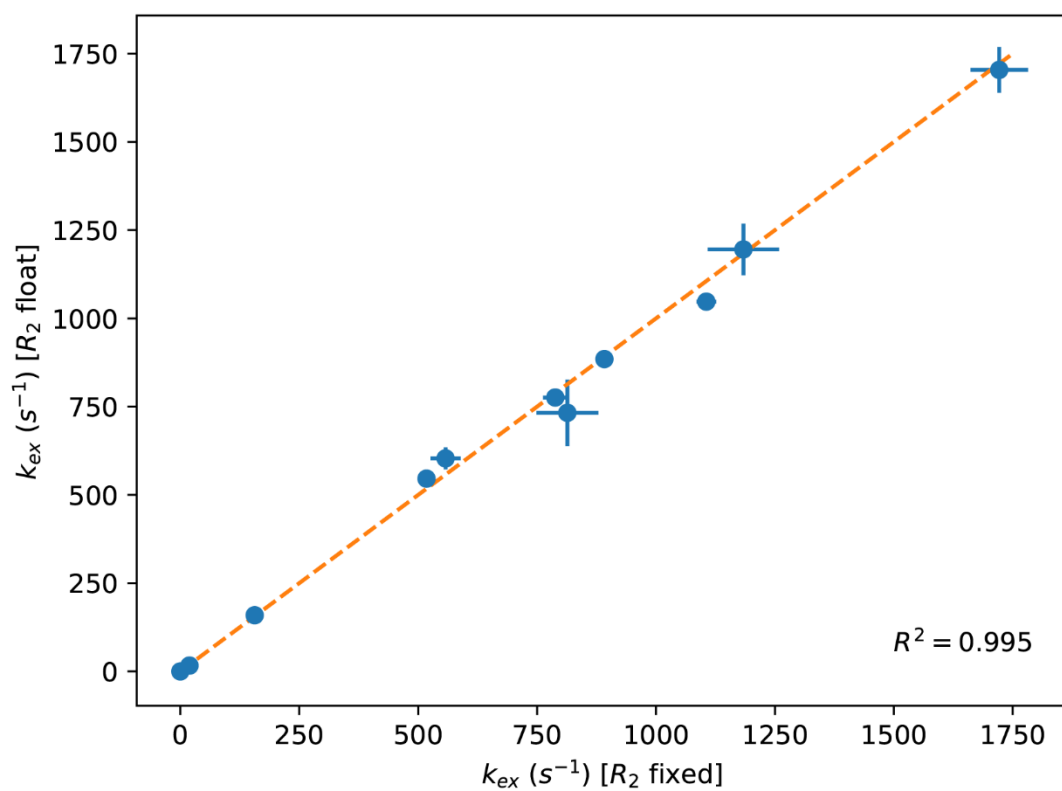
**Fig S6.** MQ-CEST data for L99A T4 Lysozyme collected at 293 K. In all cases the CEST field is applied for  $T_{\text{CEST}} = 250$  ms with a field strength of approximately 10 Hz (the exact  $B_1$  field is calibrated in all cases using the method of Guenneugues<sup>4</sup>). The data is additionally fitted with an experiment recorded with a CEST field of 18 Hz at 14.1 T (data not shown for clarity).



**Fig S7.** Calculated transverse relaxation rate of  $^{15}\text{N}^n$  as a function of the static magnetic field strength ( $B_0$ ) and correlation time ( $\tau_c$ ). The calculated relaxation rates are the sum of dipolar relaxation with the two  $^1\text{H}^n$  nuclei<sup>12</sup> and relaxation due to chemical shift anisotropy (CSA) of the  $^{15}\text{N}^n$  nuclei (the CSA is set to 75 ppm, which is estimated from DFT calculations). Dipolar relaxation between  $^{15}\text{N}^n$  and other nuclei is not included and is confirmed to have a minimal impact on the results. Overall, the calculated relaxation rates show very little dependence on the  $B_0$  field strength.



**Fig S8.** Contour plot showing  $\chi^2$  values as a function of  $R_2$  and  $k_{\text{ex}}$ . The colors indicate different confidence intervals calculated by taking the difference between the minimum  $\chi^2$  value and the  $\chi^2$  value at each point in the grid and then drawing contour lines for  $\Delta\chi^2$  at the indicated confidence intervals for two degrees of freedom<sup>16</sup>. Overall, the fitted exchange rate,  $k_{\text{ex}}$ , is only very weakly correlated with the transverse relaxation rates,  $R_2$ . This means that accurate  $k_{\text{ex}}$  are obtained although the obtained  $R_2$  values may be uncertain. The horizontal dashed lines represent the confidence interval for the experimentally measured  $R_2$  values ( $R_2 \pm \sigma_{R_2}$ ).



**Fig S9.** Comparison of fitted  $k_{\text{ex}}$  rates when  $R_2$  is fixed to the experimentally determined value ( $x$ -axis) and when it is allowed float in the fitting process ( $y$ -axis). The orange dashed line is  $x = y$ .



## Pulse Sequences

```
; Filename: arg_hczne_cest.gk
;
; (He)CzNe HSQC with CEST
; 1H excitation, 13C detection
; semi-constant time evolution in t1
; Gogulan Karunanithy 05/11/19
;
; This pulse sequence is for recording MQ CEST profiles of the guanidinium groups of arginine residues
; in uniformly labelled (13C-15N) samples. In this experiment magnetisation is excited on 1Hepsilon and
; detected on 13Czeta.
;
; WARNING: This sequence does not work with 'getprosol'. If you type 'getprosol' by accident when using
; this sequence all parameters must be checked carefully manually.
;
; start on He->Ne(semi-constant time evolution)->
;         2CzetaNe -> 4CzetaNHNh (CEST) ->
;         C(x) for detection
;
; How to:
; -----
; -----
; Set 13C carrier frequency to 157 ppm (C zeta)
; Set 1H carrier frequency to 7.15 ppm (Heps)
; Set 15N carrier frequency to 84 ppm (Neps)
;
; Initial 1Heps-15Neps INEPT:
; - Set cnst2 = 92 (Heps-Neps J-coupling)
;
; Semi Constant Time Evolution:
; - Set cnst3 = 20 (Neps-Czeta J-coupling)
; - Set cnst12 = 40.5 (Cdelta chemical shift)
; - Apply 300 us Seduce inversion pulse at 16.4 T on Cdelta (set by cnst12) and Czeta (on resonance) to
;   refocus and evolve respective J-couplings
; - Apply 80 us WALTZ64 decoupling on 1H to suppress J-coupling revolution
; - At the end of this period we will have 2Cz(zeta)Nz(eps) magnetisation - note that sweep widths may need
;   to be adjusted slightly to avoid negative delays.
;
; Selective 13Czeta Excitation
; - 1.5 ms Eburp2 pulse at 16.4 T
;
; CEST period
; - The saturation frequencies are taken from fqllist. If the absolute frequency value is set to >10000Hz a
;   references plane is recorded
; - The CEST pulse length is set by d17. The strength of the CEST pulse (pl37) must be carefully
;   calibrated: we use the method of Guenneugues et al. Journal of Magnetic Resonance, 136, 118-126, 1999
; - 40 us Composite decoupling (90x240y90x) is applied to 1H during CEST
;
; During final INEPT a 4.5 ms ReBurp inversion is applied to Neta at 16.4 T. cnst32 should be set to 70 ppm
; (position of Neta protons)
;
; 80 us WALTZ64 decoupling and 350 us GARP4 decoupling are applied to 1H and 15N respectively during
; acquisition

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

;DEFINE PULSES

define pulse pwc
    "pwc=p1" ;13C hard pulse at p11
define pulse pwc_selr1
    "pwc_selr1=p11" ;13C selective seduce-pulse at p111 (acts on C zeta)
define pulse pwc_selr2
    "pwc_selr2=p12" ;13C selective seduce-pulse at p112 (acts on C delta)
define pulse pwc_sele
    "pwc_sele=p13" ;13C selective eburp-pulse at p113

define pulse pwh
    "pwh=p2" ;1H hard pulse at p12

define pulse pwn
    "pwn=p3" ;15N hard pulse at p13
define pulse pwn_selr
    "pwn_selr=p31" ;15N selective r-pulse at p131

;DEFINE DELAYS

define delay taua
    "taua=1s/(cnst2*4)" ;1/4JHN
define delay taub
    "taub=1s/(cnst3*4)" ;1/4JCN
```

```

"d11= 30m" ;Delay for disk
"d12= 4u" ;Delay for power switching
"d16= 200u" ;Delay for gradient recovery

"in0=in0/2" ;incremental delay

#ifdef HALFDWELL
"d0=in0/2"
#else
"d0=0.0"
#endif /*HALFDWELL*/

;DEFINE OFFSETS

define list<frequency> N15sat = <$FQ1LIST>

"spoffs11=0" ;Czeta seduce
"spoffs12=(cnst12*bf1)/1000000-o1" ;Cdelta seduce
"spoffs13=0" ;Czeta eburp2
"spoffs31=0" ;15N reburp

;DEFINE ZERO POWER ON ALL CHANNELS USING SHAPED PULSES

"plw10=0"
"plw20=0"
"plw30=0"

;DEFINE CONSTANTS

"cnst11=o1/bf1"
"cnst21=o2/bf2"

"cnst31=o3/bf3" ; Ne
"cnst33=0.5*(cnst31+cnst32)" ;15N decoupling position

;PULSE PROGRAM BEGINS

1 ze
2 d11 do:f2 do:f3 ;decoupling off
if "abs(N15sat)>10000Hz" {
d12 p137:f3 p122:f2 ; set appropriate power level for 15N CEST pulse and hydrogen decoupling
30u fq=N15sat:f3 ; put frequency at right place

30u fq=0:f2 ;
d12 cpd8:f3 cpd5:f2 ; turn on CEST (N15) and decoupling (1H)
d17 ; delay for CEST
d12 do:f2 do:f3
}

50u UNBLKGRAD
d12 p12:f2
(pwh ph10):f2

2u
p51:gp1 ;cleaning gradient
d16

50u BLKGRAD

d12 p129:f2

d1

;start purge equilibrium 15N magnetisation

50u UNBLKGRAD ;gradient amp on
d12 fq=cnst33(bf ppm):f3 ;set 15N carrier
d12 p12:f2 p13:f3 ;power to high (1H, 15N)
(pwn ph10):f3 ;90x on Nitrogen to purge
2u
p51:gp1 ;cleaning gradient
d16

d12 fq=cnst31(bf ppm):f3 ;set 15N carrier, Nepsilon
d12 fq=cnst11(bf ppm):f1
d12 fq=cnst21(bf ppm):f2

;end purge block

;start HN INEPT (Non-selective)

(pwh ph1):f2
"DELTA = taua-0.6366*pwh-2u-p52-d16-0.5*larger(pwh*2,pwn*2)"
DELTA
2u
p52:gp2
d16

```

```

( center (pwh*2 ph10):f2 (pwn*2 ph10):f3 )
2u
p52:gp2
d16
"DELTA = taua-0.5*larger(pwc*2,pwn*2)-2u-p52-d16-0.6366*pwh"
DELTA
(pwh ph11):f2
; end INEPT now on HzNz

2u
p53:gp3                                ;cleaning gradient
d16
d12 pl10:f1 pl29:f2                    ;power to 0(13C), decouple (1H): prepare for what's coming

;now go from 2HzNz to 2CzNz with semi-constant time evolution
(pwn ph2):f3                            ;make 2HzNy (phase cycle for 2D)

if "d0<(2*taua - pwc_selr2)"
{
"DELTA = d0"
DELTA
(pwc_selr2:sp12 ph10):f1                ; 180 on Cdelta
"DELTA = 2*taua - d0 - pwc_selr2"
DELTA
d12 cpd2:f2
"DELTA = taub + d0 - pwc_selr1 - d12 - 2*taua"
DELTA
(pwc_selr1:sp11 ph10):f1                ; 180 on Czeta
}
else
{
"DELTA = 2*taua"
DELTA
d12 cpd2:f2
"DELTA = d0 - 2*taua"
DELTA
(pwc_selr2:sp12 ph10):f1                ; 180 on Cdelta
"DELTA = taub - pwc_selr2 - pwc_selr1 - d12"
DELTA
(pwc_selr1:sp11 ph10):f1                ; 180 on Czeta
}

if "d0<taub"
{
(pwn*2.0 ph10):f3
"DELTA = taub - d0"
DELTA
}
else
{
"DELTA = d0 - taub"
DELTA
(pwn*2.0 ph10):f3
}

(pwn ph10):f3                            ; go back to 2CzNz
d12 do:f2                                ; turn off proton decoupling

2u
p54:gp4                                ;cleaning gradient
d16
30u fq=cnst33(bf ppm):f3
d12 pl10:f1
d12
(pwc_sele:sp13 ph10):f1                  ;13C selective excitation (eburp pulse)
;start Nh selective block
"DELTA = taub-2u-p52-d16-0.5*larger(pwc*2,2*pwn) "
DELTA
2u
p55:gp5                                ;p55 gradient time
d16 pl1:f1                              ;power to high (13C) d16 is 200 us delay for grad
recovery
( center (pwc*2 ph10):f1 (pwn*2 ph10):f3 ) ; 15N hard
2u
p55:gp5                                ;p53: gradient time
d16
"DELTA = taub-2u-p52-d16-0.5*larger(pwc*2,2*pwn)-0.6366*pwc"
DELTA
;end INEPT
(pwc ph11):f1                            ;We should now be on 4CzNhZNhZ (

; do CEST here

if "abs(N15sat)>10000Hz" {
d12 pl30:f3                            ; for reference plane
} else {

```

```

    d12 pl37:f3 pl22:f2      ; set appropriate power level for 15N CEST pulse and hydrogen decoupling
  }
  30u fq=N15sat:f3          ; stick frequency at right place
  d12 cpd8:f3 cpd5:f2       ; turn on CEST (N15) and decoupling (1H)

  if "abs(N15sat)>10000Hz" {
    d12                      ; set delay v short for reference plane
  }
else {
  d17                        ; delay for CEST
}
  d12 do:f3 do:f2           ; turn off decoupler and cest pulse
  30u fq=cnst32(bf ppm):f3 ; place carrier back in right spot for N15 i.e. Nh

; end CEST

2u
p56:gp6                      ;cleaning gradient
d16

d12 pl30:f3 pl29:f2         ;power for 1H decoupler and 15N shape
d12

(pwc ph10):f1                ;-4CzNh1zNh2z to 4CyNh1zNh2z

"DELTA = taub-0.6366*pwc-2u-p55-d16-0.5*larger(pwc*2,pwn_selr)"
DELTA
2u
p57:gp7
d16
( center (pwc*2 ph10):f1 (pwn_selr:sp31 ph10):f3 )
2u
p57:gp7
d16
30u fq=cnst33(bf ppm):f3    ;set 15N carrier to decouple (in middle)
d12 pl39:f3                 ;power to decouple(15N)
50u BLKGRAD                 ;gradient amp off
"DELTA = taub-0.5*larger(2*pwc,pwn_selr)-2u-p55-d16-30u-d12-50u"
DELTA
                                ;end Nh selective block

;end second INEPT, acquire Cy magnetisation with 15N, 1H decoupling

go=2 ph31 cpd2:f2 cpd3:f3
  d11 do:f2 do:f3 mc #0 to 2
  F2QF(calclist(N15sat,1))
  F1PH(calph(ph2,+90), caldel(d0,+in0))

exit

;PHASE PROGRAMS

ph1= 0 2
ph2= 0 0 2 2
ph31= 0 2 2 0

ph10= 0
ph11= 1
ph12= 2
ph13= 3

;DEFINITIONS

;p11      : f1 channel - power level for hard pulse
;p12      : f2 channel - power level for hard pulse
;p13      : f3 channel - power level for hard pulse
;p122     : f2 channel - power level for composite 1H decoupling
;p129     : f2 channel - power level for CPD/BB decoupling
;p137     : f3 channel - power level for CEST pulse
;p139     : f3 channel - power level for CPD/BB decoupling
;sp11     : f1 channel - shaped pulse 180 degree (selective for Czeta)
;spnam11  : Seduce.1000
;sp12     : f1 channel - shaped pulse 180 degree (selective for Cdelta)
;spnam12  : Seduce.1000
;sp13     : f1 channel - shaped pulse 90 degree (selective for Czeta)
;spnam13  : Eburp2.1000
;sp31     : f3 channel - shaped pulse 180 degree (selective for Ne)
;spnam31  : Reburp.1000
;p1       : f1 channel - 90 degree high power pulse
;p10      : setup for composite decoupling [40 us]
;p11      : f1 channel - 180 degree shaped pulse [300 us at 18.8T]
;p12      : f1 channel - 180 degree shaped pulse [300 us at 18.8T]
;p13      : f1 channel - 90 degree shaped pulse [1.5 ms at 18.8T]
;p2       : f2 channel - 90 degree high power pulse
;p25      : f2 channel - composite decoupling 90 degree pulse [40 us]
;p3       : f3 channel - 90 degree high power pulse
;p31      : f3 channel - 180 degree shaped pulse [4.5 ms at 18.8T]

```

```

;p51      : homospoil pulse
;p52      : gradient pulse
;p53      : homospoil pulse
;p54      : homospoil pulse
;p55      : gradient pulse
;p56      : homospoil pulse
;d0       : incremented delay
;d1       : relaxation delay
;taua    : 1/(4JHN)
;taub    : 1/(4JCN)
;d11     : delay for disk I/O [30 ms]
;d12     : delay for power switching [2 us]
;d16     : delay for homospoil/gradient recovery [200 us]
;d17     : delay for CEST [250 ms]
;cnst2   : J(HN) [92 Hz]
;cnst3   : J(CN) [20 Hz]
;cnst12  : Cdelta chemical shift offset [40.5 ppm]
;cnst31  : Ne chemical shift offset [= o3p, 84.0 ppm]
;cnst32  : Nh chemical shift offset [70 ppm]
;cnst33  : average of Nh and Ne shifts [~77 ppm]
;o1p     : Czeta chemical shift offset [156 ppm]
;o2p     : He/Hh chemical shift offset [7 ppm]
;o3p     : Ne chemical shift offset [84 ppm]
;inf2    : 1/SW = 2 * DW
;in0     : 1/(2 * SW) = DW
;NS      : 8 * n
;DS      : 64
;td1     : number of experiments
;FnMODE  : States-TPPI
;cpd2    : decoupling according to sequence defined by cpdprg2 (WALTZ64)
;pcpd2   : f2 channel - 90 degree pulse for decoupling sequence [80 us]
;cpd3    : decoupling according to sequence defined by cpdprg3 (GARP4)
;pcpd3   : f3 channel - 90 degree pulse for decoupling sequence [350 us]
;cpd5    : decoupling according to sequence defined by cpdprg5 (composite)
;cpd8    : sequence for CEST (cwp)

;for z-only gradients:
;gpz1    : 23% (spoil)
;gpz2    : 11%
;gpz3    : 31% (spoil)
;gpz4    : 19% (spoil)
;gpz5    : 13%
;gpz6    : 29% (spoil)
;gpz7    : 17%

;use gradient files:
;gpnam1  : SMSQ10.100
;gpnam2  : SMSQ10.100
;gpnam3  : SMSQ10.100
;gpnam4  : SMSQ10.100
;gpnam5  : SMSQ10.100
;gpnam6  : SMSQ10.100
;gpnam7  : SMSQ10.100

;preprocessor-flags-start
;HALFDWELL: for initial sampling delay of half a dwell-time with
;           option -DHALFDWELL (eda: ZGOPTNS)
;preprocessor-flags-end

```

```

; Filename: arg_hnhcesth_cest.gk
;
; H-Ne HSQC with CEST on 4Cz2Nh2NhZ
; 1H excitation and detection
; semi-constant time evolution in t1
; Gogulan Karunanithy 20/11/19
;
; start on He->Ne(semi-constant time evolution)->
;      2CzNe -> 4CzNhNh (CEST) -> 2CzNe -> Ne -> He Detection
;
; How to:
; -----
; -----
; Set 1H carrier frequency to 4.77 ppm (Water)
; Set 13C carrier frequency to 157 ppm (C zeta)
; Set 15N carrier frequency to 84 ppm (Neps)
;
;
; Initial 1Heps-15Neps INEPT:
; - Set cnst2 = 92 (Heps-Neps J-coupling)
;
; Semi Constant Time Evolution:
; - Set cnst3 = 20 (Neps-Czeta J-coupling)
; - Set cnst22 = 40.5 (Cdelta chemical shift)
; - Apply 300 us Seduce inversion pulse at 16.4 T on Cdelta (set by cnst12) and Czeta (on resonance) to
;   refocus and evolve respective J-couplings
; - Apply 80 us WALTZ64 decoupling on 1H to suppress J-coupling revolution
; - At the end of this period we will have 2Cz(zeta)Nz(eps) magnetisation - note that sweep widths may need
;   to be adjusted slightly to avoid negative delays.
;
; Selective 13Czeta Excitation
; - 1.5 ms Eburp2 pulse at 16.4 T
;
; CEST period
; - The saturation frequencies are taken from fqllist. If the absolute frequency value is set to >10000Hz a
;   references plane is recorded
; - Set cnst12 = 8.0 (move 1H carrier to middle of amide region)
; - The CEST pulse length is set by d17. The strength of the CEST pulse (p137) must be carefully
;   calibrated: we use the method of Guenneugues et al. Journal of Magnetic Resonance, 136, 118-126, 1999
; - 40 us Composite decoupling (90x240y90x) is applied to 1H during CEST
;
; 1000 us Sinc pulses are employed for water suppression at 16.4 T
;
; 350 us GARP4 decoupling is applied 15N respectively during acquisition

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

;DEFINE PULSES

define pulse pwc
    "pwc=p2" ;13C hard pulse at p11
define pulse pwc_selr1
    "pwc_selr1=p21" ;13C selective seduce-pulse at p111 (acts on C zeta)
define pulse pwc_selr2
    "pwc_selr2=p22" ;13C selective seduce-pulse at p112 (acts on C delta)
define pulse pwc_sele
    "pwc_sele=p23" ;13C selective eburp-pulse at p113

define pulse pwh
    "pwh=p1" ;1H hard pulse at p12

define pulse pwn
    "pwn=p3" ;15N hard pulse at p13
define pulse pwn_selr
    "pwn_selr=p31" ;15N selective r-pulse at p131

;DEFINE DELAYS

define delay taua
    "taua=1s/(cnst2*4)" ;1/4JHN
define delay taub
    "taub=1s/(cnst3*4)" ;1/4JCN

"d11= 30m" ;Delay for disk
"d12= 4u" ;Delay for power switching
"d16= 200u" ;Delay for gradient recovery

"in0=inf1/2" ;incremental delay

#ifdef HALFDWELL
    "d0=in0/2"
#else
    "d0=0.0"
#endif /*HALFDWELL*/

;DEFINE ZERO POWER ON ALL CHANNELS USING SHAPED PULSES

```

```

"plw10=0"
"plw20=0"
"plw30=0"

;DEFINE CONSTANTS
"cnst11=o1/bf1"
"cnst21=o2/bf2"
"cnst31=o3/bf3" ;Nepsilon
"cnst33=0.5*(cnst31+cnst32)" ;15N decoupling position

;DEFINE OFFSETS

define list<frequency> N15sat = <$FQ1LIST>

"spoffs21=0" ;Czeta seduce
"spoffs22=(cnst22*bf2)/1000000-o2" ;Cdelta seduce

;PULSE PROGRAM BEGINS

1 ze
2 d11 do:f3 ;decoupling off

if "abs(N15sat)>10000Hz" {
  d12 pl37:f3 pl12:f1 ; set appropriate power level for 15N CEST pulse and hydrogen decoupling
  30u fq=N15sat:f3 ; put 15N frequency at right place

  4u fq=cnst12(bf ppm):f1
  (p15 ph11):f1
  d12 cpd8:f3 cpds:5f1 ph10 ; turn on CEST (N15) and decoupling (1H)
  d17 ; delay for CEST
  d12 do:f1 do:f3
  (p15 ph13):f1
}

50u UNBLKGRAD
d12 pl1:f1 ;
(pwh ph10):f1 ; purge any 1H magnetisation prior to d1: always start from same place

2u
p51:gp1 ;cleaning gradient
d16

(pwh ph11):f1 ; purge any 1H magnetisation prior to d1: always start from same place

2u
p51:gp8 ;cleaning gradient
d16

50u BLKGRAD

d1

;start purge equilibrium 15N magnetisation

50u UNBLKGRAD ;gradient amp on

d12 fq=cnst33(bf ppm):f3 ;set 15N carrier
d12 fq=0:f1 ;set 1H onto water

d12 pl1:f1 pl3:f3 ;power to high (1H, 15N)
(pwn ph10):f3 ;90x on Nitrogen to purge

2u
p51:gp1 ;cleaning gradient
d16

d12 fq=cnst21(bf ppm):f2 ;set to Czeta

d12 fq=0:f2
d12 fq=0:f3

;end purge block

;start HN INEPT (Non-selective)

d12 pl1:f1

(pwh ph10):f1

"DELTA = taua-0.6366*pwh-2u-p52-d16-larger(pwh,pwn)"
DELTA

2u
p52:gp2
d16

```

```

( center (pwh*2 ph10):f1 (pwn*2 ph10):f3 )

2u
p52:gp2
d16

"DELTA = taua-larger(pwn,pwh)-2u-p52-d16-0.6366*pwh"
DELTA

(pwh ph11):f1
d12 pl10:f1
(pl1:sp11 ph10):f1 ;water suppression pulse
2u

; end INEPT now on HzNz

2u
p53:gp3 ;cleaning gradient
d16

d12 pl20:f2 pl19:f1 ;power to 0(13C) decoupl on 1H

;now go from 2HzNz to 2CzNz with semi-constant time evolution
(pwn ph2):f3 ;make 2HzNy (phase cycle for 2D)

if "d0<(2*taua - pwc_selr2)"
{
"DELTA = d0"
DELTA
(pwc_selr2:sp22 ph10):f2 ; 180 on Cdelta
"DELTA = 2*taua - d0 - pwc_selr2"
DELTA
(p19 ph11):f1
d12 cpds1:f1 ph10
"DELTA = taub + d0 - pwc_selr1 - p19 - d12 - 2*taua"
DELTA
(pwc_selr1:sp21 ph10):f2 ; 180 on Czeta
}
else
{
"DELTA = 2*taua"
DELTA
(p19 ph11):f1
d12 cpds1:f1 ph10
"DELTA = d0 - 2*taua"
DELTA
(pwc_selr2:sp22 ph10):f2 ; 180 on Cdelta
"DELTA = taub - pwc_selr2 - pwc_selr1 - p19 - d12"
DELTA
(pwc_selr1:sp21 ph10):f2 ; 180 on Czeta
}

if "d0<taub"
{
(pwn*2.0 ph10):f3
"DELTA = taub - d0"
DELTA
}
else
{
"DELTA = d0 - taub"
DELTA
(pwn*2.0 ph10):f3
}

(pwn ph10):f3

d12 do:f1
(p19 ph13):f1

2u
p54:gp4 ;cleaning gradient
d16

(pwc_sele:sp23 ph1):f2 ;13C selective excitation (eburp pulse)

"DELTA = taub-2u-p55-d16-larger(pwc,pwn)"
DELTA

2u
p55:gp5 ;p55 gradient time
d16 pl2:f2 ;power to high (13C) d16 is 200 us delay for grad
recovery

( center (pwc*2 ph10):f2 (pwn*2 ph10):f3 ) ; 15N hard

```



```

2u
p55:gp5                                ;p53: gradient time
d16

"DELTA = taub-2u-p55-d16-larger(pwc,pwn)-0.6366*pwc"
DELTA
;
(pwc ph11):f2                            ;We should now be on 4CzNzNz (for N etas)
; CEST here

if "abs(N15sat)>10000Hz" {
  d12 pl30:f3 pl10:f1                    ; for reference plane
} else {
  d12 pl37:f3 pl12:f1                    ; set appropriate power level for 15N CEST pulse and hydrogen decoupling
}
30u fq=N15sat:f3                          ; stick frequency at right place
4u fq=cnst12(bf ppm):f1
d12 cpd8:f3 cpds5:f1 ph10                ; turn on CEST (N15) and decoupling (1H) - we want high pwr (10kHz)
composite (90x240y90x) decoupling on proton

if "abs(N15sat)>10000Hz" {
  d12                                      ; set delay v short for reference plane
}
else {
  d17                                      ; delay for CEST
}
d12 do:f3 do:f1                            ; turn off decoupler and cest pulse
4u fq=0:f1
30u fq=cnst33(bf ppm):f3 ; place carrier back in right spot for N15
; end CEST

2u
p56:gp6                                ;cleaning gradient
d16

d12 pl3:f3                                ;reset 15N power
; now go to 2CzNeZ
(pwc ph10):f2                            ;-4CzNh1zNh2z to 4CyNh1zNh2z

"DELTA = taub-0.6366*pwc-2u-p57-d16-larger(pwc,pwn) "
DELTA

2u
p57:gp7
d16

( center (pwc*2 ph10):f2 (pwn*2 ph10):f3 )

2u
p57:gp7
d16

"DELTA = taub-larger(pwc,pwn)-2u-p57-d16-0.6366*pwc"
DELTA

(pwc ph11):f2 ; bring Czeta back up: now should be 2CzNz (wrt N epsilon)

2u
p58:gp8
d16
; go from 2CzNz to pure in phase N epsilon

d12 pl20:f2 pl19:f1
(p19 ph11):f1

d12 cpds1:f1 ph10
(pwn ph10):f3 ; bring down Ne

"DELTA = taub - 0.5*larger(pwc_selr1,2.0*pwn)"
DELTA

( center (pwc_selr1:sp21 ph10):f2 (pwn*2 ph10):f3 ) ; refocus coupling to Cdelta and evolve Czeta

"DELTA = taub - 0.5*larger(pwc_selr1,2.0*pwn) - 2.0*taua - d12"
DELTA

d12 do:f1                                ; turn off 1H decoupler
(p19 ph13):f1                            ; preserve water mag

"DELTA = 2.0*taua - p19"
DELTA

(pwn ph10):f3 ; bring up HezNez

```

```

2u
p58:gp8 ; purge anything that's not transverse
d16

d12 fq=0:f1 ;set 1H to water

d12 p110:f1
(p11:sp11 ph12 ):f1 ;water suppression pulse
d12 p11:f1

(pwh ph10):f1 ; transverse antiphase on proton
"DELTA = taua - 0.6366*pwh - 2u - p52- d16 - p11 - d12 - larger(pwh,pwn)"
DELTA
2u
p52:gp2
d16 p110:f1
(p11:sp11 ph12):f1 ;water suppression pulse
d12 p11:f1
( center (pwh*2 ph10):f1 (pwn*2 ph10):f3 )
d12 p110:f1
(p11:sp11 ph12):f1 ;water suppression pulse
2u
p52:gp2
d16 pl39:f3 ;set power for 15N decoupling
50u BLKGRAD ;gradient amp off
;end second INEPT, acquire Hy magnetisation with 15N decoupling
"DELTA = taua - larger(pwh,pwn)- d12 - p11 - 2u - p52- d16 - 50u"
DELTA

go=2 ph31 cpd3:f3
d11 do:f3 mc #0 to 2
F2QF(calclist(N15sat,1))
F1PH(calph(ph2,+90), caldel(d0,+in0))

exit

;PHASE PROGRAMS

ph1= 0 0 2 2
ph2= 0 2
ph31= 0 2 2 0

ph10= 0
ph11= 1
ph12= 2
ph13= 3

;DEFINITIONS

;p11 : f1 channel - power level for hard pulse
;p12 : f2 channel - power level for hard pulse
;p13 : f3 channel - power level for hard pulse
;p112 : f2 channel - power level for composite 1H decoupling
;p119 : f1 channel - power level for CPD/BB decoupling
;p137 : f3 channel - power level for CEST pulse
;p139 : f3 channel - power level for CPD/BB decoupling
;sp11 : f1 channel - shaped pulse 90 degree (for water suppression)
;spnam11 : sinc pulse
;sp21 : f2 channel - shaped pulse 180 degree (selective for Czeta)
;spnam21 : Seduce.100
;sp22 : f2 channel - shaped pulse 180 degree (selective for Cdelta)
;spnam22 : Seduce.100
;sp23 : f2 channel - shaped pulse 90 degree (selective for Czeta)
;spnam23 : Eburp2.1000
;sp31 : f3 channel - shaped pulse 180 degree (selective for Nepsilon)
;spnam31 : Reburp.1000
;p1 : f1 channel - 90 degree high power pulse (1H)
;p10 : setup for composite decoupling [40us]
;p11 : f1 channel - water suppression sinc pulse (1000 us)
;p15 : pulse for 1H CEST decoupling [40us]
;p19 : pulse for Waltz decoupling [80us]
;p2 : f2 channel - 90 degree high power pulse (13C)
;p21 : f2 channel - 180 degree shaped pulse [300 us at 18.8T]
;p22 : f2 channel - 180 degree shaped pulse [300 us at 18.8T]
;p23 : f2 channel - 90 degree shaped pulse [1.5 ms at 18.8T]
;p3 : f3 channel - 90 degree high power pulse (15N)
;p31 : f3 channel - 180 degree shaped pulse [4.5 ms at 18.8T]
;p51 : homospoil pulse
;p52 : gradient pulse
;p53 : homospoil pulse
;p54 : homospoil pulse
;p55 : gradient pulse
;p56 : homospoil pulse
;p57 : gradient pulse
;p58 : homospoil pulse
;p59 : gradient pulse
;p60 : gradient pulse

```

```

;d0      : incremented delay
;d1      : relaxation delay
;taua    : 1/(4JHN)
;taub    : 1/(4JCN)
;d11     : delay for disk I/O [30 ms]
;d12     : delay for power switching [2 us]
;d16     : delay for homospoil/gradient recovery [200 us]
;d17     : CEST Delay [250 ms]
;cnst2   : J(HN) [92 Hz]
;cnst3   : J(CN) [20 Hz]
;cnst12  : amide chemical shift [8.0 ppm]
;cnst22  : Cdelta chemical shift offset [40.5 ppm]
;cnst31  : Nepsilon chemical shift offset [= o3p, 84.0 ppm]
;cnst32  : Neta chemical shift offset [70 ppm]
;cnst33  : average of Neta and Nepsilon shifts [~77 ppm]
;o1p     : Czeta chemical shift offset [156 ppm]
;o2p     : He/Hh chemical shift offset [7 ppm]
;o3p     : Ne chemical shift offset [84 ppm]
;inf2    : 1/SW = 2 * DW
;in0     : 1/(2 * SW) = DW
;NS      : 4 * n
;DS      : 64
;td1     : number of experiments
;FnMODE  : States-TPPI
;cpd1    : decoupling according to sequence defined by cpdprg1 (WALTZ64)
;cpd1    : f1 channel - 90 degree pulse for decoupling sequence [80 us]
;cpd3    : decoupling according to sequence defined by cpdprg3 (GARP4)
;cpd3    : f3 channel - 90 degree pulse for decoupling sequence [350 us]
;cpd5    : decoupling according to sequence defined by cpdprg5 (composite 90x240y90x)
;cpd8    : sequence for CEST (cwp)

;for z-only gradients:
;gpz1    : 41% (spoil)
;gpz2    : 11%
;gpz3    : 31% (spoil)
;gpz4    : 19% (spoil)
;gpz5    : 13%
;gpz6    : 29% (spoil)
;gpz7    : 17%
;gpz8    : 37% (spoil)

;use gradient files:
;gpnam1  : SMSQ10.100
;gpnam2  : SMSQ10.100
;gpnam3  : SMSQ10.100
;gpnam4  : SMSQ10.100
;gpnam5  : SMSQ10.100
;gpnam6  : SMSQ10.100
;gpnam7  : SMSQ10.100
;gpnam8  : SMSQ10.100
;gpnam9  : SMSQ10.100
;gpnam10 : SMSQ10.100

;preprocessor-flags-start
;HALFDWELL: for initial sampling delay of half a dwell-time with
;          option -DHALFDWELL (eda: ZGOPTNS)
;preprocessor-flags-end

```

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