Determination of $(\Delta S/S_0)^{cor}$ for REDOR and fpCTDQBU analyses

This material considers determination of $(\Delta S/S_0)^{cor}$, the contribution to $(\Delta S/S_0)^{exp}$ due only to the labeled nuclei. $(\Delta S/S_0)^{cor}$ is equivalently described by the parameter "f", the S_1/S_0 ratio for the labeled ^{13}CO nuclei considering only other labeled nuclei. Comparison of $(\Delta S/S_0)^{cor}$ to $(\Delta S/S_0)^{sim}$ yields the labeled nuclei internuclear dipolar couplings and distances. In the equations in this section, the subscript i (referring to dephasing time τ_i) is not written. Contributions to S_0 and S_1 are considered for ^{13}CO nuclei in different environments.

A. REDOR analysis of I4 peptide

The following parameters/approximations are used:

A1. There is 99% labeling of the Ala-9 ^{13}CO and Ala-13 ^{15}N sites. $S_1 = S_0$ for a labeled Ala-9 ^{13}CO in a molecule with a Ala-13 ^{14}N.

A2. Effects of natural abundance ^{15}N on ^{13}CO S_1 signals are evaluated using the following criteria: (1) $S_1 = 0$ for a labeled Ala-9 ^{13}CO separated by one or two bonds from a natural abundance ^{15}N at Ala-10 or Ala-9. Ala-9 S_1 is not affected by other natural abundance ^{15}N. (2) $S_1 = 0$ for natural abundance backbone ^{13}COs at Glu-12 or Ala-13 which are separated by one or two bonds from the labeled Ala-13 ^{15}N. $S_1 = S_0$ for other natural abundance backbone ^{13}CO sites. Criteria (1) and (2) are based on the close distance (≤ 2.5 Å) and consequent strong (≥ 200 Hz) dipolar coupling of ^{13}CO and ^{15}N nuclei separated by one or two bonds.

A3. Ten percent of the Ala-9 ^{13}CO S_0 is due to molecules in random coil structures. The Ala-9 ^{13}CO in these structures have $S_1 = 0$ if criterion A2-(1) is satisfied and have $S_1 = S_0$ otherwise. Previous solid-state NMR experiments suggest that the I4 peptide has 17 ± 6%

random coil structure but a lower random coil population is chosen in our analysis because some of the random coil ^{13}CO shifts are outside the 1 ppm integration range used to calculate S_0 and S_1 intensity values (*J. Am. Chem. Soc.*, **120**, 1998, 7039-7048).

The $(\Delta S/S_0)^{cor}$ expression is calculated using the following parameters: U_C and U_N, the fractions of Ala-9 ^{12}CO sites and Ala-13 ^{14}N sites, respectively; A_C and A_N, the ^{13}C and ^{15}N natural abundances, respectively; n, the total number of unlabeled peptide backbone CO sites in an I4 molecule; and h, the fraction of the Ala-9 ^{13}CO S_0 signal due to molecules with regular secondary structure. A flow chart for the determination of $(\Delta S/S_0)^{cor}$ is given in Figure S1.

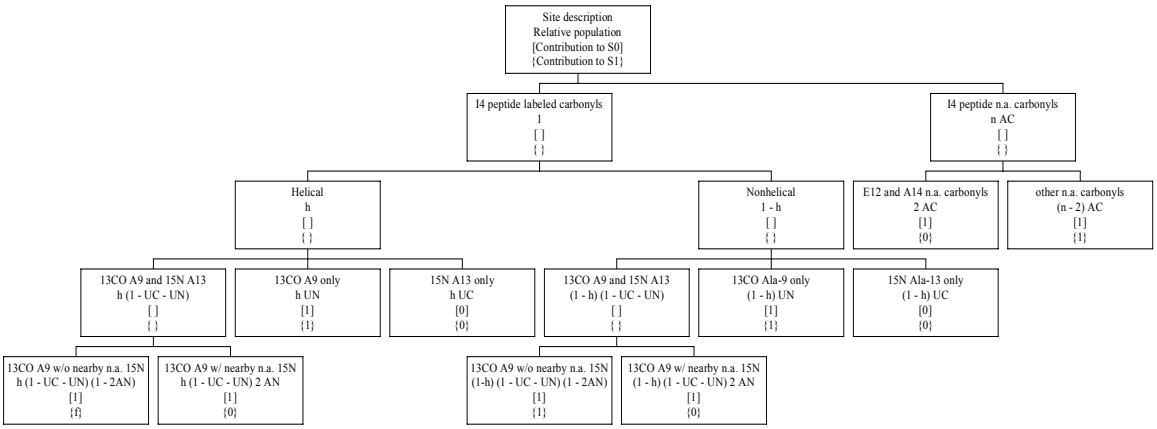

FIGURE S1. Flow chart of derivation of $(\Delta S/S_0)^{cor}$ for REDOR of the I4 peptide. The four rows in each box are in sequence: the site description, its relative population, and its contributions to S_0 and S_1.

Derivation

Express S_0^{exp} as the sum of contributions from labeled ^{13}CO nuclei (S_0^{lab}) and from natural abundance ^{13}CO nuclei ($S_0^{n.a.}$):

$$S_0^{\exp} = S_0^{lab} + S_0^{n.a.} = 1 - U_C + n A_C \qquad \text{(S1)}$$

with:

$$S_0^{lab} = h\left(1-U_C-U_N\right) + hU_N + \left(1-h\right)\left(1-U_C-U_N\right) + \left(1-h\right)U_N \tag{S2}$$

and:

$$S_0^{n.a.} = 2\,A_C + \left(n-2\right)A_C \tag{S3}$$

Express $S_1^{\exp}$ as the sum of contributions from labeled ^{13}CO nuclei (S_1^{lab}) and from natural abundance ^{13}CO nuclei ($S_1^{n.a.}$):

$$S_1^{exp} = S_1^{lab} + S_1^{n.a.} \tag{S4}$$

with:

$$S_1^{lab} = h\left(1-U_C-U_N\right)\left(1-2A_N\right) f + hU_N + \left(1-h\right)\left(1-U_C-U_N\right)\left(1-2A_N\right) + \left(1-h\right)U_N \tag{S5}$$

and:

$$S_1^{n.a.} = \left(n-2\right)A_C \tag{S6}$$

and the parameter *f*:

$$f = \frac{S_1^{cor}}{S_0^{cor}} = 1 - \frac{S_0^{cor} - S_1^{cor}}{S_0^{cor}} = 1 - \left(\frac{\Delta S}{S_0}\right)^{cor} \tag{S7}$$

Incorporate Eq. S7 into Eq. S5:

$$\begin{aligned} S_1^{lab} &= h\left(1-U_C-U_N\right)\left(1-2A_N\right)\left[1-\left(\frac{\Delta S}{S_0}\right)^{cor}\right] + \left(1-h\right)\left(1-U_C-U_N\right)\left(1-2A_N\right) + U_N \\ &= \left(1-U_C-U_N\right)\left(1-2A_N\right) - h\left(1-U_C-U_N\right)\left(1-2A_N\right)\left(\frac{\Delta S}{S_0}\right)^{cor} + U_N \end{aligned} \tag{S8}$$

U_C, U_N, and $2A_N$ are much less than 1 so that:

$$\left(1-U_C-U_N\right)\left(1-2A_N\right) \cong 1-U_C-U_N-2A_N \tag{S9}$$

and:

$$S_1^{lab} \cong 1 - U_C - 2A_N - h\left(1 - U_C - U_N - 2A_N\right)\left(\frac{\Delta S}{S_0}\right)^{cor} \qquad \text{(S10)}$$

Incorporate Eqs. S6 and S10 in Eq. S4:

$$S_1^{\exp} = 1 - U_C - 2A_N - h\left(1 - U_C - U_N - 2A_N\right)\left(\frac{\Delta S}{S_0}\right)^{cor} + \left(n - 2\right)A_C \,. \qquad \text{(S11)}$$

Combine Eqs. S1, S2, S3, and S11:

$$S_0^{\exp} - S_1^{\exp} = \left[1 - U_C + n\,A_C\right] - \left[1 - U_C - 2A_N - h\left(1 - U_C - U_N - 2A_N\right)\left(\frac{\Delta S}{S_0}\right)^{cor} + \left(n - 2\right)A_C\right] \qquad \text{(S12)}$$

and simplify:

$$S_0^{\exp} - S_1^{\exp} = 2A_C + 2A_N + h\left(1 - U_C - U_N - 2A_N\right)\left(\frac{\Delta S}{S_0}\right)^{cor} \qquad \text{(S13)}$$

Combine Eqs. S1 and S13:

$$\left(\frac{\Delta S}{S_0}\right)^{\exp} = \frac{2A_C + 2A_N + h\left(1 - U_C - U_N - 2A_N\right)\left(\frac{\Delta S}{S_0}\right)^{cor}}{1 - U_C + n\,A_C} \qquad \text{(S14)}$$

and rewrite:

$$\left(\frac{\Delta S}{S_0}\right)^{cor} = \frac{1 - U_C + n\,A_C}{h\left(1 - U_C - U_N - 2A_N\right)}\left(\frac{\Delta S}{S_0}\right)^{\exp} - \frac{2A_C + 2A_N}{h\left(1 - U_C - U_N - 2A_N\right)} \qquad \text{(S15)}$$

Incorporate $U_C = 0.01$, $U_N = 0.01$, $A_C = 0.011$, $A_N = 0.0037$, $n = 17$, and $h = 0.9$:

$$\left(\frac{\Delta S}{S_0}\right)^{cor} = 1.345\left(\frac{\Delta S}{S_0}\right)^{\exp} - 0.034 \qquad \text{(S16)}$$

B. REDOR analysis of HFPtr-L7$_C$F11$_N$ samples

The following parameters/approximations are used and B1 and B2 are based on A1 and A2 for the I4 peptide.

B1. There is 99% labeling of the Leu-7 ^{13}CO and Phe-11 ^{15}N sites. $S_1 = S_0$ for a labeled Leu-7 ^{13}CO in a peptide strand with a Phe-11 ^{14}N.

B2. (1) $S_1 = 0$ for a labeled Leu-7 ^{13}CO separated by one or two bonds from a natural abundance ^{15}N at Phe-8 or Leu-7. The Leu-7 S_1 is not affected by other natural abundance ^{15}N. (2) $S_1 = 0$ for natural abundance backbone ^{13}COs at Gly-10 or Phe-11 which are separated by one or two bonds from the labeled Phe-11 ^{15}N. $S_1 = S_0$ for other natural abundance backbone ^{13}CO sites.

B3. In the HFPtr-L7$_C$F11$_N$/PC-PG sample, the natural abundance lipid ^{13}CO signal is resolved from the Leu-7 labeled ^{13}CO signal and does not contribute to S_0 or S_1.

Using $h = 1$, Eq. (S15) is modified:

$$\left(\frac{\Delta S}{S_0}\right)^{cor} = \frac{1 - U_C + n A_C}{1 - U_C - U_N - 2A_N}\left(\frac{\Delta S}{S_0}\right)^{\exp} - \frac{2A_C + 2A_N}{1 - U_C - U_N - 2A_N} \qquad \text{(S17)}$$

Consider n as the average number of unlabeled backbone CO sites per strand. Using $U_C = 0.01$, $U_N = 0.01$, $A_C = 0.011$, $A_N = 0.0037$, and $n = 29.33$:

$$\left(\frac{\Delta S}{S_0}\right)^{cor} = 1.350\left(\frac{\Delta S}{S_0}\right)^{exp} - 0.030 \qquad \text{(S18)}$$

C. fpCTDQBU analysis of the D-GFF SSNMR sample

The following parameters/approximations are focused on the Gly-1 ^{13}CO nuclei because S_0 and S_1 signals from these nuclei are used in the experimental data analysis.

C1. ^{13}CO signals from Gly-1, Phe-2, and Phe-3 are completely resolved.

C2. Intermolecular ^{13}C-^{13}C dipolar coupling is not considered. For Gly-1 ^{13}CO, the closest intermolecular carbon nucleus is > 4 Å away.

C3. For D-GFF, there is 99% labeling of Gly-1 ^{13}CO and Phe-3 ^{13}CO. $S_1 = S_0$ for a molecule with a labeled Gly-1 ^{13}CO and a Phe-3 ^{12}CO.

C4. S_1 values for a molecule with a labeled Gly-1 ^{13}CO and nearby natural abundance ^{13}C are set with the following criteria: (1) $S_1 = 0$ when $\tau_i \leq 32$ ms and the labeled Gly-1 ^{13}CO/natural abundance ^{13}C nuclei are separated by one or two bonds. (2) $S_1 = 0$ when $\tau_i > 32$ ms and the labeled Gly-1 ^{13}CO/natural abundance ^{13}C nuclei are separated by one, two, or three bonds. (3) S_1 is not affected by the natural abundance ^{13}C if neither criterion (1) nor (2) is satisfied. The criteria are based on the ~1.5 Å, ~2.5 Å and ~3.8 Å distances for one-, two- and three-bond ^{13}C-^{13}C separations, respectively, and the consequent 2200 Hz, 500 Hz, and 140 Hz dipolar couplings.

C5. $S_1 = S_0$ for a natural abundance Gly-1 ^{13}CO in an unlabeled GFF molecule.

Each $(\Delta S/S_0)^{cor}$ value is calculated using the following parameters: U_{C1} and U_{C2}, the fractions of Gly-1 and Phe-3 ^{12}CO sites in D-GFF, respectively; A_C, the fractional ^{13}C natural abundance; n, the ratio of unlabeled GFF to D-GFF molecules in the crystal; and m, the number of unlabeled carbon nuclei which satisfy either criterion C4-(1) or C4-(2).

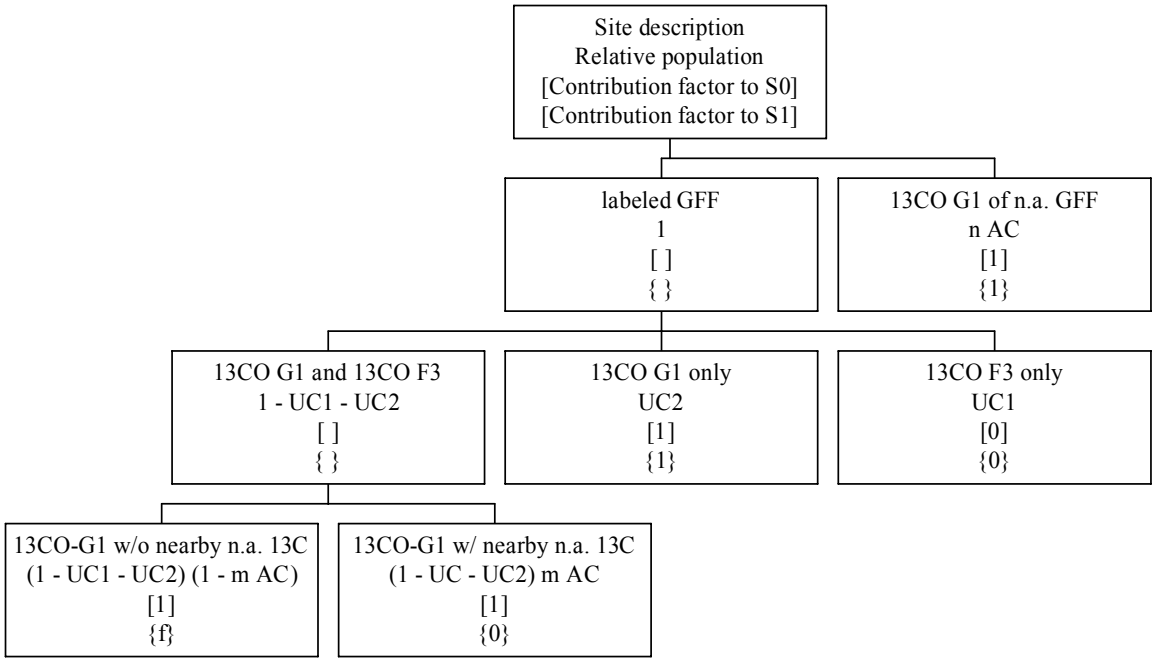

FIGURE S2. Flow chart of derivation of $(\Delta S/S_0)^{cor}$ for fpCTDQBU of the D-GFF sample.

The fpCTDQBU expression for $(\Delta S/S_0)^{cor}$ is:

$$\left(\frac{\Delta S}{S_0}\right)^{cor} = \frac{1 - U_{C1} + n A_C}{1 - U_{C1} - U_{C2} - m A_C}\left(\frac{\Delta S}{S_0}\right)^{\exp} - \frac{m A_C}{1 - U_{C1} - U_{C2} - m A_C} \qquad \text{(S19)}$$

The values of U_{C1}, U_{C2}, A_C, and n are 0.01, 0.01, 0.011, and 49. For $\tau_i \le 32$ ms, $m = 2$ and numerical evaluation of Eq. S19 yields:

$$\left(\frac{\Delta S}{S_0}\right)^{cor} = 1.596\left(\frac{\Delta S}{S_0}\right)^{exp} - 0.023 \qquad \text{(S20)}$$

For $\tau_i > 32$ ms, $m = 4$ and numerical evaluation of Eq. S19 yields:

$$\left(\frac{\Delta S}{S_0}\right)^{cor} = 1.634\left(\frac{\Delta S}{S_0}\right)^{exp} - 0.047 \qquad \text{(S21)}$$

D. fpCTDQBU analysis of the HFPtr-L7$_C$F11$_N$ sample

The following parameters/approximations are used:

D1. There is 99% labeling of Leu-7 ^{13}CO.

D2. S_1 values for a Leu-7 ^{13}CO with nearby natural abundance ^{13}C are determined by the following criteria: (1) $S_1 = 0$ for $\tau_i \leq 32$ ms and Leu-7 ^{13}CO/natural abundance ^{13}C separated by one or two bonds. (2) $S_1 = 0$ for $\tau_i > 32$ ms and Leu-7 ^{13}CO/natural abundance ^{13}C separated by one, two, or three bonds. (3) S_1 is not affected by natural abundance ^{13}C if neither criterion (1) nor (2) is satisfied.

D3. The following criteria are used to set S_1 values for natural abundance backbone ^{13}CO sites: (1) For the Ala-6 and Phe-8 sites, $S_1 = S_0$ for $\tau_i \leq 32$ ms and $S_1 = 0$ for $\tau_i > 32$ ms. These values are in accord with D2-(1) and D2-(2). (2) For other sites, $S_1 = S_0$ for all values of τ_i.

Each $(\Delta S/S_0)^{cor}$ value is calculated using the parameters U_C, A_C, n and m where: U_C is the fraction of Leu-7 ^{12}CO sites; A_C is the fractional ^{13}C natural abundance; n is the average number of unlabeled backbone CO sites per peptide strand; and m is the number of natural abundance sites which satisfies either D2-(1) or D2-(2).

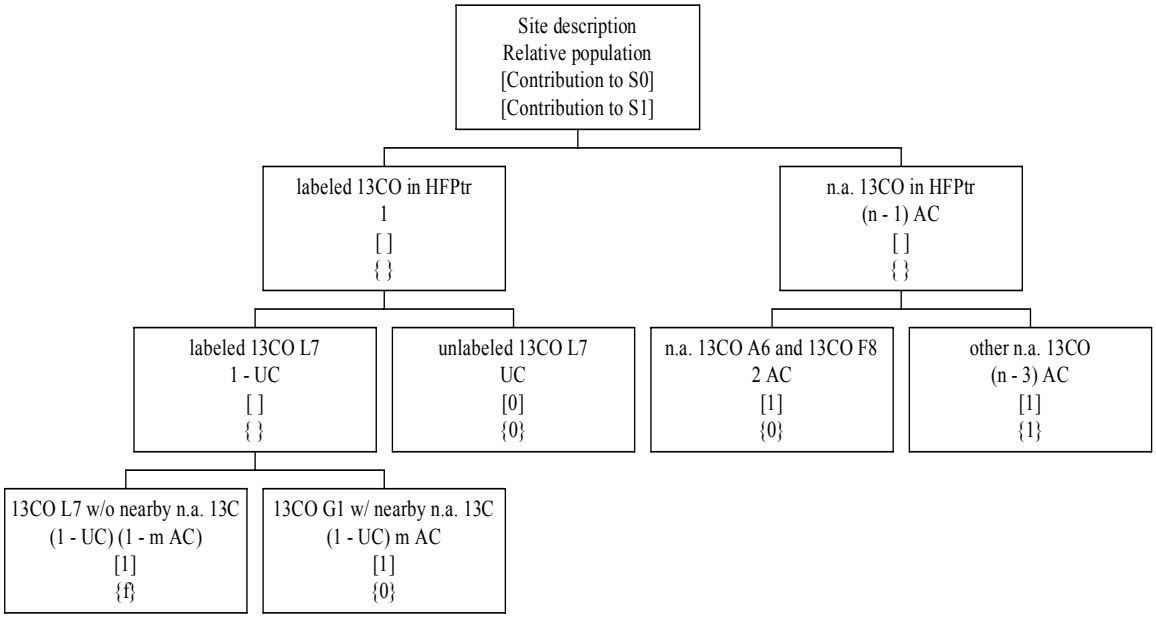

FIGURE S3. Flow chart of derivation of $(\Delta S/S_0)^{cor}$ for fpCTDQBU of the HFPtr-$L_{7C}F_{11N}$ sample for $\tau_i > 32$ ms.

A derivation similar to that for Eq. S19 yields an expression for $\tau_i \leq 32$ ms:

$$\left(\frac{\Delta S}{S_0}\right)^{cor} = \frac{1 - U_C + n A_C}{1 - U_C - m A_C}\left(\frac{\Delta S}{S_0}\right)^{\exp} - \frac{m A_C}{1 - U_C - m A_C} \tag{S22}$$

The values of U_C, A_C, n, and m are 0.01, 0.011, 29.33, and 3, respectively, and numerical evaluation of Eq. S22 yields:

$$\left(\frac{\Delta S}{S_0}\right)^{cor} = 1.372\left(\frac{\Delta S}{S_0}\right)^{exp} - 0.034\,; \tag{S23}$$

For $\tau_i > 32$ ms, the contribution to S_1 of natural abundance Ala-6 and Phe-8 ^{13}COs slightly modifies Eq. S22:

$$\left(\frac{\Delta S}{S_0}\right)^{cor} = \frac{1 - U_C + n A_C}{1 - U_C - m A_C}\left(\frac{\Delta S}{S_0}\right)^{\exp} - \frac{(m + 2) A_C}{1 - U_C - m A_C} \tag{S24}$$

The values of U_C, A_C, n, and m are 0.01, 0.011, 29.33, and 7, respectively, and numerical evaluation of Eq. S24 yields:

$$\left(\frac{\Delta S}{S_0}\right)^{cor} = 1.438\left(\frac{\Delta S}{S_0}\right)^{exp} - 0.108 \qquad \text{(S25)}$$

In Eqs. S22 and S24, the small fraction of Leu-7 ^{12}CO (denoted by the parameter U_C) is considered in the calculation of total signal but is neglected in the calculation of the effect of ^{13}C-^{13}C dipolar coupling on S_1. This latter approximation neglects the possibilities of a Leu-7 ^{13}CO being near either one or two Leu-7 ^{12}COs in adjacent strands. The approximation makes a much smaller contribution to the uncertainty in $(\Delta S/S_0)^{cor}$ than does uncertainty in $(\Delta S/S_0)^{exp}$.

The experimental S_0 and S_1 intensities are determined by integration over a defined region and for the I4 and HFPtr samples, the contribution from a particular labeled or natural abundance ^{13}CO site depends on its chemical shift distribution which is influenced by amino-acid identity as well as conformation. Deconvolution of individual site contributions is not feasible with the medium-linewidth (~2 ppm full-width at half-maximum) and medium signal-to-noise spectra and in the above derivations, we made the approximation in the that labeled and natural abundance sites contribute fully to the S_0 and S_1 signals. For the HFPtr samples, the experimental signal-to-noise ratios are ~10 and this approximation makes a much smaller contribution to the uncertainty in $(\Delta S/S_0)^{cor}$ than does uncertainty in $(\Delta S/S_0)^{exp}$.