

# Spin State Selective Carbon-Detected HNCO with TROSY Optimization in all Dimensions and Double Echo-Antiecho Sensitivity Enhancement in Both Indirect Dimensions

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## 1. Appendix

There are a variety of conventions for the nomenclature employed for describing spin states, and the chemical shift and chemical shielding tensors (as well as the symbols  $\sigma$  and  $\delta$  for chemical shift tensor and chemical shielding tensor) and their principal axes. To calculate spin state specific relaxation rates of single transition product operators, we have used a uniform definition of single transition product operators and the corresponding formulae to calculate their relaxation rates.

The spherical product operators are defined as:  $S_{\pm} = \frac{1}{\sqrt{2}}(S_x \pm iS_y)$ . The single transition product operators are:  $S_{\pm}I^{(\alpha)} = S_{\pm}(\frac{1}{2}E + I_z)$  with chemical shift frequency appearing at  $\pm(\omega_S + \pi J_{IS})$  and  $S_{\pm}I^{(\beta)} = S_{\pm}(\frac{1}{2}E - I_z)$  with chemical shift frequency appearing at  $\pm(\omega_S - \pi J_{IS})$ , where  $\pm$  corresponds to the spin order +1 or -1. It should be emphasized that  $\omega_S$  can be positive or negative depending on the sign of the gyromagnetic ratio  $\gamma_S$ , and that  $J_{IS}$  can also be positive or negative. The relaxation rates for the single transition product operators are given as:

$$R(S_{\pm}I^{(\alpha)}) = \overline{R_{S_{\pm}}^I} + \Gamma_{DD,CSA}^S \quad (A1)$$

$$R(S_{\pm}I^{(\beta)}) = \overline{R_{S_{\pm}}^I} - \Gamma_{DD,CSA}^S \quad (A2)$$

where  $\overline{R_{S_{\pm}}^I}$  is the average transverse relaxation rate of spin S in-phase and anti-phase with respect to spin I,  $\overline{R_{S_{\pm}}^I} = \frac{R(S_{\pm}) + R(2S_{\pm}I_z)}{2}$ , and  $\Gamma_{DD,CSA}^S$  is the cross-correlated relaxation rate.

$$\Gamma_{DD,CSA}^S = \Gamma_{DD(IS)CSA^*(S)} = -\frac{1}{6} \rho_{IS} \Delta\sigma_S^* \omega_S \{4J(0) + 3J(\omega_S)\} \quad (A3)$$

with the dipolar interaction amplitude  $\rho_{IS} = \frac{\mu_0 \gamma_I \gamma_S}{4\pi} \frac{h}{\langle r_{IS}^3 \rangle 2\pi}$ , and

$$\Delta\sigma_S^* = P_2(\cos\theta^{IS,XX\sigma_S}) \Delta\sigma_{S,XX} + P_2(\cos\theta^{IS,YY\sigma_S}) \Delta\sigma_{S,YY} \quad (A4)$$

$\Delta\sigma_{S,XX} = \sigma_{S,ZZ} - \sigma_{S,XX}$ ,  $\Delta\sigma_{S,YY} = \sigma_{S,ZZ} - \sigma_{S,YY}$ , where  $\sigma_{S,XX}$ ,  $\sigma_{S,YY}$ , and  $\sigma_{S,ZZ}$  are the principal values of the traceless chemical shielding anisotropy (CSA\*, where the \* is used to differentiate from CSA, acronym for chemical shift anisotropy) tensors of spin S with  $|\sigma_{S,ZZ}| > |\sigma_{S,XX}| > |\sigma_{S,YY}|$ .  $\theta^{IS,XX\sigma_S}$ ,  $\theta^{IS,YY\sigma_S}$  are the angles between the internuclear IS vector and the principal axes XX and YY of the CSA\* tensor of spin S. When the CSA\* tensor is axially symmetric, that is  $\sigma_{S,XX} = \sigma_{S,YY}$ , on the basis of

$$P_2(\cos\theta^{IS,XX\sigma_S}) + P_2(\cos\theta^{IS,YY\sigma_S}) + P_2(\cos\theta^{IS,ZZ\sigma_S}) = 0 \quad (A5)$$

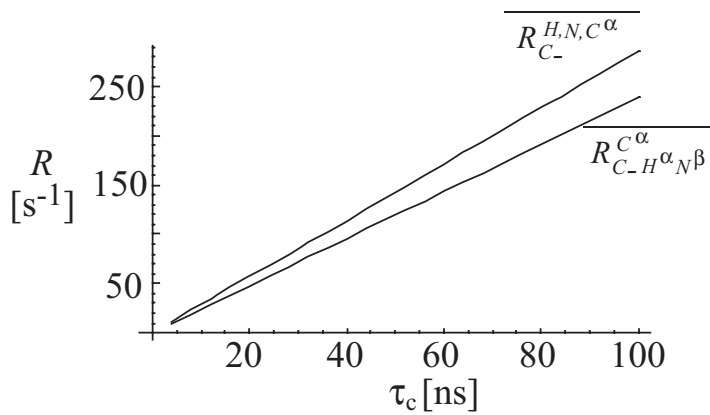
then,

$$\Delta\sigma_S^* = -\Delta\sigma_S P_2(\cos\theta^{IS,ZZ\sigma_S}). \quad (A6)$$

Conventionally, for an approximately axially symmetric traceless tensor, the chemical shielding tensor anisotropy  $\Delta\sigma_S$  is defined as:  $\Delta\sigma_S = \sigma_{S,\parallel} - \sigma_{S,\perp} = \sigma_{S,ZZ} - (\sigma_{S,XX} + \sigma_{S,YY})/2$  (in fact  $\Delta\sigma_S = \Delta\sigma_{S,XX} = \Delta\sigma_{S,YY}$  when  $\sigma_{S,XX} = \sigma_{S,YY}$ ). For these conditions, the axis ZZ is usually described as the main axis of the tensor, and the angle between the IS vector and the tensor main axis,  $\theta^{IS,ZZ\sigma_S}$  can be written as  $\theta^{IS,\Delta\sigma_S}$ . Thus, for an approximately axially symmetric traceless CSA\* tensor,

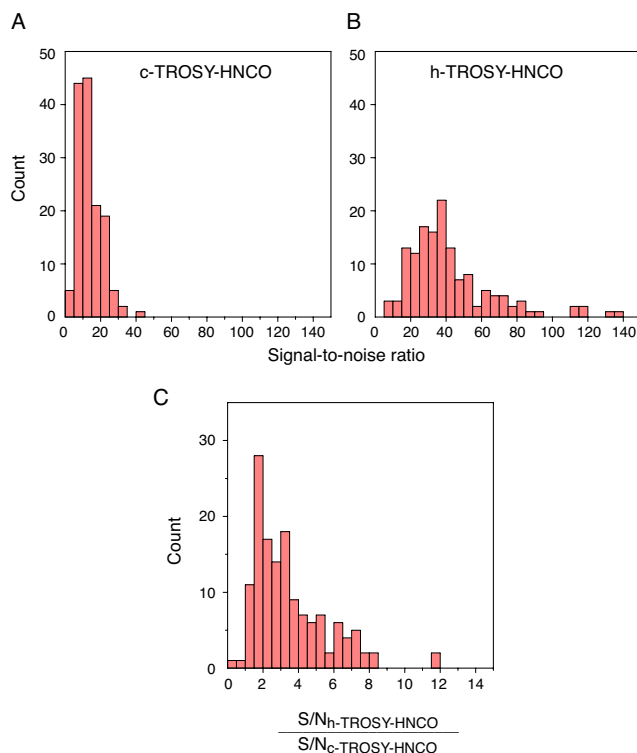
$$\Gamma_{DD,CSA}^S = \Gamma_{DD(IS)CSA^*(S)} = \frac{1}{6} \rho_{IS} \Delta\sigma_S P_2(\cos\theta^{IS,\Delta\sigma_S}) \omega_S \{4J(0) + 3J(\omega_S)\} \quad (A7)$$

## 2. Carbonyl relaxation



**Figure S1.** Theoretical comparison of the  $R_{C-H^{(\alpha)}N^{(\beta)}}^{C^{\alpha}}$  relaxation rate of a doubly TROSY optimized carbonyl, as in the c-TROSY-HNCO experiment with the  $R_{C-}^{H,N,C^{\alpha}}$  relaxation rate of a carbonyl under  $^{15}\text{N}$  decoupling, as in c-HNCO experiments as a function of the correlation time  $\tau_c$  of the macromolecule. The relaxation rate of the carbonyl in the c-TROSY-HNCO is decreased by about 15% to that in the c-HNCO. Relaxation rates are calculated using Eqs. (A1) and (A2) using the parameters provided in Fig. 3 of the main text, for a spectrometer  $^1\text{H}$  frequency of 800 MHz.

### 3. Comparison of S/N ratios for carbon-detect c-TROSY-HNCO and the conventional out-and-back <sup>1</sup>H-detect h-TROSY-HNCO.



**Figure S2.** Histograms showing an experimental comparison of the distribution of signal-to-noise ratios for (A) the carbon-detected c-TROSY-HNCO and (B) the conventional out-and-back <sup>1</sup>H-detect TROSY-HNCO<sup>S1</sup> experiments, recorded on a sample of 0.75 mM <sup>15</sup>N/<sup>13</sup>C/<sup>2</sup>H/[Leu,Val]-methyl-protonated IIB<sup>Man</sup> in 20 mM phosphate buffer, pH 6.5. (C) Histogram of the pairwise ratio of the signal-to-noise ratios for the h-TROSY-HNCO and c-TROSY-HNCO experiments. The total measurement time for both experiments was about 17.5 hours. For the c-TROSY-HNCO experiment (A) 24(*t*<sub>1</sub>) x 40(*t*<sub>2</sub>) x 2048(*t*<sub>3</sub>) complex points were acquired with 16 scans per increment and maximum acquisition times of 7 ms (<sup>1</sup>H<sub>N</sub>), 16.44 ms (<sup>15</sup>N) and 127.8 ms (<sup>13</sup>C'). Linear prediction of size 36 was applied in the <sup>1</sup>H dimension, followed by the application of 90° shift sine-bell square apodization functions in all three dimensions prior to Fourier transformation. For the h-TROSY-HNCO experiment (B) 96(*t*<sub>1</sub>) x 40(*t*<sub>2</sub>) x 512(*t*<sub>3</sub>) complex points were acquired with 4 scans per increment and maximum acquisition times of 39.7 ms (<sup>13</sup>C'), 16.44ms (<sup>15</sup>N) and 45.88ms (<sup>1</sup>H<sub>N</sub>). 90° shift sine-bell square apodization functions were applied in all three dimensions prior to Fourier transformation.

#### Reference

S1. Yang, D. W.; Venters, R. A.; Mueller, G. A.; Choy, W. Y.; Kay, L. E. *J. Biomol. NMR* **1999**, 14, 333-343.