Using multiple quantum coherence to increase the $^{15}$N resolution in a three-dimensional TROSY HNCO experiment for accurate PRE and RDC measurements

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1. Introduction

In recent years, several NMR methods, which complement traditional NOE-based approaches, have been developed to facilitate three-dimensional structure determination, to characterize molecular motions, and to visualize lowly-populated transient species. In particular, residual dipolar couplings (RDC) yield bond vector orientations relative to an external alignment tensor that are particularly useful for structure determination of multidomain proteins, protein complexes and nucleic acids [1–4]. In addition, accurate RDC data can potentially be used to study protein dynamics [5–13]. Paramagnetic relaxation enhancement (PRE) provides long-range distance information (up to ~35 Å in suitable cases) that is particularly useful in the study of macromolecular complexes [14–16] and has been recently exploited to characterize highly transient, lowly-populated species [17–24], as well as unfolded and disordered states of proteins [25–27].

In cases where resolution permits, conventional backbone amide RDCs and PREs can be measured using 2D $^1$H–$^{15}$N HSQC- or TROSY-[28] based experiments [29,20], allowing the rapid measurement of a large number of RDCs and PREs. However, for larger proteins, or moderately-sized proteins with high α helical or unstructured coil content, cross-peak overlap greatly decreases the accuracy of the measured peak splittings and intensities.

Yang et al. extended the 2D $^1$H–$^{15}$N correlation experiment to a 3D TROSY-based HNCO to separate overlapped peaks along an additional $^{13}$C dimension for the measurement of $^1$D$_{NH}$ RDCs [30]. In principle, a similar HNCO scheme can also be implemented for PRE $\Gamma_2$ measurements by simply replacing the first INEPT element by a PRE measuring block [20]. However, the conventional TROSY-based HNCO [31] suffers from extremely low resolution along the $^{15}$N dimension because the maximum $^{15}$N evolution time is limited by the constant time period (1/2$\nu_{HC}$ ~ 32 ms), for coherence back transfer from $^{13}$C to $^{15}$N, and resolution is inversely proportional to this evolution time. As a result, RDCs and PREs measured along the $^{15}$N dimension in a conventional TROSY HNCO would result in lower precision compared to the 2D $^1$H–$^{15}$N HSQC counterpart.

Here, we present a high-resolution version of the TROSY HNCO, which we refer to as HR-TROSY HNCO, in which both constant time periods (~32 ms each) for coherence forward and backward transfer between $^{15}$N and $^{13}$C are utilized to maximize the $^{15}$N evolution time of 64 ms. This experiment separates cross-peaks along the $^{13}$C dimension while maintaining high resolution along the $^{15}$N dimension. An HNCO pulse scheme with improved resolution along the $^{15}$N dimension was previously proposed by Madsen and Sørensen [32]. In the latter implementation only the $^{15}$N to $^{13}$C (forward) INEPT transfer period (~32 ms) is utilized for $^{15}$N chemical shift evolution, and improved resolution along the $^{15}$N dimension is realized by varying the evolution period from ~32 to 32 ms by shifting the 180° pulses from one end of the constant time period to the other. This concept can also be easily incorporated into our HR-TROSY HNCO pulse sequence which would further extend the evolution time from ~64 to 64 ms. We demonstrate the utility of the 3D HR-TROSY HNCO...
2. Experimental

2.1. Materials

A 29 residue peptide (pKID) comprising the kinase-inducible domain of the cAMP response-element binding protein (CREB, residues 119–146) was synthesized by solid state methods with Ser133 phosphorylated, the N-terminus acetylated, and the C-terminus amidated (Biopeptide Co., San Diego). U-[15N, 13C, 2H]-labeled mouse KIX domain (residues 586–672 of the CREB binding protein) was expressed and purified essentially as described previously [33].

2.2. NMR experiments

All NMR spectra were recorded at 27 °C on a Bruker 600 MHz spectrometer equipped with a z-gradient triple resonance cryo-probe. A 2D 1H–13N HSQC spectrum (non-constant time) was recorded as a reference with 128(t1) × 512(t2) complex points along the 13N and 1H dimensions corresponding to acquisition times of 64 and 63.9 ms, respectively. The data matrix for the HR-TROSY HNCO comprises 100(t1) × 10(t2) × 512(t3) complex points along the 15N, 13C and 1H dimensions corresponding to acquisition times of 63.24, 8.28 and 77.4 ms, respectively. The HR-TROSY HNCO spectrum was recorded with 16 scans per increment and an interscan delay of 1.1 s, resulting in ~18 h of total measurement time. For comparison, a conventional TROSY HNCO was recorded with 50(t1) × 10(t2) × 512(t3) complex points along the 15N, 13C and 1H dimensions corresponding to acquisition times of 31.62, 8.28 and 77.4 ms, respectively, using 32 scans per increment, resulting in the same total measurement time as the HR-TROSY HNCO. For both the HR-TROSY HNCO and conventional TROSY experiments the 15N, 13C and 1H carrier frequencies were placed at 117, 176 and 4.75 ppm, respectively, and the sweep widths in the corresponding dimensions were 26, 8 and 11.02 ppm, respectively. (The same carrier frequencies and sweep widths for 1H and 15N were also used for the 1H–13N HSQC.) The conventional TROSY and HR-TROSY HNCO spectra were processed identically using linear prediction and zero-filling along the 15N dimension, giving final spectra with 256 and 512 frequency data points, respectively. Replacing the first INEPT element (dashed block in Fig. 1A) with the PRE measuring block (Fig. 1B) adapts the HR-TROSY HNCO pulse sequence to one suitable for PRE F2 measurements. By setting the transverse relaxation delay T1 to be 0 and 14 ms and acquiring the data for both delays in an interleaved manner [20], the total experimental time is ~39 h. All data sets were processed using the NMRPipe package [34].

3. Results and discussion

Fig. 1A provides a schematic of the 3D HR-TROSY HNCO pulse scheme. After the first INEPT, magnetization on 15N is transferred to 13C and MQ coherence between 13N and 13C is generated by the 90° 13C pulse (φ2). By synchronously shifting the 180° 15N and 13C pulses (as indicated by the arrows), the chemical shift of 15N is encoded during both constant time periods for the coherence forward and backward transfer between 15N and 13C, thereby extending the maximum 15N evolution time to as long as 11 JNCA × 64 ms. This allows the resolution along the 15N dimension of the 3D HR-TROSY experiment to be comparable to that of the 2D 1H–15N HSQC counterpart. A similar approach has been previously reported for the 3D HNCA TROSY used for sequence specific backbone assignments [31], in which the maximum 15N evolution time was 1/(JNCA + 1/JHNC) ~ 44 ms. The 3D HR-TROSY HNCO offers even higher resolution along the 15N dimension, which results in better separation of amide cross-peaks and yields clearer 2D planes and strips.

Fig. 2A shows the overall 2D 1H–13N HSQC spectrum for the KIX/pKID complex, and an expansion of the most crowded region is provided in Fig. 2B. 1H–13C planes of the HR-TROSY HNCO and conventional TROSY HNCO spectra are displayed in Figs. 2C and D, respectively, taken at the 13N chemical shifts corresponding to the dashed lines a–e in Fig. 2B. A comparison of Figs. 2C and D clearly demonstrates that the HR-TROSY HNCO spectrum is cleaner
and better resolved than the conventional TROSY HNCO, which exhibits significant peak leakage between different $^{14}$N-$^{13}$C planes due to the lower $^{15}$N resolution. Indeed, the $^{14}$N-$^{13}$C planes taken from the conventional TROSY HNCO at the $^{15}$N chemical shifts $b$ and $c$ are completely overlapped (identical). Fig. 3 provides a comparison of 1D traces along the $^{15}$N dimension for the reference 2D $^1$H-$^{15}$N HSQC (taken at the $^1$H chemical shifts indicated by the red dashed lines in Fig. 2), the 3D HR-TROSY HNCO (taken at the cross-peak positions indicated by the green stars in Fig. 2C) and the conventional 3D TROSY HNCO (taken at the cross-peak positions indicated by the blue stars in Fig. 2D). These traces correspond to peaks for W6, V50, E80, E81, K82 and R83 (all peaks in the green traces have narrower linewidths than those in the blue traces). The increased resolution along the $^{15}$N dimension makes the HR-TROSY HNCO particularly well suited for RDC and PRE measurements because peak positions and intensities can be measured more accurately in the 3D HR-TROSY HNCO than in the conventional 3D TROSY HNCO or 2D $^1$H-$^{15}$N HSQC experiments.

During the constant time periods of the HR-TROSY HNCO, no decoupling or 180° pulse is applied on $^1$H to maintain the $^{15}$N coherence under the TROSY state. To minimize intensity attenuation on MQ coherence due to passive $J$ couplings between $^1$H and
$^{15}\text{N}$ (e.g., $^{2}J_{\text{HN}}$ and $^{2}J_{\text{HN}}^{(1)}$) during the long $^{15}\text{N}$ evolution period (64 ms) and between $^{1}\text{H}$ and $^{13}\text{C}$ (e.g., $^{2}J_{\text{HC}}$ and $^{2}J_{\text{HC}}^{(1)}$) during the $^{13}\text{C}$ evolution period ($\tau_{2}$), we recommend perdeuterated samples for this type of experiment even for proteins of moderate molecular size. This is especially important when measuring peak intensities. Clear recognition of resolved cross-peaks with nearly undistorted peak shape is critical for measuring both peak position and intensity, which are important for accurate measurement of $^{1}\text{D}_{\text{HN}}$ RDCs and $^{1}\text{H}_{\text{N}}$ PRE relaxation rates, respectively.

Indeed, this basic 3D HR-TROSY HNCO pulse scheme is easily adapted to pulse sequences for the measurement of $^{1}\text{H}_{\text{N}}-^{15}\text{N}$ PRE $\Gamma_{2}$ rates [20] (Fig. 1B) and $^{1}\text{D}_{\text{HN}}$ RDCs (Fig. 1C). For PRE measurements, the INEPT element (delineated by the dashed block in Fig. 1A) is replaced by the PRE measuring block (Fig. 1B). For RDC measurements, two alternative schemes are available. For proteins of moderate molecular size, where the anti-TROSY component of $^{15}\text{N}$ is not extremely broad and the peak shape remains undistorted, the anti-TROSY component can be directly selected by simply swapping the phases of $\phi_{A}$ and $\phi_{B}$ in the pulse scheme shown in Fig. 1A; selection of the TROSY and anti-TROSY components of $^{15}\text{N}$ can be run in an interleaved mode to obtain a pair of peaks, from which the splitting $J$ or $(J+D)$ is measured. When the relaxation of the anti-TROSY component of $^{15}\text{N}$ is too fast or the peak shape is severely distorted, a $(J+D)$ coupling scaling element $\kappa_{1}\text{t}$ is inserted [35] as indicated by the dashed lines in Fig. 1, and no other changes are necessary. In this case, the relevant coherence is under the TROSY state during the two constant time periods (total as long as 64 ms), which better optimizes the relaxation properties of the pulse scheme. Insertion of the $\kappa_{1}\text{t}$ element allows the relevant coherence to evolve under the Hamiltonian $(J+D)$. The measured splitting between the peak of this scheme and that of the original HR-TROSY HNCO along the $^{15}\text{N}$ dimension is $(J+D)$ scaled by a factor of $\kappa_{1}$. In most cases, the optimal value of the scaling factor $\kappa_{1}$ is 1. Lerche et al. [36] previously suggested a scheme for measuring $^{1}\text{D}_{\text{HN}}$ RDCs along the $^{1}\text{H}_{\text{N}}$ dimension in which the anti-TROSY and TROSY components of $^{1}\text{H}_{\text{N}}$ or the neutral (decoupled or refocused) $^{1}\text{H}_{\text{N}}$ chemical shift are chosen for the measurement of the couplings $(J$ or $J+D)$. To avoid potential linewidth broadening along the $^{1}\text{H}_{\text{N}}$ dimension due to passive $^{1}\text{H}-^{1}\text{H}$ RDCs, we made use of an ST2-PT element [37] in our pulse sequence to select the TROSY or anti-TROSY component for measuring $^{1}\text{D}_{\text{HN}}$ RDCs along the $^{15}\text{N}$ dimension while detecting $^{1}\text{H}_{\text{N}}$ under the TROSY state.

Acknowledgments

This work was supported by the intramural program of NIDDK, National Institutes of Health, and the Intramural AIDS Targeted Antiviral Program of the Office of the Director of the National Institutes of Health (to G.M.C.).

References


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