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Communication

Unraveling long range residual dipolar coupling networks in strongly aligned proteins

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ABSTRACT

Long-range residual dipolar couplings (lrRDCs) have the potential to serve as powerful structural restraints in protein NMR spectroscopy as they can provide both distance and orientation information about nuclei separate in sequence but close in space. Current nonselective methods for their measurement are limited to moderate alignment strengths due to the sheer abundance of active couplings at stronger alignment. This limits the overall magnitude and therefore distance across which couplings can be measured. We have developed a double resonance technique for the inversion of individual coupled spin pairs, called Selective Inversion by Single Transition Cross Polarization (SIST-CP). This technique enables the selective recoupling of lrRDCs, thus allowing the complex multiplets occurring in strongly aligned systems to be disentangled. This technique is demonstrated in the context of an application to the measurement of $^{13}C'$–$^{1}H^{N}$ lrRDCs in strongly aligned proteins.

1. Introduction

The measurement of NMR residual dipolar couplings (RDCs) in weakly aligned molecules can provide valuable information on molecular structure and dynamics. When measured between directly bonded nuclei, RDCs assume large magnitudes in even weakly aligned conditions and are thus highly sensitive to bond orientations. Presently, the measurement of couplings such as $^{1}D_{NH}$ in proteins is routine for structure refinement or to establish long range ordering of extended or multi-domain systems [1–3]. When RDCs are measured between non-bonded nuclei, they depend on the distance between nuclei in addition to the orientation and thus, for non-scalar coupled nuclei in particular, can provide long range distance restraints similar to the NOE while not suffering from spin diffusion effects [4]. Yet the measurement of these long range residual dipolar couplings (lrRDCs) is quite challenging, and so applications have been limited to date [5–7].

A major obstacle to lrRDC measurement is that the coupling partner giving rise to a specific lrRDC is in general not known. In specialized cases, one can transfer magnetization between covalently linked nuclei in order to control the identity of the coupling partner but this limits measurements to interactions between nuclei proximal in sequence [8–10]. Alternatively, one can transfer magnetization across the lrRDC in order to identify coupling partners [4,11,12], but such an approach fails if the magnitudes of the lrRDCs are too small. Employing a stronger degree of alignment might allow more weakly coupled (and hence more distant) nuclear spin pairs to be identified, but in practice the rapid increase in the number of coupling partners causes dephasing of the active magnetization and a correspondingly smaller fraction of the magnetization that is transferrable to any individual coupling partner. Such dipolar broadening effects can be mitigated but not overcome by dilution of the spin density or in some cases by broadband decoupling of undesired coupling interactions [13–16]. Yet these approaches still require nonselective preservation and transfer of coherence across the coupling interactions of interest and thus remain unsuitable for conditions of strong alignment when many couplings are present.

It has long been recognized that for small molecules with well resolved resonances, selective irradiation of individual spins can allow all coupling interactions to that spin to be mapped out [17–19]. Following this rationale, we propose a strategy here whereby the selective inversion of individual spins enables the selective (de-/re-) coupling of specific spin–spin interactions within a coupling network of arbitrary complexity. But in order to employ such a strategy, one must be able to achieve the highly selective inversion of individual spins. We describe here a pulse double resonance technique suitable for application to proteins that enables the selective inversion of a coupled heteronuclear spin pair. This technique is demonstrated in an application in which individual amide ^{15}N–$^{1}H^{N}$ spin pairs are inverted during a dephasing period inserted within a ^{13}C-detected CACO–COSY experiment. This enables the measurement of $^{1}H^{N}$–$^{13}C'$ lrRDCs originating from specific $^{1}H^{N}$ targets under conditions of strong alignment in which dipolar splittings are abundant.

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2. Theoretical

Shown in Fig. 1A is our proposed double resonance pulse element designed for application to amide N–H spin pairs. It enables the selective inversion of a single coupled pair of spins depending not on their individual but rather their joint two dimensional chemical shift resolution properties. This is accomplished by selectively interchanging the populations of the $\alpha\tilde{\alpha}/\beta\beta$ and $\alpha\tilde{\beta}/\beta\alpha$ states using the Single Transition Cross Polarization (ST-CP) technique developed by Bodenhausen and coworkers [20]. In ST-CP, the application of very low power Hartmann–Hahn matched contact-pulses (i.e. $\omega_H = \omega_N = \omega_1 \ll J_{NH}$) at frequencies corresponding to individual transitions drives the "quasi-isotropic" selective transfer of magnetization between the coupled spins [21]. It can be shown that when the ST-CP contact time, τ_{CP}, is chosen to be $\sqrt{2}\pi/\omega_1$, longitudinal magnetization for either spin is entirely transferred to the other spin according to,

$$\begin{pmatrix} I_z \\ S_z \end{pmatrix} \xrightarrow{SE-ST-CP} \begin{pmatrix} S_z \\ I_z \end{pmatrix} \xrightarrow{NE-ST-CP} \begin{pmatrix} -I_z \\ -S_z \end{pmatrix} \quad (1)$$

More generally it can be shown that a ST-CP element in fact achieves the selective inversion of $|\alpha\alpha\rangle$ and $|\beta\beta\rangle$ populations or $|\alpha\beta\rangle$ and $|\beta\alpha\rangle$ populations depending on whether the irradiated transitions are progressive or regressive, respectively (Fig. 1B). As such, sequential application of SE- followed by NE-ST-CP elements achieves the coupling dependent inversion of both target spins. The NE and SE multiplet components are chosen as in general, they possess more favorable relaxation properties. Each individual applied RF field will still have collateral single resonance effects for any nontarget spins that are close to resonance. There are a number of potential schemes (none absolutely perfect) to account for this. For the sequence shown in Fig. 1, these undesired single resonance effects are largely removed by executing echoes on the ^{15}N channel and by inverting the phase of the second CP pulse on the ^{1}H channel. This will selectively invert the targeted ^{1}H spin while leaving all other ^{1}H spins unperturbed except for those ^{1}H spins which are off-resonance by less than ca. $2J$ (SI). We correct for these non-ideal perturbations to off-resonant ^{1}H spins by recording a reference experiment with the just ^{1}H RF on (ie. the ^{15}N RF is turned off). The effect of the ^{15}N RF is to cause all ^{15}N spins to undergo a 360° pulse except for the target ^{15}N spin, which is not perfectly inverted by the entire SIST-CP sequence (SI).

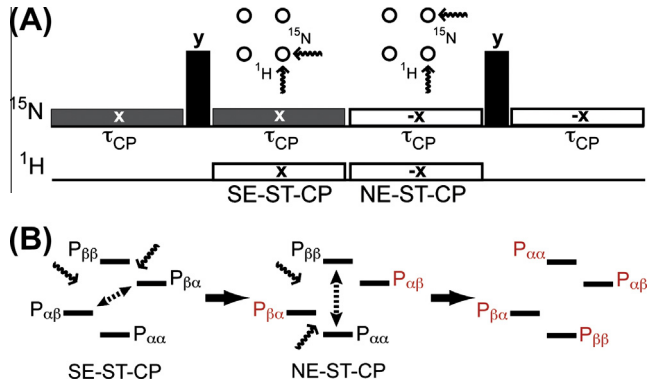

Fig. 1. The selective inversion by single transition cross polarization (SIST-CP) element (A). Low power RF pulses applied at ^{1}H and ^{15}N frequencies must be Hartmann–Hahn matched and applied with strength $\omega_1 \ll J$. The shaded/open low power pulses are applied at frequencies corresponding to the downfield/upfield doublet components of the targeted multiplet (see inset). The black high power pulses are nonselective 180° pulses applied at the center of the spectral bandwidth. (B) Illustration of the effects of the SE-ST-CP and NE-ST-CP elements on the spin state populations. The dashed arrows denote the population inversion being driven by the specific ST-CP element with inverted populations denoted in red. Energy levels are represented assuming a positive γ in keeping with convention for the ST-CP experiment [20]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The rate of spin state selective coherence transfer is independent of the strength of the coupling between spins provided that the RF field is much weaker than the operative coupling. In highly aligned systems, the strength and sign of the $^{1}D_{NH}$ of the target spin pair could have meaningful effects on the inversion efficiency if ω_1 is no longer much less $^{1}(J + D)_{NH}$. In the current work an ω_1 field strength of 35 Hz has been employed which will maintain good efficiency of inversion in the presence of $^{1}D_{NH}$ couplings of up to ca. +20 Hz. For cases in which $^{1}D_{NH}$ assumes even larger magnitudes, "parasitic" spin mixing is expected to occur, thereby reducing the efficiency of inversion though not abolishing it. This can be mitigated, if feasible, by reducing the strength of the ω_1 field (thus lengthening τ_{cp}). On the other hand for residues with large negative $^{1}D_{NH}$ values, increasingly shorter τ_{cp} may be employed while still maintaining efficient inversion.

3. Experimental

3.1. Sample preparation

$^{13}C,^{15}N,^{2}H$ (70%)-labeled GB1 was prepared as previously described [22,23]. Isotropic experiments were conducted on a 2 mM sample in 20 mM phosphate buffer at pH 6.8% and 10% D_2O. Concentrated bicelle stock solutions (ca. 50% w/v) were prepared by mixing three parts DMPC and one part DHPC (Avanti Polar Lipids) in the sample buffer according to the procedure of Ottiger and Bax [24]. GB1 was added to the bicelles stock solutions and diluted in sample buffer to a final protein concentration of 1 mM. GB1-bicelle solutions ranging from 15% to 25% w/v were employed in spin relaxation studies, while a 30% w/v solution was employed for measuring lrRDCs. All bicelle solution concentrations were determined from deuterium residual quadrupolar splittings.

3.2. SIST-CP optimization

$^{1}(J + D)_{NH}$ values and ^{15}N carrier offsets for the up and downfield frequencies of targeted residues in GB1 were determined directly from a 2D [^{1}H, ^{15}N] coupled-HSQC experiment while ^{1}H carrier offsets of the upfield frequency were determined by difference between the observed decoupled frequency and half the measured $^{1}(J + D)_{NH}$ coupling value. CP contact times were set to 15 ms corresponding to a nutation frequency of 47 Hz. ^{1}H and ^{15}N CP power levels were initially estimated from their respective hard 90° pulse widths (pw_{90}) according to

$$\Delta_{pwr} = 20\log(\omega_{cp}4pw_{90}) \quad (2)$$

where ω_{cp} is the desired CP nutation frequency and Δ_{pwr} is the attenuation (in dB) from hard to CP pulse power level. The CP power levels and frequency offsets were then optimized from 1D spectra using a modified 2D [^{1}H, ^{15}N]-HSQC in which the SIST-CP element has been inserted prior to the initial polarizing ^{1}H 90° pulse. Recording of spectra with and without applied ^{15}N CP RF along with concomitant inversion of the receiver phase allows selection of just the target resonance with parameters systematically adjusted to achieve maximum signal amplitude. The ^{1}H inversion efficiency, ε, is determined from the ratio of intensities observed with both ^{1}H and ^{15}N CP power on (I_{on}) and with only the ^{1}H CP power on (I_{off}) according to,

$$\varepsilon = \frac{1}{2}\left(1 - \frac{I_{on}}{I_{off}}\right) \quad (3)$$