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High-resolution heteronuclear multi-dimensional NMR spectroscopy in magnetic fields with unknown spatial variations

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ABSTRACT

Heteronuclear NMR spectroscopy is an extremely powerful tool for determining the structures of organic molecules and is of particular significance in the structural analysis of proteins. In order to leverage the method's potential for structural investigations, obtaining high-resolution NMR spectra is essential and this is generally accomplished by using very homogeneous magnetic fields. However, there are several situations where magnetic field distortions and thus line broadening is unavoidable, for example, the samples under investigation may be inherently heterogeneous, and the magnet's homogeneity may be poor. This line broadening can hinder resonance assignment or even render it impossible. We put forth a new class of pulse sequences for obtaining high-resolution heteronuclear spectra in magnetic fields with unknown spatial variations based on distant dipolar field modulations. This strategy's capabilities are demonstrated with the acquisition of high-resolution 2D gHSQC and gHMBC spectra. These sequences' performances are evaluated on the basis of their sensitivities and acquisition efficiencies. Moreover, we show that by encoding and decoding NMR observables spatially, as is done in ultrafast NMR, an extra dimension containing *J*-coupling information can be obtained without increasing the time necessary to acquire a heteronuclear correlation spectrum. Since the new sequences relax magnetic field homogeneity constraints imposed upon high-resolution NMR, they may be applied in portable NMR sensors and studies of heterogeneous chemical and biological materials.

1. Introduction

Multidimensional heteronuclear NMR spectroscopy is an indispensable tool for structural studies of macromolecules that regularly enables the facile determination of organic molecules' three dimensional structure in solution. In order to detect subtle differences in the chemical environments of nuclear spins, structure determination efforts often rely on the use of very homogeneous magnetic fields (with variations below one part-per-billion). Unfortunately, conventional methods cannot be applied to inherently heterogeneous samples, such as materials with voids that produce variations in magnetic susceptibility throughout the sample volume [1,2]. Furthermore, bulky samples often have to be investigated in inhomogeneous magnetic fields because of the impracticality of inserting them into the magnet bore [3,4].

A variety of methods have been proposed to collect high-resolution spectra in inhomogeneous magnetic fields, including the use of spin echoes [5], the use of phase varying RF pulses and pulsed field gradients to compensate for field inhomogeneities [6,7], and the use of long lived coherences [8,9]. In addition, methods based on distant dipolar field (DDF) modulation [10–13], an effect which arises due to the incomplete averaging of dipolar couplings between spins separated by a distance comparable to or greater than the distance molecules diffuse on an NMR time scale (typically microns) [14], were proposed. These methods, including HOMOGeneity Enhancement by Intermolecular ZEro-quantum Dectection (HOMOGENIZED) [10] and Intermolecular Dipolar-interaction Enhanced All Lines (IDEAL) [15], have usually been designed to obtain 1D ^{1}H and homonuclear ^{1}H correlation spectra. However, recently, Warren and co-workers proposed a modified version of the heteronuclear CRAZED sequence to narrow ^{13}C linewidths in the presence of magnetic field inhomogeneities [16]. Nevertheless, obtaining heteronuclear chemical shift correlations and *J* couplings in inhomogeneous fields is still a challenging endeavor.

In this study, we develop a strategy based on DDF modulation to acquire high-resolution heteronuclear NMR correlation spectra in

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magnetic fields with unknown spatial variations. The excitation and modulation of the DDF is discussed in detail. We present two alternative acquisition schemes to be used in conjunction with the strategy developed: three-dimensional (3D) acquisition and "ultrafast" acquisition utilizing spatial encoding and decoding. These schemes are evaluated on the basis of their acquisition efficiencies and sensitivities. The acquisition of high-resolution 2D gHSQC and gHMBC spectra in inhomogeneous fields is demonstrated.

2. Theory

An overview of the proposed strategy for acquiring high resolution NMR spectra in inhomogeneous magnetic fields is shown in Fig. 1. Without loss of generality, we make a ^{13}C-^{1}H heteronuclear system in an aqueous environment the subject of our theoretical analysis. Let I_1 be the ^{1}H nuclei spins of water, and S and I_2 be the ^{13}C and ^{1}H nuclei spins of the ^{13}C–^{1}H heteronuclear spin system, respectively. The chemical shifts of I_1, I_2, and S in the absence of field inhomogeneity are Ω_{I_1}, Ω_H, and Ω_C, respectively. All gradients are assumed to be applied along the z direction. Solvent protons are selectively excited prior to the execution of a traditional heteronuclear sequence (PENDANT, gHSQC, gHMQC, or gHMBC) [17–20] using a DDF excitation module (shown as a box in Fig. 1a and b; the detailed operations of the available DDF modules are shown in Fig. 2). To prevent the dephasing of signals caused by field inhomogeneities before the DDF takes effect, a π RF pulse is inserted in the middle of the 2Δ delay before acquisition.

2.1. The DDF excitation module

As pointed out by Bloembergen in 1948 [23], diffusion averages the dipolar couplings between nearby spins in a solution environment. However, the dipolar couplings between spins

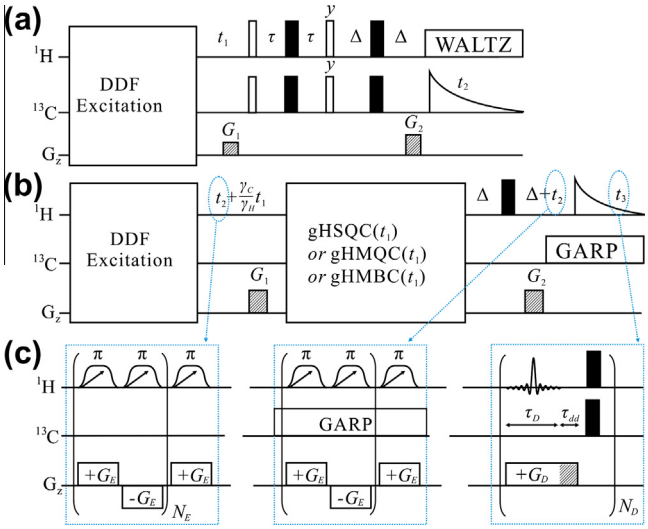

Fig. 1. Proposed strategy for acquiring DDF-modulated high-resolution heteronuclear NMR spectra in inhomogeneous fields. All radiofrequency pulses are x-phase unless otherwise noted. Empty (filled) rectangles represent $\pi/2$ (π) pulses. The delay τ is set to $1/4J_{CH} \approx 2$ ms, and the delay $\Delta = 60$ ms. (a) DDF-1D heteronuclear sequence. $G_1 = 4.8$ G/cm, $G_2 = \frac{\gamma_C}{\gamma_H} G_1 = 19.2$ G/cm, and the gradients are applied for 1.2 ms. (b) DDF-2D heteronuclear sequence with the 3D acquisition scheme. $G_1 = G_2 = 21$ G/cm, and the gradients are applied for 1.2 ms. (c) modules for the DDF-2D heteronuclear sequences employing the "ultrafast" acquisition scheme [21,22]. Shapes with sloping arrows indicate adiabatic refocussing pulses. The "ultrafast" acquisition scheme entails the use of an "ultrafast" constant-time (CT) encoding of the t_2 dimension (left and middle panels) and J-detection decoding (right panel). These encoding and decoding modules are substituted into (b) as indicated by the blue arrows. $N_E = 1$, $G_E = 1.0$ G/cm, $G_D = 3.0$ G/cm. The phases of the π pulses during the J-detection decoding module are alternated between y and $-y$ every two pulses (N_D is a multiple of four).

separated by distances at least as large as the relevant molecular diffusion radii are not completely averaged [10]. If the magnetic moments of the spins in the sample are arranged in a spherically symmetric manner, the sum of the spin dipole magnetic fields vanishes. However, this spherical symmetry can be broken by magnetic field gradients, generating spatially modulated longitudinal magnetization and producing a DDF [24,25]. Methods for producing DDFs may be categorized into two groups: those that use non-selective excitations, thereby spatially modulating *all* the spins in the sample (group I) [10,11,26,27], and those that use selective excitation, only spatially modulating *solvent* spins (group II) [12,15,28,29]. Group I methods are best suited to large field inhomogeneities because these methods avoid the difficulty of selectively exciting only the solvent resonance when spectral lines are substantially broadened. However, when utilizing group I methods, all of the resonances in the sample are excited and each of these resonances, in turn, produce a DDF, which may complicate spin dynamics and make interpreting the resulting spectra difficult. In cases where the DDF originating from the solvent is much stronger than the DDF originating from the solute, solute DDFs can be ignored and the interpretation of the resulting spectra is greatly simplified. Another difficulty associated with the use of group I methods lies in separating traditional coherence transfer pathway signals from DDF-modulated signals. When it is possible efficiently selectively excite only solvent spins, applying group II methods avoids the abovementioned problems.

Fig. 2 shows the group I and group II approaches we propose for DDF excitation in high-resolution heteronuclear correlation sequences. The non-selective excitation (group I, Fig. 2a) approach uses a Testing for Adjacent Nuclei with a Gyration Operator (TANGO) module [30] to achieve excitation of the solvent resonance and other protons not bound to ^{13}C. On the other hand, the group II (Fig. 2b) method uses a simple selective excitation to excite a DDF. A Bilinear Rotation Decoupling (BIRD) module [31] flanked by pulsed field gradients is optionally inserted to dephase the transverse magnetization of any ^{13}C-bonded protons excited due to imperfections of the selective pulse or TANGO block. Note that the DDF excitation modules we propose share the advantages and drawbacks mentioned earlier for group I and group II DDF producing strategies. However, ignoring protons bound to nuclei other than carbon, in uniformly ^{13}C-labelled samples a non-selective excitation module affects only the solvent resonance. Therefore the non-selective (group I) approach behaves, in effect, similar to the group II selective excitation approach when applied to uniformly ^{13}C-labelled samples and avoids the difficulties that normally attend the use of group I methods.

2.2. DDF-modulated 1D heteronuclear spectroscopy

In advance of the acquisition of 2D heteronuclear correlation spectra, we require high-resolution reference 1D proton and carbon spectra. In order to obtain a reference proton spectrum, we utilize a high-resolution method previously developed for use in inhomogeneous fields [32]. The technique used to obtain high-resolution carbon spectra is illustrated in Fig. 1a. The DDF excitation block puts solvent spins in the $-I_{1y}$ state. Solvent spins then evolve according to Ω_{I_1} and the magnetic field inhomogeneity at the location of the spin, $\gamma_H \Delta B$, where γ_H is the proton gyromagnetic ratio. A gradient of strength G_1 is applied for a duration of δ, so that the state of solvent spins becomes

$$-I_{1y}\cos[(\Omega_{I_1} + \gamma_H \Delta B)t_1 + \gamma_H \delta G_1 \cdot z] + I_{1x}\sin[(\Omega_{I_1} + \gamma_H \Delta B)t_1 + \gamma_H \delta G_1 \cdot z] \quad (1)$$

Following the $(\pi/2)_x$–τ–$(\pi)_x$–τ–$(\pi/2)_y$ sequence of pulses and delays, the x-phase component of solvent magnetization is converted to longitudinal magnetization. The solvent magnetization thus