Fast acquisition of high-resolution NMR spectra in inhomogeneous fields via intermolecular double-quantum coherences

Zhong Chen,^{1,a),b)} Shuhui Cai,¹ Zhiwei Chen,¹ and Jianhui Zhong^{2,a),c)}

¹Department of Physics, Fujian Key Laboratory of Plasma and Magnetic Resonance, State Key Laboratory of Physical Chemistry of Solid Surface, Xiamen University, Xiamen 361005, China

²Department of Imaging Sciences and Department of Biomedical Engineering, University of Rochester, Rochester, New York 14642, USA

(Received 13 December 2008; accepted 7 January 2009; published online 27 February 2009)

A pulse sequence, IDEAL-II, is proposed based on the concept of intermolecular dipolar-interaction enhanced all lines [Z. Chen *et al.*, J. Am. Chem. Soc. **126**, 446 (2004)] for obtaining one-dimensional (1D) high-resolution liquid NMR spectra in inhomogeneous fields via two-dimensional acquisitions. With the new acquisition scheme, the range of magnetic field inhomogeneity rather than chemical shift is sampled in the indirect dimension. This enables a great reduction in acquisition time and amount of data, much improved over the original IDEAL implementation. It is applicable to both isolated and J -coupled spin systems in liquid. For the latter, apparent J coupling constants are magnified threefold in spectra obtained with this sequence. This allows a more accurate measurement of J coupling constants in the cases of small J coupling constants or large inhomogeneous fields. Analytical expression was derived based on intermolecular multiple-quantum coherence treatments. Solution samples that were purposely deshimmied and biological samples with intrinsic field inhomogeneities were tested. Experimental results demonstrate that this sequence retains useful structural information including chemical shifts, relative peak areas, and multiplet patterns of J coupling even when the field inhomogeneity is severe enough to almost erase all spectroscopic information with conventional 1D single-quantum coherence techniques. This sequence is more applicable to weakly coupled and uncoupled spin systems, potentially useful for studying metabolites in *in vivo* NMR spectroscopy and for characterizing technologically important new materials in combinatorial chemistry.

© 2009 American Institute of Physics. [DOI: 10.1063/1.3076046]

I. INTRODUCTION

High-resolution nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for analyzing molecular structures and compositions through the measurements of physical parameters such as chemical shifts, scalar coupling constants, multiplet patterns, and relative peak areas. The linewidths of conventional liquid NMR spectra usually depend on the overall magnetic field inhomogeneity that the sample experiences as well as transverse relaxation processes. For proton NMR of a liquid sample, homogeneity of the magnetic field of the order of 10^{-8} over the sample volume is often required to obtain chemical shift and scalar coupling information. Although magnetic fields used in NMR and magnetic resonance imaging (MRI) are designed to maintain a high degree of field homogeneity, there are many circumstances where the spatial and temporal homogeneities of the magnetic field are degraded, and the testing objects are subject to intrinsic variations in magnetic susceptibility over the sample volume and among various structural components. The examples include heterogeneous tissues in human or animal *in vivo* NMR spectroscopy, rock samples

for oil exploration, and porous resin beads in combinatorial chemistry. Tissues with microscopic air inclusion such as the lung of mammals or leaves of plants, and interfaces between tissue and air or bone, are generally recognized to be inaccessible or very difficult for NMR spectroscopy. Line broadening due to inhomogeneous field leads to severe signal overlapping and loss of fine spectral features, which precludes NMR spectral analysis based on the interpretation of the familiar NMR parameters mentioned above.

In addition to magic-angle spinning, which is applicable to some but not to other systems mentioned above, four main types of NMR experimental techniques have been developed to address the above problems related to inhomogeneous magnetic fields.^{1–24} The first approach is to use the scalar and dipolar coupling interactions originated from proximity of spins within a molecule. Weitekamp *et al.*¹ employed total coherence transfer echoes based on intramolecular multiple-quantum coherences (MQCs) to obtain high-resolution NMR spectra. Hall and Norwood proposed to use intramolecular zero-quantum coherences² (ZQCs) and constant-time spin-echo correlated spectroscopy³ for *in vivo* metabolic measurements to allow for refocusing of field inhomogeneity effects while retaining chemical shift and scalar coupling information. Intramolecular ZQCs were also used to study rat brain *in vivo*.²³ Recently, high-resolution correlation spectra of dis-

^{a)}Authors to whom correspondence should be addressed.

^{b)}Electronic mail: chenz@xmu.edu.cn.

^{c)}Electronic mail: jianhui.zhong@rochester.edu.

ordered solids were obtained in the presence of magnetic susceptibility broadening.⁴ Although intramolecular ZQC techniques can remove inhomogeneous broadening partially, they cannot provide correct multiplet patterns and relative peak areas, especially for isolated spins (singlets) which do not result in any ZQC signals under usual experimental conditions.

The second approach is based on nutation echo and/or z -rotation pulse techniques to extract chemical shift information in strongly inhomogeneous fields.^{4–6,18–20,24,25} A high-resolution *ex situ* NMR technique was proposed to enable the recovery of chemical shift in the presence of field inhomogeneity by nutation echoes.^{5,24} It lets the radio-frequency (rf) pulse field precisely counteract the phase dispersion originating from the inhomogeneity of the static magnetic field. To realize “active matching,” Topgaard *et al.*²⁵ put forward an advanced concept of “shim pulses,” which corresponds to the role of “shim coils” in a normal high-resolution spectrometer. The shim pulses compensate the imperfection of experimental environments and hardware through a real-time tuning of the adjustable parameters of applied static field gradients and adiabatic rf pulses for high-resolution NMR and MRI. Blümich and co-workers^{18,19,26} developed a unilateral and mobile NMR sensor for high-resolution NMR spectra at low magnetic field strengths. This approach can be used in highly inhomogeneous fields and does not rely on any special properties of a given substrate, such as intra- or intermolecular interactions. However, it cannot mitigate the effects of magnetic susceptibility broadening, thus not able to recover spectral information of scalar coupling constants and multiplet patterns.

The third approach is based on spatial encoding and phase correction. The method is an extension of single-scan two-dimensional (2D) NMR spectroscopy suggested by Frydman *et al.*²⁷ Spatial encoding NMR spectroscopy is different from the Fourier transform (FT) NMR in that the spatial encoding method can make direct phase compensation for dephasing caused by inhomogeneous fields.^{21,22} The method can recover chemical shift information in quite large inhomogeneity, but the J coupling multiplet structure may remain unresolved since its spectral resolution usually is not high enough.

The fourth approach is based on intermolecular dipolar interactions among spins in different molecules. The main advantage of the method lies in its ability to detect identical interactions for different spin groups in a molecule, therefore simultaneously retaining all features of chemical shifts, multiplet patterns, coupling constants, and relative peak areas, which are key parameters for analyzing and characterizing molecular structures in NMR spectroscopy. In principle, intermolecular nuclear Overhauser effect (NOE) from short-range dipolar interactions can be employed to extract spectral information in inhomogeneous fields.⁷ However, the signals from intermolecular NOE are usually very weak, so very long acquisition is required.⁷ Recently, several research groups have explored various properties of intermolecular MQCs (iMQCs) for their use in NMR, ranging from high-resolution NMR spectroscopy in inhomogeneous fields to MR imaging.^{15,16,28–41} Although intermolecular dipolar inter-

actions occur among spins in the whole sample, the predominant contribution in an isotropic sample comes from spins on different molecules separated by a correlation distance, which is in the range of 5–500 μm and far smaller than typical sample dimensions or the range of the overall field inhomogeneity in a typical sample. In principle, only the inhomogeneous field in the range of this dipolar correlation distance affects the linewidth of the iMQC spectra, so it is intuitively attractive to apply the iMQCs to NMR spectroscopy in inhomogeneous fields. Vathyam *et al.*⁹ first proposed homogeneity enhancement by intermolecular zero-quantum detection (HOMOGENIZED) method based on a 2D intermolecular ZQC (iZQC) pulse sequence. Their theoretical analyses and numerical simulations show that the intermolecular cross peaks in the indirect detection dimension are almost independent of magnetic field inhomogeneity. Lin *et al.*¹⁴ reported that a composite CPMG-HOMOGENIZED sequence could be adopted to achieve high-resolution spectra even in a 25 T electromagnet, which is inherently of poor stability and homogeneity. Recently, several groups explored the *in vivo* applications of iZQC localized spectroscopy.^{30–32} The first attempt of iZQC localized spectroscopy was made by Mescher *et al.*⁴² Resolution enhanced *in vivo* iZQC NMR spectra were presented by Faber and co-workers.^{23,30,32} Galiana *et al.*³¹ extended the HOMOGENIZED sequence for 2D spectroscopy which acquires several echoes with a train of refocused gradients after one excitation to achieve faster acquisition. Although 2D iZQC spectrum offers some very attractive features, three inherent drawbacks limit its application in inhomogeneous fields. First, the iZQC measurements suffer from a very strong solvent signal, which results in large ridge noise in the indirect dimension of a 2D spectrum. This undesired signal mostly comes from the residual conventional single-quantum coherences (SQCs) and from solvent-solvent iZQCs. Second, it cannot distinguish positive and negative frequency differences in intermolecular cross peaks in the indirect dimension. Third, there exist many insignificant solute-solute intermolecular cross peaks for concentrated solutes. These inherent drawbacks may obstruct truthful regeneration of high-resolution one-dimensional (1D) spectra from 2D iZQC spectra acquired in an inhomogeneous field. It has been reported that intermolecular DQCs (iDQCs) possess features similar to those of iZQCs but also some distinctive advantages.^{29,43} Recently, we proposed intermolecular dipolar-interaction enhanced all lines¹⁰ (IDEAL) (hereafter referred to as IDEAL-I), selective HOMOGENIZED¹¹ (SEL-HOMOGENIZED), and intermolecular double-quantum filter HOMOGENIZED¹⁵ (iDQF-HOMOGENIZED) sequences with selective rf pulses to simplify NMR spectra in inhomogeneous fields, which result in spectra with chemical shifts, J coupling constants, multiplet patterns, and relative intensities comparable to conventional 1D spectra in homogeneous fields. Similar improved versions of the iZQC-based HOMOGENIZED sequence were also achieved by Faber and co-workers.^{44,45} These sequences provide enhanced sensitivity compared to the original HOMOGENIZED sequence, and the use of selective rf pulses and iDQF efficiently suppresses solvent signal. However, both the IDEAL-I and HOMOGENIZED-alike sequences re-

FIG. 1. Pulse sequence for high-resolution spectra acquisition in inhomogeneous fields via iQCs. Full vertical bars stand for nonselective rf pulses, Gauss-shaped pulses are I -spin selective rf pulses, and dash rectangles represent CSGs. The phases of the rf pulses are indicated with subscripts.

quire a long acquisition time for a 2D data acquisition since the whole chemical shift range needs to be covered in the indirect dimension F1. This limitation likely restricts their practical applications. Therefore, improvement of experimental schemes for more efficient acquisition is necessary.

The aim of this work is to design a new iMQC pulse sequence that allows more efficient acquisition of high-resolution spectra in inhomogeneous fields. A modified version of IDEAL sequence, hereby referred to as IDEAL-II, is proposed. Unlike conventional NMR spectra in inhomogeneous fields, the reconstructed 1D spectra extracted from the 2D data sets using IDEAL-II sequence retain almost all features of chemical shifts, scalar coupling constants, multiplet patterns, and relative peak areas. Similar to the IDEAL-I and HOMOGENIZED-alike sequences, IDEAL-II is applicable in a moderately inhomogeneous field (for example, 500 Hz in a 500 MHz spectrometer). For samples with large inhomogeneous fields or sharp changes in field strength, the performance of IDEAL-II will break down. Most importantly, although a 2D measurement is still needed for the IDEAL-II pulse sequence, the acquisition time for this new sequence can be shortened substantially in comparison to the IDEAL-I and HOMOGENIZED-alike sequences, with the time saving proportional to the ratio of the range of chemical shift over the range of field inhomogeneity, which can be an order of magnitude or more. The pulse sequence thus provides an attractive and efficient way to achieve 1D high-resolution NMR spectra in inhomogeneous fields. Experimental results are presented for several samples to demonstrate the feasibility of the method.

II. THEORETICAL FORMALISM

The new sequence is shown schematically in Fig. 1. The evolution time t_1 is divided into two equal parts by the second and third rf pulses which are applied one after the other. The first and third rf pulses are nonselective, while the second rf pulse is selective for solvent spins. In the following, theoretical expressions will be derived from the iMQC treatments.¹³ Without loss of generality, a homogeneous liquid mixture, consisting of an AX spin-1/2 system of S component (including S_k and S_l spins with a scalar coupling constant J_{kl}) and a single spin-1/2 system of I component is considered. It is assumed that I spin (corresponding to solvent) is abundant and S spins (corresponding to solutes) are either abundant or dilute, and ω_m is the frequency offset of spin m ($m=I, S_k, S_l$) in the rotating frame in the absence of field inhomogeneity. For simplification, the effects of radiation damping, diffusion, relaxation, and intermolecular NOE

will be ignored, and the coherence selection gradients (CSGs) are applied along the z direction. Since theoretical derivation is very difficult or even impossible for spatially varying resonance frequencies in general, we will only deduce the theoretical expressions for the case of a homogeneous B_0 magnetic field and subsequently discuss the impact of field inhomogeneity.

For the pulse sequence with coherence order $n=+2$, as shown in Fig. 1, the first rf pulse $(\pi/2)_y$ rotates the equilibrium density operator into the transverse plane. After dropping unimportant factors and terms, we can express the double-quantum term $S_{kx}I_{xj}$, excluding $S_{kx}S_{lx}$ term in the reduced density operator as⁴⁶

$$\begin{aligned}\sigma(0^+) &= \sum_{i=1}^{N_S} \sum_{j=1}^{N_I} S_{kxi} I_{xj} \\ &= \frac{1}{4} \sum_{i=1}^{N_S} \sum_{j=1}^{N_I} [(S_{ki}^+ I_j^+ + S_{ki}^- I_j^-) + (S_{ki}^+ I_j^- + S_{ki}^- I_j^+)],\end{aligned}\quad (1)$$

where S_{kxi} represents the x component of the i th S_k spin operator, I_{xj} represents the x component of the j th I spin operator, and N_S and N_I are the total molecule numbers of S and I spins, respectively. The spin density operators evolve under chemical shift, scalar coupling, and magnetic field gradients during the evolution period $t_1/2$. It is well known that all density operator components except coherence order $p=-1$ are unobservable and can be disregarded during the detection period. In the case of $n=+2$, only the term $S_{ki}^+ I_j^+ / 4$ in Eq. (1) is chosen by the CSGs shown in Fig. 1. We have

$$\begin{aligned}\sigma_{\text{eff}}(0.5t_1^-) &= \frac{1}{4} \sum_{i=1}^{N_S} \sum_{j=1}^{N_I} [S_{ki}^+ \cos(\pi J_{kl} t_1 / 2) \\ &\quad - i 2 S_{ki}^+ S_{lzi} \sin(\pi J_{kl} t_1 / 2)] \\ &\quad \times I_j^+ e^{-i[\varphi(r_i) + \varphi(r_j)]} e^{-i[\omega_{S_k} + \omega_l] t_1 / 2}.\end{aligned}\quad (2)$$

Phase angles at positions $\mathbf{r}_i$ and $\mathbf{r}_j$ for S_{ki}^+ and I_j^+ due to the first CSG are $\varphi(\mathbf{r}_i) = \gamma G \delta s_i$ and $\varphi(\mathbf{r}_j) = \gamma G \delta s_j$, where γ is the gyromagnetic ratio, s_i and s_j are the locations of the i th S_k spin and the j th I spin along the gradient direction s , G and δ are the strength and duration of the CSG, respectively. The second $(\pi/2)_y$ rf pulse which is selective for I spin only transforms I_j^+ into $(0.5I_j^+ - 0.5I_j^- - I_{zj})$. The third rf pulse $(\pi)_y$ transforms the coherence order from $S_{ki}^+ I_{zj}$ to $S_{ki}^- I_{zj}$. Since there are no subsequent pulses, we only need to consider $p=-1$ terms that contribute to the observable signals. For signals of the S spin which is of interest, we have

$$\begin{aligned}\sigma_{\text{eff}}(0.5t_1^+) &= -\frac{1}{4} \sum_{i=1}^{N_S} \sum_{j=1}^{N_I} [S_{ki}^- \cos(\pi J_{kl} t_1 / 2) \\ &\quad + i 2 S_{ki}^- S_{lzi} \sin(\pi J_{kl} t_1 / 2)] \\ &\quad \times I_{zj} e^{-i[\varphi(r_i) + \varphi(r_j)]} e^{-i[\omega_{S_k} + \omega_l] t_1 / 2}.\end{aligned}\quad (3)$$

In the high-field approximation, for long-range homonuclear interactions the dipolar Hamiltonian can be approximated to^{13,46}

$$H_{d,\text{eff}}^{\text{sec}} = \sum_{i=1}^{N_S} \sum_{j=1}^{N_I} D_{ij} 3S_{ki} I_{zj}. \quad (4)$$

The residual intermolecular dipolar coupling constant is defined by⁴⁶

$$D_{ij} = \frac{\mu_0}{4\pi} \frac{\gamma^2 \hbar}{4r_{S_{ki}I_j}^3} (1 - 3 \cos^2 \theta_{S_{ki}I_j}), \quad (5)$$

where μ_0 is the vacuum permeability, $r_{S_{ki}I_j}$ is the distance between the S_{ki} spin and the I_j spin, and $\theta_{S_{ki}I_j}$ is the angle

between the S_{ki} and I_j internuclear vector and the static magnetic field.

Terms for the gradient pulse, background gradient, chemical shift, scalar coupling, and effective residual dipolar coupling in the Hamiltonian during the detection period, $t_1/2 + t_2$, are all commutable with each other for a weakly coupled spin system. Therefore, their effects can be evaluated in a cascade of evolutions. The evolution under the scalar coupling between S_{ki} and S_{li} spins in one molecule can be written as

$$\text{Eq. (3)} \longrightarrow -\frac{1}{4} \sum_{i=1}^{N_S} \sum_{j=1}^{N_I} \{S_{ki}^- \cos[\pi J_{kl}(t_1 + t_2)] + i2S_{ki}^- S_{lzi} \sin[\pi J_{kl}(t_1 + t_2)]\} \cdot I_{zj} e^{-i[\varphi(r_i) + \varphi(r_j)]} e^{-i[\omega_{S_k} + \omega_l]t_1/2}. \quad (6)$$

Since the $2S_{ki}^- S_{lzi} I_{zj}$ term cannot evolve into directly observable one-spin, one-quantum operator, S_k^- or S_l^- , under the subsequent gradient, chemical shift, scalar coupling, and effective residual dipolar coupling, Eq. (6) can be simplified to

$$\text{Eq. (6)} \xrightarrow{\text{Simplification}} -\frac{1}{4} \sum_{i=1}^{N_S} \sum_{j=1}^{N_I} S_{ki}^- \cos[\pi J_{kl}(t_1 + t_2)] I_{zj} e^{-i[\varphi(r_i) + \varphi(r_j)]} e^{-i[\omega_{S_k} + \omega_l]t_1/2}. \quad (7)$$

The evolution of Eq. (7) under the second CSG can be expressed as

$$\text{Eq. (7)} \longrightarrow -\frac{1}{4} \sum_{i=1}^{N_S} \sum_{j=1}^{N_I} S_{ki}^- \cos[\pi J_{kl}(t_1 + t_2)] I_{zj} e^{i[\varphi(r_i) - \varphi(r_j)]} e^{-i[\omega_{S_k} + \omega_l]t_1/2}. \quad (8)$$

The evolution of Eq. (8) under the chemical shift can be written as

$$\text{Eq. (8)} \longrightarrow -\frac{1}{4} \sum_{i=1}^{N_S} \sum_{j=1}^{N_I} S_{ki}^- \cos[\pi J_{kl}(t_1 + t_2)] I_{zj} e^{i[\varphi(r_i) - \varphi(r_j)]} e^{-i[\omega_{S_k} t_1/2 - \omega_{S_k} t_2]}. \quad (9)$$

The evolution of Eq. (9) under the residual dipolar coupling can be described as

$$\begin{aligned} \text{Eq. (9)} \longrightarrow & -\frac{1}{4} \sum_{i=1}^{N_S} \sum_{j=1}^{N_I} \{S_{ki}^- I_{zj} \cos[1.5D_{ij}(t_1/2 + t_2)] + i0.5S_{ki}^- \sin[1.5D_{ij}(t_1/2 + t_2)]\} \\ & \times \cos[\pi J_{kl}(t_1 + t_2)] e^{i[\varphi(r_i) - \varphi(r_j)]} e^{-i[\omega_{S_k} t_1/2 - \omega_{S_k} t_2]}. \end{aligned} \quad (10)$$

Since only S_k^- term is directly observable, we can further simplify Eq. (10) to

$$\text{Eq. (10)} \xrightarrow{\text{Directly observable}} -\frac{i}{8} \sum_{i=1}^{N_S} \sum_{j=1}^{N_I} S_{ki}^- \sin[1.5D_{ij}(t_1/2 + t_2)] \cos[\pi J_{kl}(t_1 + t_2)] e^{i[\varphi(r_i) - \varphi(r_j)]} e^{-i[\omega_{S_k} t_1/2 - \omega_{S_k} t_2]}. \quad (11)$$

The restriction $|1.5D_{ij}(0.5t_1 + t_2)| \ll 1$ is satisfied for a liquid in usual case,^{13,37} which leads to tremendous simplification for calculating the time evolution. Using $\sin[1.5D_{ij}(0.5t_1 + t_2)] \approx 1.5D_{ij}(0.5t_1 + t_2)$ for Eq. (11), we have

$$\begin{aligned} \sigma_{\text{eff}}(t_1 + t_2) = & -\frac{i3(0.5t_1 + t_2)}{16} \sum_{i=1}^{N_S} \sum_{j=1}^{N_I} \{S_{ki}^- \cos[\pi J_{kl}(t_1 + t_2)] D_{ij} e^{i[\varphi(r_i) - \varphi(r_j)]} e^{-i[\omega_{S_k} t_1/2 - \omega_{S_k} t_2]}\} \\ = & -\frac{i3(0.5t_1 + t_2)}{16} \sum_{i=1}^{N_S} \{S_{ki}^- e^{-i(\omega_{S_k} t_1/2 - \omega_{S_k} t_2)} \cos[\pi J_{kl}(t_1 + t_2)] \sum_{j=1}^{N_I} D_{ij} e^{i\gamma_G \delta(s_i - s_j)}\}. \end{aligned} \quad (12)$$

Since we are free to choose the origin of the coordinate system, we can set $s_i=0$, so

$$\sum_{j=1}^{N_I} D_{ij} e^{i\gamma G \delta(s_i - s_j)} = \sum_{j=1}^{N_I} D_{ij} [\cos(\gamma G \delta s_j) - i \sin(\gamma G \delta s_j)]. \quad (13)$$

Summation over dipolar-coupled spins can be replaced by an integral if the distribution of the spins can be considered continuous. Since sine is an odd function, the term containing $\sin(\gamma G \delta s_j)$ in Eq. (13) vanishes after the sum over the sample. Hence Eq. (13) can be simplified to

$$\begin{aligned} \sum_{j=1}^{N_I} D_{ij} \cos(\gamma G \delta s_j) \\ \approx \frac{-\mu_0 \hbar \gamma^2 N_I}{16\pi V} \int_V \frac{3 \cos^2 \theta - 1}{r^3} \cos(\gamma G \delta s) r^2 \sin \theta dr d\theta d\phi. \end{aligned} \quad (14)$$

If the volume of the sample is large enough, the integral converges as

$$\begin{aligned} \int_{r=r_{\min}}^{\infty} \int_{\theta=0}^{\pi} \int_{\phi=0}^{2\pi} \frac{3 \cos^2 \theta - 1}{r^3} \cos(\gamma G \delta s) r^2 \sin \theta dr d\theta d\phi \\ = -\frac{8\pi}{3} \Delta_s, \end{aligned} \quad (15)$$

where $\Delta_s \equiv [3(\hat{s} \cdot \hat{z})^2 - 1]/2$, $\hat{s}$ and $\hat{z}$ represent unit vectors along the directions of the field gradient and static magnetic field respectively. Following Eq. (15), the sum in Eq. (14) becomes

$$\sum_{j=1}^{N_I} D_{ij} \cos(\gamma G \delta s_j) \approx \frac{N_I \mu_0 \hbar \gamma^2}{V 6} \Delta_s. \quad (16)$$

The equilibrium magnetization per unit volume of I spin is defined as $M_0^I = -(N_I \hbar \gamma / 4V) (\hbar \omega_I / kT)$, where $(\hbar \omega_I / kT)$ is the Boltzmann factor. The dipolar demagnetizing time is defined as $\tau_d^I \equiv 1/(\mu_0 \gamma M_0^I)$. Therefore, Eq. (12) can be written as

$$\begin{aligned} \sigma_{\text{eff}}(t_1 + t_2) \\ = \frac{i}{8} \left(\frac{kT}{\hbar \omega_I} \right) \frac{(0.5t_1 + t_2) \Delta_s}{\tau_d^I} \sum_{i=1}^{N_S} S_{ki}^- e^{-i(\omega_I t_1/2 - \omega_{S_k} t_2)} \\ \times \cos[\pi J_{kl}(t_1 + t_2)]. \end{aligned} \quad (17)$$

We can use this expression to calculate the observable signal,

$$\begin{aligned} M_+^{S_k}(t_1 + t_2) &= \text{Tr}[\sigma_{\text{eff}}(t_1 + t_2) \gamma \hbar S_k^+ / V] \\ &= \frac{i \Delta_s (0.5t_1 + t_2)}{2 \tau_d^I} \left(\frac{kT}{\hbar \omega_I} \right)^2 M_0^S e^{-i(\omega_I t_1/2 - \omega_{S_k} t_2)} \\ &\quad \times \cos[\pi J_{kl}(t_1 + t_2)]. \end{aligned} \quad (18)$$

Equation (18) can be further simplified to

$$\begin{aligned} M_+^{S_k}(t_1 + t_2) &= \frac{i \Delta_s (0.5t_1 + t_2)}{4 \tau_d^I} \left(\frac{kT}{\hbar \omega_I} \right)^2 \\ &\quad \times M_0^S [e^{-i(\omega_I/2 - \pi J_{kl})t_1} e^{i(\omega_{S_k} + \pi J_{kl})t_2} \\ &\quad + e^{-i(\omega_I/2 + \pi J_{kl})t_1} e^{i(\omega_{S_k} - \pi J_{kl})t_2}]. \end{aligned} \quad (19)$$

Equation (19) provides the quantitative relationship of intermolecular cross peak between the k spin of the S component and the I component. It shows that the cross peak is a doublet locating at $(\omega_I/2 - \pi J_{kl}, \omega_{S_k} + \pi J_{kl})$ and $(\omega_I/2 + \pi J_{kl}, \omega_{S_k} - \pi J_{kl})$ due to J splitting. In an inhomogeneous field, the linewidth of the projected spectrum in the F1 or F2 dimension relies on several factors such as transverse relaxation, diffusion, and inhomogeneous field distribution. It is impossible to quantitatively translate an inhomogeneous field with unknown distribution into an iMQC linewidth. On the other hand, we can deduce simply the apparent shape of the intermolecular cross peak in a magnetic field with inhomogeneous deviation $\Delta B(\mathbf{r})$. When the influences of relaxation, diffusion and radiation damping are ignored, the resonance frequency for the intermolecular cross peak in the F2 dimension will be $\omega_{S_k} + \gamma \Delta B(\mathbf{r}) + \pi J_{kl}$ and $\omega_{S_k} + \gamma \Delta B(\mathbf{r}) - \pi J_{kl}$. Since the spins on two molecules coupled by long-range dipolar interactions are physically close to each other and should experience almost the same magnetic field over the distance between the two spins, the frequency of the intermolecular cross peak in the F1 dimension then will be $(\omega_I + \gamma \Delta B(\mathbf{r}))/2 - \pi J_{kl}$ and $(\omega_I + \gamma \Delta B(\mathbf{r}))/2 + \pi J_{kl}$. If the spectrometer reference frequency is set to ω_I , i.e., $\omega_I=0$, the intermolecular cross peak will locate at $(\gamma \Delta B(\mathbf{r})/2 - \pi J_{kl}, \omega_{S_k} + \gamma \Delta B(\mathbf{r}) + \pi J_{kl})$ and $(\gamma \Delta B(\mathbf{r})/2 + \pi J_{kl}, \omega_{S_k} + \gamma \Delta B(\mathbf{r}) - \pi J_{kl})$ respectively. Thus the peak appears as two streaks along a specific direction $\phi = \arctan(\Delta B(\mathbf{r}) / \Delta B(\mathbf{r})/2) = \arctan(2) = 63.4^\circ$, where ϕ is the angle of spectral streaks with respect to the F1 axis (assuming the linewidth is dominated by the inhomogeneous broadening while the influences of relaxation and diffusion on the linewidth can be ignored). It indicates that the ratio of the projected lengths of the streak in the F1 and F2 dimensions is 1:2 due to the inhomogeneous field. Although this analysis is rough, the conclusion that the frequency range of the streak in the F1 dimension is about half of that in the F2 dimension is verified by our experimental observations. It implies that all the streaks in the 2D spectrum occupy only a narrow range of frequency in the F1 dimension. This feature enables a substantial decrease in the F1 spectral width that needs to be sampled and greatly reduces experimental time and data size. Although the ranges of the streaks are susceptible to inhomogeneity in both F1 and F2 dimensions, a projection of the cross peaks to the F2 dimension after rotating the streaks by 63.4° counterclockwise results in a 1D high-resolution spectrum with inhomogeneous broadening greatly suppressed. The linewidth of the projected spectrum relies on the signal attenuation induced by several factors, including transverse relaxation, diffusion, and the residual background inhomogeneous field within correlation distance. Furthermore, J coupling is independent of the spatial distribution of field inhomogeneity, so all solute

FIG. 2. NMR spectra of 1M solution of tyrosine in water: (a) conventional SQC spectrum in a well-shimmed field, (b) conventional SQC spectrum in an inhomogeneous field of about 40 Hz linewidth, (c) accumulated projection of the sheared experimental spectrum from (d), (d) 2D iDQC spectrum in the same inhomogeneous field using the IDEAL-II sequence, and (e) five expanded regions after rotation of (d) and their accumulated projection. The insets are magnified in both vertical and horizontal directions.

spins evolve under J couplings in both F1 and F2 dimensions. The projected spectrum in the F2 dimension has the same multiplet patterns as a conventional 1D spectrum with a scale factor of $1 + \cot \phi = 3$ of the J coupling constants. All these features are verified by our experimental results.

III. MATERIALS AND METHODS

All experiments were performed at 298 K using a Varian Unity plus 500 MHz NMR spectrometer equipped with a 5 mm HCN triple-resonance probe having a rf coil of 1.5 cm effective length and a self-shielded z -gradient coil. The pulse sequence shown in Fig. 1 was used. Pulse width of a rf $\pi/2$ hard pulse was extended from 6 to about 110 μ s by detuning the probe to suppress the radiation damping during the evolution and detection periods.⁴⁷ Selective Gaussian $\pi/2$ pulse width was 5 ms. The CSGs were applied just before the second and after the third pulses with the same duration of $\delta = 3.0$ ms but strengths of 0.1 and 0.2 T/m, respectively.

The IDEAL-II sequence was first employed to a sample of 1M tyrosine in water. To produce a broadened linewidth (~ 40 Hz) for the peak of solvent, the magnetic field was intentionally deshimmmed by adjusting the linear and quadratic shim currents. The spectral width of the F1 dimension was set to 100 Hz in 128 increments and 2500 Hz for the F2 dimension. The pulse repetition time was 6.0 s and the acquisition time t_2 was 1.0 s. The total experimental time was 20 min. The signal was zero filled to $16\,384 \times 2048$ before regular fast FT (FFT).

The ability of the IDEAL-II for spectral resolving was also tested using the same sample with larger field inhomogeneity (~ 500 Hz linewidth). The spectral width of the F1 dimension was set to 350 Hz in 150 increments and 3500 Hz for the F2 dimension. The pulse repetition time was 6.0 s and the acquisition time t_2 was 1.0 s. Phase cycling of the first rf

pulse between 0° and 180° was employed. The total experimental time was 30 min. The signal was zero filled to $16\,384 \times 2048$ before regular FFT.

As we have mentioned above, a major improvement of the IDEAL-II over the IDEAL-I sequence is that it can shorten the experimental acquisition time. To see how fast a high-resolution spectrum of reasonable quality can be obtained, a mixture of histidine, malic acid (solute), and water (solvent) was measured. The spectral width of the F1 dimension was set to 45 Hz in 24 increments and 4000 Hz for the F2 dimension. The pulse repetition time was 2.1 s and the acquisition time t_2 was 0.6 s. The total experimental time was within 1 min. The signal was zero filled to $16\,384 \times 2048$ before regular FFT.

To test the feasibility of the IDEAL-II sequence for samples with intrinsic macroscopic susceptibility gradients, a piece of grape sarcocarp was fit inside a 5 mm NMR tube. 1D and 2D NMR spectra were obtained under the same experimental conditions. The spectral width was 200 Hz in 64 increments for the F1 dimension and 4000 Hz for the F2 dimension. To remove other coherence transfer pathways and suppress the water peak, an eight-step phase cycling was used: the phases for the first and third rf pulses were $(x, y, -x, -y, x, y, -x, -y)$ and (x, x, x, x, y, y, y, y) , respectively, and the phases for the receiver were $(x, -x, x, -x, -x, x, -x, x)$. The pulse repetition time was 3 s and the acquisition time t_2 was 0.6 s. The total experimental time was about 30 min. The signal was zero filled to $16\,384 \times 2048$ matrix before regular FFT.

IV. EXPERIMENTAL RESULTS AND DISCUSSION

Improvement of the IDEAL-II over the IDEAL-I sequence is demonstrated with the sample of tyrosine aqueous solution (Fig. 2). For the IDEAL-I sequence, the intermolecular cross peaks lie along the diagonal, and their positions

FIG. 3. NMR spectra of tyrosine in water in an inhomogeneous field of 500 Hz linewidth: (a) 2D iDQC spectrum using the IDEAL-II sequence, together with the conventional single-pulse SQC 1D spectrum under the same field inhomogeneity above it, and (b) expanded regions of iDQC spectrum in (a) after rotation.

in the F1 dimension are given by the sum of the respective chemical shifts, scalar couplings, and field inhomogeneity.¹⁰ For the IDEAL-II sequence, the intermolecular cross peaks lie along the horizontal axis, and their relative positions in the F1 dimension are given by the sum of scalar couplings and field inhomogeneity [see Fig. 2(d)]. The length of the streak projected in the F1 dimension is 1/2 of that in the F2 dimension, in agreement with the theoretical prediction about the ratio of peak extension in two dimensions brought by field inhomogeneity. The slope of all streaks of resonance peaks is thus 0.5 due to the field inhomogeneity (i.e., if the same scales are used for both axes, the lines are clockwise rotated by 63.4° angle from the F1 axis). Although the length of the streaks are susceptible to the inhomogeneity in both F1 and F2 dimensions, the projection of the 2D spectrum into the F2 axis after a proper rotation results in narrow and well resolved *J*-multiplets [Fig. 2(c)]. For the IDEAL-I spectrum, the rotation is $90^\circ - \arctan(2) = 26.6^\circ$ counterclockwise around the centers of *J*-multiplets. For the IDEAL-II spectrum, it is a counterclockwise rotation of 63.4° , which is easily processed with VNMR or VNMRJ software equipped with Varian spectrometer. Five expanded regions after this shearing operation are shown in Fig. 2(e). The scale factor of the *J* coupling in the projection spectrum from the IDEAL-II is 3. This makes it possible to retain multiplet information in spin systems with small *J* coupling constants using the IDEAL-II sequence. Furthermore, since the spatial correlation via dipolar field of the solvent affects all the spins of the solutes equally, the relative areas of iDQC peaks for all resonances are the same as those in the conventional 1D NMR spectrum. Therefore, the resulted 1D spectrum after proper projection of the 2D spectrum along the F2 axis maintains chemical shifts, relative peak areas, and multiplet patterns while inhomogeneous broadening is suppressed [Fig. 2(c)]. The linewidth of the projection spectrum is reduced from 40 to 3 Hz, a value remarkably similar to the conventional high-resolution ^1H spectrum [Fig. 2(a)].

In order to estimate the range of inhomogeneity within which the IDEAL-II sequence can be used to achieve high-resolution spectra, the magnetic field was further deshimm

to produce a 500 Hz linewidth for the peak of solvent of the same sample. The result suggests that the method is robust in eliminating the effect of field inhomogeneity, as long as the peaks of solute and solvent can still be separated (Fig. 3). Because of the use of a selective pulse for solvent peak in the IDEAL-II sequence, the upper limit of the field inhomogeneity resolvable by the sequence depends on the minimum difference in chemical shifts between the solvent and solute peaks of the interest. In principle, if this minimum difference is, e.g., 4 ppm, the sequence would be able to resolve field inhomogeneity of less than 2 kHz on a 500 MHz spectrometer. Although the method still works in the case of moderate inhomogeneous fields when the solvent peak partly overlaps some of solute resonances, it will fail with large field inhomogeneity, with the applicable range greatly depending on the samples. This is in contrast to its iZQC counterpart, the HOMOGENIZED sequence, which can be applied to larger field inhomogeneity since there is no selective pulse in the sequence. However, nonselective excitation also brings some disadvantages, such as strong t_1 ridge noises at the solvent peak position and interferences from conventional solute signals and solute-solute and solute-solvent iZQC signals. The strong solvent resonance would cover the resonances from low-concentration solutes, which hinders applications of the HOMOGENIZED method. On the other hand, although intramolecular ZQC approaches suffer less interferential signals, they are strictly limited to scalar-coupled spin systems and cannot provide correct multiplet patterns and relative peak areas. Intra- and intermolecular ZQCs may play a complementary role to each other in high-resolution NMR in inhomogeneous field.

In order to evaluate the time saving in acquisition with the IDEAL-II sequence, a mixture of histidine and malic acid in water was placed in an inhomogeneous field deshimm to a linewidth of 30 Hz [see Fig. 4(b)]. A 2D iDQC spectrum was acquired within 1 min [Fig. 4(d)]. This 2D spectrum also resulted in a high-resolution 1D NMR spectrum [see Fig. 4(c)]. Note that since the *J* couplings are threefold magnified, the fine structures of the multiplets are better resolved than the conventional ^1H spectrum acquired from the same

FIG. 4. Partial NMR spectra of histidine and malic acid in water: (a) conventional SQC spectrum in a well-shimmed field (with individual multiplets expanded above the full spectrum), (b) conventional SQC spectrum in an inhomogeneous field of about 30 Hz linewidth, (c) accumulated projection of the rotated 2D spectrum from (d), and (d) 2D iDQC spectrum in the same inhomogeneous field using the IDEAL-II sequence. The insets are magnified in both vertical and horizontal directions.

sample in a relative homogeneous field [see Fig. 4(a)]. Since with the IDEAL-I sequence the spectral width in the F1 direction has to cover at least the range of the total chemical shift, whereas with the IDEAL-II sequence only half of the

range of field inhomogeneity needs to be sampled in the F1 dimension, theoretically when the IDEAL-II is used instead of the IDEAL-I sequence,

$$\text{The time saving} \propto \frac{\text{range of chemical shift}}{1/2 \text{ range of field inhomogeneity}}. \quad (20)$$

For the example given in Fig. 4, with chemical shift range of 1500 Hz and field inhomogeneity range of 30 Hz, the time saving can be as large as a factor of 100. For samples with a large chemical shift range, the time saving can be more substantial. This estimate does not consider changes in signal-to-noise ratio (SNR) when different frequency ranges are sampled. Compared to the ultrafast iZQC pulse sequence proposed by Galiana *et al.*,³¹ the IDEAL-II is simpler and can be used in conventional NMR spectrometers with single-axis gradients.

Figure 5 shows the NMR spectra of a piece of grape sarcocarp, representing a biological sample with intrinsic field inhomogeneity. The linewidth of the water resonance (near 4.8 ppm) is about 100 Hz in the conventional 1D NMR spectrum [Fig. 5(a)]. After a projection of the 2D IDEAL-II spectrum onto the horizontal axis, several separated resonances of glucose are observed [Fig. 5(b)], which are similar to the result from grape juice.⁴⁸ The linewidths are reduced from 100 to about 4 Hz. In addition, a weak triplet peak from the methyl group of ethanol with low concentration is observed at 1.1 ppm. Its *J*-coupling constant can be determined to be 7 Hz since the triplet is well resolved (the apparent one in the spectrum is 21 Hz). Two peaks of β - and α -glucose, which are about 0.2 and 0.4 ppm away from the water resonance, respectively,⁴⁸ are also observed. The results from this experiment suggest that line broadening due to intrinsic macroscopic susceptibility gradients can be almost completely removed with the IDEAL-II method, similar to the cases when linewidth was artificially broadened in the examples given earlier.

FIG. 5. 2D iDQC spectra of a piece of grape sarcocarp: (a) conventional 1D NMR spectrum with the linewidth for water of about 100 Hz, (b) 2D iDQC spectrum using the IDEAL-II sequence after a counterclockwise rotation of 63.4° and projection. The insets are magnified 50 times for (a) and 10 times for (b) in the vertical direction.

TABLE I. Comparison of pulse sequences for high-resolution NMR in inhomogeneous fields via iMQCs.

Pulse sequence	Coherence order	Sampling range in the F1 dimension	Scale factor of J coupling	Location of cross peaks	Angle between the F1 axis and streaks (deg)	Ref.
HOMOGENIZED	iZQC	Chemical shift	1.0	Diagonal	90	9
SEL-HOMOGENIZED	iZQC	Chemical shift	1.0	Diagonal	90	11
iDQF-HOMOGENIZED	iZQC	Chemical shift	1.0	Diagonal	90	15
IDEAL-I	iDQC	Inhomogeneous field and chemical shift	3.0	Diagonal	26.6	10
IDEAL-II	iDQC	1/2 range of inhomogeneous field	3.0	Horizontal	63.4	This work

As can be seen from Fig. 5(b), since a double-quantum filter is built in the IDEAL-II sequence, the conventional SQC signal is effectively suppressed. Moreover, the signals originated from intramolecular MQCs are not observable. The line broadening due to susceptibility gradients is essentially eliminated and spectral resolution is significantly enhanced. Since the IDEAL-II sequence is insensitive to shimming, a high-resolution spectrum can be obtained without a time-consuming shimming procedure. The method may be applied to *in vivo* tissues with intrinsic susceptibility gradients. It is noted that the inhomogeneity is only refocused over a scale larger than a typical correlation distance, which is of the order of 120 μm in our experiments. Therefore, the IDEAL-II sequence, similar to the HOMOGENIZED sequence, cannot alleviate inhomogeneous line broadening due to susceptibility gradients (such as susceptibility differences of air inclusions in some tissues) within 10–100 μm or smaller scales.³⁰

To facilitate comparisons of different pulse sequences for high-resolution NMR spectra in inhomogeneous fields via iMQCs, techniques published so far as we are aware of and their main features are listed in Table I.

For *in vivo* NMR spectroscopy, the magnetic environment is often inhomogeneous. Inhomogeneous line broadening due to magnetic susceptibility gradients often leads to severe resonance peak overlapping. The apparent chemical shift of one nucleus at a specific position is correlated with the apparent chemical shift of the nearby nucleus of another molecule even in an inhomogeneous field. Since the signals originating from solvent-solute short-range NOEs are very weak, long-range intermolecular dipolar interactions will be the main mechanism competing against the NOE mechanism of close intermolecular dipolar interactions for high concentrated solvent. In a 500 MHz spectrometer, ^1H signal intensity from iDQCs between the solvent and the solute is about 1/10 of the equilibrium proton magnetization, which is much larger than those from intermolecular NOEs between the solvent and the solute. The IDEAL-II sequence overcomes the major disadvantage in the IDEAL-I and HOMOGENIZED-alike sequences of need for a long acquisition time for a 2D data set. The potential time saving in using IDEAL-II sequence is proportional to the range of chemical shift over the range of field inhomogeneity, which can be significant for samples with large chemical shift range and/or relatively small field inhomogeneity. Since the radiation damping effects in human MRI (low field, 1.5–3 T) are much weaker than those in high-field NMR, it is not necessary to detune the probe to suppress the radiation damping effects. Thus

there is no related loss of SNR. Although the SNR of signals from the IDEAL-II sequence is still substantially lower than conventional SQC methods since it relies on the signals from solvent-solute iDQCs, the gain in time offered by this sequence makes it possible for more acquisitions within a definite time, which will partly compensate for the poorer sensitivity in low field. Our preliminary results on a 3 T whole-body MRI scanner indicate its feasibility.

The 1D spectral resolution is primarily limited by the T_2 relaxation attenuation effect under a well-shimmed magnetic field. The 1D projected spectral resolution of a 2D spectrum obtained using the IDEAL-II sequence is mainly dependent on the signal attenuation due to T_2 relaxation and diffusion effects as the evolution time t_1 increases. In principle, diffusion effect influences the signal intensity as well as the spectral resolution of the IDEAL-II sequence. In comparison to the evolution time in the indirect dimension (a period of a few hundred milliseconds), the diffusion effects due to the selective pulses are ignored in discussing the mechanism of high-resolution spectra under inhomogeneous fields. However, it is noted that diffusion decays due to the background field gradient (inhomogeneous field) will increase gradually when t_1 increases. In the case of a highly inhomogeneous field, diffusion decays due to background field gradient and relatively long selective pulses become obviously. It lowers the resolution of 1D projected spectra obtained by the IDEAL II sequence. Further work is needed to quantify the limit of the size and concentration of solute molecules to which the method can be applied. Further development of the IDEAL methods is also necessary for better water suppression for practical applications.

The IDEAL-II sequence is an extension of the IDEAL-I sequence we have proposed previously. To shorten acquisition time, the idea of dividing the evolution time in indirect dimension into two equally parts is adopted, as in the case of 2D J -resolved spectroscopy. In this aspect, the IDEAL-II sequence can be taken as the iMQC version of 2D J -resolved spectroscopy. However, the IDEAL-II sequence takes the advantage of yielding J -coupling constants in inhomogeneous fields as well as high-resolution chemical shift information. Although the 2D J -resolved spectroscopy can yield J -coupling constants of spin systems, it cannot provide high-resolution chemical shifts information in inhomogeneous fields due to the line broadening along the F2 dimension (chemical shift axis), which may even cause overlapping of neighboring streaks when the line broadening is larger than their chemical shift difference. The methods based on the IDEAL sequences do not require the spin species to have J

coupling or with NOE process. The only requirements are that one component such as solvent has a concentrated proton magnetization and other components are intermingled with it on micron or millimeter scale. Therefore, they are applicable to both coupled and uncoupled spin systems in liquid.

By means of intermolecular long-range dipolar interactions, the IDEAL techniques can be used to effectively enhance the resolution in inhomogeneous magnetic fields with abundant component such as solvent and dilute component such as solutes. The method may be applied to a wider range of samples than those relying on scalar coupling or intermolecular NOEs. Potential applications of the method include *in vivo* NMR spectroscopy, where the field homogeneity is always degraded by the wide distributed magnetic susceptibilities among various tissues and cellular structures, and other areas where large magnetic field inhomogeneity are unavoidable such as *in situ* NMR in porous media. Many elegant applications are concerned with solute molecules in highly-polarized solvent. The features of the IDEAL-II sequence may make *in situ* NMR a powerful analytical tool to study the metabolism of organisms in *in vivo* NMR spectroscopy.

V. CONCLUSIONS

In this paper, a new pulse sequence, IDEAL-II, was designed to obtain 1D high-resolution NMR spectra in inhomogeneous fields with fast 2D acquisitions. Based on the theoretical analysis of the evolution of iMQC signals, selective rf excitation was employed to greatly decrease the ridge noise artifacts and to effectively suppress undesired trivial signals. Theoretical formalism provides quantitative analysis of the scalar coupling scale. The resulting spectrum retains all useful spectral information including chemical shifts, coupling constants, multiplet patterns, and relative peak areas. Experimental results demonstrate that 1D high-resolution spectrum may be extracted from the 2D IDEAL data under the field inhomogeneity severe enough to completely erase all spectroscopic features with conventional methods. Since the IDEAL-II sequence can effectively suppress residual solvent signals, undesired solute-solute and solute-solvent iMQC cross peaks, only the intermolecular solvent-solute and solvent-solvent cross peaks are maintained in the 2D spectra.

The IDEAL techniques can be used to effectively enhance the NMR spectral resolution of samples in inhomogeneous magnetic fields with abundant component such as solvent and dilute component such as solutes. Potentials of the IDEAL sequences have been demonstrated in this work, but intrinsic low SNR and strong residual water signal remains challenging. Improvement of these pulse sequences and optimization of experimental parameters are still needed for practical applications.

ACKNOWLEDGMENTS

This work was partially supported by the NNSF of China under Grant Nos. 10774125 and 10875101, Research Fund for the Doctoral Program of Higher Education of China under Grant No. 200803840019, NCET of MOE of China, and the NIH of United States under Grant No. NS41048.

- ¹D. P. Weitekamp, J. R. Garbow, J. B. Murdoch, and A. Pines, *J. Am. Chem. Soc.* **103**, 3578 (1981).
- ²L. D. Hall and T. J. Norwood, *J. Chem. Soc., Chem. Commun.* 44 (1986).
- ³L. D. Hall and T. J. Norwood, *J. Chem. Soc., Chem. Commun.* 1508 (1986).
- ⁴D. Sakellariou, S. P. Brown, A. Lesage, S. Hediger, M. Bardet, C. A. Meriles, A. Pines, and L. Emsley, *J. Am. Chem. Soc.* **125**, 4376 (2003).
- ⁵C. A. Meriles, D. Sakellariou, H. Heise, A. J. Moulé, and A. Pines, *Science* **293**, 82 (2001).
- ⁶B. Blümich, J. Perlo, and F. Casanova, *Prog. Nucl. Magn. Reson. Spectrosc.* **52**, 197 (2008).
- ⁷J. J. Balbach, M. S. Conradi, D. P. Cistola, C. G. Tang, J. R. Garbow, and W. C. Hutton, *Chem. Phys. Lett.* **277**, 367 (1997).
- ⁸J. Jeener, in *Encyclopedia of Nuclear Magnetic Resonance*, edited by D. M. Grant and R. K. Harrispp (Wiley, New York, 2002), Vol. 9, p. 642.
- ⁹S. Vathyam, S. Lee, and W. S. Warren, *Science* **272**, 92 (1996).
- ¹⁰Z. Chen, Z. W. Chen, and J. H. Zhong, *J. Am. Chem. Soc.* **126**, 446 (2004).
- ¹¹Z. Chen, T. Hou, Z. W. Chen, D. W. Hwang, and L. P. Hwang, *Chem. Phys. Lett.* **386**, 200 (2004).
- ¹²V. Demas, D. Sakellariou, C. A. Meriles, S. Han, J. Reimer, and A. Pines, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 8845 (2004).
- ¹³S. Lee, W. Richter, S. Vathyam, and W. S. Warren, *J. Chem. Phys.* **105**, 874 (1996).
- ¹⁴Y. Y. Lin, S. Ahn, N. Murali, W. Brey, C. R. Bowers, and W. S. Warren, *Phys. Rev. Lett.* **85**, 3732 (2000).
- ¹⁵X. Chen, M. J. Lin, Z. Chen, S. H. Cai, and J. H. Zhong, *Phys. Chem. Chem. Phys.* **9**, 6231 (2007).
- ¹⁶Y. Q. Lin, Z. Chen, S. H. Cai, and J. H. Zhong, *J. Magn. Reson.* **190**, 298 (2008).
- ¹⁷D. Z. Balla and C. Faber, *Concepts Magn. Reson. Part A* **32A**, 117 (2008).
- ¹⁸A. Wiesmath, C. Filip, D. E. Demco, and B. Blümich, *J. Magn. Reson.* **149**, 258 (2001).
- ¹⁹J. Perlo, V. Demas, F. Casanova, C. A. Meriles, J. Reimer, A. Pines, and B. Blümich, *Science* **308**, 1279 (2005).
- ²⁰J. Perlo, F. Casanova, and B. Blümich, *Science* **315**, 1110 (2007).
- ²¹B. Shapira and L. Frydman, *J. Am. Chem. Soc.* **126**, 7184 (2004).
- ²²B. Shapira and L. Frydman, *J. Magn. Reson.* **182**, 12 (2006).
- ²³R. A. de Graaf, D. L. Rothman, and K. L. Behar, *J. Magn. Reson.* **187**, 320 (2007).
- ²⁴D. Topgaard, D. Sakellariou, and A. Pines, *J. Magn. Reson.* **175**, 1 (2005).
- ²⁵D. Topgaard, R. W. Martin, D. Sakellariou, C. A. Meriles, and A. Pines, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 17576 (2004).
- ²⁶J. Perlo, F. Casanova, and B. Blümich, *J. Magn. Reson.* **180**, 274 (2006).
- ²⁷L. Frydman, A. Lupulescu, and T. Scherf, *J. Am. Chem. Soc.* **125**, 9204 (2003).
- ²⁸W. S. Warren, S. Ahn, M. Mescher, M. Garwood, K. Ugurbil, W. Richter, R. R. Rizi, J. Hopkins, and J. S. Leigh, *Science* **281**, 247 (1998).
- ²⁹J. H. Zhong, Z. Chen, and E. Kwok, *Magn. Reson. Med.* **43**, 335 (2000).
- ³⁰C. Faber, E. Pracht, and A. Haase, *J. Magn. Reson.* **161**, 265 (2003).
- ³¹G. Galiana, R. T. Branca, and W. S. Warren, *J. Am. Chem. Soc.* **127**, 17574 (2005).
- ³²D. Z. Balla and C. Faber, *MAGMA (N.Y.)* **20**, 183 (2007).
- ³³D. Z. Balla and C. Faber, *J. Chem. Phys.* **128**, 154522 (2008).
- ³⁴R. Bowtell, R. M. Bowley, and P. Glover, *J. Magn. Reson.* **88**, 643 (1990).
- ³⁵R. Bowtell and P. Robyr, *Phys. Rev. Lett.* **76**, 4971 (1996).
- ³⁶P. Robyr and R. Bowtell, *J. Chem. Phys.* **107**, 702 (1997).
- ³⁷C. Ramanathan and R. Bowtell, *J. Chem. Phys.* **114**, 10854 (2001).
- ³⁸J. P. Marques and R. Bowtell, *Magn. Reson. Med.* **51**, 148 (2004).
- ³⁹C. Faber, C. Heil, B. Zahneisen, D. Z. Balla, and R. Bowtell, *J. Magn. Reson.* **182**, 315 (2006).
- ⁴⁰Y. Y. Lin, N. Lisitza, S. D. Ahn, and W. S. Warren, *Science* **290**, 118 (2000).
- ⁴¹S. Datta, S. Y. Huang, and Y. Y. Lin, *J. Chem. Phys.* **124**, 154501 (2006).
- ⁴²M. Mescher, W. Richter, L. DelaBarre, M. Garward, and W. S. Warren, *Proceeding of the International Society for Magnetic Resonance in Medicine Eighth Scientific Meeting and Exhibition, Denver Colorado, USA, April 1–7, 2000*.
- ⁴³Z. Chen, Z. W. Chen, and J. H. Zhong, *J. Chem. Phys.* **115**, 10769 (2001).

⁴⁴D. Balla and C. Faber, [Chem. Phys. Lett.](#) **393**, 464 (2004).

⁴⁵C. Faber, *J. Magn. Reson.* **176**, 120 (2005).

⁴⁶W. Richter and W. S. Warren, [Concepts Magn. Reson.](#) **12**, 396 (2000).

⁴⁷S. Y. Huang, C. Anklin, J. D. Walls, and Y. Y. Lin, [J. Am. Chem. Soc.](#)

126, 15936 (2004).

⁴⁸G. E. Pereira, J. P. Gaudillere, C. van Leeuwen, G. Hilbert, O. Lavialle, M. Maucourt, C. Deborde, A. Moing, and D. Rolin, *J. Agric. Food Chem.* **53**, 6382 (2005).