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Communication

Hyperpolarized carbon–carbon intermolecular multiple quantum coherences

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ABSTRACT

Intermolecular multiple quantum coherences (iMQCs) can provide unique contrast with sub-voxel resolution. However, the characteristic growth rate of iMQCs mostly limits these effects to either hydrogen or hydrogen-coupled systems for thermally polarized samples. Hyperpolarization techniques such as dynamic nuclear polarization (DNP) allow for significant increases in the carbon signal (even more signal than that from hydrogen), making carbon iMQCs achievable. We present the first intermolecular multiple quantum signal between two carbon nuclei.

1. Introduction

During the last decade, dipolar field interactions between different spins in solution have allowed for the detection of new types of magnetic resonance signals, arising from intermolecular multiple quantum coherences (iMQCs). These coherences have unique and fundamentally different properties than conventional signal, in particular an intrinsic sensitivity to sub-voxel structure. This sensitivity makes these signals particularly suitable for a wide range of applications, such as temperature imaging [1], novel contrast in human brain imaging [2], and detection of molecular anisotropy[3].

iMQC experiments in a test tube of water can exhibit strong signals (within a factor of two of the equilibrium magnetization). However, applications to water in more complex samples, such as tissue, are limited by relaxation effects resulting in a smaller signal (typically 10–20% of the conventional signal). For other nuclei the drop is even more dramatic, essentially because the signal scales as the square of the magnetization density. For physiologically reasonable concentrations of molecules with low γ nuclei such as carbon with normal thermal polarization, the low magnetization density makes it nearly impossible to detect iMQC signals. The solution discussed here is to employ hyperpolarization techniques, which provide even more dramatic gains in iMQC experiments than in conventional images. One such technique is dynamic nuclear polarization (DNP) which transfers the large spin polarization present in the electron spin reservoir to the nuclei. This transfer is routinely done by microwave irradiation at or near the electron Larmor frequency in the presence of a large magnetic field at low temperatures. DNP techniques have been used to see increases in signal to noise of >10,000 [4]. By using hyperpolarization to enhance the initial magnetization of the sample, we were able to acquire the first multi-CRAZED FID of carbon–carbon coherences.

2. Results and discussion

The first demonstrations of iMQCs were over a decade ago, and so the theory behind the CRAZED experiment [5,6] will not be explained here. The multi-CRAZED experiment, which acquires multiple iMQCs, was first outlined in [7] but it is important to briefly revisit that theory to better understand why iMQCs between low γ nuclei (with thermal polarization) is impractical.

To understand why low γ nuclei give very weak multiple quantum signals, it is important to look at the expression for the signal generated by the multi-CRAZED experiment. The pulse sequence for the multi-CRAZED experiment is shown in Fig. 1. The standard pulse sequence for acquiring the signal from *n*-quantum intermolecular coherences is the "CRAZED" sequence 90 – {delay τ} – {gradient pulse – area GT} – θ – {gradient pulse area nGT} – {delay TE} – 180 – {delay TE – nτ} – acquire. The multi-CRAZED sequence takes advantage of differences in echo timings of the various coherences to acquire multiple echoes [7,8]. Neglecting diffusion and T_1 relaxation effects, the signal for a multi-CRAZED experiment is [7,9]:

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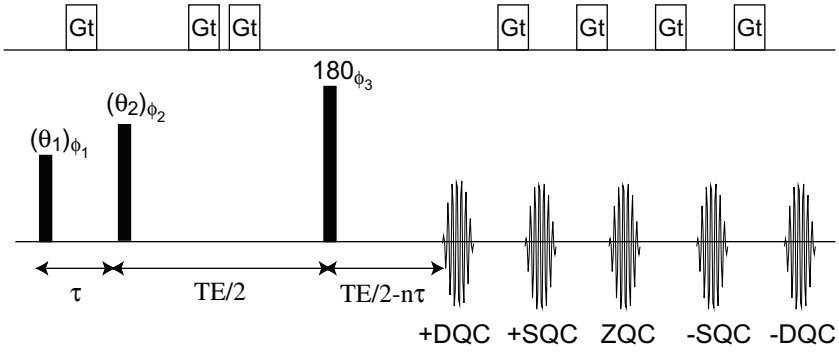

Fig. 1. The multi-CRAZED sequence takes advantage of differences in the echo timing to separate the different echoes at full intensity. The +DQC, −DQC and ZQC (+2, −2 and 0 quantum coherences, respectively) have contrast from sub-voxel variations in the magnetization density or resonance frequency. The +SQC and –SQC (+1 and −1 quantum coherences, respectively) signals have conventional contrast.

$$M^{+}(\tau,TE) = i^{n-1}e^{i\Delta\omega TE}e^{-TE/T_2}e^{-in\Delta\omega\tau}e^{-in\phi_1}e^{i(n+1)\phi_2} \times e^{-2i\phi_3}M_0\sin(\theta_1)\times\left[\frac{n\tau_d}{TE\Delta_s\sin(\theta_1)\sin(\theta_2)}J_n\left(\frac{-TE\Delta_s\sin(\theta_1)\sin(\theta_2)}{\tau_d}\right) - \frac{\cos(\theta_2)}{2}J_{n+1}\left(\frac{-TE\Delta_s\sin(\theta_1)\sin(\theta_2)}{\tau_d}\right) + \frac{\cos(\theta_2)}{2}J_{n-1}\left(\frac{-TE\Delta_s\sin(\theta_1)\sin(\theta_2)}{\tau_d}\right)\right] \quad (1)$$

where $\Delta\omega$ is the resonance offset, $\tau_d = (\gamma\mu_0 M_0)^{-1}$ is the dipolar demagnetizing time, $\Delta_s = 3((s\cdot z)^2-1)/2$ where s is the direction of the gradient pulse, θ_1 and θ_2 are the flip angles of the first two pulses, φ_1 is the phase of the first (excitation) pulse, φ_2 is the phase of the mixing pulse, and φ_3 is the phase of the refocusing pulse.

Note that the argument of the Bessel functions is proportional to TE/τ_d, which can be made large in a test tube of water, but which is limited by the relaxation time T_2 to small values in tissue (Table 1 below). For $x<<1$, $J_n(x)$ is proportional to x^n. This implies that the multiple quantum terms ($n = -2, 0, 2$) grow in linearly with TE. Table 1 compares these values for water in tissue (80 M) and water in the brain, to thermally polarized 1 M ^{13}C urea with at T_2 of 36 ms (measured using a spin echo sequence, with the echo time varied), and the same urea sample hyperpolarized (20% polarization) at 7 T. From these values it is clear that for reasonable values of T_2, we expect essentially no iMQC signal from a thermally polarized carbon sample, since by the time the multiple quantum signal has grown in, T_2 has dephased the spins.

Using the Hypersense hyperpolarizer from Oxford Instruments, we polarized a sample of urea (^{13}C labeled, 1 M). The sample was polarized for 4 h at a microwave frequency of 94.105 GHz. The dissolution was done in 3 mL of DI water with a 25 mM EDTA. This sample was inserted in a 7 T small animal imager using a Bruker console. The experimental parameters were: TE = 7.232 ms, $\theta_1 = 90$, $\theta_2 = 135$, 45–180 delay = 19.080 ms, 1228 points, SW = 90090.09 Hz.

In Fig. 2 the carbon–carbon multi-CRAZED fid shows both double quantum (DQC) and zero quantum (ZQC) signal, demonstrating that hyperpolarization techniques can be used to acquire multiple quantum signal between low γ nuclei. For comparison, a multi-CRAZED fid of water is shown in the top corner of the figure, illustrating the standard multi-CRAZED fid. Control experiments were done on a sample of water, with the correlation gradients along the *z*-axis and along the magic angle. When the correlation gradients are on the magic angle, the distant dipolar field is disabled, and no iMQC signals should appear. The experiments with the correlation gradient along the magic angle show a dramatic decrease in the amount of +iDQC signal (the signal seen in the first acquisition, the +iDQC acquisition period, is .8% of the signal seen with the gradients just along *z*). A similar decrease is seen for the −iDQC acquisition window. The drop in iZQC signal is less dramatic, which is also expected because some contamination arises from pulse imperfections.

In the case of hyperpolarized urea, the enhancement of the iMQC signal is hampered by the short T_2 of the sample as illustrated in Fig. 3. It is also clear from the Table 2, which compares signal intensities, that the T_2 of the carbon sample is short enough to significantly hamper even the −SQC signal intensity. In the case of water (inset on Fig. 2) the −SQC signal is 23.6% of the +SQC signal. Fig. 3 compares the calculated signal intensity (from Eq. (1)) for SQC and ZQC signals for water, and two hyperpolarized carbon samples with a short (36 ms) T_2 and a long (300 ms) T_2. The ZQC signal intensity is hurt much more by the shortening of T_2 than the intensity of the SQC signal. It is clear from the figures that the short T_2 of the carbon nuclei has a profound effect on the amount of iMQC signal that can be obtained. An increase of T_2 from 36 to 300 ms results in a seven fold increase in maximum signal intensity. Unfortunately, T_2 for urea and radical is quite short (approximately 36 ms), severely limiting the amount of iMQC signal possible from this molecule. Nonetheless, the demonstration of observable multi-CRAZED signal even under these unfavorable conditions bodes well for imaging applications.

Table 1
Comparison of predicted iMQC signal for protons, thermally polarized carbon and hyperpolarized carbon.

	^{1}H, 80 M water, 7 T	^{1}H water, in the brain (T_2 = 25 ms)	^{13}C, 1 M, thermally polarized, 7 T	^{13}C, 1 M, hyperpolarized, 7 T
τ_d	185 ms	185 ms	933,976 ms	298 ms
T_2/τ_d	10.81	.135	.00003854	.123
$M_0{}^*T_2/\tau_d$	1	.0249	2.8×10^{-9}	.3859

3. Conclusion

In summary, we have demonstrated that carbon hyperpolarization permits intermolecular multiple quantum signals with intensities comparable to those previously seen in water. In general, hyperpolarized techniques can give useful metabolic information complementary to the anatomical information obtainable with standard proton MR. One pitfall of these techniques is the inherently short acquisition time, which limits the spatial resolution in MR images and the spectral resolution in NMR spectra. It has been previously demonstrated that iMQC experiments can provide subvoxel resolution and inhomgeneity compensation in MRI and MRS of thermally polarized proton samples. Experiments that are currently done using single quantum coherences, could be done

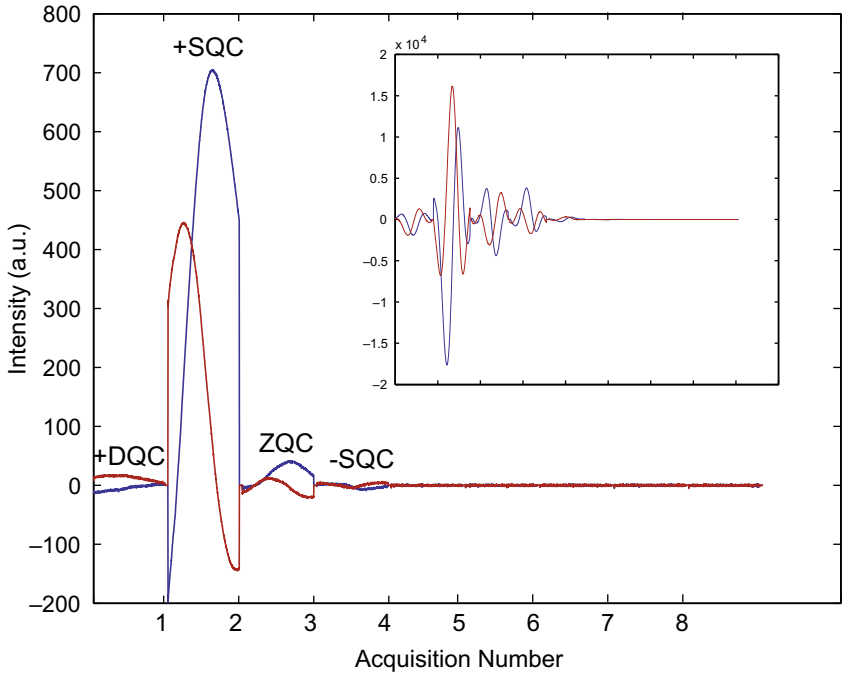

Fig. 2. Multi-CRAZED FID of hyperpolarized urea compared to the multi-CRAZED FID of water (top corner). It is important to note that this is not one long FID, but a series of consecutive FIDs separated by a gradient pulse.

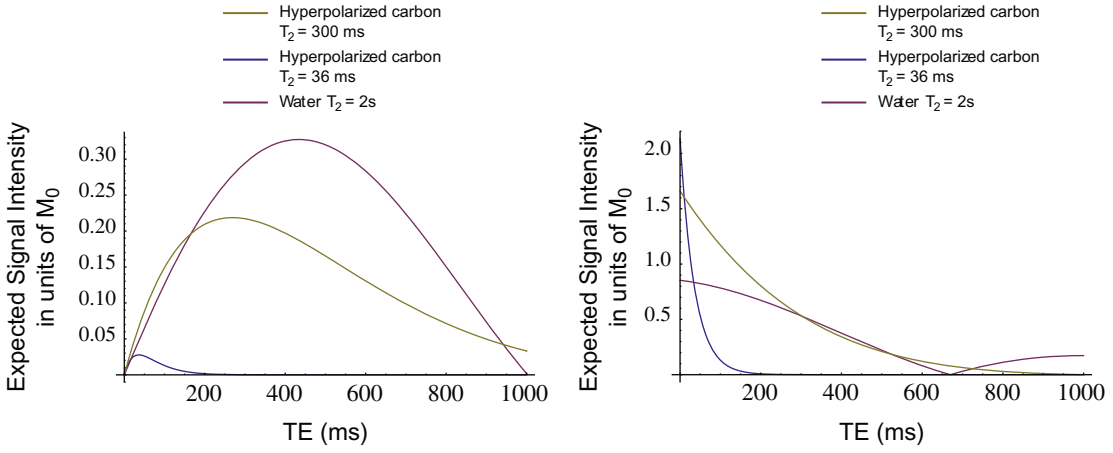

Fig. 3. Comparison of the calculated signal (from Eq. (1)) for a ZQC (left) experiment with an SQC (right) experiment for water (T_2 = 2 s), and two hyperpolarized carbon samples with T_2 s of 300 and 36 ms with the following parameters: $\Delta\omega = 0$, τ = 7 ms, $\varphi_1 = \varphi_2 = \varphi_3 = 0$, M_0 (hyperpolarized carbon) = 2.5, M_0 (water) = 1, $\theta_1 = \pi/2$, $\theta_2 = 3\pi/4$, τ_d = 298 ms (hyperpolarized carbon), τ_d = 185 ms (water) and $\Delta_s = 1$.

Table 2
Signal intensities for the coherences observed in the multi-CRAZED experiment.

	+DQC	+SQC	ZQC	−SQC
Maximum signal intensity, ^{13}C urea	25	700	50	10
Percent of +SQC maximum signal intensity, ^{13}C urea	3.59%	100%	7.14%	1.43%
Maximum signal intensity, ^{1}H water	1920	16,150	3720	3815
Percent of +SQC maximum signal intensity, ^{1}H water	11.9%	100%	23.0%	23.6%

with iMQCs to achieve better spatial and spectral resolution. A particular example of this is that the iMQC signal could be tuned to detect intracellular and extracellular compounds. For low concentration thermally polarized samples of low γ nuclei such as carbon, the exceedingly small magnetization makes iMQC detection nearly impossible; however, for hyperpolarized samples, the demagnetization time becomes reasonably short, and in fact the theoretical signal gain is vastly larger for iMQC sequences than for conventional imaging. The demonstration that carbon–carbon iMQC signal can be observed has been shown in this paper, and opens the door to the possibility of future applications of non-proton iMQC experiments. For example, fast 2D NMR spectroscopy has been demonstrated for iMQCs [10], and for hyperpolarized samples [11], and the combination of these types of experiments would al-

low for inhomogeneity compensated 2D spectra. In addition, iMQC signals are proportional to M_0^2 instead of M_0, as in standard experiments, and this difference could be used to amplify the concentration variation of different metabolites in vivo. Since this signal is intrinsically different than the standard MRI signal, we expect this approach will enhance the utility of hyperpolarized experiments.

Acknowledgment

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References

[1] G. Galiana, R.T. Branca, E.R. Jenista, W.S. Warren, Accurate temperature imaging using intermolecular coherences, Proceedings of the International Society for Magnetic Resonance in Medicine (2007).
[2] R.R. Rizi, S. Ahn, D.C. Alsop, S. Garrett-Roe, M. Mescher, W. Richter, M.D. Schnall, J.S. Leigh, W.S. Warren, Intermolecular zero-quantum coherence imaging of the human brain, Magnetic Resonance in Medicine 43 (2000) 627–632.
[3] L.S. Bouchard, W.S. Warren, Multiple-quantum vector field imaging by magnetic resonance, Journal of Magnetic Resonance 177 (2005) 9–21.
[4] J.H. Ardenkjaer-Larsen, B. Fridlund, A. Gram, G. Hansson, L. Hansson, M.H. Lerche, R. Servin, M. Thaning, K. Golman, Increase in signal-to-noise ratio of >10,000 times in liquid-state NMR, Proceedings of the National Academy of Sciences of the United States of America 100 (2003) 10158–10163.
[5] W.S. Warren, W. Richter, A.H. Andreotti, B.T. Farmer, Generation of impossible cross-peaks between bulk water and biomolecules in solution NMR, Science 262 (1993) 2005–2009.
[6] W.S. Warren, W. Richter, S. Qiuhong He, Imaging with intermolecular multiple-quantum coherences in solution nuclear magnetic resonance, Science 267 (1995) 654–657.
[7] X.P. Tang, H. Ong, K. Shannon, W.S. Warren, Simultaneous acquisition of multiple orders of intermolecular multiple-quantum coherence images, Magnetic Resonance Imaging 21 (2003) 1141–1149.
[8] K.L. Shannon, R.T. Branca, G. Galiana, S. Cenzano, L.S. Bouchard, W. Soboyejo, W.S. Warren, Simultaneous acquisition of multiple orders of intermolecular multiple-quantum coherence images in vivo, Magnetic Resonance Imaging 22 (2004) 1407–1412.
[9] J.H. Cho, S. Ahn, C. Lee, K.S. Hong, K.C. Chung, S.K. Chang, C. Cheong, W.S. Warren, Magnetic resonance microscopic imaging based on high-order intermolecular multiple-quantum coherences, Magnetic Resonance Imaging 25 (2007) 626–633.
[10] G. Galiana, R.T. Branca, W.S. Warren, Ultrafast intermolecular zero quantum spectroscopy, Journal of the American Chemical Society 127 (2005) 17574–17575.
[11] L. Frydman, D. Blazina, Ultrafast two-dimensional nuclear magnetic resonance spectroscopy of hyperpolarized solutions, Nature Physics 3 (2007) 415–419.