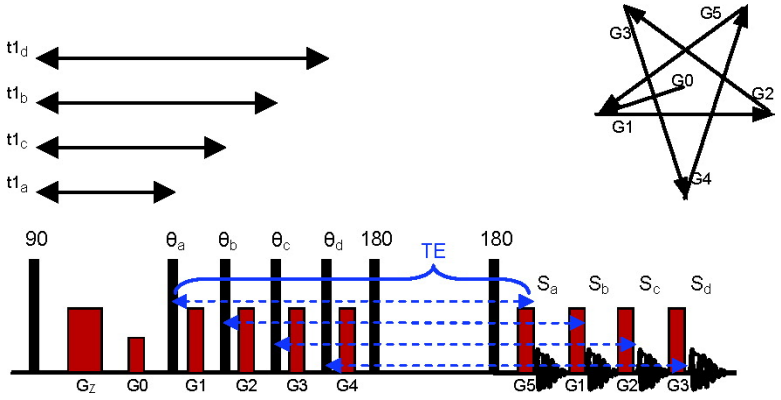
Communication

Ultrafast Intermolecular Zero Quantum Spectroscopy

Gigi Galiana, Rosa T. Branca, and Warren

J. Am. Chem. Soc., **2005**, 127 (50), 17574-17575• DOI: 10.1021/ja054463m • Publication Date (Web): 19 November 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 5 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 11/19/2005

Ultrafast Intermolecular Zero Quantum Spectroscopy

Gigi Galiana, Rosa T. Branca, and Warren S. Warren*,†

Princeton University, Department of Chemistry, Princeton, New Jersey, and Duke University, Department of Chemistry, Durham, North Carolina

Received July 6, 2005; E-mail: warren.warren@duke.edu

Magnetic resonance spectroscopy in vivo could empower diagnostics with the richness of NMR familiar to the lab chemist. Unlike liquid state NMR, however, spectra in vivo are acutely broadened by inhomogeneous susceptibility gradients making the spectra from most organs broad and illegible.[1] Intermolecular zero quantum coherences (iZQCs) are intrinsically insensitive to magnetic field inhomogeneities and, despite such variations, yield sharp lines along the indirect dimension.[2] This communication presents the first high-resolution iZQC spectrum in vivo which reveals that motion has restricted previous attempts.[3] To average away motion artifacts, we demonstrate a new approach to ultrafast two-dimensional (2D) spectroscopy, as well as extensions to speed up a broad range of 2D sequences.

All zero quantum coherences evolve at the difference frequency of the spins involved in the coherence. For intermolecular zero quantum coherences, this is the difference frequency between spins that are a correlation distance apart, typically around 100 μm.[2] Despite a broad range of absolute frequencies over the sample, this difference frequency will remain uniform and most $T2^*$ broadening is refocused.

While the insensitivity of iZQCs to inhomogeneous broadening has been demonstrated in vitro many times, previous in vivo work has shown only a modest increase in resolution.[3] However, our work on cold-blooded animals at low temperatures has increased resolution an order of magnitude over the direct acquisition.

In the spectrum of a live earthworm at 4 °C (Figure 1), the iZQC frequencies are far narrower than the inhomogeneously broadened line width along the direct dimension.

This implies that muting the physiological fluctuations significantly enhances resolution in the indirect dimension. Each heartbeat and breath irregularly distorts the spectrum from scan to scan and contributes t_1 noise that ultimately broadens the indirect dimension. The ultrafast 2D methods[4] such as those developed by Frydman et al. can overcome this problem in principle. However, their approach (time offset excitations of many different narrow slices) will not work for our application, since the molecules involved in an iZQC inevitably suffer some chemical shift misregistration, and their coupling would thus be eliminated. Instead, we propose an approach that enables the acquisition of 2D iZQC spectra within a single data acquisition scan.

A conventional HOMOGENIZED experiment resembles a COSY sequence with a gradient applied during t_1 and a spin−echo in t_2. The sequence for ultrafast iZQC spectroscopy (Figure 2) can be seen as a superposition of four steps in a HOMOGENIZED experiment with different t_1 values.

A 90° excitation pulse excites the zero quantum coherences. During the ZQC evolution time a correlation gradient is applied along the z axis with a small component in the x,y plane. As in the approach proposed by Bax et al., a low flip angle pulse transfers only a small fraction of the ZQC signal into detectable magnetization.[5] This leaves most coherences evolving in t_1 still available for the next mixing pulses. In this way, the magnetization is partitioned in small amounts which are each characterized by a different t_1 evolution time.

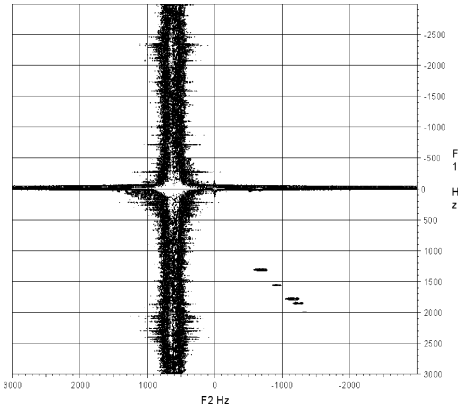


Figure 1. In vivo spectra of worms show a significant improvement in resolution is possible when the t_1 noise from physiological fluctuations is reduced.

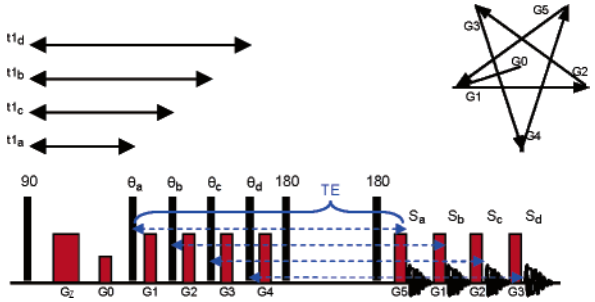


Figure 2. The sequence for fast iZQC with 4-fold speedup can be seen as a superposition of four t_1 steps in a HOMOGENIZED experiment.

Spoiling gradients separate the different t_1 increments as in the Bax method, but for intermolecular signals these gradients play an important role as the correlation gradients for the concurrent experiments. A small gradient following each mixing pulse winds each SQC into a different helix, and during the acquisition only one of these helices is rephased at any given time. If this were achieved with collinear gradients, each excitation would experience a unique diffusion weighting as ZQC and SQC, distorting the spectrum. Instead, the gradients form a star pattern that rotates the net correlation gradient along a cone, and the net diffusion gradient along a circle, restoring a similar diffusion weighting to each echo. Since the vectors of the star pattern (G_1−G_5) sum to zero, the excitations consecutively refocus when they have experienced a full star of gradients as SQC. The use of a double spin−echo for localized spectroscopy ensures that each excitation evolves for exactly TE before it is refocused and acquired. This allows us to acquire the same position of each echo, preserving the phase

† Duke University.

10.1021/ja054463m CCC: $30.25

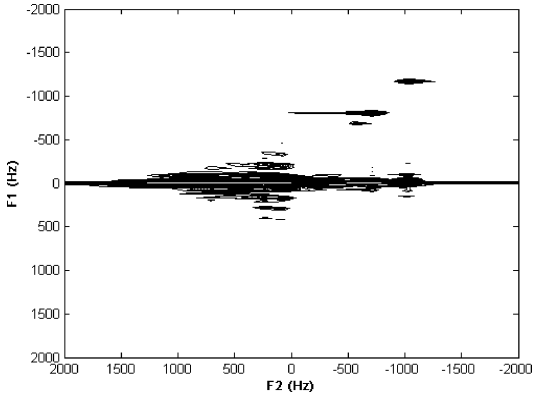


Figure 3. Spectrum of a 5% MEK solution in water, acquired with the fast iZQC pulse sequence with eight indirect acquisition points per scan. Water is suppressed with selective inversion pulses.

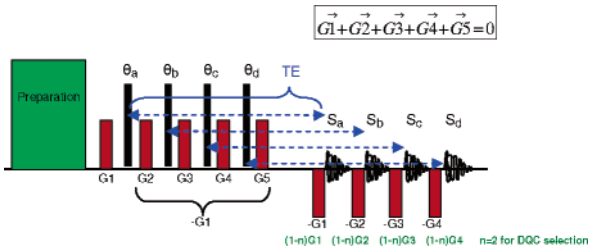


Figure 4. General scheme to speed up a standard *n*-quantum 2D pulse sequence. After the appropriate preparation period, a train of gradients refocuses each signal in the correct acquisition windows.

information critical to 2D analysis. It also eliminates modulation that would result from different time evolution under dipolar interaction.

Figure 3 demonstrates the fast iZQC pulse sequence on a solution of 5% methyl ethyl ketone (MEK) in water with eight indirect acquisition points per scan. To acquire the full data set, the time between the 90° and the first mixing pulse is stepped until it has covered all the t_1 points up to the initial position of the second mixing pulse. During these steps the second mixing pulse has covered all the t_1 points up to the initial position of the third mixing pulse, and so on. Using this approach we have scaled the sequence to as many as 31 t_1 points per scan.

The principles of the fast iZQC experiment have been used to speed up other standard 2D-spectroscopy pulse sequences, such as intra- or intermolecular SQC and DQC pulse sequences. For a double quantum pulse sequence (Figure 4), magnetization excited by the first pulse has seen G_1 during the t_1 evolution period. During the mixing pulses that follow, that magnetization experiences $G_2 + G_3 + G_4 + G_5 = -G_1$, since a full star sums to zero. Another gradient of $-G_1$ is then applied to create a collinear +2-quantum filter for this excitation. Subsequently applying a gradient of $-G_2$ creates a +2-quantum filter for magnetization excited by the second pulse, and so on. In general, a refocusing sequence of gradients with components $(1 - n)$ will create an *n*-quantum gradient filter for each excitation (Figure 4).

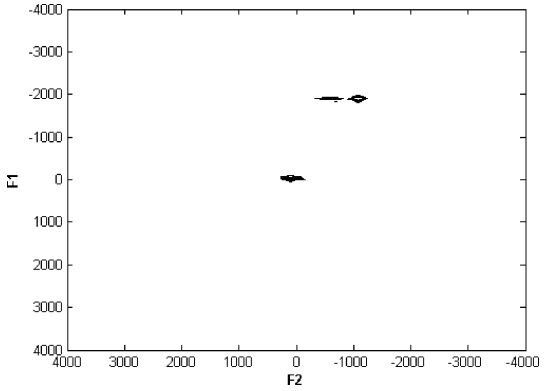


Figure 5. Application of the pulse sequence in Figure 4 to 5% MEK in D_2O.

We have demonstrated such a sequence for fast double quantum spectroscopy with 4-fold speedup, as seen in Figure 5. The pairing of low flip angle pulses with carefully chosen gradients provides an alternative method for a fast spectroscopy, which may allow for the successful application of iZQC spectroscopy in vivo to organs that are inaccessible by standard techniques.

Acknowledgment. We are grateful to the National Institute of Health (NIH Grant EB 2122) and the New Jersey Commission on Science and Technology for support of this work. We acknowledge useful discussion with Marlene Richter and Istvan Pelczer.

References

(1) (a) Smith, I. C. P.; Stewart, L. C. *Prog. Nucl. Magn. Reson. Spectrosc.* **2002**, *40*, 1−34. (b) Mountford, C. E.; Doran, S.; Lean, C. L.; Russell, P. *Chem. Rev.* **2004**, *104*, 3677−3704. (c) De Graaf, R. A. *In Vivo NMR Spectroscopy: Principles and Techniques*; Wiley: New York, 1998.

(2) (a) Vathyam, S.; Lee, S.; Warren, W. S. *Science (Washington, D.C.)* **1996**, *272*, 92−96. (b) Lin, Y.-Y.; Ahn, S.; Murali, N.; Brey, W.; Bowers, C. R.; Warren, W. S. *Phys. Rev. Lett.* **2000**, *85*, 3732−3736. (c) Garrett-Roe, S.; Warren, W. S. *J. Magn. Reson.* **2000**, *146*, 1−13. (d) Chen, Z.; Chen, Z.; Zhong, J. *J. Am. Chem. Soc.* **2004**, *126*, 446−447. (e) Chen, Z.; Chen, Z.; Zhong, J. *Proc. Int. Soc. Magn. Reson. Med.* **2004**, *11*, 2300.

(3) (a) Faber, C.; Pracht, E.; Haase, A. *J. Magn. Res.* **2003**, *161*, 265−274. (b) Mescher, M.; Richter, W.; Delabarre, L.; Garwood, M.; Warren, W. S *Proc. Int. Soc. Magn. Reson. Med.* **2000**, *8*, 600.

(4) (a) Frydman, L.; Scherf, T.; Lupulescu, A. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15858−15862. (b) Shrot, Y.; Frydman, L. *J. Am. Chem. Soc.* **2003**, *125*, 11385−11396. (c) Frydman, L.; Lupulescu, A.; Scherf, T. *J. Am. Chem. Soc.* **2003**, *125*, 9204−9217. (d) Freeman, R.; Kupce, E. *J. Biomol. NMR* **2003**, *27*, 101−113.

(5) Bax, A.; Mehlkopf, A. F.; Smidt, J.; Freeman, R. *J. Magn. Reson.* **1980**, *41*, 502−506.

JA054463M