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Spurious signals in DQF spectroscopy: two-shot stimulated echoes

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Abstract The most widely used technique for double-quantum filtered (DQF) single-voxel spectroscopy (SVS) is based on a symmetric PRESS sequence with two additional spatially unselective $\pi/2$ pulses, one of which is usually frequency selective. The actual filtering, rejecting signals from all uncoupled resonances, can be done by suitable phase cycling of the rf pulses in successive shots, but in practice gradient filtering is always used. Under usual conditions the sequence repetition time is comparable to the spin-lattice relaxation time, and a stimulated echo is formed by five out of the ten rf pulses in two consecutive shots. This echo is not filtered out by the gradients, and additional phase cycling is needed to eliminate it. Its spatial origin is the full transverse slice selected by the last pulse of the PRESS sequence. The SVS shimming procedure may create an important field variation in this slice (outside the volume of interest VOI). Water singlet signals therefore appear in a band of frequencies other than 4.7 ppm, and remain unaffected by water suppression pulses. In practice phase-alternation schemes can reduce these spurious signals by several orders of magnitude, but even then they may mask the weak metabolite signals of interest. We describe a strategy to minimize these spurious signals and propose a 16-step phase cycling scheme that attenuates the stimulated echo in every two-step subcycle.

Keywords GSH · Shimming · Magnetic field profile

Introduction

The motivation for our work is the search for a method of quantitative determination of glutathione (GSH) levels in the brain. The DQF spectrum of GSH in buffered aqueous solution [1] has a dominant signal at 2.9 ppm (βCH_2 cys), and lesser ones at 3.7 ppm (gly), 2.5 and 2.1 ppm (CH_2 glu). The multiplet around 4.6 ppm (CH cys) is formed by weaker lines. The pulse sequence used in [1] consists of the usual symmetric $\pi/2-\pi-\pi/2$ sandwich to create the double quantum coherence, followed by a binomial $1\bar{3}3\bar{1}$ composite $\pi/2$ pulse to read the coherence back, and a π to rephase the multiplet. Crusher gradients were placed around the first π, and coherence selection was by gradient filtering, with a gradient of moment G during the DQF evolution period, and another of moment $2G$ between the final π and the start of data acquisition. That sequence can be made volume selective by forming a PRESS sequence out of the first two and the last pulses [2, 3], and this modification was first applied to the in-vivo determination of the 2.9 ppm GSH signal in the brain by Trabesinger et al. [4]. In that work the read pulse is a binomial $1\bar{1}$. Later they have shown that an improved discrimination against GABA can be obtained by using a DANTE pulse as read pulse [5].

Some other strategies have been developed for quantitative in-vivo spectroscopy of those brain metabolites that lack a characteristic singlet resonance. At 3 T, after

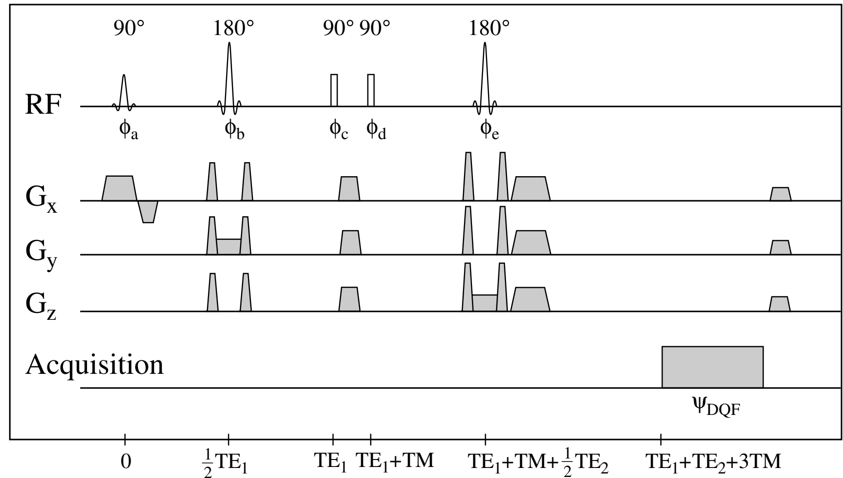

Fig. 1 Timing diagram for a PRESS-DQF sequence. The five RF pulses $a, \ldots, e$ are labeled with their nominal flip angle and a phase variable ϕ. The acquisition phase variable is Ψ_{DQF}. For the relation between these phases, see the Discussion section

shimming the water peak to 0.1 ppm width, difference-edited spectra from a PRESS sequence could be very well fitted using LCModel [6]. A similar fitting procedure has been applied to short echo time spectra from brain tumors at 1.5 T [7]. However double quantum filtering has been used more frequently.

In one series of such experiments at 3 T, a rather mysterious spurious signal was reported, that had some characteristics of a dipolar-coupled water resonance, except that it could occur at frequencies different from 4.75 ppm [8]. We have found at 1.5 T in-vivo spurious signals with similar erratic frequency behavior, but with otherwise the characteristics of a singlet stimulated echo, created by five pulses from two successive shots of the PRESS-DQF sequence (which contains five pulses per shot). The spin dynamics leading to this stimulated echo is very similar to that described in [9].

We analyze the reason for the erratic frequency behavior, and consider several cycling schemes for the phases of the rf pulses. We show that it is possible to suppress the echo after every second shot, rather than after a cycle of eight, as previously proposed [9].

Methods

The timing of the DQF sequence is defined in Fig. 1, where the pulses are supposed of negligible length. For simplicity, the read pulse (one before last in the sequence) is shown non-selective, but in actual experiments a frequency-selective read pulse is used. The preceding water suppression is not shown. The encoding and decoding filter gradients have the same direction and amplitude, but lengths of TG and 2TG, respectively. For hardware reasons, usually TM > TG. All filter and spoiler gradients are along the magic angle (i.e., have equal components along the x, y, z axes). The DQF echo rephases at time $\mathrm{TE}_1 + \mathrm{TE}_2 + 3\mathrm{TM}$. Referring to Fig. 1, this timing can be understood by considering $90_c - (\mathrm{TM} + (1/2)\mathrm{TE}_2) - 180_e - (2\mathrm{TM} + (1/2)\mathrm{TE}_2)$ as the refocusing path. The TM − 2TM asymmetry in the timing of this path occurs because during the first TM period the signal is in a DQ state and therefore dephases twice as fast as the single quantum state rephases during the second, 2TM, period.

We have implemented this sequence on a 1.5-T Siemens Symphony with 22 mT/m gradients, using the body coil as transmitter and the standard head coil as receiver. Our sequence is derived from a product sequence for asymmetric PRESS, and uses the same water suppression and field shimming techniques. Usually the repetition time TR was set to 2 s. The in-vivo experiments were performed on healthy volunteers, following procedures laid out by the relevant local authorities. The VOI was usually placed mid-sagittally in the prefrontal cortex, with a volume of $20 \times 20 \times 20$ mm. Some spectra have been obtained from spherical phantoms, containing metabolites in concentrations of 10–20 mM, and a VOI of comparable volume was used. The usual length of TG is 4 ms.

The timing in Fig. 1, the mechanism of stimulated echo formation to be explained later in this paper, as well as the phase cycling schemes mentioned in the Discussion section have all been checked by analytical calculations on an AX spin system, using the Mathematica computer algebra platform.

Results

Water resonances anywhere

Usual field shimming routines in single voxel spectroscopy set the shim region (or adjust volume (AV)) equal to the voxel region (or volume of interest (VOI)), and will do a good job inside the voxel, but care little about the magnetic field profile elsewhere. As a slightly unfair example, we have worked with a composite phantom consisting of two large cylindrical bottles, plus a small spherical container, arranged as sketched in Fig. 2. Each individual region has a simple geometry, and can therefore be shimmed very well, but the sharp susceptibility discontinuities in between them mean that their optimum settings

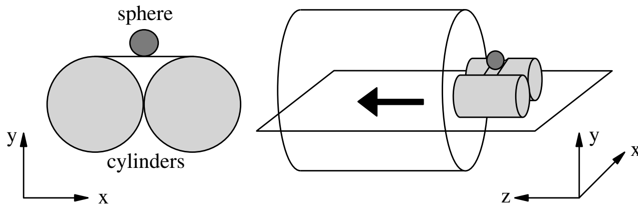

Fig. 2 Geometry for the composite phantom experiments

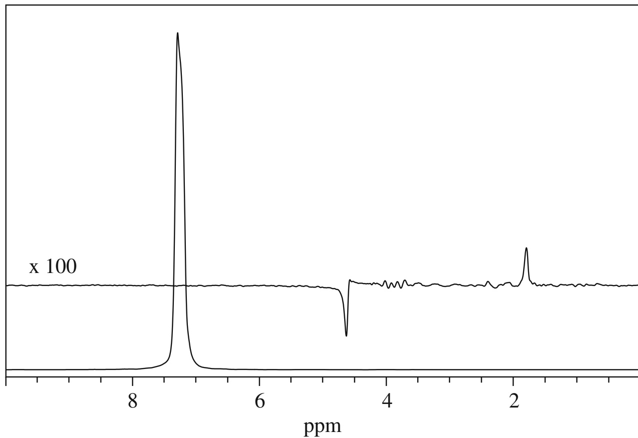

Fig. 3 *Top* the shimming adjust volume AV is the same as the observation volume of interest. The peak near 2 ppm is the NAA singlet. *Bottom* same VOI, but now the AV is the full transverse slice

are different, so that shimming on the total slice will be determined mainly by the "largest" contribution to the water signal. Since the routine does not have our prior knowledge about geometrical arrangements and does not know that there should be just one shift value for all of the signal, it is not shocked by finding several resonances, as in Fig. 3.

For that experiment, we ran our shimming routine twice, both times with the VOI inside and centered at the small sphere, but once with the AV equal to this VOI, and next with the AV (nonstandard) defined as the full transverse slice, including sections of the two cylinders. As can be seen from the PRESS result in Fig. 3, the shimming routine in the latter case focuses on the cylinders, so that the water in the sphere (our real VOI) now resonates at 7.4 ppm, thereby becoming immune to water saturation pulses, and erasing any hope of detecting the small metabolite signal shown in the top trace. After small changes in the initial conditions (shifting the slice by a few millimetres) and reshimming, the resonant frequency in the sphere will be different from the 7.4 ppm of this particular example, which explains the apparent erratic behavior of this signal.

For usual SVS, the phenomena in Fig. 3 do not matter at all, but the case of DQF sequences is different. They contain spatially unselective pulses, that do indeed excite

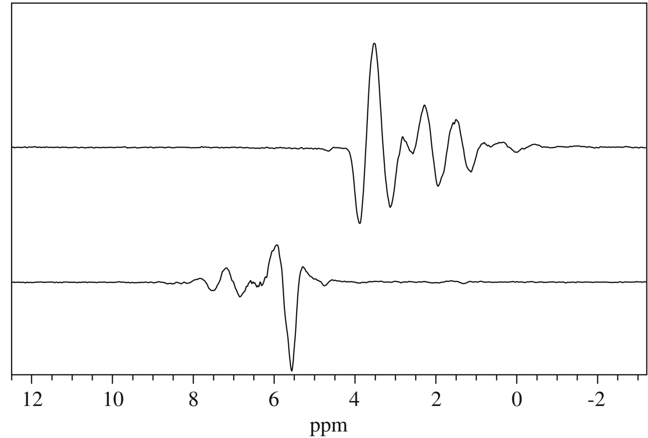

Fig. 4 DQF sequences in vivo without phase cycling, four accumulations. The two traces have been taken for VOIs 10 mm apart

a spin-echo signal from all of the transverse slice. Suitable phase cycling of the acquisition sequence will greatly reduce these outside signals, but in general, reduction of water signals by just phase cycling will rarely be better than a factor of a few hundred. Outer volume suppression (OVS) will not always work in these cases, because of the shift in resonance frequency.

We think that a similar variation of the magnetic field profile outside the VOI can occur in in-vivo experiments, and that this explains the signals observed at "arbitrary" frequencies in [8], and in the present paper. The actual spin dynamics however seems to be different in the two cases.

Diffusion-attenuated stimulated echoes

To determine the properties of this in-vivo spurious signal, we typically run between four and 16 accumulations of the PRESS-DQF sequence, with phase cycling switched off. The shimming AV is equal to the acquisition VOI. The presence of cavities in the head close to the VOI makes the shimming more difficult, not unlike the problem posed by our composite phantom. We show in Fig. 4 the effect of shifting the VOI over 10 mm (on the same volunteer in the same session). Two sequence parameters have an important effect: the magnitude of the filter gradient G and the repetition time TR, as shown in Fig. 5 (TR fixed at 2 s) and Fig. 6 (G fixed at 10 mT/m).

The TR-dependence in Fig. 6 shows that we are dealing with a signal created by the RF pulses from (at least) two different shots. From experiments in which selected pulses are switched off, it is found that the important pulses are the two spatially-unselective $\pi/2$ pulses (c and d in Fig. 1) in the first shot, plus the c and d pair in the second shot, followed by the transverse-slice selecting π pulse (e in Fig. 1). Such a five-pulse sequence can generate up to 81 signals [10], three of which are stimulated

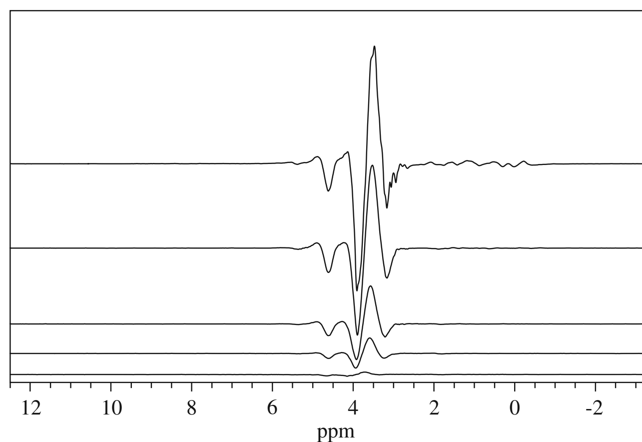

Fig. 5 Effect of gradient amplitude on spurious signal in vivo. The filter gradients are 4 and 8 ms long, and from *top to bottom* 1, 5, 10, 15 and 22 mT/m on each axis

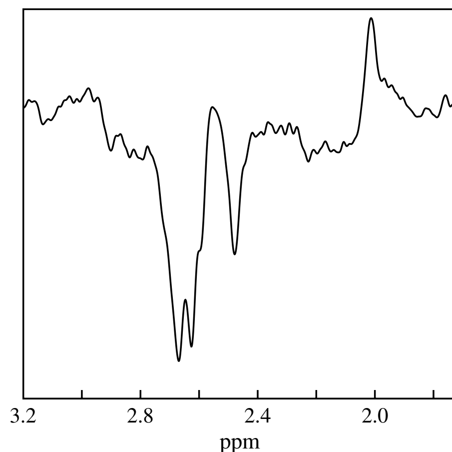

Fig. 7 The NAA singlet at 2 ppm, as observed on a phantom using a DQF sequence without phase cycling

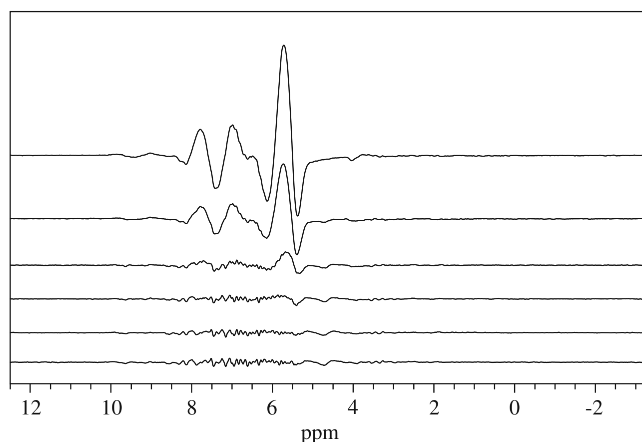

Fig. 6 Spurious signal in vivo. *Top to bottom* TR = 1.5, 2, 4, 8, 14 and 20 s

echoes that occur at around the same time as the refocused DQF signal. Only one of the three can survive the filter and post-acquisition spoiler gradients, and refocuses at the same time as the DQF signal. The elementary refocusing path is $90_{c,1} - (2\text{TM} + (1/2)\text{TE}_2) - 180_{e,2} - (2\text{TM} + (1/2)\text{TE}_2)$, with the magnetization stored along the longitudinal axis by $90_{d,1}$ and recalled by $90_{c,2}$. (Here the additional subscripts 1 and 2 refer to the first and second shots).

The variation with G in Fig. 5 shows how the stimulated echo is attenuated by diffusion during the TR period between the two shots. Because of the diffusional motion, the total gradient moment seen by a nucleus no longer balances to exactly zero. (The remaining spurious signal for TR = 20 s in Fig. 6 is probably due to residual dipolar coupling; but now it is small enough to be effectively eliminated by phase cycling).

Discussion

It is sometimes stated that gradient-filtered DQF is an interesting technique because it eliminates singlet signals in a single shot. Under usual signal averaging, however, strong singlets like the NAA peak at 2 ppm will form a stimulated echo that in principle will contribute to the spectrum, see Fig. 7. As before, this unwanted signal is attenuated when TR increases, or the gradients are made stronger. The data in Fig. 7 have been obtained using signal averaging without phase cycling, thus preserving the "single-shot" character of the experiment. The simplest way to eliminate the spurious signal is to increase TR to several times the relevant T_1, but this is suboptimal for the time efficiency of the desired DQF spectrum. The other possibility is very strong gradients, but this has not only practical but also theoretical limits. Correct formation of the DQF signal requires that the variation across the VOI of the phase shift accumulated by the chemical shift difference between the coupled nuclei during the filter gradients can be neglected. Furthermore, diffusion of the molecule between the double-quantum encoding and decoding periods will lead to increased attenuation of the DQF signal. In systems with maximum gradients of the order of 20 mT/m however, these effects remain negligible. The best strategy is to use gradients just strong enough that the remaining amplitude of the stimulated echo can be correctly eliminated through appropriate phase cycling. Since the success of such an elimination depends on the stability of certain, not always well known, experimental parameters over the length of the cycle, the shortest possible cycle should be used.

There exists [9] an eight-step phase cycle that coherently adds the DQF signal, and attenuates the stimulated echo. The DQF phase cycling scheme used in [4] was not specifically designed to suppress stimulated echoes, and

requires its full sixteen steps to do so. The ideal shortest cycle of course has just two steps, and we will show that such a cycle is indeed possible. To be specific, we consider in the following a two-spin system, with coupling constant J (in hertz). We neglect relaxation effects.

The double-quantum coherences just after the pulse at TE_1 are maximized when the pulse phases (see Fig. 1) obey

$$\phi_a - 2\phi_b + \phi_c = k\pi, k \text{ integer} \quad (1)$$

If the first three pulses obey (1), then the overall phase of the final DQF signal depends on the five pulse phases through

$$\psi_{\mathrm{DQF}} = \phi_a - 2\phi_b - \phi_c + \phi_d + 2\phi_e \quad (2)$$

and (under weak coupling) it will be an in-phase doublet with maximum amplitude when the timing is chosen such that

$$|J|\mathrm{TE}_1 = \frac{1}{2}$$
$$\mathrm{TE}_2 = \mathrm{TE}_1 - 2\mathrm{TM} \quad (3)$$

For the stimulated echo, we need to consider two consecutive shots (labeled with subscripts 1 and 2). Its phase depends on the pulse phases through

$$\psi_{\mathrm{STE},2} = -\phi_{c,1} + \phi_{d,1} - \phi_{c,2} + 2\phi_{e,2} \quad (4)$$

Usual phase cycling schemes switch all ϕ and ψ through multiples of $\pi/2$, even if modern hardware can do arbitrary phases. A simple version is then to set the phases in the n-th step of the cycle to

$$\phi_a = \phi_b = \phi_c = (n \bmod 4)\frac{\pi}{2}$$
$$\phi_d = \phi_e = 0 \quad (5)$$

In theory, this phase scheme is a DQF all by itself: even without applying gradients, singlet signals will be suppressed after every two-step subcycle. In practice, it is hard to obtain good nulling of all singlet signals through phase cycling only. But the superposition of the gradient and phase-cycling filters can be expected to improve selectivity.

This simple cycling n modulo 4 does not eliminate the stimulated echo, since according to (2) and (4)

$$\psi_{\mathrm{DQF},2} - \psi_{\mathrm{STE},2} = -\frac{\pi}{2} \quad (6)$$

which is why the authors of [9] propose to alternate cycling modulo 4, as in (5) and modulo −4, where the sign in the right hand side of (4) changes. This requires an eight-step cycle to attenuate the stimulated echo. The DQF phase cycling scheme used in [4] can be reordered to suppress stimulated echoes every second step, but this scheme does not combine the gradient and phase DQF mechanisms.

A 16-step DQF cycle that suppresses (ideally even without the need for filter gradients) both single-shot singlets and two-shot stimulated echoes in every two-step subcycle is e.g. the following:

$$\phi_a = \phi_c = (n \bmod 4)\frac{\pi}{2}$$
$$\phi_d = 0$$
$$\phi_b = (n \operatorname{div} 4)\pi$$
$$\phi_e = -\phi_a + (n \operatorname{div} 8)\pi \quad (7)$$

Here (n div 4) is the integer part of the division $n/4$ with $n = 0, \ldots, 15$, a sequence of four zeroes, followed by four ones, four twos and four threes. As usual, (n mod 4) repeats the sequence 0, 1, 2, 3, 0, 1, ... The required setting of the detection phase ψ_{DQF} can be found from (2) and turns out to be a simple alternation of addition and subtraction.

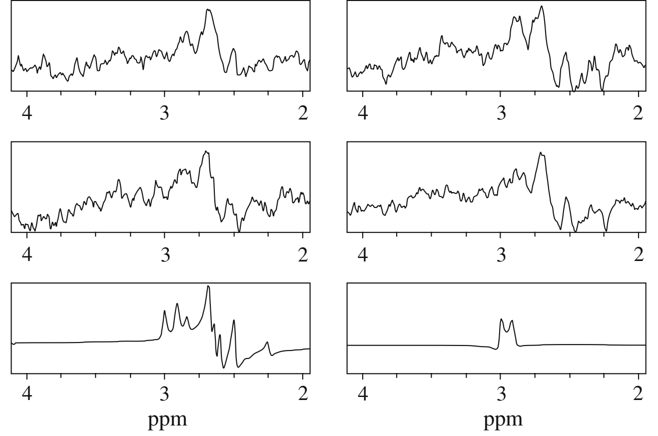

Fig. 8 Reproducibility of the 2–4 ppm spectral range using DQF. *Top two* lines: in-vivo spectra from frontal (*left column*) and occipital (*right column*) brain regions in two volunteers. *Bottom line* simulated spectra for a NAA/GSH mixture (*left*), and for GSH only (*right*). For details, see text

Conclusion

The shimming procedure for single-voxel spectroscopy may lead to a complicated magnetic field profile in the transverse slice outside the volume of interest (VOI). In that case, water signals from outside the VOI are not suppressed by CHESS, and in a DQF sequence stimulated echoes may form that appear at arbitrary (and seemingly irreproducible) frequencies in the spectrum. Relative to a metabolite signal, these echoes may be so large that they are not adequately suppressed by rf phase cycling. Increasing the amplitude of the filter gradients helps to attenuate the stimulated echoes through diffusion.

Experimentally therefore, the first DQF-specific step after the usual preparation for in-vivo spectroscopy is to locate the frequency region of the spurious signal, with an experiment as in Fig. 4. If that signal is centered in the 3 ppm region (supposing that the goal is GSH detection), then we go back to the SVS preparation stage, shift the VOI by a small amount, and reshim. Once the spurious signal has been shifted out of the region of interest, switching on the phase cycling will lead to a sufficiently clean background. The optimum phase cycling suppresses

both single-shot singlets and two-shot stimulated echoes every second step, while the full 16-step cycle compensates pulse errors, as usual.

When the spurious signals are eliminated, the signals in the 2–4 ppm spectral region become fairly reproducible, as shown for voxels in the frontal and occipital brain regions of two volunteers in the top four panels of Fig. 8. It seems however that with the parameters used here ($TE_1 = TE_2 = 70$ ms, TM = 4 ms, TR = 2 s, filter gradients 22 mT/m on each axis, DANTE read pulse as in [5], 256 scans, VOI $30 \times 30 \times 30$ mm), the signal is dominated by NAA, as suggested by the spectral simulations shown at the bottom. The left spectrum is for a 10:1 molar ratio of NAA:GSH, the right spectrum shows the GSH contribution only. These spectra have been calculated using the GAMMA platform [11], available at http://gamma.magnet.fsu.edu. Since neither NAA nor GSH can be considered a simple AX spin system, we are currently exploring a wider range of parameter values to find whether a better discrimination between the two can be obtained.

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