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Communication

Quenching homonuclear couplings in magnetic resonance by trains of non-refocusing pulses

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ABSTRACT

Trains of 2π or 4π pulses fail to refocus offsets but can suppress the effects of bilinear interactions such as homonuclear scalar couplings.

One of the fundamental tenets of NMR states that 180° pulses refocus offsets and inhomogeneous static fields, but fail to refocus homonuclear dipolar or scalar couplings. The latter lead to echo modulations that can be exploited to determine coupling constants, but make it difficult to determine accurate transverse relaxation rates $R_2 = 1/T_2$. A non-trivial corollary of this common wisdom, which will be demonstrated in this work, is that trains of 2π or 4π (i.e., 360° or 720°) pulses fail to refocus offsets but can suppress the effects of bilinear interactions such as homonuclear scalar couplings that can be represented by Hamiltonian terms such as I_zS_z.

The basic motivation of refocusing [1–3] is to cancel the effects of inhomogeneous static fields and remove some other interactions (notably chemical shifts and *heteronuclear* scalar or dipolar interactions) by time reversal. Hahn originally suggested using a single refocusing pulse, which Carr and Purcell later referred to as "Method A". The cancellation of deleterious effects of translational diffusion was the main motivation for the use of trains of 180° refocusing pulses ("Method B" in the parlance of Carr and Purcell). For slowly tumbling molecules like proteins, which have inherently broad lines, the inhomogeneous contributions to the linewidths can be neglected in modern high-resolution magnets. In this case, diffusion does not interfere with refocusing, and one can dispense with multiple refocusing ("Method B") to revert to single refocusing ("Method A"), since this suffices to suppress the effects of offsets and chemical shifts. Indeed, common sequences such as Insensitive Nuclei Enhanced by Polarization Transfer [4] and Incredible Natural Abundance Double-Quantum Transfer Experiment [5] are rarely adorned with multiple refocusing pulses, and for good reason: in reasonably homogeneous static fields, there is little advantage to be gained from multiple refocusing. In modern high-resolution magnets, the main use of multiple refocusing is to identify contributions to echo decays that arise from chemical exchange, which depend on the pulse repetition rates if these are comparable with the exchange rates ('relaxation dispersion' in the parlance of biomolecular NMR.) [6,7].

Consider a conventional multiple-refocusing Carr–Purcell–Meiboom–Gill (CPMG) sequence $(\pi/2)_y - [\tau - (\pi)_x - \tau]_{2n}$, where a spin I is on-resonance ($\Omega_I = 0$) and a scalar-coupled spin S (with $J_{IS} \neq 0$) is off-resonance ($\Omega_S \neq 0$). The pulse repetition rate $\nu_{rep} = 1/(2\tau + \tau_\pi)$ is the inverse of the duration of the cycle, 2τ being the pulse interval and τ_π the length of the π pulse. It has long been known that echo modulations due to homonuclear scalar couplings can be quenched by using refocusing pulses with strong radio-frequency (*rf*) amplitudes $\omega_1 = \gamma B_1 \gg \Omega_S$, provided the pulse repetition rates are fast $\nu_{rep} \gg \Omega_S$ [8,9], or alternatively by using selective pulses $\omega_1 \ll \Omega_S$ [10]. More recently, it has been discovered that multiple refocusing can *quench* the effects of homonuclear scalar couplings [11–17]. This effect occurs when the refocusing pulses have a moderate *rf* field strength ω_1, so that they induce an ideal rotation through an angle π for a spin I on resonance ($\Omega_I = 0$), but a non-ideal rotation through an angle $\pi + \varepsilon$ about a tilted effective field for a coupling partner S that is off-resonance. In multiple pulse sequences, these non-ideal rotations have a cumulative effect. To quench the effects of homonuclear scalar couplings, the pulse repetition rate ν_{rep} must however be chosen so as to avoid the harmonic conditions $\nu_{rep} \approx \Omega_S/(2k\pi)$,

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where k is an integer. (These conditions are reminiscent but not exactly equal to the sidebands encountered when using 'Delays Alternating with Nutation for Tailored Excitation' or DANTE [18–20].) For the quenching of homonuclear scalar couplings to be effective, the ratio $\gamma = \Omega_S/\omega_1$ which determines the tilt of the effective field experienced by the neighboring spin S, should typically lie in a range $8° < \theta < 27°$, where the angle θ is the tilt of the effective field $\omega_{eff} = (\omega_1^2 + \Omega_S^2)^{1/2}$ with respect to the transverse plane. The most effective range of γ depends on the magnitude of the scalar couplings [16].

All schemes discussed so far are based on the use of *refocusing* pulses, i.e., pulses that bring about a nutation through an ideal angle π on resonance, and $\pi + \varepsilon$ for off-resonance spins. In the present work, we demonstrate that homonuclear scalar couplings can be quenched with sequences of 2π or 4π pulses, i.e., pulses that bring about a nutation through a multiple of 2π on resonance, and $2(\pi + \varepsilon)$ for off-resonance spins. Such sequences obviously fail to refocus offsets, and cannot lead to the formation of spin echoes, but they can be quite effective in quenching homonuclear scalar couplings.

The new scheme has similar properties as quadrupolar echoes based on $(\pi/2)_y - [\tau - (\pi/2)_x - \tau]_{2n}$ sequences, which can refocus quadratic I_z^2 terms that are involved in quadrupolar or homonuclear dipolar interactions between like spins in solids, but fail to refocus bilinear I_zS_z terms between unlike spins [21,22].

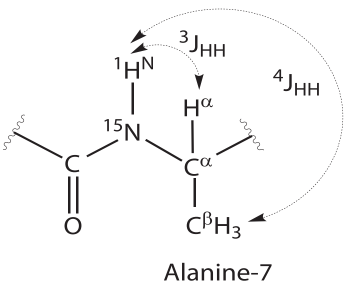

Fig. 1. Fragment of Cyclosporin A (CsA) containing the proton $I = H^N$ of Alanine-7. The only resolved coupling is $^3J(H^NH^\alpha) = 7.4$ Hz that leads to a doublet of the $I = H^N$ signal in the proton NMR spectrum. The long-range coupling $^4J(H^NH^b)$ contributes to line broadening.

In earlier work [13–15], we advocated the use of so-called 'hybrid' experiments, using a traditional Carr–Purcell–Meiboom–Gill (CPMG) sequence $(\pi/2)_y - [\tau - \pi_x - \tau]_{2n}$, where the delay τ is incremented stepwise while keeping the number of cycles $2n$ fixed. The integral of the multiplet of a spin I that is on-resonance ($\Omega_I = 0$), obtained by Fourier transformation of the signal starting at the top of the $2n$th echo, can be plotted as a function of the interval τ. Once a favourable τ value where scalar modulations are quenched has been identified, a conventional CPMG sequence is

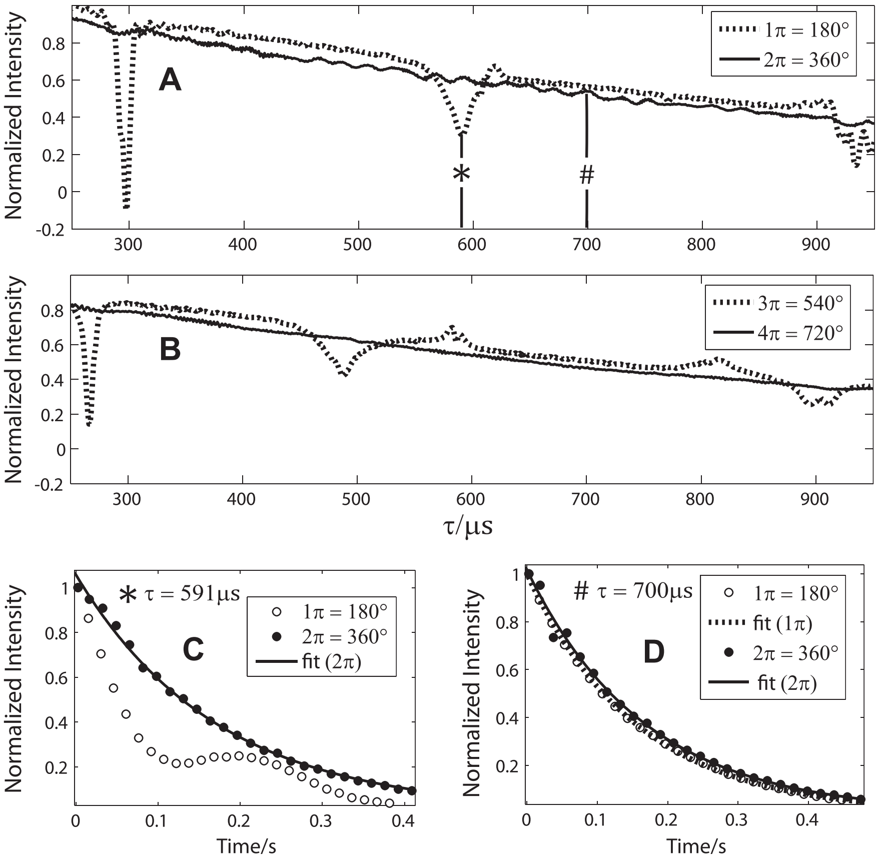

Fig. 2. (A) Amplitude of the H^N proton doublet of Alanine-7 in CsA recorded with the hybrid sequence $(\pi/2)_y - [\tau - m\pi_x - \tau]_{2n}$ for $n = 50$ (100th echo) as a function of $280 < \tau < 950$ μs (in steps of 0.5 μs). The dashed and the solid lines correspond to 'proper' echoes with $m = 1$ ($\pi = 180°$), and to $m = 2$ ($2\pi = 360°$), respectively. The curves are normalized by the initial amplitude of the decay for $m = 1$. The vertical lines at $\tau = 591$ μs ($*$) and $\tau = 700$ μs (#) indicate the conditions chosen for the CPMG sequence and its $2\pi = 360°$ equivalent in (C) and (D). (B) The dashed and the solid lines correspond to normal echoes with $m = 3$ ($3\pi = 540°$) and to $m = 4$ ($4\pi = 720°$), respectively. Their ampitudes are normalized by the signals for $m = 1$ in (A). (C) Decays of the H^N proton doublet of Alanine-7 recorded with the sequence $(\pi/2)_y - [\tau - m\pi_x - \tau]_{2n}$ for $\tau = 591$ μs as a function of the number of cycles n. Empty circles ($m = 1$): $n = 1, 10, 20, \ldots, 200$, so that the time axis $T = n(4\tau + 2m\tau_\pi)$ extends over $0 < T < 381$ ms. No meaningful R_2^{app} rate could be extracted from the modulated decay. Filled circles ($m = 2$): the fit of the unmodulated decay leads to $R_2^{app} = 5.78\ s^{-1}$. (D) Decays of the H^N proton doublet of Alanine-7 recorded with the sequence $(\pi/2)_y - [\tau - m\pi_x - \tau]_{2n}$ for $\tau = 700$ μs as a function of the number of cycles n. Empty circles: echoes with $m = 1$. Fitting the unmodulated decay leads to $R_2^{app} = 6.43\ s^{-1}$. Filled circles: $m = 2$ and $0 < T < 474$ ms. The decay rate is $R_2^{app} = 5.98\ s^{-1}$.

used by incrementing n. The mono-exponential echo envelope allows one to determine the apparent transverse relaxation rate R_2^{app}. In this work, the π pulses are simply replaced by 2π or 4π pulses, and hybrid experiments are again carried out. The old and new schemes can be written $(\pi/2)_y - [\tau - m\pi_x - \tau]_{2n}$, where $m = 1, 3, \ldots$ for sequences that refocus linear Zeeman terms like Hahn, Carr and Purcell intended, and $m = 2, 4, \ldots$ for our (slightly iconoclastic) scheme.

The cyclic peptide Cyclosporin A (CsA) comprises 11 amino acids. [14] By way of example, we focus attention on the doublet of proton $I = H^N$ of Alanine-7 (Fig. 1). The only resolved coupling is $^3J(H^NH^\alpha) = 7.4$ Hz; the long-range couplings $^4J(H^NH^\beta)$ to the three methyl protons merely contribute to broaden the lines. The *rf* carrier was placed on the chemical shift (7.68 ppm) of proton $I = H^N$, hence $\Omega_I = 0$. In a field $B_0 = 11.74$ T (500 MHz for protons), the offset of the coupling partner $S = H^\alpha$ is $\Omega_S/(2\pi) = 1.578$ kHz. The *rf* amplitude was set at $\omega_1/(2\pi) = 5.5$ kHz, so that $\gamma = \Omega_S/\omega_1 = 0.29$, corresponding to $\theta = 16°$. Echoes were recorded with 'hybrid' schemes using $m = 1, 2, 3$, or 4 with $n = 50$, i.e., the amplitudes of the $2n$th = 100th "echoes" were observed as a function of the interval τ in each case.

In Fig. 2A, we compare signal amplitudes observed using this hybrid scheme with traditional refocusing pulses with $m = 1$ (π or 180° pulses, dashed line) and with a sequence with $m = 2$ (2π or 360° pulses, solid line). Fig. 2B shows a comparison between $m = 3$ (3π or 540° pulses, dashed line) and $m = 4$ (4π or 720° pulses, solid line). The sequences with $m = 1$ and 3 lead to nearly flat responses when the modulations are quenched, but to fairly sharp 'dips' at the recoupling conditions. The dips observed with 540° pulses are shifted compared to those seen with 180° pulses because the *rf* pulse lengths are different. Remarkably, our scheme (solid line in 2A for 360° pulses, and solid line in 2B for 720° pulses) does *not* lead to any recoupling effects. Yet, the overall decay rates are very similar. Since the dips have disappeared, there is no longer any need to run any hybrid experiments.

With reference to Fig. 2A, a conventional refocusing sequence with 180° pulses ($m = 1$) leads to *recoupling* when using $\tau = 591$ μs (*) and to *quenching* at $\tau = 700$ μs (#). The latter τ value allows one to determine $R_2^{app} = 6.43$ s^{-1} by incrementing n while keeping τ fixed. In the former case, one observes modulated echoes (empty circles in Fig. 2C). By contrast, the use of a sequence with 360° pulses ($m = 2$) with the same interval $\tau = 591$ μs (filled circles in Fig. 2C) leads to a clean mono-exponential decay with $R_2^{app} = 5.78$ s^{-1}. Remarkably, the modulations are quenched for *arbitrary* τ delays if one uses 360° pulses ($m = 2$). For $\tau = 700$ μs, both schemes with $m = 1$ and 2 show monoexponential decays (Fig. 2D) with $R_2^{app}(m = 1) = 6.43$ s^{-1} and $R_2^{app}(m = 2) = 5.98$ s^{-1} (empty and filled circles). In the latter case, the apparent rate may be affected by the inhomogeneity of the static field. Surprisingly, the rate obtained with the sequence $R_2^{app}(m = 2)$ is slightly smaller than the $R_2^{app}(m = 1)$ obtained with conventional refocusing, i.e., the life-time of the transverse magnetization is actually prolonged by using trains of 2π rather than π pulses. This can be attributed to the suppression of unresolved modulations due to long-range scalar couplings.

Thus, quenching and recoupling are characteristic of sequences with even and odd numbers of π pulses. For $m = 2$ the quenching effect *cannot* be attributed to a simple spin locking effect arising from to the fact that 360° pulses last twice as long as 180° pulses. Indeed, for $m = 3$ recoupling effects re-appear. Clearly, the quenching mechanism depends on whether m is odd or even.

Our observations could be corroborated by numerical simulations [11,12], by considering the propagation of the density operator in subspaces spanned by triads of commuting operators [11,23], in the framework of average Hamiltonian theory [12] or by analytical calculations of the 'overshoot' of nutations under off-resonance pulses [11].

In conclusion, we have explored effects associated with sequences of 2π or 4π pulses, which have different properties than traditional Carr–Purcell echoes. They can be used to suppress the effects of homonuclear scalar couplings, in particular of unresolved long-range scalar couplings.

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