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An improved method for measurement of lifetime of long-lived coherences in NMR

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An improved method for the measurement of lifetime of long-lived coherences in NMR

Maninder Singh and Narayanan D Kurur[a]

Rotating frame zero quantum or Long-Lived Coherences among singlet triplet states in two weakly coupled protons are known to have longer lifetime than Zeeman magnetization.
10 The usual method for measuring these lifetimes involves fitting a damped oscillatory function which is lengthy and ineffective for various NMR studies and in turn restricts its usefulness. Here we introduce a new robust strategy to overcome this problem.

15 The worth of zero quantum coherences (ZQC) in NMR is well known for decades because of the special property that they are immune to inhomogeneous broadening due to local static field variations and dipole-dipole fluctuations. This difference in behavior from other types of coherences, like single and double 20 quantum coherences, has long been recognized and utilized in various studies[1,2] including Magnetic Resonance Imaging (MRI).[3] Although in the early stages of NMR development ZQC were thought to be undetectable with the advent of modern NMR pulsed techniques it became possible to detect them by various 25 indirect methods which helped recognize their importance.[4]

In the last few years, for a pair of spin-1/2 nuclei some states better known as singlet states, were introduced where nuclear population is stored for unusually long times.[5,6,7] Their unique relaxation property can be understood as arising due to the 30 ineffectiveness of the major relaxation mechanism i.e., intra molecular dipole-dipole coupling, in relaxing it because of symmetry constraints.[5,6,7] Along similar lines was the finding of the unusually long lifetime of the coherence between the long lived singlet state "$|S_0\rangle = 1/\surd2(|\alpha\beta\rangle - |\beta\alpha\rangle)$" and central 35 triplet state "$|T_0\rangle = 1/\surd2(|\alpha\beta\rangle + |\beta\alpha\rangle)$" in a pair of two weakly coupled protons resulting in narrow linewidth.[8,9,10] This coherence which is rotating frame zero quantum coherence (RF-ZQC) was as a result called as Long-Lived Coherence (LLC).[8,9,10] Numerous studies exploiting the long lifetime associated with 40 these long lived singlet states and coherences have appeared recently.[11,12,13,14,15,16]

Sarkar et al. (see Fig. 1a)[8] demonstrated a way to observe and detect the RF-ZQC or LLC in a weakly coupled two spin-1/2 system. The operator $(I_x - S_x)$ is created by applying a 45 nonselective $(\pi/2)_x$ pulse followed by delay $\tau = 1/2\Delta\nu$. Spin locking this state, by using low power continuous *rf* irradiation, removes the chemical shift difference between the two spins making them equivalent. During this spin locking period coupling
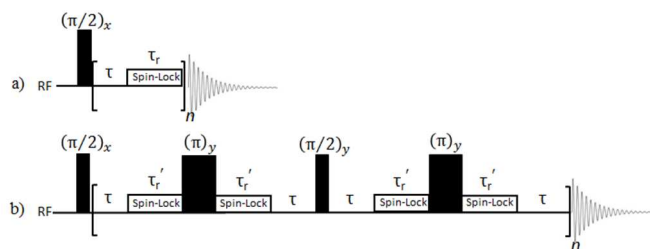

Fig. 1 Pulse sequences for (a) the normal LLC experiment (b) LLC
50 with a centrally placed 90_y^0 coherence transfer pulse. The spin lock time $\tau_r = 1/J$ which is divided into four parts as $\tau_r' = 1/4J$ in new sequence. The delay τ in both cases is $1/2\Delta\nu_{IS}$, where $\Delta\nu_{IS}$ is the chemical shift difference between two spins.

55 evolution results in a mixture of in-phase and anti-phase components which is detected later by switching off the *rf* irradiation. The coupling evolution is monitored point-by-point by incrementing the spin locking interval in several hundred experiments to obtain complete time domain sustained induction 60 decay (SID) signal. Fourier transformation of the SID yields a high resolution 2J - LLC spectrum, with the linewidth $\Delta\nu$ being a measure of the inverse of the lifetime $(\Delta\nu = 1/\pi T_{LLC})$. Alternatively, the SID is fitted to an exponentially decaying function modulated by a cosine with frequency J to obtain the 65 relaxation time of LLC and the coupling constant. Either way the procedure to obtain LLC relaxation time is lengthy, laborious and may contain fitting errors. Also, the method may prove to be inefficient for *in vivo* studies when changes that occur at faster rate than total required experimental time are monitored.

70 Recently, a method for "windowed acquisition" of LLC[7] to determine the lifetime of LLC's in a single scan was proposed. Although it alleviates many of the above mentioned problems, it requires fast sampling hardware in the spectrometer which is not routinely available on many NMR machines. Also, the sensitivity 75 of the method is poor at low sample concentration, as in many *in cell*/*in vivo* studies, due to signal loss during each windowed acquisition cycle.

Therefore, a solution which perfectly works under all experimental conditions is desirable. Along these lines, we report 80 here a new pulse sequence to monitor the relaxation of LLC as an exponentially decaying function free from any type of modulation
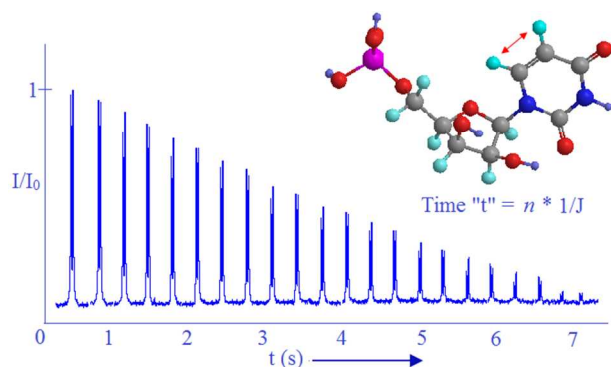
Fig. 2 Pure exponential decay of LLC signal of (0.01M) UMP measured experimentally with the sequence in Fig. 1b. The intensity is normalized with respect to the first data point. The set of experiments took 10 minutes to perform.
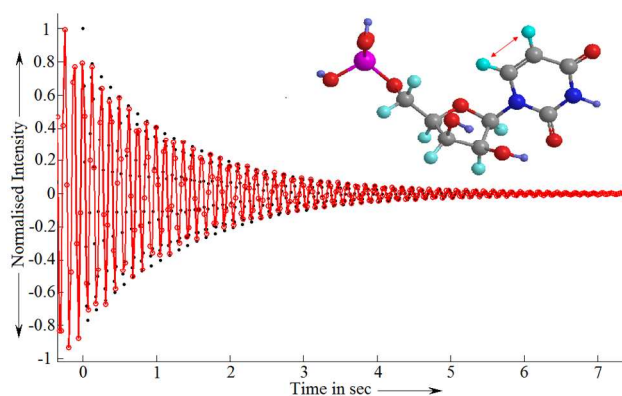

5 **Fig. 3 The normal SID of (0.01M) UMP at 300 MHz. The black dots represents the intensity at particular variable time "τ_r" and the solid red line is the fitting curve of these points. The experimental time required to obtain the complete SID was 300 minutes, 30 times longer than the experiment in Fig. 2.**

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. It employs the recent observation[17] that the J modulation can be refocused in a CPMG experiment by inserting a 90^0 refocusing pulse at the midpoint of a double spin echo ("PROJECT") by planar mixing of coherences. In the same way, the new LLC 15 sequence involves multiple pulses and delays to completely refocus chemical shift while allowing coupling evolution without the accompanying modulation of the SID. The sequence is shown in Fig. 1b. It has two spin echoes separated by a 90^0_y coherence transfer pulse interleaved by four spin lock pulses. The complete 20 product operator description of new LLC sequence, simulations and also the pulse program used are given in ESI†. The method works perfectly for weakly coupled two spin system at Boltzmann equilibrium.

The utility of the new sequence is demonstrated in a weakly 25 coupled two spin-1/2 systems, the nucleotide Uridine-5'-monophosphate (UMP) and 2, 3, 6 tri-chlorobenzaldehyde (TCB). Fig. 2 shows the mono-exponential decay of in-phase LLC peak obtained by using sequence of Fig. 1b, whereas Fig. 3 shows the cosine modulated decay of LLC created among a pair 30 of two coupled protons in nucleotide base of UMP by using sequence in Fig. 1a. Similar decay pattern was observed in the case of TCB. Fig. 2 shows complete refocusing of J modulation

which is reminiscent in normal procedure (as shown in Fig. 3) for determining LLC lifetime. The signal in this method is a pure 35 exponential in contrast with the existing method which has decaying oscillatory signal. Hence, only a few experiments are required which effectively reduces the experimental time from several hours to few minutes.

For TCB and UMP, the LLC lifetime of $5.75 \pm 0.1s$ and $1.41 \pm$ 40 0.02s is obtained in the case of new LLC sequence (using WALTZ-16 for spin locking) by globally fitting the exponentially decaying intensities of both peaks. In comparison LLC lifetimes of $6.15 \pm 0.3s$ and $1.22 \pm 0.03s$ were obtained from the normal LLC sequence. The delay τ in both the cases is $(1/2\Delta\nu_{IS})$ where 45 $\Delta\nu_{IS}$ is the chemical shift difference of two coupled spins and $\tau_r = (1/J)$ is the spin lock time which is divided into four parts as $\tau_r' = (1/4J)$ for sequence in Fig. 1b. As the $J \ll \Delta\nu_{IS}$ for weakly coupled spin system, radiofrequency "*rf*" duty cycle is therefore low. In both cases, the carrier frequency is positioned in 50 the center of both the peaks. Continuous wave (CW) and WALTZ-16 were tested for spin locking with WALTZ-16 outperforming CW.

All the experiments were performed on a 300 MHz Bruker Avance-III spectrometer equipped with a 5mm multinuclear 55 probe at 298K. Sample concentrations were 10mM in D_2O and DMSO-d_6 for UMP and TCB respectively. The data processing was performed off-line using the "curve fitting" tool in MATLAB R2013b (The MathWorks Inc., Natick, MA, 2000).

In summary, we conclude that the new LLC pulse sequence 60 works perfectly for determining the relaxation time of RF-ZQC or LLC at any sample concentration and condition. The technique has been applied to a pair of weakly coupled spins. The effect of strong coupling on the sequence is under investigation. This technique allows the study of slow conformational exchange in 65 large deuterated biomolecules by exciting LLC in one of its non-deuterated molecular component with this improved sequence. Because LLCs are sensitive to random field fluctuations in the immediate environment than any other coherence,[11] we believe that they should be a sensitive probe for various in-cell/in-vivo 70 studies involving molecules having a pair of weakly coupled protons.

Notes and references

*[a]Indian Institute of Technology Delhi, Hauz Khas, New Delhi – 10016* 75 *India. Fax:+91 11 26582037; Tel: +91 11 26591378*
E-mail: nkurur@chemistry.iitd.ac.in

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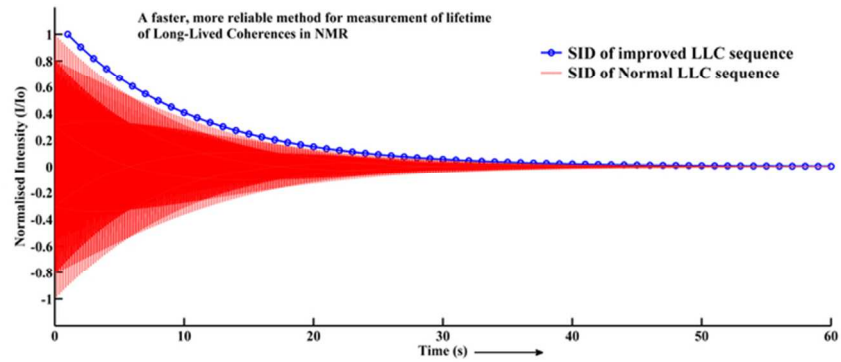
A faster, more reliable method for measurement of lifetime of long-lived coherences in NMR
67x26mm (300 x 300 DPI)