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Mechanisms of coherent re-arrangement for long-lived spin order

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This article is dedicated to Geoffrey Bodenhausen for his 70th birthday.

ABSTRACT

Long-lived spin order-based approaches for magnetic resonance rely on the transition between two magnetic environments of different symmetry, one governed by the magnetic field of the spectrometer and the other where this strong magnetic field is inconsequential. Research on the excitation of magnetic-symmetry transitions in nuclear spins is a scientific field that debuted in Southampton in the years 2000. We advanced in this field carrying the baggage of pre-established directions in NMR spectroscopy. We propose to reveal in this text the part of discoveries that may have been obscured by our choice to only look at them through the experience of such pre-established directions, at the time. Focussing on potential applications, we may have insufficiently emphasised in the manuscripts the methodological developments that necessitated most scientific effort. Such methods developments foster most of the progress in NMR. Thus, we present the contributed mechanisms of translation between the symmetric and non-symmetric environments with respect to the main magnetic field $\boldsymbol{B}_0$, free of any utilitarian perspective. The concept of zero-quantum rotations in the starting blocks of long-lived state populations, magnetisation transfers between hyperpolarised heteronuclei and protons, and selective inversion for long-lived coherences are discussed, as well as hybrid 2D methods based on both insensitive nuclei excitation ('INEPT') and long-lived spin order. We can see at this point that these magnetic wheels will take a longer time than we initially thought to set in motion new applications in studies of slow diffusion, angiography, or large-protein structure. However, these pulse sequences seed subsequent magnetic mechanisms that are sure to contribute to applications. For instance, some of the introduced coherence rotations were combined with classical pulse blocks to obtain 2D

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correlations between protons and heteronuclei. We hope the pulse sequence building blocks discussed herein open further perspectives for magnetic resonance experiments with long-lived spin order.

KEYWORDS

Long-lived states/ Long-lived coherences/ NMR methods

1. Introduction

This paper is an opportunity to present several magnetic resonance concepts free of particular application-specific introductions. This may allow the community to evaluate such concepts for what they are worth simply as magnetisation transfer mechanisms and comment on their potential usefulness in further experiments. We point out that all concepts presented herein were already addressed, albeit concisely, in references (Sarkar, Vasos and Bodenhausen, 2007; Vasos *et al.*, 2009; Ahuja *et al.*, 2010; Sarkar *et al.*, 2010) or in the supporting material of these papers.

Proposing that a presentation free of application-specific introductions for readers with a background in chemistry, biology, or physics may reveal magnetic resonance progress to the fullest implies that the drive for traditional discipline-oriented applications may have obscured part of the concepts in the original papers. A legitimate question is whether these articles would have been accepted by the journal editors without the applications in mind, or whether they would have been worth accepting. With hindsight, doubts raised by whether our work in Lausanne or in Paris brought actual progress for applications were far more severe than any doubts regarding the soundness of the work itself. For instance, in our search for new excitation sequences on the route to long-lived spin states (Carravetta, Johannessen and Levitt, 2004; Pileio, 2020) (LLS), we were never tormented by the question '*is transport of hyperpolarisation really long-lived?*' (Vasos *et al.*, 2009). However, '*is LLS-based polarisation storage in peptides better than the mere longitudinal relaxation time constant of heteronuclei with which peptides are often isotopically enriched, $T_1(^{15}N)$, $T_1(^{13}C)$?*' was a harrowing question. Equally tormenting was the doubt: "*are long-lived states, with their complicated excitation and sustaining mechanism, really a better way of measuring slow diffusion, slow exchange constants than heteronuclei* (Ferrage *et al.*, 2003) *such as ^{15}N?*" or '*are long-lived coherences (LLC's) actually a good route to improved spectral resolution in NMR?*'.

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When we dedicated the first of a series of papers (Sarkar, Vasos and Bodenhausen, 2007) to Anatole Abragam along with a letter expressing our hopes that the discoveries may be useful for diffusion studies, he appeared to ignore most of the letter only to seize the essence of our work in his answer (mainly addressed to Geoffrey Bodenhausen): '*nice to see a way of skillfully sending spins to sleep in their soft bed', 'envoyer les spins se reposer dans leur lit douillet'*. The remark, thus rhythmed by alliteration, was as concise as it was exact, since the singlet state we were searching for is magnetically inactive, i.e., the spins are *'sleeping'*. This commentary alone may have replaced the introduction to our original paper.

2. Zero-quantum rotation in the starting block of long-lived states

The structure of singlet-triplet population differences, or long-lived states operators, Q_{LLS}:

$$Q_{LLS} = |S_0\rangle\langle S_0| - \frac{1}{3}(|T_{-1}\rangle\langle T_{-1}| + |T_0\rangle\langle T_0| + |T_1\rangle\langle T_1|) \quad (1)$$

was first discussed in formulae adapted to the zero-field magnetic structure for a two-spin system, as first created (Carravetta, Johannessen and Levitt, 2004) in non-equivalent nuclei. While the drive to express highly-symmetrical long-lived states in spherical tensor operators is natural, we recurred in Lausanne, however, to Cartesian operators (Sørensen *et al.*, 1984) in the Liouvillian space:

$$Q_{LLS} = -N_{LLS}(I_xS_x + I_yS_y + I_zS_z) \quad (2)$$

with $N_{LLS} = 4/3$.

The more relevant form of this operator, in order to understand the structure of coherences prone to evolution, is:

$$LLS = -\frac{4}{3}ZQ_x - \frac{2}{3}2I_zS_z \quad (3)$$

where $ZQ_x = \frac{1}{2}\left(2I_xS_x + 2I_yS_y\right)$ is a zero-quantum coherence.

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Under such a configuration, the system is immune to the scalar-coupling evolution and also to chemical shift evolution, provided the two spins are rendered identical (Cavanagh *et al.*, 1995). Equation (3) connects the singlet-states area of interest to research in homonuclear and heteronuclear magnetisation transfer, while Eq. (1) proved to be one the most useful formulae in developing the general theory of long-lived states by pointing out the very nature of their extended lifetime: the population imbalance between states or manifolds of different symmetries with respect to spin permutations (Stevanato, 2015, 2020, p. 1) which cannot be interconverted by relaxation mechanisms with certain symmetries.

The first method of excitation for long-lived populations was developed by Levitt and collaborators (Carravetta and Levitt, 2004), yet it only worked for a pair of spins *I,S* at that time with carefully chosen delays, as it was dependent on the chemical shifts, making a sweep through frequencies necessary to excite different pairs of coupled spins (I,S), (R,K)… in different experiments, just like 1D magnetic resonance spectroscopy necessitated a sweep of the main field through resonance conditions for different chemical environments before the introduction of Fourier transform (Ernst and Anderson, 1966; *Richard R. Ernst – Nobel Lecture. NobelPrize.org. Nobel Media AB 2021. Sun. 14 Mar 2021.*, no date). The chemical-shift dependency of long-lived states rendered impossible any 2D investigations of phenomena involving two or more spin pairs or several chemical environments of spin pairs with encoded LLS, such as exchange or interaction dynamics, in the same experiment.

The first concept introduced in the Lausanne paper (Sarkar, Vasos and Bodenhausen, 2007) was the broadband excitation of singlet states. The topic may have deserved, in retrospect, a paper on its own. Our way towards broadband LLS excitation passed through zero-quantum coherences, as explained below. The first attempts to excite Q_{LLS} in Lausanne (Fig. 1) posed challenges regarding the evolution and relative orientation of zero-quantum coherences (ZQ_x, ZQ_y) and ZZ-magnetization ($2I_zS_z$).

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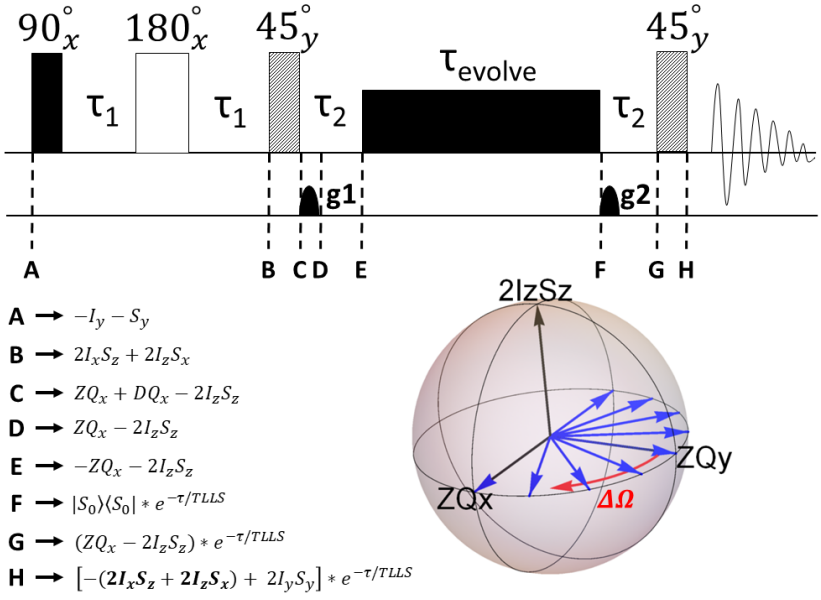

Figure 1. Pulse sequence adapted from reference(Sarkar, Vasos and Bodenhausen, 2007) showing the evolution of the density operator at different stages with an emphasis on the evolution of zero-quantum coherences between stages C and E (figure generated with SpinDynamica (Bengs and Levitt, 2018)).

After the first 45° pulse, at point (C) in Fig. 1, the density operator takes the following expression:

$$\rho_{pre-LLS}^{C} = 2I_xS_x - 2I_zS_z = ZQ_x + DQ_x - 2I_zS_z \quad (4)$$

At first, we expected to indirectly sense the presence of Q_{LLS} at point (C) in this sequence, due to the presence of projections on Q_{LLS}, such contributions being brought by both longitudinal two-spin order and $2I_xS_x$. However, we realized these two contributions exactly annihilate one another, leaving us at a loss on how to excite singlets in a broadband manner. We could have anticipated the mutual cancellation by expressing the operator at point (C) in the singlet-triplet basis, relevant upon application of a 'sustaining' radio-frequency (rf) field:

$$\rho_{pre-LLS}^{C} = -\frac{1}{2}(|T_{-1}\rangle\langle T_{-1}| + |T_1\rangle\langle T_1| - 2|T_0\rangle\langle T_0| - |T_{-1}\rangle\langle T_1| - |T_1\rangle\langle T_{-1}|) \quad (5)$$

indicating there is no singlet order to be found there.

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By applying the pulse field gradient g_1, the double-quantum term dissipates, and the density operator becomes:

$$\rho^{D}_{pre-LLS} = (I_x S_x + I_y S_y) - 2I_z S_z = ZQ_x - 2I_z S_z \quad (6)$$

which possesses all the component of the long-lived state (Eq. (1)), but displays an opposite orientation of zero-quantum and ZZ components with equal projections on Q_{LLS}. Therefore, these projections cancel each other. To better understand this apparent conundrum, we can write the operator in the basis of singlet-triplet operators:

$$\rho^{D}_{pre-LLS} = \frac{1}{2}(|T_0\rangle\langle T_0| - |S_0\rangle\langle S_0|) - \frac{1}{2}(|T_{-1}\rangle\langle T_{-1}| + |T_1\rangle\langle T_1| - |T_0\rangle\langle T_0| - |S_0\rangle\langle S_0|) =$$
$$\frac{1}{2}(2|T_0\rangle\langle T_0| - |T_{-1}\rangle\langle T_{-1}| - |T_1\rangle\langle T_1|) \quad (7)$$

The next step was figuring out how to interchange the singlet and central triplet populations in order to get the expression for *LLS*, a task which is not immediately obvious in this form. To do that, a reconversion in the cartesian product basis proved fruitful: $|T_0\rangle\langle T_0| - |S_0\rangle\langle S_0| = I_x S_x + I_y S_y = ZQ_x$. After several weeks of calculations, a group seminar was dedicated to the otherwise well-known evolution of ZQ_x under a scalar coupling interaction (Cavanagh *et al.*, 1995). The new aspect was that the rotation axis was this time also apparent in the density operator expression, so effectively one of the constituents of spin order was rotating around the other, thus changing the chirality of the magnetic system and yielding the sought-after Q_{LLS} (Fig. 2). As a side note, these physical exercises of magnetisation succeeded to captivate more attention on paperback than in the coffee-table setting around a group-meeting whiteboard. However, the atmosphere in the magnetic resonance laboratory in Lausanne should be credited for a substantial contribution to the birth of these concepts.

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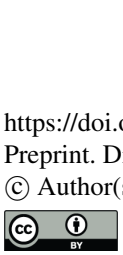

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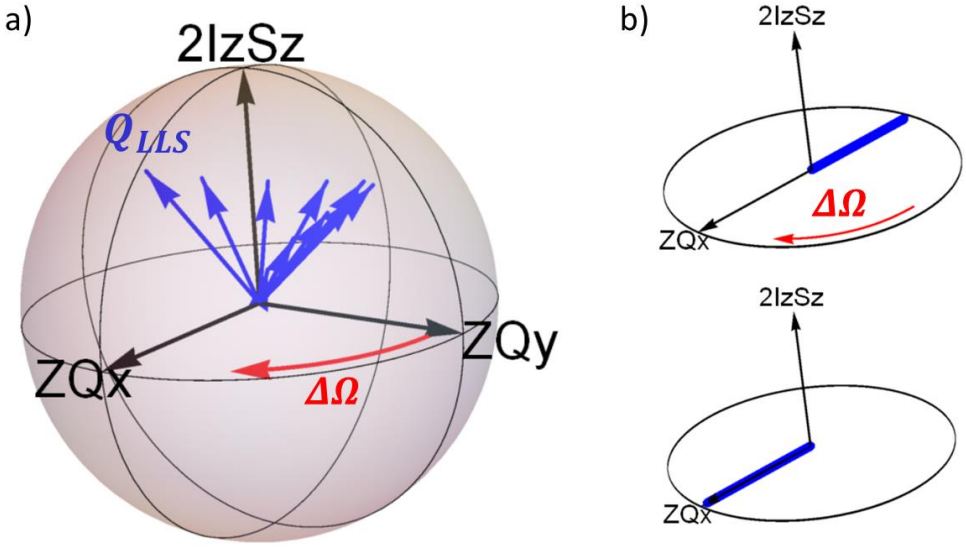

Figure 2. a) Increase in the spin-order projection onto Q_{LLS} as the ZQ_x changes sign during the time interval $\tau_2 = 1/(2\Delta\upsilon)$; b) Magnetisation rotation around longitudinal two-spin order, $2I_zS_z$, to yield non-null projection on Q_{LLS}, changing triplet-triplet into singlet-triplet population differences.

The evolution of the ZQ_x during free precession is:

$$ZQ_x \xrightarrow{(\Omega_1 I_z + \Omega_2 S_z + 2\pi J * I_z S_z)t} ZQ_x \cos \Delta\Omega t + ZQ_x \sin \Delta\Omega t \quad (8)$$

where $\Omega_1$is the Larmor pulsation of spin *I*, $\Omega_2$is the Larmor pulsation of spin *S*, $\Delta\Omega = \Omega_1 - \Omega_2 = 2\pi\Delta\upsilon$ is the chemical shift difference (in terms of angular frequency) between the two spins and *J* is the scalar coupling constant between spins *I* and *S*. Thus, after an evolution period $\tau_2 = 1/(2\Delta\upsilon)$, where $\Delta\upsilon = \frac{\Delta\Omega}{2\pi}$, the ZQ_x will change sign such that $\frac{1}{2}(|T_0\rangle\langle T_0| - |S_0\rangle\langle S_0|) \xrightarrow{(\Omega_1 I_z + \Omega_2 S_z + 2\pi J * I_z S_z)t} \frac{1}{2}(|S_0\rangle\langle S_0| - |T_0\rangle\langle T_0|)$ and the density operator will be:

$$\rho_{LLS}^{E} = |S_0\rangle\langle S_0| - \frac{1}{2}(|T_{-1}\rangle\langle T_{-1}| + |T_1\rangle\langle T_1|) \quad (9)$$

Juggling with operators in order to drive the spin system in its '*soft bed*' we realized we should always look at Nature from various perspectives. We learned that if only one of the longitudinal

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two-spin order and zero-quantum components could be selected at time point (C) (Fig. 1), Q_{LLS} would have been present already. For instance, ZZ-magnetization alone projects on the long-lived state, given that $2I_zS_z = \frac{1}{2}(|T_{-1}\rangle\langle T_{-1}| + |T_1\rangle\langle T_1| - |T_0\rangle\langle T_0| - |S_0\rangle\langle S_0|)$. Thus, during the sustaining period, only the singlet population will survive for a period much longer than longitudinal magnetization. In order to do so, we employed a Thrippleton-Keeler (Thrippleton and Keeler, 2003) filter to wipe out the troubling zero and double quantum coherences and obtained a singlet state with an amplitude two-times lower than using both zero-quantum and ZZ-magnetization. Other groups employed the so-called "pseudo singlet order" (Pileio, 2017) which is just $ZQ_x = \frac{1}{2}(|T_0\rangle\langle T_0| - |S_0\rangle\langle S_0|)$ as the source for long-lived state obtaining similar results.

Though broadband excitation of singlet states would have deserved publication as a discovery in its own right, we were cautious to avoid publication of our research in slices of 'salami science' (Sweedler, 2019). However, this advance proved relevant for the advancement of long-lived state order (Pileio, 2017, 2020; Bengs *et al.*, 2020; Teleanu, Sadet and Vasos, 2021) and was more challenging to obtain than the 2D spectroscopy application for the study of singlet-state-based exchange we describe in the same paper (Sarkar, Vasos and Bodenhausen, 2007) (SS-EXSY). In the tradition of finding low-key names for sequences such as 'INEPT' (Morris and Freeman, 1979) or 'INADEQUATE' (Bax, Freeman and Kempsell, 1980), we could have named the zero-quantum rotation block of the pulse sequence in Fig. 1 a '(ZZ-)ZEROTATION'.

3. Heteronuclei or proton long-lived states for conserving hyperpolarisation

In order to maximise the magnetisation lifetime, heteronuclear longitudinal spin-order (mainly on carbon-13) can be excited and used during evolution periods in both room-temperature (Bermel, Bertini, Duma, *et al.*, 2005; Richter *et al.*, 2010) and hyperpolarised NMR. We strived to also preserve hyperpolarization on a pair of hydrogens entwined in a long-lived state (Vasos *et al.*, 2009).

Since the invention of dissolution-Dynamic Nuclear Polarisation (dissolution-DNP) (Ardenkjær-Larsen *et al.*, 2003; Comment *et al.*, 2008; Balzan *et al.*, 2016) and its development in Lausanne by the team of A. Comment, S. Jannin, and J. van der Klink in the Functional Imaging Laboratory at EPFL (Ardenkjær-Larsen *et al.*, 2003; Comment *et al.*, 2008; Balzan *et al.*, 2016), the topic was

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associated with our research due to its conjunction with long-lived spin order (Ardenkjær-Larsen *et al.*, 2003; Comment *et al.*, 2008; Balzan *et al.*, 2016). The preservation of hyperpolarised magnetisation obtained by dissolution-DNP in long-lived states raised fewer challenges than the comparison of LLS with heteronuclear lifetimes in terms of performance as polarisation batteries. The hyperpolarised magnetisation in samples stemming from a polarizer such as the one developed in Lausanne and the similar one installed as the first dissolution-DNP system in France (Ardenkjær-Larsen *et al.*, 2003; Comment *et al.*, 2008; Balzan *et al.*, 2016) followed intently the rf pulses in our high-resolution magnets. Inhomogeneities due to fast dissolution and injection could be tamed to run pulse sequences for LLS excitation and decoding and observe the signal. To us, the main issue remained that the benefits of a procedure consisting of Q_{LLS} excitation on protons compared to simply preserving hyperpolarised magnetisation in heteronuclei had to be carefully considered. We scrutinised this issue in terms of magnetisation lifetimes in the given conditions (room temperature, molecular size) as well as in the case of extreme molecular sizes or crowding of the environment and came to the conclusion that proton-based LLS were valuable for storing magnetisation even when the molecules contained isotope-enriched heteronuclei like ^{15}N or ^{13}C. In this analysis, we were inspired by similar comparisons between proton and heteronuclear magnetisation carried out for relaxation rates of heteronuclei and protons in large or paramagnetic proteins (Bermel, Bertini, Felli, *et al.*, 2005).

A particularity surely due in large part to the interest of the research field, more than to our particular research skills, was that we hardly underwent any uphill sisyphean battles to publish papers (Molinié and Bodenhausen, 2013; *The Myth of Sisyphus - Wikiwand*). Journal editors believed in these developments as sincerely as we did. The only significant delays in publication were incurred, paradoxically, for a paper submitted directly to a specialised journal (Balzan *et al.*, 2017), since the paper was one of the first obtained with our freshly-installed DNP system in France. The reason may have been that, since hyperpolarisation lifetimes showed we had a kinship with time, we could afford to wait.

Potential applications of symmetry-adapted states as magnetisation reservoirs for various 2D experiments led us to investigate the polarization transfer from long-lived state of protons towards heteronuclei across proteins' backbones (Fig. 3) (Laetitia Fernandes *et al.*, 2013; L. Fernandes *et al.*, 2013). The particular relaxation rates of LLS reflect conformational exchange and act as probes for unravelling protein's inner dynamics, while 2D correlations (e.g., ^{1}H-^{15}N) help disentangle

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246 complex spectra, which is acutely needed in the analysis of unfolded or intrinsically disordered
247 proteins. The field of 2D heteronuclear experiments using long-lived spin order (Fig. 3) is likely
248 to develop further. As the field advances, long-lived states-based explorations of inter-molecular
249 (Bornet *et al.*, 2011; Stavarache *et al.*, 2017) and intra-molecular (Ahuja *et al.*, 2007; Tayler *et al.*,
250 2010) interactions become important, and so becomes spectral resolution for the study of systems
251 of increasing complexity.
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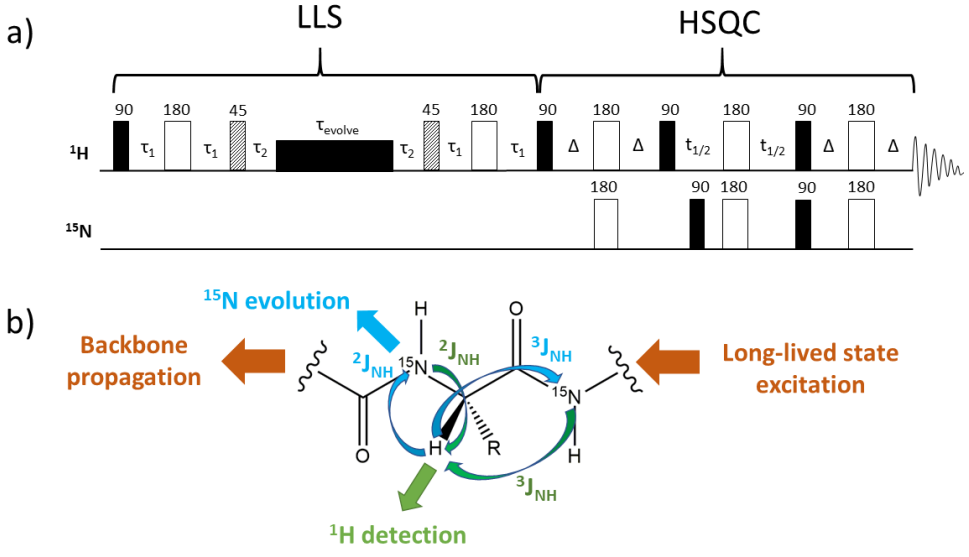

254

255 Figure 3. a) Pulse sequence designed to generate a two-dimensional correlation spectrum between
256 ^{1}H and ^{15}N via scalar coupling of the type $^{2}J_{NH}$ and $^{3}J_{NH}$ starting from a proton long-lived state. b)
257 Schematic representation of polarization transfer along protein's backbone which generates a two-
258 dimensional correlation spectrum via the $^{2}J_{NH}$ and $^{3}J_{NH}$ coupling constants. Protons are excited via
259 the first part of the pulse sequence from (A) into a long-lived state spin order. Figure adapted from
260 reference (Fernandes *et al.*, 2013).
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4. Long-lived coherences, eppur' si muove

Aware of the potential of long magnetisation lifetimes for line-narrowing in NMR, we strived to obtain some type of magnetisation akin to LLS to rotate. Long lifetimes of magnetisation had been traded for spectral resolution, e.g., for ^{1}H-^{15}N pairs in cross-correlated relaxation experiments and for ^{15}N heteronuclei for narrowing spectroscopic lines (Goldman, 1984; Pervushin *et al.*, 1997; Vasos *et al.*, 2006).

Our search for 'moving' long-lived configurations first involved complicated coherences in alanine, serine, and other molecules with up to five coupled spins (Ahuja *et al.*, 2009). When we finally resorted to our favourite paired Gly aliphatic protons of AlaGly, the *Ix-Sx* configuration was deduced from the diagonalized Liouvillian (Carravetta and Levitt, 2005). The next hurdle appeared in fitting the exotic long-lived states with products of oscillating and decaying functions and translating them to signals in 2D spectra via Fourier transform-adapted spectroscopy (prior experience with non-conventional heteronuclear 2D experiments (Bertini *et al.*, 2004; Vasos, Hall and Fushman, 2005) helped at this point). The simple scheme involving a 180 deg pulser for creating a Q_{LLC} observable in the indirect dimension of a 2D experiment, where:

$$Q_{LLC} = (I_x - S_x)\,cos(\pi Jt) + (2I_yS_z - 2I_zS_y)\,sin(\pi Jt) \qquad (10)$$

was further refined by different approaches (Fig. 4), of which the most advanced itches coherences with spins pointing in opposite ways in molecules with almost-equivalent nuclei (Sheberstov *et al.*, 2019).

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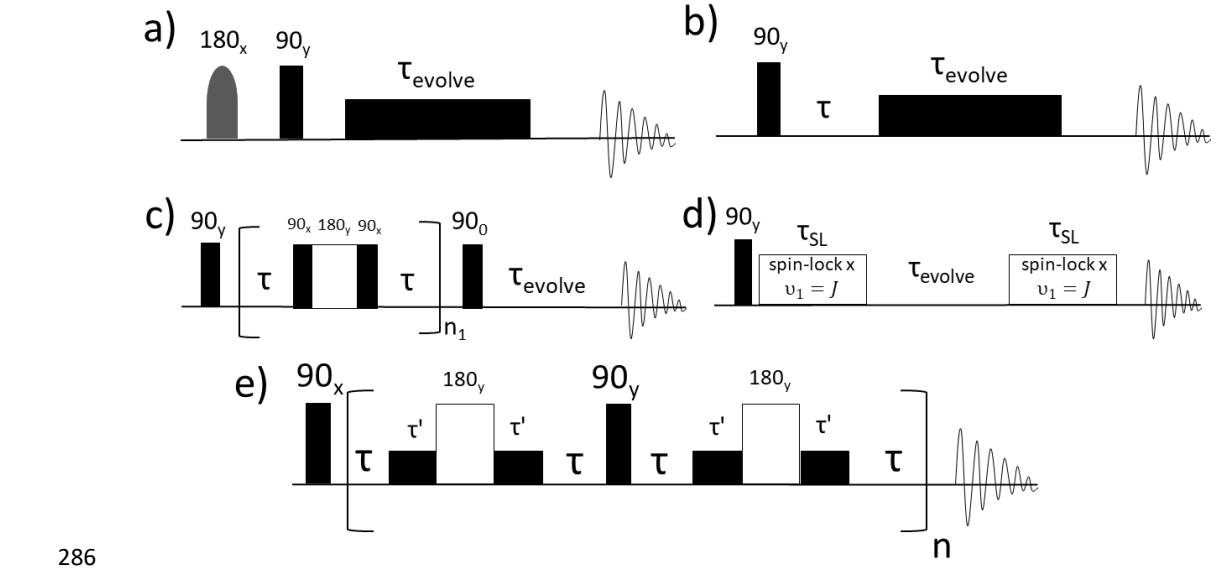

Figure 4. Experimental methods known so far for converting thermal equilibrium nuclear spin polarization into long-lived coherences. a) The first pulse sequence used to excite LLC, with a selective π-pulse followed by a non-selective π/2-pulse and spin-lock (Sarkar *et al.*, 2010); b) a similar pulse sequence, but the relative orientation of spin angular momentum operators is given by an evolution period under the effect of different chemical shifts ($\tau = 1/2\Delta\upsilon$) ; c) First half of the M2S pulse sequences, with trains of pulses whose repetition number n_1 is optimized as a function of scalar coupling and chemical shift difference (Tayler and Levitt, 2011); d) SLIC pulse sequence that also excite LLC during the spin-lock with amplitude equal to the scalar coupling between the two nuclei[5] (sequences c and d are particularly adapted for molecules with 'quasi-equivalent' spins, i.e., where differences in chemical shifts between the spins involved are less than the J-couplings between these spins); e) Improved LLC pulse sequence with a refocussing π/2-pulse train that allows for a better determination of the coherence's lifetime ($\tau = 1/2\Delta\upsilon$; $\tau' = 1/4J$) (Singh and Kurur, 2015).

When we sought for long-lived coherences in high fields, we were not aware yet that the contemporary work on extremely low-frequency oscillations (Pileio, Carravetta and Levitt, 2009) in low magnetic fields developed at Southampton involved, practically, the same operators.

Just as in the case of LLS for hyperpolarisation safekeeping was compared to heteronuclear storage, once LLC's were developed comparisons with zero-quantum coherences came to mind.

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For the case of two *J*-coupled, non-identical spins, *I* and *S*, the evolution of $\rho_1(0) = I_x - S_x$ and $\rho_{ZQx}(0) = I_xS_x + I_yS_y$ during free-precession (without any radio-frequency 'sustaining' applied) is given by:

$$\rho_1(t) = [(I_x - S_x)\cos(\pi Jt) + (2I_yS_z - 2I_zS_y)\sin(\pi Jt)]\cos(\tfrac{\Delta\Omega t}{2}) - [(I_y + S_y)\cos(\pi Jt) - (2I_xS_z + 2I_zS_x)\sin(\pi Jt)]\ \sin(\tfrac{\Delta\Omega t}{2}) \quad (11)$$

$$\rho_{ZQx}(t) = (I_xS_x + I_yS_y)\cos(\Delta\Omega t) + (I_yS_x - I_xS_y)\sin(\Delta\Omega t) = ZQx\cos(\Delta\Omega t) + ZQy\sin(\Delta\Omega t) \quad (12)$$

while in presence of rf fields with the carrier placed at the middle of their offsets ($\Omega_1 = -\Delta\Omega/2$ and $\Omega_2 = \Delta\Omega/2$) and an amplitude $\omega >> \Delta\Omega$, the evolutions for the LLC and ZQ_x are:

$$\rho_{LLC}(t) = (I_x - S_x)\cos(\pi Jt) + (I_yS_z - I_zS_y)\sin(\pi Jt) \quad (13)$$

$$\rho_{ZQx}(t) = \tfrac{1}{J^2+16\omega^2}\Big\{(I_xS_x)(J^2 + 16\omega^2) + (I_yS_y)\left(8\omega^2\cos\left(\pi t\sqrt{J^2 + 16\omega^2}\right) + J^2 + 8\omega^2\right) + \omega\Big[J * I_x\left(\cos\left(\pi t\sqrt{J^2 + 16\omega^2}\right) - 1\right) + J * S_x\left(\cos\left(\pi t\sqrt{J^2 + 16\omega^2}\right) - 1\right) + 2\left(\sqrt{J^2 + 16\omega^2}(I_yS_z + I_zS_y)\sin\left(\pi t\sqrt{J^2 + 16\omega^2}\right) + 8\omega(I_zS_z)\sin^2\left(\tfrac{1}{2}\pi t\sqrt{J^2 + 16\omega^2}\right)\right)\Big]\Big\} \quad (14)$$

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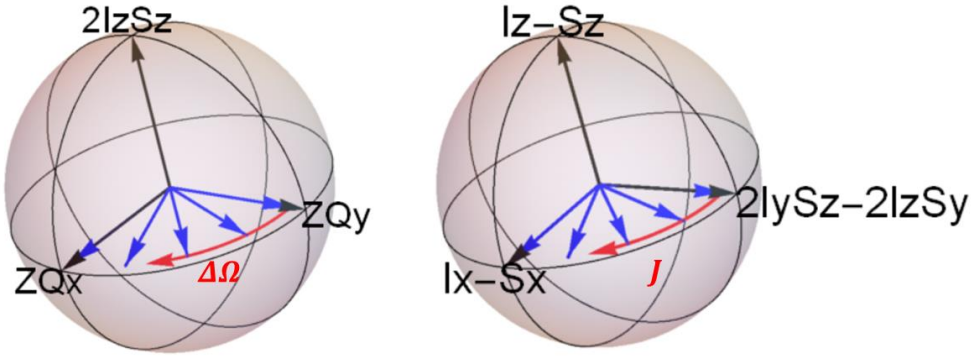

Figure 5. Evolution of zero-quantum coherences during free precession with an oscillating frequency of $\Delta\Omega$ and evolution of long-lived coherences during sustaining period with an oscillating frequency equal to the scalar coupling constant *J*.

In the absence of sustaining rf fields, differences of single-quantum transverse coherences, the source of LLC's, evolve under the chemical shift difference and the scalar coupling, while *ZQ* are immune to the latter (Cavanagh *et al.*, 1995). In the second scenario, LLC's evolve only under the effect of *J*-coupling (Sarkar *et al.*, 2010), oscillating between in-phase ($I_x - S_x$) and anti-phase ($2I_yS_z - 2I_zS_y$), with the coherence order equal to 1. Thus, the scalar coupling evolution sets LLC's aside from ZQ's (Fig. 5).

Broadband excitation of LLC's in molecules with broadly different *J*-couplings and chemical shifts is still a challenge, despite the progress. We explored part of the territory by exciting with a series of selective 180 degrees pulses (Sarkar *et al.*, 2011) and by sustaining with various pulse trains (Sadet *et al.*, 2014), but we can safely say that LLC's benefit from their simple and parametric-free excitation scheme which consists of a selective π pulse and non-selective $\pi/2$ hard pulse followed by spin-lock.

Conclusions

We present the challenging aspects of the mechanisms of zero-quantum inversion with respect to longitudinal two-spin order in homonuclear spin systems, of ^{1}H long-lived states for conserving hyperpolarised magnetisation, and of generating and sustaining entwined spins pointing opposite

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ways for long-lived coherences. We hope that these concepts, as explained herein, may find use as building blocks in different applications.

Author contributions

F.T and P.V performed the theoretical investigations and wrote the paper.

Competing interests

The authors declare that they have no conflict of interest.

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