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 symmetries, 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 herein 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. The methodological developments that emphasised herein are the mechanisms of translation between the symmetric and non-symmetric environments with respect to the main magnetic field $\boldsymbol{B}_0$. More specifically, we look again thoroughly at zero-quantum rotations in the starting blocks of long-lived state populations, magnetisation transfers between hyperpolarised heteronuclei and protons. These pulse sequences seed subsequent magnetic mechanisms that contribute to further applications. For instance, we show how some of the introduced coherence rotations were combined with classical pulse blocks to obtain 2D 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 to 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(Ahuja et al., 2010; Sarkar et al., 2007, 2010; P. R. Vasos et al., 2009) or in the supporting material of these papers.

Proposing that a presentation free of application-specific introductions 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 editors whether our work in Lausanne and Paris brought actual progress for applications were far more severe than any doubts regarding the soundness of the work itself. For instance, in the search for new singlet-based excitation sequences (Marina Carravetta et al., 2004) on the route of hyperpolarised magnetisation to long-lived spin states ; (LLS), we were never tormented by the question '*is transport of hyperpolarisation really long-lived?*'(P. R. Vasos et al., 2009) (Pileio, 2020). 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 present 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?*'.

When we dedicated the first of a series of papers(Sarkar et al., 2007) to Anatole Abragam along with a letter expressing our hopes that the discoveries may be useful for diffusion studies, he seized 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(Marina Carravetta et al., 2004) in non-equivalent nuclei. While the preference of writing 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 form of this operator allowed us to understand the structure of coherences prone to evolution:

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

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

Under such a configuration, the system is immune to the scalar-coupling evolution and also to chemical shift evolution, provided the chemical shift difference between the two spins is eclipsed by ample radio-frequency radiation or by cycling the main field (Cavanagh et al., 1995).

Equation (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) which cannot be interconverted by relaxation mechanisms with certain symmetries. The novelty of Equation (3) was that it strongly connected the singlet-states explorations to research in the I. Solomon - consecrated (I,S) homonuclear and heteronuclear magnetisation transfer(Solomon, 1955).

The first method of excitation for long-lived populations, developed by Levitt and collaborators(Marina Carravetta & Levitt, 2004), worked for a pair of spins *I*,*S* provided carefully-chosen delays dependent on the chemical shifts, ν_I and ν_S, were used, 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 & Anderson, 1966; *Richard R. Ernst – Nobel Lecture.*, n.d.). 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 et al., 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 (Figure 1) posed challenges regarding the evolution and relative orientation of zero-quantum coherences (ZQ_x, ZQ_y) and ZZ-magnetization ($2I_zS_z$).

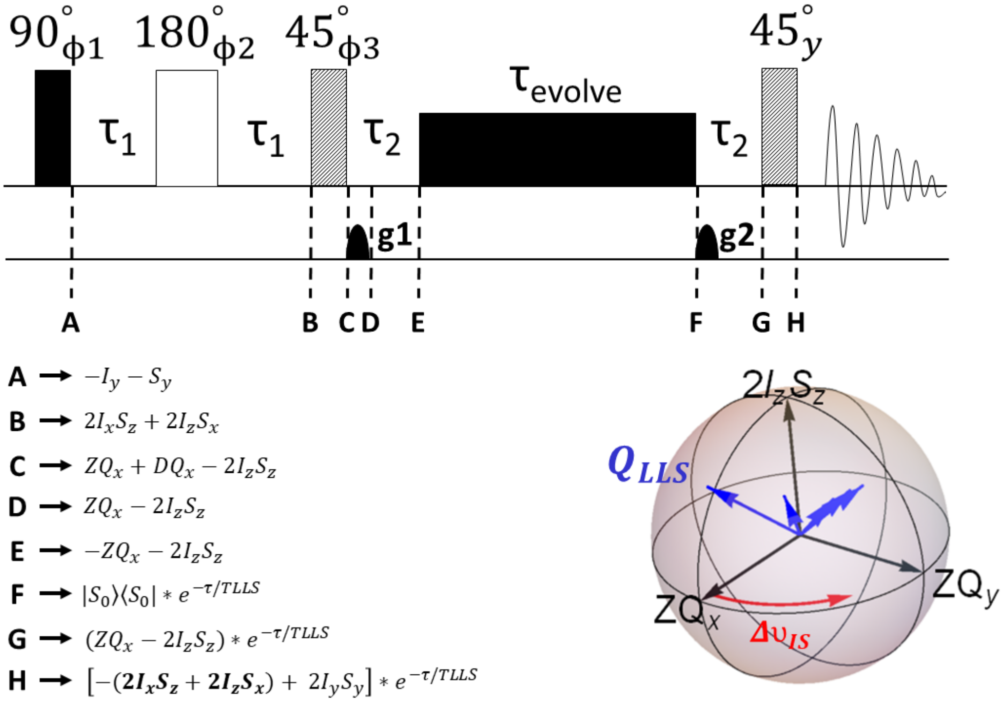

Figure 1. Pulse sequence adapted from reference(Sarkar et al., 2007) showing the evolution of the density operator at different stages with an emphasis on its three projections ($ZQ_x, ZQ_y, 2I_zS_z$)between time points C and E (figure generated with SpinDynamica(Bengs & Levitt, 2018)). Here, $\tau_1 = 1/(4 \cdot J_{IS})$ and $\tau_2 = 1/(2 \cdot \Delta\nu_{IS})$ where J_{IS} is the scalar coupling constant (Hz) between spins I and S, $\Delta\nu_{IS} = \nu_I - \nu_S$ is the chemical shift difference (Hz) between the two spins. The phase cycling is $\phi 1 = (x, -x)$, $\phi 2 = x$, $\phi 3 = 2(y), 2(-y)$ and $\phi rec = (x, -x, -x, x)$.

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

$$\rho^{C}_{pre-LLS} = 2I_xS_x - 2I_zS_z = ZQ_x + DQ_x - 2I_zS_z \qquad (4)$$

At first, we expected to induce the presence of Q_{LLS} at point (C) in this sequence, due to the presence of projections on Q_{LLS} 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:

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

indicating there was no singlet order to be found there.

By applying the pulsed field gradient *g₁*, the double-quantum term dissipates, and the density operator becomes:

$$\rho^{D}_{pre-LLS} = (I_xS_x + I_yS_y) - 2I_zS_z = ZQ_x - 2I_zS_z \qquad (6)$$

which possesses all the component of the long-lived state (Equation 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 relative sign of the constituent product operators ZQ_x and $2I_zS_z$ and yielding the sought-after *LLS* (Figure 2). The atmosphere in the magnetic resonance laboratory in Lausanne should be credited for a substantial contribution to the birth of these concepts. However, as a side note, the physical exercises of magnetisation succeeded to captivate more attention on paperback than in the coffee-table setting around a group-meeting whiteboard.

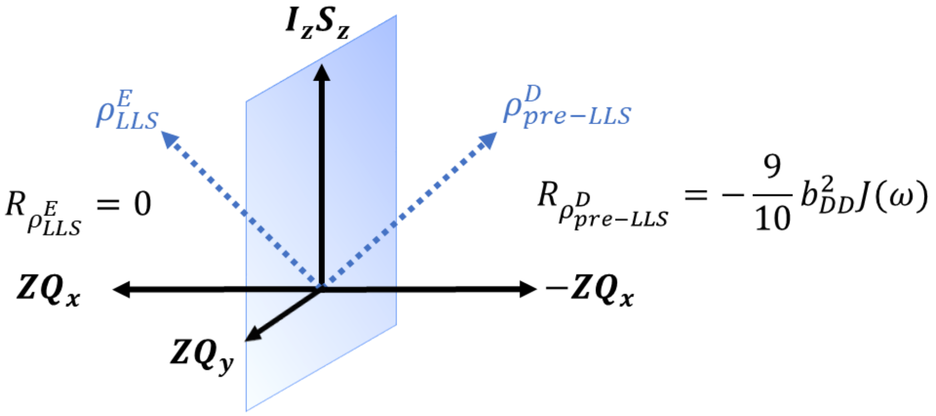

Figure 2. Position of coherence prior to and after zero-quantum rotation with auto-relaxation rate constant corresponding to the density operator given by the two linear combinations of $ZQ_x$and I_zS_z. Only the dipolar relaxation mechanism was considered for a pair of two spins where b_{DD} is the dipolar coupling constant and $J(\omega)$ is the spectral density

The evolution of the ZQx during free precession is:

$$ZQ_x \xrightarrow{(2\pi\nu_I I_z + 2\pi\nu_S S_z)t} ZQ_x \, cos \, 2\pi\Delta\nu_{IS}t + ZQ_y \, sin \, 2\pi\Delta\nu_{IS}t \qquad (8)$$

where ν_I is the Larmor frequency of spin I, ν_S is the Larmor frequency of spin S, $\Delta\nu_{IS} = \nu_I - \nu_S$ is the chemical shift difference (Hz) between the two spins and J_{IS} is the scalar coupling constant (Hz) between spins I and S. Thus, after an evolution period $\tau_2 = 1/(2\Delta\upsilon)$,, the ZQ_x will change sign such that $\frac{1}{2}(|T_0\rangle\langle T_0| - |S_0\rangle\langle S_0|) \xrightarrow{(2\pi\nu_I I_z + 2\pi\nu_S S_z)t} \frac{1}{2}(|S_0\rangle\langle S_0| - |T_0\rangle\langle T_0|)$. During the evolution represented in the coherence clock in Figure 2, the right-side hand in the first quadrant is transformed into its symmetric image on the other side of the $(2I_zS_z, ZQ_y)$ plane. The initial $\rho^D_{pre-LLS}$ and its plane-symmetric image ρ^E_{LLS} have quite different relaxation rates triggered by different symmetry rules. The density operator evolves into:

$$\rho^E_{LLS} = |S_0\rangle\langle S_0| - \frac{1}{2}(|T_{-1}\rangle\langle T_{-1}| + |T_1\rangle\langle T_1|) \qquad (9)$$

An note can be made on the time dependence of the relaxation rate constant for the case of free precession of the LLS. Given the evolution of zero-quantum coherences under the chemical shift difference, the density operator, starting from $ZQ_x + I_zS_z$, will have an oscillatory evolution between the two ZQ_x and ZQ_y as:

$$ZQ_x + I_zS_z \xrightarrow{(2\pi\nu_I I_z + 2\pi\nu_S S_z)t} ZQ_x \, cos \, 2\pi\Delta\nu_{IS}t + ZQ_y \, sin \, 2\pi\Delta\nu_{IS}t + I_zS_z \qquad (10)$$

Considering a relaxation superoperator only for the dipolar interaction between two coupled spins:

$$\hat{\Gamma} = -\frac{6}{5} b_{DD}^2 \sum_{m=-2}^{2} (-1)^m J(m * \omega)[T_{2,m}[T_{2,-m}\_]] \qquad (11)$$

where b_{DD} is the dipolar coupling constant, $J(\omega)$ is the spectral density and $T_{2,m}$ are the spherical tensor spin operators of rank 2, the computed relaxation rate constant for the density operator $ZQ_x \, cos \, 2\pi\Delta\nu_{IS}t + ZQ_y \, sin \, 2\pi\Delta\nu_{IS}t + I_zS_z$ is

$$R(t) = -\frac{6}{5} b_{DD}^{2}((1 + \cos 2\pi\Delta\nu_{IS}t) \cdot J(0) + 3 \cdot J(\omega)) \cdot (\sin\frac{2\pi\Delta\nu_{IS}t}{2})^{2} \tag{12}$$

Thus, only for $t = \frac{2n}{2\pi\Delta\nu_{IS}}$ the relaxation rate constant is null.

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 two-spin order and zero-quantum components could be selected at time point (C) (Figure 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 & 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(Bengs et al., 2020; Pileio, 2017, 2020; Teleanu et al., 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 et al., 2007) (SS-EXSY). In the tradition of finding low-key names for sequences such as 'INEPT'(Morris & Freeman, 1979) or 'INADEQUATE'(Bax et al., 1980), we could have named the zero-quantum rotation block of the pulse sequence in Figure 1 a '(ZZ-)ZEROTATION'.

As singlet-based applications ambition to store magnetisation for ever longer time periods, the most adapted systems to this purpose, quasi-equivalent spin pairs with *J*-couplings far overweighing the differences between the chemical shits of the components, became increasingly studied. Spin dynamics that shift the magnetisation of the two spins differentially to create the singlet state are particularly challenging in such systems.

Pulse sequences of particular interest include the Magnetization-to-Singlet(Tayler & Levitt, 2011a) and Spin-Lock Induced Crossover(DeVience et al., 2013). These methods are suited for strongly-coupled spins where the scalar coupling is larger than the chemical shift difference, while the pulse sequence described in Figure 2, which we identify herein as “$ZZ+ZQ_X$” (Sarkar et al., 2007), performs better in terms of long-lived states excitation in the weakly-coupled regime. Several attempts to efficiently excite singlet order on broader domains of coupling regimes have been recently devised(Bengs et al., 2020; Mamone et al., 2020). Figure 3 depicts numerical simulations performed with SpinDynamica(Bengs & Levitt, 2018) for singlet population excitation using the aforementioned pulse sequences for both weakly and strongly coupled regime outlining the difference in excitation efficiency.

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(P. R. Vasos et al., 2009). Since the invention of dissolution-Dynamic Nuclear Polarisation (dissolution-DNP)(Ardenkjær-Larsen et al., 2003; Balzan et al., 2016; Comment et al., 2008) 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; Balzan et al., 2016; Comment et al., 2008), the topic was associated with our research due to its conjunction with long-lived spin order(Ardenkjær-Larsen et al., 2003; Balzan et al., 2016; Comment et al., 2008).

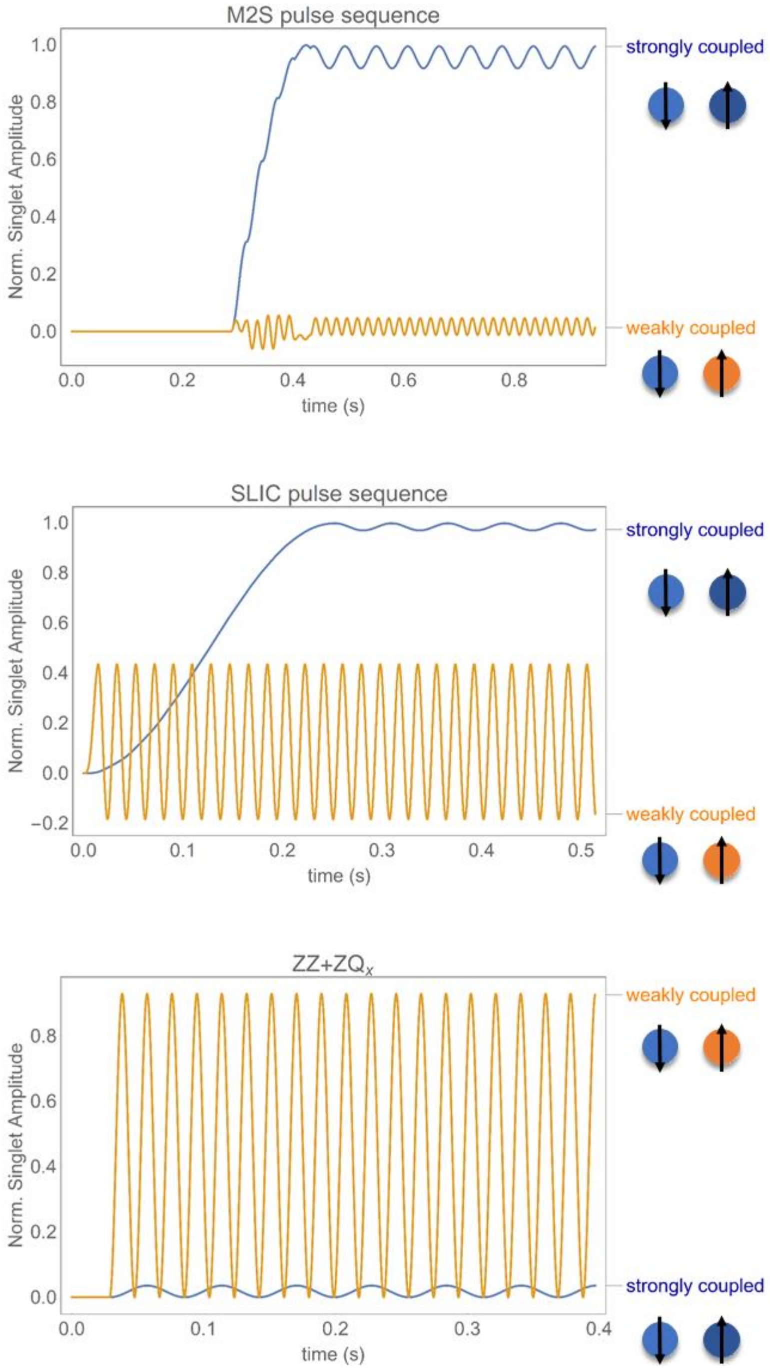

Figure 3. Numerical simulations using SpinDynamica outlining the efficiency of singlet polarisation for M2S, SLIC and ZZ+ZQ$_x$ pulse sequences in a two-spin system (by projecting the density operator during the pulse sequence onto the singlet population). The weakly-coupled system is described by $\{\Delta\nu_{IS} = 50\ Hz; J_{IS} = 17.4\ Hz\}$, while the strongly coupled system features $\{\Delta\nu_{IS} = 2.8\ Hz; J_{IS} = 17.4\ Hz\}$. For each scenario, only the coherent evolution was considered in simulations (no relaxation dampening of the amplitude of source operators or LLS is taken into account). The excitation period is followed by a free-precession evolution after the maximum amplitude for the singlet population was reached.

The preservation of hyperpolarised magnetisation obtained by dissolution-DNP in long-lived states raised fewer challenges than the comparison of the 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; Balzan et al., 2016; Comment et al., 2008) 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 & Bodenhausen, 2013; *The Myth of Sisyphus - Wikiwand*, n.d.). 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 specialized 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 (Figure 4)(L. Fernandes et al., 2013; Laetitia 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 complex spectra, which is acutely needed in the analysis of unfolded or intrinsically disordered proteins. The field of 2D heteronuclear experiments using long-lived spin order (Figure

4) is likely to develop further. As the field advances, long-lived states-based explorations of inter-molecular(Bornet et al., 2011; Stavarache et al., 2017) and intra-molecular(Ahuja et al., 2007; Tayler et al., 2010) interactions become important, and so becomes spectral resolution for the study of systems of increasing complexity.

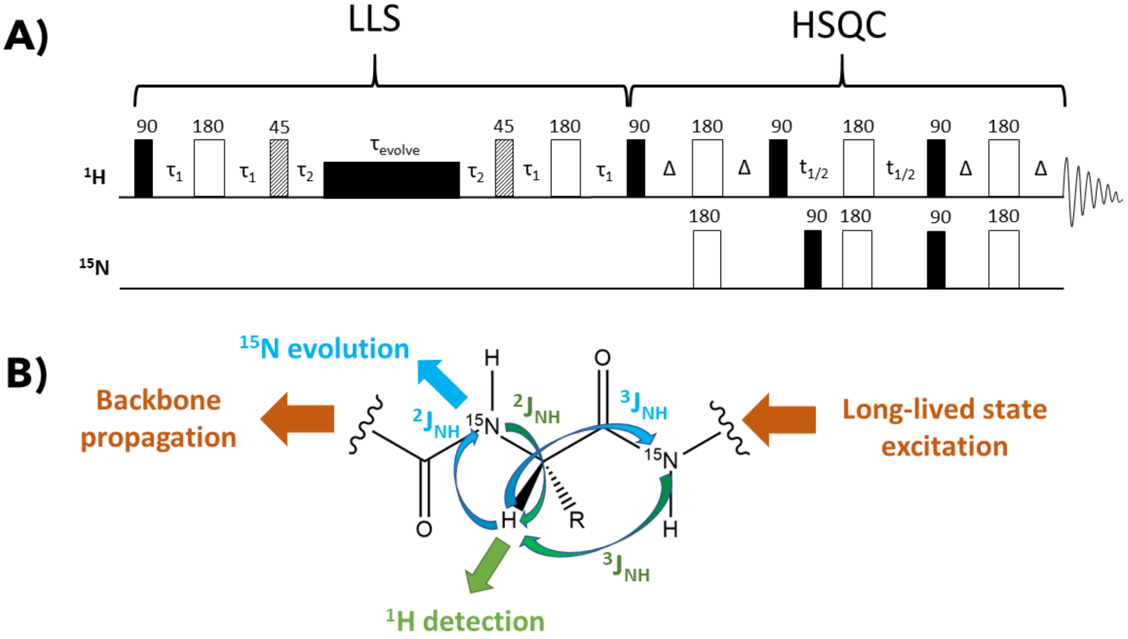

Figure 4. A) Pulse sequence designed to generate a two-dimensional correlation spectrum between ^{1}H and ^{15}N via scalar coupling of the type $^2J_{NH}$ and $^3J_{NH}$ starting from a proton long-lived state. B) Schematic representation of polarization transfer along protein's backbone which generates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

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; Paul R. 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 favorite paired Gly aliphatic protons of AlaGly, the *Ix*-*Sx* configuration was deduced from the diagonalized Liouvillian(M. Carravetta & 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 non-conventional heteronuclear 2D experiments(Bertini et al., 2004; P.R. Vasos et al., 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:

$$Q_{LLC} = (I_x - S_x)\, cos(2\pi J_{IS} t) + (2I_y S_z - 2I_z S_y)\, sin(2\pi J_{IS} t) \qquad (13)$$

was further refined by different approaches (Figure S1), of which the most advanced itches coherences with spins pointing in opposite ways in molecules with almost-equivalent nuclei(Sheberstov et al., 2019).

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

$$\rho_1(t) = [(I_x - S_x)\cos(\pi J_{IS} t) + (2I_y S_z - 2I_z S_y)\sin(\pi J_{IS} t)]\cos(\tfrac{2\pi\Delta\nu_{IS} t}{2}) - [(I_y + S_y)\cos(\pi J_{IS} t) - (2I_x S_z + 2I_z S_x)\sin(\pi J_{IS} t)]\ \sin(\tfrac{2\pi\Delta\nu_{IS} t}{2}) \qquad (14)$$

$$\rho_{ZQx}(t) = \left(I_xS_x + I_yS_y\right)\cos(2\pi\Delta v_{IS}t) + \left(I_yS_x - I_xS_y\right)\sin(2\pi\Delta v_{IS}t) = ZQx\cos(2\pi\Delta v_{IS}t) + ZQy\sin(2\pi\Delta v_{IS}t) \qquad (15)$$

while in presence of rf fields with the carrier placed at the middle of their offsets ($\upsilon_1 = -\Delta\upsilon_{IS}/2$ and $\upsilon_2 = \Delta\upsilon_{IS}/2$) and an amplitude $\upsilon_1 >> \Delta\upsilon_{IS}$, the evolutions for the LLC and ZQ_x are:

$$\rho_{LLC}(t) = (I_x - S_x)\cos(2\pi J_{IS}t) + (I_yS_z - I_zS_y)\sin(2\pi J_{IS}t) \qquad (16)$$

$$\rho_{ZQx}(t) = I_xS_x + I_yS_y * \cos(2\pi\upsilon_1 t)^2 + I_zS_z * \sin(2\pi\upsilon_1 t)^2 + \left(I_yS_z + +I_zS_y\right)\sin(2\pi\upsilon_1 t)\cos(2\pi\upsilon_1 t) \qquad (17)$$

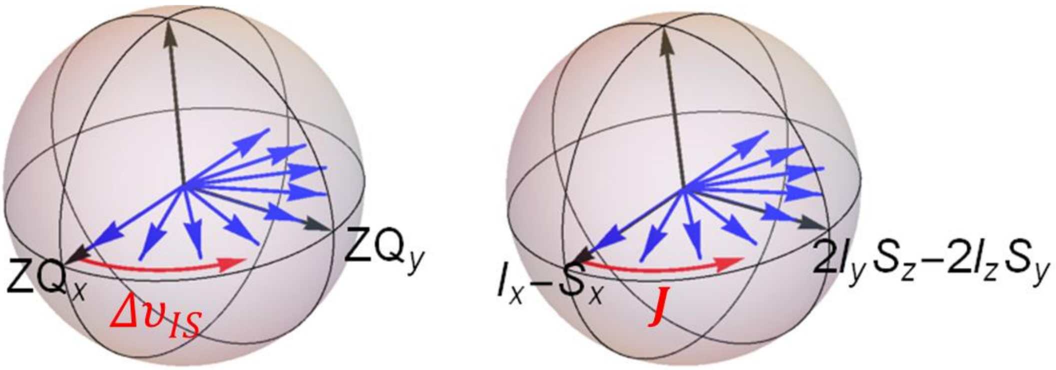

Figure 5. Evolution of zero-quantum coherences during free precession with an oscillating frequency of $\Delta\upsilon_{IS}$ and evolution of long-lived coherences during sustaining period with an oscillating frequency equal to the scalar coupling constant J_{IS}.

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's* 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 (Figure 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 in detail and in perspective several challenging aspects of the mechanisms of zero-quantum inversion with respect to longitudinal two-spin order in homonuclear spin systems. Numerical simulations outlining the efficiency of different pulse sequences to create long-lived states in different coupling regimes are discussed. The concepts are presented in order to find use as building blocks in different applications.

Author contribution

FT and PV designed the study, FT performed the numerical simulations, FT and PV wrote the paper.

Competing interests

The authors declare that they have no conflict of interest.

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Code availability

The Mathematica notebook used for the simulations is provided as supplementary information.

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