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RESEARCH ARTICLE

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A magnet moment silenced: A tribute to my friend and mentor Alex D. Bain

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This article is a contribution to the special issue in honor of Alex Bain.

Abstract

Alex D. Bain was an exceptional NMR spectroscopist who played an important role in the development of modern NMR methods and whose keen intellect and wonderful personal qualities endeared him to faculty and students alike who often sought him out for NMR advice. In this brief recollection, I will focus on a number of seminal contributions that Alex made that greatly influenced my research, illustrating how they changed the practice of modern NMR spectroscopy and laid the foundation for new experiments that are currently in widespread use.

KEYWORDS

Alex D. Bain, cross-correlated spin relaxation, phase cycling, TROSY

1 | INTRODUCTION

For 10 years, I had the great fortune to share office space with one of the most NMR savvy and personable individuals that I have ever met. He always had a smile and an encouraging word and he was a fantastic optimist. That person was Alex D. Bain and as is often the case one does not fully appreciate one's fortunes until they disappear. This happened on December 28, 2017 with Alex's untimely death from heart failure. He leaves a legacy of contributions to NMR that will continue to inspire its practitioners and lessons about how to live life to its fullest. Like other modest scientists, just interested in doing work and not in the self-aggrandizement that accompanies too many accomplished individuals these days, Alex never received the recognition that he deserved. This was noted in a short historical piece by another NMR pioneer, Geoffrey Bodenhausen, where he reflects on coherence transfer pathways in NMR experiments,[1] an area where Alex contributed seminal insights (see below). Yet, those of us who care about the details behind NMR experiments know full well of Alex's contributions, and he was greatly admired by a wide NMR community. Alex and I shared a great interest in spin dynamics, in chemical exchange and in methyl groups, although Alex, of course, knew much more than I about all these subjects.

Alex joined my laboratory a decade ago as an unpaid post doc, after he became an Emeritus Professor of Chemistry at McMaster University, contributing to our group meetings, providing insight into subtle NMR nuances that we needed to understand, and mentoring students and postdoctoral fellows. My office space seems too lonely now and the laboratory days a little less sunny. Alex is sorely missed, but in the pages of this special issue, his profound influences on us all become readily apparent. In what follows I have chosen a pair of topics that illustrate Alex's NMR prowess and how this influenced my research and that of many others. The first topic centers on Alex's famous 1984 JMR paper that laid the mathematical foundations for understanding phase cycling[2] where I show how his work could be used to construct an NMR experiment for measuring protein conformational exchange using the protons of methyl groups.[3] A second topic focuses on Alex's earlier work on spin relaxation in methyl groups[4] that served, in part, as a basis for developing the methyl-TROSY effect for studies of molecular machines.[5,6] I lay no claim of rigor nor of completeness here; the goal is simply to remember Alex and his accomplishments with great respect.

2 | DISCUSSION

2.1 | Coherence and the phases of pulses

Consider a coherence of order k as represented by a density matrix ρ^k. Then, the effect of a pulse P of phase x on ρ^k can be denoted as

$$P\rho^k P^{-1} = \sum_{k'} a_{k'}\rho^{k'}, \tag{1}$$

where the superscript "−1" denotes inverse. It can be seen, therefore, that P creates a series of coherences of order k' from ρ^k. In the case where a pulse of phase ϕ is applied to ρ^k Alex derived a famous expression between the phase of the resulting k' coherence and ϕ.[2] Denoting the application of a pulse of phase ϕ on coherence k as

$$P_\phi \rho^k P_\phi^{-1} \tag{2}$$

and recalling that

$$P_\phi = \exp(-i\phi I_z) P \exp(i\phi I_z) \tag{3}$$

where I_z is the z-angular momentum operator, it follows that

$$\begin{aligned} P_\phi \rho^k P_\phi^{-1} &= \exp(-i\phi I_z) P \exp(i\phi I_z)\rho^k \\ &\quad \times \exp(-i\phi I_z) P^{-1} \exp(i\phi I_z) \\ &= \exp(ik\phi)\exp(-i\phi I_z) P\rho^k P^{-1} \exp(i\phi I_z) \\ &= \exp(ik\phi)\exp(-i\phi I_z)\sum_{k'} a_{k'}\rho^{k'} \exp(i\phi I_z) \\ &= \exp(ik\phi)\sum_{k'} a_{k'}\rho^{k'}\exp(-ik'\phi) \\ &= \sum_{k'} a_{k'}\rho^{k'}\exp(-i(k'-k)\phi) \end{aligned} \tag{4}$$

In the derivation of Equation (4), I have made use of the fact that

$$\exp(i\phi I_z)\rho^k \exp(-i\phi I_z) = \exp(ik\phi)\rho^k \tag{5}$$

Equation (4) leads us to the important conclusion that application of a P_ϕ pulse converts coherence order k to coherence order k' with a phase factor of $\exp(-i(k'-k)\phi)$. As described in some detail in the original paper,[2] and subsequently in standard NMR textbooks,[7-9] by judicious choice of a set of ϕ values (phase cycling), one can select the desired coherence order at each step in a pulse sequence and in so doing generate the coherence pathway(s) of interest. As is often the case for groundbreaking science, a similar result was also presented by Bodenhausen, Kogler, and Ernst at approximately the same time,[10] and one of the authors of this famous paper speaks about this in a beautiful short historical perspective on the development of this work.[1]

The importance of the result of Equation (4) and the implications for modern NMR spectroscopy can hardly be overemphasized, as nearly every pulse sequence takes advantage of phase cycling and the selection of particular transfer pathways. To illustrate this in more detail, I choose one example from my own research because it so nicely illustrates the power of the approach and because it is an example that involves studies of chemical exchange, an area of research that was of great interest to Alex.[11]

Figure 1A shows the pulse sequence used to measure ^{1}H Carr-Purcell-Meiboom-Gill (CPMG) relaxation dispersion profiles of methyl groups in proteins.[3] As with many spin relaxation applications involving large biomolecules, the experiment is recorded in pseudo-3D mode as a series of ^{13}C (F_1)-^{1}H (F_2) planes, where the intensities of cross peaks are modulated by the CPMG pulse train that is applied for duration T_{relax} prior to ^{13}C chemical shift evolution (t_1). Central to this experiment is that ^{1}H triple-quantum coherences (3Q) are evolved during the CPMG element, between points B and C in the scheme of Figure 1 because these are three times more sensitive to chemical shift differences than their single-quantum (SQ) counterparts. Thus, in the fast exchange limit, the resulting CPMG dispersion curve is nine fold larger than the corresponding profile recorded using ^{1}H SQ coherences.[3] The part of interest to us here is the interval from A to B in the pulse diagram where the 3Q ^{1}H coherences are created and selected.

In the interest of pedagogy, we focus first on the ^{1}H channel, neglecting the ^{13}C pulses and the delays that are, of course, critical to the experiment, so as to illustrate a central point. Thus, we consider a ^{1}H pulse train $90_{\phi 1}$-$180_{\phi 1}$-$180_{\phi 1}$-$90_{\phi 1}$ and we write the effective rotation introduced by the pulse train on starting z-magnetization, following the notation introduced above, as

$$\begin{aligned} &P_T I_z P_T^{-1}, \\ &\quad P_T = P_{90_{\phi 1}} P_{180_{\phi 1}} P_{180_{\phi 1}} P_{90_{\phi 1}} \\ &\quad P_{\theta_{\phi 1}} = \exp(-i\phi_1 I_z) P_{\theta_x} \exp(i\phi_1 I_z) \end{aligned} \tag{6}$$

where $P_{\theta_{\phi 1}}$ is a pulse of flip angle θ applied along an axis making an angle ϕ_1 with respect to the x-axis of the rotating frame. It is straightforward to show that P_T simplifies to $P_{90_{\phi 1}} P_{90_{\phi 1}}$. Thus, in our consideration of the appropriate phase cycle to generate 3Q coherences, it will be sufficient to consider how cycling of the phases of the flanking 90° pulses in the pulse train above affects the various coherence orders of interest. Next, we consider a description of the sequence from points A to B. This can be written succinctly as

$$I_z^j \rightarrow I_y^j \xrightarrow{J} 2I_x^j C_z \rightarrow 2I_x^j C_y \xrightarrow{J} 8I_x^j I_z^k I_z^l C_y \rightarrow 8I_x^j I_y^k I_y^l C_z \tag{7}$$

where we have focused on the case where ϕ_1 = x, neglected the effects of pulse imperfections and relaxation,

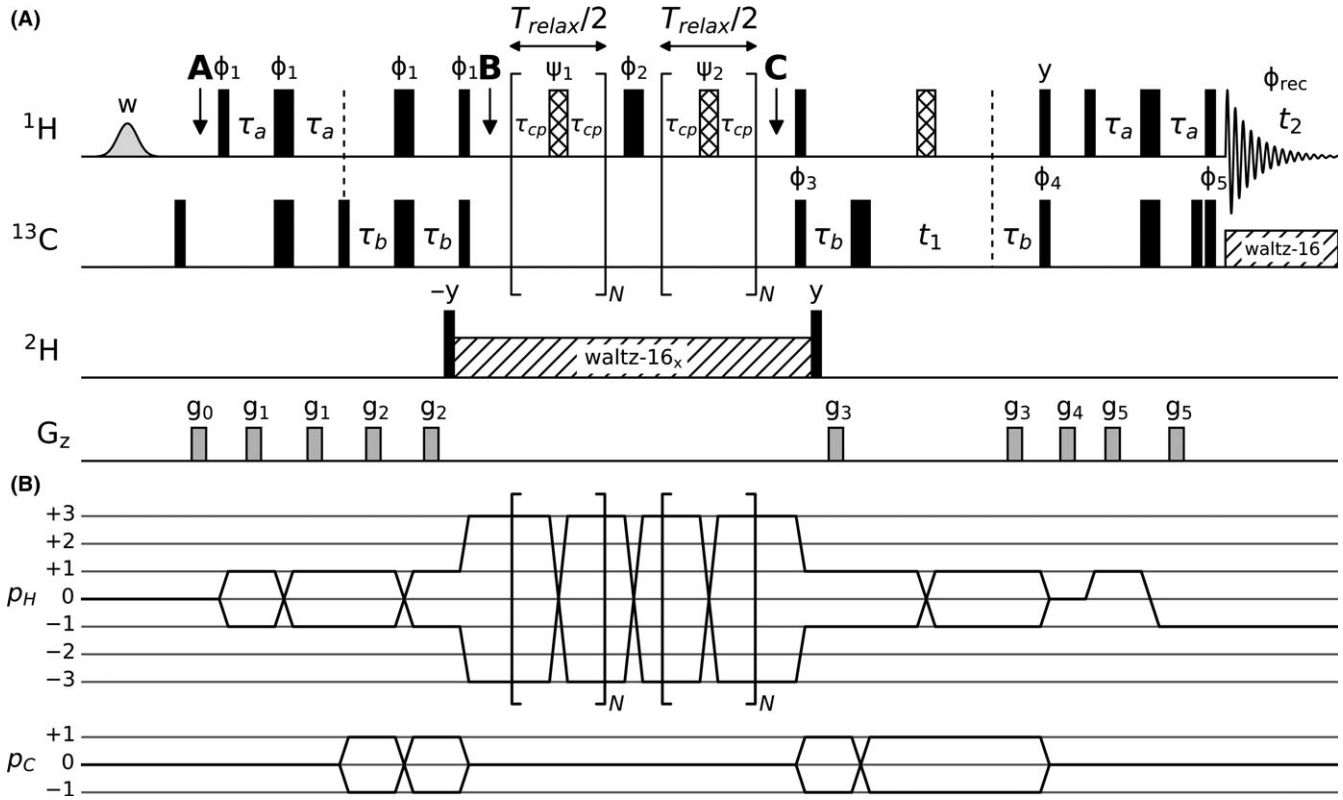

FIGURE 1 A, A 3Q-based ^{1}H CPMG pulse scheme for quantifying exchange dynamics using $^{13}CH_3$ methyl group probes. B, Coherence transfer diagram illustrating the relevant pathways that pertain to the pulse scheme, focusing on both ^{1}H and ^{13}C coherences and the relevant coherence orders p_H and p_C. Details of the experiment can be found in the original literature[3]

assumed that $\tau_a = \tau_b = 1/(4J_{CH})$, where J_{CH} is the one-bond ^{13}C-^{1}H scalar coupling constant, and ignored trivial minus signs for clarity. In the derivation of Equation (7), we have considered the path originating from ^{1}H magnetization associated with spin j, but of course equivalent pathways exist for ^{1}H spins k and l as well. It is convenient to recast the Cartesian operators in terms of raising and lowering operators from which coherence orders become readily apparent. Thus,

$$\begin{aligned} 8I_x^jI_y^kI_y^lC_z &= 8\left(\frac{I_+^j+I_-^j}{2}\right)\left(\frac{I_+^k-I_-^k}{2i}\right)\left(\frac{I_+^l-I_-^l}{2i}\right)C_z \\ &= \frac{-1}{8}8(I_+^jI_+^kI_+^l - I_+^jI_+^kI_-^l - I_+^jI_-^kI_+^l + I_-^jI_+^kI_+^l \\ &\quad - I_-^jI_-^kI_+^l - I_-^jI_+^kI_-^l + I_+^jI_-^kI_-^l + I_-^jI_-^kI_-^l)C_z \end{aligned} \tag{8}$$

where the 3Q(SQ) elements are highlighted in red (black). Although only two of the eight terms are of the desired 3Q variety, they contribute ¾ of the resulting signal so that the selection of 3Q coherences does not lead to a significant attenuation of the resulting magnetization.[3]

In addition to providing an intuitive feel for the flow of magnetization during the scheme extending from points A to B, Equation (7) also provides insight into how phase ϕ_1 might be cycled to select the desired 3Q terms. To this end, we note that the $I_\pm^j$ term in Equation (8) originates from application of the first ^{1}H 90° pulse, while the second ^{1}H 90° pulse generates $I_\pm^kI_\pm^l$ and $I_\pm^kI_\mp^l$ from $I_z^kI_z^l$. Using Alex's formula in Equation (4), and noting that $k = 0$, $k' = \pm1$ for the first ^{1}H $90_{\phi1}$ pulse, we see that the effect of the pulse is to add an additional phase factor exp $(\mp i\phi)$ to the single-quantum coherences that are created. In a similar manner, the phase of the second ^{1}H 90° pulse leads to an additional factor of exp$(\mp 2i\phi)$ for the 3Q terms since $k = \pm1$, $k' = \pm3$ (so that $k'-k = \pm2$) while no additional factor is obtained for the remaining SQ terms. Thus, factors of exp$(\mp 3i\phi)$ and exp$(\mp i\phi)$ are accrued for the 3Q and SQ elements, respectively, that can be exploited to select one coherence type over the other. In the present case by choosing ϕ = [0°, 60°, 120°, 180°, 240°, 300°] and inverting the receiver ever second scan, the signal from the pathway involving 3Q coherences adds constructively as is seen by the fact that

$$\sum_{j=1-6}(-1)^j\exp(\mp 3i\phi_j) = -6 \tag{9}$$

The summation in Equation (9) denotes the addition of signal from six scans where ϕ_j is phase cycled as above and the $(-1)^j$ term takes into account the consecutive

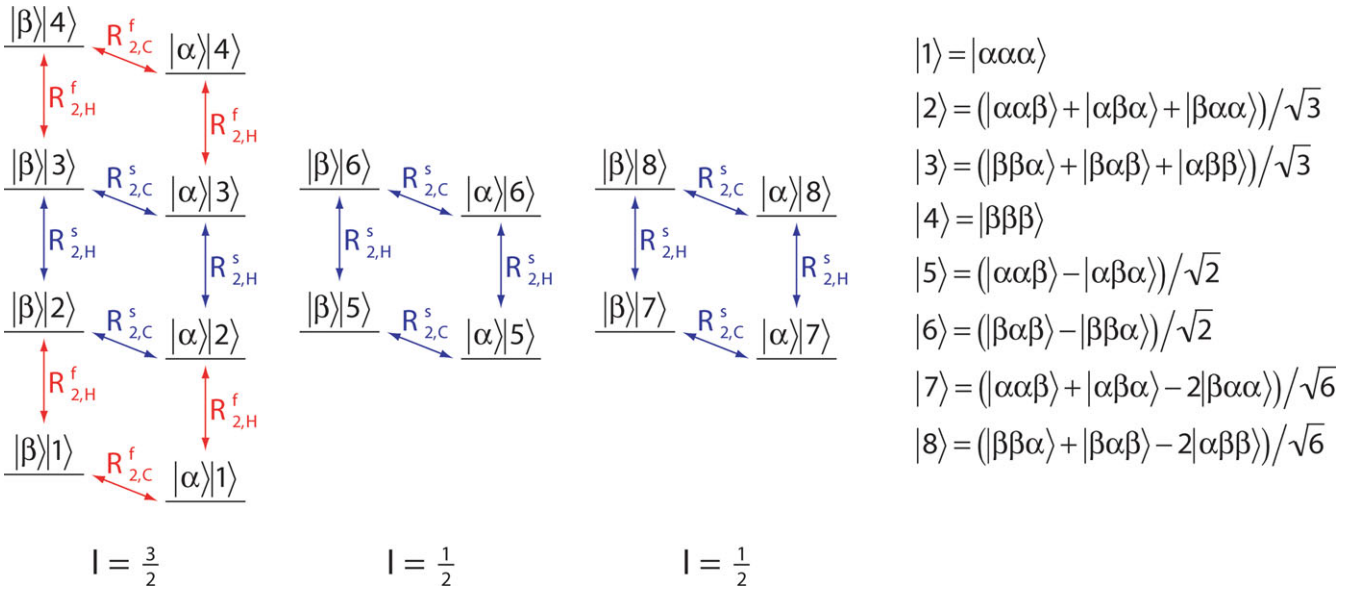

FIGURE 2 Energy level diagram of an isolated $^{13}CH_3$ methyl group, as illustrated in Ollerenshaw et al.[27] Wave functions are written in an irreducible basis representation above each horizontal line, with the ^{13}C and ^{1}H spin states denoted by the first α or β and |j>, j ∈ [1,8], respectively. Single-quantum ^{1}H and ^{13}C transitions are depicted with vertical and horizontal arrows, respectively, colored either red (fast-relaxing components) or blue (slow-relaxing components). All density elements corresponding to single-, double-, and zero-quantum transitions relax in a single exponential manner in the limit discussed in the text and in references.[5,27] ^{1}H-^{13}C double- and zero-quantum transitions are those that correct states |β>|4> with |α>|3> (double) and |α>|4> with |β>|3> (zero), for example. Both of these coherences decay rapidly, while those coupling |β>|2> with |α>|3> or |β>|3> with |α>|2> decay very slowly.[5,27] Expressions for $R^f_{2,H}$, $R^S_{2,H}$, $R^f_{2,C}$, and $R^S_{2,C}$ as well as for the decay of multiple-quantum elements are in the literature[5,27]

inversion of the phase of the receiver. In contrast, for the SQ pathway

$$\sum_{j=1-6} (-1)^j \exp(\mp i\phi_j) = 0 \qquad [10]$$

so that it does not contribute to observed magnetization. Figure 1B shows the relevant coherence pathway diagrams for both ^{1}H and ^{13}C channels that follow from the elegant work of Alex[2] and Bodhenhausen and coworkers[10] during a very special time in the development of modern multidimensional NMR spectroscopy. The ease by which phase cycling can be achieved to select the pathway(s) of interest (Equation (4)) has significantly advanced the NMR field and those of us practitioners owe Alex (as well as Geoffrey, Herbert, and Richard) our gratitude for making our professional lives so much easier!

2.2 | Relaxation of AX_2 and AX_3 spin systems

One of the tremendous strengths of NMR spectroscopy is that it is possible to obtain a detailed, site-specific description of molecular dynamics.[12-14] The importance of NMR to studies of molecular motion is made clear by the continued development of experiments that probe dynamics over a very broad range of time scales, extending from picoseconds to seconds. Perhaps equally important is that relaxation effects can be exploited to improve both spectral resolution and sensitivity in applications to macromolecules via a TROSY effect that was first illustrated by the Wüthrich group with applications to backbone amide positions in proteins.[15] Alex was a pioneer in spin relaxation[4,16,17] and his work, along with other important studies by Werbelow and Grant[18] and Vold and Vold,[19] led to a firm understanding of spin relaxation in multispin systems such as those of methylene and methyl groups. Alex recognized very early on that cross-correlation effects could be significant in AX_2 and AX_3 spin systems and in a landmark paper, he tabulated the requisite relaxation elements that included contributions of dipolar, chemical shift anisotropy, spin rotation, and external field interactions to the relaxation of the A spins in these spin systems.[4] This work, along with similar contributions from the other researchers mentioned above, focused on molecules tumbling in the extreme narrowing limit since in the early 1970s, applications to macromolecules were relatively less frequent. Yet, these studies paved the way for the investigations of macromolecules that were to follow because they provided a rigorous approach for calculating relaxation elements for molecules of arbitrary size that are

necessary in the description of spin dynamics in large biomolecules.

My foray into NMR spin relaxation began in the mid-1980s with the studies of molecular dynamics using methyl group probes, but it was only later in the early 1990s that my work closely paralleled what Alex had done, when Tom Bull and I derived expressions for the transverse relaxation of spin A in AMX, AX_2, and AX_3 spin systems that are general for any tumbling regime.[20] Unlike the case of Alex's studies that included a whole range of different relaxation effects, our work focused exclusively on dipolar interactions. An important result emerged from our calculations on $^{13}CH_3$ spin systems which were to form the basis of many years of fruitful research in my laboratory involving studies of high-molecular weight proteins. In the limit of an isolated methyl attached to a molecule tumbling very slowly, and assuming that the rotation of the methyl group about its three-fold symmetry axis is rapid, the relaxation of the single- and multiple-quantum transitions in a $^{13}CH_3$ spin system become single exponential (Figure 2) and can effectively be divided evenly between fast- and slow-decaying components.[5,21] Notably, the simple HMQC pulse scheme[22,23] is ideally suited to take advantage of this situation because magnetization transfer pathways sequester the fast- and slow-relaxing components, leading to enhanced spectral quality in studies of very large protein systems.[5] This so-called methyl-TROSY effect and its backbone amide counterpart have significantly extended biomolecular NMR studies to a wide range of protein and nucleic acid systems whose studies were heretofore intractable due to their sizes.[6,24] Yet, it is important to emphasize that the underlying theoretical understanding of cross-correlation effects in AX, AX_2, and AX_3 spin systems ultimately formed the basis for the design of our current experiments that lead to these important applications and that Alex was an early pioneer in this area. Bill Reynolds, a long-time colleague and friend of Alex, tells the interesting story of how in the early 1980s, long before the TROSY effect was popularized, he approached Alex with a ^{13}C spectrum of fluorobenzene containing asymmetric doublet components which Alex immediately recognized as the telltale sign of cross correlation between dipolar and chemical shift anisotropy interactions. Thus, Alex recognized the essential elements of the famous Wüthrich/Pervushin experiment[15] long before its development.

3 | CONCLUDING REMARKS

The two short examples that I illustrate here do little justice to Alex's many contributions, but they illustrate his role as an extremely clear and original thinker whose work has significantly moved the NMR field forward. Alex's scientific contributions were often of a theoretical nature focusing on spin physics, yet the practical implications of his work were at least as significant. There is not a single NMR experiment that does not take advantage of the phase cycling rules that he taught us, while his work on spin relaxation and chemical exchange have laid the foundations for exploring molecular dynamics, currently an area of extensive focus in the NMR field. Equally important is his early recognition of the importance of relaxation interference effects that have been exploited in TROSY-based experiments that form the basis of current NMR studies of large biomolecules.[6,25]

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This is a personal account but I know the sentiments that I have expressed resonate with all of my laboratory members. In particular Ranjith Muhandiram and Ashok Sekhar enjoyed close relationships with Alex. Ashok and I would like to thank Alex for his clear explanations of aspects of chemical exchange involving reactions more complex than unimolecular which lead to a recent publication with him.[26] This work was supported through grants from the Canadian Institutes of Health Research and the Natural Sciences and Engineering Research Council of Canada. L.E.K. holds a Canada Research Chair in Biochemistry and is a member of the Canadian Institute for Advanced Research. L.E.K. thanks Tairan Yuwen for making Figure 1.

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