High-Resolution NMR in Magnetic Fields with Unknown Spatiotemporal Variations

Philippe Pelupessy,²* Enrico Rennella,² Geoffrey Bodenhausen¹,³

Nuclear magnetic resonance (NMR) experiments are usually carried out in homogeneous magnetic fields. In many cases, however, high-resolution spectra are virtually impossible to obtain because of the inherent heterogeneity of the samples or living organisms under investigation, as well as the poor homogeneity of the magnets (particularly when bulky samples must be placed outside their bores). Unstable power supplies and vibrations arising from cooling can lead to field fluctuations in time as well as space. We show how high-resolution NMR spectra can be obtained in inhomogeneous fields with unknown spatiotemporal variations. Our method, based on coherence transfer between spins, can accommodate spatial inhomogeneities of at least 11 gauss per centimeter and temporal fluctuations slower than 2 hertz.

Nuclear magnetic resonance (NMR) is arguably one of the most versatile and ubiquitous forms of spectroscopy. Year after year, magnetic resonance imaging (MRI) reveals surprising insights into morphology, function, and metabolism. Most applications, regardless of whether they are concerned with inanimate solids or liquids or with living organisms, rely...
The coherence can then be transferred (in the example of Fig. 1) by a simple \(\pi/2\) pulse from \(S_1\) to \(I\). With coherence order \(p = -1\) through any spin-spin coupling, exploiting (for example) a scalar interaction \(J_{GS}\). If there are no scalar couplings, it might be possible to use de-magnetizing fields, which can manifest themselves as internuclear dipolar interactions (23, 24).

As in the two-dimensional (2D) single-scan experiments, the evolution under the chemical shifts needs to be intertwined with gradient encoding (22). Let \(S\) and \(I\) constitute a homonuclear pair of spins. When a linearly swept adiabatic refocusing pulse is applied in the presence of a gradient to a coherence \(S\), with coherence order \(p = +1\) belonging to the manifold of \(S\)-spin transitions (Fig. 1A), the resulting phase at time \(t_1\) is given by

\[
\varphi_1 = \alpha (\Omega_S + \delta\omega(r) + \Gamma_E(r))^2
\]

(20), where \(\Omega_S\) is the chemical shift of the \(S\) spin, \(\delta\omega(r) = -\gamma S B_d(r)\) is the (unknown) spatially dependent frequency induced by the inhomogeneous \(B_0\) field, \(\Gamma_E(r) = \gamma_S G_E(r)\) is the (known) frequency induced by the encoding gradient \(G_E = \{G_E,k,G_E,i,G_E,l\}\), and \(\alpha = \tau_{ad}/\Delta\omega_{ad}\) is the ratio of the duration \(\tau_{ad}\) and sweep width \(\Delta\omega_{ad}\) of the adiabatic inversion pulse. A second identical pulse then leads to a phase at time \(t_2\):

\[
\varphi_2 = 4\alpha (\Omega_S + \delta\omega(r))\Gamma_E(r)
\]

Fig. 1. Pulse sequences to obtain high-resolution spectra in a single scan in arbitrarily inhomogeneous, slowly fluctuating magnetic fields with unknown spatiotemporal distributions. Solid and open vertical rectangles represent \(\pi/2\) and \(\pi\) pulses. The rectangles with sloping arrows indicate adiabatic frequency-swept refocusing pulses. \(G_x\) and \(G_y\) are encoding and decoding gradients. The gradients \(G_x\) and \(G_y\) of equal area serve to select the desired coherence pathways (they must be identical for (A) and opposite for (B) and (C)). The delay \(\tau_c\) serves to compensate for the evolution under the inhomogeneous \(B_0\) field during \(G_x\) and \(G_y\). Increasing \(G_y\) and prolonging \(\tau_c\) by \(\pi/2\) shifts the signals at zero frequency toward the center of the gradient \(G_y\). Sequences (D) and (E) are adaptations of (B) and (C), respectively, for situations where a permanent gradient is dominant. Adiabatic pulses with down-pointing arrows have reversed sweep directions. The phases of the \(\pi\) pulses during decoding are alternated between \(x\) and \(x\) every two pulses (21). In these examples, \(\pi/2\) pulses are used to achieve transfer of coherence, so that the spins need to be scalar-coupled. Other sequences can be used, such as total correlation spectroscopy (TOCSY). The signals during even (or odd) decoding gradients are arranged in sequential order to yield a 2D array. The gradient echoes that are formed during each decoding gradient appear in a temporal sequence that corresponds to the peaks in the spectrum. The scalar coupling pattern in the other dimension is obtained by performing a Fourier transformation as a function of the index of the decoding gradients.

---

1Département de Chimie Associé au CNRS, Ecole Normale Supérieure, 24 rue Lhomond, 75231 Paris Cedex 05, France.
2Department of Biomedical Sciences and Technologies, Università degli Studi di Udine, I-33100 Udine, Italy.
3Institut de Sciences et Ingénierie Chimiques, École Polytechnique Fédérale de Lausanne, Batochime, 1015 Lausanne, Switzerland.
4*To whom correspondence should be addressed. E-mail: philippe.pelupessy@ens.fr
that evolve during the encoding and decoding blocks of Fig. 1; their scalar coupling patterns appear along the horizontal dimension and carry the same labels (A → B). The arrows in (A) indicate all difference frequencies between scalar-coupled spins that can be observed; the top arrows correspond to the outer lines in the spectrum, the bottom arrows to the smallest frequency differences (inner lines). The line at zero frequency corresponds to coherences that are refocused but not transferred from one spin to another. Both spectra were recorded with $\gamma = 1$; gradients applied simultaneously along directions $z$ and $y$ with strengths $G_z = 0.8$ G/cm and $G_y = 2.4$ G/cm. The adiabatic pulses of 6 ms had a wideband uniform rate and smooth truncation (WURST) profile (28) and sweep widths of 14 and 20 kHz for propanol and butenol, respectively. (B) Columns taken parallel to the vertical $\Omega_0$ or $\Omega_1$ domain, taken along the dotted lines of the 2D spectra of propanol in homogeneous (red lines) and inhomogeneous fields (black lines), showing differences of chemical shifts in the spectrum of propanol. (C) Rows taken parallel to the horizontal $\Omega_2$ domain, showing a multiplet due to scalar couplings with a full width at half height of 3.5 Hz, for $n_0 = 64$ ($t_2^{\text{max}} = 337$ ms).

The difference $\Omega_S - \Omega_I$ between the chemical shifts of spins $I$ and $S$ is now encoded not only by the gradient $G_E$ but also by the $B_0$ inhomogeneity. During a decoding gradient $G_D$ with the same amplitude as the encoding gradient $G_E$, the phase is then

$$\varphi_D(t_D) = \alpha(S)\varphi \pm \alpha(I) \pm 2\alpha(S)\Omega_S \pm \alpha(I)\Omega_I \pm 2\alpha(S)\Omega_S \pm \alpha(I)\Omega_I$$

(4)

At $t_D = 2\alpha(S) - \Omega_S$, an echo results because the phase is independent of $\delta\Omega(r)$. Thus, a spectrum of frequency differences is obtained in the time domain in a single scan. The phase of this echo is

$$\varphi_D(t_D) = 2\alpha(S)\Omega_S \pm 2\alpha(I)\Omega_I + 2\alpha(S)\Omega_S \pm 2\alpha(I)\Omega_I$$

(5)

A hybrid scheme is shown in Fig. 1C. A bipolar gradient pair with adiabatic pulses that is repeated $n_t$ times is inserted before each adiabatic pulse of Fig. 1B. The resulting phase at time $t_D$ is

$$\varphi_g = 2\alpha(S)\Omega_S \pm \alpha(I)\Omega_I \pm 2\alpha(S)\Omega_S - \alpha(I)\Omega_I + 2\alpha(S)\Omega_S - \alpha(I)\Omega_I$$

(7)

The decoding gradient should have an amplitude that is $(2n_t + 1)$ times the encoding gradient—$G_D = (2n_t + 1)G_E$—in order to cancel the effects of the inhomogeneities $\delta\Omega(r)$. The sequence of Fig. 1C is less sensitive to translational diffusion than the experiment of Fig. 1B (25).

Fig. 2. (A to D) Two-dimensional spectra, plotted in absolute-value mode, of propanol $\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$ (left panels) and butenol $\text{CH}_3=\text{CH}\text{CH}_2\text{CH}_2\text{OH}$ (right panels), both taken in ~500 ms with the single-scan method of Fig. 1C. (B) and (D) were obtained in homogeneous and inhomogeneous $B_0$ fields, respectively, as evidenced by the conventional proton spectra in (A) (with linewidths on the order of 2 Hz, recorded in a well-shinned static field) and in (C) (where the linewidths were degraded to ~4 ppm or 2.4 kHz at 600 MHz). The resonances of spins $S$ and $I$, labeled a, b, c, and d, correspond to single-quantum coherences and $\text{CH}_2$ resonances, respectively. This improvement scheme is shown in Fig. 1B. Instead of applying two adiabatic pulses before and after coherence transfer, only one is applied, which leads to a phase:

$$\varphi_0 = \alpha(S)\Omega_S \pm \delta\Omega(r) + 2\alpha(I)\Omega_I \pm 2\alpha(S)\Omega_S \pm \delta\Omega(r)$$

(4)
could have implications for measurements in stray magnetic fields (26) or other permanent gradients. Shown in Fig. 1, D and E, are adaptations of Figs. 1, B and C, for situations with a permanent gradient. In Fig. 1E the sweep direction of the second adiabatic pulse in each repeated block is inverted. Both schemes lead to a phase $\varphi_0$ of Eq. 4, but the latter limits losses due to diffusion (25).

All schemes can be extended from one to two dimensions by appending $n_D$ repetitions of a block comprising a decoding gradient followed by a $\pi$ pulse in order to observe a train of spin echoes (21). A Fourier transformation of this echo train reveals (convoluted) multiplets due to scalar couplings in a second dimension. This option requires $\sim 500$ instead of $\sim 50$ ms for the basic 1D experiment. The simplest 1D spectrum corresponds to the signal acquired during the first decoding gradient.

Shapira et al. (4) proposed schemes for measurements in fluctuating resistive magnets. The resolution of correlation spectra can be improved, either by compensating for (known) inhomogeneities with tailored rf fields, or by exploiting echoes following coherence transfer from carbon-13 to proton nuclei. The latter scheme is related to our method, although in their case the effects of inhomogeneities are canceled only at one point in the acquisition period.

The experiments of Fig. 1C have been tested by applications to samples containing 5% 1-propanol (CH$_3$CH$_2$CH$_2$OH) and 5% 3-buten-1-ol (CH$_2$=CHCH$_2$OH, henceforth simply called butanol) in CDC$_3$. The spectra in Fig. 2, A and B, have been obtained in a carefully shimmed homogeneous field. The resonances labeled a through d give rise to combination lines (differences in chemical shift) $I = 0$, because each transfer from spin $S$ to $F_1$s is accompanied by a transfer in the opposite direction. The central peak at $\omega_2 = 0$ arises from magnetization that is refocused but not transferred from one spin to another.

Shown in Fig. 2, C and D, are spectra obtained with the same method in an inhomogeneous field after deliberately missetting the shim currents. Yet the results of our single-scan experiments are virtually indistinguishable. The experiments have been repeated using several shim settings, invariably leading to similar spectra, as can be seen from the cross sections in Fig. 2, E and F. As with all 2D single-scan experiments, the signal-to-noise ratio decreases in proportion to the square root of the number of points observed in the indirect dimension, relative to a 1D spectrum taken under properly shimmed conditions. If the signal-to-noise ratio is poor, the experiments can be repeated for signal averaging, even if the $B_0$ inhomogeneities vary in time, provided the fluctuations are slow relative to the time it takes to acquire each scan. For a simple 1D spectrum, about 50 ms is sufficient. Thus, if we assume that the information must be acquired within 10% of the period of the fastest fluctuations, the spectrum of the stochastic variations must be limited to 2 Hz.

This should be useful for NMR spectra obtained in resistive Bitter or hybrid magnets, which suffer from random fluctuations due to the power supply and to low-frequency vibrations associated with the circulation of cooling water.

The resolution, as measured by the full linewidth at half height in the vertical (chemical shift) dimension ($\omega_1$ or $\omega_0$) of 1-propanol in Fig. 2E, is 60 $\mu$s, for a decoding gradient $G_D$ of 4 ms. This corresponds to a resolution of 76 Hz (0.13 ppm) over a frequency range of $\pm 2500$ Hz ($\pm 4.2$ ppm). The resolution of the spectra can be improved by increasing the amplitudes of both encoding and decoding gradients (provided the frequency sweep of the adiabatic pulses covers the frequency range induced by the encoding gradients) or, as shown in Fig. 3, by increasing $n_D$ and the strength of the decoding gradients. The frequency range can be increased by extending the sweep width of the adiabatic pulses, albeit at the expense of the resolution, unless one also increases the strength of both encoding and decoding gradients (27). The resolution in the $\omega_2$ dimension is determined by the number $n_D$ of points acquired in the $t_2$ dimension, as in other forms of 2D spectroscopy.

To mimic a gradient that cannot be switched off, we have applied the scheme of Fig. 1D in the presence of a permanent gradient, or more accurately, a pulsed field gradient that was switched on before the experiment and stopped immediately after observing the signals. The echo corresponding to zero frequency appears at a delay $\tau_c$ after the start of signal acquisition. Only 1D spectra revealing combinations of chemical shifts in propanol were recorded (to avoid using prolonged strong gradients). The sample height was decreased to 18 mm, so that the line shape was further deteriorated by susceptibility effects at the edges. Figure 4A shows the resulting spectra in the presence of a gradient $G_z = 2.75$ G/cm ($\sim 35$ ppm or 21 kHz across the sample). Signals arising from coherence transfer are indicated by arrows. The duration of the adiabatic pulses was 18 ms and their sweep width was 40 kHz. When the gradient strength was doubled to 5.5 G/cm ($\sim 70$ ppm or $\sim 42$ kHz) together with a doubling of the adia-
batic sweep width, there was some loss of intensity due to diffusion (Fig. 4B). These losses could be reduced with the scheme of Fig. 1E, as shown in Fig. 4C for a total of six adiabatic pulses of 6-ms duration (instead of two otherwise identical pulses of 18 ms each, as in Fig. 4B). The linewidth in Fig. 4C is about 70 Hz. Increasing the gradient strength further to 11 G/cm (~140 ppm or ~84 kHz) led to the spectrum in Fig. 4D. In this case, the signal amplitude suffers not only from diffusion losses, but also from the limited rf amplitude (25 kHz) of the initial π/2 pulse. In stronger gradients, one should use a large number of short inversion pulses that cover a very broad bandwidth with the desired phase profile. In addition, the (possibly frequency-swept) π/2 pulses should excite the full bandwidth uniformly, bearing in mind that a linear dependence of the phase with respect to offset is allowed.

References and Notes
1. G. Eidmann, R. Savelberg, P. Blumler, B. Blümich, J. Magn. Reson. A 122, 104 (1996).
2. B. Blümich, J. Perlo, F. Casanova, Prog. Nucl. Magn. Reson. Spectrosc. 52, 197 (2008).
3. Y.-Y. Lin et al., Phys. Rev. Lett. 85, 3732 (2000).
4. B. Shapira, K. Shetty, W. W. Brey, Z. Gan, L. Frydman, Chem. Phys. Lett. 442, 478 (2007).
5. T. C. M. Cox et al., J. Magn. Reson. 70, 163 (1986).
6. C. E. Mountford, S. Doran, C. L. Lean, P. Russell, Chem. Rev. 104, 3677 (2004).
7. E. L. Hahn, Phys. Rev. 80, 580 (1950).
8. H. Y. Carr, E. M. Purcell, Phys. Rev. 94, 630 (1954).
9. E. L. Hahn, D. E. Maxwell, Phys. Rev. 88, 1070 (1952).
10. R. L. Vold, S. O. Chan, J. Chem. Phys. 53, 449 (1970).
11. B. Shapira, L. Frydman, J. Am. Chem. Soc. 126, 7184 (2004).
12. B. Shapira, L. Frydman, J. Magn. Reson. 182, 12 (2006).
13. A. Wokaun, R. R. Ernst, Chem. Phys. Lett. 52, 407 (1977).
14. K. Nagayama, K. Wüthrich, R. R. Ernst, Biochem. Biophys. Res. Commun. 90, 305 (1979).
15. V. Demas et al., Concepts Magn. Reson. B 29B, 137 (2006).
16. J. Perlo et al., Science 308, 1279 (2005); published online 7 April 2005 (10.1126/science.1108944).
17. B. Shapira, L. Frydman, J. Am. Chem. Soc. 126, 7184 (2004).
18. A. Wokaun, R. R. Ernst, Chem. Phys. Lett. 52, 407 (1977).
19. K. Nagayama, K. Wüthrich, R. R. Ernst, Biochem. Biophys. Res. Commun. 90, 305 (1979).
20. L. Frydman, T. Scherf, A. Lupulescu, Proc. Natl. Acad. Sci. U.S.A. 99, 15858 (2002).

White Phosphorus Is Air-Stable Within a Self-Assembled Tetrahedral Capsule

Prasenjit Mal,1 Boris Breiner,1 Kari Rissanen,2 Jonathan R. Nitschke1*

The air-sensitive nature of white phosphorus underlies its destructive effect as a munition: Tetrahedral P4 molecules readily react with atmospheric dioxygen, leading this form of the element to spontaneously combust upon exposure to air. Here, we show that hydrophobic P4 molecules are rendered air-stable and water-soluble within the hydrophobic hollows of self-assembled tetrahedral container molecules, which form in water from simple organic subcomponents and iron(II) ions. This stabilization is not achieved through hermetic exclusion of O2 but rather by constricting of individual P4 molecules; the addition of oxygen atoms to P4 would result in the formation of oxidized species too large for their containers. The phosphorus can be released in controlled fashion without disrupting the cage by adding the competing guest benzene.

Microenvironment alters behavior in chemical systems; encapsulation within the inner phase (1–3) of a molecular container can accelerate a reaction (4–7), change a reaction’s course (7–11), perturb equilibria (11–13), or prevent the oligomerization of a reactive species such as a cyclooctasiloxane (14) or cyclobutadiene (15). The lifetimes of such fleeting molecules as hemiaminals (16), benzylene (17), and cycloheptatriene (18) may also be extended long enough to permit spectroscopic observation. Here, we show how encapsulation can render molecules of white phosphorus (P4) air-stable through a constrictive mechanism.

The P-P bonds of tetrahedral P4 (white phosphorus) are weak (200 kJ mol−1) (19), leading to a low activation barrier to oxidation, and the P-O bonds of combustion products are strong (330 to 650 kJ mol−1) (19), releasing substantial energy during their formation. P4 is thus violently pyrophoric; its slow chemiluminescent combustion when O2 is limited [“phosphorescence,” the meaning of which has since changed (20)] defined the element’s essence for the first chemists.

We have described the preparation of tetrahedral cage 1 in aqueous solution from the subcomponents shown in Fig. 1 (21). When an aqueous solution of 1 was left in contact with solid white phosphorus, uptake of P4 converted 1 into the host-guest complex P4⊂1 (Fig. 1) (22). Vapor diffusion of acetone into an aqueous solution yielded crystalline P4⊂1 in 91% yield.

Incorporation of P4 into 1 was marked by changes in the 31P nuclear magnetic resonance (NMR) chemical shifts of 1 (Fig. S1) and by the appearance of a single resonance in the 31P NMR spectrum at −510 parts per million (ppm). Single crystals of sufficient quality for x-ray diffraction were grown by vapor diffusion of 1,4-dioxane into an aqueous solution of P4⊂1. Its x-ray crystal structure is shown in Fig. 2.

The quality and resolution of the diffraction data, although limited, allow for unambiguous assignment of the cage’s conformation and the placement of the guest within the cage, where it is disordered between two orientations (only one of 211 and subsequent incorporation of P4.

Fig. 1. Synthesis of tetrahedral cage 1 and subsequent incorporation of P4.

M. D. Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK. 2Department of Chemistry, Nanoscience Center, University of Jyväskylä, Post Office Box 35, 40014 JYU, Finland.

*To whom correspondence should be addressed. E-mail: jrn34@cam.ac.uk