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Reversible Interactions with para-Hydrogen Enhance NMR Sensitivity by Polarization Transfer**

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Supporting information:
Supporting Online Material including Materials and Methods; Figs. S1 and S2; References; Movies S1 and S2 is available at www.sciencemag.org/cgi/content/full/323/5922/1705/DC1

Abstract

The sensitivity of both nuclear magnetic resonance (NMR) spectroscopy and magnetic resonance imaging (MRI) is very low because the detected signal strength depends upon the small population difference between spin states even in high magnetic fields. Hyperpolarization methods can be used to increase this difference and thereby enhance signal strength. This has been achieved previously by incorporating the molecular spin singlet para-hydrogen into hydrogenation reaction products. We show here that a metal complex can facilitate the reversible interaction of para-hydrogen with a suitable organic substrate such that up to an 800 fold increase in proton, carbon, and nitrogen signal strengths are seen for the substrate without its hydrogenation. These polarized signals can be selectively detected when combined with methods that suppress background signals.

Main text

The wide variety of applications of nuclear magnetic resonance (NMR) (*1-3*) are limited by the technique's extremely low inherent sensitivity. Here we describe a new approach that uses hyperpolarized spins derived from para-hydrogen (para-H_2) (*4*) to sensitize the NMR experiment without actually incorporating para-H_2 into the molecule that is to be probed. Specifically, we show that high-resolution NMR spectra can be collected for a range of molecules and nuclei where the detected signal strengths are up to 800 times greater than would be normally achievable with an unpolarized sample. This improvement facilitates the collection of diagnostic high resolution ^{1}H, ^{13}C, ^{15}N, ^{19}F NMR spectra and magnetic resonance images of selected signals in a fraction of the time that would normally be necessary. When optimised, this route is predicted to increase proton sensitivity by up to four orders of magnitude (*5*) such that the routine single shot characterization of materials, even at picomole levels, will become possible (*6*).

The relative weakness of NMR signals exhibited by nuclei with a non-zero magnetic moment results from the way the original energy levels split in a magnetic field (*7*). The bulk magnetic moment for an ensemble of such nuclei is determined by the Boltzmann population of each energy level. In general, the difference in the energy between these levels is so small that almost equal spin populations exist across them. For example, in a magnetic field of 9.4 T such as that found in routine high-resolution NMR spectrometers, the difference in spin population will only be around 1 in 32,000 for ^{1}H. Unfortunately, ^{1}H nuclei are the most sensitive and for ^{19}F, ^{31}P, ^{13}C, and ^{15}N, the next most common nuclei to be studied, the sensitivity problem is even more acute, with the associated signal decreasing by factors of 1.2, 15, 64 and 10^4 respectively. The problem is further exacerbated when the natural abundance of ^{13}C (1.108%) and ^{15}N (0.37%) isotopes are taken into account, meaning the effective differences in sensitivity scale from 1 in 32,000 for ^{1}H to 1 in 120 million and 1 in 8.7 billion in these nuclei respectively. As a result of this, general routine human imaging experiments are

restricted to measuring the ^{1}H signals coming from water and lipid in tissues. Furthermore the direct detection of non-proton signals can require many hours of measurement even in NMR.

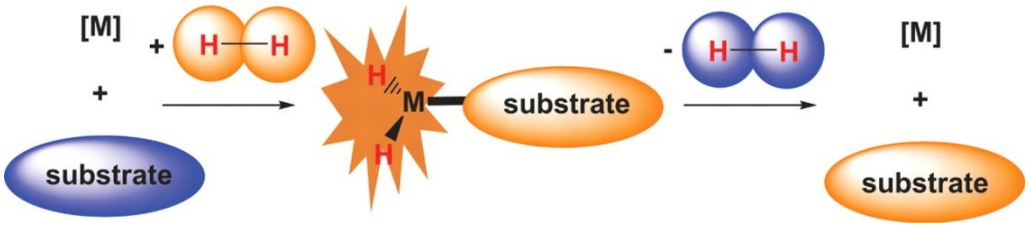
A number of "hyperpolarization" methods have been developed (*8-18*) to enhance signal strength by transferring non-equilibrium nuclear spin polarizations. The method of dynamic nuclear polarization (DNP), as reviewed in (*10)*, creates a non-Boltzmann spin population by transfer of polarisation from an unpaired electron. Recently this approach has been demonstrated to usefully enhance ^{13}C and ^{15}N signals by factors that exceed 10,000 (*11, 12, 13, 14*). Currently, however, this method requires long polarization times (often over 6 hours), normally employs water and methanol as solvents and is unable to detect enhanced proton signals routinely.

Here we tackle the sensitivity problem in a different way by using para-H_2 as the source of polarization. Para-H_2 has the advantage that it can be prepared easily and stored at room temperature for months. Previously, studies with para-H_2 have been limited to those involving the formation of hydrogenation products containing para-H_2 derived protons (*15*). For example, Pines *et al.* have recently used it to image heterogeneous hydrogenation reactions through the detection of polarized protons of propane (*16*). More usually, however, it is the imaging of ^{13}C-based magnetization that is targeted because such nuclei can be polarized by para-H_2 based hydrogenation reactions in low magnetic field, as demonstrated by Bargon *et al.* (*17*) and exploited by Golman *et al*. (*12*). In these hydrogenative processes the newly formed reaction products contain protons originating from a single para-H_2 molecule and they can produce strongly enhanced NMR signals in the reaction product provided the reaction does not change the magnetic arrangement of these coupled atoms (*15*).

The need for a suitable hydrogen-acceptor, however, reflects a significant limitation of the existing approach. Nonetheless, the ability to increase proton signal strengths in such products by 32,000 with para-H_2 and hence detect pico-moles of material in a single scan has been established (*6, 18*). In order to generalize the use of para-H_2 as a source of polarization, a method for the transfer of polarization without the direct hydrogenation of materials is needed. Here we show that the temporary association of a substrate and para-H_2 via a transition metal center in low magnetic field can achieve just this. Thus, NMR spectral amplification by reversible exchange (NMR-SABRE) is achieved without any chemical modification of the hyperpolarized material. As an example, we use the labile complex $[Ir(H)_2(PCy_3)(substrate)_3][BF_4]$, which is formed by the reaction of $[Ir(COD)(PCy_3)(MeCN)][BF_4]$ (where Cy is cyclohexyl and COD is cyclooctadiene) with para-H_2 and an excess of the substrate to be polarized (*19*, *5*). Notably, the same observations can be made on a range of materials and metal templates according to the concept illustrated in Scheme 1.

We first illustrate this effect using pyridine as the substrate where the iridium dihydride complex $[Ir(H)_2(PCy_3)(pyridine)_3][BF_4]$ is formed. The single scan ^{1}H NMR spectrum shown in Fig. 1A was recorded after the sample was first polarized in a magnetic field of around 2×10^{-2} T and contains signals with enhanced intensity for the three proton sites of the pyridine substrate. All that is necessary to achieve this result is to shake the sample in low magnetic field (Movie S1). This dissolves fresh para-H_2 from the head

space above the solvent, allowing it to associate with the metal complex, thereby activating the polarisation transfer process in the solution. Specifically, the signals for free pyridine at δ 7.84, 8.54, and 7.43 appear in the downward direction that is most simply described as emission. After the sample is then left in the NMR spectrometer for ten minutes, the resulting NMR spectrum demonstrates that the pyridines magnetic states have returned to their more usual Boltzmann arrangement distribution through relaxation. We note that the resonances illustrated for free pyridine in Fig. 1A can be described as 'hyperpolarized'.



Scheme 1. Schematic representation of magnetization transfer process.

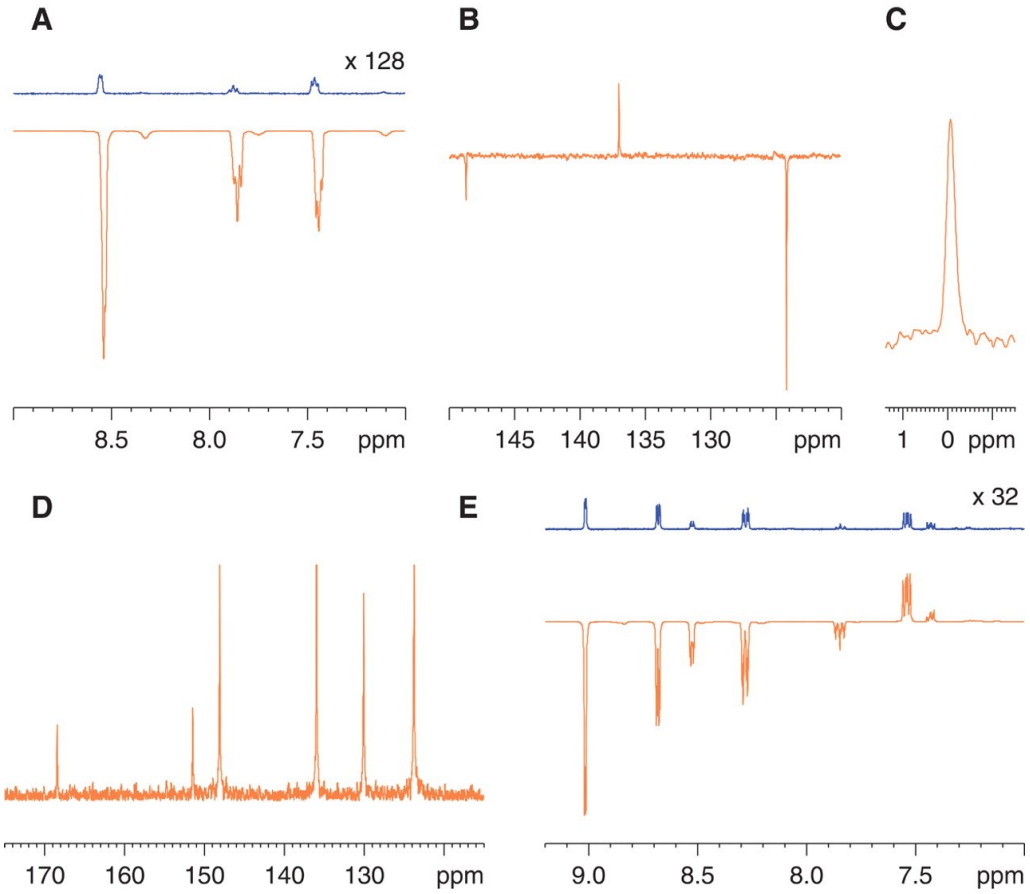


Figure 1. Single scan NMR spectra of samples containing a templating medium, the indicated substrate, and para-H_2 at 295 K in d_4-methanol where the polarisation transfer step was achieved in a 2 x 10^{-2} T field. (A) ^{1}H control trace (upper) of 6 nanomoles of pyridine with 128 fold vertical expansion relative to the lower ^{1}H trace

that was recorded immediately after polarisation transfer; (B) Polarized ^{1}H decoupled ^{13}C trace of the same sample after refocusing. (C) Polarized ^{1}H decoupled ^{15}N trace, with refocusing, of a sample containing 25 nanomoles of ^{15}N labelled pyridine; (D) Polarized ^{1}H decoupled ^{13}C trace after refocusing, in magnitude, of a sample containing 50 μmoles of nicotinamide; (E) ^{1}H NMR spectrum of the same nicotinamide sample showing the control trace (upper), 32 fold vertical expansion relative to the polarized trace (lower) with transfer in a 5 x 10^{-4} T field.

When the region of the ^{1}H NMR spectrum containing the pyridine signals is examined in more detail, weaker signals for the bound pyridine ligands within the host-ligand complex $[Ir(H)_2(PCy_3)(pyridine)_3][BF_4]$ are also seen to show this emission character. We conclude therefore that whilst at low field, spontaneous polarization transfer occurs between para-H_2 and the pyridine substrate that is in temporary association with the metal template. Furthermore, substrate exchange with that bound in the host-ligand template during this period leads to the build-up of hyperpolarization in free pyridine. When these enhanced signals are compared to those without enhancement, up to 550 fold increase in signal strength is observed. The enhancement achieved using this simple process is realised after just a few seconds of contact when the sample in low field (sMovie 1).

This enhancement effect is not just limited to proton signals since the corresponding ^{13}C (Fig. 1B) and ^{15}N resonances (Fig. 1C) of pyridine are also polarized. Furthermore, these effects can be regenerated by simply removing the sample from the spectrometer and bringing it into contact with fresh para-H_2 in low magnetic field. When the hyperpolarized ^{13}C NMR spectrum is compared to that obtained when a standard ^{13}C NMR spectrum is recorded, it would take 670,000 scans to achieve equal signal intensity to that seen with only one scan after the para-H_2 enhancement process (*5*). The time saved through this 823-fold signal enhancement has been estimated to exceed 3 months assuming that the individual measurements are separated by a 20 second recovery delay and use 90^{o} observation pulses. If the NMR spectra of 100% ^{13}C enriched materials were compared to those obtained from para-H_2 enhancement of the un-enriched material, an eight fold gain in sensitivity would still be apparent. Of course these enriched materials could themselves be easily polarized and therefore yield even larger signals.

These methods can be extended beyond pyridine and, in Fig. 1D, we illustrate a ^{13}C spectrum of nicotinamide that is collected in the same way; 345 fold ^{1}H signal enhancements have been quantified for this system based on spectra such as that shown in Fig 1E. All of the ^{13}C resonances for nicotinamide are enhanced, although not to the same degree (*5*). We note that 3-fluoropyridine, nicotine, pyridazine, quinoline, quinazoline, quinoxaline and dibenzothiophene also show enhancement and that ^{19}F and ^{31}P signals can also be detected (*5*). These substrates associate weakly with the metal complex through their basic donor sites thereby facilitating the polarisation transfer step. NMR spectra representative of these materials can be found in the supplementary information (sFig. 2 and sFig. 3).

We have recently described a gradient-based NMR method called Only Parahydrogen Spectroscopy (OPSY) that suppresses signals derived from nuclei with thermally equilibrated spin state populations whilst allowing the observation of signals from para-H_2 derived protons found in hydrogenation products (*20*). This method works by selectively probing the longitudinal two-spin order term that is generated for coupled spins derived from para-H_2 whilst dephasing terms from the usual thermal magnetisation through the use of pulsed field gradients. When it is applied here, all thermal signals are successfully suppressed and those for the hyperpolarized molecules still remain. As a result, NMR spectra of polarized substrates can be recorded in the presence of a protio solvent. The corresponding ^{1}H selected OPSY spectral trace showing signals for polarized pyridine, collected in protio methylenechloride, is shown in Fig. 2A.

This polarisation transfer process is predicted to generate spin states that are unaffected by dipole-dipole relaxation mechanisms and as such to have long lifetimes in low field (*5*). The potential impact of long lived states in NMR has been highlighted by Levitt in a different context (*21*). The OPSY-based ^{1}H NMR spectrum in Fig. 2B was recorded 150 seconds after polarization. This illustrates the presence of the longitudinal two-spin order magnetic state associated with a pair of coupled hydrogen atoms within polarized pyridine.

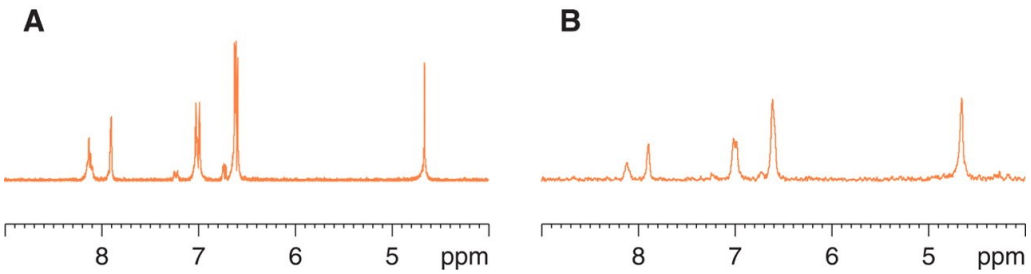


Figure 2. (A) Unlocked ^{1}H NMR spectrum recorded in protio-CH_2Cl_2 using the OPSY filtration sequence to suppress background signals illustrating the detection of 60 μmoles of polarized pyridine in a single scan. (B) ^{1}H NMR spectrum recorded on the same sample using the same pulse sequence but recorded 150 s after the low field polarisation step.

These results suggest a route for the use of naturally occurring molecules as contrast agents in MRI. In this regard, Fig. 3 shows two separate single-average True-FISP based MRI images of pyridine collected over 0.7 seconds on an 8 mm sample tube containing cylinders of 1 mm internal diameter (*22*). We collected the first trace on a 0.5 mm slice using polarized pyridine in d_4-methanol while the second was collected on the same sample after the pyridine polarisation had decayed, albeit over a 20 mm slice thickness with half the in-plane resolution in both directions; this introduces an inherent 160 fold increase in signal strength between the two measurements before any additional signal enhancement is considered. Even under the latter conditions, no

image is visible illustrating both the viability of this approach in MRI imaging and that the sensitivity gain is over 160 fold.

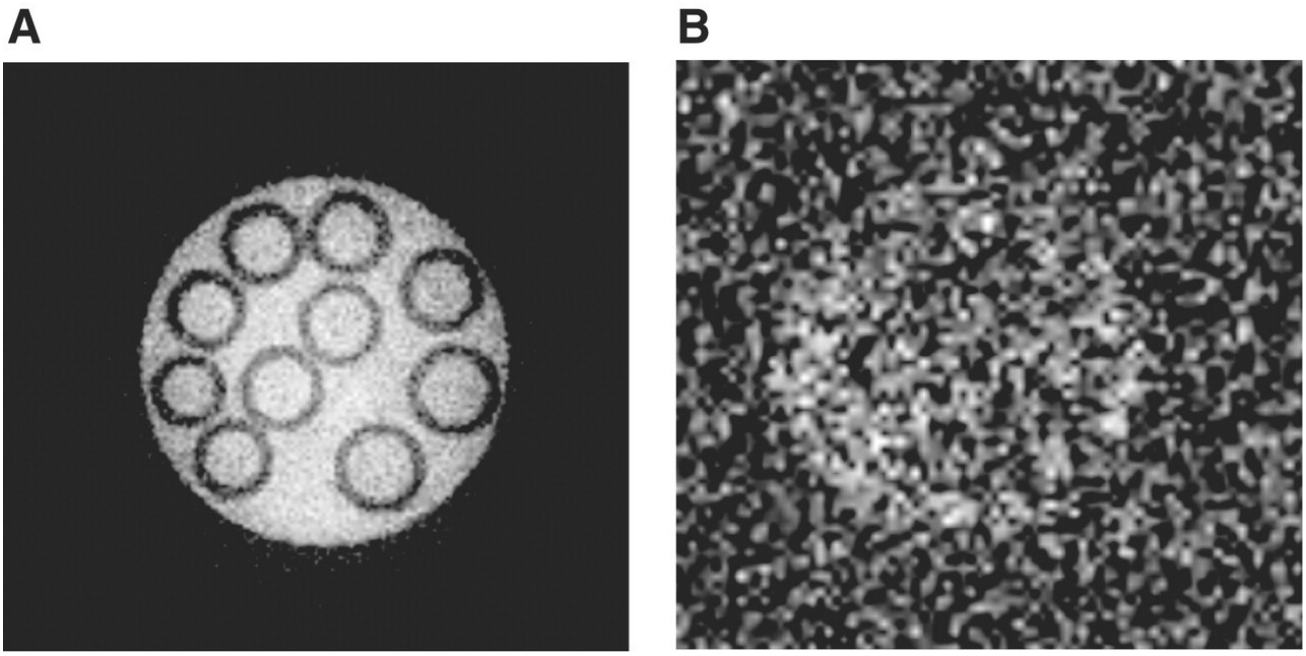


Figure 3. Single average ^{1}H True-FISP MRI images of an 8 mm sample tube containing glass cylinders with 1 mm internal diameter showing: (A) signals from polarized pyridine in d_4-methanol within a 0.5 mm slice; (B) signals from the same sample after the decay of polarisation for a 20 mm slice thickness.

The procedures described in this paper are relatively cheap to implement and can produce large amounts of hyperpolarized materials in a short time. Furthermore, the video sequence [see supplementary information (Movie S1)] illustrates the simplicity of the measurement process we have described. The method can be used on routine proton based MRI instruments without the need to exploit other magnetically active nuclei that provide much weaker signals. Given the opportunities that hyperpolarized methods have been shown to offer for the development of real-time metabolic imaging applications, the results presented here take us a step further toward the goal of responsive high sensitivity MR based methods for the diagnosis of disease (*10*).

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