Micron-scale SABRE-enhanced NV-NMR Spectroscopy

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Optically-probed nitrogen-vacancy (NV) quantum defects in diamond can detect nuclear magnetic resonance (NMR) signals with high-spectral resolution from micron-scale sample volumes of about 10 picoliters. However, a key challenge for NV-NMR is detecting samples at millimolar concentrations. Here, we demonstrate an improvement in NV-NMR proton concentration sensitivity of about 10^5 over thermal polarization by hyperpolarizing sample proton spins through signal amplification by reversible exchange (SABRE), enabling micron-scale NMR of small molecule sample concentrations as low as 1 millimolar in picoliter volumes. The SABRE-enhanced NV-NMR technique may enable detection and chemical analysis of low concentration molecules and their dynamics in complex micron-scale systems such as single-cells.

Nitrogen vacancy (NV) quantum defects in diamond are a leading modality for sensitive magnetometry with high spatial-resolution and operation under ambient conditions [1, 2], including for nuclear magnetic resonance (NMR) spectroscopy at small length scales (nanometers to microns) [3–8]. Initial work on NV-NMR spectroscopy [3–8] suffered from low spectral resolution (kHz), due to the short decoherence time of the NV centers. To overcome this problem, Glenn et al. [9] implemented a coherently averaged synchronized readout (CASR) technique and demonstrated an NV-NMR spectral resolution of a few Hz at 88 mT on a micron-scale sensing volume. However, due to the finite sensitivity of the NV-NMR sensor, its application is restricted to highly concentrated pure samples, which limits its utility for most chemical and biological problems.

Recently, Bucher et al. [10] employed dynamic nuclear polarization (DPN) based on the Overhauser mechanism [11], where polarization is transferred to the sample nuclear spins from the electronic spins of dissolved molecular radicals, and obtained two orders of magnitude proton number sensitivity enhancement for micron-scale CASR NV-NMR. However, the DNP sensitivity enhancement is limited by the finite electronic spin polarization at the low magnetic fields and ambient temperatures used for NV-NMR. Higher sample nuclear spin polarization and thereby improvement in NMR sensitivity can be achieved through a parahydrogen-based signal amplification by reversible exchange (SABRE) process [12–14]. To date SABRE has been shown to enhance conventional inductive NMR sensitivity in mL to µL scale sensing volumes [12–17].

Here, we integrate SABRE hyperpolarization with CASR NV-NMR to realize five orders of magnitude enhancement in concentration sensitivity relative to thermal nuclear spin polarization - in a micron-scale sample. Using our method, we measure the NMR spectrum from small molecule samples with concentrations as low as 1 millimolar and at a sensing volume of 10 picoliters. The high spectral resolution of SABRE NV-NMR enables the measurement of J-couplings in dilute molecules, thereby providing chemical specicity at a low magnetic eld of about 6.6 mT.

The experimental set up is shown in Fig. 1(a). The NV-NMR sensor is a (2 × 2 × 0.5) mm³ high purity diamond chip with 13 µm thick NV layer and an NV concentration of 3 × 10^15 cm^-3. The [111] axis of the NV-NMR sensor is oriented parallel to the bias magnetic field (B₀ ≈ 6.6 mT), which is generated by a feedback-stabilized electromagnet [9]. A green optical beam (λ = 532 nm) in a total internal reflection configuration with a spot diameter of ∼15 µm is used to initialize and readout the electronic spins of the NV-NMR sensor [9]. A single-
FIG. 1. **NV-NMR sensor integrated with signal amplification by reversible exchange (SABRE).** (a) Experimental schematic of the ensemble NV-NMR sensor. A 532 nm optical beam illuminates the diamond chip via a total internal reflection configuration. A microwave antenna on the diamond chip drives the electronic spins of the NV centers. Parahydrogen diffused into the sample through the capillary tube, initiates the SABRE reaction, and thereby hyperpolarizes sample proton spins via an Iridium-based catalyst in the sample solution. The hyperpolarized NMR signal is sensed using the electronic spins of the ensemble of NV centers. (b) Energy level diagram for the nitrogen vacancy (NV) centers in diamond. The expanded view shows the Zeeman splitting of the ground triplet state $^3A_2$ in the presence of a magnetic field. (c) SABRE hyperpolarization process. The SABRE catalyst is in reversible exchange (indicated by red arrows) with parahydrogen (left side) and a small molecule substrate (e.g., pyridine, right side). In the transient (ms) bound state of the catalyst-substrate complex (center), spin order flows from the hydrides to the substrate leading to polarization built up on the free substrate in solution, which is the sample to be probed with NV-NMR. (d) Pulse sequence for SABRE hyperpolarization and NV-NMR detection. The parahydrogen, bubbled into the sample solution, activates the catalyst and hyperpolarizes the small molecule substrate (the sample). A $\pi/2$ pulse induces a free nuclear precession (FNP) signal from the hyperpolarized sample, which is detected by NV sensor spins via a coherently-averaged synchronized readout (CASR) pulse sequence.

coil wire loop antenna [18], placed directly above the NV surface of the diamond is used to drive the electron spin resonance transitions of the NV centers. At the proton NMR frequency of 280 kHz for a 6.576 mT bias field, the AC magnetic field sensitivity ($\eta_B$) of the NV ensemble sensor is $35(2)$ pT/$\sqrt{\text{Hz}}$ [19]. The liquid sample is placed directly on top of the diamond surface. The thickness of the NV layer and the spot diameter of the optical beam provides an effective NMR sensing volume of about 10 pL [9, 10].

Hyperpolarization of proton spins in the sample molecules is obtained through SABRE. Parahydrogen gas is first dispersed into the sample solution (Fig. 1(a)) for about 20 minutes to activate an Iridium-based catalyst [19], which then mediates reversible exchange of spin order between the parahydrogen and the small molecule substrate - the sample to be probed with NV-NMR - as shown in Fig. 1(c). Once activated, about 30 s of additional parahydrogen bubbling is sufficient to establish hyperpolarization on the substrate. During the transient lifetime of the catalyst-substrate complex (on the order of ms), proton spin order flows from the hydrides (in parahydrogen) to protons in the bound small molecule substrate (e.g., pyridine). Lastly, the hyperpolarized substrate dissociates, to give free hyperpolarized small molecules in solution with polarization lifetime $T_1 \sim 5$ s. The polarization transfer process is resonant at about 6.6 mT, where the $J$-coupling between the hydrides equals the frequency difference between hydride and substrate proton spins, leading to a level-anti-crossing between the singlet state of the hydrides and the proton spin-down states of the substrate. In summary, spin evolution and chemical exchange continually pump hyperpolarization into free small molecules in solution, as long as the parahydrogen is periodically refreshed by bubbling between NV-NMR measurements. Details of the home-built parahydrogen generation, its integration with the NV-NMR sensor, and the SABRE polarization transfer mechanism are discussed in the supplementary material [19].

After a one second wait time following SABRE hyperpolarization, a $\pi/2$ RF pulse is applied resonant with the nuclear spins of the sample. The induced Larmor precession of the nuclear spin results in a decaying oscillatory magnetic field called free nuclear precession (FNP). The NV-NMR sensor is then probed using a CASR pulse se-
sequence [9], which detects the FNP signal and maps it onto a population difference of the NV ensemble electron spin states. The population difference is read out optically by spin state-dependent fluorescence for 1 µs, followed by optically reinitializing the NV electronic spins for 4 µs.

**FIG. 2. SABRE-enhanced NV-NMR spectra of pyridine.** Comparison of measured NV-NMR spectra of 100 mM pyridine sample with (red circles, 1 acquisition) and without (blue circles, $10^4$ acquisitions) SABRE hyperpolarization using a coherently-averaged synchronized readout (CASR) pulse sequence duration of 2 seconds. The solid red line is a Lorentzian fit to the SABRE-enhanced NV-NMR spectrum, giving a linewidth of 2.3(5) Hz and a signal enhancement of about $2.22(3) \times 10^5$, with a proton number sensitivity of 66.48(15) fmol/$\sqrt{Hz}$ for a signal to noise ratio (SNR) of 3. Inset: CASR resonance frequency $F_{res}$ of hyperpolarized pyridine (green squares) obtained by varying the bias magnetic field $B_0$. A linear fit (green line) of $F_{res}$ versus $B_0$ gives $\gamma_p = 42.5355(40)$ MHz/T, consistent with the gyromagnetic ratio.

As a first demonstration we detect the SABRE-enhanced NV-NMR spectrum of a sample of pyridine, a weakly-alkaline heterocyclic organic molecule. The sample solution is made with 100 mM concentration of pyridine and 5 mM concentration of catalyst dissolved in methanol. We also apply a calibrated test AC magnetic signal using a coil antenna [19]. The observed NV-NMR spectrum with (red dots) and without (blue dots) SABRE hyperpolarization is shown in Fig. 2. The expected thermally-polarized NV-NMR signal amplitude (without SABRE hyperpolarization) is 32 fT at a bias magnetic field of 6.6 mT [19]. The measured SABRE-enhanced NV-NMR signal has a FWHM line width of 2.3(5) Hz and an amplitude of 7.1(1) nT (by comparing with the amplitude of the test signal [19]), which is an enhancement of about $2.22(3) \times 10^5$ in signal amplitude over the expected thermally-polarized signal. The signal to noise ratio (SNR) of this single-shot hyperpolarized NMR signal is 320(3) for a measurement duration of 2 seconds. This result corresponds to a molecule number sensitivity of 13.3(3) fmol/$\sqrt{Hz}$ for pyridine and a proton number sensitivity of 66.48(15) fmol/$\sqrt{Hz}$, which is a two orders of magnitude improvement in proton number sensitivity compared to the Overhauser DNP technique applied to NV-NMR [10]. The sensitivity is defined relative to a signal to noise ratio (SNR) of 3, which is typical in conventional NMR [20]. The pressure, flow rate, and parahydrogen bubbling duration are optimized to achieve this enhancement in sensitivity [19]. The NV-NMR signal without hyperpolarization (blue dots in Fig. 2) is too weak to observe even after $10^4$ averages. We verify the hyperpolarized pyridine NV-NMR signal by measuring the signal resonance frequency $F_{res}$ as a function of applied bias magnetic field $B_0$, yielding a variation of $\gamma_p = 42.5355(40)$ MHz/T, consistent with the gyromagnetic ratio of the proton.

We next perform SABRE-enhanced NV-NMR spectroscopy (Fig. 3) by further diluting pyridine in methanol. Samples are prepared at concentrations of 100 mM, 30 mM, 10 mM, 5 mM, and 1 mM of pyridine dissolved in methanol. The ratio of pyridine concentration to the catalyst concentration (20:1) is kept constant [21]. A hyperpolarized NV-NMR spectrum is observed even at a concentration of 1 mM (10 femtomoles of pyridine molecules) with a signal-to-noise ratio (SNR) of 50 after

**FIG. 3. SABRE-enhanced NV-NMR measurement for variable pyridine concentrations.** (a) CASR detected NV-NMR spectra (blue circles) for hyperpolarized pyridine samples and its associated Lorentzian fits (solid red line) at concentrations of 100 mM, 30 mM, 10 mM, 5 mM, and 1 mM in methanol. (b) CASR detected NV-NMR signal amplitude (red dots) of hyperpolarized pyridine at various concentrations. The solid blue curve is a power function model of the form $ax^b + c$ with fit parameters $a = 0.042$, $b = 0.6855$, $c = 0.012$. 
averaging for 300 s (Fig. 3a). The red dots in Fig. 3b denote the detected NV-NMR signal amplitude at various pyridine concentrations. The error bars represent the standard deviation of the NV-NMR signal measured across three independent trials. A power function model of the form $ax^b + c$ is fit to the experimental data (Fig. 3b solid blue curve), in excellent agreement with the measurements for fit parameters $a = 0.042, b = 0.6855$, and $c = 0.012$. Deviations from a linear dependence are expected since SABRE hyperpolarization of fewer substrate molecules is more efficient than hyperpolarization of more substrate molecules [13, 21, 22], and thus the relative hyperpolarization decreases with increasing pyridine concentration. The model fit in Fig. 3b is repeatable and could be used, for a given SABRE NV-NMR system and within a calibrated concentration range, to quantify samples of unknown pyridine concentration.

To illustrate the versatility of our technique, we acquire SABRE-enhanced NV-NMR signals from two additional molecules (Fig. 4). First, we study $^{15}$N-labeled pyridine, which has a $J$-coupling of about 10 Hz between the nuclear spins of the protons and the $^{15}$N [17, 22]. The sample is prepared with a 100 mM concentration of $^{15}$N-labeled pyridine and a 5 mM concentration of catalyst dissolved in methanol. The hyperpolarized NV-NMR spectrum has an SNR of 150(5) (Fig. 4a) for a CASR pulse sequence duration of 3 seconds. This high-resolution spectrum has a linewidth of 3(1) Hz and shows well resolved peaks due to the $J$-coupling [23], with a splitting of $\Delta f_J \approx 10(1)$ Hz (Fig. 4a, solid red line) determined from a double Lorentzian fit. Finally, we measure the SABRE-enhanced NV-NMR spectrum of nicotinamide, a water-soluble form of vitamin B$_3$ (niacin), at 100 mM concentration. The observed NMR spectrum has an SNR of 200(4) (Fig. 4b) for a CASR pulse sequence duration of 2 seconds. The spectral linewidth from the Lorentzian fit (Fig. 4b, solid red line) is 4(1) Hz.

Nicotinamide has important functions in mammalian metabolism and is a metabolic precursor to NAD+/NADH [24–26]. With further development, we envision using our technique to observe the conversion of Nicotinamide to NAD+/NADH, which could allow NMR measurement of the redox status in cells. Hyperpolarized NV-NMR may also enable metabolic studies of healthy and diseased cells with dysregulated metabolism on the single-cell level. To increase the chemical specificity necessary for such applications, the SABRE technique can be implemented at a tesla-scale bias magnetic field. For example, in RF-SABRE methods [27–29], NMR pulse sequences are applied to the catalyst-substrate complex spins, allowing polarization transfer from parahydrogen derived hydrides to substrate molecules at any magnetic field. The current hyperpolarization enhancement can be further increased by implementing the SABRE method on a microfluidic-diamond chip, thereby increasing the contact area between the substrate and the parahydrogen [30, 31]. A factor of three times improvement in hyperpolarization can also be obtained by utilizing pure parahydrogen instead of the 50% parahydrogen produced by our home-built system [19].

In summary, we demonstrate about a $10^3$ improvement in NV-NMR proton concentration sensitivity over thermal polarization at 6.6 mT by hyperpolarizing sample proton spins through the technique of signal amplification by reversible exchange (SABRE). This advance augments the growing toolbox of techniques for sensitive, high-resolution NMR spectroscopy in micron-scale samples using NV quantum defects in diamond. Compared to other signal enhancement methods, such as room temperature Overhauser DNP [10] or direct flow-based prepolarization [31], SABRE provides significantly higher concentration sensitivity while being applicable to a wide range of small molecule analytes [21, 32, 33]. With planned extension to tesla-scale magnetic fields, SABRE-enhanced NV-NMR may become a high-impact tool for

FIG. 4. SABRE-enhanced molecular NV-NMR spectra. (a) Single-shot CASR spectrum of hyperpolarized $^{15}$N-labeled pyridine (blue circles) for a sensing duration of 3 seconds at a concentration of 100 mM. The double Lorentzian fit (solid red line) indicates a splitting of $\Delta f_J \approx 10(1)$ Hz (indicated by the vertical dashed lines) due to $J$-coupling between the $^{15}$N nucleus and the protons. Inset: Chemical structure of $^{15}$N-labeled pyridine (b) Single-shot CASR spectrum of hyperpolarized nicotinamide (blue circles) for a sensing duration of 2 seconds at a concentration of 100 mM. The Lorentzian fit (solid red line) gives a spectral linewidth $\approx 4(1)$ Hz. Inset: Chemical structure of nicotinamide.