Hyperpolarization of “Neat” Liquids by NMR Signal Amplification by Reversible Exchange

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**Supporting Information**

**ABSTRACT:** We report NMR Signal Amplification by Reversible Exchange (SABRE) hyperpolarization of the rare isotopes in “neat” liquids, each composed only of an otherwise pure target compound with isotopic natural abundance (n.a.) and millimolar concentrations of dissolved catalyst. Pyridine (Py) or Py derivatives are studied at 0.4% isotopic natural abundance $^{15}$N, deuterated, $^{15}$N enriched, and in various combinations using the SABRE-SHEATH variant (microTesla magnetic fields to permit direct $^{15}$N polarization from parahydrogen via reversible binding and exchange with an Ir catalyst). We find that the dilute n.a. $^{15}$N spin bath in Py still channels spin order from parahydrogen to dilute $^{15}$N spins, without polarization losses due to the presence of $^{14}$N or $^2$H. We demonstrate $P_{15N} \approx 1\%$ (a gain of 2900 fold relative to thermal polarization at 9.4 T) at high substrate concentrations. This fundamental finding has a significant practical benefit for screening potentially hyperpolarizable contrast agents without labeling. The capability of screening at n.a. level of $^{15}$N is demonstrated on examples of mono- and dimethyl-substituted Py (picolines and lutidines previously identified as promising pH sensors), showing that the presence of a methyl group in the ortho position significantly decreases SABRE hyperpolarization.

Nuclear spin polarization can be temporarily enhanced by >4 orders of magnitude through the process of hyperpolarization with corresponding improvements in sensitivity or signal-to-noise ratio (SNR).1–3 This significant sensitivity gain can also be used to decrease NMR acquisition time or analyte concentration,4 but more importantly hyperpolarization techniques enable preparation of large batches of hyperpolarized (HP) compounds, which can be used as contrast agents5 for various applications involving dynamic tracking in living organisms, including functional pulmonary imaging with HP $^{129}$Xe,5,6 metabolic cancer imaging with HP $^{13}$C agents,7,8 and many others.5 Advances in hyperpolarization techniques have enabled production of HP agents with sufficient payload (the product of nuclear spin polarization $P$ and concentration/quantity) for in vivo applications and ultimately for human clinical trials, as demonstrated using HP $^{13}$C-pyruvate for prostate cancer detection10 in men and HP $^{129}$Xe for lung imaging.6 Signal Amplification by Reversible Exchange (SABRE)11,12 is a particularly cost-efficient and fast hyperpolarization method that relies on exchange of a to-be-hyperpolarized substrate and parahydrogen ($\text{para-H}_2$) on a catalyst. Similarly to conventional parahydrogen-induced polarization (PHIP),13 SABRE utilizes para-$\text{H}_2$ as the source of spin order,14 and the entire hyperpolarization procedure can be completed in seconds; however, unlike PHIP, it does not require the irreversible chemical modification of the substrate.11,12 Until recently, demonstrations achieving efficient hyperpolarization via SABRE were generally limited to protons; while in some cases the resulting $^1$H polarization values were relatively high (e.g., $P \approx 8\%$15), such nonequilibrium polarization is relatively short-lived ($T_1 \approx 1$ min).15N hyperpolarization include LIGHT-SABRE (Low-Irradiation Generation of High Tesla-SABRE)16 and SABRE-SHEATH (SABRE in SHield Enables Alignment Transfer to Heteronuclei)17,18 using RF-irradiation-based and field-cycling-based approaches, respectively. SABRE-SHEATH17–19 is an advantageous approach because it only requires that the exchange reaction with para-$\text{H}_2$ be performed in a microTesla field. This condition can be created easily by shielding the Earth’s magnetic field using a mu-metal chamber and is therefore simple and inexpensive. $^{15}$N polarization levels

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of up to 10% were shown in the proof-of-principle demonstration. However, such hyperpolarization was achieved in dilute (4–45 mM) alcohol solutions; that is, the payload (the product of concentration and polarization) of the HP agents was not optimized, and alcohol solutions have limited biocompatibility.

Here, we demonstrate the feasibility of SABRE-SHEATH hyperpolarization of “near” liquids—each comprised only of an otherwise pure target compound and millimolar concentrations of dissolved catalyst, without any additional diluting solvent. In principle, such liquids could be used directly as hyperpolarized MRI contrast agents; the use of organic solvents is obviated, and we observe greater payload for the concentrated agents.

The previously developed setup for SABRE-SHEATH was utilized, wherein \( \text{para-H}_2 \) is bubbled through a liquid agent (e.g., pyridine (Py) or others) containing an activated catalyst (formed from the precursor: [IrCl(COD)(IMes)] (IMes = 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene; COD = cyclooctadiene). To date, this is the best catalyst for SABRE exchange processes involving \( \text{para-H}_2 \) and the substrate (e.g., Py) shown schematically in Figure 1 (this figure also describes the sample manipulation during \( ^{15}\text{N} \) SABRE-SHEATH process). Proton hyperpolarization via SABRE at milliTesla magnetic fields (6 ± 4 mT) was performed as described previously, with \( \text{para-H}_2 \) bubbling performed in the fringe field (6 ± 4 mT) of the 9.4 T magnet (instead of steps 1 and 2 shown in Figure 1A). See the Supporting Information (SI) for additional experimental details. We note that SABRE of para-H2 (conducted conventionally at 9.4 T) yielded very small signal enhancement of \( \epsilon \approx 4 \) (Figure 2B). The \( ^{15}\text{N} \) signal exhibited a strong, nearly linear dependence on the flow rate of \( \text{para-H}_2 \) in the range studied (the flow-rate of 150 standard cubic centimeters (sccm) represents an experimental limitation of our setup at \( \sim 7 \) atm), which was metered independently of the applied pressure and hence solution \( \text{para-H}_2 \) concentration (Figure 2C). Note that the \( ^{15}\text{N} \) signal enhancement was approximately independent of the \( \text{para-H}_2 \) pressure (and solution concentration according to Henry’s law), indicating that the flux of the available \( \text{para-H}_2 \) spin bath (the source of spin order) was indeed the limiting factor; that is, the potential possibility of exchanging more \( \text{para-H}_2 \) per unit time would likely yield greater \( ^{15}\text{N} \) signal enhancements. Larger \( \text{para-H}_2 \) exposure can be attained by higher pressures and smaller bubbles/better gas-phase—liquid-phase mixing.

The other important effect limiting the maximum achievable hyperpolarization is spin–lattice relaxation. The \( ^{15}\text{N} \) spin–lattice relaxation time is significantly shorter in microTesla fields than at high field (9.4 T), 5.5 ± 0.5 versus 60.8 ± 0.6 s, respectively (Figures 2E,F), and such efficient relaxation results in SABRE-SHEATH \( ^{15}\text{N} \) enhancements reaching significantly lower steady-state levels after the hyperpolarization procedure. More importantly, the supply of \( \text{para-H}_2 \) is limited because only \( \sim 0.1 \) mmol/s pass through the tube at the maximum flow rate of 150 sccm, whereas 90 mM catalyst ([~0.4 mL volume) alone is capable of exchanging of \( \sim 0.2 \) to 0.4 mmol/s of \( \text{H}_2 \) because the hydrogen exchange rate is \( \sim 5–10 \) per s. However, Ir-hydride protons do not have 100% exchange efficiency with \( \text{para-H}_2 \) gas. Instead, this exchange is further constricted by at least two major bottlenecks: (i) exchange of \( \text{H}_2 \) between gas and liquid phases and (ii) exchange of dissolved \( \text{para-H}_2 \) with Ir-hydride. Note that equilibrium \( \text{H}_2 \) concentration in organic solvents is <4 mM/atm;24–26 that is, even at the maximum \( \text{para-H}_2 \) pressure used (~7 atm), \( \text{para-H}_2 \) concentration is <30 mM, that is, at least three times lower than that of the Ir-hydride catalyst at 90 mM concentration. Moreover, when \( \text{para-H}_2 \) singlet spin order is transferred to Py via SABRE, \( \text{para-H}_2 \) becomes ortho-H2, manifesting as an HP byproduct,27 and this resulting ortho-H2 can no longer serve as a source of hyperpolarization in conventional ex situ SABRE. Furthermore, hydride proton exchange rates are on the order of 10 per second,23 therefore, each \( \text{para-H}_2 \) molecule on average experiences >30 exchanges per second under these conditions ([catalyst] of ~90 mM results in the total of ~900 \( \text{para-H}_2 \) exchanges per second for <30 mM [\( \text{para-H}_2 \) dissolved]. The main implication of the above two bottlenecks, the fast hydrogen exchange and the limited flux of para-H2 gas, is that \([\text{ortho-H}_2]\) \( \gg \) \([\text{para-H}_2]\). Furthermore, the additional feature of the complex interplay of microTesla \( ^{15}\text{N} \) effective \( T_1 \), and limited access to \( \text{para-H}_2 \) is that it should imply the existence of an optimal catalyst concentration and an optimal ratio of Py to catalyst concentrations. Taken together, these results indicate that greater signal enhancements are potentially feasible, provided that engineering issues limiting \( \text{para-H}_2 \) access for SABRE hyperpolarization are solved.

The additional condition that the finite \( \text{para-H}_2 \) spin bath is limiting the SABRE processes is also seen when n.a. Py (\( \epsilon \approx 2900 \)) was replaced by 99% \( ^{13}\text{N} \) enriched Py (\( ^{13}\text{N-Py}, \epsilon \approx 33 \), Table 1. \( ^{13}\text{N} \) signal enhancement decreases by nearly 2 orders of magnitude (88-fold), while the concentration of \( ^{15}\text{N} \) spins is increased by 278 fold (= 1/0.0036); however, note that the total Py concentration and quantity is maintained the same. In summary, the observed signal (given by \( ^{13}\text{N} \) and \( \epsilon \)) only decreases by 3 fold when working with n.a. Py.
Another important aspect in this context is that $^{15}$N microTesla effective $T_1$ of $^{15}$N-Py (10.2 ± 1.1 s) is actually longer than that of n.a. Py (5.5 ± 0.5 s); see Table 1. Furthermore, achieving such significantly greater (by 88-fold) $^{15}$N $\epsilon$ in n.a. Py with respect to $^{15}$N-Py under the conditions of limited access to para-$H_2$ has a major significance for the mechanistic understanding of the SABRE-SHEATH phenomenon. In particular, this result indicates that the hyperpolarization para-$H_2$ spin bath is not depleted when the exchanging substrate on Ir-hydride catalyst is $^{14}$N-Py. If no interaction between para-state of hydride and $^{15}$N-Py occurs (i.e., the exchanging partner is $^{14}$N-Py), para-state of hydride should exchange back into para-$H_2$ with preservation of the para-$H_2$ hyperpolarization pool (Figure 4B). This is an important conclusion because the spin order residing in the entire pool of para-$H_2$ can be selectively channeled to hyperpolarize $^{15}$N nuclei of the exchangeable substrate (e.g., n.a. Py) rather than (say) being depleted by rapidly relaxing $^{14}$N sites acting as hyperpolarization sinks. This finding fundamentally enables achieving relatively high levels of $^{15}$N hyperpolarization (e.g., $P_{^{15}N} \approx 1\%$), even when performing SABRE-SHEATH in the high substrate concentration regime encountered with effectively neat solutions and when the supply and transport of para-$H_2$ are restricted. The $^{14}$N species likely do not deplete the para-$H_2$ state because the quadrupolar relaxation rate of the $^{14}$N spins is faster than the $J$-coupling.

Figure 2. SABRE of “neat” natural abundance $^{15}$N (0.36%) pyridine (Py). (A) $^{15}$N SABRE-SHEATH hyperpolarized spectrum (red) and the corresponding thermally polarized reference spectrum (blue) after 192 signal averages. (B) $^1$H SABRE spectrum of hyperpolarized (red) sample in milliTesla magnetic field (~6 mT) and the corresponding NMR spectrum using thermally polarized sample (blue). (C) Effect of the para-$H_2$ flow rate (measured in standard cubic centimeters per minute or sccm) on $^{15}$N signal enhancement at ~90 mM catalyst concentration under five para-$H_2$ pressure values. (D) Effect of [Py] to [catalyst] ratio on $^{15}$N signal enhancement using 120 sccm flow rate under ~7 atm of para-$H_2$ pressure. (E) $^{15}$N SABRE-SHEATH dependence (modeled as exponential decay) as a function of the sample exposure to the microTesla magnetic field after stopping para-$H_2$ bubbling time. (F) $^{15}$N $T_1$ decay at 9.4 T. The experiments in panels E and F are conducted using ~90 mM catalyst concentration (~140:1 [Py] to [catalyst] ratio) at 120 sccm flow rate and ~7 atm para-$H_2$ pressure.
interactions that would otherwise transfer hyperpolarization to the target spins, hence, the $^{14}\text{N}$ spins are effectively (self-)decoupled from the bound para-H$_2$.

Our previous theoretical model of SABRE-SHEATH, while appropriate for $^{15}\text{N}$-enriched substrates, no longer applies for n.a. Py, and hence we present an amended theoretical model to describe the polarization transfer in the n.a. case. The original model invokes an AA′BB′ four spin system, where AA′ represents the parahydrogen-derived hydrides and BB′ represents the equatorial (exchangeable) $^{15}\text{N}$ spins depicted in Figure 3A. For this case, we originally derived that the strong quadrupolar interaction decouples the $^{14}\text{N}$ spin from the depicted spin systems.

In the SI we show that for the three-spin system the resonance condition is

$$\Delta \nu_{\text{HN}} = |J_{\text{HH}} - (J_{\text{HN}} + J_{\text{HN}})/4|$$

(3)

In the three-spin system it is also the NH-J couplings that drive the hyperpolarization transfer; here it is specifically the term $(J_{\text{HN}} - J_{\text{HH}})/(2\sqrt{2})$, which determines the rate of hyperpolarization transfer. Full derivation and additional details are given in the SI.

Next, we also performed conventional homonuclear $^1\text{H}$-SABRE experiments. The $^1\text{H}$ signal enhancements, which are optimized in the milliTesla regime (Table 1) followed the general trend seen for $^{15}\text{N}$ SABRE-SHEATH, with signal enhancements being greater when the proton spin bath of to-be-hyperpolarized substrate was reduced. For example, $\varepsilon \approx (−)60$ was observed for Py-$d_5$ versus $\varepsilon \approx (−)4.2$ for n.a. Py, which is in agreement with previous studies.$^{15}$

Because $^{14}\text{N}$ and other quadrupolar nuclei could have the potential to act as direct or indirect hyperpolarization sinks (e.g., polarization transfer from Ir-hydride protons to $^{14}\text{N}$, D, etc. or from $^{15}\text{N}$ (after hyperpolarization transfer from para-H$_2$) to $^{14}\text{N}$, D, etc.) at such low magnetic fields (analogous to interaction between $^{129}\text{Xe}$ and $^{131}\text{Xe}$ in xenon lattices$^{27}$), and because the local molecular environment can significantly alter the $^{15}\text{N}$ effective $T_1$ in the microTesla field regime, $^{15}\text{N}$ SABRE-SHEATH of deuterated Py (Py-$d_5$) was studied as well as various mixtures of $^{15}\text{N}$-Py and Py-$d_5$ with $^{15}\text{N}$-Py and n.a. Py (Table 1). Note that the Py type (i.e., n.a. Py, Py-$d_5$, or 15N-Py) used during the activation period determines the spin configuration of Py in the axial nonequivalence position of the hexacoordinate Ir-hydride complex, whereas the abundance of the Py type in the mixture determines the most probable type of exchangeable Py in the two equatorial positions. (The corresponding most-probable configurations are summarized in the SI.) Deuteration of to-be-polarized $^{15}\text{N}$-substrate had the most detrimental effect on microTesla $^{15}\text{N}$ effective $T_1$, a decrease from 5.5 ± 0.5 to 2.2 ± 0.1 s for n.a. Py versus Py-$d_5$ (row 1 vs row 2 of Table 1). A similar but slightly larger decrease (from $\varepsilon \approx (−)2900$ to $\varepsilon \approx (−)850$) was observed for the corresponding SABRE $^{15}\text{N}$ enhancement values, indicating that the majority of deuteration-induced depolarization is due to indirect transfer, for example, from $^{15}\text{N}$ to $^1\text{H}$.$^{28}$ However, the direct depolarization losses are likely to have a

![Figure 3](image-url)
significant contribution as well. For example, in cases when nondeuterated $^{15}$N-Py was used in combination with Py-$d_5$, microTesla $^{15}$N effective $T_1$ is actually greater when the catalyst is first activated with Py-$d_5$ versus that when catalyst is first activated with $^{15}$N-Py, $15.1 \pm 2.3$ versus $10.1 \pm 0.8$ s (remembering that the activation order defines the non-exchangeable ligand in the axial position), but the $^{15}$N signal enhancements were somewhat lower, $\varepsilon \approx (\sim)400$ vs $(\sim)520$, indicating that at least some polarization losses occurred on the hyperpolarized Ir-hydride due to the presence of deuterium in the catalyst structure. This particular finding at first seems to contradict recent studies where deuterium was incorporated in both catalyst$^{29}$ and exchangeable substrate$^{15,30}$ in the original homonuclear SABRE but can be explained by the different field regimes (microTesla vs several milliTesla) involved and by the fact that the previous efforts involved hyperpolarization of protons in exchangeable substrate instead of the present focus on $^{15}$N nuclei polarized using $^{15}$N SABRE-SHEATH$^{17}$.

The effect of $^{14}$N presence in the catalyst structure as a potential relaxation or polarization sink was studied by comparing two samples prepared using a mixture of $^{15}$N-Py and n.a. Py (consisting mostly of $^{14}$N-Py), rows 6 and 7 of Table 1. Activation of SABRE catalyst with $^{15}$N-Py versus n.a. Py resulted in a slight increase in the microTesla $^{15}$N effective $T_1$ ($9.9 \pm 1.1$ vs $8.2 \pm 1.1$ s) as well as the $^{15}$N signal enhancement ($\varepsilon \approx (\sim)450$ vs $(\sim)380$), indicating that $^{15}$N presence indeed can act as a weak relaxation or polarization sink, likely through contributions from both mechanisms; that is, direct transfer from hyperpolarized Ir-hydrides and from exchangeable $^{15}$N-Py. To summarize, the above evidence advocates for avoiding the utilization of quadrupolar nuclei (e.g., deuterium and $^{14}$N studied here) for $^{15}$N SABRE-SHEATH hyperpolarization processes, whose presence can result in reduced hyperpolarization in microTesla fields.

As previously described, the $^{15}$N SABRE-SHEATH of neat liquids is an advantageous tool for efficient hyperpolarization of $^{15}$N spins, particularly at their low natural abundance level. One potential use is for rapid compound screening, which we demonstrate here on a series of picolines and lutidines shown in Figure 5. We find that the presence of a methyl group in position 2 or 6 results in no detectable $^{15}$N hyperpolarization via SABRE-SHEATH, whereas the substituents in other positions result in $^{15}$N signal enhancements levels similar to those of Py. The obvious explanation is that steric hindrance induced by the presence of methyl groups in ortho positions significantly alters the time scale of the SABRE exchange process or reduces the association constant.

We chose to examine picolines and lutidines because it was previously shown that pH-mediated protonation of N-heterocyclic compounds can be useful for in vivo pH imaging using conventional proton-based nonhyperpolarized sensing, where the difference in $^{15}$N chemical shift induced by the agent protonation can be useful for pH imaging provided that the agent’s $pK_a$ is in the physiologically relevant range$^{31,32}$. $^{15}$N centers of the Py class screened here were also identified as promising hyperpolarized pH sensors with potential biomedical application to noninvasively image local variances in tissue pH$^{33}$. Unlike previously demonstrated pH imaging with hyperpolarized H$^{13}$CO$_3^-$/$^{13}$CO$_2$ that relies on the measurement of the ratio of two exchanging species, pH imaging using hyperpolarized $^{15}$N heterocycles relies on the modulation of $^{15}$N chemical shift, which changes by up to 100 ppm between

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**Figure 4.** Diagrams of para-$H_2$ exchange and $^{15}$N SABRE-SHEATH hyperpolarization in the absence (A) and in the presence (B) of $^{14}$N-Py excess. Note that the exchange with $^{15}$N-Py does not cause a significant reduction in the spin order of the para-$H_2$ pool. It should also be noted that both equatorial pyridines of the active complex undergo the chemical exchange with free Py in solution, while the axial pyridine (labeled as “Py”) is not exchangeable.
protonated and deprotonated states.33,35 This feature offers a significant sensitivity advantage because only one species requires detection (i.e., ratimetric measurements are not needed), and low signal-to-noise ratio would not affect the accuracy of the measurement because the chemical shift reports on the pH. Moreover, hyperpolarized 15N sites have significantly longer $T_1$ in aqueous media ($>30$ s)33 compared with 13C bicarbonate ($<10$ s),34 which can also be a significant advantage for in vivo applications (especially relevant for applications involving cancer, given the known hallmarks of elevated glycolysis and mildly acidic microenvironments).36,37 We note that the 15N signal enhancements reported in Figure 5 are obtained in a nonoptimal setup, and thus they could potentially be increased through improved apparatus design, allowing for better access to the hyperpolarization source of para-H2 (as well as reduced transit times to high field for detection). Moreover, the combination of heterogeneous SABRE catalysts with the method presented here may allow preparation of pure hyperpolarized liquids because such solid-phase catalysts can be separated38 and recycled.39 Nevertheless, the reported 15N signal enhancement values are already comparable to 1H enhancements previously reported using DNP technology and a commercial DNP hyperpolarizer.35 However, the method reported here achieves the steady-state maximum hyperpolarization level in <1 min without sophisticated equipment, versus ∼2 h using expensive DNP hyperpolarizers.10 It should be noted that unlike PHIP or DNP technologies, which have been successfully tested in vivo using relevant biomolecules, SABRE technology has not yet been demonstrated for in vivo use. SABRE for hyperpolarization of 129Xe has been demonstrated on a model Py molecule and applied to human lung.30–32

In conclusion, 15N SABRE-SHEATH of neat liquids was successfully demonstrated on a model Py molecule and applied as a screening technique for mono- and dimethyl-substituted Py compounds shown to be promising for minimally invasive pH imaging.33 Catalyst access to para-H2 was found to be the limiting factor for achieving 15N polarization levels beyond 1%. The 15N SABRE-SHEATH process was found to be selective for utilizing the spin order of the para-H2 spin bath for hyperpolarization of 15N versus 14N, enabling efficient hyperpolarization of neat liquids containing a naturally abundant level of 15N. Deuterium and 14N nuclei can act as direct and indirect hyperpolarization sinks and should be avoided or minimized where possible. While the NMR signal and polarization enhancements are relatively modest for 1H SABRE of n.a. Py, (Py in miliTesla regime, $\epsilon \approx 4$) and 15N SABRE ($^{15}$N-Py in microTesla regime $\epsilon \approx 33$) due to the finite capacity of the parahydrogen/Ir-hydride spin bath, the resulting payload of 15N hyperpolarization (the product of agent concentration and its hyperpolarization) is more than doubled in this proof-of-principle demonstration compared with previous demonstrations of 15N SABRE in methanol-$d_4$ solutions.

ASSOCIATED CONTENT

* Supporting Information

Experimental details and $T_1$ simulations based on experimental data at microTesla and 9.4 T magnetic fields. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpclett.5b00782.

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Notes

The authors declare no competing financial interest.

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