Parahydrogen Hyperpolarized Multi-Nuclear Contrast Agents For Potential MRI Applications

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PARAHYDROGEN HYPERPOLARIZED MULTI-NUCLEAR CONTRAST AGENTS FOR POTENTIAL MRI APPLICATIONS

by

NUWANDI M. ARIYASINGHA

DISSERTATION

Submitted to the Graduate School
of Wayne State University,
Detroit, Michigan
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

2020

MAJOR: CHEMISTRY (Physical)

Approved By:

_______________________________
Advisor

_______________________________
Date
DEDICATION

My roots;
“thaththa and amma”, to my father who always told me “You can go to the top of the world, therefore you should” and to my mother, my first science teacher for shaping me into who I am today.

And

My wings;
to my husband Asiri & son Ārya, with love!
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CHAPTER 1 INTRODUCTION

Biomedical imaging is a vital tool that is used today to study anatomy and physiology of humans to be able to perform disease diagnosis, tracking the progress of a health condition like cancer, and even in providing information about the progress of a certain treatment. Many of the worlds’ top killer diseases such as heart diseases (Coronary Artery Disease, CAD) and Chronic Obstructive Pulmonary Disease (COPD), and more recently viral illnesses like COVID-19 require effective early diagnosis for successful initiation of treatment. Therefore, obtaining visual representation of tissues and organs inside the body is the key to therapeutic applications. Many different imaging modalities are used today, including X-ray radiography, ultra-sound, endoscopy, positron emission topography, magnetic resonance imaging etc. Some of these methods involve a form of ionization radiation. Although, a great amount of information can be obtained, some of the imaging technologies suffer from different limitations such as penetration depths, tissue/ patient accessibility, limited operating window, inability of real time imaging and very long detection times etc. Our research interest is particularly on developing advanced Magnetic Resonance Imaging methods.

MRI is a non-invasive technique used in the biomedical field for study of organ functions, metabolism, detection of health-related issues such as tumors, cancer, abnormalities, injuries lung/ heart/liver related health issues and others, by taking images of the organs and tissues inside human body. Unlike many other modalities,
MRI can provide information based on the physical and biochemical properties of the subject tissues. In most cases, clinical MRI scanners use the ability of protons to signal in the presence of a radio frequency pulse, when placed in a magnetic field. The concept behind magnetic resonance imaging is Nuclear Magnetic Resonance (NMR). Although, MRI is widely used in clinical imaging where large substrate concentrations are feasible, detection of void spaces, gases, and low-concentration metabolites remain a major challenge due to the inherently low sensitivity of NMR technique. This limitation is due to low degree of nuclear spin alignment (termed polarization) with the applied field of the MRI scanner. Techniques to achieve substantial enhancement of polarization or hyperpolarization were introduced as means of artificial transient increase of the signal, which can boost the NMR sensitivity by up to several orders of magnitude. The boost in sensitivity enables the use of inhalable or injectable hyperpolarized (HP) media as MRI contrast agents. Among various hyperpolarization techniques that were developed, my research has focused on development of parahydrogen-induced polarization a.k.a. PHIP methods.

PHIP detection uses a chemical reaction between a substrate molecule and the parahydrogen state of molecular hydrogen (p-H$_2$) where the spin order of the p-H$_2$ is transferred into the HP substrate via a pairwise addition reaction or a reversible exchange reaction of p-H$_2$. p-H$_2$ provides cheap and convenient access to hyperpolarization. Throughout this work described here, several compounds
were investigated which can be used as HP contrast agents with potential biomedical applications.

I report the study of proton-hyperpolarized hydrocarbons such as HP propane and HP diethyl ether that can be developed into and validated as inhalable imaging agents in the future. The lifetime of these HP compounds is also explored enabling their preparation and potential administration to subjects before undergoing substantial relaxation of HP state during potential pulmonary imaging scans. Imaging studies using a 0.35 T clinical MRI scanner will be performed in the near future in a large animal models (sheep) as a part of future clinical translation efforts of our laboratory.

Furthermore, heteronuclear atoms $^{15}$N and $^{19}$F of N-heterocycles were also studied for their utility as injectable contrast agents towards hypoxia sensing. FDA approved antibiotic metronidazole, and fluorinated pyridine derivatives were successfully hyperpolarized via a non-hydrogenative variant of PHIP technique, and the results will be discussed in detail later.

In addition, we also report on a creation of Radio Amplification of Stimulated Emission of Radiation (RASER) at for low concentration of HP substrate molecules prepared by PHIP using a commercially available bench-top NMR spectrometer operating at clinically relevant magnetic field of 1.4 T. The RASER effect is reported for a wide range of substrates. The RASER detection paves new opportunities in magnetic resonance applications, data encryption, quantum computing and beyond.
CHAPTER 2 BACKGROUND

2.1 Theory of magnetic resonance

Magnetic resonance (MR) phenomenon was first observed in the early 19th century. The observation of nuclear energy level splitting in the presence of a magnetic field dates back to 1897 when Pieter Zeeman detected an alternation of the period of light in the presence of an external magnetic force; that is the atomic emission line splitting when experiments are conducted in an external static magnetic field.\(^1\) Pieter Zeeman received 1902 Nobel Prize in Physics for the discovery of Zeeman effect. First direct measurements of nuclear magnetic moment was pioneered by Rabi and co-workers in 1939.\(^2\) They reported on a molecular beam resonance method to measure magnetic moments by inducing transitions between the energy levels depending on the orientation of the nuclear spins of the atoms. Rabi later received the Nobel Prize in Physics in 1944 for this work. Only a couple of years after, several other scientists working on similar research observed nuclear spin energy transitions when paraffin was irradiated with radio frequency (RF) pulses making it the very first such observation of nuclear magnetic resonance in solid materials.\(^3\) Simultaneously, nuclear induction experiments\(^4\) were performed towards nuclear magnetic resonance of liquids and solids. A couple of decades after the first observation of NMR in solid materials, Ernst et al. took a giant step by inventing Fourier Transform Nuclear Magnetic Resonance Spectrometer (FT-NMR), which led to many spectroscopy applications and beyond.\(^5\) Later, rapid developments of hardware and electronics paved the
way to the design of high-resolution NMR spectrometers used by chemists, physicists and radiologists towards numerous applications ranging from chemical structure elucidation of compounds to development of magnetic resonance imaging, and thereby claiming other Noble prizes in physics, chemistry and medicine along the way.

The canonical NMR model treats the nucleus of an atom as a positively charged sphere spinning around on an axis analogous to a spinning top. Similar to an electric current, spinning action of a charge (electrons and nuclei) therefore creates a small magnetic field with direction given by the right-hand grip rule. A nucleus with a non-zero spin angular momentum (I) is identified as an NMR active isotope. Spin angular momentum is a combined effect originating from electrons, protons and neutrons of a particular atom. Although in many scenarios, the net effect of the spin is zero due to cancellation of individual spin pairs, some atoms exhibit a net non-zero spin I, thereby causing the nucleus to be NMR active. Examples of commonly used NMR active nuclei are given in Table 1. One can use the below stated selection rules to find if an atomic nucleus of interest is NMR active.

1. Atoms with odd mass number (A) have a half-integer nuclear spin value. (e.g. $^1$H, I = 1/2)

2. Atoms with even mass numbers (A) and odd atomic numbers (Z), have integer nuclear spin values. (e.g. $^2$H, I = 1)
3. Atoms with both mass and atomic numbers (A and Z respectively) are even, there is no nuclear spin value.

**Table 1.** A list of commonly used NMR active nuclei and their properties

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<th>Spin Number(I)</th>
<th>Abundance (%)</th>
<th>Sensitivity</th>
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<tr>
<td>(^1)H</td>
<td>1/2</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>(^2)H</td>
<td>1</td>
<td>0.015</td>
<td>0.0000015</td>
</tr>
<tr>
<td>(^{13})C</td>
<td>1/2</td>
<td>1.1</td>
<td>0.016</td>
</tr>
<tr>
<td>(^{15})N</td>
<td>1/2</td>
<td>0.37</td>
<td>0.001</td>
</tr>
<tr>
<td>(^{19})F</td>
<td>1/2</td>
<td>100</td>
<td>0.83</td>
</tr>
<tr>
<td>(^{129})Xe</td>
<td>1/2</td>
<td>26.44</td>
<td>0.0057</td>
</tr>
</tbody>
</table>

An NMR active nucleus (with \(I\) = non-zero value) possesses a magnetic dipole moment (\(\mu\)), which is directly proportional to its nuclear spin as indicated by equation 1 below.

\[
\mu = \gamma \frac{\hbar I}{2\pi}
\]

(1)

\(\gamma\) is the nucleus-specific gyromagnetic ratio, which is a measure of the strength of the magnetic nucleus, and \(\hbar\) is the Planck’s constant. When a positively charged atomic nucleus is placed inside of an external static magnetic field, the atomic nucleus will align with the applied magnetic field. As stated above, when an ensemble of nuclei with magnetic dipole moments is inserted into a magnetic field
$B_0$, it orients their spins either in the direction of the applied field (hence called parallel) or opposed (anti-parallel) to the field. Parallel spins have lower energy, and therefore have a more energetically favorable orientation. The anti-parallel spins have higher energy and thus is called the less energetically preferred orientation. The energy created ($E$) by the magnetic moment in a field of $B_0$ which is called as Zeeman coupling is given by,

$$E = -\mu B_0$$ \hfill (2)

In addition to the magnetic moment, the nucleus also has an angular momentum due to its spinning motion. The torque generated by the external field results the nucleus to precess very similarly to the motion of a spinning top placed in the Earth’s magnetic field (Figure 1).

![Diagram of a spinning nucleus](image)

**Figure 1. Schematic diagram of a spinning nucleus.** Magnetic field strength is $B_0$ and the precession frequency is noted by $\nu_0$.

The rate of precession given by Larmor frequency $\nu_0$ is proportional to the applied field $B_0$ with the following relationship.
\[ \nu_0 = \gamma B_0 / 2\pi \quad (3) \]

Since the resonance frequency of a sample falls into the RF regime, an RF pulse is applied to the NMR active nuclei and the energy absorbed by the sample is detected.

To better understand MR, a quantum mechanical view is also desired. A typical nucleus with a given \( I \) value has \( (2I+1) \) number of degenerate Zeeman energy levels when an external magnetic field is not used. However, when a field with \( B_0 \) strength is applied, nuclear Zeeman splitting takes place which is simply the separation of Zeeman energy levels of a given ensemble of nuclei, \( i.e., \) for \(^1\text{H} \) like nuclei with \( I=1/2 \), energy level is split into two different Zeeman levels in the presence of a magnetic field; \( B_0 \neq 0 \). The two different energy levels are associated with the two different spin state orientations, parallel and anti-parallel (Figure 2). The gap (\( \Delta E \)) between two energy states is denoted by,

\[ \Delta E = h\nu_0 \quad (4) \]

The resonant frequency (\( \nu_0 \)) is the same as the Larmor frequency obtained from classical mechanics. Therefore, using equations (3) and (4), we can calculate the difference in energy between Zeeman energy levels to be proportional to the external field \( B_0 \),

\[ \Delta E = \gamma hB_0 / 2\pi \quad (5) \]
Figure 2. Zeeman effect. Zeeman splitting of the energy levels for a nucleus with $I = 1/2$, when inserted in a magnetic field. The two spin states are shown as spin $-1/2$ (aligned opposed to the magnetic field direction, hence the “upper” state and spin $+1/2$ (aligned with the magnetic field, hence the “lower” state.

At thermal equilibrium, the population of the number of spins between these Zeeman levels follow Boltzmann distribution as indicated below.

$$\frac{N_{\text{upper}}}{N_{\text{lower}}} = e^{-\Delta E / kT}$$

(6)

, where $N_{\text{upper}}$ is the population in the spin down state (higher energy state) and $N_{\text{lower}}$ is the spin population of the spin up state (lower energy state) and $k$ and $T$ are Boltzmann constant ($1.380 \times 10^{-23}$ J/K) and temperature in Kelvin respectively. Since more spins prefer to occupy the lower energy state in line with second law of thermodynamics, the population of the lower energy level is slightly higher than the population of the higher energy level. However, the energy gap between the two states, as indicated in equation (5), is very small at the room temperature compared to the average energy of the sample at thermal equilibrium as obtained by Boltzmann energy ($E \propto kT$). In fact, the two energy levels are almost equally
populated under thermal conditions. The NMR signal is generated by this small population difference between the Zeeman energy levels. Therefore, NMR signal for different nuclei is strongly dependent on their $\gamma$ values (equations 1 and 5).

Nuclear spin polarization ($P$) is defined as the ratio between the population difference of the two Zeeman energy levels and the total population of the levels. Of important note, the NMR signal is directly proportional to $P$. The mathematical formula to calculate spin polarization is given below,

$$\frac{N_{\text{lower}} - N_{\text{upper}}}{N_{\text{lower}} + N_{\text{upper}}} = P \quad (7)$$

Equation (7) can be rearranged to equation (8) by substitution of equation (6),

$$P = \frac{(1 - e^{-\mu B_0/kT})/(1 + e^{-\mu B_0/kT})}{1 - e^{-\mu B_0/kT}} \quad (8)$$

It is also important to calculate the net magnetization of a sample of nuclei at thermal equilibrium. For this we use Boltzmann equation (eq. 6), and applying $e^{-x} = 1 - x$ approximation for low values of $x$, which is indeed the case for minute energy differences at thermal conditions, and arrive at,

$$\frac{N_{\text{upper}}}{N_{\text{lower}}} \sim 1 - \frac{\Delta E}{kT} \quad (9)$$

By expansion, above equation can further be simplified to,

$$N_{\text{lower}} - N_{\text{upper}} = \frac{N_{\text{upper}}}{2kT} \Delta E \quad (10)$$

$N_{\text{upper}}$ and $N_{\text{lower}}$ are very close to each other and thereby can be simply noted as
N/2 and by comparing eq. (10) with eq. (5) we obtain,

\[ N_{\text{lower}} - N_{\text{upper}} = \frac{N\hbar y B_0}{2kT} \]  

(11)

, where \( h = \frac{\hbar}{2\pi} \). This concludes that the population difference is directly related to the total number of spins, the gyromagnetic ratio and field strength, while it is inversely related to the absolute temperature, at which the spin ensemble is kept. Spins in the upper state cancel with the spins in the lower state when the summation of all the spins is performed and we are left with few spins in the lower state; \( N_{\text{lower}} \) that are oriented with \( B_0 \) resulting in a net magnetization, \( M_0 \). Net magnetization is defined as the magnetic strength of each individual spins times the number of spins pointing up giving an overarching equation for \( M_0 \) as below, where the net magnetization of a sample is directly proportional to the number of spins in the sample, the external magnetic field and to the square of the gyromagnetic ratio and is inversely proportional to the absolute temperature in Kelvin.\(^6\) Note the NMR signal is directly proportional to \( M_0 \).

\[ M_0 = \gamma (N_{\text{lower}} - N_{\text{upper}}) = \frac{N\hbar y^2 B_0}{2kT} \]  

(12)

Moreover, substantial research has been executed to develop bigger, stronger magnets with high fields (in Tesla region) to achieve better levels of net magnetization values with ultimate goal of increasing \( M_0 \) and by extension the sensitivity of NMR experiment.
2.2 Typical NMR spectrometer operation

A basic NMR spectrometer consists of the following main parts: a superconducting magnet to generate a static magnetic field, an RF transmitter to provide the alternating field and an RF receiver for amplified signal detection, along with analog-digital converters (ADC) and computers for visualization and analysis of the signal (Figure 3). Additionally, there are some other optional components, which can be employed to enhance the signal to noise (S/N) ratio, which usually include cryostats and temperature control devices. Although initial developments of NMR spectrometers involved the designing of continuous wave (CW) NMR spectrometers, nearly all modern NMR spectrometers are pulsed devices. The development of FT-NMR led to fast and convenient acquisition of NMR spectra making it the most commonly used type of NMR spectrometer today.

A standard FT-NMR procedure begins with the sample insertion into the large magnet with a field of $B_0$ (which is applied in the z direction and we use $z$ component of the equations (2), (3) and (4)) creating the bulk magnetization vector ($M_0$) arising from all the individual nuclear spins in the sample, to be collinear with the $z$ direction of $B_0$. A pulsed magnetic field of $B_1$ is then applied using the RF coil at the resonance frequency of NMR spins. This pulse tips the net magnetization vector ($M_0$) from $z$ direction into the $x$-$y$ plane. Tipping angle can vary depending on the experiment of interest. Similar to a gyroscope, the tipped magnetization continues precession along the $z$- axis eventually relaxing its magnetization back to the $z$- axis. Detailed description of the relaxation of magnetism will be given later.
in the section 2.13. Free induction decay (FID) is collected, and Fourier transformed into a frequency domain signal, which can be conveniently displayed on the computer as NMR spectrum of the nucleus of interest. A schematic representation of this entire process is given in Figure 4. One of the key advantages of using FT-NMR is the rapid acquisition of data, which enables pulse excitation and recording of FID to be frequently repeated several times and the sum of added FIDs are transformed to create a signal. Each of the nuclear spins in the sample with a different resonant frequency contributes to a separate peak in the spectrum.

![Figure 3. Schematic representation of a typical NMR spectrometer hardware.](image)

The sample is placed inside of a radio frequency coil sitting inside a magnet. A RF pulse is sent to the sample and the receiver collects the FID, directs it to analog-to-digital converter (ADC) and the FT spectrum of the sample is generated.
Figure 4. Fundamentals of the NMR experiment. a) the application of the RF pulse (with \( \omega \) angular velocity which matches the Larmor frequency at \( B_1 \) field) to sample with the bulk magnetization vector \( (M_0) \) oriented along z-direction. \( M_0 \) will precess both around the static magnetic field and the applied pulsed magnetic field. b) This creates the tipping of \( M_0 \) to x-y plane resulting in \( M_{xy} \). c) After a certain pulse duration, \( B_1 \) is turned off and \( M_{xy} \) will remain precessing about \( B_0 \) until it slowly relaxes back to \( M_0 \). d) A signal free induction decay (FID) is generated, which is Fourier transformed to obtain a NMR spectrum.

When choosing an NMR spectrometer for a particular study or when operating a spectrometer, the following important factors and parameters should be considered: magnetic field strength, RF frequency range, spectrometer frequency, pulse width, sweep width, recycle delay, etc. As indicated above, the magnetization generated is directly proportional to the strength of the magnetic
field of the NMR spectrometer. Therefore, NMR spectrometers / MRI scanners of various strengths ranging up to 21 T are used to study living subjects. The spectrometer frequency is the frequency of the RF pulse applied, and it depends on both the magnetic field strength of the spectrometer and the observed nucleus. For example, SpinSolve Carbon60, Magritek’s bench-top spectrometer operating at 1.4 T has a resonance frequency of 61 Hz*ppm⁻¹. Although a sample of NMR active nucleus does not have one resonant frequency at any given time, due to the differences in the chemical environment, resulting in slight different frequencies (chemical shifts in ppm units), NMR spectrometer is designed in a way to distribute the RF power over a range of frequencies by applying RF excitation pulse. If a wide excitation bandwidth is desired, a more intense RF pulse is applied. Similarly, for a frequency-selective RF excitation, RF pulse with of lower strength can be used. Another key parameter when acquiring NMR spectra is the tipping angle (flip angle), which causes flipping of the magnetization from z-axis (M₀) to x-y plane (Mₓy; Figure 4b). The flip angle can be tuned by changing the width of the RF pulse depending on the nature and the objective of the experiment. Selection of the correct spectral width is equally important when performing a NMR experiment. Spectral width translates to the width in Hz / ppm selected to observe transitions of interest, and it is usually calculated using the spectrometer frequency, e.g., for the spectrometer mentioned in the previous example with a frequency of 61 Hz*ppm⁻¹, an RF pulse with B₁ strength of ~300 Hz should be applied to measure NMR peaks in a 5-ppm range. Although, the detection parameters can be carefully
tuned to maximize signal-to-noise ratio (SNR), some applications of NMR/ MRI are still limited due the sensitivity of the signal.

2.3 Factors affecting the NMR signal intensity

NMR spectroscopy differs from other common spectroscopic modalities mainly due its low sensitivity. One reason is the sensitivity of the technique and the other reason is the low SNR. This is directly related to the low spin polarization due to the approximately equally populated Zeeman energy levels with a minute energy gap compared to thermal energy calculated by Boltzmann factor (kT, equation 11). Combination of equations (7), (5) and (10) present the mathematical relationship between \( P \) and thermal energy by,

\[
P = \frac{\Delta E}{2kT} = \frac{\gamma hB_0}{2kT}
\]

Nuclear spin polarization \( P \) is directly proportional to the gyromagnetic ratio of the nucleus and the applied magnetic field while it is inversely proportional to the absolute temperature. NMR signal can be enhanced experimentally either by using stronger magnets or by lowering the temperature of the sample. Atomic nuclei with high \( \gamma \) values yield better signals compared to those with lower values.

Concentration, natural abundance of the isotope and the Larmor frequency can also affect the sensitivity of the NMR signal. The total number of spins giving rise to a signal increases with increase in concentration. In combination with \( \gamma \), natural abundance of the nuclei also affects the signal strength. Nuclei precessing at
higher Larmor frequencies can also improve the signal on the receiver.\textsuperscript{7} But, even for nuclei with high $\gamma$ values (e.g., $^{1}$H and $^{19}$F), the equilibrium $P$ values however remain in the order of $10^{-6}$-$10^{-4}$ at biologically important temperatures (~ 300 K) and at high magnetic field.\textsuperscript{8,9}

2.4 NMR polarization and signal enhancement

Although the nuclear spin polarization (equation 13) and by extension the NMR signal can be improved by lowering the temperature or by using magnets with stronger fields, these approaches have limited utility in the context of biomedical applications (3 T clinical scanner field strength and body temperature). Proton polarization values fall in the range of $10^{-5}$ even with high field clinical MRI scanners operating at 3 T when such image acquisition is performed \textit{in vivo}.\textsuperscript{10} One other possibility is to use very high concentrations of samples to get high-resolution spectra, which is the common practice of MRI using the water molecules as the proton source for image acquisition.\textsuperscript{8} However, image acquisition of low concentrated samples is essential when studying tissue metabolism, pulmonary imaging, imaging of gases, etc. Consequently, numerous efforts\textsuperscript{11-16} have been taken to artificially increase the polarization of a sample. This increase of polarization well above its thermal equilibrium level is called as \textit{hyperpolarization}.

2.5 Fundamentals of NMR Hyperpolarization

The theory related to hyperpolarization was first reported by Overhauser\textsuperscript{14} stating that a saturation of the conduction electrons cause an increase in the
Zeeman energy level population difference in metals by several orders of magnitude thus serving as a potential method of hyperpolarizing nuclear spins. First experimental observation of hyperpolarization dates back to 1953, and it was illustrated by Carver et al. in a study of metals.\textsuperscript{16} For lithium \(^{7}\text{Li}\), they reported an increase in nuclear resonance (calculated value of \(10^3\) times increase if nuclear relaxation processes are neglected, and an experimental value of 140-fold increase) which is caused by saturation of free electrons.\textsuperscript{17} A polarization enhancement factor (\(\varepsilon\)) is introduced to compute the increase in polarization.

\[
\mathcal{E} = \frac{p_{\text{HP state}}}{p_{\text{Thermal state}}} 
\]  

(14)

Polarization enhancement factor is defined as the spin polarization of the HP state with respect to that of the thermally polarized equilibrium state. Hyperpolarization yields enhanced SNR through the increase of the NMR signal, because it is directly proportional to \(P\). Thus, low-concentration HP samples can be employed to acquire spectra and images.

Hyperpolarization also enables rapid spectral and imaging acquisition. There is no time and the necessity for long durations of data averaging due to the already amplified SNR. Imaging sequences can also be accelerated (e.g., 3D image acquisition time of \(\sim 1-5\) seconds) because waiting time for magnetization recovery is not required, thereby making it a more useful technique for biomedical imaging compared to conventional NMR spectroscopy and imaging.\textsuperscript{7,18}
Hyperpolarization process is usually performed outside the human body requiring the HP state of the contrast agent to last for a time period from preparation to \textit{in-vivo} administration.\textsuperscript{7} Moreover, rapid data acquisition is desired in order to minimize polarization losses because HP state is not replenishable. The use of expensive hardware and software is another translational challenge of hyperpolarization techniques. Development of faster and more cost-effective hyperpolarization methods is one of the main foci in NMR and MRI research today.

2.6 Methods of NMR hyperpolarization

Since the first attempt of artificial increase of nuclear spin polarization, many methods have been successfully developed for efficient hyperpolarization. Some of these hyperpolarization techniques allow $P$ enhancement to the order of unity ($%P=100\%$). Some commonly used techniques will be briefly discussed in this section (Figure 5) including dynamic nuclear polarization (DNP), spin exchange optical pumping (SEOP), brute force (BF), magnetic field cycling (MFC) methods, signal amplification by reversible exchange (SABRE) and parahydrogen induced polarization (PHIP).
Figure 5. Common hyperpolarization techniques. SABRE and PHIP techniques use parahydrogen (p-H₂) and the work reported throughout this dissertation used these two techniques.

Dynamic Nuclear Polarization also known as DNP is based on the studies of Overhauser\textsuperscript{14} and Slichter\textsuperscript{16, 17} where polarization is transferred from electron spins to nuclear spins. Near 100% electron polarization is achievable at high magnetic fields and low temperatures due to the inherent high $\gamma$ value of the electrons, \textit{i.e.} $\gamma(e) = 660\gamma(\text{^1H})$.\textsuperscript{8} Although DNP involves a broad range of variants, the dissolution DNP (d-DNP) is often used in the context of biomedical applications.\textsuperscript{9, 19} It should be noted that biomedical applications of hyperpolarization is the key driver behind the development of hyperpolarization techniques today. D-DNP is composed of mixing of the contrast agent of interest with a sample of free electrons, which is usually a stable free radical, at a low
temperature and high magnetic field. The mixture is then exposed to microwave source with matching frequency of the electron resonance enabling electron spin transfer to the nuclei. Finally, the sample is used on the subject via intravenous injection after dissolution, radical removal and warming have completed within a few seconds.

![Diagram of DNP process](image.jpg)

**Figure 6. Schematic representation of DNP process.** Biomolecule (1-13C-pyruvic acid) is allowed to thermally equilibrate with the paramagnetic sample at low temperature and a high magnetic field followed by irradiation of high-power microwaves. Polarization transfer from electrons to 13C nuclei is achieved and can now be used as a contrast agent for imaging. Adapted with permission from Ref. #8. Copyright (2014) John Wiley and Sons.

While polarization transfer from electrons in DNP is a rather slow process\(^9\), the further research developments have afforded almost six times faster polarization transfer to the 13C nuclei by transferring spin polarization from electrons to \(^1\)H first and then relaying the proton polarization to 13C nucleus compared to direct transfer from electrons to the 13C.\(^{20, 21}\) Several biomolecules containing 13C and 15N have been studied\(^{22-25}\) using DNP hyperpolarization methods, and have shown successful *in vivo* applications towards clinical trials for prostate cancer\(^{26}\) in men as an example. HP MRI using DNP has shown promising advantages compared
to other imaging approaches as a hyperpolarization technique with already undergoing clinical trials. However, the high cost (>\$2M ca. 2020) associated with the installation and maintenance of the DNP hyperpolarizer equipment is a major drawback limiting its use. Moreover, the commercial polarization process requires at least 30 mins.

First introduced by Bouchiat\textsuperscript{27} and co-workers, \textbf{Spin Exchange Optical Pumping (SEOP)} is a technique (Figure 7) used for the hyperpolarization of noble gases such as \textsuperscript{129}Xe and \textsuperscript{3}H. The optical pumping process is designed to generate highly polarized electrons of an alkali metal (typically Rubidium) via an irradiation of the small fraction of Rb in the OP cell mixed with the noble gas of interest such that the circularly polarized light is in resonance with the electronic transition of Rb. The next step, however, is the spin polarization transfer from Rb vapor electrons to the nuclear spins of the noble gas through gas phase collisions. The transfer of spin polarization from Rb electrons to noble gas nuclei, \textit{e.g.}, \textsuperscript{129}Xe is called as the spin exchange process which ultimately creates highly polarized noble gas nuclei. Despite of the high $\gamma$ value, \textsuperscript{3}He applications are not popular because of the scarcity of \textsuperscript{3}He sources in the world: it is a product of tritium decay.\textsuperscript{8} However, the use of \textsuperscript{129}Xe for SEOP towards biomedical applications\textsuperscript{10, 28-30} has experienced a promising growth due to its compatibility with biological subjects, solubility in blood, and the previous history of use as anesthetic. \textsuperscript{129}Xe chemical shift is very sensitive to its local environments meaning that a small change in the chemical environment can result in (100-200) ppm chemical shift changes making it a good candidate for
MR imaging. Moreover, the long relaxation times (of up to tens of seconds \textit{in vivo}) of $^{129}$Xe permits convenient access to \textit{in vivo} applications.\textsuperscript{31} Additionally, the absence of $^{129}$Xe in human body yields background-free spectral/ image acquisition. Numerous research projects are conducted toward the development of $^{129}$Xe as a contrast agent for MRI, which can be administered into humans either via inhalation or through intravenous injection (IV).\textsuperscript{31-33} Similar to DNP methods one of the major drawbacks of SEOP is the high cost ($\sim$0.3-0.6M ca. 2020) and complexity of the hyperpolarizer hardware.\textsuperscript{34}
Figure 7. Illustration of SEOP method. a) the optical cell with Rb, $^{129}\text{Xe}$ and buffering gas. b) absorption of circularly polarized laser light in Rb. c) exchange of polarization from Rb electrons to $^{129}\text{Xe}$ via collisions. Adapted with permission from Ref. #8. Copyright (2014) John Wiley and Sons.
**Brute Force (BF)** method, among all of the other hyperpolarization techniques, is the oldest and first discovered technique in 1934.\(^{35, 36}\) This approach involves substantial splitting of nuclear sub-levels via the application of an external magnetic field and at low temperature such that the sublevels are occupied by spin population in accordance with Boltzmann distribution. However, it is not a very popular technique due to the limitations of operating at low temperature values (mili Kelvin range) and at very high magnetic fields (~10 T). Nonetheless, brute force does not employ complex hardware requirements such as powerful lasers (as for SEOP) or microwave radiation like in DNP. Although, it is not a frequently used technique, promising enhancement values have been reported for metabolites such as 1-\(^{13}\)C-pyruvic acid, 1-\(^{13}\)C-sodium lactate by conducting brute force experiments at 14 T and 2.3 K temperatures with spectral detection at 1 T and 40 °C temperature values.\(^{37-39}\)

The history of Field Cycling (FC) experiments goes back to experiments conducted at Harvard University and Columbia University.\(^{3, 13, 40, 41}\) A thorough descriptive history on FC can be found in the review by Anoardo et al.\(^{41}\) The very first FC instrumentation was developed by Johannesson and co-workers,\(^{42}\) when polarization was converted into \(^{13}\)C nuclei from \(^{1}\)H. Since then, FC experiments showed great benefits towards cross relaxation studies, spin thermodynamic studies etc.\(^{41}\) While FC remains as a complimentary technique for hyperpolarization among others, it has been shown to have significant advantages when NMR is performed at low fields. The sample can be pre-polarized at a higher
field and the generated magnetization is then detected as it evolves in the low field followed by detection of the evolved signal using a third field. The other promising characteristic of using FC methods is the detection of indirect magnetic resonances. Typical field cycling experiments consist of two different magnets. One higher-field magnet is the polarizing device. The other lower-field magnet is the detection magnet/ NMR spectrometer magnet with a stable and more homogeneous magnetic field. The ability to quickly transition from high to low field is frequently required in order to minimize the polarization losses due to relaxation and also to accelerate the experiments in case if multiple repetitions are required.

Today, FC techniques and experiments have evolved to applications ranging from solid-state NMR to magnetic resonance imaging (MRI) applications, details of which can be found elsewhere.

In addition to all hyperpolarization methods discussed above, chemical transformation of a spin order from \( p-H_2 \) molecules to a substrate molecule via a chemical reaction is also feasible. This technique in general is called as ParaHydrogen Induced Polarization (PHIP) due to involvement of a hydrogenation reaction of the substrate of interest with the singlet form of molecular hydrogen, \( i.e. \) parahydrogen state, via its pairwise addition or chemical exchange. Conventional PHIP requires pairwise parahydrogen addition, whereas SABRE is a non-hydrogenative variant of PHIP technique relying on reversible \( p-H_2 \) exchange. The key feature of PHIP and SABRE is the involvement of \( p-H_2 \). \( p-H_2 \) provides a fast and convenient access to hyperpolarization. These two methods as well as
production of p-H$_2$ gas will be thoroughly reviewed below.

2.7 Production of parahydrogen (p-H$_2$)

The very first observations of NMR signal enhancement were recorded in 1981$^{48}$ when a tricobaltalkylidyne complex resulted in increase of the NMR signal when stored with hydrogen at $-40$ °C, but the reason was unknown until five years later, when Bowers and Weitekamp$^{15}$ published their work on parahydrogen-based hyperpolarization technique.

Dihydrogen molecule has two different spin states: the ortho- spin state and the para- spin states. Of these states, orthohydrogen (o-H$_2$) is when both of the spins in hydrogens are oriented in the same direction or triplet spin state and parahydrogen (p-H$_2$) is when the two nuclear spins are oriented in opposite directions thereby creating a singlet spin state (Figure 8). The triplet state is triply degenerate, and the singlet state is non-degenerate. Under normal conditions, all of these four states are populated equally in accordance with Boltzmann distribution, resulting 75:25 abundance of o-H$_2$ to p-H$_2$. Interesting feature of using p-H$_2$ for hyperpolarization modalities is that, the lifetime of the singlet state can be very long lived (up to weeks to months in the absence of paramagnetic impurities) thereby facilitating the storage of p-H$_2$ gas once produced. However, the production of p-H$_2$ is not a simple task, because at room temperature (~20 °C), normal hydrogen gas (a mixture of 3:1 of o-H$_2$ to p-H$_2$) cannot be easily converted to p-H$_2$. Inter-conversion of o-H$_2$ to p-H$_2$ is a forbidden transition resulting in the
extremely slow conversion rates. Nevertheless, it was shown that such conversion acceleration or catalysis is feasible in the presence of a paramagnetic materials such as hydrated iron oxide.\textsuperscript{49, 50} \( \text{p-H}_2 \) has lower energy than \( \text{o-H}_2 \), and therefore, \( \text{p-H}_2 \) is favored at sufficiently low temperatures. As a result, under low temperatures, normal hydrogen (consisting from 75\% ortho- and 25\% para-) can be converted to \( \text{p-H}_2 \)-enriched mixture by simply using a catalytic acceleration of interconversion in the singlet-triplet equilibrium,\textsuperscript{15, 51} making the conversion kinetically feasible. Once prepared and isolated from the catalyst, \( \text{p-H}_2 \) to \( \text{o-H}_2 \) conversion at the room temperature is a very slow process in the gas phase given the absence of paramagnetic impurities such as steel.\textsuperscript{51, 52} Since \( \text{p-H}_2 \) has the net spin of zero, it cannot be detected using NMR spectroscopy but, the remaining orthohydrogen fraction can be readily detected by NMR spectroscopy. Consequently, para- fraction can be determined due to the decrease of the orthohydrogen NMR signal enabling us to obtain the \( \text{p-H}_2 \) enrichment fraction for the production,\textsuperscript{15, 53} Figure 8. Alternatively, para-/ortho- ratio can be determined by IR spectroscopy.\textsuperscript{49, 50, 54, 55}
Figure 8. Schematic representation of o-H₂ and p-H₂ spin states of H₂. Using the parahydrogen generators built in our lab, we can generate p-H₂ with 87 % and > 99 % enrichment.

In our laboratory, p-H₂ is produced on site using a p-H₂ generator consisting of three main parts, a source of normal hydrogen gas, the generator for the catalytic conversion from the triplet to the singlet state and the storage container (Figure 9). Normal dihydrogen (ultra-high purity grade: >99.999% content) from the source with a statistical distribution of -ortho to -para states is cooled down at cryogenic temperatures (25-27 K) in the presence of a catalyst.¹⁵,⁵¹ When the hydrogen gas passes through the iron oxide catalyst at <70 K temperatures, the kinetics of the interconversion can be improved to seconds versus months when a catalyst is not present. The chemical equilibrium of ortho- to para- is thermodynamically in favor of the para- state at those low temperatures. The gas mixture enriched in p-H₂ is
then slowly warmed up to the room temperature although the conversion p-H$_2$ is the less thermodynamically favorable state at the room temperature.$^{56-58}$ Therefore, p-H$_2$ spin order is kinetically trapped in the singlet state with enrichments of 50-100% depending on the generator used. p-H$_2$ can be stored for months without rapid conversion back to the equilibrium state at room temperature. We used two different p-H$_2$ generators which have been designed in our lab for the work reported here. One produces p-H$_2$ with $\sim$87% enrichment,$^{59}$ and the other generator is capable of making $>99\%$ enriched p-H$_2$.$^{57}$ The enrichment factor (f) can be obtained as below, where $S^{\text{rt}}$ and $S^{\text{en}}$ are the signals of the gas mixture before and after parahydrogen formation.$^{57,60,61}$

$$f = 1 - (3S^{\text{en}}/4S^{\text{rt}})$$ \hspace{1cm} (15)
Figure 9. p-H$_2$ generation. a) Normal hydrogen is sent through a catalytic converter at cryo-cooled temperatures facilitate the triplet to singlet transformation of hydrogen. b) the temperature dependence of the p-H$_2$ percentage in the mixture. Adapted with permission from Ref. #8. Copyright (2014) John Wiley and Sons.

2.8 Parahydrogen Induced Polarization

Hydrogenative PHIP$^{15, 51, 62}$ operates by incorporation of the spin order from p-H$_2$ into the substrate molecule of interest. PHIP methods can be divided into different sub-categories depending on the number of hydrogens transferred to the substrate compound. If both hydrogens are involved in the transfer, PHIP effect can either be “parahydrogen and synthesis allow dramatically enhanced nuclear alignment” (PASADENA method) or “adiabatic longitudinal transport after dissociation engenders net alignment” (ALTADENA method) depending on the field of the performed reaction.$^{15, 51, 62-64}$ When one proton undergoes the transfer,
it is called as one hydrogen PHIP (OneH-PHIP).\textsuperscript{65} In SABRE method\textsuperscript{66}, the transfer of p-H\textsubscript{2}-derived polarization occurs via reversible exchange without transferring any p-H\textsubscript{2} protons into the substrate molecule. SABRE will be discussed later in this chapter (section 2.9). Although PHIP was initially developed as a technique of signal enhancement, additional uses such as the study of reaction intermediates and mechanisms have shown a rise in popularity; \textit{e.g.}, a study reports one H transfer where the CHO proton indicated a single H net polarization during hydroformylation reaction was investigated using p-H\textsubscript{2}.\textsuperscript{65}

PHIP is based on the fast reaction of p-H\textsubscript{2} pairwise addition to an unsaturated bond (most frequently carbon-carbon) through a hydrogenation reaction. The key requirements for this type of hydrogenation to occur are: (i) a substrate molecule with a double or triple (most frequently carbon-carbon) bond (ii) hydrogenation reaction that occurs faster than the spin relaxation and, (iii) magnetic inequivalency (a.k.a. symmetry breaking) of the product molecule making it NMR visible after hydrogenation—note the product molecule may remain chemically symmetric.\textsuperscript{15} A typical p-H\textsubscript{2} pairwise addition reaction is presented in Figure 10.
Figure 10. Pairwise addition of p-H$_2$ into the substrate molecule with a double bond. p-H$_2$ derived protons are labeled as H$_A$ and H$_B$ in the final product to distinguish their spins due to symmetry breaking of nascent p-H$_2$ singlet.

Since p-H$_2$ is a symmetric state and therefore NMR invisible, it is essential to break the symmetry of the singlet state in order to detect magnetization of the product. This occurs via the addition of the two p-H$_2$ derived protons to the unsaturation in a pairwise manner preserving the spin correlation between p-H$_2$ and the product thereby resulting a magnetically inequivalent compound with distinct chemicals shifts for H$_A$ and H$_B$ protons, that can be visualized using NMR, Figure 10.$^{15}$

Although, the requirement of an unsaturated substrate molecule limits the application of PHIP, it has been still employed in many biologically related applications ranging from hyperpolarization of important metabolites such as $^{13}$C-glucose$^{67}$ derivatives, $^{1-13}$C-succinic acid,$^{68-70}$ $^{1-13}$C-phospholactate,$^{71, 72}$ $^{15}$N-propargylglycine$^{73}$ to gas phase MRI contrast agent developments.$^{74, 75}$ New methods of using different targeted molecules for PHIP is carried out by many researchers today to expand the number of potential contrast agents in biomedical imaging applications.
Early discoveries of PHIP used Wilkinson’s catalyst/ rhodium (Rh) based catalysis and, the practice remained for decades because they provided good conversion efficiencies\textsuperscript{15, 51} Details of reaction mechanism of PHIP using Wilkinson’s catalyst can be found in the work reported by Duckett and coworkers\textsuperscript{76}. Most commonly used catalysts apart from Wilkinson’s are, Vaska’s complex; Ir(CO)Cl(PPh\textsubscript{3})\textsubscript{2} and [(COD)Ir(PCy\textsubscript{3})(Py)]\textsuperscript{PF\textsubscript{6}−} where COD= 1,4-cyclooctadiene and PCy\textsubscript{3} = tricyclohexylphosphine\textsuperscript{77}. A great deal of research has been conducted using different ligand systems but the major problem associated was their insolubility in aqueous media\textsuperscript{77, 78}. Recently developed new catalysts with water soluble ligands permitted new ways of generating HP contrast agents with potential \textit{in vivo} applications\textsuperscript{68, 69, 79}. Some work reported in this dissertation was performed using homogeneous PHIP catalysis, and these findings will be discussed in the chapters below. We will also discuss heterogeneous PHIP in section 2.10, which broadened the scope of PHIP biomedical applications.

\section*{2.9 SABRE variant of PHIP technique}

Introduced by Duckett and co-workers in 2009\textsuperscript{80}, SABRE is dependent on the transfer of the spin from p-H\textsubscript{2} into the substrate compound, but the main difference from PHIP is its non-hydrogenative nature. Moreover, SABRE does not require an unsaturated substrate to interact with p-H\textsubscript{2}, but the spin order from p-H\textsubscript{2}-derived hydrides is transferred onto the substrate compound using an organometallic catalyst. In SABRE, there is no chemical reaction between the
substrate and p-H₂, but parahydrogen is chemically exchanged on a metal complex instead. The simultaneous exchange of the substrate molecule between the solution and the organometallic complex facilitates the transfer of spin order from p-H₂-derived hydrides to the substrate molecule of interest. Schematic representation of SABRE approach is given in Figure 11.

Figure 11. Schematic representation of SABRE technique. Hyperpolarization is transferred from p-H₂ to the substrate molecule (denoted in S) via reversible exchange. Final step is the delivery of the free substrate. M is the metal and ligands are represented by L₁-L₃. Adapted with permission from Ref. #⁸¹. Copyright (2018) John Wiley and Sons.

First, an inorganic polarization transfer complex (PTC) is formed by substrate molecule binding to the catalytic complex to which reversible binding of p-H₂ occurs. Once the p-H₂-PTC complex is generated, spin order is delivered to the substrate from p-H₂, followed by an exchange process, where the HP substrate compound is then released to the free state in solution.⁸¹ Symmetry breaking of p-H₂-derived hydrides into observable magnetization occurs through the substrate spin-spin coupling (J coupling) creating a magnetic inequivalence.⁸⁰, ⁸² For this entire process to work successfully, the choice of catalyst type plays a crucial role. The
important factors to consider when selecting an efficient organometallic catalyst are, (i) a temporary binding of the catalyst to both p-H$_2$ and substrate is required, (ii) PTC complex should be neither too short-lived or too long-lived to ensure both the efficient spin order transfer. The lifetime of the PTC complex should have sufficient time to exist for successful spin-spin-coupling-mediated transfer to occur (i.e., PTC lifetime should be on the order of 1/J). On the other hand, PTC lifetime should not be overly lengthy to minimize the depletion of gained hyperpolarization by relaxation processes on the catalyst and in the bulk solution.$^7,^8,^{80}$ Therefore, the lifetime of the PTC complex is crucial in the context of SABRE efficiency optimization. Duckett et al. used [Ir(COD)(PCy$_3$)(MeCN)][BF$_4$]; Cy= cyclohexyl and COD= cyclooctadiene catalyst with excess amounts of pyridine as the substrate.$^{80}$ A number of different catalysts have been studied for their efficient spin exchange behavior which could be used both on high field and low field NMR/ MRI instruments. [Ir-(IMes)H$_2$Py$_2$S]$^+$ catalyst has shown best performance thus far(IMes = 1,3-bis(2,4,6-trimethylphenyl)imidazolium ylium.$^{83-85}$ A mechanistic representation of the SABRE process is given in Figure 12 below.

Multiple substrates have also been successfully hyperpolarized by SABRE technique as potential candidates for biomedical applications such as pyridine, pyrazinamide, isoniazid, metronidazole, compounds with fluorine containing moieties and many others.$^{55, 84, 86-89}$
Since the polarization transfer is mainly dependent upon $J$ coupling and the magnetic field, magnetic field cycling methods or RF pulses are used to obtain the transfer process via creating level anticrossings (LACs). Spin transfer mechanisms are of two different types; (i) coherent spin mixing mechanism and (ii) cross-relaxation mechanism. Coherent spin mixing occurs in low magnetic fields (micro- to milli- Tesla), when $p$-$H_2$ is bubbled into the substrate catalyst solution. To achieve this condition, field cycling techniques have been used to switch between low and high fields until different approaches were suggested by Theis et al. and Pravdivtsev et al. creating similar resonance conditions with the use of a new pulse sequencing method known as Spin-Lock Induced Crossing (SLIC, will be discussed later in this chapter). Coherent spin mixing can be understood as follows. Energy LACs provide the location of the crossing of the energies between Zeeman levels and $J$ couplings. When these two states are coupled, LAC occurs enabling coherent mixing of the spins thus allowing transfer of spin population from $p$-$H_2$–derived hydrides to the nucleus of interest in the exchangeable substrate. The homonuclear condition for hydride-to-proton polarization transfer is met at a few millitesla, whereas the heteronuclear condition for hydride-to-heteronucleus is met in the sub-microtesla fields (as discussed in details below). Cross relaxation mechanism usually occurs in the high field, and it can simply be understood as transfer of spins from one nuclei to another; when the polarization is dramatically changed for one spin, the neighboring spin polarization can also deviate from the equilibrium condition.
Figure 12. Schematic representation of SABRE mechanism. The transfer of hyperpolarization from p-H_2 derived hydrides to the substrate compound is illustrated. Adapted with permission from Ref. #55 Copyright (2020) John Wiley and Sons.

Depending on where SABRE polarization process occurs, two types of SABRE experiments exist, (i) ex-situ SABRE, when the sample is transferred in to the spectrometer after SABRE process and (ii) in-situ SABRE when SABRE process also occurs within the detection magnet. During ex-situ SABRE experiments, prior to physically moving the sample in to detection magnet, it is first placed in a magnetic field small enough (~6 mT for ^1H) that matches the energy difference between the p-H_2-derived hydrides and the substrate spins with the J coupling frequencies between the two to induce successful spontaneous spin order transfer. In contrast, for in-situ SABRE experiments, dipolar cross-
relaxation mechanisms between the two different spin systems are expected to aid the spin order transfer at higher magnetic fields (e.g., ~9 T).\textsuperscript{85, 98, 100}

Another variation of conventional homonuclear SABRE is called SABRE-SHEATH\textsuperscript{99} (signal amplification of reversible exchange in shield enables alignment transfer to heteronuclei). Theis and co-workers\textsuperscript{99} developed a new way of SABRE technique to hyperpolarize $^{15}$N nuclei at magnetic fields as low as in µT regime using field cycling methods with $P > 20\%$ on $^{15}$N.\textsuperscript{101} Demonstration of their SABRE-SHEATH apparatus is shown in Figure 13. This technique brings cost effective, direct polarization of biomedically relevant compounds with heteronuclei. Additionally, we also pioneered an alternative version of SABRE called as QUASR-SABRE (Quasi-resonance SABRE), which will be discussed in chapter 7.

Both PHIP and SABRE techniques we discussed so far employed homogeneous catalysis. In addition to homogeneous catalysis, heterogeneous PHIP\textsuperscript{102, 103} can also be successfully used, which will be the main topic explained in the section below.
Figure 13. Illustration of the SABRE-SHEATH experiment. a) the experimental setup used for SABRE-SHEATH. b) Substrate ($^{15}$N pyridine and p-H$_2$ chemical exchange with the catalyst. Adapted with permission from Ref. # 74. Copyright (2015). https://pubs.acs.org/doi/full/10.1021/ja512242d Further permissions related to the material excerpted should be directed to the ACS.

2.10 Heterogeneous PHIP catalysis

The history of heterogeneous PHIP catalysis goes back to 2007, when Koptyug et al. first illustrated the hydrogenation of styrene using a polymer supported Wilkinson’s catalyst. Three different catalyst types were studied including Wilkinson’s catalyst supported on styrene-divinylbenzene supported silica gel and RhCl(PPh$_3$)$_2$PPh$_2$(CH$_2$)$_2$ supported on silica and the deoxygenated styrene in deuterated benzene was then mixed with the catalyst followed by hydrogenation reaction with 50% enriched p-H$_2$. NMR spectra were recorded under two different conditions. When hydrogenation reaction occurs inside the magnet and when hydrogenation is performed prior to the detection (differences of these two PASADENA and ALTADENA conditions will be focused in the following section). A
similar study was also performed in the gas phase with successful hydrogenation reactions, which were confirmed by control studies thus ruling out possibilities of homogeneous hydrogenation due to the separation of the catalyst from the solid support.\textsuperscript{53} A study done by the same group after the discovery of heterogeneous catalysis in PHIP reactions (HET-PHIP), reported first gas phase imaging studies using propylene via pairwise p-H\textsubscript{2} reactions using silica supported catalysts, paying way to applications like lung imaging and beyond.\textsuperscript{104} Compared to homogeneous catalysis, HET-PHIP is favored due to the ability to obtain pure product material without traces of the catalyst after hydrogenation, which avoids time consuming and tedious separation purification processes otherwise enabling new directions to \textit{in vivo} applications. HET-PHIP also allows for catalyst recycling. Growing interest of research on heterogeneous catalysis\textsuperscript{102} of PHIP led to successful alkene hydrogenation using supported metal catalysts such as Pt/Al\textsubscript{2}O\textsubscript{3} and Pd/Al\textsubscript{2}O\textsubscript{3} resulting in enhancement of hyperpolarized signal which was later followed by the study of numerous types of supported metal catalysts\textsuperscript{66, 105-107} including the study of the use of different metals, type of the support material, particles size and shape to tune the hydrogenation reaction parameters to achieve higher polarization values.\textsuperscript{103, 108-111} Polarization transfer from \textsuperscript{1}H into heteronuclear atoms like \textsuperscript{15}N and \textsuperscript{13}C have also been investigated\textsuperscript{112-114} using HET-PHIP with long lifetime of the resultant transferred polarization on \textsuperscript{13}C and \textsuperscript{15}N nucleus. Therefore, the discovery of HET-PHIP allowed fast-growing research\textsuperscript{55} area of
studying HP gases and liquids that can be used as MRI contrast agents (Figure 14).\textsuperscript{74}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure14}
\caption{MRI imaging using HP propane. Pairwise p-H\textsubscript{2} addition to propylene via HET-PHIP (left). A 2D MRI image of HP propane gas (right). Adapted with permission from Ref. \textsuperscript{74}. Copyright (2019) American Chemical Society.}
\end{figure}

2.11 NMR signal of a parahydrogen polarized molecule

The first predictions of the NMR signal due to parahydrogen induced hyperpolarization falls back to 1986.\textsuperscript{15, 51} Although, some observations of antiphase nature of NMR signals were recorded few years before, at the time PHIP had not been introduced thus those observations were not correctly attributed to PHIP effect. Bowers and Weitekamp proposed hyperpolarization of substrate molecules by reacting with p-H\textsubscript{2} enriched molecular hydrogen gas, creating a different spin population in the product molecule compared to its thermally equilibrium state thereby resulting a signal enhancement. This process is achieved via transferring the magnetically equivalent spin order of p-H\textsubscript{2} into observable
magnetization in the HP product molecule. The effect was discovered in Pasadena, California and was named the “Parahydrogen And Synthesis Allow Dramatically Enhanced Nuclear Alignment” (PASADENA) effect.\textsuperscript{51} A year after PASADENA was invented, Pravica \textit{et al.}\textsuperscript{62} reported a different observation, when the hydrogenation reaction was performed outside of the spectrometer and detection of HP product as done by physically placing the HP sample into the magnet after hydrogenation is completed. This effect was named “Adiabatic Longitudinal Transport After Dissociation Engenders Nuclear Alignment” (ALTADENA) effect. Of note, the city of Altadena borders Pasadena in Southern California. PASADENA was experimentally observed for acrylonitrile hydrogenation in the presence of Wilkinson’s catalyst where antiphase signals were obtained for the HP propionitrile with distinct chemical shift difference ~1 ppm and with high enhancement values.\textsuperscript{51} First ALTADENA spectrum detection was conducted on HP ethylbenze by reacting p-H\textsubscript{2} with styrene.

Theoretical background behind these two phenomena can be explained by using a simple AX type spin system\textsuperscript{48} as shown in Figure 15. For an example if we compare a coupled spin system of AX type, it has four different states coming from four possible spin combinations; αα (the lowest energy state which is aligned with the magnetic field and the m= -1/2), αβ, βα and ββ (the highest energy state opposed to the magnetic field direction) which are almost equally populated except for the small difference in population in the range of 10\textsuperscript{-5} in accordance with Boltzmann distribution. Four distinct signals are expected due to four allowed
transitions by the selection rule, \( \Delta m = \pm 1 \), where \( m \) is the spin quantum number. The chemical shift difference of the two transitions is obtained by the difference in the centers of the two doublets. Resonance line splitting occurs due to \( J \) coupling between the spins, which means each nucleus will feel the difference of the applied magnetic field and the field exerted by the neighboring nucleus. The populations of these levels undergo an abrupt change when PASADENA type of experiment is conducted inside the magnetic field. Since hydrogenation occurs inside NMR magnet, this is considered as the weakly coupled regime. A weakly coupled regime is when the spin systems are weakly coupled with each other with the chemical shift difference of the two spins \((\delta_A - \delta_X)\) being greater than the \( J \) coupling (measured in Hertz units) between the two spins \((\delta_A - \delta_X > J)\). Therefore, during hydrogenation, spin order transferred from \( p\text{-H}_2 \) will populate \( \alpha \beta \) and \( \beta \alpha \) levels creating a much stronger signal with two antiphase doublets. In contrast, ALTADENA experiments are done by performing the hydrogenation in a low magnetic field (< 10 mT) followed by quick transfer of the HP sample into magnet for detection (> 1T).\(^{81}\) In this strongly coupled regime, the \( J \) coupling dominates the chemical shift difference \((\delta_A - \delta_X < J)\). One level of the two \( \alpha \beta \) or \( \beta \alpha \) becomes overpopulated giving rise to two transitions. A nice illustration is presented in Figure 15 comparing these effects. Therefore, by selecting the magnetic field of hydrogenation, one can perform PHIP experiments either in a weakly coupled regime (ALTADENA) or a strongly coupled
regime (PASADENA) and control the line shape appearance of HP substrates.

**Figure 15.** Representation of the population difference in energy levels for an AX type spin system. a) AX spin system at thermal equilibrium and its NMR spectrum. b) AX spin system under PASADENA conditions and the typical signature of the spectrum. c) AX spin system under ALTADENA conditions and its typical spectral signature.

2.12 Low-field NMR detection of PHIP signals

Numerous studies have been performed with the NMR spectral or imaging detection of HP molecules in high field NMR devices. However, the ability to carry out hyperpolarization experiments in the low field offers many advantages: (i) low cost of the magnet, RF coils and spectrometer, (ii) convenience of siting and operation, and more importantly (iii) opportunity to perform NMR detection in vivo using much longer HP state decay constants. Low-field detection has thus become an interesting area of research, but development of different detection methods
and technologies are required. As mentioned in the section above, high-field NMR experiments are performed in the weakly coupled regime, where the chemical shift difference is much higher than the $J$ coupling between the spin systems. Therefore, the spectral lines are well resolved thus structural information of molecules can be easily obtained. However, problems arise when spectral acquisition is done at the low magnetic fields where the chemical shift difference is much lower than the $J$ coupling of the two nascent $p$-$H_2$-derived spins. Let’s consider the HP propane spectrum at 1.4 T field and 0.0475 T field. Spinsolve 1.4 T spectrometer (Magtritek, New Zealand) employs 61 Hz per ppm at $^1H$ resonance frequency of circa 61 MHz. Since the chemical shift difference between the two HP protons of propane (methyl and methylene protons as shown in Figure 14) is ~0.4 ppm, it corresponds to (0.4 ppm*61 Hz*ppm$^{-1}$) 24 Hz, the $J$ coupling between the two protons is approximately 7.3 Hz, i.e., much smaller than the 24 Hz chemical shift difference. As a result, clear distinct spectral lines can be observed in PASADENA or ALTADENA experiments. But, using a low field 0.0475 T NMR spectrometer (with a resonance frequency of ~ 2 Hz*ppm$^{-1}$), we reach a strongly coupled regime, where 0.4 ppm equals to (0.4 ppm*2 Hz*ppm$^{-1}$) 0.8 Hz, which is substantially smaller than the spin-spin coupling of 7.3 Hz. Therefore, the two spectral lines effectively cancel each other in ALTADENA regime, and no substantial HP NMR signal can be observed in the low field. Moreover, since magnet homogeneity is only ~20 Hz over 5 cm density volume sphere the signal cancelation will happen even in the case of PASADENA experiment. As a consequence of PHIP signal
cancelation, the low-field detection of PHIP were not investigated. However, over
the past decade, various methods$^{115-119}$ have been developed for low-field
detection of PHIP resonances. In our studies, we have employed SLIC (Spin-
Locked Induced Crossing, explained in section 2.14)$^{95}$ RF pulse sequence to
transform the nascent singlet into observable magnetization in order to obtain NMR
spectra of the HP samples at 0.0475 T magnet, i.e., in the strongly-coupled regime.

2.13 Decay of the HP signal and long-lived spin states (LLS)

An extensive amount of research has been done on the study of singlet states
in the context of HP compounds.$^{120, 121}$ To understand the lifetime of singlet states,
we first need to discuss the decay processes of a HP molecule. As described in
section 2.1, during precession process of a tipped magnetization of a nucleus, it
suffers two different relaxation processes. One is the relaxation of the net
magnetization to the initial thermal state, which is governed by its spin-lattice
relaxation constant $T_1$. The other is the spin–spin relaxation due to the dephasing
arising from different precessions of the neighboring nuclei in a sample, i.e., a $T_2$
time constant. Spin–lattice relaxation and spin-spin relaxation are also called as
longitudinal relaxation and transverse relaxation processes respectively. $^1$H $T_1$ and
$T_2$ values are different for different type of molecules and their environments, and
for biological systems they usually vary from millisecond to second scale.
Paramagnetic impurities such as cellular molecular oxygen only enhance the $T_1$
relaxation.$^{122}$ As stated above for a conventional NMR experiment, the tipped
magnetization will begin to relax after the sample is placed inside the NMR magnet,
eventually recovering to its original thermal equilibrium value (in the z direction, Figure 4c). Typically, in the context of thermal polarization, nuclear spin magnetization (measured in the z direction) increases with time to its equilibrium value. However, due to non-equilibrium polarization created by hyperpolarization techniques, the initial polarization is significantly greater than the thermal equilibrium value, causing it to eventually decrease the thermal magnetization with the corresponding decrease of MRI signal. As a result, when once a HP state is generated, the polarization will always decrease to thermal equilibrium in accord to $T_1$. Thus, the pool of nuclear spin hyperpolarization is not replenishable. Although, the produced singlet states render longer lifetimes of the HP state, they also follow the same trend of overall spin order decay due to nuclear spin relaxation.

In relation to all the applications of HP compounds, the $T_1$ polarization relaxation typically follows a mono-exponential decay (e.g., Figures 23a, c, e and Figure 39c).

Therefore, ideally rapid acquisition following production and administration of HP contrast agent is essential before the HP state relaxes back to its thermal equilibrium state. In the context of proton-hyperpolarized contrast agents, it is challenging for biomedical applications due to short in vivo $T_1$ values of protons. Different ways of improving the relaxation constants thus slowing down the relaxation process are widely studied. One approach is the creation of singlet spin orders, where the HP molecule can be effectively trapped in a higher spin order frequently with a much longer relaxation value. Similarly, to the p-H$_2$ singlet state, most HP molecules have longer lifetimes in singlet states compared to faster
relaxation otherwise. As a result, these states are referred to as long-lived singlet states or LLS. Therefore, preparation of HP molecules in singlet spin pairs has drawn much attention over the past decade because that way spin hyperpolarization can be stored for much longer and subsequently a greater fraction of HP state can be detected – thus, maximizing the SNR of HP scan. The existence of singlet spin pairs has been reported when their $J$ couplings are much higher than the chemical shift difference, i.e., in the strongly coupled regime. The ultimate decay of a LLS can occur due to many reasons.\textsuperscript{128, 129} To summarize, $T_{LLS}$ can be orders of magnitude greater than the corresponding $T_1$ values thus enabling preparation and storage of some HP molecules for biomedical applications.\textsuperscript{126, 127, 130-134}

Various approaches have been undertaken in order to prepare singlet spin orders of HP molecules.\textsuperscript{133, 135, 136} Once created LLS cannot be readily detected by low-field NMR without converting the singlet spin order into NMR-observable magnetization. In my work, we employ two approaches for singlet state visualization at low magnetic field. The first one is molecular deuteration of the substrate, which additionally breaks the symmetry of the singlet state in low magnetic field (due to additional spin-spin couplings), and thus makes ALTADENA spectra readily seen even in the nascent singlet state.\textsuperscript{137} The second approach is the new technique introduced by DeVience et al.\textsuperscript{95} in 2013 called Spin-Locked Induced Crossing (SLIC) to achieve magnetization transfer from non-observable singlet state to observable triplet state.
2.14 Spin Locked Induced Crossing (SLIC)

Although the LLS of an HP molecule benefits the lifetime of the state, the conversion of the triplet state to a singlet state cannot be readily achieved by a simple hard RF excitation pulse. A few different approaches have been employed\textsuperscript{133, 135} for the triplet-singlet transformation based on the application of different pulse schemes but, in 2013, DeVience and co-workers introduced a new way of applying a continuous spin lock pulse with a \( B_1 \) strength similar to that the \( J \) coupling of the two spins of interest -- thus creating a similar energy of singlet and triplet state allowing for successful polarization interconversion between the singlet and the triplet states.\textsuperscript{95} This approach was named as Spin-Lock Induced Crossing or SLIC. A schematic representation of the SLIC experiment is given in Figure 16. In its original implementation, the approach was developed to convert the observable magnetization into the singlet state. As a result, a 90° RF pulse is applied first to create a transverse magnetization followed by a spin locking (SL) pulse with a \( B_1 \) frequency matching the \( J \) coupling of the spins. Now, the singlet order has been created, and the generated singlet spin magnetization evolves for a period of time (\( \tau_{\text{evolve}} \)) in accord to T\(_{\text{LLS}}\). Finally, the second SL pulse converts the singlet polarization back to the triplet state for detection.\textsuperscript{95}
**Figure 16. A schematic diagram of the typical SLIC experiment.** First the creation of triplet polarization is achieved via a 90 pulse and SL pulse is sent to convert the triplet to the singlet state. A similar SL applied again to convert the singlet back to the triplet state for detection. Please note the nutation frequency ($\nu_n$) of SL pulse matches the $J$ coupling.

SLIC opened up new ways of creating triplet-singlet transfers with applications such as selective ways of spin preparation in desired magnetic fields, solid-state spin dynamic studies, long lived quantum memories etc. In the work reported in this dissertation, we used SLIC as a tool to detect HP NMR spectra in the low magnetic field. Spin singlet states are already generated at low magnetic fields via the PHIP chemical reaction naturally due to the nature of the strongly coupled regime. SLIC pulses were utilized to convert those singlet states into observable triplet polarization for visualization.

### 2.15 Hyperpolarized compounds as MRI contrast agents

Generally, HP molecules are studied to investigate their ability to be used as a contrast agent in various biomedical applications. Even though the polarization values obtained for these HP molecules approach the order of unity or 100%, the
SNR is still low for in vivo studies of all biocompatible molecules due to low concentration of many of them. As a result, the hyperpolarization community has focused on key metabolites and drugs that can be injected or inhaled in safe large doses suitable for HP MRI. Furthermore, relaxation process can induce significant losses in polarization during administration and delivery to the specific tissue / organ / cell. Moreover, as discussed above in Chapter 2.13, these HP contrast agents cannot be regenerated once their relaxation is complete. These limitations make the translation of HP contrast agents into clinical applications challenging. Various approaches are therefore taken in order to minimize relaxation losses: (i) introduce rapid administration of HP contrast agents to the subject, (ii) increase the lifetime of the HP contrast agent, (iii) attempt the polarization transfer from one nucleus to another thereby increasing the lifetime of the HP molecule and others. A broad range of HP molecules have been investigated for their potential use as MRI contrast agents ranging from simple hydrocarbons to hetero-nuclear atoms.

Typically, HP molecules can be categorized into different groups according to their biological function of interest. Some behave as inert contrast agents, e.g., $^{129}\text{Xe}$. Others may undergo metabolic conversion, and therefore act as metabolic contrast agents, e.g., HP pyruvate converts to lactate.$^{69, 79, 142-145}$ The main goal throughout this work was to design inhalable and injectable longer-lived contrast agents towards pulmonary imaging and metabolism studies respectively.
Experiments by various groups have shown successful reports on the use of injectable contrast agents. The use of $^{13}$C labeled hydrocarbon molecules such as 2-hydroxyethyl [1-$^{13}$C]-propionate (HEP) has been analyzed as PHIP based inert contrast agent to monitor and locate the heart and lung contours in rats and to study coronary angiography and myocardial perfusion in pigs.$^{142, 143, 146-148}$ Numerous experiments *in vivo* have been done on the use of metabolic contrast agents such as $^{13}$C-succinate, $^{13}$C-phospholactate, $^{13}$C-acetate, $^{13}$C-pyruvate, $^{13}$C-lactate using PHIP; studies using HP $^{15}$N-nicotinamide, $^{15}$N-nitroimidazole and many more are underway.$^{43, 80, 82, 99, 100, 144, 149-157}$ Considerable efforts are advancing towards exploring heteronuclear contrast agents labeled with HP fluorine-19,$^{158-162}$ phosphorous-31,$^{163}$ silicon-29,$^{164, 165}$ etc. Further information on PHIP based contrast agents can be found in the comprehensive review provided by Hovener *et. al.*$^{81}$

Nitroimidazole group of molecules has been of a particular interest to us because of its established use as a biomarker for hypoxia cell detection thus enabling cancer diagnosis and treatment. $^{18}$F labeled fluoromisonidazole (FMISO) is a popular example of nitroimidazole-based contrast agent, which is successfully employed as an imaging tracer in positron emission topography (PET) for tumor hypoxia detection. Our interest is to develop such contrast agents that can be successfully employed for MR imaging.$^{69, 166}$ HP MRI using such agents offers three critical potential advantages: (i) fast examination time (minutes versus hours), (ii) low-cost contrast agent, and (iii) no use of ionizing radiation (a typical PET-CT
exam exposes a human to ~8-year equivalent of natural level of radiation on planet Earth)—low-cost in particular has been recognized as the deal-breaker for the widespread use of FMISO and other similar radioactive tracers in the context of cancer hypoxia imaging. The $^{15}$N SABRE studies reported here were performed with the goal of developing HP $^{15}$N metronidazole for hypoxia detection in cancer cells and tumors. We envision the use of HP metronidazole as an injectable contrast agent with $^{15}$N of up to 10 minutes. If successful, our new contrast agent may accelerate hypoxia imaging (down to 10-minute long exam) without the use of ionizing radiation as noted above.

Our other interest is the development of PHIP-based inhalable (gaseous) contrast agents for pulmonary imaging applications. Although HP $^{129}$Xe and $^3$He are very popular gaseous contrast agents, they have several disadvantages such as, high cost of the inert gases, high production cost (~ $0.5M equipment) and slow production rate of HP gas, i.e. a few liters of the HP gas can be produced per hour at the best efficiency. Therefore, new area of interest is drawn towards the use of alternative gaseous contrast agents like simple hydrocarbon molecules. Apart from the low cost associated with hydrocarbon proton-hyperpolarized molecules, the other advantage is their ability to be used on the commercially available proton detection MRI scanners thereby limiting the requirements of special detection hardware. The most widely studied inhalable contrast agent to date is HP propane after its first HP detection using HET-PHIP technique. Significant progress on HP propane is reported on its production and MR imaging
with reasonable enhancement values.\textsuperscript{75, 169-171} Non-hyperpolarized propane was employed for pulmonary imaging in rats by Hane and co-workers.\textsuperscript{172} We have extended the applications of HP propane by reporting methods of creating LLS at low fields as well as clinical-scale production of HP propane with significantly higher throughput than that of HP \textsuperscript{129}Xe: a 0.3 L batch of HP propane can be produced every 2 seconds, thus enabling access to rapid imaging.\textsuperscript{74, 75, 138}

\subsection*{2.16 Clinical-scale production of HP propane}

Previous studies showed successful polarization of propane via pairwise addition of p-H\textsubscript{2} to propylene using HET-PHIP with reports on LLS of HP propane at 0.05 T magnetic fields.\textsuperscript{102, 138} Salnikov \textit{et al} reported on the production of \textasciitilde 0.2 L HP propane in a short duration as \textasciitilde2 s opening up ways of clinical-scale production using a clinical-scale propane hyperpolarizer as shown by Figure 17.\textsuperscript{74, 173} A gas mixture of p-H\textsubscript{2} and propylene was sent through a catalytic reactor filled with solid Rh/ TiO\textsubscript{2} as the heterogeneous hydrogenation catalyst at high temperature. The HP propane exiting the reactor was then studied using a 4.7 T MRI scanner.\textsuperscript{74} Using this approach, a batch mode production of HP propane was conducted with average flow rates of \textasciitilde18 SLM (standard liters per minute) for a 1:1 HP propane: p-H\textsubscript{2} mixture at temperature of \textasciitilde40 °C.\textsuperscript{74}
2.17 Radio Amplification by Stimulated Emission of Radiation (RASER)

RASER is a recently discovered phenomenon, which is an RF equivalent of LASER reported by Suefke and co-workers in 2017. RASER process is similar to the well-known phenomena MASERs. Both LASERs and MASERs show
stimulated emission of radiation in the optical and microwave frequencies of the electromagnetic spectrum respectively. Stimulated emission happens due to the population inversion of energy levels. Therefore, RASER effect is also due to the creation of population difference in energy levels which result in emission of radiation but, in the RF region using Zeeman energy levels. Coupling of an LC frequency resonator with coherent oscillations in the RF region due to spin polarization of a molecule generates a RASER signal. For this coupling to happen, high level of spin magnetization (and by extension polarization) is required compared to the thermal magnetization. Therefore, first observations of liquid state RASER was reported for HP molecules with high spin polarizations values up to unity using SABRE experiments in the mT field using specialized detection with ultra-high quality factor (Q) resonator values. The following equations provide mathematical insights to the RASER process. Spin–spin relaxation constant follows the below equation for RASER to occur,

\[
\frac{1}{\tau_{RD}} > \frac{1}{T_2^*}
\]

(16)

where \(\frac{1}{T_2^*}\) is the modified spin- spin relaxation rate and \(\frac{1}{\tau_{RD}}\) is defined as the radiation damping rate (radiation damping is recognized as the damping of the MR signal introduced by the coupling of the rotating magnetization of spins with an electrical resonance circuit).

\[
\frac{1}{\tau_{RD}} = -\mu_0\gamma\eta QM_0/2 = -\mu_0\eta Q\gamma^2 h n_s P/4
\]

(17)
given, \( M_0 = \gamma P n_s / 2 \) with \( \mu_0, \eta, Q, \gamma, h, n_s \) and \( P \) are permeability of vacuum, the filling factor of the resonator, the quality factor of the resonator, gyromagnetic ratio, Planck’s constant, spin number density and degree of spin polarization respectively.\(^{174, 178}\) When the degree of spin polarization \( P \) is greater than zero, damping rate becomes negative (equation 17) resulting in broadened spectral lines. But, when \( P \) is smaller than zero, population inversion takes place with a positive damping rate. RASER activity is initiated when equation 16 satisfies the following condition, \( 1 / T_2^* - 1 / \tau_{RD} < 0 \).\(^{178}\) Therefore, the first demonstration of RASER involved high Q resonators to lower the requirements of RASER effect conditions for low frequency proton spins.\(^{174, 177}\) A recent study by Joalland et al.\(^{178}\) revealed that the RASER is a common phenomenon and RASER activity was reported for substrate molecule concentrations as low as 40 mM under both ALTADENA and PASADENA conditions without the use of high Q resonators. Various molecular substrates\(^{141, 178}\) showed RASER activity under above mentioned conditions and the spectra were recorded by a commercial 1.4 T NMR spectrometer device.

Indeed, the RASER effect opens new ways of applications\(^{178, 181}\) ranging from magnetic resonance to quantum computing and cryptography, and beyond.
CHAPTER 3 Relaxation Dynamics of Nuclear Long-Lived Spin States in Propane and Propane-d6 Hyperpolarized by Parahydrogen


In this chapter, “we” and other first-person plural pronouns are used in reference to all authors of this publication. My individual contributions to the research include conduction of all the experiments, analyzing data and writing parts of the manuscript. Figures and tables containing data contributed by researchers other than me will contain a disclaimer in the caption; figures and tables with no disclaimer in the caption contain data collected by me. I also would like to acknowledge all of the co-authors listed in here who provided with chemicals and materials.

In the United States, lung cancer and related lung diseases is one of the leading causes of death for both men and women. Different methods of imaging technologies are under investigation for fast detection in early stage diagnostic procedures. Hyperpolarized propane, which is a low toxic HP molecule, can be potentially developed as an MRI contrast agent for pulmonary image acquisition on a single patient breath hold. We present a study on HP propane hyperpolarization relaxation dynamics to show HP state lifetime can be increased through the use of long-lived spin state (LLS), benefiting its preparations and applications as an inhalable imaging agent in the future.

3.1 Abstract

We report a systematic study of relaxation dynamics of hyperpolarized (HP) propane and HP propane-d6 prepared by heterogeneous pairwise p-H2 addition to propylene and propylene-d6 respectively. Long-lived spin states (LLS) created for
these molecules at the low magnetic field of 0.0475 T were employed for this study. The p-H₂ induced overpopulation of a HP propane LLS decays exponentially with time constant (T_{LLS}) approximately 3-fold greater than the corresponding T₁ values. Both T_{LLS} and T₁ increase linearly with propane pressure in the range from 1 atm (the most biomedically relevant conditions for pulmonary MRI) to 5 atm. The T_{LLS} value of HP propane gas at 1 atm is ~3 s. Deuteration of the substrate (propylene-d₆) yields hyperpolarized propane-d₆ gas with T_{LLS} values approximately 20% shorter than those of hyperpolarized fully protonated propane gas, indicating that deuteration does not benefit the lifetime of the LLS HP state. The use of p-H₂ or Xe/N₂ buffering gas during heterogeneous hydrogenation reaction (leading to production of 100% HP propane (no buffering gas) versus 43% HP propane gas (with 57% buffering gas) composition mixtures) results in (i) no significant changes in T₁, (ii) decrease of T_{LLS} values (by 35±7% and 8±7% respectively); and (iii) an increase of the polarization levels of HP propane gas with a propane concentration decrease (by 1.6±0.1-fold and 1.4±0.1-fold respectively despite the decrease in T_{LLS}, which leads to disproportionately greater polarization losses during HP gas transport). Moreover, we demonstrate the feasibility of HP propane cryo-collection (which can be potentially useful for preparing larger amounts of concentrated HP propane, when buffering gas is employed), and T_{LLS} of liquefied HP propane reaches 14.7 seconds, which is greater than the T_{LLS} value of HP propane gas at any pressure studied. Finally, we have explored the utility of using a partial Spin-Lock Induced Crossing (SLIC) radio frequency (RF) pulse sequence for converting
the overpopulated LLS into observable $^1$H nuclear magnetization at low magnetic field. We find that (i) the bulk of the overpopulated LLS is retained even when the optimal or near-optimal values of SLIC pulse duration are employed, and (ii) the overpopulated LLS of propane is also relatively immune to strong RF pulses—thereby, indicating that LLS is highly suitable as a spin-polarization reservoir in the context of NMR/MRI detection applications. The presented findings may be useful for improving the levels of polarization of HP propane produced by HET-PHIP via the use of an inert buffer gas; increasing the lifetime of the HP state during preparation and storage; and developing efficient approaches for ultrafast MR imaging of HP propane in the context of biomedical applications of HP propane gas, including its potential use as an inhalable contrast agent.

3.2 Introduction

Hyperpolarization increases the nuclear spin polarization by several orders of magnitude over its thermal equilibrium value.\textsuperscript{182, 183} This dramatic polarization increase results in corresponding gains in NMR and MRI signals.\textsuperscript{79, 184} Biomedical use of hyperpolarized (HP) spin states enables a variety of new applications, such as probing metabolism and organ function on the time scale of tens of seconds.\textsuperscript{81, 152, 182} In the case of gases and lung MRI, the potential of HP noble gases like $^3$He and $^{129}$Xe for such applications was demonstrated over 20 years ago,\textsuperscript{185-188} and they have been shown safe in clinical trials (e.g. Reference\textsuperscript{189}). Although HP $^3$He MRI was demonstrated before $^{129}$Xe,\textsuperscript{190-192} the supply of $^3$He is limited (it is a product of tritium decay), thus presenting a significant obstacle to a sustainable
widespread clinical use. As a consequence $^{129}$Xe is the leading HP noble gas for prospective use as an inhalable contrast agent for imaging COPD, emphysema, brownfat, and other applications. However, while progress has been made, the clinical-scale hyperpolarization hardware for HP $^{129}$Xe preparation remains relatively complex and costly. More importantly, clinical MRI scanners are not equipped with the hardware or software required for HP $^{129}$Xe imaging, because they are designed to image the $^1$H spins from water and lipids in the body, whereas $^{129}$Xe resonates at a frequency that is several-fold lower than that of $^1$H. Due to the above limitations of HP $^{129}$Xe production and imaging technologies, the search for biomedically useful inhalable HP contrast agents remains an active area of basic and translational research.

HP gaseous hydrocarbons potentially obviate the shortcomings of HP $^{129}$Xe in the context of biomedical applications, because protons of hydrocarbons can be hyperpolarized quickly and cheaply via the $p$-$H_2$ utilization. Using heterogeneous catalysts, gaseous hydrocarbons can be hyperpolarized via pairwise addition of ($p$-$H_2$) to a suitable unsaturated substrate via a process termed heterogeneous parahydrogen-induced polarization (HET-PHIP). Although gas-phase proton $T_1$ values are generally short—ca. 1 second at 1 atm—a number of approaches have been developed to extend the lifetime of HP states in hydrocarbons, including the use of high operating pressure and reversible dissolution. Most importantly, the pioneering works of Levitt, Bodenhausen, Warren and others have demonstrated that the
relaxation decay of HP state can be significantly prolonged when two HP nuclear spins are entangled in a singlet state. More broadly in spin systems with three\textsuperscript{217} and more spins,\textsuperscript{210} long-lived spin states (LLS) can exist due to the symmetry properties of the spin Hamiltonian. The exponential decay constant of LLS $T_{\text{LLS}}$ can significantly (by up to several orders of magnitude) exceed $T_1$.

HP propane has garnered our attention because it is relatively inert,\textsuperscript{218, 219} has low in vivo toxicity,\textsuperscript{220} and it is approved by the FDA for the use in the food industry as a propellant and for food storage.\textsuperscript{221} More broadly, it is used as a food additive (E944). Therefore, we envision its potential use as an inhalable HP gas in a manner similar to that of HP $^{129}\text{Xe}$. However, unlike HP $^{129}\text{Xe}$, HP propane can be produced using relatively simple, low-cost, and high-throughput hyperpolarization hardware,\textsuperscript{222} and HP propane can be readily imaged using conventional clinical MRI scanners which can readily detect HP protons of propane gas,\textsuperscript{134, 137, 169, 223} representing clear advantages for biomedical use. Recently, we have demonstrated a clinical-scale hyperpolarization process for production of pure (from catalyst) HP propane gas capable of producing $\sim$0.3 standard liters of HP hydrocarbon gas in $\sim$2 seconds.\textsuperscript{222} As a result, access to HP propane for potential use in humans and large animals is enabled. The work presented here is focused on systematic relaxation studies of HP propane in the gas and liquid states with the key focus on the biomedical application of this potential inhalable contrast agent and extending the lifetime of HP state as much as possible for potential bioimaging applications.
3.3 Materials and methods

Parahydrogen Generation and Experimental Setup. Two different NMR spectrometer systems were used in this study: One setup used a dual-channel Kea-2 low-field NMR spectrometer (Magritek, New Zealand) to study HP propane at 0.0475 T (Figure 19) with a previously built radio-frequency (RF) coil. The other setup employed a bench-top 1.4 T NMR spectrometer (NMRPro 60, Nanalysis, Canada) (Figure 51, appendix A).

Figure 19. HET-PHIP polarizer apparatus and low-field NMR setup. This setup consists of three major components (outlined by dashed lines): p-H₂ generator, propane hyperpolarizer, and the 0.0475 T NMR spectrometer/pulse-programmable automated gas manifold controlled by Kea2 NMR spectrometer with a pressurized phantom (~17.5 mL).

Parahydrogen was prepared using a custom-made p-H₂ generator using 99.9995% hydrogen (Airgas) producing a p-H₂ enrichment fraction of ~87%. During operation p-H₂ is produced continuously and collected in a storage chamber (0.5-liter size) prior to its use in the experiments. p-H₂ was combined with propylene (Sigma-Aldrich 295663-300G) or propylene-d₆ (99% atom D, Sigma-
Aldrich 455687) gas in a mixing chamber to achieve a given desired ratio of the reagents. In some experiments, an additional cylinder containing a buffering gas (extra p-H₂ was added or 3:1 Xe/N₂ mixture was added in parallel, Figure 19). The prepared reaction mixture was then sent through a mass flow controller (MFC) set to approximately 2,000 standard cubic centimeters per minute (sccm) flow rate (unless otherwise stated), and into the reactor. The catalytic reactor (44-cm-long copper tubing with ¼ in. outer diameter, OD) contains ~280 mg of 1% (by weight) Rh/TiO₂ catalyst mixed with 12 g of copper particles (10-40 mesh size, >99.90% purity, Sigma-Aldrich) in the gas-reaction section, Figure 19. The gas heating and gas cooling sections of the reactor were also filled with 12 g of the copper particles in each section (with Cu particles added for even heat distribution), giving a total of ~36 g of copper particles in this copper tube; the sections were separated into three stages by glass wool. In the first section, the gas mixture is heated using cartridge heaters connected to a PID temperature controller. The second stage is heated similarly but contains the catalyst (with Cu particles added for even heat distribution) to perform substrate hydrogenation with p-H₂ (Figure 20a and Figure 20d). The third / final section of the reactor is for gas cooling, where a third PID temperature controller / heater / cooling fan combination is employed to regulate the cooling of the gas, which is facilitated by passing it through another ~12 g of Cu particles (Figure 19). The gas mixture exiting the reactor is then directed to either the 0.0475 T or the 1.4 T NMR system to collect enhanced ¹H NMR spectra of HP propane or propane-d₆. The following scheme was used for the 0.0475 T
system. The HP gas exiting the reactor was sent through a valve (#2') into the small phantom (17.5 mL) located within the RF coil of the 0.0475 T magnet. The flow of the gas is then directed through another valve (#3') after which the gas is vented to atmosphere (i.e. within a hood) via a safety valve operating at 0-60 psi overpressure. The gas inlet and outlet are also connected via a normally-open (NO) valve (#1') through a bypass connection to enable the gas flow, when the phantom is closed for the gas flow—this way, the production of HP gas remains uninterrupted.
Figure 20. SLIC detection of HP propane. a) Diagram of the pairwise addition of p-H₂ to propylene over the Rh/TiO₂ catalyst. b) the sequential steps of the signal acquisition method using the 0.0475 T setup for the measurement of HP propane T_{LLS} (achieved by varying the relaxation delay τ_R), optimization of the chamber-refill time τ_CR, and optimization of SLIC transformation (varying RF amplitude, power, and frequency offset). c) Corresponding pulse sequence used for HP propane T₁ measurements using 90° RF pulse (to create z-magnetization) after SLIC irradiation followed by a small angle (α) RF pulse; note the SLIC pulse and chamber refill are performed once in the sequence shown in c) versus multiple refills employed in sequence shown in b). d) Diagram of the pairwise addition of p-H₂ to propylene-d₆ over the Rh/TiO₂ catalyst. e) Pulse sequence used for the measurement of HP propane-d₆ T_{LLS}. Note the p-H₂ symmetry breaking is achieved by the chemical reaction, with nascent protons H_A and H_B placed in methylene and methyl chemical groups in a) and d).
The details of the 1.4 T apparatus are provided in Figure 51, appendix A. For these experiments, the HP propane gas exiting the reactor system was directed to the bench-top NMR spectrometer. The inlet was connected to the bottom of a standard 5-mm NMR tube. This NMR tube was cut at the bottom, and glued with epoxy to 1/8 in. OD polyethylene tubing. The NMR tube designed in this fashion was placed inside the NMR spectrometer. The top of the NMR tube was connected to a manual valve (via ¼ in. OD Teflon tubing), which was then vented via two manual valves and a safety valve. Here we also employed a bypass between the inlet and the outlet lines of the NMR tube. A receiver gain of 4 dB was used for all the spectra recorded using this 1.4 T NMR spectrometer system. The 1.4 T bench-top NMR spectrometer arrangement was used to directly measure the polarization enhancement values and chemical conversion of HP propane under the following experimental conditions: a 1.2:1 mixture of p-H₂: propylene was used at a gas flow rate of 2000 sccm at variable reactor temperature. NMR spectra of HP propane were acquired in two flow regimes: continuous-flow (Figure 24b, under continuous flow) and stopped-flow (Figure 24d, when the flow was terminated). The overpressure was measured using a pressure gauge connected downstream of the NMR tube setup (Figure 51, appendix A). These experiments were performed by flowing the HP gas mixture from the exit of the catalytic reactor into the 5 mm NMR tube via the bottom side of the bench top NMR spectrometer (Figure 51, appendix A). The gas exiting at the top of the NMR tube was then flowed through manual valves (#1” and #2”) to the vent, and the spectrum was recorded under
continuous-flow conditions. In a stopped-flow condition, after flowing the gas for at least 20-40 seconds the gas flow was stopped by dialing zero from the MFC and closing the manual valve #2", so the HP propane gas would be trapped (and stopped) throughout the duration of the NMR spectrum acquisition (Figure 24d). The time delay between stopping the gas flow and the NMR acquisition was 0.5-1 s. After the relaxation of hyperpolarization, NMR spectra of stopped-flow thermally polarized propane gas were recorded (an example is shown in Figure 24c). Although the stopped-flow mode exhibited better spectral resolution because the gas-flow artifacts were eliminated, we generally used the continuous-flow mode for data acquisition owing to the less complicated experimental procedure, more reproducible data, and greater HP signals.

For the HP propane condensation, a slightly modified version of the setup shown in Figure 19 was employed: the phantom inside the RF coil was replaced by a 5 mm NMR tube via a Wye-connector in which the reaction mixture was cryo-cooled inside a dry ice / ethanol bath at a flow rate of 1300 sccm for ~20 s in the Earth’s magnetic field. Then the NMR tube was rapidly placed inside the 0.0475 T magnet, and spectra were collected using a RF SLIC pulse (200 ms).

**NMR pulse sequences.** Both the filling of the pressurized phantom and the acquisition of NMR spectra were fully automated in Prospa software (Magritek, New Zealand) using a custom-made pulse program and hardware of the pulse-programmable polarizer described previously. This setup was used for most of
the experiments reported here to study the effects of varying the refill duration, spin-lock induced crossing (SLIC) pulse duration, etc. All experiments were conducted by first filling the phantom with HP gas, terminating the flow by closing valves #2' and 3', and then applying the sequence of RF pulses of interest on the static HP gas mixture (i.e., under stopped-flow conditions, in contrast with our previous work on HP propane, where RF pulses were applied to continuously flowing HP gas) and finally collecting the FID as shown in Figure 20b, Figure 20c, and Figure 20e.

The exponential decay of the long-lived spin state (LLS) in HP propane is characterized by the exponential decay constant $T_{LLS}$, which was measured by varying the relaxation delay time period as shown in Figure 20b. The corresponding NMR spectrum of HP propane after SLIC is shown in Figure 21a. After the SLIC transformation of the p-H$_2$-induced overpopulation of the LLS (denoted schematically as a singlet in Figure 22a; it should be noted that the spin eigenstates of protonated propane have eight protons, and therefore the resulting spin system is different from the prototypical 'singlet' and 'triplet' states—after pairwise addition of p-H$_2$ singlet (as the two protons belonging to the CH$_2$ and CH$_3$ groups respectively), many collective states of the proton spin system can be populated, and some of these states can be long-lived due to the symmetry properties of the spin Hamiltonian; therefore, strictly speaking, LLS of propane cannot be called a 'singlet' state), the observable magnetization is aligned along the axis of the SLIC irradiation (i.e. x or y axis of the rotating frame, which is atypical
approach employed in NMR to describe the effect of RF pulses), and a 90° pulse is applied to align the magnetization of HP propane along the z-axis (i.e. along the applied static magnetic field referred to as z-axis of the rotating frame), Figure 20c. After these RF-induced transformations, the resulting z-magnetization decays exponentially according to the spin-lattice decay time constant $T_1$, which is measured by applying a small-angle excitation pulse $\alpha$ (~10°) followed by FID detection, applied several (N) times to observe the decay (as shown in Figure 20c).

Although LLS exists for HP propane-d$_6$, the application of a SLIC pulse for transformation of LLS into observable magnetization is not required due to the spin-spin coupling of nascent protons with deuterons—see Reference $^{137}$ for details. As a result, $T_{LLS}$ can be conveniently measured by applying a small-angle excitation pulse (~10°) followed by FID detection, repeated several times to observe the decay (Figure 20e). The corresponding NMR spectrum of HP propane-d$_6$ is shown in Figure 21b.

**Figure 21. Low field spectra of HP propane and propane-d$_6$.** a) $^1$H 0.0475 T NMR spectrum of HP propane after SLIC transformation (as shown in Scheme 2b). b) $^1$H 0.0475 T NMR spectrum of HP propane-d$_6$ acquired using a small-angle (~10°) hard RF pulse (as shown in Figure 20e).
3.4 Results and discussion

Optimization of SLIC RF Pulse Sequence and Reaction Temperature, and Tests of Reproducibility. The effects of different experimental conditions toward the HP propane signal intensity induced by the SLIC pulse were tested in order to optimize the pulse parameters and thereby maximize the HP propane signal (Figure 22). All the data were recorded using the 0.0475 T experimental setup (Figure 19) via the sequence shown in Figure 20b. A 1:1 gas mixture of p-H₂ and propylene was used for the temperature variation experiments in order to determine the optimal temperature for the reactor, particularly in stage two of the hyperpolarizer setup. The gas flow rate was 2000 sccm, and the refill time was 5 s for each run. For refilling the phantom, first the gas was allowed to flow through the phantom for ~5 seconds with valve #1′ closed and valves #2′ and #3′ open, and after ~5 seconds of gas flow valves #2′ and 3′ were closed, and the bypass valve #1′ was opened. First, the consistency of the HP propane signal was tested (Figure 22b) using a SLIC pulse duration of 500 ms; over numerous scans a standard deviation of ~9% was found, indicating good shot-to-shot reproducibility for the hyperpolarizer. Next, the SLIC sequence was used for the experiment, and three HP propane spectra were collected at each reaction temperature (recorded as the temperature of the reactor’s aluminum jacket). The average signal value and the corresponding standard deviation are plotted for each temperature in Figure 22c. The strength of the SLIC RF pulse also has a negligible effect on the signal, as identified in previous studies. Results from SLIC pulse power
optimization are shown in Figure 22d; from this SLIC power plot, the highest SLIC signal corresponds to a power setting of -51.25 dB (this value likely corresponds to 20-30 Hz of B1 power\textsuperscript{138}), and this optimal power was used for the remaining studies using the SLIC sequence. The SLIC pulse duration was varied from 2 to 1802 ms, and the signal was found to increase with the SLIC pulse duration and was well-reproduced by a sinusoidal function (in a manner similar to the B1 nutation curve, which is predicted by the similar works of Rosen and co-workers\textsuperscript{95} and the previous study of the HP propane system\textsuperscript{138} note that data acquisition at long SLIC times is impractical due to T2 relaxation effects\textsuperscript{138}) (Figure 22e), yielding a SLIC period (t\textsubscript{SLIC}) of 4.0±0.2 s\textsuperscript{95}. The last parameter optimized was the SLIC pulse frequency offset\textsuperscript{138}. The offset was swept from ~10 Hz to 80 Hz while monitoring the signal strength, and the resulting signal intensity data indicated an optimal offset of ~45 Hz for the system under study (Figure 22f) using the experimental setup presented here.
Figure 22. HP propane SLIC pulse optimization data acquired using the 0.0475 T NMR spectrometer setup shown in Figure 19 at production/reactor/reaction temperature of 60 ± 1 °C (except for display c) where the temperature was varied). a) Diagram showing the transformation of LLS of HP propane (denoted schematically as a singlet) into observable magnetization achieved using a SLIC pulse. b) Results from a test of the shot-to-shot reproducibility of the intensity of the HP propane signal. c) Temperature dependence of the average (over three data points) HP propane SLIC-induced signal using a 1:1 gas mixture of p-H₂ and propylene. d) Optimization of RF power of the SLIC pulse (note the x-axis is provided in db units of the Kea NMR spectrometer due to the non-linearity of the RF amplifier at low power levels; the maximum is expected at B₁ RF strength between 10 Hz and 30 Hz\textsuperscript{138}). e) The dependence of the HP propane signal on the SLIC pulse duration (experimental data: black points; a fit using a sinusoidal function is shown by the solid red curve). f) The dependence of the HP propane signal on the SLIC pulse frequency offset (the solid lines are added to guide the eye). Note a SLIC pulse duration of 500 ms was employed for the data acquisition in panels b), c), d), and f). Connecting lines in displays b), c), d), and f) are meant only to guide the eye.
**1H Relaxation Dynamics of HP Propane at 0.0475 T.** First, we measured $T_1$ and $T_{LLS}$ values for HP propane gas in the pressure range between 1 and 4.6 atm formed using a 1:1 mixture of p-H$_2$ and propylene at 75 °C and 2000 sccm flow rate. As discussed below, the chemical conversion was ~100% at these conditions, i.e. yielding a nearly 100% propane gas product. We note that a previous $T_1$ and $T_{LLS}$ study of HP propane$^{138}$ was performed within a significantly higher pressure regime (3-7.6 atm total pressure), whereas the present study is focused on a lower pressure regime relevant for the future *in vivo* studies (wherein the gas would need to be imaged at 1 atm). In the overlapping range of the pressure values, the present results generally agree with those of the previous study.$^{138}$ Although the $T_1$ and $T_{LLS}$ values reported here are somewhat larger (by approximately 10%), this difference likely reflects the fact that the previous study employed reaction conditions where the chemical conversion of substrates was incomplete. The examples of the signal decay curves associated with $T_1$ and $T_{LLS}$ of HP propane are shown in Figure 23a and Figure 23c respectively. Figure 23b shows the dependence of HP propane $T_1$ time on the propane pressure, yielding values in the range of ~1.05 seconds (at 1.6 atm) to 3.4 seconds (at 4.5 atm). Figure 23d shows the corresponding dependence of HP propane $T_{LLS}$ time on the propane pressure, showing values in the range of ~3.1 seconds (at 1.0 atm) to 9.4 seconds (at 4.5 atm). On average, the $T_{LLS}$ values were approximately 3 times greater than the corresponding $T_1$ values, with nearly linear dependence on pressure, which is in accord with the previous report.$^{138}$ Such a linear $T_1$ dependence is consistent
with the major contribution of the gas-phase nuclear spin relaxation being from the spin-rotation mechanism in the intermediate-density (here, multi-amagat) regime.\textsuperscript{226, 227} Under such conditions, different relaxation times for $T_1$ and $T_{\text{LSS}}$ (and slopes with respect to density) would not be surprising as spin states with different symmetries would likely couple to different rotational states.\textsuperscript{226} Future theoretical studies are certainly warranted to provide a more-detailed understanding of the observed trends.

**Effect of HP Propane Deuteration on LLS Decay at 0.0475 T.** Since $T_{\text{LSS}}$ is considerably greater than $T_1$ in HP propane, the LLS of HP propane may be better suited for *in vivo* experiments, which would likely require at least several seconds of HP gas handling for inhalation and MR imaging. Given the desire to further lengthen the lifetime of the non-equilibrium spin order endowed by hyperpolarization,\textsuperscript{71, 228} we also studied the effect of deuteration on $T_{\text{LSS}}$ in HP propane-$d_6$ (the corresponding effective $T_1$ data is shown in Figure 54, appendix A), acquiring data under conditions similar to the HP propane $T_{\text{LSS}}$ measurements discussed above (Figure 23c). We note that the symmetry of nascent p-H$_2$ protons is broken in propane-$d_6$ in a way that the magnetization is already observable, even in the absence of significant chemical shift differences (Figure 21b) at 0.0475; for additional details the reader is referred to Ref. #\textsuperscript{137}. Therefore, the data acquisition for HP propane-$d_6$ was performed differently from that for HP propane (i.e. using the sequence shown in Figure 20e), and small tipping-angle ($\sim$10°) RF excitation pulses followed by NMR signal detection were employed. An example of LLS
decay of HP propane-d₆ is shown in Figure 23e. Such $T_{\text{LLS}}$ values were plotted against the gas pressure and a linear fit was performed for the data obtained (Figure 23f). According to Figure 23f, $T_{\text{LLS}}$ for propane-d₆ increases linearly with pressure, with $T_{\text{LLS}}$ values being ~20% lower (on average) than the corresponding $T_{\text{LLS}}$ values of HP propane (Figure 23d). We note that this small difference is likely a combination of two effects: some depolarization losses due to RF excitation pulses (not taken into account) employed for data collection for HP propane-d₆, as well as the effect of deuterium labeling of the substrate; future theoretical studies are certainly warranted to provide the understanding of the observed trends. Taking into account the RF pulse excitation (which we did not perform due to concerns described by Kharkov and co-workers²²⁹) would lengthen $T_{\text{LLS}}$ values by less than 8%, which is insufficient to account for the ~20% difference between $T_{\text{LLS}}$ values of HP propane and HP propane-d₆. We also note a different slope of the curves shown in Figures 23d and 23f.
Figure 23. HP propane decay data. Examples of $T_1$ (at ~3.6 atm pressure) and $T_{LLS}$ (at ~4.5 atm pressure) signal decays of HP propane and mono-exponential fitting are shown in a) and c); e) shows a corresponding example of LLS decay of HP propane-d$_6$. Dependences of $T_1$ (b) and $T_{LLS}$ (d) of propane hyperpolarization on its pressure. f) Dependence of $T_{LLS}$ of propane-d$_6$ hyperpolarization on its pressure (an example of experimental data reporting on effective $T_1$ for HP propane-d$_6$ is shown in Figure 54, appendix A). All data is acquired at 0.0475 T using production/reaction conditions of 75 °C and 2000 sccm flow rate with near-100% chemical conversion of p-H$_2$ and unsaturated substrates. See Figure 19 for additional details.
It should be noted that although HP propane-d$_6$ $T_{LLS}$ values are generally lower than the corresponding values for HP propane, the detection of HP propane-d$_6$ offers an advantage of direct detection using a hard, short RF excitation pulse, whereas a long soft SLIC pulse is required to obtain observable magnetization in case of using HP propane at low magnetic fields. This advantage can be useful in the context of MRI applications, because RF excitation pulses can be very short (compared to SLIC RF pulses).

**NMR Spectroscopy of HP Propane Gas at 1.4 T.** We note that the methylene and methyl proton resonances still partially overlap at 1.4 T; the spectral appearance was well-reproduced by spectral simulation. No traces of unreacted propylene were seen (monitored by the lack of methine proton resonances after multiple averaging) consistent with a full (i.e. near 100%) chemical conversion of the unsaturated substrate in the hydrogenation reaction with p-H$_2$ under these experimental conditions: a corresponding NMR spectrum of thermally polarized propylene is provided in Figure 53, appendix A.

We note that SLIC-based detection does not allow obtaining the true value of polarization enhancement of the overpopulated LLS at 0.0475 T,\textsuperscript{138} because SLIC transformation does not offer a 100% conversion efficiency of the LLS into observable magnetization. On the other hand, direct detection of HP propane gas at 1.4 T was employed to measure polarization enhancement values. For this purpose, the integral values of the signals from H$_A$ and H$_B$ protons (the continuous-
flow mode) (Figure 24, we note that the absolute values for \( H_A \) and \( H_B \) were similar) were compared to the corresponding integral values for thermally polarized propane under the same pressure and multiplied by a factor of 8 (to account for eight protons contributing to the NMR signal of thermally polarized propane). The enhancement values recorded in such manner for the \( H_A \) proton were nearly unchanged (\( \varepsilon \)) ranging from 950 to 1150, Figure 24e) over the range of reactor temperatures studied (between 20 °C to 140 °C), in agreement with our similar studies using the 0.0475 T setup (Figure 22a). This trend is important for two reasons. First of all, it indicates that a robust catalyst performance in production of HP propane gas can indeed be obtained over a wide range of temperatures with effectively ~100% chemical conversion (note: 20% excess p-H\(_2\) was employed for data collection in Figure 24e). Second, the highest levels of polarization enhancement were observed at 40-60 °C, suggesting that reactor temperatures near that of the human body (ca. 40 °C) can be readily employed without sacrificing polarization efficiency. This finding bodes well for future in vivo use of HP propane gas, because the produced HP propane can be immediately inhaled by the subject without the need for significant additional cooling.
Figure 24. $^1$H NMR single-scan spectroscopy of HP propane gas using the 1.4 T setup (see Figure 28 for details). a) Schematic of heterogeneous pairwise p-H$_2$ addition. b) $^1$H NMR spectrum of HP propane acquired with the apparatus in continuous-flow mode. c) $^1$H NMR spectrum of thermally polarized propane spectrum (blue) and spectral fitting (red) using the Bruker Daisy software package. d) $^1$H NMR spectrum of HP propane acquired in a stopped-flow mode. e) Plot of NMR signal enhancement values of the HP methyl proton (H$_A$) at different operating temperatures obtained via continuous-flow operation (reactor prepared by mixing ~62 mg of the Rh/TiO$_2$ catalyst and 6.6 g of Cu in the 2$^{nd}$ stage of the hyperpolarizer). Connecting lines in display e) are meant only to guide the eye.
Effects of Buffering Gases on Propane Hyperpolarization Level and Decay. The effect of the buffering gas on the relaxation constants and polarization values of HP propane was also studied using the 0.0475 T NMR spectrometer setup (Figure 19). For these experiments, we used a flow rate of 2000 sccm, a reaction temperature of 75 °C, and 38 psi overpressure (total pressure of 3.6 atm); SLIC sequences were used for all acquisitions. The results obtained with p-H$_2$ buffering gas and with variable propane concentrations are shown in Figures 25a, 25b and 25c, whereas those obtained with Xe/N$_2$ mixture (3:1 ratio) as the buffering gas and with variable propane concentration are shown in Figures 25d, 25e, and 25f. The rationale for the mixture of Xe and N$_2$ was to test the effects of a reduced gas diffusion by using a dense gas (indeed, we note that previous attempts employing pure Xe were challenging because of high gas viscosity). The results indicate that T$_1$ decay of HP propane is not significantly impacted by the presence of light (H$_2$) or heavy (Xe/N$_2$) buffering gases, as shown in Figures 25a and 25d respectively. However, LLS decay of HP propane decreases significantly (by 35±7% from 100% to 43% mixture) with the increased presence of a light buffering gas, H$_2$ (Figure 25b). Yet in the presence of a heavy buffering gas (Xe/N$_2$, 3/1), the observed decrease in T$_{LLS}$ is far more modest (by 8±7% from 100% to 43% mixture, Figure 25e). Note, if we consider polarization levels, p-H$_2$ is not truly a buffering gas, because its concentration can influence the processes occurring on the catalyst surface including the percentage of pairwise hydrogen addition. On the other hand, Xe/N$_2$ mixture is inert relative to the catalyst operation (except the
dilution of the reactants leading to decrease of their partial pressures). The \(^1\text{H}\) polarization values increase in the presence of the buffering gas by 1.6±0.1 fold (100% propane mixture versus 43% propane mixture) in case of \(\text{H}_2\) buffering gas (in agreement with previous studies\(^{207, 230}\)) and by 1.4±0.1 fold (100% propane mixture versus 43% propane mixture) in case of \(\text{Xe/N}_2\) (3:1) buffering gas, as shown in Figures 25c and 25f respectively. We note that the actual increase in (initial) polarization of HP propane gas may be significantly greater, because in the case of more dilute mixtures, HP propane has lower corresponding \(T_{LLS}\) values (see Figures 25c and 25f), and therefore, likely experiences disproportionately greater polarization losses during more than 5-10-second transport time from the reactor to the detector.

The observation that the buffering gas boosts propane hyperpolarization in HET PHIP process is important in the context of biomedical applications with the goal of maximizing the levels of hyperpolarization. Although the produced HP gas is diluted with the buffering gas, it can potentially be separated by rapid cryo-condensation of HP propane gas, as discussed below.
Figure 25. Effects of using different buffering gases on the hyperpolarization decay constants and polarization levels of HP propane determined using the 0.0475 T magnetic field NMR spectrometer setup (Figure 19). $T_1$ (a) and $T_{LLS}$ (b) dependence of HP propane on the propane fraction in the resultant gas mixtures with the use of p-H$_2$ buffering gas. c) Dependence of propane $^1$H polarization (arbitrary units, a.u.) on the propane fraction in the resultant gas mixtures with the use of H$_2$ buffering gas. $T_1$ (d) and $T_{LLS}$ (e) dependence of HP propane on the propane fraction in the resultant gas mixtures with the use of Xe/N$_2$ (3:1) buffering gas mixture. f) Dependence of propane $^1$H polarization (arbitrary units, a.u.) on the propane fraction in the resultant gas mixtures with the use of Xe/N$_2$ (3:1) buffering gas mixture. All data were obtained at 38 psi backpressure (~3.6 atm total pressure), and the p-H$_2$ to propylene ratio was 1:1 for the experiments shown in displays d, e, and f. Connecting lines are meant only to guide the eye.
Partial SLIC RF excitation of HP propane gas at 0.0475 T. We carried out a series of experiments with a variable SLIC pulse duration on HP propane gas using the 0.0475 T spectrometer setup (Figure 19) for a 1:1 reaction mixture of propylene and p-H₂ at 38 psi overpressure (~3.6 atm total pressure). Once the phantom was filled with fresh HP propane gas, the SLIC pulse was applied followed by immediate signal detection, and these two steps were repeated for N times on a single fill of HP propane (Figure 26a). The signal of HP propane obtained in this fashion falls exponentially as a result of a combination of LLS decay and the application of SLIC pulses. Several different SLIC RF pulse durations (varying between 50 and 1600 ms) were employed to record NMR spectra, the intensities of which are plotted against time in Figure 26b. We note that in case of longer SLIC durations, the RF pulses were applied more sparsely, resulting in a longer repetition time (TR) but in greater signal intensities (Figure 26c). An effective T_{LLS} value was determined for each experimental series in Figure 6b, and these effective T_{LLS} values (detailed in Table 2, appendix A) are plotted in Figure 26d. As expected, when the SLIC pulse duration is increased, the effective decay time constant decreases, indicating a faster depleting of the HP state. All effective T_{LLS} values measured in this fashion were in the range of 4.7 s to 8.1 s (due to decay and RF depletion), which is less than the corresponding T_{LLS} value (~8.4 s) obtained at otherwise-identical conditions using the procedure described above (cf. Figure 23d). This finding indicates that partial SLIC can be applied to HP propane to convert only a fraction of the hyperpolarization pool at one time (prolonging the
useful lifetime of the enhanced spin order). We note that even though near-optimal values of SLIC pulses are applied (i.e. 800-1600 ms durations, making an analogy with a 90° excitation pulse—note the corresponding signal produced by SLIC pulse increases with duration, Figure 22e and Figure 26c), the overpopulated LLS remains largely intact, because subsequent RF excitation pulses produce comparable signal levels, Figure 26a. This finding is useful for several reasons. First, a short SLIC pulse (i.e., with the duration significantly shorter than the optimal value) can be employed in a manner similar to a small tipping-angle excitation pulse for applications ranging from measurements of relaxation of HP species to MRI encoding. In case of MRI applications, we note that the resulting magnetization (after SLIC pulse) is in the x-y plane, and therefore, can be conveniently combined with echo-planar imaging (EPI) readout. As a result, SLIC can provide both partial excitation of overpopulated LLS to record 2D slices, and also enable singlet order selection (SOS) filtering$^{231}$ for selective excitation of the singlet states in the presence of proton background signal of tissues.

There are at least two possible explanations for the observed retention of overpopulated LLS even after the application of a SLIC pulse of optimal duration (which in principle is designed to convert all singlet order into observable magnetization$^{95}$). First, gas convection and $B_1$ inhomogeneities may lead to subpar performance of the SLIC pulse sequence, leaving the bulk of the HP LLS unaffected by the SLIC pulse sequence.$^{229}$ Second, the HP propane spin system cannot be, strictly speaking, treated as a true singlet,$^{134, 137, 138}$ because there are
eight spin-spin coupled protons, and therefore, the application of the SLIC sequence may indeed generate observable magnetization while retaining some overpopulated LLS in the spin system of HP propane gas. Future studies using stronger magnets and more homogeneous excitation RF coils are certainly warranted to delineate the relative contribution of these two effects.

Figure 26. Partial SLIC data. a) Pulse sequence comprising a series of partial SLIC pulses with variable duration each followed by NMR signal acquisition; note the sequence is repeated N times on a single batch of static HP propane gas. b) The decay of HP propane signals obtained using the partial SLIC excitation scheme (with variable SLIC duration); note the color coding of the SLIC pulse duration in the figure legend (see Table 2, appendix A for additional details). c) The intensity of the first data point in display b) plotted as a function of the SLIC pulse duration. Connecting lines in displays c) and d) are meant only to guide the eye.
We have also tested the immunity of the overpopulated LLS with respect to irradiation by hard RF pulses. The applied sequence is shown in Figure 27a, and the corresponding decay data is presented in Figure 27b (red curve). Specifically, we have employed a SLIC pulse duration of 200 ms, which was followed by signal acquisition. This partial SLIC detection was repeated four times. Next, 64 hard 90° RF pulses were applied back-to-back. This process was repeated three times, and the integral intensity of the HP signals (obtained via partial SLIC and shown as red squares in Figure 27b) is plotted versus time. The resulting data were fit to give an effective $T_{LLS}$ constant of $5.3\pm0.1$ s. This value is close to a corresponding value obtained when no hard RF pulses were employed: effective $T_{LLS}$ of $6.3\pm0.4$ seconds (Figure 27b, blue curve), indicating that the overpopulated LLS is relatively immune to the application of hard RF pulses. This observation is useful in the context of future MR imaging studies, where the application of strong RF pulses may be desirable for background signal suppression, while retaining the overpopulated LLS of HP propane gas.
Figure 27. Partial SLIC with hard RF pulses. a) Pulse sequence comprising a series of partial SLIC pulses with 200 ms duration followed by NMR signal acquisition. The 200 ms SLIC pulse is followed by NMR detection; this loop is repeated 4 times, and a train of sixty-four (equally spaced) hard 90° RF pulses is applied and the entire sequence is repeated three times. b) The recorded SLIC NMR signal intensities (comprising twelve data points). The mono-exponential fitting (red) yielded an effective $T_{\text{LLS}}$ constant of 5.3±0.1 seconds. The corresponding decay curve without the use of hard RF pulses is shown by blue trace with triangles. All experiments were performed at 38 psi of overpressure (~3.6 atm total pressure)

Feasibility of Condensation of HP Propane Gas and LLS Decay of Liquefied HP Propane at 0.0475 T. Cryo-collection of HP gas is a common practice in a continuous-flow production of HP noble gases, a practice that is designed to make the contrast agent more concentrated.\textsuperscript{202, 232, 233} Since the addition of buffering gas increases the degree of propane hyperpolarization (Figure 25c and Figure 25f), we have investigated the feasibility of HP propane cryo-collection. A slight modification to the 0.0475 T experimental setup was made to perform HP propane condensation (Figure 19 and Methods section for details). The phantom inside the RF coil was replaced by a 5 mm NMR tube wherein the reaction mixture was cryo-cooled. The cryo-cooling was performed at a flow rate
of 1300 sccm for ~20 s inside a cooling bath with dry ice and ethanol (ca. -78 °C) outside the magnet, i.e. at the Earth’s magnetic field. Then the NMR tube containing liquid HP propane was carefully placed inside the magnet, and a series of NMR spectra were collected using partial SLIC excitation with 200 ms pulse duration. The typical NMR spectrum of the liquefied propane (Figure 52a, appendix A) was similar to the one of HP propane gas (Figure 21a). The fitting to mono-exponential decay (Figures 52b-d, appendix A) revealed an effective $T_{\text{LLS}}$ time constant of 14.7±0.5 s. We note that this value is actually a lower-limit estimate, because the partial SLIC excitation reduces the pool of HP LLS (Table 2, appendix A), and the effect of the RF pulse-associated losses was not taken into account in our data fitting. Nevertheless, this relatively long $T_{\text{LLS}}$ value is significantly greater than any $T_{\text{LLS}}$ value of HP propane in the gas phase measured here or elsewhere.\textsuperscript{138} This lower-limit $T_{\text{LLS}}$ value for liquid HP propane is somewhat lower than the $T_1$ value of HP propane dissolved in deuterated organic solvents (28-35 s).\textsuperscript{208}

Because higher polarization values can be obtained via HET-PHIP using buffering gases, and because buffering gases can be potentially separated from cryo-collected HP propane, this approach may be a viable option for future experimentations to boost propane hyperpolarization via HET-PHIP. Moreover, the relatively long $T_{\text{LLS}}$ value of cryo-collected HP propane may be a useful means for temporary storage of HP propane gas prior to its use as an inhalable contrast agent.
3.5 Conclusions

A systematic study of nuclear spin relaxation dynamics at 0.0475 T of HP propane prepared by HET-PHIP is reported using a clinical-scale hyperpolarizer device under conditions providing nearly 100% chemical conversion. We find that the HP propane $T_{LLS}$ is $\sim$3 seconds at 1 atm, i.e. under clinically relevant conditions. $T_{LLS}$ and $T_1$ values generally exhibit a linear dependence on propane pressure. At the pressures studied, the $T_{LLS}$ values are approximately 3 times greater than the corresponding $T_1$ values. The use of deuterated propylene as HP propane precursor (i.e. production of HP propane-d$_6$) reduces $T_{LLS}$ by as much as $\sim$20%, indicating that deuteration of the precursor maybe somewhat detrimental to the lifetime of the HP state. The use of light and heavy buffering gases (p-H$_2$ and a 3:1 mixture of Xe: N$_2$) is found to have a negligible effect on $T_1$ and generally results in somewhat lower $T_{LLS}$ values: the $T_{LLS}$ reduction is concentration dependent. At the same time, the use of buffering gases increases the polarization levels of HP propane gas during HET-PHIP production, which is welcome in the context of biomedical applications. Although the buffering gas dilutes the HP propane gas, it can be separated through a process of HP propane cryo-collection, the feasibility of which was demonstrated here. The added benefit of HP propane cryo-collection is the increase of the $T_{LLS}$ value to 14.7 seconds in the liquid state, which is significantly greater than any reported $T_{LLS}$ value for HP propane gas at any pressure, and which can be useful for temporary storage of produced HP propane prior to its in vivo administration. We have also investigated the possibility
of applying a partial SLIC pulse to HP propane LLS, and we find that most of the pool of the over-populated LLS is retained even after application of an optimized SLIC pulse. The overpopulated LLS of propane is relatively immune to the application of hard RF pulses. This behavior of the HP propane spin system with respect to application of partial SLIC and strong RF pulses can be potentially useful for developing efficient ultra-fast MR imaging approaches, especially those involving EPI readout. To summarize, the results presented in this study may be beneficial for guiding future work studying the preparation of highly polarized batches of HP propane and designing efficient MR imaging approaches.

3.6 Acknowledgements

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Associated content can be found in appendix A.
CHAPTER 4 The Search for Ultra-long-lived Spin States in Hyperpolarized Propane Gas: Cyclopropane Deuteration Reveals Symmetric Spin Isomers

Successful production of HP propane can be achieved using cyclopropane, an alternate precursor to propylene. We hypothesize that HP propane prepared by p-H₂ pairwise addition to cyclopropane can have ultra-long-lived spin states thus providing an advantage for biomedical applications (Ariyasingha et al. manuscript in preparation for ChemPhysChem)

4.1 Introduction

We have demonstrated that HP propane can have LLS that benefit the preparation of the HP gas as well as potentially offers in vivo applications due to the sufficient lifetime for subject inhalation and imaging. In addition to propylene, cyclopropane can also be used as an alternative precursor molecule to create HP propane using PHIP. Moreover, cyclopropane has been previously used as an anesthetic thus making it more suitable for future clinical translations. While cyclopropane does not have a double/ triple carbon-carbon bond, the ring opening leads to three possible reaction products, where only one isotopologue is NMR active. Salnikov et al. reported high levels of proton polarization (~2.4%) for HP propane preparation using cyclopropane, where they also demonstrate a possible reaction mechanism of hydrogenation. Although two of the hydrogenation products are NMR invisible, they may potentially possess substantially longer lifetimes than the visible hydrogenation product. Our interest was to therefore to investigate the
fraction of those NMR-invisible isotopologues of cyclopropane hydrogenation reaction by studying the complementary deuteration reaction.

4.2 Materials and methods

Homogeneous hydrogenation. Hydrogenation reaction of propylene (PE-Sigma Aldrich; 115-07-1) with D$_2$ (Isotec) were studied using homogeneous catalysts. Propylene was bubbled into the deuterated methanol solution (CD$_3$OD) in the NMR tube with 5 mmol of the bicyclo[2.2.1]hepta-2,5-diene][1,4-bis(diphenylphosphino) butane]rhodium(I) tetrafluoroborate catalyst prepared in CD$_3$OD for about 2-3 minutes. Then, D$_2$ gas was bubbled into the NMR tube as shown in the schematic diagram in (Figure 41). After thorough mixing of the solution, the sample tubes were allowed to stand for about 30-minutes to 1 hour, and their $^1$H NMR spectra were recorded at 700 MHz as explained below.

Heterogeneous hydrogenation. Heterogeneous hydrogenation of cyclopropane (CP) and propylene was performed using the schematic diagram shown in Figure 28. A gas mixture was prepared using the substrate and D$_2$ in the storage cylinder (unless otherwise stated) before sending it through the reactor. Cyclopropane gas and D$_2$ gas mixture (20% excess for each reactant excess conditions) was passed through the reactor with Rh/TiO$_2$ catalyst$^{235}$ at 150 °C at a flow rate of 200 sccm for about 3-5 minutes and the product mixture was collected into a small HPLC column containing ~ 1.0 mL of deuterated methanol. Liquid sample product mixtures were then transferred into NMR tubes rapidly by opening
the exit port of the column. For CP hydrogenation studies, two different sets of sample tubes were prepared with either excess cyclopropane (by a factor of 2) or with 20% excess D₂. A similar experimental procedure was carried out for the heterogeneous catalysis of propylene and D₂. The expected common hydrogenation reaction of CP and PE with D₂ is the production of propane either by ring opening hydrogenation for CP or addition reaction for PE respectively (Figure 29). The product propane by heterogeneous reaction of cyclopropane is indicated in green color and the possible product propane of the PE reaction is given in blue, which will be used throughout this manuscript to elaborate the reaction routes resulting in different isotopologues.

**NMR detection and spectral simulations.** 700 MHz Bruker Avance NMR spectrometer was used for the detection of ¹H NMR spectra in this work. The gas product of heterogeneous hydrogenation which was collected in to an HPLC column was immediately transferred into a 5 mm NMR tube prior to their spectral detection. All the samples collected were allowed to sit for 20-30 minutes prior to recording their ¹H NMR spectra using 700 MHz Bruker Avance NMR spectrometer. Simulations of ¹H NMR spectra were also performed for PE and CP deuteration reaction products using Daisy simulation package of Bruker Topspin 3.1 software. Details for the spectral simulations are provided in the appendix B.

**Control experiments.** Control experiments were conducted as follow. PE and CP were tested separately for any possible contaminants of propane/ H₂ by directly
bubbling the gas into CD$_3$OD and their $^1$H NMR spectra were recorded. D$_2$ gas was also tested for possible contaminants of H$_2$.

**Figure 28. Schematic diagram of the experimental set up** used to study heterogeneous deuteration of cyclopropane reaction. Experimental conditions used are as follow. Cyclopropane and D$_2$ gas mixture was passed through the reactor at a 200 sccm flow rate at a temperature of 150 °C and the product mixture exiting the reactor was collected into an HPLC column filled with ~1 mL of deuterated methanol prior to NMR detection.

**Figure 29. Deuteration reactions of a) cyclopropane and b) propylene** in the presence of heterogeneous Rh/ TiO$_2$ catalyst. The general product is propane. Different colors (green for cyclopropane deuterated product and blue for propylene deuterated product) used are in consistence with the other figures throughout this chapter.
4.3 Results and discussion

**Homogeneous hydrogenation of PE.** $^1$H NMR spectrum obtained from homogeneous hydrogenation of PE with D$_2$ is indicated in the magenta trace in Figure 30 where one would assume to see the deuterium added product CH$_3$CHDCH$_2$D$^{207,138,206}$. Surprisingly, we did not observe the classical $^1$H NMR spectral pattern for CH$_3$CHDCH$_2$D. However, initial inspection revealed an asymmetric multiplet structure (Figure 30). The product spectrum pattern reveals the presence of some propane (PA) in addition to the expected product of CH$_3$CHDCH$_2$D. Propane formation is most likely due to an isomerization reaction of propylene itself or residual traces of H$_2$ in the system (subject to ongoing investigations). Please refer to the section below for spectral simulations and discussions.

**Heterogeneous deuteration of PE.** Heterogeneous deuteration of PE also resulted in approximately similar products as obtained by homogeneous hydrogenation conditions as shown by their $^1$H NMR spectra (Figure 30). $^1$H NMR product signatures surprisingly agree well with each other for both methylene region and methyl region except for the additional peak observed around 1.06 ppm (Figure 30a and Figure 30b). Both spectra were obtained under the homogeneous and heterogeneous reaction conditions are likely to result of a mixture of products of CH$_3$CHDCH$_2$D and CH$_3$CH$_2$CH$_3$. The spectral simulations (Figure 31) performed here largely failed to give a fine simulation for the product distribution but we were able to simulate key spectral signatures. The initial partial of the
spectral simulation points that the asymmetry is likely due to the presence of a fully protonated propane species (please refer to the appendix B for simulation details). Therefore, it is very interesting to note that we observe some propane formation as we discussed above, which could be due to (i) isomerization of propylene, or (ii) due to the presence of some mysterious side reaction that leads to propane formation under homogeneous and heterogeneous reaction conditions employed in this study, or (iii) due to residual impurities of H\textsubscript{2} in the bubbling system, or (iv) residual deposition of H\textsubscript{2} in heterogeneous catalytic reactor. However, we were only able to fit the spectral signatures of propane and the targeted spin isomer, CH\textsubscript{3}-CHD-CH\textsubscript{2}D. This fact represented a challenge for us, because we could not employ simulations to be able to delineate the contribution of each reaction route as described by Salnikov \textit{et al.}, and we also could not make a complete spectra simulation of overlapping multiplets). Additionally, our observations of propane detection are supported by their \textsuperscript{1}H NMR spectra simulations of HET-PHIP spectra (Figure 31), where spectral signatures of non-deuterated propane and partially deuterated spin isomer are in good agreement with the experimental observations. All reactants used in the studies were also tested carefully for any traces of H\textsubscript{2} or propane to investigate for any possible contaminants in the starting gas mixtures. However, all the reactants tested showed no detectable contaminations confirming the occurrence of an additional isomerization reaction. The other spectral signatures observed approximately in the range of ~1.08-1.04 ppm (Figure 31b and Figure 31c) are likely due to the less concentrated catalyst, which were not
attempted for simulations due to their complexity and irrelevancy. The key observation we report by PE deuteration experiments and spectral simulations is that we obtain propane, the expected spin isomer CH$_3$CHDCH$_2$D and some other additional isomer/s.

![Diagram](image)

**Figure 30.** $^1$H NMR spectrum of the product mixture of propylene deuteration reactions. a) proton NMR spectrum of methylene region of the propane products of propylene and D$_2$ reaction under heterogeneous (blue trace) and homogeneous (magenta trace) reactions. b) $^1$H NMR spectrum of the methyl region of the propane products of propylene and D$_2$ reaction under heterogeneous (blue trace) and homogeneous (magenta trace) reactions.

This observation is important because the presence of this additional isomerization reaction/s may explain low chemical conversions and lower than the anticipated % polarization levels of HP propane in previously reported propylene
hydrogenation reaction studies.\textsuperscript{75, 206} Moreover, this suggests that better catalytic developments for HET-PHIP studies for propylene hydrogenation reactions are indeed desirable in the future in order to prevent molecules undergoing unwanted reaction pathways.

\textbf{Figure 31. Product simulation of propylene deuteration.} a) Generic reaction of propylene deuteration resulting in propane. b) \textsuperscript{1}H NMR spectrum of methylene protons of the product mixture obtained after heterogeneous deuteration of propylene (blue) and the simulated spectrum (red) of non-deuterated propane with isotopologue CH\textsubscript{3}-CHD-CH\textsubscript{2}D. c) \textsuperscript{1}H NMR spectrum of methylene protons of the product mixture obtained after heterogeneous deuteration of propylene (blue) and the simulated spectra of two separate products i.e. non-deuterated propane (red) and isotopologue CH\textsubscript{3}-CHD-CH\textsubscript{2}D (black). Note that although the presence of non-deuterated propane was unexpected in our studies, and it complicates the spectral appearance, this is not a significant limitation of the reported studies since our approach relies on the assignment of spectral signatures to specific isotopologues or groups of isotopologues.
**Heterogeneous hydrogenation of cyclopropane with D₂.** Cyclopropane hydrogenation under homogeneous catalysts were not attempted in because previous efforts did not lead to formation of any appreciate amount of the products. Cyclopropane hydrogenation in the presence of a heterogeneous catalyst produces the generally expected product propane which consists of three different isotopologues such as CH₃-CHD-CH₂D, CH₂D-CH₂-CH₂D and CD₂H-CH₂-CH₃ depending on the reaction mechanism (Figure 32a) while propylene deuteration results in a single product (CH₃-CHD-CH₂D) by adding deuterium into the double bond. However, one of the isotopologues, i.e., CH₃-CHD-CH₂D can be detected via ¹H NMR spectroscopy, while the two are NMR-invisible products. The ¹H NMR spectral region of methylene protons of the isotopologues product mixture obtained by the HET-PHIP cyclopropane deuteration reaction (green grace in Figure 32c), is in great agreement with that of the CH₃-CHD-CH₂D product of propylene (blue trace in Figure 32c), offering clear evidence for the presence of one of the product channels (route # 1 in Figure 32a). This observation is well supported by the appearance of both methylene and methyl regions of product ¹H NMR spectra of cyclopropane D₂ reaction (Figure 32b and Figure 32c). Note that some features of the complex multiplets (especially in the methylene region) are overlapping very well, indicating that the cyclopropane product mixture contains ALL spin isomers present in propylene deuteration and some additional spin isomers. The additional complex proton NMR spectral features are presumably due to the presence of other more symmetric spin isomers. Simulations of ¹H NMR spectra in the
presence of all the isotopologues were not exhausted in this work. This interesting observation of complex NMR spectral nature is attributed to apparent reaction routes of cyclopropane deuteration. Detection and spectral assignment of the CH$_3$-CHD-CH$_2$D isotopologue allows us to get branching ratio information of route #1 and branching ratio of the sum of the route #2 and route #3 via the comparison of the integrated spectral intensities of the blue and green traces in Figure 32. Note that in homogeneous hydrogenation, hydrogenation leads to product formation only via route #1, whereas in case of heterogeneous hydrogenation, the observed signal is the sub of the signals from the products obtained via all three routes. Heterogeneous deuteration of cyclopropane shows similar ratio ($\chi$ defined as the ratio of NMR signal obtained from products formed via route #1 (obtained as relative intensity from the blue trace) divided by the NMR signal obtained from routes #1-3 (obtained as relative intensity from the green trace)) under two different reaction conditions employed in this study, i.e., in the presence of excess cyclopropane, average value (averaged using both methyl and methylene $^1$H NMR spectral intensities) of $\chi$ (route#1) is found to be 17.5% and $\chi$ (route#2+#3) is 82.5% with a chemical conversion of 1.3%, while average value of $\chi$ (route#1) is 21.5% and average value of $\chi$ (route#2+#3) is 78.5 % with a chemical conversion of 12% were obtained for excess deuterium conditions. Please note that cyclopropane spectra were normalized with respect to data obtained for the propylene samples by subtracting the normalized intensity of the spin isomers produced by propylene from those produced by the reaction of cyclopropane and
by dividing the remaining signal integral by the total integral of the spectral lines (details of computations are provided in appendix B). This is a very important observation for several reasons. First, it confirms that this heterogeneous cyclopropane hydrogenation reaction primarily proceeds in accord to the routes #2 and #3, which form NMR-invisible spin isotopologues, where p-H₂ is employed. As a result, the bulk of the formed HP propane gas may indeed be locked in ultra-long-lived spin states (ULLSS). Therefore, it may be possible to design HP propane with ULLLSS in the future. Second, the previous estimate for reported propane polarization values of ~2.4% needs to be revisited. In the previous studies, it was assumed that all produced HP propane isotopologues contributes to HP NMR signal. Now, when we know that the visible NMR signal is created by ~1/5th of the HP sample, the apparent value of ~2.4% needs to be multiplied by factor of ~4-5. This large correction factor means that the created proton polarization exceeded the value of 10% in the previous studies.
Figure 32. $^1$H NMR spectra of the products via heterogeneous catalysis. a) shows the possible product channels of hydrogenation of propylene using para hydrogen and deuteration reactions for both precursor molecules. b) $^1$H NMR spectrum of the methylene spectral region of cyclopropane (green) and propylene (blue). c) $^1$H NMR spectrum of methyl spectral region of both cyclopropane (green) and propylene (blue).
Mechanism of hydrogenation. Cyclopropane deuteration reaction is a ring opening addition reaction of D$_2$ molecule in the presence of heterogeneous titania supported metal catalyst$^{236}$. Our choice of catalyst was Rh/TiO$_2$ is dictated by its proven high efficiencies in alkene para hydrogenation reactions,$^{237,238}$ where three types of HP propane isotopologues can be generated depending on the reaction routes (Figure 33a). The schematic of the potential reaction mechanism for the addition of D$_2$ on the surface of the supported metal catalyst is illustrated in Figure 33b. Please note that two shades of green color have been used in order to distinguish between two separate D$_2$ molecules in the reaction mechanism. The NMR-visible isotopologue, CH$_3$-CHD-CH$_2$D is produced via route #1 by the addition of deuterium atoms into a methyl group and a methylene group over the heterogeneous titania supported Rh metal catalyst via a series of mechanistic steps that involves cleavage of C-H bond/s. The other two isotopologues CH$_2$D-CH$_2$-CH$_2$D and CD$_2$H-CH$_2$-CH$_3$ produced via routes #2 (direct addition of D$_2$ after ring opening, which is inherently the most favorable step because that does not involve any C-H bond cleavage) and #3 are spectroscopically invisible due to their magnetically equivalent nature. We report clear evidence using experimental and simulation observation discussed above (Figure 31 and Figure 32) in support of both NMR-visible and NMR-invisible routes.
**Figure 33. Reaction mechanisms for cyclopropane deuteration.** a) possible different products of heterogeneous catalytic deuteration reaction of cyclopropane. b) proposed mechanism of cyclopropane deuteration in the presence of Rh/ TiO$_2$ catalyst surface. Please note, two different shades of green color are used to guide different steps in the catalytic process resulting in formation of three isotopologues.

Although the symmetric spin states of cyclopropane hydrogenation reaction are not possible for direct detection by their conventional $^1$H NMR spectroscopy, this work on observation of the complex spin isomers of HET-PHIP cyclopropane deuteration reaction, provides clear confirmation of the creation of potentially ultra-long-long-lived spin states of more symmetric HP propane isotopologues obtained via pairwise p-H$_2$ addition to cyclopropane. This discovery brings the opportunities for the preparation of highly symmetric spin states, which may indeed be longer lived, and hence can be used as hyperpolarization storage reservoirs for inhalation of HP propane gas for potential biomedical application as an inhalable contrast agent.
4.4 Acknowledgements

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Associated SI content can be found in appendix B.
CHAPTER 5 Parahydrogen-Induced Radio Amplification by Stimulated Emission of Radiation


In this chapter, “we” and other first-person plural pronouns are used in reference to all authors of this publication. My individual contributions to the research include conduction of ~ 50% experiments, ~40-50 % of data analysis and writing parts of the manuscript. Figures and tables containing data contributed by researchers other than me will contain a disclaimer in the caption; figures and tables with no disclaimer in the caption contain data collected by me.

RASER is a recently discovered phenomenon due to coherent coupling of spin polarization with a radio frequency resonator that was first detected in SABRE experiments using a high-quality resonator and at low magnetic fields. We present that RASER activity is a much more common phenomenon. RASER was detected using a commercial bench-top NMR spectrometer without using an RF excitation pulse. RASER effect can be used for many applications and beyond.

5.1 Abstract

Radio amplification by stimulated emission of radiation (RASER) was recently discovered in a low field NMR spectrometer incorporating a highly specialized radio frequency resonator where a high degree of proton spin polarization was achieved by reversible p-H₂ exchange. RASER activity, which results from the coherent coupling between the nuclear spins and the inductive detector, can overcome the limits of frequency resolution in NMR with potentially important
opportunities in fundamental and applied physics. Here we show that this phenomenon is not limited to low magnetic fields or the use of resonators with high quality factors. Specifically, we use a commercial, bench-top 1.4 T NMR spectrometer in conjunction with p-H$_2$ pairwise addition producing proton-hyperpolarized molecules in the Earth’s magnetic field (aka. ALTADENA condition) or in a high magnetic field (aka. PASADENA condition) to induce RASER without any radio-frequency excitation pulses. The results demonstrate that RASER activity can be observed on virtually any NMR spectrometer and measures with high precision most of the important NMR parameters, such as chemical shifts and $J$-coupling constants. These findings are important for future applications of RASER in many different fields of science and technology, in particular for the development and quality assurance of hyperpolarization techniques such as parahydrogen-induced polarization.

5.2 Introduction

Suefke and co-workers reported recently (in 2017) on the first experimental observation of radio amplification by stimulated emission of radiation (RASER) of protons.$^{174, 239}$ Unlike lasers and masers, which employ self-organizing systems emitting coherent optical- and micro-waves, RASER is induced by continuous coherent oscillation of radio waves at much lower frequencies through the coupling between nuclear spin magnetization and an LC resonance circuit.$^{174}$ Because the required magnetization, i.e., the product of the concentration of nuclear spins and
their nuclear spin polarization \((P)\) or the degree of the spin alignment with the static magnetic field of the NMR magnet, is very high, RASER-based NMR spectroscopy is difficult to achieve using thermal nuclear spin polarization. Hyperpolarization techniques allow for enhancing the nuclear spin polarization by several orders of magnitude\(^8, 9, 96, 183, 240\) up to unity.\(^241, 242\) Suefke and co-workers thus employed the technique of Signal Amplification by Reversible Exchange (SABRE) to provide a highly magnetized sample in their pioneering RASER demonstration.\(^80\) SABRE relies on the simultaneous exchange between parahydrogen \((p-H_2\), the source of hyperpolarization\) and a substrate on a metal complex.\(^82\) With SABRE, the spontaneous polarization of proton spins is efficient in the magnetic field range of 1 to 10 mT.\(^83\) Therefore, Suefke and co-workers employed NMR detection at magnetic fields of several milli-Tesla with corresponding resonance frequencies in the range of 41-512 kHz.\(^174, 239\) These authors also employed SABRE because it allows for continuously regenerating the proton polarization via the sustained delivery of \(p-H_2\) gas to the sample placed inside the NMR detector.\(^174\) Mathematical expressions for RASER activity are given in section 2.17 using equations (16) and (17). Note that if \(P > 0\), the rate \(1/T_{RD}\) is negative, and the associated line is additionally broadened by radiation damping with a total damping rate \(\kappa_{tot} = (1/T_2^* - 1/T_{RD}) > 1/T_2^*\). If \(P < 0\), which corresponds to a population inversion, \(1/T_{RD}\) is positive, and the line is narrowed due to the decreased total damping rate \(\kappa_{tot} < 1/T_2^*\). RASER activity starts if \(\kappa_{tot} < 0\), as described by equation (16). To fulfill this condition for proton spins at low frequencies, Suefke and co-workers employed
high-quality \( (Q \sim 300) \) resonators,\(^{177} \) which reduce significantly the RASER threshold requirements for polarization and concentration.\(^{174} \) Hence, the experimental conditions reunited for this first demonstration RASER, i.e., low magnetic field, high-\( Q \) resonator, and continuous regeneration of hyperpolarization, seem very peculiar. As a matter of fact, RASER activity is a much more common phenomenon than one could expect, as we will show below.

5.3 Results and discussion

Here, we show evidence that a commercial, high-field NMR system with standard inductive detection (i.e., without specialized, high-\( Q \) resonators) can readily detect RASER when combined with the parahydrogen-induced polarization (PHIP) technique.\(^6^3\) Specifically, we employ a 1.4 T (61.7 MHz) bench-top NMR spectrometer (SpinSolve Carbon 60, Magritek, New Zealand) with \( Q = 68 \) to induce RASER of protons in hyperpolarized (HP) ethyl acetate (EA) and 2-hydroxyethylpropionate (HEP). These HP compounds are formed through the pairwise addition of \( \text{p-H}_2 \) onto the unsaturated C=C chemical bonds of the substrates vinyl acetate (VA) and 2-hydroxyethyl acrylate (HEA), respectively (Figure 34 a,b). The symmetry breaking of the nascent \( \text{p-H}_2 \)-derived protons allows for the hyperpolarization to become observable.\(^{15,18^3} \) The solutions were prepared with \( \sim 0.4 \text{ M} \) of substrates and \( 4 \text{ mM} \) of catalyst (Bicyclo[2.2.1]hepta-2,5-diene)[1,4-bis(diphenylphosphino)butane]rhodium(I) tetrafluoroborate (Sigma-Aldrich, P/N 341126-100MG) in CD\(_3\)OD. Nearly 100\% \( \text{p-H}_2 \) was employed using a home-built cryogenic generator.\(^{5^7} \) At 75 °C and 100 psi, the substrates were fully converted.
into their respective HP products via bubbling p-H₂ for 15 second with a 150 sccm flow rate controlled by a mass-flow controller, as described previously (Figure 34 c). The chemical conversion was monitored by comparing the ¹H NMR spectra of thermally polarized samples taken before and after the PHIP reactions (Figures 67 and 68, appendix C). The polarization of Hₐ and Hₐ was estimated to be between 10% and 20% based on previous study and their T₁ relaxation times was measured to be ~16-25 seconds (Figure 69, appendix C).

Two main experimental protocols were followed. The first protocol corresponds to the ALTADENA condition. In this case, the samples were hydrogenated in the Earth’s magnetic field (~ 50 µT). The p-H₂ flow was then interrupted, the sample depressurized, the catheter removed, the “pulse-and-acquire sequence” initiated, and the sample inserted in the NMR spectrometer (Figure 34 d). To make sure that the sample does not experience any RF excitation, the sample was inserted several seconds after an RF pulse with a flip angle lower than 0.01° was applied. The detector channel was opened for up to 32 seconds. The second protocol corresponds to the PASADENA condition, in which the hydrogenation reaction was performed at 1.4 T, i.e., inside the spectrometer. During the hydrogenation reaction, the sample was positioned 4-5 cm above the RF coil so the catheter could be removed without interfering with the signal detection, which was initiated before the hydrogenation reaction was completed. Once the reaction was completed and the catheter removed, the sample was pushed inside the RF coil (Figure 34 e).
Figure 34. Experimental RASER protocols. a,b) Reaction schemes for the $p$-$H_2$ pairwise addition to vinyl acetate (VA) yielding to ethyl acetate (EA) and to 2-2-hydroxyethyl acrylate (HEA) yielding to 2-hydroxyethyl propionate (HEP), respectively. c) Schematic of the experimental setup. d,e) ALTADENA and PASADENA protocols used to evidence parahydrogen-induced RASER activity, respectively.
The main results obtained for EA and HEP are illustrated in Figure 35 and Figure 36, respectively. For both ALTADENA and PASADENA experiments, the NMR signal exhibits the characteristic features of RASER activity: Signal persistence for significantly longer periods of time than $T_2^*$ of $\sim 0.6$ s.\textsuperscript{174,177} The Fourier transformed spectra of selected regions with defined duration of the RASER active signals, e.g., Figure 35b,c for ALTADENA and Figure 35i-l for PASADENA, clearly show evidence for the enhanced spectral resolution due to RASER activity with sharp peaks with FWHM < 0.2 Hz, whereas the resolution of the spectrometer is $\sim 0.5$ Hz after full shimming and $\sim 2$ Hz in case of conventional HP experiments. Immediately after these RASER active signals were recorded, additional NMR spectra were acquired using $\sim 3.3^\circ$ excitation RF pulse. The first of those spectra show partially RASER active NMR lines (see Figure 35e in case of ALTADENA and Figure 36l in case of PASADENA), while the subsequent acquisitions correspond to normal, hyperpolarized ALTADENA (Figures 35g and 36g) and PASADENA (Figures 35n and 36n) spectra.
Figure 35. $^1$H NMR spectroscopy of solution-phase PHIP of 0.4 M HP EA (ethyl acetate) probed at 1.4 T. a) ALTADENA RASER signal recorded without RF pulse after hydrogenation in the Earth’s magnetic field. b,c) Fourier spectra of the regions outlined by red and purple boxes in display a), respectively. d,e) Partial ALTADENA RASER signal and Fourier spectrum obtained with ~3° RF pulse. f,g) Canonical ALTADENA (non-RASER) FID and Fourier spectrum recorded after further polarization decay. h) PASADENA RASER signal recorded without RF pulse after hydrogenation at 1.4 T. i,j,k,l) Fourier spectra of the regions outlined by red, purple, green and orange boxes in display h), respectively. m,n) Canonical PASADENA (non-RASER) FID and Fourier spectrum acquired with ~3° RF pulse after further polarization decay (~30 s)
Figure 36. $^1$H NMR spectroscopy of solution-phase PHIP of 0.4 M HP HEP (2-hydroxyethyl propionate) probed at 1.4 T. a) ALTADENA RASER signal recorded without RF pulse after hydrogenation in the Earth’s magnetic field. b,c) Fourier spectra of the regions outlined by red and purple lines in display b), respectively. d,e) Partial ALTADENA RASER signal and Fourier spectrum acquired using $\sim 3^\circ$ RF pulse. f,g) Canonical ALTADENA (non-RASER) FID and Fourier spectrum recorded after further polarization decay ($\sim 30$ s). h) PASADENA RASER signal recorded without RF pulse after hydrogenation at 1.4 T. i,j) Fourier spectra of the regions outlined by red and purple lines in display h), respectively. k,l) Partial PASADENA RASER signal and Fourier spectrum acquired using $\sim 3^\circ$ RF pulse. m,n) Canonical PASADENA (non-RASER) FID and Fourier spectrum acquired with $\sim 3^\circ$ RF pulse after further polarization decay ($\sim 30$ s).
With ALTADENA, the Fourier spectra of the time slices of the RASER active signals (displayed in Figures 35a and 36a) show a doublet (Figures 35b and 36b). These two RASER resonances can be attributed to lines in the PHIP spectra depicted in Figures 35g and 36g respectively. In particular, each of the three triplet lines corresponding to proton H\textsubscript{B} are population inverted (have negative sign) and its two most intense lines are RASER active. The doublet is separated by splitting corresponding to the spin-spin coupling $J_{HA-HB}$ of 7.0 Hz between proton H\textsubscript{A} and H\textsubscript{B} in EA and HEP (Figure 35b and 36b respectively).\textsuperscript{43} While the HP state decays, the number of RASER active lines changes, for example from two RASER active lines in Figures 35b and 36b to one single in Figures 35c and 36c correspondingly. This could be explained by different transverse relaxation rates and multiplicities of each RASER line. For instance, at low polarization only one line with the highest amplitude in the NMR spectrum and with the smallest linewidth overcomes the RASER threshold and is RASER active.

All ALTADENA-hyperpolarized RASER spectra from Figures 35 and 36 differ significantly from the corresponding PHIP spectra in Figures 35g and 36g. The latter feature the HP resonances of H\textsubscript{A} and H\textsubscript{B} with the lines of the quartet and the triplet spectrum of opposite signs. These quartet and triplet are separated by $\sim$2.8 ppm ($\sim$174 Hz) for EA and $\sim$1.2 ppm ($\sim$74 Hz) for HEP. Specifically, the linewidth of the quartet FWHM of $\sim$4 Hz in Figure 35g is significantly broader compared to the linewidth of each of the triplet lines with FWHM of $\sim$2 Hz. This is more pronounced in Figure 35e, where the difference in linewidth is more than one order
of magnitude. The same trend is observed in Figures 36e and 36g. The reason for this is the sign and the magnitude of the HP state, which introduce a broadening with $\kappa_{\text{tot}} > 1/T_2^*$ for the quartet lines and a narrowing with $\kappa_{\text{tot}} < 1/T_2^*$ of the triplet lines. For the RASER lines in Figures 35b and 36b $\kappa_{\text{tot}}$ is negative and the linewidth in principal is only limited by the finite measurement time and ultimately by the Cramér–Rao condition.$^{245}$ We conclude that the RASER spectra of VA and HEP hyperpolarized by ALTADENA allow the $J$-coupling constant $J_{HA-HB}$ to be determined with enhanced precision but the chemical shift difference between $H_A$ and $H_B$ is not measurable in this RASER experiment.

The analysis of the RASER active signals in the PASADENA case (Figure 35h and 36h) renders other interesting observations in addition to the anticipated line narrowing. In particular, the Fourier spectra of the RASER active signals (Figures 35i and 36i) exhibit two large central RASER lines separated by the chemical shift difference ($\delta_{HA} - \delta_{HB}$) between the $H_A$ and $H_B$ protons, i.e., $\delta_{HA} - \delta_{HB} = 2.8$ ppm ($\sim 174$ Hz) for EA and $1.2$ ppm ($\sim 74$ Hz) for HEP. The two central lines are accompanied by evenly spaced small sidebands, and the distance between two consecutive lines is exactly ($\delta_{HA} - \delta_{HB}$). This can be explained by the non-linear interaction between different RASER active modes (here two), leading to a frequency comb like spectrum.$^{239}$ We also found even frequency comb like spectra in the case of the ALTADENA pumped RASER where the two central modes and all sidebands are spaced by $J_{HA-HB}$. Moreover, the resonance frequencies of the RASER active protons (Figures 35b, 35c, 36b, and 36c) are sometimes shifted by about 1 ppm
when compared with the partial RASER and hyperpolarized ones. We speculate that this is due to the magnetic field fluctuations induced by RASER. A detailed evaluation of these and other nonlinear phenomena will be published elsewhere.

A series of additional experiments were performed, demonstrating further that the experimental conditions necessary for observing RASER through PHIP reactions are not stringent at all. RASER bursts can indeed be observed with more dilute samples, as illustrated by the NMR signal shown in Figure 70, appendix C and obtained with a 40 mM VA solution. This indicates that PHIP RASER occurs at relatively low concentrations of HP substrate; in particular lower concentrations than those reported previously for spontaneous emission of NMR signals with the dissolution Dynamic Nuclear Polarization (d-DNP) technique. ALTADENA RASER activity was also observed while leaving the catheter (1/16” outer diameter, 1/32” inner diameter) inside the NMR tube, thus creating more stringent conditions for its occurrence because the presence of the catheter leads to significant susceptibility induced $B_0$ gradient and effectively shorter $T_2^*$ (see Figures 71 and 72, appendix C). Note that in additional PASADENA experiments, the hydrogenation reactions were performed within the RF coil and RASER was detected immediately after the cessation of p-H$_2$ bubbling because the bubbles induce a significant degradation of $T_2^*$ (likely below 1 ms) that prevents RASER activity at this magnetic field. These findings are crucial in the context of PHIP studies and biomedical applications. The HP substrates used here can indeed be employed as in vivo contrast agents. For example, HP HEP has been extensively
studied as a potential contrast agent in angiography studies. Because some of the PHIP techniques rely on the application of RF pulses, especially in the case of polarization transfer from protons to $^{13}$C nuclei, the RF coils may interact with the highly proton-hyperpolarized compounds resulting in complicated nonlinear effects and depletion of hyperpolarization via RASER activity. Therefore, RASER effects may be considered as an obstacle in this context. The use of untuned RF coils may help mitigate the occurrence of RASER, so that the hyperpolarized proton pool is not depleted prior its utilization during contrast agent preparation. Moreover, the recent advent of PHIP via side-arm hydrogenation (SAH) significantly expanded the range of biomolecules (including ethyl acetate) that can be hyperpolarized via PHIP. With this technique, a wide range of carboxylic acids have been hyperpolarized and employed in vivo for metabolism tracking.

Amid the peer-review evaluation of the present work, another interesting study regarding PHIP-RASER by Pravdivtsev and co-workers was reported. In contrast with the common and somewhat ordinary conditions we have presented here, Pravdivtsev et al. designed a specific experiment dedicated to observe $p$-$H_2$-induced RASER activity under PASADENA conditions at a magnetic field of 14 T (600 MHz) and with a cryogenically-cooled coil (Q~500). The catalyst activity was tuned for building up polarization throughout an extended period of time (about 10 min) with a continuous delivery of $p$-$H_2$. The $p$-$H_2$ pairwise addition was performed with two substrates incorporating $C\equiv C$ triple bonds. Without RF pulse...
excitation, RASER activity was detected only after bubbling p-H$_2$ for about 90 s. Only chemical shifts but no J-couplings could be determined in these very high field experiments and the reported linewidths (in the order of 1 Hz) of the RASER active lines do not differ significantly from the linewidth obtained by a corresponding standard NMR spectrum (a few ppb at 600 MHz). The work presented in this article differs in many regards. First and foremost, the equipment employed here is a commercially available NMR spectrometer with unaltered room-temperature RF coil with Q of ~68—such instrumentation is widely available making our observations possible and easy to replicate by others interested in using PHIP-RASER effect. Second, we demonstrated PHIP-RASER under both PASADENA and ALTADENA conditions and showed intriguing J-coupling and chemical-shift controlled dynamics of RASER signal evolution. No RF stimulation was required to induced RASER effect. Third, we employed two molecular moieties (acetate$^{256,257}$ and propionate$^{79}$ via pairwise p-H$_2$ addition to double C=C bond) that have been previously employed in in vivo bio-imaging studies of perfusion and metabolism. Importantly, we employed the process of batch hyperpolarization,$^{151,225}$ when a bolus of material is hyperpolarized over a short period of time (ca. 10 s), and such bolus could be employed for in vivo imaging applications—paving the way to potential future use of RASER in bio-imaging applications, which have the potential to revolutionize MRI and medical imaging.
5.4 Conclusions

To summarize, RASER activity of two PHIP-hyperpolarized compounds is reported here using standard NMR hardware at concentrations as low as 40 mM and at estimated proton polarization values of over 10% at the time of the detection. RASER activity is observed with and without applications of RF excitation pulses and under both ALTADENA and PASADENA conditions. $J$-coupling constants as well as chemical shift differences could be measured with increased precision. As the field of PHIP continues to grow, researchers using standard, commercial NMR spectrometers are therefore expected to experience RASER activity and radiation damping or line narrowing phenomena routinely. Appropriate considerations must be made when performing NMR experimentations with such highly polarized compounds. Our observations are especially important for PHIP studies that aim at providing highly polarized contrast agents for imaging of metabolism, $i.e.$, where high levels of polarization at high substrate concentrations are desired. Despite its complications the p-H$_2$ -induced RASER phenomenon described here could enable new applications in magnetic resonance, quantum computing, data encryption and beyond. Further studies are underway in our laboratories to provide additional theoretical and experimental insights about this intriguing phenomenon and its applications.

5.5 Acknowledgements

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Associated content can be found in appendix C.
CHAPTER 6 Parahydrogen-Induced Polarization of Diethyl Ether Anesthetic


In this chapter, “we” and other first-person plural pronouns are used in reference to all authors of this publication. My individual contributions to the research include conduction of ~50 % experiments, 50-55 % data analysis and writing parts of the manuscript. Figures and tables containing data contributed by researchers other than me will contain a disclaimer in the caption; figures and tables with no disclaimer in the caption contain data collected by me.

Diethyl ether was the first commercial anesthetic in practice although it is no longer employed as a medical anesthetic in the USA due to its low flammability, but still in practice in some other countries. HP diethyl ether was successfully prepared in both gas and liquid states via a low cost pairwise p-H\textsubscript{2} addition method to ethyl vinyl ether, with high polarization values and complete chemical conversion. HP diethyl ether is envisioned to be used as an inhalable contrast agent in the future, after modifications are taken to address its flammability issues.

6.1 Abstract

The growing interest in magnetic resonance imaging (MRI) for assessing regional lung function relies on the use of nuclear-spin hyperpolarized gas as a contrast agent. The long gas-phase lifetimes of hyperpolarized $^{129}$Xe make this inhalable contrast agent acceptable for clinical research today despite limitations such as high cost, low throughput of production and challenges of $^{129}$Xe imaging
on clinical MRI scanners, which are normally equipped with proton detection only. We report on low-cost and high-throughput preparation of proton-hyperpolarized diethyl ether, which can be potentially employed for pulmonary imaging with a non-toxic, simple, and sensitive overall strategy using proton detection commonly available on all clinical MRI scanners. Diethyl ether is hyperpolarized by pairwise $p$-$H_2$ addition to vinyl ethyl ether and characterized by $^1$H NMR spectroscopy. Proton polarization levels exceeding 8% are achieved at near complete chemical conversion within seconds, causing the activation of radio amplification by stimulated emission radiation (RASER) throughout detection. Although gas-phase $T_1$ relaxation of hyperpolarized diethyl ether ($p=0.5$ bar) is very efficient with $T_1$ of ca. 1.2 second, we demonstrate that at low magnetic fields, the use of long-lived singlet states created via pairwise $p$-$H_2$ addition extends the relaxation decay by approximately 3-fold, paving the way to biomedical applications and beyond.

6.2 Introduction

The degree of nuclear spin alignment with applied static magnetic field, termed nuclear spin polarization ($P$), is typically on the order of $10^{-5}$ for protons at clinically and physiologically relevant conditions. Because NMR detection sensitivity is directly proportional to $P$,\textsuperscript{258} NMR spectroscopy and imaging are generally regarded as relatively low-sensitivity techniques. Thus, \textit{in vivo} MRI is limited primarily to highly concentrated compounds such as water and lipids. However, NMR hyperpolarization techniques can transiently increase $P$ to the order of unity resulting in corresponding gains in detection sensitivity.\textsuperscript{8, 10, 152, 183} Hyperpolarized
(HP) compounds can be employed as injectable or inhalable contrast agents. To date, the two groups of HP contrast agents that have transitioned to clinical trials are HP $^{13}$C–labeled biomolecules (most notably $[1^{-13}C]$-pyruvate) and $^{129}$Xe gas, which can be employed for spectroscopic imaging of in vivo metabolism (e.g., glycolysis) and organ function (e.g., lung ventilation and perfusion). The key motivation of the biomedical HP community that has historically focused on $^{13}$C and $^{129}$Xe nuclei is the significantly longer lifetime of their HP states compared to those of protons. For example, in vivo $T_1$ are on the order of 1 min (in favorable cases) and 4-20 s for $^{13}$C and $^{129}$Xe, respectively, versus 1-2 s for protons.

Despite some success and a number of ongoing clinical trials, there are several translational limitations that $^{13}$C and $^{129}$Xe HP contrast agents face to address critical medical needs. First and foremost, $^{13}$C and $^{129}$Xe detection is not available on most clinical MRI scanners, which are limited to proton detection only. Second, $^{13}$C and $^{129}$Xe have ~4-fold lower magnetic moments and ~4-fold lower resonance frequencies compared to those of protons, resulting in a factor of (~4)$^2$ less NMR signal for $^{13}$C and $^{129}$Xe nuclei. Besides, MRI spatial encoding also requires a factor of $4^2$ more gradient power to achieve the same spatial resolution.

Third, clinical-scale $^{13}$C and $^{129}$Xe hyperpolarization techniques have been demonstrated using dissolution Dynamic Nuclear Polarization (d-DNP) and Spin-Exchange Optical Pumping (SEOP), respectively, which have limited production throughput (approximately 4 doses per hour) and highly complex and
expensive instrumentation ($0.5-$2M cost, circa 2020). More affordable methods as well as new indirect detection schemes in clinical-scale d-DNP may eventually emerge for hyperpolarization of [1-\(^{13}\)C]-pyruvate and other agents, although alternatives for HP \(^{129}\)Xe gas as an inhalable HP contrast agent are still rather limited.

Levitt and co-workers have provided numerous examples of the existence of long-lived singlet states (LLS), with \(T_{\text{LLS}}\) values significantly greater than corresponding \(T_1\). This new concept rekindled the interest in HP proton contrast agents, yet no LLS have been translated to \textit{in vivo} demonstration on biologically suitable carriers. Lately, we have demonstrated the existence of LLS in p-H\(_2\)-hyperpolarized propane gas at low magnetic fields, \textit{i.e.}, in the strong coupling regime where the spin-spin coupling \(J\) between the protons of interest is greater than the difference between their chemical shifts. The LLS of HP propane was found immune to O\(_2\), indicating that it may be a useful contrast agent for pulmonary imaging applications—efforts are in progress in our laboratory to demonstrate the utility of HP propane in a large animal model.

Here we report on a simple and fast approach to prepare HP diethyl ether (DE), the first anesthetic produced on a commercial scale. We employ parahydrogen-induced polarization (PHIP) for pairwise p-H\(_2\) addition to ethyl vinyl ether (EVE) to form HP DE in the gas and liquid phases (Figure 37). DE was on the World Health Organization (WHO) essential list of drugs until 2005. The substrates EVE (a.k.a. Vinamar) and divinyl ether (a.k.a. Vinethene) have also been employed as
inhalable anesthetics in clinical anesthesiology.\textsuperscript{279} Although these ethers have been phased out in the US and other countries due to the availability of nonflammable alternatives (Desflurane, Isoflurane, Sevoflurane, etc.), they remain approved for medical use as anesthetics in many countries\textsuperscript{280} including Russia.

\textbf{Figure 37. Pairwise p-H\textsubscript{2} addition to ethyl vinyl ether (EVE) to form HP diethyl ether (DE). Note the symmetry breaking of protons H\textsubscript{A} and H\textsubscript{B}.}

\textbf{6.3 Materials and methods}

Liquid-phase hyperpolarization studies were performed with solutions of \textasciitilde200 mM of EVE (Sigma Aldrich, 99\%) in CD\textsubscript{3}OD and 4 mM of rhodium catalyst (1,4-Bis(diphenylphosphino)butane(1,5-cyclooctadiene) rhodium(I) tetrafluoroborate, STREM, 79255-71-3). 0.5 mL samples were placed in 5 mm NMR tubes under argon atmosphere. NMR tubes were pressurized with p-H\textsubscript{2} at \textasciitilde8 bar and heated at 80 °C for 30 seconds. p-H\textsubscript{2} (enriched at >99\%\textsuperscript{57}) was bubbled through the solution at a flow rate of 150 standard cubic centimeters per minute (sccm). Pairwise p-H\textsubscript{2} addition to EVE was performed in the Earth’s magnetic field followed by detection at 1.4 T via a benchtop NMR spectrometer (SpinSolve Carbon60, Magritek), corresponding to ALTADENA condition.\textsuperscript{62} \textsuperscript{1}H NMR spectra of neat
thermally polarized ethyl acetate were used as signal reference to evaluate polarization levels (Figure 38a,b). For each sample, the chemical conversion and residual DE concentration in CD$_3$OD (liquid fraction) were evaluated from the thermal NMR spectra acquired before and after the reaction (Figure 73, appendix D). $^1$H polarization levels were corrected accordingly (see calculations in appendix D).

After cessation of p-H$_2$ flow, the NMR sample was left for additional 15 seconds in the Earth’s magnetic field (~50 μT) before insertion in the 1.4 T spectrometer to collect NMR spectrum using 8° excitation pulse. This delay was necessary to avoid strong radio amplification by stimulated emission radiation (RASER, Figure 38c).$^{174, 239}$ This recently discovered phenomena, due to the coupling of the inductive detector with the pool of HP proton spins, is manifested here by the appearance of non-linear effects such as multi-mode RASER with both H$_A$ and H$_B$ emitting (Figure 38c). RASER activity can last for more than 30 s in molecules hyperpolarized by p-H$_2$, preventing polarization level measurement immediately after the reaction completion. The observation of PHIP-RASER indicates the production of highly magnetized DE samples and may be useful in the context of future imaging studies. HP DE polarization levels were back calculated to account for the relaxation losses (in the Earth’s magnetic field).
Figure 38. HP DE spectroscopy data in CD$_3$OD. a) $^1$H NMR spectrum of HP DE in CD$_3$OD solution acquired using $8^\circ$ pulse angle with polarization of 8.4% after correction for evaporation and the Earth's field relaxation. b) Corresponding NMR spectrum of neat thermally polarized ethyl-acetate-1-$^{13}$C. c) ALTADENA RASER signal of HP DE recorded without excitation pulse (red), along with Fourier transform (FT) spectra of the regions outlined by purple (H$_B$/ H$_A$ two-mode RASER) and orange (H$_B$ single-mode RASER) boxes.
6.4 Results and discussion

A maximum polarization of 8.4% was obtained in the liquid phase after bubbling p-H$_2$ for 10 s (Figures 38a and 39a). At that time, the chemical conversion of EVE to DE was complete and more than 80% of DE remained in the liquid phase (Figure 39b). $^1$H polarization levels were fitted to:

$$P_H(t) = \frac{P_{\text{max}}}{T_{\text{cat}}/T_{\text{LLS}} - 1} \left( \exp\left(-\frac{t-t_0}{T_{\text{cat}}}\right) - \exp\left(-\frac{t-t_0}{T_{\text{LLS}}}\right) \right)$$

(18)

to determine $P_{\text{max}}$, the theoretical maximum polarization neglecting relaxation, and $T_{\text{cat}}$, the time constant for the catalytic reaction (or polarization build-up), while leaving $T_{\text{LLS}}$ and $t_0$ fixed to 14 s and 1 s, respectively. These latter values correspond to independent measurements performed at 50 µT (Figure 39c). $\%P_{\text{max}}$ of 12.8±0.6% and $T_{\text{cat}}$ of 4.5±0.6 s were obtained, indicating that highly polarized DE can be prepared via PHIP with production speed significantly exceeding relaxation decay. $\%P_{\text{max}}$ can be potentially further improved via the rational design of a PHIP catalyst; nevertheless, the polarization levels of HP DE and the speed of the reaction derived here are promising for in vivo applications. Moreover, the lifetime of HP state can be further extended at higher magnetic field: the $T_1$ values are 29 ± 1 s and 24 ± 1 s for $H_A$ and $H_B$ protons at 1.4 T, respectively.
Figure 39. Reaction kinetics, conversion and relaxation studies of HP DE. a) $^1$H polarization levels of HP DE in CD$_3$OD as a function of reaction time in the Earth’s magnetic field (50 $\mu$T). Dashed lines indicate 95% confidence boundaries of the fit b) DE liquid fraction (pink crosses) and chemical conversion (magenta circles) of EVE to DE as a function of reaction time. c) Exponential decays of HP DE NMR signals at 1.4 T (black circles and squares) and 50 $\mu$T (blue circles and squares).

For gas-phase polarization and relaxation experiments, p-H$_2$ was bubbled in a glass column filled with ~ 5-10 mL of neat EVE at a flow rate of 4000 sccm. The
resulting gas mixture comprising p-H\(_2\) saturated with EVE vapor was directed through a Rh/TiO\(_2\) catalytic reactor (300 mg of Rh/TiO\(_2\) in 42 g of Cu particles) maintained at 170 °C for heterogeneous pairwise p-H\(_2\) addition\(^{282}\) (Figure 74), and collected at 3.3 bar and \(\sim\)35 °C in (i) a 5 mm NMR tube for NMR detection using 1.4 T benchtop NMR device (Figure 40a), (ii) a 17 mL phantom sphere for NMR detection with a 47.5 mT Kea2-based MRI scanner (Magritek, New Zealand) (Figure 74). The concentration of DE in the gas phase (22 mM) was evaluated at 1.4 T against reference spectra of neat ethyl acetate. Inversion recovery experiments with thermally polarized DE vapor yielded \(T_1 = 1.21 \pm 0.03\) s at 1.4 T (Figure 40c), a typical value under these low partial pressure conditions (0.5 bar).\(^{283}\) Even though the LLS of HP DE is NMR invisible at 47.5 mT, it can be converted into observable magnetization by singlet-to-triplet conversion with the spin-lock induced crossing (SLIC) radio-frequency pulse sequence (Figure 40b).\(^{95}\) SLIC was optimized by tuning pulse amplitude, power, duration and frequency in the gas and liquid phases (Figures 75 and 76, appendix D). \(T_{\text{LLS}}\) was determined by applying a “partial” (200 ms duration) SLIC pulse every second to a batch of HP DE vapor and recording the produced signal—corresponding data points were employed for mono-exponential fitting (Figure 40d), yielding \(T_{\text{LLS}} = 2.8 \pm 0.4\) s. The “partial” SLIC approach is known to induce an underestimation of the \(T_{\text{LLS}}\) value, because some fraction of the magnetization is lost in each SLIC transformation.\(^{283}\) Comparative partial SLIC experiments on HP DE in CD\(_3\)OD suggest correcting the measured \(T_{\text{LLS}}\) by a factor of 1.4 (Figure 76, appendix D). Our estimate is \(T_{\text{LLS}} = \)
$4.0 \pm 0.7 \text{ s}$ for HP DE at 0.5 bar partial pressure, i.e., ~3 times greater than the corresponding $T_1$ at high field—in line with the overall trends of LLS lifetime in HP propane.$^{138,283}$

**Figure 40. HP DE spectroscopy data at 1.4 T.** a) $^1\text{H}$ spectrum at 1.4 T of 22 mM [DE] / 110 mM [p-H$_2$] at 3.3 bar total pressure. b) $^1\text{H}$ spectrum at 47.5 mT of 22 mM [DE] / 110 mM [p-H$_2$] at 3.3 bar using SLIC. c) Inversion recovery of thermal DE vapor at 1.4 T. d) Exponential decay of LLS of HP DE at 47.5 mT.
6.5 Conclusions

In summary, hyperpolarization of DE anesthetic was successfully achieved. High levels of proton polarization (>8%) were obtained on two hydrogen sites along with 100% conversion of the EVE precursor—the produced magnetization was sufficiently high for inducing RASER activity. We also report on the existence of LLS in gas phase HP DE under clinically relevant conditions. Alternative precursor divinyl ether could be potentially employed to double the payload of p-H$_2$-derived polarization. All three compounds: DE, EVE and divinyl ether have very safe toxicity profile, which, combined with the ease and scalability of HP DE preparation and existence of long-lived states in the gas phase, bode well for future bioimaging applications—especially for functional 3D pulmonary imaging in a manner similar to that with HP $^{129}$Xe. The flammability of HP DE should be addressed in the context of potential biomedical use as an inhalable contrast agent: for example, through the use of small inhalation doses or by capturing the exhaled gas with carbon filters. Alternatively, the use of partially fluorinated PHIP precursors (e.g., Fluroxene) can possibly mitigate some or all flammability issues.

6.6 Acknowledgements

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Associated content can be found in appendix D.
Fluoromisonidazole known as FMISO, is an emerging radiotracer drug used for tumor molecular imaging via PET. Molecules with nitroimidazole moieties have shown promising results towards detection of tissues with hypoxic conditions. We show that QUASR-SABRE can produce HP metronidazole with good $^{15}$N polarization values allowing for this FDA-approved antibiotic to be used as a novel molecular imaging probe for HP MRI hypoxia sensing.

7.1 Abstract

Here we present the feasibility of NMR Signal Amplification by Reversible Exchange (SABRE) using radio-frequency irradiation at low magnetic field (0.05 T) in the regime, where the chemical shifts of free and catalyst bound species are similar. In SABRE, the $^{15}$N-containing substrate and $p$-$\text{H}_2$ perform simultaneous chemical exchange on an Iridium hexacoordinate complex. Shaped Spin-Lock Induced Crossing (SLIC) radio-frequency pulse sequence followed by a delay is
applied at QUASi-Resonance (QUASR) condition of $^{15}$N spins of $^{15}$N-enriched substrate. As a result of this pulse sequence application, $^{15}$N z-magnetization is created from the spin-order of p-H$_2$ derived hyperpolarized hydrides. The repetition of the pulse-sequence block consisting of shaped radio-frequency pulse and the delay leads to the build-up of $^{15}$N magnetization. The modulation of this effect by the irradiation frequency, pulse duration and amplitude, delay duration, and the number of pumping cycles was demonstrated. Pyridine-$^{15}$N, acetonitrile-$^{15}$N, metronidazole-$^{15}$N$_2$-$^{13}$C$_2$ substrates were studied representing three classes of compounds (five- and six-membered heterocycles and nitrile) showing the wide applicability of the technique. Metronidazole-$^{15}$N$_2$-$^{13}$C$_2$ is an FDA-approved antibiotic that can be injected in large quantities promising non-invasive and accurate hypoxia sensing. The $^{15}$N hyperpolarization levels attained with QUASR-SABRE on metronidazole-$^{15}$N$_2$-$^{13}$C$_2$ were more than two-fold greater than with SABRE-SHEATH (SABRE in SHield Enables Alignment Transfer to Heteronuclei) demonstrating that QUASR-SABRE can deliver significantly more efficient means of SABRE hyperpolarization.

7.2 Introduction

Conventional NMR relies on equilibrium thermal nuclear spin polarization $P$ dictated by the Boltzmann distribution among Zeeman energy levels in dependence of the applied static magnetic field $B_0$. Although $P$ can be boosted significantly by applying stronger magnetic field (because $P \propto B_0$), $P$ is typically on
the order of $10^{-5}$ to $10^{-6}$ for a conventional high-field NMR spectrometer (ca. 9.4 T) or MRI scanner (ca. 3 T) at room temperature, i.e. when the high temperature approximation holds. Hyperpolarization techniques increase $P$ to the order of unity increasing NMR sensitivity increase by 4-5 orders of magnitude.\textsuperscript{8, 10, 183}

Several hyperpolarization techniques exist.\textsuperscript{8, 10, 183} Signal Amplification by Reversible Exchange (SABRE) is one of more recent techniques pioneered by Duckett and co-workers in 2009.\textsuperscript{80, 82, 97, 284} SABRE relies on simultaneous chemical exchange of parahydrogen ($p$-$H_2$) and to-be-hyperpolarized substrate (Figure 41a).\textsuperscript{285} When the transient complex is formed, the $p$-$H_2$ symmetry is broken,\textsuperscript{15} and the network of spin-spin couplings can enable transfer polarization from $p$-$H_2$-derived hydrides to the nuclear spins of the substrate.\textsuperscript{80, 82, 97, 284} Two major groups of approaches have been developed for SABRE polarization transfer: the first group employs a matching static magnetic field $B_{evo}$,\textsuperscript{77, 80, 286-288} and the second group applies radio-frequency (RF) pulse sequences,\textsuperscript{92} to approach Level Anti-Crossings (LAC)\textsuperscript{94, 289} and induce polarization transfer. Both approaches have merit depending on the application. For biomedical applications, which represent the main driver for development of hyperpolarization technology,\textsuperscript{8} the key is to achieve high degrees of polarization with the long lifetimes in a suitable biomolecular motif.\textsuperscript{81} So far, approaches relying on static magnetic fields such as SABRE-SHEATH (SABRE in SHield Enables Alignment Transfer to Heteronuclei)\textsuperscript{99, 150, 290} have been the most efficient for preparation of long-lived
$^{15}$N hyperpolarized spin states with exponential decay constant of more than 20 minutes$^{216}$ and $P_{^{15}N}$ exceeding 30%.$^{101, 291}$

One likely explanation as to why RF-based methods are lagging behind in the context of SABRE is the reliance on conventional high-field NMR spectrometers with small coils that only encompass a small fraction of the liquid sample in a 5 mm NMR tube,$^{92}$ which is continuously bubbled with p-$\text{H}_2$.$^{100}$ These factors result in major RF-inhomogeneities. In contrast, a previous approach used in hydrogenative Parahydrogen Induced Polarization (PHIP$^{115, 51, 63}$), employs low-field (ca. 5-50 mT) magnets and RF excitation coils,$^{119, 134, 137, 151, 225, 292}$ which encompass the entire sample volume, sometimes in excess of 50 mL.$^{58, 252, 293}$ Moreover, the hardware behind such low-field devices is significantly less complex and less costly compared to that of the high-field NMR spectrometers.$^{81, 151, 225, 293, 294}$ Here it is demonstrated that these advantageous features can also be translated to SABRE.

RF-based polarization transfer such as Low-Irradiation Generation of High Tesla (LIGHT)-SABRE employs RF irradiation of the catalyst-bound substrate (Figure 41a), which typically has a chemical shift difference of 30-50 ppm with respect to the free substrate.$^{92, 295}$ Irradiation of the catalyst bound species allows for polarization transfer from p-$\text{H}_2$-derived hydrides.$^{92}$ When the complex dissociates, the $^{15}$N nuclear spin polarization is preserved in the free substrate, because it is not affected by the frequency-selective RF pulses.$^{92}$ Achieving this frequency selective irradiation at high magnetic fields is trivial due to large
chemical shift dispersion. For example, 50 ppm difference equals to ~2 kHz at 9.4 T. However, this difference vanishes at low magnetic fields: for example, 50 ppm difference equals 10 Hz at 0.05 T (the magnetic field employed in this Letter), and selective RF-excitation becomes challenging. We demonstrate that this challenge can be overcome through the use of quasi-resonance (QUASR) spin-lock induced crossing (SLIC)\(^95\) irradiation to polarize \(^{15}\)N spins from \(\text{p-H}_2\)-derived hydrides.

7.3 Materials and methods

The QUASR-SABRE experiment (Figure 41b) is performed at 0.05 T magnetic field using previously described \(\text{p-H}_2\) bubbling setup in a medium-walled 5-mm NMR tube.\(^{100, 296, 297}\) During \(\text{p-H}_2\) bubbling a triangular-shaped pulse is applied for duration \(\tau_{\text{PULSE}}\) followed by a delay period \(\tau_{\text{DELAY}}\). The process is repeated, and the net z-magnetization increases during this “rf-pumping” process. The resulting magnetization can be conveniently assessed by applying a broad-band excitation (\(\pi/2\)) RF pulse, Figure 41c. We compare the performance of this QUASR-SABRE approach with SABRE-SHEATH approach (Figure 41d), which has been employed previously to obtain record-high \(^{15}\)N polarization in excess of 30%.\(^{101, 291}\)
Figure 41. SABRE hyperpolarization experimental set up. a) the diagram of molecular exchange with p-H₂ in SABRE hyperpolarization. b) the experimental setup for QUASR-SABRE, c) the RF pulse sequence for QUASR-SABRE, d) corresponding experimental setup for SABRE-SHEATH experiment. The data for this figure was collected by Eduard Chekmenev.
The previously described SABRE-SHEATH setup\textsuperscript{298, 299} \textsuperscript{15}N RF coil and 0.05 T magnet have been employed here.\textsuperscript{298} Samples of three substrates and IrILMes catalyst precursor in perdeuterated methanol were prepared as follows: pyridine-\textsuperscript{15}N/catalyst, ~20 mM/\textsim\texttildelow1 mM, acetonitrile-\textsuperscript{15}N/catalyst, ~40 mM/\textsim\texttildelow1-2 mM, metronidazole-\textsuperscript{15}N\textsubscript{2}-\textsuperscript{13}C\textsubscript{2}/catalyst, ~20 mM/\textsim\texttildelow1 mM. All \textsuperscript{15}N-enriched compounds were purchased from Isotec. The RF pulse sequences was coded and applied on Kea-2 NMR spectrometer (Magritek, New Zealand) using Tomco RF amplifier. The employed Kea-2 spectrometer was operated in the signal averaging mode, where the signal is averaged during multi-scan acquisitions version being added. As a result, the signal integral value from multi-scan spectrum is similar to that acquired using 1-scan acquisition, e.g. the integral values of spectra shown in corresponding displays in Figures 4-44 can be compared directly without any scaling even though the spectra were recorded using different numbers of scans. A Three-layered mu-metal magnetic shield was employed (Magnetic Shield Corp., Bensenville, IL, P/N ZG-206). All data was acquired employing 75-80% p-H\textsubscript{2} prepared using home-built p-H\textsubscript{2} generator based on Sunpower cryo-chiller at a flow rate of 150 standard cubic centimeters per minute (sccm). For the SABRE experiments, the following conditions were used: 75 psi back-pressure and 70 sccm p-H\textsubscript{2} flow rate (Figure 41b and Figure 41d). The flow rate was maintained by via a mass flow controller (MFC, Sierra Instruments, Monterey, CA, P/N C100L-DD-OV1-SV1-PV2-V1-S0-C0).
7.4 Results and discussion

Low-field detection of hyperpolarized (HP) compounds offers sufficient detection sensitivity in the context of NMR detection of HP states. However, detection of thermal polarization is challenging due to low $P$ even at high concentrations (see appendix E for details). As a result, the quantification of $^{15}$N enhancements ($\varepsilon_{15N}$) and polarization ($P_{15N}$) relied on signal to noise measurements (see appendix E for details) in order to determine the minimum values achieved.

$^{15}$N NMR spectra with high SNR were obtained for all three studied molecules: pyridine-$^{15}$N, acetonitrile-$^{15}$N, and metronidazole-$^{15}$N$_2$-$^{13}$C$_2$ using SABRE-SHEATH (Figure 42a, Figure 43a, and Figure 44a respectively) and QUASR-SABRE (Figure 42c, Figure 43c, and Figure 44c respectively). We note that while p-H$_2$ bubbling through the sample placed in 0.05 T leads to $^{15}$N signal even without RF pulses (Figure 42b, Figure 43b and Figure 44b respectively), this $^{15}$N signal is distinctly anti-phase, and has significantly lower intensity compared to those obtained using SABRE-SHEATH and QUASR-SABRE protocols.
Figure 42. Pyridine-$^{15}$N data. The other experimental conditions were as follows: 20 mM pyridine-$^{15}$N, 1 mM catalyst in CD$_3$OD. Note the width of the signal in displays b and d. a) the $^{15}$N spectrum obtained after performing SABRE-SHEATH; b) The $^{15}$N spectrum recorded using 90-degree excitation pulse when bubbling p-H$_2$ in situ of the 0.05 T magnet; c) the $^{15}$N spectrum obtained after performing QUASR-SABRE; d) $^{15}$N QUASR-SABRE signal dependence on the applied radio frequency offset from the actual resonance condition; e) the build-up of $^{15}$N QUASR-SABRE signal as a function of the number of pumping cycles. Note the individual spectra employed for figures in displays d and e were auto-phased, and the data is presented in the magnitude mode.
**Figure 43. Acetonitrile-$^{15}$N data.** The other experimental conditions were as follows: 40 mM acetonitrile-$^{15}$N, 1 or 2 mM catalyst in CD$_3$OD. Note the width of the signal in displays a and c is nearly the same as opposed to pyridine-$^{15}$N case.

a) the $^{15}$N spectrum obtained after performing SABRE-SHEATH; b) The $^{15}$N spectrum recorded using 90-degree excitation pulse when bubbling p-H$_2$ in situ of the 0.05 T magnet; c) the $^{15}$N spectrum obtained after performing QUASR-SABRE; d) the build-up of $^{15}$N QUASR-SABRE signal as a function of the number of pumping cycles; Note the individual spectra employed for figures in display d were auto-phased, and the data is presented in the magnitude mode.
Figure 44. Metronidazole-$^{15}$N$_2$-$^{13}$C$_2$ data. The other experimental conditions were as follows: ~20 mM metronidazole-$^{15}$N$_2$-$^{13}$C$_2$, 1 or 2 mM catalyst in CD$_3$OD. Note the width of the signal in displays a and c is nearly the same as opposed to pyridine-$^{15}$N case. a) The $^{15}$N spectrum obtained after performing SABRE-SHEATH; b) The $^{15}$N spectrum recorded using 90-degree excitation pulse when bubbling p-H$_2$ in situ of the 0.05 T magnet; c) the $^{15}$N spectrum obtained after performing QUASR-SABRE; d) $^{15}$N QUASR-SABRE signal dependence on the frequency of the applied RF shaped pulse; e) the build-up of $^{15}$N QUASR-SABRE signal as a function of the number of pumping cycles N. Note the individual spectra employed for figures in displays d and e were auto-phased, and the data is presented in the magnitude mode.
The key distinct feature of QUASR-SABRE phenomenon is strong RF offset frequency dependence: Figure 42d and Figure 44d demonstrate the dependence of the $^{15}$N QUASR-SABRE signal for pyridine-$^{15}$N and metronidazole-$^{15}$N$_2$-$^{13}$C$_2$ respectively. Note that very small signal is obtained at the resonance frequency. The maximum intensity of $^{15}$N QUASR-SABRE was compared to that of SABRE-SHEATH and is reported here as the ratio of $^{15}$N QUASR-SABRE and SABRE-SHEATH signal, $\eta$. We find $\eta$ of $\sim$1.0 for pyridine-$^{15}$N, $\eta$ of $\sim$0.44 for acetonitrile-$^{15}$N, and $\eta$ of (at least) $\sim$2.4 for metronidazole-$^{15}$N$_2$-$^{13}$C$_2$. The relatively low $\eta$ value for acetonitrile-$^{15}$N is in part explained by the fact that frequency optimization was not performed, and the data was recorded using the frequency offset parameter optimized for pyridine-$^{15}$N, which unfortunately did not provide a fair comparison. Based on the range of on the frequency optimization data for pyridine-$^{15}$N (Figure 42e) and for metronidazole-$^{15}$N$_2$-$^{13}$C$_2$ (Figure 44d), we estimate that this optimization may potentially yield an improvement of up ten-fold. We note that $\eta$ value for metronidazole-$^{15}$N$_2$-$^{13}$C$_2$ assumes that both $^{15}$N sites are hyperpolarized via SABRE-SHEATH and QUASR-SABRE protocols. While SABRE-SHEATH indeed yields hyperpolarization of both $^{15}$N sites due to spin-relay of polarization at very low magnetic fields (the Earth’s field (ca. 50 $\mu$T) and below), QUASR-SABRE may yield hyperpolarization of only one (N$_3$) site, Figure 44. If that is the case, then $\eta$ would be effectively doubled to 4.8, because only one $^{15}$N site contributes to the QUASR-SABRE signal (Figure 44c) versus two $^{15}$N sites to the SABRE-SHEATH NMR signal (Figure 44a). We note that the Full Width at the Half-
Height (FWHH) was approximately the same for SABRE-SHEATH and QUASR-SABRE NMR spectra for acetonitrile-$^{15}$N and metronidazole-$^{15}$N$_2$-$^{13}$C$_2$: Figure 43 and Figure 44 respectively, whereas QUASR-SABRE spectrum FWHH was approximately half of that for SABRE-SHEATH spectrum for pyridine-$^{15}$N (Figure 42c and Figure 42a respectively). The latter observation may in part explain pyridine-$^{15}$N $\eta$ value, which is significantly lower than that of metronidazole-$^{15}$N$_2$-$^{13}$C$_2$.

All three molecules studies exhibited a clear and strong dependence of $^{15}$N QUASR-SABRE signal on the duration of the pulse ($t_{\text{PULSE}}$) and the duration of the delay ($t_{\text{DELAY}}$): appendix E Figure 78a, Figure 79a and Figure 79b, Figure 80a and Figure 78b, Figure 79c, Figure 80b respectively. This strong dependence is likely due to the dynamics and kinetics of the substrate and p-H$_2$ exchange on the catalyst. When the $t_{\text{PULSE}}$ and $t_{\text{DELAY}}$ are in sync with chemistry of exchange, the maximum QUASR-SABRE signal may be achieved. However, we note that QUASR-SABRE signal has very complex dependence on $t_{\text{PULSE}}$ and $t_{\text{DELAY}}$. For example, the $t_{\text{PULSE}}$-curves for acetonitrile-$^{15}$N are vastly different at $t_{\text{DELAY}}$ of 2 ms (Figure 79a, appendix E) and $t_{\text{DELAY}}$ of 33 ms (Figure 79b, appendix E). Future theoretical work is certainly warranted to study this complex behavior of QUASR-SABRE effect, which is outside the scope of this pioneering phenomenological report.

The second key feature of QUASR-SABRE effect allows for continuous “RF-pumping” of $^{15}$N z-magnetization: Figure 42e, Figure 43d, and Figure 44e for the
three studied compounds respectively. This fitting of the exponential dependence of the build-up processed yielded $T_b$ of $0.5\pm0.05$ s for acetonitrile-$^{15}$N, $T_b$ of $1.26\pm0.02$ s for pyridine-$^{15}$N, and $T_b$ of $2.17\pm0.09$ s for metronidazole-$^{15}$N$_2$-$^{13}$C$_2$.

The $T_b$ values correlate well with $\eta$ values with $R^2$ of 0.93, Figure 45, suggesting that the build-up rate may be affecting the efficiency of QUASR-SABRE polarization process. A likely explanation of this observation in the contribution of the polarization destruction processes to $T_b$: when the destruction due to RF pulses is significant, $T_b$ is reduced resulting in lower $^{15}$N signals in a manner analogous to that of batch-mode Spin Exchange Optical Pumping (SEOP). Further experimental and theoretical studies are certainly warranted in the future to maximize the efficiency of QUASR-SABRE approach described here.

All experiments were performed at room temperature (ca. 298 K). We note that the rf pumping of QUASR-SABRE process needs to be effectively matched to the chemical exchange dynamics in order to maximize the polarization transfer efficiency to yield the highest $P_{^{15}N}$ value. Therefore, it is expected that the optimal values of $t_{\text{PULSE}}$ and $t_{\text{DELAY}}$ (Figure 78, Figure 79, and Figure 80, appendix E) would exhibit temperature dependence, because temperature modulates the rates of substrate and p-H$_2$ exchange. Moreover, it is entirely possible that optimum temperature (i.e. yielding the highest value of $P_{^{15}N}$) may be significantly different from room temperature and may be different from optimal temperature of the SABRE-SHEATH process (note that the optimal SABRE-SHEATH temperature for pyridine-$^{15}$N and for metronidazole-$^{15}$N$_2$-$^{13}$C$_2$ corresponds to approximately room
temperature, i.e. SABRE-SHEATH experiments were optimized with respect to temperature). As a result, some additional improvement in the maximum value of $P_{15N}$ may be potentially expected for QUASR-SABRE process.

![Graph](image)

**Figure 45.** Correlation plot of $T_b$ and $\eta$ for the three studied compounds.

We have also investigated the dependence of the QUASR-SABRE signal on the amplitude of SLIC power amplitude. Figure 80c, appendix E exhibits a plateau (with a range of approximately 6 decibels) with relatively steep slopes on both sides. This trend is expected, because LAC conditions are usually created in a relatively narrow power range.\textsuperscript{94,138}

Optimization of $P_{15N}$ was not the goal of this report. Moreover, due to lack of direct $^{15}$N signal reference (due to insufficient thermal equilibrium signal), we can only report the low limit of $e_{15N}$ and $P_{15N}$ values. Metronidazole-$^{15}$N$_2$-$^{13}$C$_2$ QUASR-SABRE estimates were $e_{15N} \sim 9 \times 10^5$ and $P_{15N} \sim 1.5\%$ (these values are doubled if only N$_3$ site is hyperpolarized). Metronidazole-$^{15}$N$_2$-$^{13}$C$_2$ SABRE-SHEATH estimates were $e_{15N} \sim 3.7 \times 10^5$ and $P_{15N} \sim 0.6\%$. Note these lower-limit estimates are
in good agreement with $P_{15N} \approx 1.5\%$ reported for SABRE-SHEATH under similar conditions using detection provided by high-resolution 9.4 T NMR spectrometer.\textsuperscript{296, 297} Pyridine-$^{15}$N lower-limit estimates were $\varepsilon_{15N} \sim 6.6 \times 10^5$ and $P_{15N} \sim 1.1\%$ for both QUASR-SABRE and SABRE-SHEATH—in line with previous SABRE-SHEATH studies.\textsuperscript{99, 290} Acetonitrile-$^{15}$N lower-limit estimates were $\varepsilon_{15N} \sim 5.3 \times 10^4$ and $P_{15N} \sim 0.09\%$ for QUASR-SABRE and $\varepsilon_{15N} \sim 1.2 \times 10^5$ and $P_{15N} \sim 0.2\%$ for SABRE-SHEATH respectively. See appendix E for details.

With regards to the limitations of the QUASR-SABRE method, it remains to be seen if QUASR-SABRE is capable of hyperpolarization of long-range spin sites in the substrate compounds. Moreover, future systematic experimental and theoretical studies are certainly needed to further optimize the efficiency of QUASR-SABRE technique. For example, more advanced shaped forms (e.g. sine, exponential, trapezoid, etc.) and strategies (adiabatic pulses) can be envisioned.

7.5 Conclusions

In summary, radio-frequency based polarization transfer approach has been presented for polarization of $^{15}$N sites. At least in some compounds, this method appears to be more efficient than the SABRE-SHEATH approach, which has already been shown to yield more than 30% $^{15}$N polarization.\textsuperscript{291} This is remarkable because in all previous demonstrations RF-SABRE approaches yielded significantly less polarization than static field matching / field cycling approaches. We hope that QUASR-SABRE may ultimately yield $^{15}$N polarization of the order of unity for a wide range of biomolecules. The employed pulse-sequence is a shaped...
variant of SLIC pulse sequence,\textsuperscript{95} which has the benefit of using low power levels. The applicability of this technique has been explored for three different types of compounds (six- and five-membered N-heterocycles and acetonitrile) including the antibiotic metronidazole. Metronidazole is an antibiotic that can be administered in large doses,\textsuperscript{301} and contains the nitroimidazole moiety, which has been exploited in a wide range of molecular contrast agents for hypoxia sensing using position emission tomography (PET).\textsuperscript{302-307} Therefore, metronidazole is a promising candidate as molecular probe for hypoxia imaging using HP MRI.\textsuperscript{297}

7.6 Acknowledgements

This work was supported by NSF under Grants CHE-1058727, CHE-1363008, CHE-1416268, and CHE-1836308. Research reported in this publication was also supported by the National Institute of Biomedical Imaging and Bioengineering of the NIH under R21EB025313 and 1R21EB020323, by National Cancer Institute under 1R21CA220137, and by DOD CDMRP under BRP W81XWH-12-1-0159/BC112431 and under W81XWH-15-1-0271.

Associated content can be found in appendix E.
CHAPTER 8 Quasi-Resonance Fluorine-19 Signal Amplification by Reversible Exchange


In this chapter, “we” and other first-person plural pronouns are used in reference to all authors of this publication. My individual contributions to the research include data analysis and writing parts of the manuscript. Figures and tables containing data contributed by researchers other than me will contain a disclaimer in the caption.

19F containing molecules offer the advantages as potential HP contrast agents due to high natural abundance of F-18 isotopope, high detection sensitivity (high γ value) of F-18 isotope, and background free detection inside the human body compared to proton spin detection. Moreover, 19F is utilized in ~20% of FDA-approved drugs used today. We report on the study of 19F hyperpolarization of fluorinated (19F) pyridine substituents using a novel SABRE hyperpolarization approach, where polarization can be effectively transferred from parahydrogen-derived hydrides directly to spin-spin coupled fluorine nucleus.

8.1 Abstract

We report on an extension of the QUASi-Resonance (QUASR) pulse sequence used for Signal Amplification by Reversible Exchange (SABRE), showing that we may target distantly J-coupled 19F-spins. Polarization transfer from the p-H2-derived hydrides to the 19F nucleus is accomplished via weak five-bond J-couplings
using a shaped QUASi-Resonance (QUASR) radio-frequency pulse at a 0.05 T magnetic field. The net result is the direct generation of hyperpolarized $^{19}$F z-magnetization, derived from the p-H$_2$ singlet order. An accumulation of $^{19}$F polarization on the free ligand is achieved with subsequent repetition of this pulse sequence. The hyperpolarized $^{19}$F signal exhibits clear dependence on the pulse length, irradiation frequency, and delay time in a manner similar to that reported for $^{15}$N QUASR-SABRE. Moreover, the hyperpolarized $^{19}$F signals of 3-$^{19}$F-$^{15}$N-pyridine and 3-$^{19}$F-$^{13}$N-pyridine isotopologues are similar, suggesting (i) that polarization transfer via QUASR-SABRE is irrespective of the nitrogen isotopologue, and (ii) the presence or absence of the spin-1/2 $^{15}$N nucleus has no impact on the efficiency of QUASR-SABRE polarization transfer. Although optimization of polarization transfer efficiency to $^{19}$F ($P_{^{19}F}$$\approx$0.1%) was not the goal of this study, we show that high-field SABRE can be efficient and broadly applicable for direct hyperpolarization of $^{19}$F spins.

8.2 Introduction

Hyperpolarization techniques increase nuclear spin polarization ($P$) by several orders of magnitude, enabling the corresponding gains in signals over that attainable via conventional NMR spectroscopy and MRI imaging.\textsuperscript{8-10, 183} This revolutionary boost in detection sensitivity enables new applications such as detection of dilute biologically relevant molecules both \textit{in vitro}\textsuperscript{18, 308, 309} and \textit{in vivo}.\textsuperscript{23, 26, 81, 152, 260} A particularly exciting hyperpolarization technique is Signal Amplification by Reversible Exchange (SABRE), pioneered by Duckett and co-
workers. SABRE relies on simultaneous chemical exchange of p-H$_2$ and substrate targeted for hyperpolarization (S) with an iridium polarization transfer catalyst (PTC), Figure 46a, during which the singlet order from the p-H$_2$-derived hydrides is allowed to transfer to the nuclear spins of the target substrates via the network of $J$-couplings. SABRE has gained the popularity because it is fast, efficient, and inexpensive relative to competing techniques. Polarization transfer is frequently accomplished in less than a minute with $P$ sometimes exceeding 30% at a cost several orders of magnitude smaller than that of dissolution Dynamic Nuclear Polarization (d-DNP), an alternative method of hyperpolarization.

There are two major approaches for polarization transfer in SABRE. The first approach is conventional SABRE, which relies on coherently-driven dynamics generated at a level anti-crossing (LACs). This is achieved by matching the static magnetic field to the resonant frequency allowing flow of spin order through $J$-couplings of nuclear spins involved in the polarization transfer. For the homonuclear case, as in polarization transfer from p-H$_2$-derived hydrides to the protons on the target substrate, the LAC condition is met at a magnetic field of a few milli-Tesla. For heteronuclear case, as in polarization transfer from p-H$_2$-derived hydrides to the substrate’s $^{13}$C, $^{15}$N, $^{31}$P, etc., the LAC condition is met at a magnetic field of a few micro-Tesla. The second approach relies on application of radio-frequency (RF) pulses, which are employed to create LAC conditions in an arbitrarily high magnetic field, e.g. the Low-Irradiation
Generation of High Tesla-SABRE (LIGHT-SABRE) pulse sequence. A number of other approaches has been demonstrated over the years such as RF-SABRE, Spin-Lock Induced Crossing-SABRE (SLIC-SABRE), Delayed Adiabatic Ramps Transfer Hyperpolarization-SABRE (DARTH-SABRE) and others. One key strength of the RF-based approach, which has remained largely unrealized, is the potential to perform SABRE at a wide range of magnetic fields. For example, one can use low-field, and potentially portable, electromagnets, high-resolution NMR spectrometers, or MRI scanners. The RF-based approaches have primarily focused on SABRE of $^{15}$N spins for two reasons. First, the dipolar relaxation of $^{15}$N magnetization is significantly longer and there is great potential for extremely long-lived ($T_s \sim 20$ minutes) $^{15}$N$_2$ singlet states. Second, isotopic enrichment of $^{15}$N heterocycles and other molecular carriers that efficiently bind iridium is relatively simple.

SABRE hyperpolarization of $^{19}$F spins is particularly attractive, because the $^{19}$F spin has the second highest detection sensitivity among stable nuclei with a gyromagnetic ratio ($\gamma_{^{19}F}$) of 0.93·$\gamma_{^{1H}}$, and approximately 9·$\gamma_{^{15}N}$. Moreover, unlike $^{15}$N and $^{13}$C, $^{19}$F is nearly 100% naturally abundant, obviating the need for isotopic enrichment needed for $^{15}$N- and $^{13}$C-labelled targets. While $^{19}$F is found in more than 20% of drugs, it has a negligible biological presence, making its spectroscopic and imaging detection relatively background free compared to that of proton spins. This allows the potential for broad applications for the theragnostic imaging and studies of drug interactions. To the best of our knowledge, there have
been five previous reports on SABRE hyperpolarization of $^{19}$F using spontaneous polarization transfer and no reported efforts using an RF-based SABRE approach. In this work, we employ a recently developed QUASI-Resonance SABRE (QUASR-SABRE) experiment, relying on SLIC pulses, for feasibility studies of RF-based hyperpolarization of $^{19}$F spins five bonds removed from the p-H$_2$-derived hydrides.

8.3 Materials and methods

For this study, we have used two compounds as SABRE substrates: 3-$^{19}$F-pyridine (196665, Sigma Aldrich) and 3-$^{19}$F-$^{15}$N-pyridine, recently synthesized by this lab, seen in Figure 46b and Figure 46d respectively. Figure 46c and Figure 46e show the network of J-couplings formed during the temporary association of p-H$_2$ and the corresponding target substrates with an activated pre-catalyst [Ir(IMes)(COD)Cl] (IMes = 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene, COD = 1,5-cyclooctadiene). A solution of 100 mM substrates (3-$^{19}$F-pyridine or 3-$^{19}$F-$^{15}$N-pyridine) and 5 mM IrIMes catalyst was prepared in methanol-d$_4$, and activated for at least 30 minutes with pH$_2$ flow of 20 standard cubic centimeters (sccm). Approximately 87% p-H$_2$, was directed into a medium-walled 5 mm NMR tube setup at a flow rate of ~70 sccm while the QUASR-SABRE pulse sequence was applied, Figure 46g.

We have employed three different $^{19}$F hyperpolarization approaches: (i) QUASR-SABRE, (ii) in-situ SABRE, the process of hyperpolarization by applying
a 90-degree hard pulse during p-H$_2$ bubbling at field (0.05 T), and (iii) conventional SABRE at 6 mT,$^{13,55}$ which should satisfy the $^{19}$F matching condition similarly to that of $^1$H. The QUASR-SABRE study of 3-$^{19}$F-pyridine and 3-$^{19}$F-$^{15}$N-pyridine was performed using a 0.05 T NMR spectrometer under similar experimental conditions$^{140}$ but with the spectrometer operating at the $^{19}$F Larmor frequency (1.94 MHz). The shaped pulse was applied with a maximum $\omega_1 = 1.24$ kHz and was ramped down to $\omega_1 = 0$ Hz for $\tau_{\text{PULSE}}$, followed by a subsequent inter-$\tau_{\text{DELAY}}$ (Figure 46f). Given significant non-linearities in the low power range of the RF amplifier, the actual applied pulse can be closely approximated with a truncated double-Gaussian pulse of the form:

$$s(t, \tau_{\text{PULSE}}) = \frac{1}{2} \left( a_1 e^{-\frac{t^2}{\tau_{\text{PULSE}}^2\sigma_1}} + a_2 e^{-\frac{t^2}{\tau_{\text{PULSE}}^2\sigma_2}} \right) \left( 1 - \tanh \left( \frac{t}{\tau_{\text{PULSE}} \delta} - a_3 \right) \right) \quad (19)$$

The values for each of the variables in equation 19 are given in appendix F. The process of pulsing and delay was repeated for N times before irradiating the sample with a hard 90-pulse similar to previous work,$^{140}$ and the free induction decay was detected (Figure 46g).
Figure 46. SABRE experiment of fluorinated pyridine. a) The schematic of simultaneous chemical exchange of parahydrogen (p-H₂) and target substrate (S) on an activated IrIMes PTC in SABRE hyperpolarization process. b) The schematic of SABRE chemical exchange with 3-¹⁹F-¹⁴N-pyridine as a substrate. c) the spin system formed on the IrIMes catalyst with 3-¹⁹F-¹⁴N-pyridine as a substrate. d) the schematic of SABRE chemical exchange with 3-¹⁹F-¹⁵N-pyridine as a substrate. e) the spin system formed on the IrIMes catalyst with 3-¹⁹F-¹⁵N-pyridine as a substrate. Note the difference between the spin systems shown in displays c) and e) due to presence of ¹⁴N (natural abundance) and ¹⁵N (isotopically enriched) nuclei. It is important to note that in both (c) and (e), that ⁵JFH ≠ ⁵JFH'. f) schematic of the experimental setup for ¹⁹F QUASR-SABRE experiment. g) the overall
schematic for quasi-resonance SABRE RF pulsing. Data for this figure was collected by Eduard Chekmenev.

Recently, a Quantum Monte Carlo (QMC) simulation approach to SABRE dynamics was introduced,\textsuperscript{38} which demonstrated the ability to predict coherent hyperpolarization dynamics for pulsed SABRE experiments with high accuracy. However, this method would have a prohibitive computational cost in the limit where there is a significant degree of coupling between each pulse, such as the case here, as the entire pulse-train must be simulated. So long as the time-step of the simulation is much smaller than the lifetime of the SABRE complex $\tau_{PTC}$, the coherent hyperpolarization dynamics are well approximated by

$$\partial_t \hat{\rho} = -i[\hat{H}, \hat{\rho}] - \frac{\hat{M} \hat{\rho}}{\tau_{PTC}}, \quad (20)$$

where $\hat{M}$ is the same operation to control exchange as used in the QMC simulation. The approximation made by equation 20 has the distinct advantage that it has a simple numerical solution that does not require iteration, making the simulation incredibly fast. This makes the explicit calculation of the effects of entire pulse sequences tractable, as opposed to approximating the dynamics as being identical and additive under each pulse, as done previously. In the simulations shown here, we have used a 10 $\mu$s step-size and have approximated the $\tau_{PTC} \approx 25$ ms. We note that this spin system is at the upper computational limit for Liouville space, however, is far from the 15-spin limit of the Hilbert-space methods utilized here.
Specifically, we have constructed this system to include the p-H$_2$ derived hydrides, the long-range $^{19}$F nuclei, and ortho-protons on pyridine, as it is assumed that all other couplings are either small or will not significantly affect the $^{19}$F hyperpolarization dynamics. This forms a six-spin AA’(BX)(B’X’) system with the Hamiltonian:

$$
\hat{H}(t, t_p) = \sum_i \Delta \omega_i \hat{I}_{iz} + \Delta \omega_F (S_{4z} + S_{6z}) + 2\pi \left[ I_{HH'} \hat{I}_1 \hat{I}_2 + I_{HH''}(\hat{I}_{1z} \hat{I}_{3z} + \hat{I}_{2z} \hat{I}_{5z}) + I_{HF}(\hat{I}_{1s} \hat{S}_{4z} + \hat{I}_{2s} \hat{S}_{6z}) + \omega_{1F}(t, t_p)(S_{4x} + S_{6x}) \right] \quad (21)
$$

The $J_{HH'}$ term is the coupling between the hydrides on the ortho-proton, $J_{HF}$ is the long range $^5J_{HF}$ coupling, $J_{HF'}$ is the ortho-proton to $^{19}$F $^3J$-coupling, and $\omega_{1F}(t, t_p)$ is the time-dependent pulse shape in the case of the QUASR-SABRE pulse sequence.

8.4 Results and discussion

The $^{19}$F QUASR-SABRE spectra of 3-$^{19}$F-pyridine and 3-$^{19}$F-$^{15}$N-pyridine are shown in Figure 47a and Figure 47b, respectively, exhibiting $\varepsilon_{19F} \sim 6,700$ ($P_F \sim 0.10\%$) and $\varepsilon_{19F} \sim 7,100$ ($P_F \sim 0.11\%$) respectively. Conventional SABRE yielded an $\varepsilon_F \sim 17,800$ ($P_F \sim 0.26\%$) for 3-$^{19}$F-$^{15}$N-pyridine, shown in Figure 47f. Spectra acquired with either QUASR-SABRE or conventional SABRE exhibit in-phase resonances in contrast to the in-situ SABRE spectra of the corresponding compounds (Figure 47c and Figure 47d), which exhibit anti-phase spin order. This anti-phase spin order arises as, in the absence of a radiofrequency pulse, there is
still a dipole-allowed transition between the $S_0^H T_0^F \rightarrow T_0^F S_0^H$ states, which rapidly decays into $I_{HF} T_2^F$ due to the ~120 kHz frequency difference between the $^1H$ and $^{19}F$ nuclei at this field. Conversely, the resonance conditions for QUASR-SABRE and conventional SABRE are determined to pump Zeeman states via the appropriate LAC. Note that the frequency shift observed in all the spectra shown in Figure 47 is a result of the magnetic field drift due to the temperature fluctuations of the $B_0$ magnet occurred during the spectral acquisition process.

The spectra shown in Figure 47a and Figure 47b suggest that the observed signals are the result of direct polarization transfer from the p-H$_2$-derived hydrides to $^{19}F$ nuclei driven by QUASR-SABRE, because the spectra have in-phase signatures versus the anti-phase signatures expected from in-situ hyperpolarization (Figure 47c and d). Moreover, we do not expect the spin-1/2 relayed mechanism through $^2J_{NH}$ to be the dominant pathway for hyperpolarization transfer, because there is little difference in the SABRE spectra of 3-$^{19}F$-pyridine and 3-$^{19}F$-$^{15}N$-pyridine.$^{101, 324}$

The enhancement values were computed as described previously$^{140}$ by taking the product of the ratio of the $^{19}F$ concentrations of the species of interest ($C_{REF}/C_{HP}$), i.e. $^{19}F$, present in the thermally polarized reference compound ($C_{REF}$, neat 3,5-$^{19}F$-pyridine; Figure 47e) and the HP substrate compound ($C_{HP}$), with the integrated signal intensity ratio of the HP substrate and the thermal signal reference compound ($S_{HP}/S_{REF}$) multiplied by a factor of 1.85 (which is the ratio of
the cross sectional areas of the thermal signal reference to the hyperpolarized arrangements as implemented by the experimental setup) as discussed in details elsewhere.\textsuperscript{100} Nuclear spin polarization $P$ was computed by multiplying $\varepsilon_{19F}$ by equilibrium $^{19}$F polarization at 0.05 T and 300 K (ca. 1.5*10^5\%).

\textbf{Figure 47. HP SABRE spectra of fluoropyridines.} $^{19}$F spectra of QUASR-SABRE hyperpolarized 3-$^{19}$F-pyridine a) and of QUASR-SABRE hyperpolarized 3-$^{19}$F-$^{15}$N-pyridine b). $^{19}$F \textit{in-situ} SABRE spectra of hyperpolarized 3-$^{19}$F-pyridine c) and 3-$^{19}$F-$^{15}$N-pyridine d): note although the signals are hyperpolarized, the NMR lines are anti-phase, which may not be suitable for some MRI sequences. $^{19}$F spectrum of thermally polarized signal reference neat 3,5-$^{19}$F-pyridine e). $^{19}$F spectrum of 3-$^{19}$F-$^{15}$N-pyridine hyperpolarized via conventional SABRE at ~6 mT
f). All NMR spectra were acquired using a single scan. Data of this figure were collected by Eduard Chekmenev.

3-^{19}F-pyridine presents an interesting chemical system, as the complex may take on four possible conformations upon association of two 3-^{19}F-pyridine ligands as seen in Figure 48. When the fluorine substituents are both positioned between the pyridine rings, the complex is highly unstable due to the proximity of two highly electronegative groups. The hydrides in this conformer are therefore short-lived and yield the broad peak labelled ‘C’ in Figure 48. When the fluorine substituents are both positioned in the periphery, the complex is stable and yields a longer lived hydride species producing the peak labelled ‘B’. The two conformers with one central and one peripheral fluorine substituent are degenerate and are not destabilized by proximity of the fluorine substituents. Because the meta-substituted pyridine is asymmetric, this final conformation induces a chemical inequivalence between the hydrides producing two anti-symmetric peaks labelled ‘A’ separated by 1.77 ppm. This chemical shift difference prevents LIGHT-SABRE⁹² hyperpolarization at high field. However, using a lower field (50 mT) as in QUASR-SABRE allows collapse of these disparate hydride resonance frequencies onto a single peak of accidental equivalence.
Figure 48. Different conformers of the PTC. a) Conformers of the activated PTC with associated ligands and hydrides inducing different chemical environments for the associated hydrides. b) $^1$H spectrum at 1T with 45° tip angle demonstrating 4 distinct hydride species coming from three inequivalent conformers with 2 pairs of equivalent hydrides (B & C) and one pair of inequivalent hydrides (A). Data for this figure was collected by Jacob R. Lindale and Warren S. Warren.

The QUASR-SABRE pulse sequence was applied in a regime where there is extreme coherent coupling between each consecutive QUASR pulse, which arises when the delay is much shorter than the lifetime of the species (typically ~40 ms). In such a regime, the dynamics generated by the pulse sequence are far from intuitive. Given that the ortho-protons of the pyridine are ~100 Hz offset in resonance from the p-$\text{H}_2$-derived hydrides, this spin system effectively reduces to
an AA'XX' spin system, for which the SABRE dynamics have been characterized extensively\textsuperscript{35}. During the first pulse, very little hyperpolarized signal is generated until the pulse power approaches the LAC condition, given by $\omega_{1F} = 2\pi^2 J_{HH}$. Given the short 2 ms inter-pulse delay, the beginning of each subsequent QUASR pulse induces large coherent oscillations at the, albeit time-dependent, nutation frequency. The relatively large signal produced with a (20 ms QUASR-pulse + 2 ms delay)$^{30}$ train correspond to when the coherent oscillations halt coincident with an effective $(2n+1)\pi$-pulse in the hyperpolarization dynamics, giving the largest deviation from equilibrium. Interestingly, the maximum observed in Figure 49c are only recovered when the non-linearities in the pulse shape are re-introduced into the simulation.

Figure 49. Coherent hyperpolarization dynamics during a triangular pulse a) and the non-linear pulse approximation used in these experiments b). The dynamics predicted during a single pulse c) vary greatly from those predicted from the explicit calculation of the entire pulse sequence d), which is shown in red overlaying the experimental (0.05 T) data in black (the black solid lines are added to guide the eye). Data for this figure was collected by Jacob R. Lindale and Warren S. Warren.
It is important to note that the QUASR-SABRE signals are by approximately 2.6-fold smaller than the signal obtained by conventional SABRE (Figure 47). This is well understood as the ramifications to various experimental details. Firstly, due to the fact that the rapid coherent oscillations induced by the QUASR-pulse will average under exchange towards the center of these oscillations, the hyperpolarization generated by each pulse is significantly damped. Also, the large nutation frequency allows for simultaneous irradiation of the free $^{19}$F-species, thus dramatically attenuating the signal to 52% of the generated hyperpolarization (see appendix F for details). Given that $^5J_{HF} = 0.34$ Hz, only small perturbations can be made to the system, leading to very slow oscillations, on the order of seconds, with very weak and long pulses, on the order of a few Hertz, as detailed by conventional SLIC theory.\textsuperscript{92, 326} Despite these aspects that complicate the experiment, hyperpolarization is generated directly through 5-chemical bonds, which is significantly further than nearly all other reported approaches for SABRE. Moreover, despite the experimental limitations of the complex polarization dynamics, our key finding of feasibility of QUASR-SABRE polarization to $^{19}$F nucleus (rather than other explanation for the observed effect) is also supported by (i) $^{19}$F signal dependency on RF frequency offset (which is expected for this polarization transfer approach,\textsuperscript{140} Figure 50a), and (ii) $^{19}$F HP signal increase with the increase of the number of pumping cycles ($N$) demonstrating a clear polarization build-up (Figure 50b, note the positive value at $n=0$ is due to residual
polarization buildup due to in-situ SABRE, Figure 47c) in a manner consistent with RF-based SABRE.$^{92,140}$

![Graph](image)

**Figure 50.** $^{19}$F QUASR-SABRE 3-$^{19}$F-$^{14}$N-pyridine. HP 3-$^{19}$F-$^{14}$N-pyridine signal dependence on the SLIC-pulse frequency offset a) and number of pumping cycles N demonstrating the build-up $^{19}$F polarization with the mono-exponential build-up (red curve) constant of 0.44±0.04 s b). All data acquired at 0.05 T magnetic field, and the black solid lines are added to guide the eye. Data for this figure was collected by Eduard Chekmenev.

### 8.5 Conclusions

We hope the pioneering results reported here can be improved, and higher levels of $^{19}$F polarization can be obtained through future work across a wide range of compounds such as $^{19}$F-containing drugs with suitable N-heterocyclic motifs (e.g. 5-fluorouracil, celecoxib, sitagliptin, fluconazole, etc.). Spectroscopy of $^{19}$F-hyperpolarized drugs is attractive because the lack of background signal and greater chemical shift dispersion of $^{19}$F compared to that of $^1$H allows for specific characterization with little to no background interference. Moreover, RF-based hyperpolarization approaches offer many practical advantages compared to low field methods as the sample does not need to be shuttled between the matching
field and the detector field, therefore enabling signal averaging and multi-dimensional HP NMR spectroscopy.\textsuperscript{327}

8.6 Acknowledgements

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Associated content can be found in appendix F
CHAPTER 9 Future Directions

We envision to use HP propane gas and HP diethyl ether gas as inhalable contrast agents in the future. Molecules with nitroimidazole moieties will be investigated further for their potential uses as intravenous contrast agents.

HP propane shows promising results for large scale catalyst free production with long lifetimes (∼ 9 s) when prepared in the low field (∼ 50 mT) in the clinically relevant pressure regime via PHIP reaction with propylene gas. Furthermore, even longer lifetimes of HP propane are expected using cyclopropane as a precursor molecule for PHIP reactions. One of our primary goals is to use HP propane on the 0.35 T MRI scanner as an illustration for the capability of high-resolution MR image acquisition. We also foresee the study of large animal models to examine mammalian lungs towards the detection of pulmonary diseases such as COPD using these inhalable contrast agents. Preliminary studies will be conducted using large phantoms (∼ 1.5 L, similar to human lung volume) filled with HP propane gas for optimization studies followed by MR image acquisition of HP propane of human sized plexi-glass cylindrical lungs using a ‘hyperfine portable’ MRI scanner under clinically relevant temperatures and pressure values. The study will then be extended to sheep models. Anesthetized sheep will be given a dose of HP propane and MR images of healthy sheep lungs will be acquired. The data will be then compared with MR images obtained from sheep models with undergoing treatments for pulmonary diseases thus enabling to monitor the effect and the progression of the treatment. After successful demonstration of HP propane as a
contrast agent for sheep models, the long-term goal is to eventually extend the applications into human clinical translations. HP propane gas is expected to be prepared using small portable reactor tubes (a device similar to a small tube with a mouthpiece for gas inhalation) where the HP contrast agent is produced at room temperature and the subject will be able to perform the imaging experiments at a single breath-hold. As illustrated in chapter 3, creation of higher polarization values of HP propane will be investigated via the use of buffering gases and HP propane is desired to be cryogenically separated from the buffering gas mixture which allows enhancements of the polarization values as well as the lifetimes thus benefiting the preparation and storage of HP gaseous contrast agent prior to subject administration.

Similar to the HP propane approach, HP diethyl ether will also be used for imaging studies as an inhalable contrast agent. Smooth clinical translations are expected for this HP molecule due to the current usage of diethyl ether as a medical anesthetic in some parts of Europe and in Russia (where some of our collaborators will be able to test HP diethyl ether for pulmonary image acquisition).

We show that PHIP- RASER activity is a common phenomenon. Precise chemical information of the HP molecules can also be extracted from RASER spectra. Our goal is to employ RASER for MR imaging acquisition. We intend to employ custom designed Q coils on clinical MRI scanners to induce RASER to obtain high resolution MR images.
Based on the QUASR-SABRE studies reported in chapters 7 and 8, a series of molecules with nitroimidazole moieties (nimorazole and others) will be explored towards their potential use as IV contrast agents for hypoxia sensing.
APPENDIX A

Supporting Information for Chapter 3, Relaxation Dynamics of Nuclear Long-Lived Spin States in Propane and Propane-d₆ Hyperpolarized by Parahydrogen.


In this chapter, “we” and other first-person plural pronouns are used in reference to all authors of this publication. My individual contributions to the research include conduction of all the experiments, analyzing data and writing parts of the manuscript. Figures and tables containing data contributed by researchers other than me will contain a disclaimer in the caption; figures and tables with no disclaimer in the caption contain data collected by me. I also would like to acknowledge all of the co-authors listed in here who provided with chemicals and materials.

1. Experimental setup for NMR spectroscopy at 1.4 T

![Experimental setup for NMR spectroscopy at 1.4 T](image)

**Figure 51.** Experimental setup for NMR spectroscopy at 1.4 T. This experimental setup is composed of three parts: custom-built parahydrogen generator, propane polarizer stages similar to those in Figure 19, and a bench-top 1.4 T Nanalysis (NMR Pro 60) NMR spectrometer.
2. Additional Figures and Tables

**Figure 52. HP propane condensation.** a) An example of a $^1$H spectrum of condensed HP propane obtained during the condensation studies performed using the 0.0475 T spectrometer setup, with some changes to gas collection as described in the experimental section. Note that since the spectrum is obtained using SLIC excitation, the polarization levels cannot be determined precisely, because the efficiency of SLIC conversion of pseudo-singlet overpopulation into observable magnetization is well below 100%. b), c), and d) are signal decay curves of the liquefied HP propane with time using RF SLIC pulses (duration of each pulse is 200 ms) applied every 5 s (b), 10 s (c) and 15 s (d) --note the effective $T_{\text{LLS}}$ values obtained vary when different durations of SLIC pulse is employed because of the polarization loss induced by the RF pulse excitation, which was not taken into account. Note the effective values of $T_{\text{LLS}}$ correspond to the lower limit value of the actual $T_{\text{LLS}}$ (see chapter 3 for details).
Figure 53. The thermally equilibrated proton NMR spectrum of propylene recorded using the 1.4 T NMR spectrometer.

Figure 54. The exponential decay constants (effective T₁) for HP propane-d₆, which were obtained using the sequence described in Figure 20c.
Table 2. Effective $T_{LLS}$ values and corresponding error bars for the partial-SLIC excitation experiment described in Figure 26a in chapter 3. The data were acquired using a 1:1 mixture of propylene and p-H$_2$ at 38 psi (or 3.6 atm) backpressure using otherwise-identical experimental conditions as those used to obtain the data in Figure 21b. The data set with “0” SLIC pulse duration corresponds to the 3.6 atm data point from Figure 21b of chapter 3. The effect of SLIC excitation on the remaining pseudo-singlet spin order pool is not taken into account in our simulations.

<table>
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<th>SLIC pulse duration in Figure 26a (milliseconds)</th>
<th>SLIC pulse application repetition time (TR) (milliseconds)</th>
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<th>Effective $T_{LLS}$ error (seconds)</th>
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<td>2030</td>
<td>5.0</td>
<td>0.1</td>
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* note this “0” value corresponds to a different measurement, when the sample chamber was refilled before each data point of the decay curved was taken using the pulse sequence shown in Figure 22b.
Figure 55. Homogeneous deuteration of propylene. Propylene gas and D₂ gas were used to prepare a gas mixture (1.2 times excess D₂) in the mixing chamber first. The mixture was then sent to the NMR tube filled with 1-2 mL of 5 mmol of (bicyclo[2.2.1]hepta-2,5-diene)[1,4-bis(diphenylphosphino)butane]rhodium(I) tetrafluoroborate catalyst in CD₃OD at a 60 sccm flow rate. The sample tube was left to react for approximately 3-4 minutes prior to the detection using Bruker NMR spectrometer at 700 MHz. Some of the samples were left for (1-8-24) hours after mixing and ¹H NMR spectra were recorded for comparison.

Some experiments were conducted with slight modifications to the scheme shown in Figure 55. Propylene gas was first bubbled bypassing the storage gas cylinder, into the deuterated methanol solution in the NMR tube with 5 mmol of (bicyclo[2.2.1]hepta-2,5-diene)[1,4-bis(diphenylphosphino)butane]rhodium(I) tetrafluoroborate catalyst in CD₃OD for about 2-3 minutes at 40 Psi backing
pressure for propylene only experiments and for $D_2$ only experiments. The gas was then allowed to mix thoroughly by shaking the tube. $^1$H NMR spectra of each sample was collected at 700 Hz using Bruker NMR spectrometer in order to investigate any presence of hydrogen gas or traces of propane as a contaminant in the reactant precursor molecules.

Figure 56. Proposed reaction mechanisms for cyclopropane hydrogenation. a) possible different products of heterogeneous catalytic hydrogenation reaction of cyclopropane via three reaction routes b) proposed mechanism of cyclopropane hydrogenation in the presence of Rh/ TiO$_2$ catalyst surface. A, B, C and D are used to distinguish different protons and two different colors (red and blue) are used to guide different steps in the catalytic process resulting in different isotopologues. Adapted with permission from Ref. # 74. Copyright (2019) American Chemical Society.
Figure 57. $^1$H NMR spectrum of the products with 20% excess deuterium. The integrated intensities (arbitrary) are also given in the figure.
Ring opening reaction of cyclopropane with D$_2$ in the presence of excess cyclopropane

Figure 58. $^1$H NMR spectrum of the products with excess cyclopropane. The integrated intensities (arbitrary) are also given in the figure.

Figure 59. Heterogeneous catalysis of propylene and D$_2$. $^1$H NMR spectrum of the products obtained from the heterogeneous reaction of propylene with D$_2$ is shown here. The integrated intensities (arbitrary) are also given in the figure.
Figure 60. $^1$H NMR spectrum of propylene precursor gas sample. The gas sample was directly bubbled into the NMR tube with CD$_3$OD solvent and its $^1$H NMR spectrum was recorded in order to check for any possible contaminants. We observe spectral signatures of propylene (assignments are given in red) but do not detect any traces on propane.

Figure 61. $^1$H NMR spectrum of D$_2$ precursor gas sample. The gas sample was directly bubbled into the NMR tube with CD$_3$OD solvent and its $^1$H NMR spectrum was recorded in order to check for any possible contaminants of H$_2$. We observe only the spectral signatures of methanol d$_4$. 
2. **Spectral simulations for the possible products**

The spectral fittings for the possible products were performed using Bruker topspin 3.0 Daisy simulation package. Few spin systems were created for some of propane isotopologues. Interestingly we observed spectral pattern for propane. Therefore, for all the simulations, two different spin systems were used i.e. propane spin system and CH$_3$-CHD-CH$_2$D spin system, and their detailed spectral simulation data are given below (Figure 62). Each of these spin systems were used to create individual simulation (Figure 31c) and one combined simulation (Figure 31b) using both of the spin system fragments. Figure 31 in the main text presents overlay $^1$H NMR spectra obtained by the spectral simulations with those observed experimentally for the propylene hydrogenation reaction.
Figure 62. a) The spin system of CH$_3$-CHD-CH$_2$D with simulated magnitude spin-spin coupling constant values and the values of $^1$H chemical shifts. b) The spin system of CH$_3$-CH$_2$-CH$_3$ with the values of chemical shift and the values of the magnitudes of spin-spin coupling constants used for spectral simulation.
Figure 63. Screenshot of the simulation data for CH$_3$-CHD-CH$_2$D spin system shown in Figure 62.

Figure 64. Screenshot of the simulation data for CH$_3$-CH$_2$-CH$_3$ spin system shown in Figure 62.

3. Equations and Calculations

Calculation of branching ratios ($\gamma$) of the products produced via routes #1, 2, 3 in Figure 33.
The branching ratios of three reactions routes (see Figure 33 for details) for cyclopropane deuteration reaction were calculated by taking the integrated intensity of the species of interest with respect to the product obtained from the propylene deuteration reaction using the same heterogeneous reactor, as indicated by the following equations.

Calculation of branching ratio $\chi$ using methylene protons' intensities,

$$\chi(\text{route #1}) = \frac{\alpha \times I_{\text{CH}_2}^\text{CP}}{I_{\text{CH}_2}^\text{PE}} \times 100\%$$ (22)

$$\chi(\text{routes #2 + #3}) = 100\% - \text{branching ratio}(\text{route #1})$$ (23)

Similarly, branching was calculated using methyl protons’ intensities via the following equation.

$$\chi(\text{route #1}) = \frac{\alpha \times I_{\text{CH}_3}^\text{CP}}{I_{\text{CH}_3}^\text{PE}} \times 100\%$$ (24)

$$\chi(\text{routes #2 + #3}) = 100\% - \text{branching ratio}(\text{route #1})$$ (25)

Where $I_{\text{CH}_2}^\text{CP}$ is the integrated intensity of the methylene peak in cyclopropane, $I_{\text{CH}_2}^\text{PE}$ is the integrated intensity of the methylene peak in propylene, $I_{\text{CH}_3}^\text{CP}$ is the integrated intensity of the methyl peak in cyclopropane and $I_{\text{CH}_3}^\text{PE}$ is the integrated intensity of the methyl peak in propane. The experimental normalization factor $\alpha$ was obtained by matching the spectral intensity of two characteristic signatures in the $^1\text{H}$ NMR
methylene spectral region for cyclopropane spectrum and corresponding propylene spectrum used for the calculations (Figure 65 and Figure 66).

The following $\chi$ values were observed for two experiments.

**Experiment with cyclopropane excess**

The contribution towards route #1 for CH$_3$-CHD-CH$_2$D (Figure 33) when cyclopropane was in excess:

a. $\chi$ was computed using equations (22) and (23) in methylene spectral region as shown below.

An example calculation is presented as $I_{CH_2}^{CP} = 0.7547$ (Figure 58) and $I_{CH_2}^{PE} = 0.2622$ (from Figure 59), $\alpha = 0.46$ (Figure 65); hence the branching ratio $\chi$ (route #1) becomes, $(0.2622*0.46) / 0.7547 = 16\%$, and $\chi$ (route #2+3) = 84%.

b. $\chi$ was computed using eqs (24) and (25) in methylene spectral region as shown below.

An example calculation is presented as $I_{CH_3}^{CP} = 1.9736$ (Figure 58) and $I_{CH_3}^{PE} = 0.8255$ (from Figure 59); hence $\chi$ becomes, $(0.8255*0.46) / 1.9736 = 19\%$, and $\chi$ (route #2+#3) = 81%.

Note the key takeaway is both approaches (via methylene and methyl peak intensities) for computing $\chi$ yield approximately the same result. In the main body
of the manuscript, the $\chi$ values obtained using methylene spectral intensities are reported.

**Experiment with $D_2$ excess**

The contribution towards route #1 for $\text{CH}_3$-$\text{CHD}$-$\text{CH}_2$D (Figure 33) when $D_2$ was in excess:

c. $\chi$ was computed using eqs (1) and (2) in methylene spectral region as shown below.

An example calculation is indicated below.

$$I^\text{PE}_{\text{CH}_2} = 0.2622 \text{ (Figure 59)}; I^\text{CP}_{\text{CH}_2} = 0.8871 \text{ (Figure 57)} \text{ and } \alpha = 0.69 \text{ (Figure 66)}:$$

therefore, the branching ratio $\chi$ (route #1) becomes $0.2622 \times 0.69 / 0.8871 = 21\%$ and $\chi$ (route #2 +#3) becomes $(100 - 21) = 79\%$.

d. $\chi$ was computed using eqs (3) and (4) in methyl spectral region as shown below.

An example calculation is indicated below.

$$I^\text{PE}_{\text{CH}_3} = 0.8255 \text{ (Figure 59)}; I^\text{CP}_{\text{CH}_3} = 2.5931 \text{ (Figure 57)} \text{ and } \alpha = 0.69 \text{ (Figure 66)}:$$

therefore, the branching ratio $\chi$ (route #1) becomes $0.8255 \times 0.69 / 2.5931 = 22\%$ and $\chi$ (route #2 +#3) becomes $(100 - 22) = 78\%$. 
Figure 65. Normalization of propane $^1$H NMR spectrum using CH$_2$ region. a) $^1$H NMR spectrum of the methylene region of propane product of cyclopropane deuteration in the presence of excess cyclopropane (red) and propylene deuteration (blue) before normalization correction is applied. Please note the normalization factor, $\alpha$, which is schematically shown in the figure. b) The corresponding $^1$H NMR spectra of the methylene region after applied intensity normalization correction $\alpha=0.46$.

Figure 66. Normalization of propane $^1$H NMR spectrum using CH$_2$ region. a) $^1$H NMR spectrum of the methylene region of propane product of cyclopropane deuteration in the presence of excess D$_2$ (red) and propylene deuteration (blue) before normalization correction is applied. Please note the normalization factor, $\alpha$, which is schematically shown in the figure. b) The corresponding $^1$H NMR spectra of the methylene region after applied intensity normalization correction $\alpha=0.69$. Note the employed the propylene deuteration (blue) is the same for Figure 65 and Figure 66.
Calculation of chemical conversion in reaction of $D_2$ addition to cyclopropane

Chemical conversion = \[ \frac{I_{PA}^{CH_2} + I_{PA}^{CH_3}}{I_{CP} + I_{PA}^{CH_2} + I_{PA}^{CH_3}} \times 100\% \] (26)

$I_{PA}^{CH_2}$ and $I_{PA}^{CH_3}$ are the integrated intensities of methylene protons and methyl protons in propylene respectively, and $I_{CP}$ is the integrated intensity of cyclopropane. Example calculations for conversion are shown below.

1. Conversion for the hydrogenation reaction in the presence of $D_2$ in excess
   
   \[ = 100\% \times \frac{0.924 + 2.59}{25.2736 + 0.924 + 2.59} = 12\% \] (Figure 57)

2. Conversion for the hydrogenation reaction in the presence of cyclopropane in excess
   
   \[ = 100\% \times \frac{0.7547 + 1.9736}{213.4691 + 0.7547 + 1.9736} = 1.3\% \] (Figure 58)
APPENDIX C

Supporting Information for Chapter 5, Parahydrogen-Induced Radio Amplification by Stimulated Emission of Radiation.


In this chapter, “we” and other first-person plural pronouns are used in reference to all authors of this publication. My individual contributions to the research include conduction of ~ 50% experiments, ~40-50 % of data analysis and writing parts of the manuscript. Figures and tables containing data contributed by researchers other than me will contain a disclaimer in the caption; figures and tables with no disclaimer in the caption contain data are collected by me.
Figure 67. $^1$H NMR spectroscopy of thermally polarized samples of HEP using 1.4 T bench-top NMR spectrometer. All spectra were collected using 90° excitation RF pulses, a single average and otherwise identical parameters. Spectral assignments of the different proton environments are labeled in red. a) Reaction scheme of parahydrogen pairwise addition to HEA resulting in production of HP HEP. b) Fourier spectrum of the thermally polarized signal reference sample of 10 M (neat) ethyl acetate-1-$^{13}$C employed for determination of concentration and chemical conversion. c,d) Fourier spectra of thermally polarized solutions recorded before and after the reaction of HEA with parahydrogen, respectively, indicating complete conversion to HEP.
Figure 68. $^1$H NMR spectroscopy of thermally polarized samples of EA using 1.4 T bench-top NMR spectrometer. All spectra were collected using 90° excitation RF pulses, a single average and otherwise identical parameters. Spectral assignments of the different proton environments are labeled in red. a) Reaction scheme of parahydrogen pairwise addition to VA resulting in production of HP EA. b) Fourier spectrum of the thermally polarized reference sample of 10 M (neat) ethyl acetate-$^{13}$C employed for determination of concentration and chemical conversion. c,d) Fourier spectra of thermally polarized solutions recorded before
and after the reaction of VA with parahydrogen, respectively, indicating complete conversion to EA.

![Figure 69. T1 relaxation values of hyperpolarized H_A and H_B protons in HP 2-hydroxyethyl propionate (HEP) (display a) and HP ethyl acetate (EA) (display b) after homogeneous pairwise p-H_2 addition in CD_3OD measured at 1.4 T.](image)

Figure 69. T_1 relaxation values of hyperpolarized H_A and H_B protons in HP 2-hydroxyethyl propionate (HEP) (display a) and HP ethyl acetate (EA) (display b) after homogeneous pairwise p-H_2 addition in CD_3OD measured at 1.4 T.

![Figure 70. 1H NMR spectroscopy of solution-phase PHIP of 40 mM HP EA (ethyl acetate) probed at 1.4 T. a) ALTADENA RASER active signal. b) Fourier spectrum.](image)

Figure 70. 1H NMR spectroscopy of solution-phase PHIP of 40 mM HP EA (ethyl acetate) probed at 1.4 T. a) ALTADENA RASER active signal. b) Fourier spectrum.
Figure 71. $^1$H NMR spectroscopy of solution-phase PHIP of 0.4 M HP EA (ethyl acetate) probed at 1.4 T. The catheter used for bubbling $p$-H$_2$ was left inside the NMR tube resulting in shorter $T_2^*$ during signal acquisition. a) ALTADENA RASER active signal. b) Fourier spectrum of the region outline by the blue box in display a). c) PASADENA RASER active signal. d) Fourier spectrum of the region outline by the blue box in display c).
Figure 72. $^1$H NMR spectroscopy of solution-phase PHIP of 0.4 M HP HEP (2-hydroxyethyl propionate) probed at 1.4 T. The catheter used for bubbling $p$-$H_2$ was left inside the NMR tube resulting in shorter $T_2^*$ during signal acquisition. a) ALTADENA RASER active signal. b) Fourier spectrum of the region outline by the blue box in display a). c) PASADENA RASER active signal. d) Fourier spectrum of the region outline by the blue box in display c).
Supporting Information for Chapter 6, Parahydrogen-Induced Polarization of Diethyl Ether Anesthetic.


In this chapter, "we" and other first-person plural pronouns are used in reference to all authors of this publication. My individual contributions to the research include conduction of ~50% experiments, 50-55% data analysis and writing parts of the manuscript. Figures and tables containing data contributed by researchers other than me will contain a disclaimer in the caption; figures contain data collected by me.

Figure 73. NMR spectra of thermally polarized samples. a) Thermal $^1$H NMR spectrum of EVE in the liquid phase. b) Thermal $^1$H NMR spectrum after hydrogenation. c) Thermal $^1$H NMR spectrum of the signal reference ethyl acetate-$^1$-$^{13}$C recorded using the same acquisition protocol. All spectra were recorded with 90° pulse single scan. Note the NMR spectra were not calibrated: e.g. CD$_3$OH resonance should occur at ~4.78 ppm.
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Figure 74. Heterogeneous hydrogenation and low field experimental setup. The PHIP polarizer incorporates a catalytic reactor of Rh/TiO$_2$ and an NMR spectrometer (Kea2, Magritek). The p-H$_2$ flow is regulated by a mass flow controller and passed through a high-pressure glass column (prepared from modified HPLC column) filled with neat EVE substrate. The saturated gas mixture is sent through the catalytic reactor for hydrogenation at 170 °C. The outgoing gas mixture ([EVE] = 22 mM, [p-H$_2$] = 110 mM) is collected in a 17 mL phantom sphere and $^1$H NMR spectroscopy performed at 2.0 MHz (47.5 mT) with Spin-Lock Induced Crossing (SLIC) radio frequency pulse sequences.
Figure 75. SLIC pulse sequences for low field detection. a) Scheme of the transformation of the long-lived singlet state of diethyl ether into triplet state with observable magnetization by a SLIC pulse sequence. b) Pulse sequence for optimizing SLIC transformation by varying RF amplitude, power, and frequency, c) Pulse sequence used for the measurement of HP DE $T_{LLS}$. 
Figure 76. SLIC detection of hyperpolarized diethyl ether. (a) Power optimization curve with liquid-phase values corrected for the relaxation losses assuming $T_{\text{LLS}} = 9.7$ s (in red). (b) Frequency optimization curve. (c) Pulse duration variation. (d) Relaxation decays of DE. SLIC data obtained with 200 ms pulse duration. The mono-exponential decay fits lead to $T_{\text{LLS}} = 9.7 \pm 0.8$ s and $T_{\text{LLS}} = 2.8 \pm 0.4$ s for HP DE in the liquid and gas phase, respectively (dashed lines). Note the effect of SLIC pulses is not taken into account for calculation of $T_{\text{LLS}}$. Given that $T_{\text{LLS}} = 14.0 \pm 0.9$ s in CD$_3$OD under ALTADENA condition (black, continuous line), i.e., ~1.4 time longer than the partial SLIC value, the LLS lifetime in the gas phase is assumed to be equally underestimated by the SLIC approach, leading to $T_{\text{LLS}} = 4.0 \pm 0.7$ s for HP DE in the gas phase (purple, continuous line). The spin-lattice relaxation at high field ($T_1$) is shown in violet for comparison.
Description of parahydrogen-induced RASER experiment

p-H$_2$ was bubbled for 10 s into a solution of ~200 mM EVE substrate and 4 mM Rh catalyst in CD$_3$OD at Earth’s magnetic field, corresponding to the maximum of polarization (8%) measured under ALTADENA condition. The pulse-acquisition sequence was initiated 2-3 seconds before inserting the NMR tube in the 61 MHz benchtop NMR spectrometer, with a pulse angle < 0.1° and the $^1$H detector channel opened for 32 s. In these conditions, no radiofrequency pulse was applied to the HP pool so that the occurrence of RASER activity was spontaneous. The relaxation dynamics of RASER shows a variety of non-linear phenomena at play, such as a two-mode RASER regime with both H$_A$ and H$_B$ emitting allowing J$_{HAHB}$ to be measured directly.

Description of low field experiments with SLIC detection

1. Liquid phase

p-H$_2$ was bubbled into a solution of 272 mM EVE substrate and 4 mM Rh catalyst in CD$_3$OD placed in an NMR tube. The NMR tube was inserted in the 47.5 mT magnet before hydrogenation. The hydrogenation reaction was carried out for ~10 seconds before running the pulse sequence and acquisition. NMR spectra were acquired using automated Prospa software (Magritek, New Zealand) with a custom-made program for running Spin-Lock Induced Crossing (SLIC) rf pulse sequences, which transform overpopulated LLS into observable magnetization. SLIC was applied immediately after hydrogenation reaction and signal optimization.
was performed for RF amplitude, power and frequency (Figure 76a-c). $T_{\text{LLS}}$ of hyperpolarized DE in CD$_3$OD (Figure 76d) was measured with 200 ms SLIC pulses (“partial” SLIC) applied every 3.2 seconds.

2. **Gas phase**

p-H$_2$ gas was bubbled at a flow rate of 4000 sccm in a high-pressure glass column at 20 °C. The column was filled with pure EVE (5-10 mL) and the gas mixture was passed through the Rh/TiO$_2$ catalytic reactor at 170 °C. The resulting gas mixture was thermalized at 35°C when filling the 17 ml phantom hollow sphere placed in the NMR spectrometer. The filling was ceased by using valves #2 and #3 (Figure 74) immediately before application of the SLIC pulse sequence. Optimization of the SLIC signal was also performed for RF amplitude, power and frequency (Figure 76a-c). $T_{\text{LLS}}$ of hyperpolarized DE gas (Figure 76d) was obtained by monitoring the signal decay every second using 200 ms SLIC duration and a single gas refill.

**Chemical conversion, liquid fraction, and polarization levels**

3. **Chemical conversion of EVE to DE, $\alpha$**

If no evaporation were taking place, it would be convenient here to monitor the chemical conversion by measuring the ratio between the integrated signal intensity of the vinyl group ($S_{\text{CH}}$), which is unique to EVE, and its value before the reaction ($S_{\text{CH}}^0$). However, the evaporation of both EVE and DE is expected to be quite significant during the reaction. For a given reaction time, the chemical conversion
must therefore be assessed only within the thermal spectrum acquired after the reaction so that DE and EVE signal intensities are compared to each other but not to the initial concentration of EVE. Assuming that the integrated signal intensity of the overlapping EVE and DE methyl groups is proportional to \([EVE] + 2 [DE]\) and cumulates 3 protons of EVE and 6 protons of DE, the chemical conversion \(\alpha = [DE]/([EVE] + [DE])\), independent of evaporation, can be determined as:

\[
\alpha = (S_{CH3} - 3 \cdot S_{CH})/(3 \cdot S_{CH} + S_{CH3})
\]  

(27)

4. Liquid fraction, \(x\)

The fraction of EVE and DE remaining in the liquid phase, i.e., the liquid fraction \(x\), corresponds to the ratio \(S_{CH3}/S_{CH3}^0\) of the intensities of the methyl groups, with \(S_{CH3}\) normalized by the numbers of protons changing while EVE (3 methyl protons) is converted into DE (6 methyl protons). Hence, using chemical conversion \(\alpha\):

\[
x = \frac{S_{CH3}}{(1+\alpha) S_{CH3}^0} 
\]  

(28)

5. Enhancement, \(\varepsilon\) and polarization, \(P\)

Enhancement \(\varepsilon\) is calculated as follow:

\[
\varepsilon = (\exp^{15/TLLS} \cdot S_{DE}^{HP} \cdot [REF] \cdot 1.167)/(S_{REF}^{REF} \cdot [EVE]_0 \cdot x) 
\]  

(29)

with \(S_{DE}^{HP}\) the signal intensity of HP DE, \(S_{REF}^{REF}\) and \([REF]\) the signal intensity and concentration of the thermal reference sample (neat ethyl acetate), respectively, and \([EVE]_0\) the concentration of EVE before hydrogenation. 1.167 is a correction due to the difference of effective detection volume in the presence of the catheter for HP samples versus no catheter for thermally polarized samples. The mono-
exponential term $exp^{15/T_{LLS}}$ accounts for the relaxation losses occurring through the delay of 15 s necessary to avoid RASER activity (HP DE $T_{LLS} = 14$ s at Earth’s field). Polarization $P$ is computed by multiplying enhancement $\varepsilon$ with the thermal polarization of protons at 1.4 T, which is equal to $4.8 \times 10^{-4}$ %.
APPENDIX E

Supporting Information for Chapter 7, Quasi-Resonance Signal Amplification By Reversible Exchange.

Reprinted (adapted) with the permission from, Thomas Theis, Nuwandi M. Ariyasingha, Roman. V. Shchepin, Jake R. Lindale, Warren S. Warren and Eduard Y. Chekmenev. Quasi-Resonance Signal Amplification by Reversible Exchange. J. Phys. Chem. Lett., 9, 6136, 2018. Copyright (2018) American Chemical Society. In this chapter, “we” and other first-person plural pronouns are used in reference to all authors of this publication. My individual contributions to the research include data analyzing and writing parts of the manuscript. Figures and tables containing data contributed by researchers other than me will contain a disclaimer in the caption; figures and tables with no disclaimer in the caption contain data collected by me.

1. Computations of $^{15}$N signal enhancement and polarization levels

Computation of signal enhancements was very challenging at 0.05 T, because even highly concentrated sample would not provide any signal. Therefore, the signal enhancements were computed using lower limit estimate as the following.

**Metronidazole-$^{15}$N$_2$-$^{13}$C$_2$:** The highest SNR achieved was 1,270 at 20 mM concentration (Figure 4c). The concentration of $^{15}$N spins in signal reference sample (imidazole-$^{15}$N$_2$ prepared as described previously$^{298}$) was ~8 M in D$_2$O. The reference sample was placed in conventional standard-wall 5 mm NMR tube. Note the reference sample contain two labeled $^{15}$N sites. SNR of at least 2 is required to see any signal, but no signal was observed with 16 scans (square root of 16 is 4 for SNR purpose calculation). 1.85 is a factor related to different effective cross-section of the NMR tubes in HP and thermally polarized experiments.$^{100}$
Assuming that both $^{15}\text{N}$ sites were polarized by QUASR-SABRE in metronidazole-$^{15}\text{N}_2^{13}\text{C}_2$: we arrive to, $\varepsilon_{15\text{N}} = ((\sqrt{\text{# of scans reference}})/(\sqrt{\text{# of scans HP sample}}))^{*}(\text{HP SNR}/(\text{SNR Reference}))*1.85*([\text{reference}]/[\text{hyperpolarized sample}])*([\# of $^{15}\text{N}$ sites per molecule in reference sample}/[\# of $^{15}\text{N}$ sites per molecule in HP sample]) = (\sqrt{16}/\sqrt{8})*(1,700/2)*1.85*[8/0.02]*[2/2] = 9.0*10^5. Note this is the lower limit of signal enhancements, because we do not know how low thermal reference truly is.

$^{15}\text{N}$ thermal polarization at 49 mT and 298 K is 1.7*10^{-6}%. Therefore, $\%P_{15\text{N}} = \%P_{\text{thermal}}*\varepsilon_{15\text{N}} = 1.7*10^{-6}^%*9.0*10^5=1.5\%$. However, if only one $^{15}\text{N}$ site was polarized, the number below would be effectively doubled!. The integral signal value in Figure 44a (SABRE-SHEATH) is 0.162. The integral signal value in display Figure 44c (QUASR-SABRE) is 0.394. Next, using integral values we arrive to the following numbers for SABRE-SHEATH: $\varepsilon_{15\text{N}} = 9.0*10^5*0.162/0.394 = 3.7*10^5$ with $\%P_{15\text{N}} \sim 0.6\%$. One again, these are the lower limit numbers. Note, under similar conditions, the average $^{15}\text{N}$ polarization using high-field NMR spectroscopy for SABRE-SHEATH on metronidazole-$^{15}\text{N}_2^{13}\text{C}_2$ was 1.4%.297 These numbers are somewhat similar, which is a good news. The efficiency defined as $S(\text{QUASR-SABRE})/S(\text{SABRE-SHEATH}) = 2.43!$. Note if we are polarizing only one site via QUASR-SABRE versus two $^{15}\text{N}$ sites in SABRE-SHEATH, the efficiency is doubled to 4.86!
**Pyridine-^{15}N:** Using the numbers above we can also compute lower-limit values for $^{15}$N signal enhancement and polarization values for pyridine-^{15}N for both SABRE-SHEATH and QUASR-SABRE (since the integral numbers are similar: **0.143** and **0.145** for Figure 42a and Figure 42c respectively): \[ \epsilon_{^{15}N} = \left( \frac{[\text{metronidazole-}^{15}\text{N}_2^{13}\text{C}_2]}{[\text{pyridine-}^{15}\text{N}]} \right) \left( \frac{\text{(# of }^{15}\text{N sites in metronidazole-}^{15}\text{N}_2^{13}\text{C}_2)}{\text{(# of }^{15}\text{N sites in pyridine-}^{15}\text{N})} \right) \left( \frac{\text{integral signal value of pyridine-}^{15}\text{N}}{\text{integral signal value of metronidazole-}^{15}\text{N}_2^{13}\text{C}_2} \right) \] \[ \epsilon_{^{15}N} (\text{metronidazole-}^{15}\text{N}_2^{13}\text{C}_2) = \frac{(20/20)(2/1)}{(0.145/0.394)}*9*10^5 = 6.6*10^5 \text{ with } \%P_{^{15}N} \sim 1.1\%. \text{ Again, this is not far from what is expected in this concentration range.}^{99,290}

**Acetonitrile-^{15}N:** Using the numbers above we can also compute low-limit values for $^{15}$N signal enhancement and polarization values for acetonitrile-^{15}N for both SABRE-SHEATH (Figure 3a, signal integral value of **0.0525**) in a manner similar to that for pyridine-^{15}N calculation detailed above. \[ \epsilon_{^{15}N} = \left( \frac{20/40}{2/1} \right) \frac{(0.0525/0.394)*9.0*10^5 = 1.2*10^5 \text{ with } \%P_{^{15}N} \sim 0.2\%. \text{ And for and QUASR-SABRE (Figure 43c, signal integral value of **0.0230**):} \] \[ \epsilon_{^{15}N} = \left( \frac{20/40}{2/1} \right) \frac{(0.023/0.394)*9.0*10^5 = 5.3*10^4 \text{ with } \%P_{^{15}N} \sim 0.09\%.}

2. Details of shaped SLIC RF pulse preparation

The rectangular $^{15}$N RF pulse was calibrated on a hyperpolarized sample prepared via SABRE-SHEATH approach at 210.0 kHz resonance frequency. The calibration yielded a value of $t_{90\degree} = 260 \mu s$ at -33 db of power setting. This value corresponded to $ \sim 960 \text{ Hz of } B_1 \text{ power or } (\omega_1/2\pi)$. The TOMCO RF amplifier was
deemed to be linear all the way to -48 db (Figure S4c). As a result, the power setting employed (-40 db for all experiments) had a $B_1$ power of $(960/2.24) = 430$ Hz.

The SLIC pulse was designed in 100 equally spaced steps with the amplitude starting from 1.00, 0.99, 0.98, ..., 0.01 as a table. The shape of the RF pulse was tested on the oscilloscope. We note a minor droop in power at the end of the shaped pulse related to the RF amplifier non-linearity at very low power settings.

![Image of two shaped pulse on oscilloscope]

**Figure 77.** The photograph of two shaped pulse on the digital oscilloscope. Data for this figure were collected by Thomas Theis, Jacob Lindale and Warren S. Warren.
Figure 78. Supplemental pyridine-$^{15}\text{N}$ data. The experimental conditions were the same as those using to obtain the data shown in Figure 42. a) $^{15}\text{N}$ QUASR-SABRE signal dependence on the duration of the shaped pulse; g) $^{15}\text{N}$ QUASR-SABRE signal dependence on the duration of the delay. Note the individual spectra employed for figures in displays a and b were auto-phased, and the data is presented in the magnitude mode. Data were collected by Thomas Theis and Eduard Chekmenev.
**Figure 79. Supplemental acetonitrile-^{15}\text{N} data.** The experimental conditions were the same as those using to obtain the data shown in Figure 43. a) ^{15}\text{N} QUASR-SABRE signal dependence on the duration of the shaped pulse; b) ^{15}\text{N} QUASR-SABRE signal dependence on the duration of the shaped pulse; c) ^{15}\text{N} QUASR-SABRE signal dependence on the duration of the delay. Note different delay duration in displays a and b. Data were collected by Thomas Theis and Eduard Chekmenev.
Figure 80. Supplemental metronidazole-$^{15}\text{N}_2$-$^{13}\text{C}_2$ data. The experimental conditions were the same as those using to obtain the data shown in Figure 44. a) $^{15}\text{N}$ QUASR-SABRE signal dependence on the duration of the shaped pulse; b) $^{15}\text{N}$ QUASR-SABRE signal dependence on the duration of the delay; c) $^{15}\text{N}$ QUASR-SABRE signal dependence on the applied radio frequency power. Note the individual spectra employed for figures in all three displays were auto-phased, and the data is presented in the magnitude mode. Data were collected by Thomas Theis and Eduard Chekmenev.
APPENDIX F

Supporting Information for Chapter 8, Quasi-Resonance Fluorine-19 Signal Amplification By Reversible Exchange.


In this appendix all of the computational work was conducted by Jacob R. Lindale and Warren S. Warren and my contribution includes comparison of the data with the experimental data given in Chapter 8.

$^{19}$F QUASR-SABRE simulations

To begin, we describe the Hamiltonian for the following system:

\[ \hat{H}(t, t_p) = \sum_i \Delta \omega_i \hat{I}_{iz} + \Delta \omega_F (\hat{S}_{4z} + \hat{S}_{6z}) + \]

\[ 2\pi \left[ J_{HH} \hat{I}_{1z} \hat{I}_{2z} + J_{HH}' (\hat{I}_{1z} \hat{I}_{3z} + \hat{I}_{2z} \hat{I}_{5z}) + \right. \]

\[ \left. 2\pi J_{HF} (\hat{I}_{1z} \hat{S}_{4z} + \hat{I}_{2z} \hat{S}_{6z}) + J_{HF}' (\hat{I}_{3z} \hat{S}_{4z} + \hat{I}_{5z} \hat{S}_{6z}) \right] + \omega_{1F} \left( 1 - \frac{t}{t_p} \right) (\hat{S}_{4x} + \hat{S}_{6x}) \]

(30)

The $J_{HH}$ term is the coupling between the hydrides on the ortho-proton adjacent the $^{19}$F, $J_{HF}$ is the long range $^5J_{HF}$ coupling, and $J_{HF}'$ is the ortho $J$-coupling. The Hamiltonian is dependent on the pulse length, such that when $t = t_p$, $\omega_{1F} = 0$. The difficulty with this pulse sequence is that $\omega_{1F}(t_0) = 1.2 \text{ kHz}$, meaning that after one pulse has been applied, there is still a large amount of $^{19}$F magnetization remaining on the PTC, which when it experiences a $1.2 \text{ kHz}$ field, will oscillate rapidly. This requires very small (10 $\mu$s) $\Delta \tau$. Given that the system is
3-fluoropyridine, I have approximated the exchange rates for this system as $k_{d,N} = 40 s^{-1}$ and $k_{a,H} = 2 s^{-1}$ and am using 100% p-H$_2$ as the initial state of the system and to replenish the system.

![Graph](image)

**Figure 81. Simulation of 3 pulses with either a 10 µs (blue) or a 100 µs (red) step-size, and we see that the solutions begin to diverge significantly with each consecutive loop. The time-step will be $\Delta t = 10 \mu s$ for these simulations.**

**Pulse length scan with fully triangular pulse**

The pulse length will be scanned in attempt to re-create the data shown in Figure 83. The simulation will use a $\tau_{\text{DELAY}} = 2$ ms (200 steps) with a Hamiltonian that is on resonance with the $^{19}$F nuclei and hydrides, having maintained the $\sim 100$ Hz frequency shift between the hydrides and the ortho-protons, and neglecting the pulse ($\omega_{1F} = 0$). The scan range for this simulation will be $\tau_{\text{PULSE}} = [1, 70, \Delta 1]$ ms.

For a single step during a 1 ms QUASR pulse, $\Delta \omega_{1F} = -12$ Hz and is exactly what is used experimentally. This means any pulse length longer and this will give a more continuous approximation to the dynamics.
This is an unfortunate result when comparing it to experimental data (Figure 83), but it indicates that the non-linearities of the end of the ramp are sufficiently large to affect the dynamics. Thus, we must attempt to reproduce non-linearities in the rf output.

We are inclined to think that the poor match of theory to experiment, and the prediction of enhancements being about 2 orders of magnitude smaller than they should be, may be due to the fact that the ramp is non-linear. The plot below shows the last 3 loops of simulations from Figure 82.

**Figure 82. Hyperpolarization dynamics** as a function of the QUASR pulse length. These results should reproduce the experimentally observed signal dependencies and assume a perfectly triangular pulse.
Figure 83. Hyperpolarization dynamics for 3 pulses assuming various pulse lengths. The highly oscillatory structure is due to the high $B_1$ power at the beginning of each QUASR-pulse. This (essentially) generates rapid nutation at the beginning of each pulse until the SLIC condition is met, near the end of the pulse.

This data assumes a perfectly linear ramp for the entire duration of the pulse, but if the ramp is non-linearly approaching zero towards the end of the pulse, the probability of stopping on one of the maxima during shorter pulse lengths is significantly higher than for the higher pulses. This will introduce a larger error in the simulation at short time points. Furthermore, the difference in polarization that would be achieved having stopped on the short-pulse maxima is significantly higher than with the longer pulses. Once the pulse is actually on resonance, it no longer can produce a large deviation from equilibrium, which is why the magnitude of the longer pulses in the experimental data is so highly attenuated.
Figure 84. The fully quantum mechanical single-pulse solution (right top) shows no significant deviation between the two solutions until later pulse lengths. The RMSD between these solutions is 3.1%.

\[ 5J_{HF} vs (4J_{HH'} + 3J_{HF}) \] coherence pathways

One of the claims in the paper that QUASR-SABRE acts only via the \( 5J_{HF} \) coupling and that population flows through no other pathways, however: \( 5J_{HF} = 0.34 \) Hz whereas \( (4J_{HH'} + 3J_{HF}) = (1.31 + 9.2) \) Hz. Therefore, it is not so simple to discern if one of the two pathways dominates.

The difference between these pathways was simulated by running the full calculation (black), which allows both pathways, and then re-running the same with \( (4J_{HH'} + 3J_{HF}) = (0 + 0) \) Hz (red), so directing all population flow through the \( 5J_{HF} \) coupling simulation with \( (4J_{HH'} + 3J_{HF}) = (0 + 0) \) Hz (red), so directing all population flow through the \( 5J_{HF} \) coupling.
Figure 85. MPSR simulation data. (Left) A 20 ms pulse/2 ms MPSR simulation was run with 3 loops with (black) and without (red) the $^4J_{HH'} + ^3J_{H'F}$ couplings and showed a 4.8% RMSD between the solutions. (Right) A follow-up 20 ms pulse/2 ms MPSR simulation was run with 5 loops with these two systems and showed a 3.4% RMSD between the solutions.

It is most likely safe to conclude that the $(^4J_{HH'} + ^3J_{H'F})$ coherence pathway contributes only a small fraction to the total signal, and that the QUASR-SABRE signal is, in fact, mostly driven through the $^5J_{HF}$ coupling.

Non-linear ramp

The possible source of the error between theory and experiment could be the non-linearity of the pulse at low powers, which given that the largest oscillations observed in the dynamics happen in this power regime, this could significantly alter the results that we are getting. We see that this pulse droops significantly and is zero for the last portion of the pulse (assuming linearity at the top of the pulse). The pulse shape is approximated by:

$$S(t) = \frac{1}{2} \left( \frac{150}{180} \exp \left[ -\left( \frac{577t}{20000} \right)^2 \right] + \frac{30}{180} \exp \left[ -\left( \frac{577t}{40000} \right)^2 \right] \right) \left( 1 - \tanh \left( \frac{577t - 175}{30} \right) \right) \right)$$  (31)
This pulse shape was generated by qualitative match to the oscilloscope trace. As predicted, the perfectly triangular pulse (black) will basically keep back-pumping the system down, whereas the non-linear pulse (red) compensates for this as it dies out.

**Nonlinear pulse length scan**

The pulse length will be scanned in attempt to re-create the data shown in Figure 83 above, now using the non-linear pulse shape. The simulation will use a \( \tau_{\text{DELAY}} = 2 \text{ ms (200 steps)} \) with a Hamiltonian that is on resonance with the \(^{19}\text{F}\) nuclei and hydrides, having maintained the \(\sim 100\) Hz frequency shift between the hydrides and the ortho-protons. \(\tau_{\text{PULSE}} = [1, 70, \Delta 1] \text{ ms.}\)

The non-linearities allow a definite maximum to be found at \(\sim 20\) ms, in accordance with experimental data. The red trace shows the inhomogeneity averaged (pick only minima) signal obtained from each trace whereas the black signal is the last point (+ the delay) of the trace. We are unsure why the data are so highly oscillatory, and why it appears as if it changes with each sampling.

The data are so oscillatory because these oscillations are present in the full quantum solution, which each loop amplifies. This makes the trace highly structured in the same way. These data were generated with 10 \(\mu\text{s}\) steps as well, just to ensure that the oscillations were real.
Excitation profile

To explore the effect of the high-powered $^{19}$F pulse on the accumulated signal, an excitation profile was calculated for a single spin-1/2 nucleus. This was done for a single 20 ms pulse and leads to a ~52% attenuation of the acquired signal with the 30 Hz offset that was used experimentally.

All of the necessary code and simulation references may be found below.

Figure 86. Five loops of a 20 ms/2 ms simulation of the QUASR-SABRE pulse sequence.
Figure 87. **Multiple and single pulse simulation.** (Top) Multiple-pulse simulation of QUASR-SABRE dynamics using a non-linear pulse profile approximated in equation 31. (Bottom) Single pulse simulation of QUASR-SABRE dynamics.

Figure 88. **The free-species excitation profile** for the linear (red) and nonlinear (blue) pulses is shown to the right. Unfortunately, there is a 52% attenuation of the free-species signal when using a 30 Hz offset.
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There have been numerous studies to develop fast, cost effective hyperpolarized (HP) contrast agents with higher polarization values that can be potentially employed for magnetic resonance clinical imaging. Some of the currently used contrast agents are based on $^{129}$Xe hyperpolarized nucleus. However, one of the problems associated with this approach, is the high cost of the produced hyperpolarized contrast agents. In addition, the other important practical problem is that the currently used clinical MRI scanners are mostly designed for proton detection as opposed to the highly specialized MRI scanners designed for research purposed with $^{13}$C, $^{129}$Xe, etc. detection capability. Therefore, it is important to investigate the possibility of using proton-hyperpolarized hydrocarbon molecules for their utility as MRI contrast agents that can be
visualized with currently available MRI scanners. One key interest in MRI research is the development of inhalable contrast agents that can be employed to a patient on a single breath hold for pulmonary image acquisition. These images can be used to probe lung functions (ventilation and diffusion) therefore identifying any abnormalities and diseases such as chronic obstructive pulmonary disease (COPD) and emphysema in early stages of these diseases, when intervention can significantly improve patients’ outcomes.

One first overarching goal of this work was to prepare cost-effective, bio-compatible, sufficiently long-lived contrast agents using scalable hyperpolarization methods relying on parahydrogen as a source of hyperpolarization. These agents can potentially be employed as inhalable contrast agents on clinically available MRI instruments with proton detection capability. I demonstrated that HP propane can be produced via propylene hydrogenation with 100% chemical conversion thus can be employed as a successful inhalable HP contrast agent with high polarizations values. Moreover, long-lived spin states (LLS) of HP propane can be created via both propylene and cyclopropane hydrogenation methods. Large animal model such as sheep will be performed to further study HP propane’s ability as an inhalable HP agent during the next three years. The study of inhalable contrast agents was extended to other molecules like, previously used anesthetic diethyl ether proving that long lived spin states of HP diethyl ether can also be generated at low magnetic fields. This demonstration proves that proton LLS of HP gases is a general phenomenon. Moreover, a low cost, high throughput
preparation method of HP diethyl ether was demonstrated. A thorough kinetic study was conducted to show that highest polarization values (~8 %) were feasible and complete chemical conversion of ethyl vinyl ether to HP diethyl ether is achieved within a few seconds. Future developments of decreasing the flammability of diethyl ether can lead to its biomedical application as an HP MRI contrast agent.

The other interest is the preparation of injectable contrast agents (intravenous (IV) contrast agents) in order to image metabolism or abnormalities of organs/tissues. Currently, hyperpolarized contrast agents for metabolism interrogation are based on the hyperpolarized $^{13}\text{C}$ nucleus. The goal of my work is to employ hyperpolarized $^{15}\text{N}$ nucleus in FDA-approved drugs. As opposed to $^{13}\text{C}$, $^{15}\text{N}$ offers the advantages of lower cost and longer lifetime of hyperpolarized state in vivo.

Therefore, the second goal is the efficient preparation of injectable MRI contrast agents, which can be used to probe metabolism and related abnormalities. e.g. metronidazole, which is a commonly used FDA-approved antibiotic. In-tumor hypoxia has been correlated with poor outcome in many cancers. Therefore, $^{15}\text{N}$-hyperpolarized metronidazole was investigated towards its feasibility as a potential contrast agent to probe hypoxia using MRI. Quasi-resonance signal amplification by reversible exchange (QUASR-SABRE) method showed successful polarization values (2-fold higher than those in SABRE-SHEATH (SABRE in shield enables alignment transfer to heteronuclei) approach) for metronidazole antibiotic thus indicating a more effective polarization transfer method via SABRE
hyperpolarization technique. This finding gives hope for achieving near-unity polarization values using QUASR- SABRE approach for a broad spectrum of biomolecules making them promising candidates of hypoxia sensing probe and probes for potentially for other applications.

In addition to the above motioned goals, there is also a need to understand approaches of successful polarization transfer methods from protons into heteronucleus in order to be able to improve the detection schemes of HP contrast agents. I investigated these approaches in my work using fluorinated N-heterocyclic molecules. I demonstrated successful polarization transfer from p-H$_2$ derived hydrides to $^{19}$F nuclei via weak five-bond H-F spin-spin couplings.

Furthermore, radio amplification of stimulated radiation (RASER) activity is reported for low concentrated (~40 mM) substrate molecules using a commercial bench-top NMR spectrometer (at 1.4 T) without an RF pulse under both adiabatic longitudinal transport after dissociation endangers net alignment (ALTADENA) and parahydrogen synthesis allows dramatically enhanced nuclear alignment (PASADENA) conditions, enabling a wide range of potential applications including MR imaging, quantum computing and beyond.
AUTOBIOGRAPHICAL STATEMENT

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